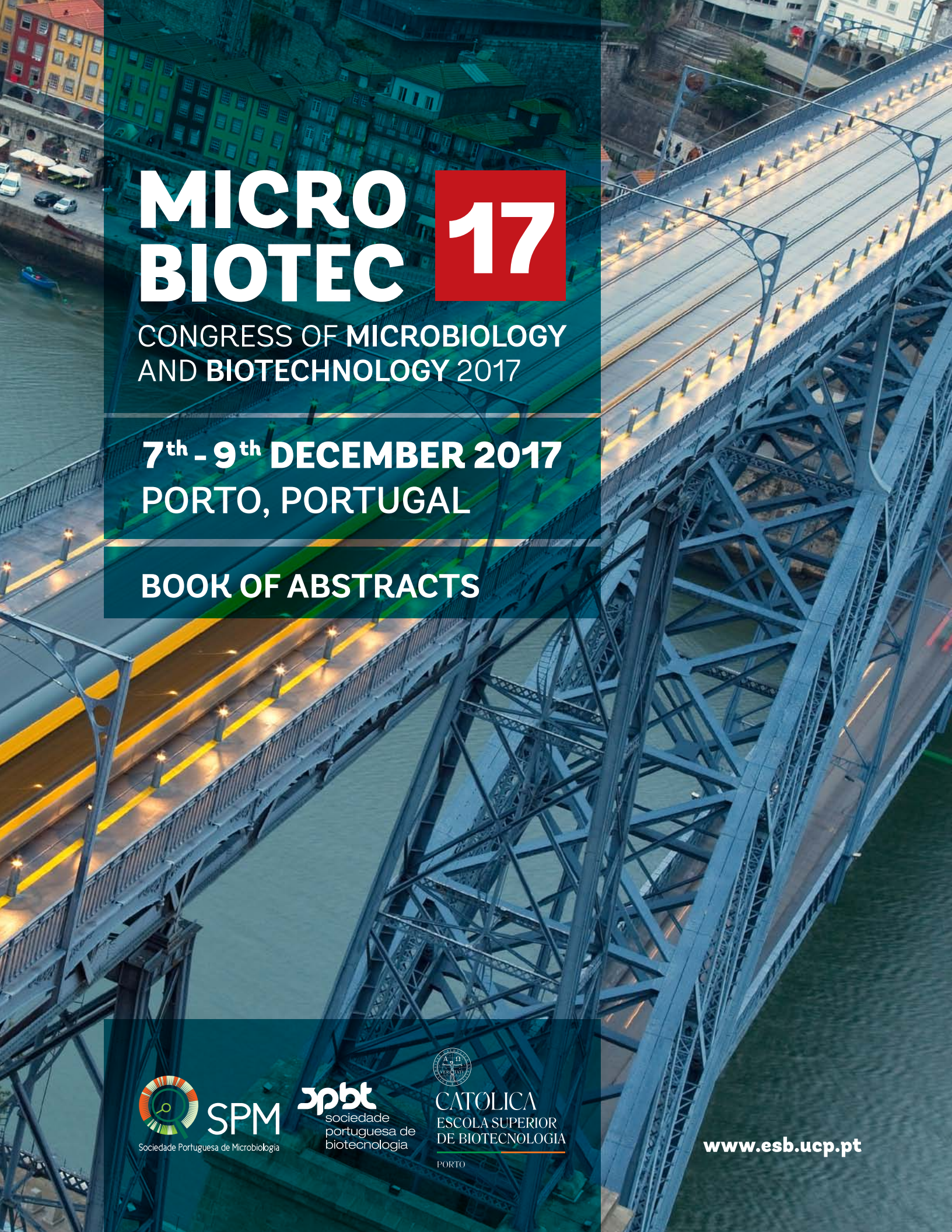

MICRO BIOTEC 17

CONGRESS OF **MICROBIOLOGY** AND **BIOTECHNOLOGY** 2017

7th - 9th DECEMBER 2017
PORTO, PORTUGAL

BOOK OF ABSTRACTS

SPM
Sociedade Portuguesa de Microbiologia

spbt
sociedade portuguesa de biotecnologia

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ESCOLA SUPERIOR DE BIOTECNOLOGIA
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CATÓLICA
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Dear MICROBIOTEC participant,

It is for us a great pleasure to host you for almost three days at the Universidade Católica Portuguesa - Porto! Every MICROBIOTEC edition is built based on the interaction of a multidisciplinary consortium, brought together by the Portuguese Societies of Microbiology and of Biotechnology. The result is the establishment of a forum of discussion where Microbiologists and Biotechnologists meet, discuss ideas and plan future projects. But, MICROBIOTEC is not only science and technology, it is also a moment to meet colleagues and friends, some of which we only see at MICROBIOTEC!

For all these reasons, we hope that MICROBIOTEC'17 will be another enjoyable Congress.

We will have 6 plenary sessions, 14 keynote speakers, 42 oral presentations, 1 round table, more than 3 hours of posters discussion, several satellite events, as well as some warm moments to praise special Microbiologists or Biotechnologists!

Welcome to MICROBIOTEC'17!

The conference Co-Chairs

Célia Manaia

Manuela Pintado

Contents

ENGINEERING WITH MEMBRANES: INSPIRED BY NATURE?

João G. Crespo

LAQV-Requimte, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Campus de Caparica, 2829-516 Caparica, Portugal
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This lecture discusses the development of synthetic membranes in order to accomplish specific tasks, which can range from selective bioconversion processes, namely when enantiomeric-specific conversions are required, to situations where target bioactive compounds are selectively transported, recovered and/or transformed.

Membranes may offer a particular adequate environment to retain or accommodate biocatalysts, providing them suitable conditions to express their bioactivity properties (solute recognition, selective bioconversion) without affecting significantly their activity / molecular conformation and resulting function(s). The challenge relies exactly on the ability to design adequate membrane environments where biocatalysts may express their bioactivity potential under stable conditions. This challenge requires the development of suitable membrane materials and operating conditions that assure a minimum change of biocatalysts' activity and structure, which should correspond to a minimum impact on their function.

Membranes offer also the possibility to organize the physical space making possible to design dedicated systems with specific transport and catalytic properties. Several examples will be presented and discussed, showing the potential of using membranes able to establish specific interaction with target solutes.

Finally, dedicated techniques able to provide non-invasive, on-line, real-time monitoring of membrane transport and biotransformation will be presented and discussed. A particular emphasis will be given to the use of natural fluorescence techniques..

PLENARY

ROLE OF RAPID DIAGNOSTICS IN PREVENTION AND CONTROL OF MULTI DRUG-RESISTANT BACTERIA

Laurent Poirel

Medical and Molecular Microbiology Unit, Dept of Medicine Swiss National Reference Center for Emerging Antibiotic Resistance University of Fribourg, Switzerland

Drug resistance among bacteria is a scourge for patients, infectious diseases and infection control specialists. Rapid detection of resistance constitutes a challenge for clinical microbiologists to prevent deleterious individual and collective consequences as (i) delaying efficient antibiotic therapy that worsens the survival of the most severely-ill patients, or (ii) delaying isolation of carriers of multidrug-resistant bacteria and promoting outbreaks. From simple cheap biochemical test to whole-genome sequencing, clinical microbiologists must select the most adequate phenotypic and genotypic tools to promptly detect and antibiotic resistance from cultivated bacteria or from clinical specimens. Many different technical approaches can be used for these purposes, including molecular, biochemical and immunologic assays. The continuous implementation of newly-developed diagnostic techniques in clinical laboratories is crucial in order to detect, control, and therefore prevent dissemination of multidrug-resistant bacteria.

PLENARY

SYNTHETIC BIOLOGY FOR FUNGI

Merja Penttilä

VTT Technical Research Centre, Finland

Yeast and filamentous fungi have traditionally been important organisms in biotechnology, and they have increased their importance in modern biorefineries in production of fuels, chemicals, material components, industrial enzymes, and high-value molecules including pharma products. The concepts and methods of synthetic biology make development of production organism much more rapid and cheap. This increases the possibilities for various companies to apply biotechnology, and also many chemical and energy companies have strong interest to biotechnology today. We have engineered various yeasts and filamentous fungi as production hosts. More recently, we have developed genome editing tools, synthetic orthogonal programmable promoters and expression circuits for a broad range of fungal species. The use of the synthetic biology tools and production of chemicals, enzymes and material proteins such as synthetic silk will be discussed.

ULTIMATE ORIGIN OF LEADING ANTIBIOTIC RESISTANT PATHOGENS REVEALED THROUGH GENOMICS: FOR ENTEROCOCCI, IT STARTED LONG AGO

Michael S. Gilmore - Sir William Osler Professor

Departments of Ophthalmology, and Microbiology and Immunology, Harvard Medical School, Boston, MA USA

Enterococci are leading causes of multidrug resistant hospital infection. However, in nature, enterococci are among the most widely distributed core components of gut flora of animals, from invertebrates and insects to mammals. Despite being numerically minor constituents of animal gut microbiota, enterococci emerged among the vanguard of multidrug resistant hospital adapted pathogens. Interestingly this happened twice: in *Enterococcus faecalis*, and in the distantly related species *E. faecium*. This raises two questions: 1) What are the core properties of enterococci that make them universal components of gut consortia of animals? and 2) Why, among the great diversity of gut microbes, did enterococci repeatedly emerge to become leading causes of multidrug resistant hospital acquired infection? With antibiotic resistance now a leading global public health threat, there is a compelling need to understand the underlying biology and genetics that led to their hospital adaptation.

To accomplish this, we examined 25 enterococcal species, representing all major phylogenetic branches of the genus, in detail for phenotype, genotype, and where possible, correlated that with host association. We further compared traits of both commensal and multidrug resistant strains of the most common human associated species, *E. faecalis* and *E. faecium*. We found that the enterococci acquired the ability to withstand episodic desiccation and starvation, among other stressors, and that the formation of new enterococcal species occurs in parallel with the evolution of new host animals with new diets. Calibration of divergence times indicates that enterococci arose at about the time of terrestrialization of animals, and parallels their radiation, including fluctuations as occurred during the Permian Extinction. We infer that in adapting to cycles of deposition on land, the enterococci acquired traits that positioned them well for survival and adaptation to the modern hospital environment. We have identified the corresponding genes, and are now exploring the underlying mechanisms for their survival in hospitals.

PLENARY

ILLUMINATION OF MICROBIAL DARK MATTER IN ENVIRONMENTAL BIOTECHNOLOGY

Per Halkjær Nielsen

Center for Microbial Communities, Department of Chemistry and Bioscience, Aalborg University, Aalborg, Denmark

Environmental biotechnology is based on the application of microbes to solve environmental challenges, and it includes systems such as biological wastewater treatment, production of bioenergy and bio-based compounds, and removal of micropollutants. Most of the microbes are, however, completely undescribed in terms of identity and function, so-called microbial dark matter (MDM). Novel DNA-tools are revolutionizing our capabilities to illuminate these microbes and how they are key players in a variety of ecosystems from human health to resource recovery. I will present some examples on how we develop and apply novel approaches to provide insight in diversity and function of bacteria, archaea and eukaryotes, primarily related to nutrient recovery and bioenergy production. The methods include meta-omics methods, and visualization techniques such as Raman microspectroscopy. Furthermore, it be demonstrated that "online" surveillance and informed control of microbial communities is now possible, and with the retrieval of complete genomes of important MDM in relevant ecosystems, information about identity, physiology, and interactions will support informed manipulations of relevant ecosystems in environmental biotechnology.

PLENARY

NEXT-GENERATION PLATFORMS FOR STRAIN OPTIMIZATION: REDUCING THE COST AND TIME FOR COMMERCIALIZING NOVEL BIOLOGICAL MOLECULES

Sunil Chandran

Senior Director, R&D, Amyris, Inc., USA

Biology is unparalleled in its molecular diversity and wide application space. There are however numerous challenges associated with realizing the full potential of this molecular diversity. Optimization of microbial production of any biological target requires repeated iterations of the design-build-test-analyze engineering cycle. The rate at which any team can execute the engineering cycle directly affects the development time for any product. Similarly, the magnitude of strain improvement in each cycle impacts overall costs and development time. At Amyris, advanced tools for strain engineering, high throughput screening, analytics, and bioinformatics have been developed over the years to rapidly accelerate the engineering cycle and reduce the number of necessary iterations. With these capabilities, scientists at Amyris can efficiently optimize multiple biological pathways simultaneously. This presentation will cover details of the next-generation strain engineering platforms that enable Amyris scientists to rapidly iterate through multiple cycles of the strain optimization process, as well as the engineering of metabolic pathways to convert naïve yeast strains into commercial scale production hosts for various molecules.

KEYNOTE SPEAKER

Genomics and Systems Biology and Emerging Technologies

ADDRESSING THE MANUFACTURING CHALLENGES OF STEM CELL-BASED THERAPIES THROUGH BIOENGINEERING

Cláudia Lobato da Silva

Department of Bioengineering and iBB-Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Lisboa, Portugal

Cell Therapy and Regenerative Medicine have progressed significantly over the last years introducing novel methods of clinical intervention to replace, repair or restore the lost function of tissues and organs. Life sciences and engineering would allow the translation from the concept of a medical intervention in a complex disease scenario, to the manufacture of safe and effective innovative medicinal products towards and realization of the economical and societal impact of these strategies for disease intervention.
The robust and scalable cell manufacturing for the cost-effective delivery of safe and potent cell-based products (either autologous (i.e. cells from the patient) or allogeneic) relies on process engineering tools to understand the impact of cellular features (biological, biochemical, etc) on cell product function and performance, and how do process variables influence the critical quality attributes of the cell product. Examples include therapies using mesenchymal stem/stromal cells, hematopoietic stem/progenitor cells or lymphocytes that were selected and cultured (*i.e.* substantially manipulated) before administration into patients. In this lecture, the main focus will be on the manufacturing of human mesenchymal stem/stromal cells using bioreactor technology.

KEYNOTE SPEAKER

Molecular Microbiology and Microbial Physiology

INVESTIGATING THE BIOTECHNOLOGICAL POTENTIAL OF THERMOPHILES FROM HOT SPRINGS THROUGH METAGENOMICS

Conceição Egas

Center for Neuroscience and Cell Biology, University of Coimbra and Biocant

The global market for industrial enzymes is highly dynamic and profitable. With $5 billion USD in 2016, it is expected to reach $6.3 billion in 2021, at a compound annual growth rate of 4.7%. The market is led by carbohydrolases, proteases and lipases, but also specialty enzymes. To achieve the expected growth, the market needs the continuous input of new or improved enzymatic activities. Microbes from extreme environments have adapted their metabolism to thrive in a range of extreme conditions representing new bioprospecting sources. Only a small fraction of these organisms can be cultivated in laboratory conditions, leaving out a significant segment of microbes unexplored.
Our studies focus on the composition and function of microbial communities from Portuguese geothermal springs through metagenomics. Samples collected from S. Pedro do Sul and Chaves had different bacterial communities, dominated by members of the Nitropirae phylum at S. Pedro do Sul and Aquificae phylum in Chaves. In S. Pedro do Sul the most abundant operational taxonomical unit was new and of the Nitrospirales order. In Chaves, the most represented operational taxonomical unit was of the Hydrogenothermaceae family. Members of the Miscellaneous Crenarchaeotal Group were the most abundant Archaea in both geothermal springs. Most of these microorganisms have been scarcely studied and provide an important resource for the discovery of new enzymes with potential industrial application.

Acknowledgments
This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement No. 685474.

KEYNOTE SPEAKER

Bioprocess Engineering

FACING THE CHALLENGES OF DOWNSTREAM PROCESS OF NEW BIOPHARMACEUTICALS

Cristina Peixoto

CICS-UBI – Health Sciences Research Centre, Universidade da Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã, Portugal

Complex biopharmaceuticals, such as virus or stem cells are gaining interest as new therapeutic agents. These novel products cannot be process with the same technology as the conventional biopharmaceuticals, such as therapeutic proteins. Therefore, a new set of challenges for process developers and regulating authorities need to be address, in order to ensure final product quality, efficacy, and safety. With the process costs shifting towards the downstream part of manufacturing, the spotlight has turned to chromatography, commonly looked as the workhorse of downstream processing and the current standard in purification of animal cell culture-based biopharmaceuticals. This unit operation can account up to 50-80% of the total processing costs, making the development/improvement of chromatographic strategies of paramount importance.

Additionally, virus or stem cells purification requires a deeper understanding the complexity of these systems. Strategic design and step by step optimization of the purification process is crucial to maximize yield and quality of the final product. Considerable progress has been perform in the area of downstream processing over the past decade. The use of scaled-down tools for characterization of product interactions, mechanistic modelling to facilitate optimization of the processing steps and establishment of single-use concepts together with the introduction of continuous DSP methods will play an important role as well as a tighter process integration in terms of the up- and downstream process.

Concepts for downstream processing of such new and large assemblies will be discussed and several case studies will be presented.

KEYNOTE SPEAKER

Environmental Microbiology and Biotechnology session

A THREE-ACT PLAY: FUNGI, CHEMICALS AND DEMISE

Cristina Silva Pereira

Instituto de Tecnologia Química e Biológica António Xavier (ITQB NOVA); Oeiras, Portugal

This lecture will be presented as a three act play, comprising Setup, Confrontation and Resolution. I will briefly review some of our major findings and current hypothesis, underlining critical steps that influence our research path. My group - Applied and Environmental Mycology - was initially launched with a strong biotech focus, particularly on the exploitation of fungi and fungal system for biotechnological applications in biodegradation and bioremediation. However, our research has evolved significantly, and, at present, our major research is focussing central questions in fungal biology and ecology. Particularly, we are investigating how chemical effectors (either natural or anthropogenic) transcriptionally modulate fungal development with emphasis on their ecological resilience and pathogenicity. Our most recent studies revealed the ecological role of the functioning of belowground fungal communities; the assignment, at a gene level, of the central pathway for the catabolism of aromatics in fungi; the impact of plant defensive polyester macromolecules in the life cycle of fungi; and chemical stimuli that augment secondary metabolite diversity by de-silencing cryptic genes and that modulate the biosynthesis of sphingolipids potentially influencing fungal resistance to antifungals. Overall, our vision is to establish knowledge foundation for the development of novel and efficient antifungal therapies. Fungi kill annually more than 1.5 million people, worldwide. The available antifungal therapies are totally insufficient to fight the emergence of novel fungal pathogens and the continuous growth of the most vulnerable population, immunocompromised/suppressed patients of all ages.

KEYNOTE SPEAKER

Cellular Microbiology and Pathogenesis

HOW *LISTERIA* MODIFIES ITS SURFACE TO PROMOTE INFECTION

Didier Jacques Cabanes

i3S - Instituto de Investigação e Inovação em Saúde

Listeria monocytogenes is a Gram-positive pathogen responsible for the occurrence of human listeriosis, an opportunistic foodborne disease with an associated high mortality rate. The key to the pathogenesis of *Listeria* is the capacity of this bacterium to trigger its internalization by non-phagocytic cells and to survive and even replicate within phagocytes. The arsenal of virulence proteins deployed by *L. monocytogenes* to successfully promote the invasion and infection of host cells has been gradually unveiled over the past decades. A large majority of them are located at the cell envelope, which provides an interface for the establishment of close interactions between these bacterial factors and their host targets. As the major scaffold for surface proteins, the cell wall and its metabolism are critical elements in listerial virulence. Conversely, the crucial physical support and protection provided by this structure make it an ideal target for the host immune system. Therefore, mechanisms involving fine modifications of cell envelope components are activated by *L. monocytogenes* to render it less recognizable by innate immunity sensors or more resistant to the activity of antimicrobial effectors. We will present our last results regarding the mechanisms used by *L. monocytogenes* to organize its surface to promote virulence.

KEYNOTE SPEAKER

Health Microbiology and Biotechnology

BIOPHARMACEUTICALS PURIFICATION BASED ON AMINO ACIDS-NUCLEIC ACIDS INTERACTIONS

Fani Sousa

CICS-UBI – Health Sciences Research Centre, Universidade da Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã, Portugal

The concept of using nonviral nucleic acid-based biopharmaceuticals as advanced medicinal products is attracting an ever-growing interest due to their unmatched safety and extraordinary potential to create breakthrough treatments for incurable diseases with worldwide prevalence. Actually, various scientific reports and ongoing clinical trials document the relevance of using genetic material, based on double-stranded DNA or RNA (iRNA and mRNA), as active biopharmaceuticals to ultimately accomplish a therapeutic effect. Gene-based therapeutics are, thus, novel and complex products that can offer unique challenges in product development. The recombinant production of biopharmaceuticals has already been established, however the envisioned therapeutic application of pDNA, mcDNA or RNA requires their recovery as highly pure products, to accomplish the strict quality criteria established by regulatory agencies. So, the challenges related to the purification of biopharmaceuticals motivated the exploitation and combination of the unmatched binding capacity of monoliths and selectivity of affinity techniques. As it will be discussed, amino acid-based affinity chromatography was implemented as a chromatographic approach to first purify pDNA, and more recently for the purification of mcDNA and RNA, using amino acids as specific ligands. In general, the selectivity achieved with the amino acids ligands towards the target nucleic acids allows their purification from complex Escherichia coli lysates. This selectivity is usually associated to the establishment of multiple non-covalent interactions, resulting in the biorecognition of the biologically active biomolecules (supercoiled isoforms of pDNA or mcDNA and target RNAs). Moreover, the use of monoliths enabled the improvement of the global performance of the purification methodology, by increasing the binding capacity and decreasing the chromatographic run-time, which contributes for the recovery of biopharmaceuticals with the required stability and biological activity.

KEYNOTE SPEAKER

Genomics and Systems Biology and Emerging Technologies

IN SILICO DESIGN OF IMPROVED CELL FACTORIES – NEW METHODS AND EXPERIMENTAL VALIDATION

Isabel Rocha

Centre of Biological Engineering, Department of Biological Engineering, University of Minho, Braga, Portugal; Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa (ITQB-NOVA), Oeiras, Portugal

Industrial Biotechnology is increasingly replacing chemical processes in numerous industrial sectors, since it allows the use of renewable raw-materials and provides a more sustainable manufacturing base. The field of Metabolic Engineering (ME) has thus gained a major importance by providing tools for the design of improved microorganisms for industrial applications. Currently, many Metabolic Engineering problems are approached using genome-scale metabolic models, which have a wide variability regarding predictive capacity.
Metabolic model predictions broadly rely upon well performed gene annotations. To aid in that task and in metabolic model reconstruction, we have previously developed the merlin framework, an open source Java software tool, distributed at www.merlin-sysbio.org. The new version of merlin allows to perform automated annotations of enzyme and transport functions, as well as protein localization based on customizable parameters.
Strain simulation is usually performed by using Genome-scale stoichiometric models and Linear or Quadratic Programing methods that assume a steady state over the intracellular metabolites. However, a systematic evaluation of the predictive capacities of the available genome-scale models and simulation tools has not been performed. We have performed a thorough analysis of in vivo data of S. cerevisiae regarding essentiality, flux distributions and auxotrophies and have concluded that many of the available ME tools do not allow to make accurate predictions, ultimately leading to ineffective ME strategies. We also propose novel tools for the reconciliation of experimental data with model predictions.
Finally, as an example of application of in silico metabolic engineering strategies, we have combined the use of genome-scale metabolic models with a multi-objective metaheuristic approach, identifying several gene deletion targets for growth-product coupling of a family of C4-dicarboxylic acids. Four multi-gene deletion strain designs, including the chassis cell and the final producer strains, were implemented and experimentally tested. Thus, we were able to generate pre-optimized backbone strains for enhanced production of different platform chemicals derived from the same metabolic pathways, hence showing that modular design strategies may contribute to accelerate metabolic engineering tasks.

KEYNOTE SPEAKER

Health Microbiology and Biotechnology

HOST-PATHOGEN ARMS-RACE: THE WITHIN-PATIENT GENETIC VARIATION OF MICROBIAL PATHOGENS ON THE COURSE OF INFECTION

João Paulo Gomes

Instituto Nacional de Saúde Doutor Ricardo Jorge

During infectious diseases, an intricate of interactions between the microbial pathogen and the host (e.g. human being) takes place. Whereas the human being uses the immune system to eliminate the threat, on the other hand, the pathogen "modifies its genome in order to escape the immune pressure and keep surviving". This is usually designated as the human-pathogen "arms-race". Besides the horizontal gene transfer of genetic material, microbial genomic mutations are generated through a random process typically associated with errors of the DNA polymerase during pathogen's replication. Only a very small fraction of these mutations turn out to be beneficial by providing the pathogen with advantageous characteristics to deal with the human being. These may include, for instance, the in vivo antigenic modification, activation of alternative metabolic capacities, modification of flagella, and acquisition of antibiotic resistance. These more fitted clones are the ones that may become more invasive and infect other organs, potentially causing septicemia and sometimes patient's death. With the recent development and application of the whole genome sequencing methodology, it is now possible to better understand the human-pathogen "arms-race" by strongly increasing our knowledge on the genetic changes that a microbial pathogen undergoes since the initial stages of infection and during disease progression. This keynote presentation aims to provide a literature review on clinically relevant cases, highlighting the extraordinary capability of the pathogen's adaptation to the human being throughout the infectious process. Unfortunately, it seems that the pathogens always finds a way…

KEYNOTE SPEAKER

Cellular Microbiology and Pathogenesis

CONVERGENT EVOLUTION OF *BURKHOLDERIA MULTIVORANS* DURING LONG-TERM CHRONIC INFECTION OF CYSTIC FIBROSIS LUNG

Leonilde M. Moreira

Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Lisboa

Chronic bacterial respiratory infections are the leading cause of morbidity and mortality among cystic fibrosis (CF) patients. The thick mucus covering the CF airway is an ideal environment for a polymicrobial community, including opportunistic pathogens such as *Pseudomonas aeruginosa*, and the *Burkholderia cepacia* complex species, to develop. Tracing bacterial evolution during these long-term infections can provide insights into how host selection pressures, such as antimicrobial therapies and the immune system, shape bacterial genomes (1). We performed genomic and phenotypic analysis of 128 longitudinally collected *Burkholderia multivorans* isolates from nine CF patients spanning a period of 7-20 years. Genome analysis within each patient showed coexisting clades with distinct evolutionary dynamics. Evidence of clonal lineages shared by some patients was observed suggesting inter-patient transmission. We also observed recurrent genome reduction, with deletions ranging from few nucleotides to 200 kb, including plasmid losses. Several loci, mostly involved in gene expression regulation, lipid metabolism, and cell wall biosynthesis were identified as likely targets of selection. Further, a broad range of phenotypes changed in association with the evolved mutations; they included antimicrobial resistance, biofilm regulation, motility, and presentation of lipopolysaccharide O-antigen repeats. This study provides the first comprehensive genome-phenome analyses of *Burkholderia multivorans* infections in CF lungs and defines the "chronic infection phenotype" as slow growth, higher adhesion to surfaces, reduced motility, greater antibiotic resistance, and reduced virulence. Identifying traits under strong selection during chronic infection not only sheds new light onto *Burkholderia* evolution, but also sets the stage for tailored therapeutics targeting prevailing lineages associated with disease progression.

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KEYNOTE SPEAKER

Genomics and Systems Biology and Emerging Technologies

INTENSIFICATION OF 2G BIOETHANOL PROCESS: YEAST DEVELOPMENT TO OVERCOME CHALLENGES DERIVED FROM LIGNOCELLULOSIC PROCESSING

Lucília Domingues

CEB - Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal

The astonishing growth of world population, the global climate changes and the depletion of fossil fuels are strong drivers for the development of a resource-efficient and sustainable economy. Lignocellulosic biomass valorization through the so-called biorefineries arises as one of the paths for such purpose. The main driver for the development of lignocellulosic biorefineries is sustainability, but to make these bioprocesses economically feasible, intensified and flexible processes, responsive to feedstock and market fluctuations, have to be considered. These lead to highly demanding operational conditions both for the cells and enzymes used in the process.
The development of robust yeast cell factories, through genetic and modern metabolic engineering tools, able to cope with the stress imposed by these processes is envisioned as a vital platform for converting the array of sugars released from biomass into biofuels and biochemicals. This seminar presents an overview of the second generation ethanol biorefineries challenges and how genetic engineering strategies can help surpass them. In this regard, examples of intensified and productive conditions attained by metabolic engineering strategies applied over robust industrial yeast chassis are given. The presented results attest the feasibility of intensifying biomass-to-ethanol processes and show how this integrated strategy has the potential to be the driver for the emergence of economical and sustainable processes for high-value chemicals' production from lignocellulosic biomass.

Acknowledgements
The financial support of the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UID/BIO/04469/2013 unit, COMPETE 2020 (POCI"01"0145"FEDER"006684), BioTecNorte operation (NORTE"01"0145 "FEDER" 000004) and MultiBiorefinery project (POCI"01"0145"FEDER"016403) is acknowledged.

KEYNOTE SPEAKER

Bioprocess Engineering

PURIFICATION OF BIOACTIVE AND VALUE-ADDED COMPOUNDS USING IONIC-LIQUID-BASED STRATEGIES

Mara G. Freire

CICECO - Aveiro Institute of Materials, Chemistry Department, University of Aveiro, 3810-193 Aveiro, Portugal. maragfreire@ua.pt

Over the past decades, ionic liquids (ILs) have been described as "greener" solvents over conventional volatile organic solvents due to their non-volatile nature at ambient conditions. Furthermore, the large number of possible ions' combinations, with highly distinct chemical structures, allows their tuning, and thus, ILs can be designed for a particular application or to present a specific set of thermophysical properties. Due to these features, ILs have been largely investigated as promising media for the separation (purification) of bioactive compounds from the most diverse origins. ILs have been studied as solvents, co-solvents, co-surfactants, electrolytes, and adjuvants, in liquid-liquid systems, as well as used in the creation of IL-supported materials [1-2]. In this work, the main results achieved by the use of IL-based processes in the separation/purification of a large range of bioactive compounds (including small organic extractable compounds from biomass, lipids, and other hydrophobic compounds, proteins, amino acids, nucleic acids, and pharmaceuticals) will be overviewed. The key accomplishments and future challenges to the field will be highlighted.

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Acknowledgements

This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (FCT Ref. UID/CTM /50011/2013), financed by national funds through the FCT/MEC and FEDER under the PT2020 Partnership M.G. Freire acknowledges the European Research Council under the European Union's Seventh Framework Programme (FP7/2007-2013) / ERC grant agreement n° 337753

KEYNOTE SPEAKER

Molecular Microbiology and Microbial Physiology

TOWARDS A NEW VACCINE AGAINST MALARIA

Miguel Prudêncio

Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Avenida Professor Egas Moniz, 1649-028 Lisboa, Portugal

Whole-sporozoite (WSp) immunization is currently the most effective strategy to induce sterile protection against malaria. However, current WSp malaria vaccines rely on the effective attenuation of deadly Plasmodium falciparum (Pf) sporozoites. In this talk, I will describe a novel approach to WSp immunization using genetically modified rodent P. berghei (Pb) parasites as malaria vaccination platforms. In a series of pre-clinical studies, we show that Pb sporozoites unabatedly infect and develop in human hepatocytes but are unable to establish a blood-stage infection, a safety requisite for an attenuated vaccine. We engineered PbVac, a Pb parasite expressing the Pf circumsporozoite (CS) protein, the major Pf immunogen, at the surface of sporozoites and hepatic parasite stages. We established an innovative rabbit model, susceptible to hepatic infection but not to blood infection by Pb, to show that PbVac elicits substantial cross-species cellular immune responses and functional PfCS-dependent antibody responses, which can efficiently inhibit Pf infection. Safety and regulatory issues were thoroughly addressed, warranting the clinical evaluation of PbVac, currently underway, towards establishing this novel immunization platform as a new paradigm in malaria vaccination.

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KEYNOTE SPEAKER

Environmental Microbiology and Biotechnology

BIOMICROTECH TO METALS - FROM MICROBIAL COMMUNITIES TO MINIATURIZED TECHNOLOGY

Paula V. Morais

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Background: Contamination of heavy metals in the environment is a major global concern because of their toxicity and threat to Humans and the environment. At the same time, metals are precious raw materials and are need to secure sustainable production of key components of many products. Microorganisms respond to metal stress using different defense systems such as metal efflux, compartmentalization, modification of the oxi-reduction state or formation of complexes and synthesis of metal binding proteins. These mechanisms confer to the microorganisms the potential to mobilize or immobilize metals and enable them to be transformed into tools for innovative technologies for bioremediation, raw materials obtainment and biosensors design.

Methods: The genetic mechanisms of microorganisms resistant to metals, and their use in the development of biomicrotechnologies, are described in three case studies. The first case describes the construction of a chromate biosensor and its use in chromate plant-biosensors. Ochrobactrum tritici strain 5bvl1 is a highly chromate resistant strain carrying the transposon TnOtChr. The developed biosensors were based on the expression of gfp under the control of the chr promoter and the chrB regulator gene of TnOtChr determinant (1). The biosensor was used for bioavailable chromate quantification (2) and the design of plant-biosensors (3). In the second case, O. tritici SCII24T with two arsenic resistant 2 operons, ars1 and ars2 and a third non-active ars3 was inactivated at the two function arsenite pumps (strain As5). A great arsenic biofilter was constructed immobilizing this new arsenic bioaccumulator on sputtered modified surfaces (4). The third case describes the characterization of a Sulfitobacter dubius tungsten bioaccumulator. This strain ability was conferred by the presence of the tup gene cluster organized as tupBCA, which is not the most usual gene arrangement (5).

Results & Conclusions: The study of the metal resistance mechanisms in bacteria is an important first step in the exploitation of new bio-based microtechnologies for recovering metals from natural or anthropogenic impacted environments.

Acknowledgements and References: This work was supported by project POCI-01-0145-FEDER-016757, PTDC/AAGREC/3839/2014 from the Programa Operacional da Região Centro e Programa Operacional da Região Lisboa and by project ERA-MIN/0002/2015 from the Fundação para a Ciência e Tecnologia (FCT).
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KEYNOTE SPEAKER

Industrial and Food Microbiology and Biotechnology

FOOD AND CLINICAL STRAINS OF *LISTERIA MONOCYTOGENES*: THE QUEST FOR DISTINCTION

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Listeria monocytogenes is one of the most feared foodborne pathogens; most frequent cause of death due from contaminated food in Europe (2008-2015); one of the highest mortalities of all bacteria (> 17%). In addition to suffering, functional disability and death, *L. monocytogenes* and listeriosis have a huge economic impact. Not only the health systems but also the food industry are severely affected. Food recalls, frequently result in the destruction of high amounts of foods – food waste and direct economic loss – brand damage and loss sales, litigation costs, etc. It is tightly controlled by regulators and companies and represents a serious barrier to the entry of a number of food products, both through official controls and by the requirements of commercial chains. Many countries, such as the USA and Russia, have a zero tolerance for the presence of *L. monocytogenes* i.e. if the organism is detected within a product, then it must be recalled, whereas current legislation in the EU state that *L. monocytogenes* cannot be present in a ready-to-eat product at levels above 100 cfu/g at the time of consumption. About 25 years ago, a group of researchers raised the following question: "Is any strain of *Listeria monocytogenes* detected in food a health risk?". Despite all the research developments since then, science still does not have a conclusive answer to this question and current legislation does differentiate between different strains of *L. monocytogenes*, lumping all strains together as a single group of pathogenic organisms. There is no doubt that the food industry craves for methodologies that can provide a reliable distinction between pathogenic from non pathogenic strains. However, this is complicated since the severity of disease i.e. virulence, is linked to individual immunity and certain people are more likely to contract listeriosis than others. Moreover it is now recognised that exposure to environmental stresses such as those encounted in foods (e.g. low pH, low temperature, high NaCl concentration) may influence the virulence expression of *L. monocytogenes*. Nevertheless the use of whole genomic sequencing and different omics approaches will in time enable the development of greater knowledge that can be useful to take a much more nuanced approach to dealing with *L. monocytogenes*, and ultimately limit the number of listeriosis outbreaks.

O-01 - CHARACTERIZATION OF THE *STAPHYLOCOCCUS AUREUS* POPULATION COLONIZING HOMELESS PEOPLE IN LISBON, PORTUGAL

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Abstract

Individuals who are homeless are more prone to methicillin-resistant *Staphylococcus aureus* (MRSA) colonization and have an increased risk for MRSA infection due to deprived living conditions, poor underlying health, common social risk behaviors, and limited access to hygiene facilities and healthcare services. There are no data on MRSA carriage among homeless in Portugal. The aim of the present study was to characterize the *S. aureus*/MRSA population colonizing homeless people in Lisbon, Portugal.

Between November 2016 and April 2017, a total of 62 homeless individuals with no fix address (n=39) or living in a homeless shelter (n=23) were nasal screened for *S. aureus* colonization. All isolates were tested for antimicrobial susceptibility by disk diffusion, and the presence of *mecA* gene and Panton Valentine leukocidine (PVL) was determined by PCR. Molecular characterization of the isolates included pulsed-field gel electrophoresis (PFGE), *spa* typing, multilocus sequence typing (MLST), and staphylococcal chromosome cassette (SCC) *mec* typing for MRSA.

A total of 31 individuals (50%) were nasally colonized with *S. aureus*, but only one was an MRSA carrier (1.6%). The majority of the carriers were males (51%) and belonged to the population with no fix address (74%). The single MRSA belonged to the EMRSA-15 clone and was characterized by PFGE D, ST15-SCC*mec* IVh and *spa* type t790. The majority (53%) of the 30 methicillin-susceptible *S. aureus* (MSSA) isolates belonged to four major clonal types: PFGE A-ST398-t1451 (n=6), PFGE B-ST15-t073/t084/t346 (n=4), PFGE C-ST45-t073/t116/t13186 (n=3), and PFGE D-ST97-t127/t693 (n=3). Although a common non-multiresistance profile was observed, a high proportion of isolates showed resistance to erythromycin (48.4%), gentamicin (41.9%), fusidic acid (29.0%), and induced resistance to clindamycin (48.4%). None of the isolates harboured PVL.

S. aureus nasal colonization is high (50%) among the homeless population in Lisbon, but MRSA carriage remains low (1.6%), close to the <1% MRSA nasal carriage rate known for the Portuguese healthy population. The single MRSA isolate belonged to clone EMRSA-15, the prevalent lineage circulating in Portuguese hospitals. The homeless population constitutes a reservoir of the livestock associated ST398 MSSA lineage in the community and a possible transmission vehicle to the hospitals.

Keywords: *Staphylococcus aureus,* MRSA, nasal carriage, homeless

O-02 - *STREPTOCOCCUS PNEUMONIAE* ASYMPTOMATIC CARRIAGE CAN LAST SEVERAL MONTHS IN THE ADULT HOST

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Abstract

Background: *Streptococcus pneumoniae* (or pneumococcus) is a common colonizer of the human nasopharynx and a leading cause of disease worldwide. Based on culture methods, colonization has been shown to be high among young children and low among immunocompetent adults. Recently, PCR based methods have challenged this observation. While there are many studies on bacterial colonization in children, much less information is available for adults, and even less from longitudinal studies. We studied the dynamics of *S. pneumoniae* colonization in the upper respiratory tract of immunocompetent adults - smokers and non-smokers - aged 25-50 years old, using real time PCR.

Methods: Between February 2015 and December 2016, 87 adults aged 25-50 years old, living in the Lisbon area, were followed for 6 months. For each participant nasopharyngeal, oropharyngeal and saliva samples were obtained monthly. Individuals found to be pneumococcal carriers were sampled weekly until two consecutive negative samples were obtained. Pneumococcal carriage was identified by culture and by real-time PCR, targeting two pneumococcal genes: *lytA* (major pneumococcal autolysin) and *piaB* (iron uptake ABC transporter lipoprotein PiaB).

Results: Twenty-five adults (28.7%) carried pneumococci at least once and ten (11.5%) were colonized during several months. Adults with regular contact with children <6 years were significantly proner to be pneumococcal carriers when compared to those with no contact [54.2% (13/24) vs 19.0% (12/63); p=0.001]. Smokers were more likely to be pneumococcal carriers when compared to those that were non-smokers [32.5% (13/40) vs 25.5% (12/47); p=0,474]. Real-time PCR increased carriage detection from 14.8% [12.1-17.9, 95% CI] to 17.1% [14.2-20.4, 95% CI] in nasopharyngeal samples, from 2.3% [1.3-3.8, 95% CI] to 20.6% [17.5-24.1, 95% CI] in oropharyngeal samples, and from 0% [0-0.6, 95% CI] to 11.2% [8.9-14.0, 95% CI] in saliva samples.

Conclusion: Our results suggest that: (i) pneumococcal carriage in adults is significant - c.a. 30% - and (ii) frequent contact with children <6 years significantly increases the risk of carriage among adults. In addition, the results obtained in this study are challenging the paradigm of pneumococcal colonization dynamics among adults, as our observations suggest that duration of carriage can be long, lasting several months.

Palavras-chave: *Streptococcus pneumoniae*, colonization, adult host, real-time PCR

O-03 - BAYESIAN ANALYSIS OF THE POPULATION STRUCTURE OF MYCOBACTERIUM BOVIS IN A HOTSPOT AREA OF ANIMAL TUBERCULOSIS, PORTUGAL

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Background

Mycobacterium bovis (*M. bovis*) is a multi-host pathogen, responsible for causing animal tuberculosis (TB) in livestock and wildlife species. Currently, in Portugal, a national eradication program is implemented in the cattle population and an epidemiological risk area for TB in red deer (*Cervus elaphus*) and wild boar (*Sus scrofa*) is also established since 2011. However, the epidemiological situation for most regions is still far from the officially TB-free status. Red deer and wild boar are described as *M. bovis* reservoirs in the Iberian Peninsula, however the specific role exerted by each of these species in transmission cascades is still under scrutiny. A deeper understanding of *M. bovis* transmission dynamics in a multi-host scenario and mechanistic insights into the ecological resilience of this pathogen are thus key to design new interventions.

Method

To study the dynamics of animal TB in Portugal, spoligotyping and MIRU-VNTR typing were used to genotype a wide collection of *M. bovis* isolated from cattle (*n*=359), wild boar (*n*=169) and red deer (*n*=206), between 2003 and 2015, from the epidemiological risk area. Furthermore, to better characterize and delineate phylogenetic groups revealed by standard genotyping methods, Minimum Spanning Trees were combined with Bayesian population structure analyses, using two clustering algorithms implemented in STRUCTURE and TESS softwares.

Results & Conclusions

Spoligotyping allowed the discrimination of more than 40 genotypes, being SB0121, SB1174 and SB0119 the most frequent in TB hotspot areas. In addition, MIRU-VNTR typing allowed detection of intra-host genotypic variability. The results demonstrate a large genotypic variety, with spoligotyping and MIRU-VNTR establishing the basis for the assessment of intra- and inter-specific genotypes. The phylogeographic reconstruction enabled by Bayesian analysis contributes to a deeper knowledge of *M. bovis* transmission cascades and resilience dynamics, which is crucial to inform new control choices.

Keywords: animal tuberculosis, *Mycobacterium bovis*, transmission dynamics, phylogeographic reconstruction, intervention

O-04 - DEVELOPING ANTIMICROBIAL MATERIALS FOR BIOTECHNOLOGICAL APPLICATIONS

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Background

Antimicrobial resistance is a serious worldwide threat to public health that is emerging due to de misuse and overuse of antimicrobials. In fact, infectious diseases are the second most frequent cause of death worldwide. Thus, it is of foremost importance to discover new therapeutic approaches against microbial pathogens. Antimicrobial peptides (AMPs) are promising alternatives to classical antimicrobials, especially because the acquisition of resistance to AMPs is significantly reduced. In general, extraction of these peptides from natural sources or by chemical synthesis is a cumbersome, complex and expensive process that does not provide an efficient method to obtain peptides in large amounts. One way to potentially overcome these drawbacks is by exploring the use of recombinant DNA technology for the biotechnological production of AMPs using microbial cell factories. Nevertheless, due to their nature, AMPs are usually expressed in insoluble inclusion bodies and are susceptible to proteolysis. The use of recombinant protein-based polymers (rPBPs) that act as polymer fusion partners presents several advantages: increases the solubility of the AMPs; function as purification tag and allows the creation of functionalized antimicrobial biomaterials. Owing to the unique balance between their mechanical properties, biocompatibility, biodegradability and thermostability, elastin-like recombinamers (ELRs) and silk-elastin like proteins (SELPs) were used as polymer fusion partners.

Method

The antimicrobial peptides were fused to the N-terminus of ELR and SELP by standard molecular genetic techniques, and confirmed by DNA sequencing. The hybrid polymers were produced using *E. coli* as expression system and purified by a simple optimized non-chromatographic approach. Minimum inhibitory concentration was determined against different bacteria in accordance with EUCAST and CLSI antimicrobial Susceptibility Testing Standards.

Results & Conclusions

Aiming at developing versatile antimicrobial biomaterials with broad bioactivity, we successfully functionalized SELP and ELR protein polymers with different antimicrobial peptides that demonstrated activity against Gram-negative and Gram-positive bacteria. This will provide the basis for the development of advanced biomaterials processed into different types of structures (e.g. hydrogels, films, fibers, particles) suitable for biomedical applications.

References & Acknowledgments

This work is supported by the strategic programme UID/BIA/04050/2013 (POCI-01-0145-FEDER-007569) funded by FCT I.P. and by ERDF through COMPETE2020–Programa Operacional Competitividade e Internacionalização (POCI) and by project EcoAgriFood (NORTE-01-0145-FEDER-000009), supported by Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF). It is also supported by FCT within ERA-NET IB, project FunBioPlas with grant number ERA-IB-15-089 and FCT reference ERA-IB-2-6/0004/2014. AMPereira acknowledges DP_AEM and FCT for the PD/BD/113811/2015 grant.

Keywords: Antimicrobial resistance, Biotechnology, Protein-based Polymers

O-05 - A QUEST FOR LYSOGENIC BACTERIOPHAGES IN *STREPTOCOCCUS DYSGALACTIAE* SUBSP. *DYSGALACTIAE*

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Background

Streptococci are often present in warm-blooded animals, including humans. Although they may assume a commensal role within the host, they can also cause localized and systemic infections with severe sequelae. Their vast virulence gene repertoire, in part encoded within mobile genetic elements, greatly contributes to their success as pathogens. Concerningly, several cases of streptococci regarded strictly as animal pathogens crossing the barrier to become zoonotic agents have been reported. *Streptococcus dysgalactiae* subsp. *dysgalactiae* (SDSD), an animal pathogen involved in bovine mastitis, seems to be undergoing the same process with instances of its involvement in human infections being described [1]. At the root of this phenomenon may be the high rate of horizontal gene transfer (HGT) observed between members of the genus, particularly involving the emerging zoonotic agents and known human pathogens, such as *Streptococcus pyogenes* [2].

Method

Several protocols for bacteriophage induction with mitomycin C from SDSD isolates were performed, producing putative phage lysates that were used in infection assays. Subsequently, phage DNA extraction and visualization of virions through Atomic Force Microscopy (AFM) were used to assess the presence of phage particles in phage lysates. Lastly, five SDSD isolates with contrasting behavior in phage infection assays, as well as differing pathogenic potential, were selected to undergo whole-genome sequencing through a nanopore sequencing technology, using Oxford Nanopore Technologies' MinION.

Results & Conclusions

Cell lysis was verified and reproducible in induction assays, but no infective bacteriophage particles were obtained in subsequent infection experiments. Although phage particle presence was confirmed through DNA extraction and AFM visualization, phage tails could not be detected pointing to the defective nature of these phages. To assess prophage genome integrity, whole-genome sequencing was employed and putative prophages were detected in all five SDSD strains, as well as bacteriophage resistance systems (abortive infection systems, CRISPR-Cas systems and restriction-modification systems) and relevant virulence factors (streptokinase, streptodornases and pyrogenic exotoxins). Some of the SDSD detected prophage sequences showed substantial homology with prophages of human pathogens such as *Streptococcus pyogenes, Streptococcus agalactiae* and *Streptococcus dysgalactiae* subsp. *equisimilis*, enlightening the role of bacteriophage-mediated HGT in the cross-talk between animal and human streptococci and in shaping their pathogenic potential.

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Keywords: *Streptococcus*, prophages, horizontal gene transfer, third-generation sequencing

O-06 - INCORPORATION OF PLGA NANOPARTICLES INTO GUAR-GUM FILMS AS A NEW BUCCAL DELIVERY

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Background
Buccal delivery offers several advantages over oral administration route, avoiding first-pass metabolism and extreme conditions that can lead to the degradation of bioactive molecules. The main goals of this work were to optimize the formulation of poly(lactide-co-glycolic acid) – PLGA – nanoparticles as carriers of an antihypertensive peptide (KGYGGVSLPEW peptide sequence) and incorporate them into the matrix of guar-gum oral films. This study allowed to obtain an optimized formulation of PLGA nanoparticles as tailored delivery systems for the antihypertensive peptide.

Method
PLGA nanoparticles (with and without peptide) were produced by a double emulsion technique evaporation method. PLGA nanoparticles physic properties were characterized by nanosizer instruments and the peptide delivery efficiency was monitored and quantified by HPLC. So, PLGA nanoparticles were optimized by Factorial Design. After optimized the nanoparticles were incorporated into guar-gum film matrix by solvent casting method. The nanoparticle into guar-gum film was characterized by physic properties and the efficiency delivery of peptide-loaded nanoparticles.

Results & Conclusions
The results showed peptide-PLGA nanoparticles had an average size of 125 nm and the zeta potential of -10 mV. The peptide-PLGA nanoparticles association efficiency was 60%.
Peptide-PLGA nanoparticles and Peptide-PLGA nanoparticles conjugated with guar-gum films showed significantly in vitro TR146 cell viability, as assessed by MTT assay. The incorporation of peptide-loaded PLGA nanoparticles into oral films led to a slower permeability across TR146 monolayer when compared with free peptide, films or nanoparticles alone. Results indicate that permeability of peptide across cells is directly correlated with peptide release from delivery systems and the peptide-loaded PLGA nanoparticles incorporated into oral films showed to be more effective as buccal delivery systems, promoting a slower and more extensive permeability. So, it is concluded PLGA nanoparticles will be valuable to deliver a bioactive peptides, to control release and to effectively protect encapsulated peptides. The combination of PLGA nanoparticles and guar-gum films showed a more effective delivery system.

Acknowledgements:
The authors acknowledge the support granted by national founds from FCT through project PTDC/BBB-NAN/3249/2014 and the scientific collaboration of CBQF under the FCT project UID/Multi/50016/2013.
Patrícia Batista would like to thanks FCT (grant PTDC/BBB-NAN/3249/2014) and Pedro Castro to thanks CCDR-N (PhD grant NORTE-08-5369-FSE-000007).

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Keywords: Bioactive peptides, Nanoparticles, PLGA, Oral film, Antihypertensive, Buccal delivery

O-07 - MULTIMODAL CHROMATOGRAPHY OF SUPERCOILED MINICIRCLES: A CLOSER LOOK INTO DNA-LIGAND INTERACTIONS

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Background

Minicircles are among the safest and most efficient gene delivery vectors. These vectors are obtained *in vivo* in *Escherichia coli* by an intramolecular recombination process that converts a replicating parental plasmid (PP) into the target minicircle (MC), which contains the eukaryotic cassette, and a miniplasmid (MP), which contains the prokaryotic backbone. Here we present a method for the purification of MCs based on multimodal chromatography (MMC) and a study of the interactions of DNA with the multimodal ligand.

Method

Nucleic acids were recovered and pre-purified from *E. coli* cells by alkaline lysis and precipitation. An *in vitro* digestion step was carried out with Nb.BbvCI, a nicking endonuclease that converts sc MP and un-recombined PP into open circular (oc) isoforms, without affecting the sc MCs. Sc MC was isolated from oc DNA molecules and RNA by MMC with a Capto™ adhere matrix using a step-wise gradient with increasing NaCl concentrations. Control experiments were performed using hydrophobic (Phenyl-Sepharose) and anion exchange (Q-Sepharose) matrices. Bioinformatics analysis of the MC sequence was performed using WebSIDD, an algorithm that predicts locations/extents of stress-induced duplex destabilization in double-stranded DNA molecules with a given base sequence and level of superhelical stress. The presence of exposed bases in sc MC and oc MP was assessed by digestion with Bal-31 nuclease.

Results & Conclusions

Gel electrophoresis reveal that sc MC-containing fractions isolated by MMC are homogeneous (>90%) and impurity-free. The sc isoforms were shown to be more susceptible to Bal-31 when compared to oc isoforms. This means that sc isoforms have exposed bases as a result of deformations induced by the torsional strain; they are thus more hydrophobic than oc isoforms and interact more strongly with the phenyl group of the multimodal ligand. An A+T rich region within the CMV promoter was identified by bioinformatics to have a higher propensity for base-pair separation. Control experiments with a Phenyl-Sepharose column showed that hydrophobic interactions are not the sole responsible for retention in the multimodal column, as all nucleic acids eluted in the flowthrough. This provided an indication that electrostatic interactions are also involved. Further experiments performed with a Q-Sepharose column confirmed that interaction of sc molecules with the charged nitrogen atom of the ligand are stronger as compared with interactions of the oc isoforms. Overall the results show that differences behind the higher retention of RNA and sc DNA isoforms as compared to oc isoforms result from a combination of hydrophobic and charge interactions.

Keywords: Multimodal chromatography, Minicircles, DNA-ligand interactions

O-08 - IMPROVING A BACTERIAL DYP-TYPE PEROXIDASE FOR LIGNIN-RELATED PHENOLICS OXIDATION AT ALKALINE PH USING DIRECTED EVOLUTION

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Background
Dye-decolorizing peroxidases (DyPs) are a family of microbial heme-containing peroxidases that show important properties for lignocellulose biorefineries due to their ability to oxidize lignin-related compounds. The bacterial PpDyP from *Pseudomonas putida* MET94 accepts a wide scope of substrates, however the specific activity for phenolic compounds is 10 to 100 lower than those measured for azo and anthraquinonic dyes or $Mn^{2+\cdot}$[1].

Method
Three rounds of directed evolution through random mutagenesis by error-prone PCR of the *ppDyP*-gene followed by high-throughput screening was used to improve the efficiency of PpDyP for lignin-related phenolic compounds [2]. Biochemical analysis of hit variants from the laboratory evolution, and single variants constructed using site-directed mutagenesis was performed.

Results & Conclusions
The 6E10 variant was identified showing a 100-fold enhanced catalytic efficiency (k_{cat}/K_m) for 2,6-dimethoxyphenol (DMP), similar to those exhibited by fungal lignin peroxidases (~10^5 $M^{-1}s^{-1}$). The evolved variant showed additional improved efficiency for a number of syringyl-type phenolics, guaiacol, aromatic amines, Kraft lignin and the lignin phenolic model dimer, guaiacylglycerol-b-guaiacyl-ether. Importantly, variant 6E10 displayed optimal pH at 8.5, an upshift of 4 units as compared to the wild-type, showed resistance to hydrogen peroxide inactivation, and was produced at 2-fold higher yields, overcoming three of the main limitations to the biotechnological applications of peroxidase enzymes. The acquired mutations in the course of the evolution affected three amino acid residues (E188K, A142V and H125Y) situated at the surface of the enzyme, in the second shell of the heme cavity. Biochemical analysis of hit variants, unveiled the critical role of acquired mutations from the catalytic, stability and structural viewpoints. We show that epistasis between A142V and E188K mutations is crucial to determine substrate specificity of 6E10. Evidence suggests that ABTS and DMP oxidation occurs at the heme access channel. Details of the catalytic cycle of 6E10 were elucidated through transient kinetics providing evidences for the formation of a reversible enzyme-hydrogen peroxide complex (Compound 0) barely detected in the majority of heme peroxidases studied to date.

Acknowledgments
This work was supported by Fundação para a Ciência e Tecnologia (FCT), Portugal (PTDC/BBB-EBB/0122/2014, RECI/QEQ-QIN/0189/2012 and REM2013) and Research Unit GREEN-it "Bioresources for Sustainability" (UID/Multi/04551/2013). V.B. holds a Post-doc fellowship (SFRH/BPD/109431/2015) from FCT, Portugal.

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Keywords: directed evolution, dye-decolorizing peroxidases, ligninolytic enzymes, enzyme specificity, epistasis, *Pseudomonas putida* MET 94

O-09 - ENHANCING THE ENZYMATIC SACCHARIFICATION OF WHOLE SLURRY FROM AUTOHYDROLYZED EUCALYPTUS GLOBULUS WOOD BY SUPPLEMENTATION WITH A RECOMBINANT CARBOHYDRATE-BINDING MODULE

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Background

Lignocellulosic biomass has a recognised potential as a sustainable platform for the production of biofuels and other biochemicals. However, lignin residues and other inhibitory compounds resulting from lignocellulosics pretreatment affect the digestibility of resulting whole slurries. The addition of synergistic proteins that can cooperate with cellulases is an emerging strategy for enhanced lignocellulosics hydrolysis. Carbohydrate-binding modules (CBMs) have been shown to improve the enzymatic hydrolysis of pure cellulose models but their effect on the enzymatic hydrolysis of lignocellulosic materials has not yet been evaluated. Thus, in this work, the potential synergistic effect of a family 3 CBM on the enzymatic saccharification of a pretreated lignocellulosic biomass was studied for the first time.

Method

The CBM3 from the *Clostridium thermocellum* scaffolding protein (CipA) was recombinantly produced in *Escherichia coli*. Different dosages of CBM3 (30-1.5 mg/g_{solids}) were incubated simultaneously with the enzymatic cocktail Cellic CTec2 (15 FPU/g_{solids}) in the whole slurry from autohydrolysed *Eucalyptus globulus* wood (EGW), containing 5% (w/v) solids and 73% (w/v) hydrolysate. Enzymatic hydrolyses were conducted at pH 4.85, 50°C and 150 rpm. Glucose yields were determined over time by HPLC. BSA effect was also evaluated using the same loads as CBM3. Additionally, the binding of CBM3 to solids and lignin of pretreated EGW was studied by fluorescence microscopy.

Results & Conclusions

CBM3 effect was found to be dose-dependent. When added at the higher dosage (30 mg/g_{solids}), CBM3 led to an increase in glucose yield from 75 to a maximum of 89% (14% increase), alleviating significantly the inhibitory effect of EGW hydrolysate on the enzymatic saccharification. BSA, which has a well-documented additive effect, led to a slightly lower glucose yield increment (11%). CBM3 was able to bind to EGW solids but not to the isolated lignin. Therefore, the CBM3 effect is not related with the typical lignin-blocking mechanism attributed to other proteins, such as BSA. CBM3 is a valid and inexpensive additive that may be included in enzymatic cocktails for improved whole slurry saccharification, thus contributing to more efficient biomass conversion bioprocesses.

Acknowledgments

C. Oliveira and D. Gomes acknowledge support from Fundação para a Ciência e a Tecnologia (FCT), Portugal (grants SFRH/BPD/110640/2015 and SFRH/BD/88623/2012, respectively). This study was supported by the FCT under the scope of the GlycoCBMs Project PTDC/AGR-FOR/3090/2012–FCOMP-01-0124-FEDER-027948 and the strategic funding of UID/BIO/04469/2013 unit and COMPETE 2020 (POCI-01-0145-FEDER-006684) and BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund under the scope of Norte2020 - Programa Operacional Regional do Norte.

Keywords: recombinant CBM3, whole slurry, enzyme inhibition, lignin, enhanced saccharification

O-10 - EXTRACTION AND CHARACTERIZATION OF EXTRACELLULAR POLYMERIC SUBSTANCES FROM AEROBIC GRANULAR SLUDGE FROM A FULL-SCALE SEQUENCING BATCH REACTOR IN PORTUGAL

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Background

Aerobic granular sludge (AGS) is a recently developed technology for wastewater treatment. This system is able to manage higher amounts of wastewater and requires less surface area than conventional systems. The granules consist of microorganisms embedded in a self-produced extracellular polymeric substances (EPS) matrix. EPS are high molecular weight polymers, which can be metabolic products of microorganisms (e.g. proteins, polysaccharides, humic substances, nucleic acids) or be due to cell lyses. Accumulation on the cells surface of such EPS forms a protective barrier for the cells from the external environment.

This work focus on the extraction and quantification of EPS from AGS from a large scale bioreactor in Portugal, during approximately 4 months. Given the environmental and chemical differences that these granules are subjected to one of the goals was to assess variability in the EPS production and characterize the granules morphology and composition in a large scale environment.

Method

EPS was extracted from AGS as described by Felz *et al.*[1] using the sodium carbonate method. EPS biochemical characterization was made using colorimetric methods to access the proteins [2], polysaccharides [3] and humic acids [4], and a fluorometric method to access the DNA content using a Qubit fluorometer. Quantitative image analysis was used to evaluate the size and several morphological parameters (e.g. roundness, robustness, compactness, and others) of the granules [5,6].

Results & Conclusions

Variations in the EPS composition were observed throughout the experience. Such variations can be due to chemical differences in the influent water and the fact that the loads can vary throughout the year. Consequently, the EPS production and composition in the granules was affected. Morphological parameters, namely roundness, compactness and robustness of the biomass are indicative factors of a stable granular biomass during the monitoring period.

In summary, despite the fact that EPS composition changed in response to external stimuli, the granules showed morphological stability throughout the analysis.

Acknowledgments

The authors thank the company SIMTEJO for supplying the granules, and the financial support of European Social Fund, under Programa Operacional under the project NORTE-08-5369-FSE-000007 and BioTecNorte operation (NORTE-01-0145-FEDER-000004). This work was also supported by Portuguese Funds from FCT - Fundação para a Ciência e a Tecnologia through the strategic funding of UID/Multi/50016/2013 and UID/BIO/04469/2013 units and COMPETE 2020 (POCI-01-0145-FEDER-006684). C.L. Amorim wishes to acknowledge the research grant from FCT (SFRH/BPD/96481/2013).

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Keywords: aerobic granular sludge, sequencing batch reactor, extracellular polymeric substances, extraction, image analysis

O-11 - STERILIZATION BY SCCO2 TECHNOLOGY: CAN WE DESTROY BACTERIAL SPORES WHILE BEING GENTLE TO ABSORBABLE BIOMATERIALS?

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Background

The development of biomaterials represents a challenge to existing medical sterilization technologies, since they are often sensitive to high temperatures. Supercritical CO_2 ($scCO_2$) has recently been identified as an effective technique for the sterilization of thermally and hydrolytically sensitive polymers [1]. $scCO_2$ sterilization can be achieved at low temperature and presents several advantages such as inertness, non-toxicity, high penetration ability and non-flammability [1]. According to EN 556–1, a guaranteed sterility assurance level (SAL) is required, or a one in a million chance of a contaminated item [2]. It has been demonstrated that $scCO_2$ combined with low molecular volatile additives can markedly improve bacterial endospores inactivation to reach the required SAL [3]. Although progress has been done in this field demonstrating the efficacy of $scCO_2$ sterilization of several absorbable polymers, many are still to be studied. In this work, $scCO_2$ was used for sterilizing 3D printed polylactic acid (PLA) and PLA/graphene composites, presently under development as surgical instruments and for other potential biomedical applications.

Method

$scCO_2$ sterilization process was conducted at different conditions of time, pressure and temperature using hydrogen peroxide as residual co-solvent. Stirring was set to 600 rpm during the process. Three types of pore strips, were used as biological indicators, containing more than 10^6 spores of the species: *Bacillus stearothermophilus*, *Bacillus pumilus* and *Bacillus atrophaeus*. Characterisation of biomaterials before/after $scCO_2$ sterilization, was performed by Scanning Electron Microscopy (SEM), Differential Scanning Calorimetry (DSC) and Fourier Transformed Infrared (FTIR).

Results & Conclusions

The different parameters of the sterilization process such as time, pressure, temperature and the amount of co-solvent have been optimized in order to ensure sterilization efficiency while minimizing its effect on the studied materials. Confirmation of sterility was assured by placing the different spore strips in the reactor and further demonstration of the achievement of SAL level. FTIR and DSC results did not present any detectable modification to the chemical structure nor to the thermal behaviour of the materials after sterilization. Studies are on-going to access the stability of the materials in physiological conditions.

Acknowledgements

"IBEROS" 0245_IBEROS_1_E, financed by Programme INTERREG V-A Spain-Portugal 2014-2020 (POCTEP). A.L. Oliveira greatly acknowledges the Portuguese Foundation for Science and Technology, for the Investigator FCT program IF/00411/2013.

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Keywords: Sterilization, Supercritical carbon dioxide, Bacterial spores

O-012 - AEROBIC GRANULAR SLUDGE REACTIVATION UNDER DIFFERENT HYDRODYNAMIC REGIMENS: MICROBIAL ECOLOGY AND BIOREACTOR PERFORMANCE

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Background

The textile industry irregularly discharges wastewaters containing high organic loads and recalcitrant azo dyes [1]. Aiming at biological reductive azo dye cleavage and aerobic degradation of the resulting, potentially toxic, aromatic amines [2], anaerobic-aerobic sequencing batch reactors (SBR) using the breakthrough aerobic granular sludge (AGS) technology [3] have been suggested for textile wastewater treatment [4]. Moreover, AGS withstands toxic compounds and prolonged storage periods [5]. This study analyzed the reactivation of stored AGS in what regards microbial ecology and operation performance in the treatment of a synthetic textile wastewater using two SBR hydrodynamic regimens: stirred anaerobic-aerobic [4] *versus* a plug-flow anaerobic-aerobic previously associated with improved AGS stability [3].

Method

Two 1.5-L SBR were seeded with AGS previously stored for 6.5 months (4°C) after a 102-day SBR operation treating synthetic textile wastewater [4]. The latter was fed to both SBR at a 12-h hydraulic retention time and a 2.0-kg $m^{-3}d^{-1}$ organic loading rate, as chemical oxygen demand (COD). The SBR were operated for 80 days in 6-h cycles: static fill (30-min in SBR1; 2-h plug-flow in SBR2), 1.5-h stirred anaerobic phase (only SBR1), 3.5-h aeration, 5-min settling, 1-min drain. Suspended solids, sludge volume index, COD, dye and metabolite levels were analyzed [4]. The microbial ecology was assessed through fluorescence *in situ* hybridization [4].

Results & Conclusions

The AGS recovered to 80% COD removal 9 days after inoculation in both SBR. SBR1 reached 80% color removal, whereas SBR2 removed under 30% due to unfavorable mass transfer conditions. However, SBR2 maintained better AGS settling properties and higher biomass concentration. Aromatic amine bioconversion was not recovered after storage. Microbial ecology analysis indicated that *Alphaproteobacteria* populations suffered with storage, namely the important group of *Defluvicoccus vanus* related GAOs, which could have been involved in amine bioconversion. While SBR1 promoted the dominance of *Gammaproteobacteria* and *Actinobacteria*, SBR2 developed a more evenly distributed microbial community along the operation.

Acknowledgments

This work was financed by Fundação para a Ciência e a Tecnologia (FCT) through projects PTDC/EBB-EBI/120624/2010 and PTDC/AAG-TEC/4501/2014 and funding received by iBB-Institute for Bioengineering and Biosciences (UID/BIO/04565/2013). R.D.G. Franca and N.D. Lourenço acknowledge FCT grants SFRH/BD/95415/2013 and SFRH/BPD/88095/2012, respectively.

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Keywords: Aerobic granular sludge, Wastewater microbiology, Aromatic amine biodegradation, Sequencing batch reactors, Textile industry wastewater

O-13 - A MULTI-ENZYMATIC APPROACH TO PRODUCE HIGH-VALUE COMPOUNDS FROM WASTEWATER

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Background

Dyeing processes produce a large amount of coloured wastewaters where the major components are azo (-N=N-) dyes, which are hazardous to the environment. An enzyme isolated from *Pseudomonas putida* MET94 (PpAzoR) showed a high efficiency in the decolourisation of several structurally different azo dyes [1]. From this degradation aromatic amines are formed that can be in some cases more toxic than the original dyes [2]. To overcome this toxicity issue, laccases can be used to oxidize aromatic amines leading to high-value compounds [3]. The use of these enzymes in multi-step reactions can promote the creation of circular economy, from wastewater to demanding products.

Method

Here, we have set-up a multi-enzymatic process to convert azo dyes into high-value products. The azoreductase PpAzoR was used in anaerobic conditions to degrade azo dyes to produce aromatic amines. Then, CotA-laccase was added in aerobic conditions to modify aromatic amines into new products. The identification of products in both steps was performed by NMR and HPLC.

Results & Conclusions

The decolourization of different dyes was very efficient reaching values up to 90-96 % in 24 h. The aromatic amines formed during the decolourization by PpAzoR can be predicted based in the original structure of the dyes. Nonetheless, some aromatic amines are further oxidised upon exposition to air [4] and are not available for the second enzymatic step. Nevertheless after adding CotA-laccase, interesting compounds like phenazines and quinones could be identified. In order to increase the sustainability of this approach, its cost-effectiveness and biotechnological applicability we have used a whole-cell system of recombinant *Escherichia coli* cells co-producing both PpAzor and CotA-laccase. Whole-cells were used to sequentially decolourize dye containing model wastewaters and produce high-value compounds.

Acknowledgments

We acknowledge financial support from FCT: project PTDC/BBB-EBB/0122/2014, Research unit GREEN-it "Bioresources for Sustainability" (UID/Multi/04551/2013), NMR Facility (RECI/BBB-BQB/0230/2012), and grant PD/BD/109637/2015 (A. F.)

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Keywords: Azo-dyes, Wastewater, Decolourisation, Multi-enzymatic systems, Azoredutases, Laccases, Circular economy

O-14 - XANTHOMONAS ARBORICOLA PV. JUGLANDIS: AN ENDEMIC WALNUT PATHOGEN IN PORTUGAL

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Background

Xanthomonas arboricola pv. *juglandis* (*Xaj*) is one of the most serious and widespread threats of walnut orchards, being globally associated with severe production losses. These concerns made *Xaj* the focus of several diversity studies to infer epidemiological patterns and contribute to improve phytosanitary practices. Regardless the high genetic diversity of *Xaj* emphasized in previous studies, comprehensive assessments of *Xaj* population diversity taking into account multiple variables thought to modulate the disease ecology of these bacteria, are still lacking.

Method

The present work proposed novel DNA markers for detection and identification of *Xaj*. Nine genomic regions were selected as promising markers using dedicated *in silico* approaches [1]. Experimental validation of putative *Xaj* markers was performed using a high throughput dot blot platform [1]. A multiplex PCR was optimized as a culture-independent detection method for *Xaj* in naturally infected walnut samples [1]. In order to unveil the genetic diversity of Portuguese *Xaj* population, 131 isolates were obtained from leaves, fruits, branches, catkins and buds of symptomatic walnut trees distributed throughout distinct climatic regions of Portugal, between 2014 and 2016. Isolates' diversity was assessed by dot blot with *Xaj*-specific DNA markers and by MLSA using four housekeeping genes (*acnB*, *fyuA*, *gyrB* and *rpoD*). Possible correlations between distinct putative *Xaj* clonal lineages and walnut cultivars, bioclimatic factors, virulence fitness and co-infection potential were determined.

Results & Conclusions

The experimental validation confirmed the specificity of the selected DNA marker for *Xaj* studies, as predicted by the initial BLAST analysis [1]. Broad and narrow ranges markers were identified within the group of *Xaj* tested. XAJ1, XAJ6 and XAJ8 markers were amplified from naturally infected walnut leaves and fruits showing the usefulness of multiplex PCR for detection of latent infections [1]. Altogether, the results showed that *Xaj* lineages present in walnut-growing regions of Portugal are genetically heterogeneous, distributed by ten clusters (I to X) inferred by the Maximum Likelihood analysis of 248 concatenated sequences and 20 different hybridization patterns. Two clusters (IX and X) were shown to diverge from the main *Xaj* lineages, and different *Xaj* lineages were isolated from the same walnut tree. The present study suggested that *Xaj* diversity is not dependent on bioclimatic regions, walnut cultivars, plant organ or isolation date, which is evocative of a cosmopolitan dispersion.

Acknowledgments

Work co-financed by the project P2020-PTDC/BIA-EVF/3635/2014, POCI-01-0145-FEDER-016600 and grant SFRH/BD/95913/2013.

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Keywords: dot blot hybridization, DNA markers, epidemiology, MLSA, Xanthomonas arboricola

O-15 - MULTI-OMICS AND LABORATORY-ASSISTED EVALUATION OF THE CHITIN-DEGRADING CAPACITIES OF GORGONIAN CORAL-ASSOCIATED BACTERIA

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Background
Chitin, a homopolymer of N-acetylglucosamine, is the second-most abundant natural polymer on Earth. It is a difficult to degrade constituent of fungal cell walls, marine diatoms, crustaceans, zooplankton and many invertebrates. Chitinases - chitin-degrading enzymes – can have applications in the medical, alimentary and agricultural industry and are therefore of increased research interest. Microbial symbionts of filter-feeding marine organisms such as corals may metabolize the chitin that is filter-concentrated from the water column and thus be a promising source for novel chitinolytic enzymes.

Method
To investigate this hypothesis, we currently explore 36 phylogenetically distinct bacterial symbionts (classes *Alphaproteobacteria*, *Gammaproteobacteria*, *Flavobacteria*) isolated from the gorgonian coral *Eunicella labiata* for their chitin-degrading capacities using phenotypic and *in-silico* assays. We employ a chitin-agar plate assay to visualize chitin degradation and a fluorometric enzyme assay to quantify exo- and endochitinase activity in the growth medium. We further explore the diversity of chitinase-enconding genes on the fully sequenced genomes available for 16 of the strains. Moreover, we inspect the full microbial metagenomes of three gorgonian species and their surrounding seawater and sediment to determine the presence and abundance of chitin-degradation related genes in the gorgonian microbiome.

Results & Conclusions
Our first results show that gorgonian-associated isolates of the genera *Sulfitobacter, Labrenzia* (*Alphaproteobacteria*) and *Vibrio* (*Gammaproteobacteria*) possess high *in-vitro* chitin-degrading activity. The genome of *Aquimarina* strain EL33 (FLRG01000001-20; *Flavobacteria*) had clearly the highest numbers (19 and 6, respectively) of exochitinase (EC 3.2.1.14) and endochitinase (EC 3.2.1.52) encoding genes. The genomes of six gorgonian-derived *Vibrio* and *Aliivibrio* (*Gammaproteobacteria*) strains also possessed several (2-5) exo- and endochitinase genes. *Alphaproteobacteria* strains of the family *Rhodbacteraceae,* however, harboured only exo-chitinase encoding genes and no endochitinases. Metagenomics revealed that the frequency of chitinase-encoding genes in the microbiomes of gorgonians, sediments and seawater were statistically similar. Yet we found an enrichment of domain III chitin-binding proteins (IPR004302) in the gorgonians which can facilitate chitin-degradation by enhancing the cells' binding capacity to chitin substrates. In contrast, chitobiases - that cleave water-soluble chitin oligomers into monomers - were significantly more abundant in seawater and sediment than in the gorgonians.

In conclusion, gorgonian corals are a valuable source of taxonomically diverse chitin-degrading bacteria which apparently display functional segregations in the types of chitinases they possess. Although chitinase abundance did not differ between habitats, the enrichment in chitin binding proteins in gorgonians suggests a higher efficiency in chitin processing in this microbiome.

Acknowledgments
Funding is acknowledged to FCT (PTDC/MARBIO/1547/2014)

Keywords: chitin, chitinase, coral-associated bacteria, gorgonian coral

O-16 - MICROBIOLOGICAL QUALITY OF TREATED WATER BY SOLAR-DRIVEN OXIDATION PROCESSES

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Background

The commonly implemented wastewater treatments have a limited capacity to remove organic micropollutants, including pharmaceuticals, and human pathogens, as well as antibiotic resistant bacteria (ARB) and related genes (ARG). As a consequence, the continuous disposal of antibiotics and related products into the environment can lead to the development and proliferation of ARB, a problem that needs urgent solutions. The present study aimed at comparing the efficiency of different solar-driven oxidation processes on the removal of undesirable microorganisms, with the simultaneous elimination of organic micropollutants, in the secondary effluent of an urban wastewater treatment plant, using a solar compound parabolic collector at pilot-scale.

Method

Wastewater samples were spiked with 100 µg L^{-1} of each selected model organic micropollutant: carbamazepine, sulfamethoxazole and diclofenac. Quantitative analysis of the organics was made by HPLC. The abundance of total and tetracycline and ciprofloxacin resistant cultivable faecal indicator bacteria and of the genes 16S rRNA, *intI1*, bla_{TEM}, *qnrS*, *sul1*, bla_{CTX-M} and *vanA* was followed by using the membrane filtration method and qPCR, respectively. Bacterial community was analysed based on the hypervariable V3/V4 region of 16S rRNA gene Illumina sequencing. Samples were analysed before treatment, immediately after treatment, and after 3-days of storage of treated wastewater at room temperature.

Results & Conclusions

The highest efficiency on the removal of the parent model organic compounds to concentrations below the limit of detection (LOD) was observed for H_2O_2-assisted photocatalysis using TiO_2 as photocatalyst. Tetracycline and ciprofloxacin resistant enterococci and faecal coliforms were removed to levels below the LOD (1 CFU 100 mL^{-1}), irrespective of the treatment applied with H_2O_2, even upon storage of the treated wastewater for 3-days. However, reactivation assessed based on 16S rRNA and *sul*1 quantification was observed in all the treatments. Regardless of the type of process, wastewater treatment and storage caused notorious bacterial communities disturbances, characterized by an increase in the relative abundance of *Proteobacteria* (classes *Beta*- and *Gammaproteobacteria*), namely of the genera *Pseudomonas*, *Rheinheimera*, *Methylotenera*.

References & Acknowledgments

This work was supported by Project nº P1404290052 (SFERA Program, EC/FP7-Integrating Activities), and by NORTE 2020 (AIProcMat@N2020 NORTE-01-0145-FEDER-000006; LEPABE-2-ECO-INNOVATION NORTE-01-0145-FEDER-000005), under the Portugal 2020 Partnership Agreement, through ERDF, Projects POCI-01-0145-FEDER-006984 (UID/EQU/50020/2013) and POCI-01-0145-FEDER-006939 (UID/EQU/00511/2013), and COMPETE2020 – and by national funds (FCT, UID/Multi/50016/2013-CBQF and Water JPI/0001/2013 STARE). NFFM, LMPM and AMTS acknowledge PD/BD/114318/2016, IF/01248/2014 and IF/01501/2013, respectively. The authors acknowledge also the COST-European Cooperation in Science and Technology, to the COST Action ES1403: New and emerging challenges and opportunities in wastewater reuse (NEREUS). Microbiological quality of treated water by solar-driven oxidation processes

Keywords: Solar-driven oxidation treatment, Organic micropollutants, Human pathogens, Antibiotic resistance, Bacterial communities

O-17 - ACCELERATION OF ANAEROBIC REACTIONS BY CONDUCTIVE CARBON NANOMATERIALS

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Abstract

Anaerobic processes are environmentally friendly solutions for the decontamination of a wide range of recalcitrant compounds, while generating energy through the production of methane, a renewable energy source. The rates of anaerobic biotransformations are often slow, but the amendment with carbon-based conductive materials (CBCM) has been reported to accelerate the microbial conversions. For example, methane production from organic compounds could be accelerated in the presence of CBCM, which has been often justified by the occurrence of direct interspecies electron transfer (DIET), between anaerobic bacteria and methanogens, over the typical electron exchange via hydrogen or formate. However, in these studies the effect of conductive materials towards individual microbial species was never determined and therefore it is difficult to conclude whether it influences the entire microbial community and changes the electron transfer mechanism between distinct microbial groups, or whether it only stimulates the activity of specific groups of microorganisms. In our laboratory, we have been investigating the effect of CBCM in two main research areas: in the anaerobic biodegradation of organic pollutants, and in the activity of pure cultures of methanogens and in syntrophic co-cultures.

Method

Different CBCM (activated carbon, carbon xerogels, carbon nanotubes (CNT) and magnetic nanomaterials (CNT@2%Fe)) were tested as catalysts during anaerobic biodegradation of azo dyes and aromatic amines, in batch assays and in continuous bioreactors. Increasing concentrations of multi-walled CNT were added to pure cultures of hydrogenotrophic or acetoclastic methanogens, and also to a syntrophic co-culture converting butyrate to methane. The rates of azo dyes reduction and methane production were determined in the presence and in the absence of CBCM.

Results and Conclusions

CBCM increased significantly the reduction rates of azo dyes and aromatic amines in both biotic and abiotic anaerobic conditions, by acting as electron shuttles. Best results were obtained with CNT@2%Fe which improved the rates of azo dyes reduction up to 79-fold [1]. Moreover, this material can be easily recycled and used as catalyst in successive cycles due to its magnetic properties. Methanogenic activity of pure cultures of methanogens was considerably enhanced in the presence of CNT [2]. Particularly, methane production rate of M. formicicum increased 17 times in the presence of CNT, showing that CBCM can directly stimulate methanogenic microorganisms. Application of CBCM revealed to be an efficient strategy to improve the anaerobic treatment of different types of wastewater.

Acknowledgments

ERC Grant nº323009; UID/BIO/04469/2013; POCI-01-0145-FEDER-006684; NORTE-01-0145-FEDER-000004; FCOMP-01-0124-FEDER-027462; SFRH/BPD/80528/2011

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Keywords: carbon materials, methane, redox potential

O-18 - UNDERSTANDING THE CROSS-TALK BETWEEN MICROBIOTA, HOST FITNESS, AND THE ENVIRONMENT USING EGYPTIAN MONGOOSE (HERPESTES ICHNEUMON) AS A MODEL

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Background

The vertebrate gastrointestinal tract is a complex ecosystem that is the habitat of an enormous density and diversity of microorganisms, whose distribution differs within gut sections. The main concentration of bacteria is found in the large intestine, wherein strict anaerobes dominate. Generally, once the mammal's gut microbiota is established, its composition is relatively stable and dominated by members of the *Firmicutes*, *Bacteroidetes* and *Proteobacteria* phyla. However, there are a variety of effects that can alter this equilibrium, such as diet, host health, host genetics, host sex, and reproductive status.

To study the microbiota and its interaction with host fitness and the environment, we used Egyptian mongoose (*Herpestes ichneumon)* as a model. This species is a medium-sized carnivore, with opportunistic feeding habits, that naturally dispersed from Africa during the Late Pleistocene. The species is under expansion in the national territory, now ranging from south to central and north-eastern regions of Portugal.

Method

We investigated the gut microbiota of Egyptian mongoose sampled in Portugal, using a wide culture-based approach to capture comprehensive microbial diversity, followed by morphological and biochemical tests and rRNA- and ITS-based molecular identification. Molecular fingerprinting was based on RAPD. We compared the microbiota of males and females (genus-based phylotypes) and tested the effect of individual bio-ecological features on the microbiota of each individual specimen.

Results & Conclusions

Extended baseline information on the microbiota of mongoose was generated, revealing that this carnivore microbiota is dominated by Gram-positive bacteria, mainly of the phylum *Firmicutes*, wherin *Enterococcus* spp. and *Bacillus* spp predominate. The microbial load of fecal samples seeded in rich medium under anaerobiosis was higher in females than in males, both in terms of total bacterial and sporobiota communities. *E. coli* presence was limited to female samples, while filamentous fungi were only isolated from male hosts.

The cross-talk between mongoose microbiota and bio-ecological features of mongoose is confirmed. A greater similarity between adult' and juvenile' microbiota was detected that contrasted with sub-adult', probably due to the higher proximity and interaction between the two first groups, since mongoose social behavior includes protection and feeding of the cubs, scent marking and social latrines, increasing diet similarity and fecal-oral transmission of microbiota.

Future work using culture-independent methods will improve our knowledge of this species microbiome and lead to a better understanding of the interplay between microbial community and host ecological patterns.

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Keywords: Egyptian mongoose, Gut Microbiota, Microbial Profiling, Carnivores, Host fitness

O-19 - DIFFERENCES IN INTRACELLULAR FATE OF TWO SPOTTED FEVER GROUP RICKETTSIA IN MACROPHAGE-LIKE CELLS

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Background
Spotted fever group (SFG) Rickettsia are recognized as important agents of emerging human tick-borne diseases worldwide such as Mediterranean spotted fever (*R. conorii*) and Rocky Mountain spotted fever (*R. rickettsii*). Recent studies in several animal models have provided evidence of non-endothelial parasitism by different Rickettsia species suggesting that the interaction of rickettsiae with cells other than the endothelium may play an important role in the pathogenesis of rickettsial diseases. These studies raise the hypothesis that the role of macrophages in rickettsial pathogenesis may have been underappreciated.

Method
We evaluated the ability of two SFG Rickettsia species, *R. conorii* (a recognized human pathogen) and *R. montanensis* (a non-virulent member of SFG), to proliferate in THP-1 macrophage-like cells and also within a non-phagocytic cell line, Vero. Growth dynamics were evaluated by quantitative PCR and the ability of the two species to associate with and invade into THP-1 and Vero cells was evaluated by immunofluorescence microscopy-based assays.

Results & Conclusions
Our results demonstrated that *R. conorii* was able to survive and proliferate in both phagocytic and epithelial cells *in vitro*. In contrast, *R. montanensis* was able to grow in Vero cells, but was drastically compromised in the ability to proliferate within THP-1 cells. Interestingly, association assays revealed that *R. montanensis* was defective in binding to THP-1-derived macrophages; however, the invasion of the remaining bacteria did not appear to be affected. Our results also demonstrated that the few *R. montanensis* that could invade the THP-1-derived macrophages were rapidly destroyed and partially co-localized with LAMP-2, a lysosomal marker. In contrast, *R. conorii* was present as intact bacilli and free in the cytoplasm in both cell types. These findings suggest that a major phenotypic difference between a non-pathogenic and a pathogenic SFG member lies in their respective ability to proliferate in macrophage-like cells and may provide an explanation as to why certain SFG rickettsial species are not associated with disease (Curto P. et al. (2016) *Front. Cell. Infect. Microbiol*. 6:80).

Acknowledgments
Pedro Curto is the recipient of FCT PhD grant SFRH/BD/96769/2013

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Keywords: Rickettsia, Macrophages, Pathogenicity, Host-pathogen interactions

O-20 - S. EPIDERMIDIS LIFECYCLE: HOW BACTERIA TRICK THE HOST AND THE CLINICIAN?

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Background
In the clinical setting, biofilms were thought to be an alternative phenotype to planktonic pure cultures but scientific evidence from the last decade has revealed that bacteria, similar to more evolved species, undergo a specific lifecycle containing: (i) biofilm formation (ii) dispersion and (iii) planktonic growth phases. This results in constant alterations in bacterial physiology, with significant consequences to the outcome of biofilm-related infections. *S. epidermidis*, a common commensal of the human skin and mucosae, is the leading causative agent of medical device-associated infections due to its tenacious ability to form biofilms. Here, we performed a multi-factorial analysis to understand how the physiological alterations associated with *S. epidermidis* biofilm lifecycle enable this bacterium to (i) evade the host immune response, (ii) tolerate higher concentrations of antibiotics, and (iii) avoid detection by standard diagnostic methods.

Methods
We developed *in vitro* models to obtain biofilms with distinct proportions of dormant bacteria (1), as well as to collect biofilm dispersed cells (2). The host immune response was assessed using an *in vivo* murine model and characterized by analyzing the transcriptome of splenocytes, cytokine levels in the serum and bacterial colonization in the host. Antibiotic tolerance to vancomycin, rifampicin and tetracycline was assessed by CFU counting and flow cytometry. Confocal laser scanning microscopy was used to assess structural differences in biofilms exposed to antibiotics.

Results & conclusions
Mouse organs colonization and splenocytes transcriptome demonstrated that bacteria in the different stages of the biofilm lifecycle presented distinct and unique adaptations to the host immune response. Biofilm cells induced a lower production of pro-inflammatory cytokines. Conversely, the cells' tolerance to antibiotics was higher. Presumably, this will allow a more efficient evasion from the host immune response and antimicrobial therapy. Furthermore, we observed that cells dispersed from biofilms, retained their high tolerance to antibiotics, confirming that tolerance is a phenomenon not only related to the tridimensional structure of biofilms. Finally, we observed that after antimicrobial treatment, significant discrepancies between CFU counts and the total load of viable but not cultivable bacteria, could explain the high rates of diagnose failure and recurrence rates.

Acknowledgments
This work was supported by national funds (FCT) by the strategic project of UID/BIO/04469/2013 and by BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by European Regional Development Fund under the scope of Norte2020. AF has an individual FCT fellowship (SFRH/BPD/99961/2014). NC and RV are Investigadores FCT.

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Keywords: biofilms, nosocomial infections, antibiotic tolerance, imune evasion

O-21 - IDENTIFICATION OF A TYPE III SECRETION EFFECTOR OF CHLAMYDIA TRACHOMATIS THAT ASSOCIATES WITH THE GOLGI COMPLEX OF HOST CELLS

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Background

Chlamydia trachomatis is an obligate intracellular human pathogen and the major cause of bacterial sexually transmitted diseases worldwide. During a *C. trachomatis* infection, the bacteria reside and multiply within a vacuole from where they modulate the host cytoskeleton and the vesicular trafficking. This is achieve by using a type III secretion (T3S) system, a mechanism by which bacteria deliver specific proteins, named T3S effectors, into the host cell. The characterization of these proteins can provide relevant information about the infection of this pathogenic bacterium.

Method

This work was based on an immunofluorescence microscopy analysis by using two different approaches. First, HeLa cells were infected with *C. trachomatis* strains expressing selected proteins fused to a double hemagglutinin (2HA) tag and the possible localization of these proteins in the host cell was evaluated. Second, the subcellular localization of the selected proteins was analyzed after ectopic expression in HeLa cells of the proteins fused to EGFP.

Results & Conclusions

From a set of previously identified *C. trachomatis* T3S substrates, a protein was identified as a potential effector. Upon infection with a *C. trachomatis* strain expressing a 2HA-tagged version of the protein, we observed its delivery into the host cell and accumulation at the Golgi complex, at 16h post infection. This Golgi localization was confirmed by treating infected cells with brefeldin A, a drug that induces Golgi fragmentation. In brefeldin A-treated cells the immunofluorescence signal of the protein was rapidly dispersed. Furthermore, we have identified the first 100 amino acids of the protein as a Golgi-targeting region. At longer times of infection, the localization of the protein at the Golgi was less evident, and at 40h post infection the protein was mostly localized at the plasma membrane. This change in localization of the protein was not dependent on intact actin filaments or microtubules. Currently, we are characterizing a mutant *C. trachomatis* strain for the gene encoding the protein to test its importance during *C. trachomatis* infection.

Acknowledgments

This work was supported by Fundação para a Ciência e a Tecnologia (FCT) through grant PTDC/IMI-MIC/1300/2014 and by Unidade de Ciências Biomoleculares Aplicadas (UCIBIO), which is financed by national funds from FCT (UID/Multi/04378/2013) and co-financed by the European Regional Development Fund (ERDF) under the PT2020 Partnership Agreement (POCI-01-0145-FEDER-007728). SVP holds PhD fellowship PD/BD/52210/2013 within the scope of the PhD program Molecular Biosciences (PD/00133/2012) funded by FCT.

Keywords: Bacterial pathogenesis, Type III secretion system, Chlamydia trachomatis

O-22 - TARGETING OF HOST CELL VESICLE TRAFFICKING BY THE LEGIONELLA PNEUMOPHILA EFFECTOR VIPA

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Background

Legionella pneumophila is a human bacterial pathogen that can infect lung macrophages causing an atypical pneumonia known as Legionnaires' disease. Inside host cells, *L. pneumophila* resides in a remodeled phagosome, the *Legionella*-containing vacuole. To escape host defense mechanisms, *L. pneumophila* injects into the eukaryotic cytosol more than 300 effector proteins via a type IV secretion system, its main virulence mechanism. In this work, we focused on the *Legionella* effector protein VipA, an actin nucleator interfering with vesicular transport, to further characterize how the pathogen targets eukaryotic cell processes. In particular, we analysed the association of VipA with key players of the endocytic pathway.

Method

In this work we analysed by microscopy the co-localization of VipA with components of the endocytic pathway, namely the lipid phosphatidylinositol 3-phosphate (PI3P) and the small Rho GTPase Rab5. Interaction with the first was also probed by *in vitro* binding assays, and with the latter was confirmed by co-immunoprecipitation assays.

Results & Conclusions

Different versions of Rab5 were expressed in mammalian CHO cells as GFP fusions: wild-type Rab5 (Rab5WT), a constitutively active mutant (Rab5CA) or a dominant-negative mutant (Rab5DN). When wildtype VipA was co-expressed in these cells, its typical localization changed and followed the one displayed by the corresponding Rab5 version present. This observation was also made for a VipA mutant lacking the C-terminal actin-binding region, but the distribution was unaltered in a VipA mutant lacking the N-terminal region. Co-immunoprecipitation assays of these double transfected cells further support an interaction of the *L. pneumophila* effector with Rab5 and confirm the involvement of the VipA N-terminal region, as all forms of GFP-Rab5 were able to pull-down VipA from cell extracts except for the mutant lacking this region of the effector.

Additionally, we observed a significant colocalization in mammalian cells of VipA-myc with the PI3P marker FYVE-GFP. An association with this lipid was further assessed by *in vitro* assays, whereby we verified the ability of the purified His-tagged effector to bind this lipid.

Acknowledgments

This work was supported by Unidade de Ciências Biomoleculares Aplicadas (UCIBIO), which is financed by national funds from Fundação para a Ciência e a Tecnologia (FCT; UID/Multi/04378/2013) and co-financed by the European Regional Development Fund (ERDF) under the PT2020 Partnership Agreement (POCI-01-0145-FEDER-007728), and by a Research Grant 2016 from the European Society of Clinical Microbiology and Infectious Diseases (ESCMID). I.S.F. is supported by a FCT post-doctoral fellowship (SFRH/BPD/102378/2014).

Keywords: Legionella pneumophila, effector protein, vesicle trafficking, Bacterial pathogenesis

O-23 - QUORUM SENSING REGULATION IN ERWINIA CAROTOVORA AFFECTS DEVELOPMENT OF DROSOPHILA MELANOGASTER UPON INFECTION

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Abstract

Insects are thought to play an important role in the dissemination of bacterial pathogens. The non-lethal interaction between the phytopagen *Erwinia carotovora* (strain Ecc15) and Drosophila has been extensively used as a model to study the molecular mechanisms that regulate host immune responses to microbes. Ecc15 is able to persist inside the gut of *Drosophila melanogaster*. This persistence is detected by the host which mounts an immune response to fight the bacterial infection[1]. The *Erwinia* virulence factor, Evf, was previously shown to be responsible for this persistence[2]. However, the genetic network involved in the regulation of this virulence factor is not known. Quorum sensing is a cell-cell signal mechanism used by bacteria to synchronize gene expression at the population level and engage in group behaviors which are thought to be important in many bacteria-host interactions. Given that in the *Erwinia* genera pectolytic enzymes, the major virulence factors necessary to infect plants, are regulated by quorum sensing, we asked if *evf* was also regulated by quorum sensing.

Results & Conclusions

To address this question we are using a conjugation of genetic, flow cytometry and biochemical techniques. We demonstrated that Ecc15 relies on quorum sensing to activate expression of *evf*. We showed that transcription of *evf* is abolished in the absence of acyl homoserine lactones a common class of quorum sensing signaling molecules. Activation of the gene was rescued by addition of these signals extracellularly. Moreover, while the production of pectolytic enzymes requires signal integration from the quorum sensing and the Gac system, a signal transduction pathway also active at high cell density, we observed that expression of *evf* is only partially regulated by the Gac system and quorum sensing is the main mechanism required for activation of *evf*. Additionally, we demonstrated that the regulation of *evf* via quorum sensing was essential for the persistence of Ecc15 in larvae. Finally, we further showed that, larvae infected with Ecc15 have a strong developmental delay, but development was not affected in larvae infected with Ecc15 quorum sensing mutants. These results highlight the importance of quorum sensing in the regulation of virulence factors involved in the establishment of host-microbe interaction.

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Keywords: Host-microbe interactions, Quorum sensing, Drosophila, Erwinia carotovora

O-24 - DEFINING THE ROLE OF BURKHOLDERIA CENOCEPACIA TRIMERIC AUTOTRANSPORTER ADHESIN BCAM2418 IN THE EARLY STAGES OF BACTERIA-HOST- CELL INTERACTIONS

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Background

Burkholderia cenocepacia is an opportunistic pathogen that belongs to *B. cepacia* complex (Bcc) and causes lung infections in Cystic Fibrosis (CF) patients. Trimeric Autotransporter Adhesins (TAAs), a class of extracellular proteins, are known for be key elements in early steps of *B. cenocepacia* infection, facilitating adherence to host epithelial cells [1,2,3]. *B. cenocepacia* J2315 genome encodes for 7 TAAs [4]. BCAM2418 is the larger TAA (2775 aa) and exhibit a unusual high percentage of serine residues (31%) organized in multiple tandem consensus repeats largely distributed across the extracellular domain of the protein. These serine-rich motifs could be targets for O-glycosylation; furthermore the repetitive DNA motifs of *bcam2418* gene may undergo both phase and antigenic variation.

Method

Expression patterns of *B. cenocepacia bcam2418* were evaluated in representative CF-related stress conditions: limited oxygen supply, elevated NaCl and H_2O_2 concentrations. Also, *bcam2418* expression was analysed in adherent vs non-adherent bacteria to polarized and non-polarized cells. Adhesion assays were performed with four human cell lines- lung, bronchial (CF and non-CF) and cervix epithelia. Expression of *bcam2418* and levels of cytokines release by host cells were characterized over time following different intervals of *B. cenocepacia* adhesion to 16HBE14o- bronchial cell line. Different strategies to create a deleterious *bcam2418* mutant are ongoing.

Results & Conclusions

The results showed that the expression of *bcam2418* vary in consequence of an external *stimuli,* and seems to be specifically triggered by pathogen-cell interaction. From all the studied cell lines, adhesion to bronchial epithelium prompted the most notorious increase in *bcam2418* expression. This increased expression seems to be dependent on the type of cell lines and is variable over time. The expression of *bcam2418* seems to follow an increasing pattern that reaches a maximum after 30 minutes of pathogen-host cell interaction, starting to decrease after 2 hours of contact. Nevertheless, the cellular response in terms of cytokines release seems to not follow the same time-line. Taken together, our findings reveal a role of the TAA BCAM2418 from *B. cenocepacia* at the early stage of the infection process. Current research focuses on understanding the relative contribution of BCAM2418 in mediating a crosstalk between the bacteria and host-cell surface components.

Acknowledgments

This research is supported by FCT: PD/BD/116940/2016 and UID/BIO/04565/2013

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Keywords: Burkholderia cenocepacia, Trimeric Autotransporter Adhesins, Bacteria-host interactions

O-25 - ASHBYA GOSSYPII RIBOFLAVIN OVERPRODUCING STRAINS ARE HIGHLY SUSCEPTIBLE TO LIGHT-INDUCED OXIDATIVE DNA DAMAGE

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Background
The overproduction of riboflavin by *Ashbya gossypii*, which is one of the most distinctive traits of this filamentous hemiascomycete, is triggered by oxidative stress [1]. In turn, riboflavin is a strong photosensitizer that upon irradiation with light has been shown to generate reactive oxygen species (ROS) and induce oxidative DNA damage in mammalian cells [2-3]. Envisioning a better understanding of this *A. gossypii* trait, here we investigated whether riboflavin overproduction is associated with increased DNA damage.

Method
The DNA damage accumulation in riboflavin overproducing and non-overproducing *A. gossypii* wild strains was assessed with a newly developed *Ashbya* Comet Assay (Single Cell Alkaline Gel Electrophoresis). This protocol is an adapted and optimized version of the Yeast Comet Assay [4] and is here shown to reproducibly measure oxidative (H2O2/menadione-mediated) and non-oxidative (camptothecin-mediated) DNA damage in *A. gossypii*. Radial growth and riboflavin production was assessed on agar-solidified AFM after incubation in the dark or under a visible fluorescent lamp for three days.

Results & Conclusions
The newly developed *Ashbya* Comet Assay allowed the reproducible measurement of H2O2/menadione-mediated (oxidative) and camptothecin-mediated (non-oxidative) DNA damage. Further assessment of the DNA damage in different *A. gossypii* wild strains with this validated protocol revealed significantly higher DNA damage accumulation in the riboflavin overproducing strain when it was exposed to light during growth. However, no significant differences were observed in terms of growth or riboflavin production by this strain. The non-overproducing strain did not display significant differences between conditions in any of the measured parameters. These evidences show that the accumulation of riboflavin in *A. gossypii* makes it highly susceptible to light-induced oxidative DNA damage, similarly to what occurs in mammalian cells [2-3]. These results thus draw attention for the importance of controlling the exposure to light of biotechnological riboflavin production processes (with *A. gossypii* or other organisms).

Acknowledgments
Study supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UID/BIO/04469/2013 unit and COMPETE 2020 (POCI-01-0145-FEDER-006684) (Post-Doc grant to T. Q. Aguiar), BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund under the scope of Norte2020 - Programa Operacional Regional do Norte, Project RECI/BBB-EBI/0179/2012 (FCOMP-01-0124-FEDER-027462) and PhD grant PD/BD/113812/2015 to R. Silva.

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Keywords: Ashbya gossypii, Riboflavin overproduction, Oxidative stress, DNA damage, Comet assay

O-26 - QUORUM SENSING SIGNAL RECOGNITION BY A NOVEL AI-2 RECEPTOR FROM CLOSTRIDIA

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Background

Quorum sensing is a cell-to-cell communication mechanism that through the exchange of small chemical molecules named autoinducers allows bacteria to monitor their population density and regulate gene expression accordingly. Autoinducer-2 (AI-2) is a quorum sensing signal that is unique in facilitating inter-species communication.[1] While it is produced and recognized by a wide variety of bacteria, to date only two classes of receptors have been identified: the LuxP-type, in the *Vibrionales,* and the LsrB-type, found in a number of phylogenetically distinct bacterial families.[2-4] Recently, AI-2 was shown to affect the colonization levels of a variety of bacteria in the microbiome of the mouse gut,[5] including members of the genus *Clostridium*, but no AI-2 receptor had been identified in this genus.

Method

To identify novel AI-2 receptors in this genus a combination of bioinformatics, biochemistry and microbiology was employed.

Results & Conclusions

Here, we demonstrate that *Clostridium saccharobutylicum* possesses a functional LsrB-type receptor. The crystal structure of the *C. saccharobutylicum* receptor shows that it binds the same form of AI-2 as the other known LsrB-type receptors, but also reveals that it has two previously unobserved variations in the amino acids of the AI-2 binding site. Isothermal titration calorimetry shows that this receptor binds AI-2 with high affinity having a dissociation constant (Kd) in the submicromolar range. Altogether, this work represents the first identification and characterization of AI-2 receptors in the *Clostridium* genus and shows previously unseen variations in the binding site of LsrB-type AI-2 receptors. These findings are important for the identification of novel AI-2 receptors in microbes of known biological importance in the mammalian gut microbiome, a therapeutically relevant niche where inter-species interactions are highly prevalent. Moreover, it provides valuable knowledge for the design of agonist or antagonists active in a wider range of quorum sensing receptors.

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Keywords: Autoinducer-2, Quorum-sensing, Microbiome, Cell signaling, Crystal structure

O-27 - GOOD BACTERIA, BAD BACTERIA – INSIGHTS FROM HEALTHY WOMEN URINARY MICROBIOTA

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Abstract

Urinary tract infections (UTI) are the most common human infections, associated with high rates of recurrence and antibiotic consumption. Current UTI diagnosis criteria assume that the urinary tract (UT) is sterile, but recent discoveries revealed the presence of a diverse microbial community that is thought to influence both normal physiology and disease susceptibilities. This study aimed to evaluate, using enhanced culturomics, the bacterial diversity from young healthy women UT, and if it may act as a reservoir of common uropathogens.

Mid-stream clean catch urine samples were collected from 12 healthy female volunteers (age:25-35). Participants had no clinical evidence of UTI or other UT disorders, and no antibiotic treatment in the previous month. Samples were processed according to a recently established enhanced culture protocol (several media and increased sample volume–100uL) slightly modified. Representatives of each colony morphology were identified by MALDI-TOF VITEK MS and/or appropriate genetic marker.

Results & Conclusions

A total of 1797 isolates (average:150 isolates/sample) were characterized. Most bacteria represented the *Firmicutes* and *Actinobacteria* phyla, with similar species diversity. A total of 86 bacterial species belonging to 38 different genera were detected among the 12 urine samples, corresponding to an average of 19 different species/sample (range:12-30). *Staphylococcus* spp., *Corynebacterium* spp., *Streptococcus* spp. and *Lactobacillus* spp. where the most prevalent genera, with high diversity at the species level. *Staphylococcus epidermidis* was detected in all samples, followed by *Staphylococcus lugdunensis* (41.67%), *Staphylococcus aureus* (25%), and *Staphylococcus saprophyticus* (16.67%), all recognized as potential uropathogens. *Enterococcus faecalis* (58.33%) and *Streptococcus agalactiae* (33,33%) were also frequently detected. Less frequently found species included *Aerococcus urinae*, *Actinotignum schaalii*, and *Corynebacterium coyleae*, involved in UTI and other UT disorders. Curiously, gram-negative bacteria which are the main agents of UTI among young women, namely, *Escherichia coli* (16.67%), *Pseudomonas putida* (8.33%), and *Stenotrophomonas maltophilia* (8.33%), were found in lower frequency, with the latter two never described in previous studies among healthy women. Species known as opportunistic pathogens, involved in bacterial vaginosis like *Gardnerella vaginalis*, *Lactobacillus iners*, *Atopobium vaginae*, *Prevotella bivia*, *Veillonella parvula,* and also associated with UTI, were detected in a lesser extent.

This study unveiled a considerable inter-individual diversity of female urinary microbiota among young healthy women, similar to previous studies. However, it is of concern the identification of potential pathogenic bacteria, in particular, those associated with UTI, vaginosis and neonates' severe infections, which will have tremendous impact on UTI and other UT disorders diagnostic and treatment approaches.

Acknowledgments

SFRH/BPD/95556/2013, SFRH/BD/132497/2017

Keywords: female urinary microbiota, culturomics, uropathogen

O-28 - EXPRESSION OF THE ABC TRANSPORTER PDR18 IS ESSENTIAL TO COUNTERACT THE NEGATIVE IMPACT OF ACETIC ACID STRESS ON YEAST PLASMA MEMBRANE

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Background

The plasma membrane (PM) ATP-binding cassette (ABC) transporter Pdr18 confers a multi-stress tolerance phenotype in *Saccharomyces cerevisiae*; its expression improves tolerance to the herbicides 2,4-D, barban and MCPA, the fungicide mancozeb, the metal cations Zn^{2+}, Mn^{2+}, Cu^{2+} and Cd^{2+}[1] and ethanol[2]. Pdr18-mediated tolerance to 2,4-D was associated to the increase of PM ergosterol content and reduction of transmembrane electrochemical potential dissipation under stress[1].

PDR18 expression also alleviates ethanol-induced PM permeabilization, reducing ethanol intracellular level and toxicity[2].

This study describes the protective role of Pdr18 against acetic acid, another relevant stress in Industrial and Food Biotechnology, and the underlying mechanisms.

Method

Susceptibility phenotypes were based on spot assays and minimum inhibitory concentration values. PM permeability was assessed based on propidium iodide uptake, PM electrochemical potential based on the uptake of ^{14}C-methyammonium or 3,3′-Dipropylthiadicarbocyanine iodide fluorescence. Laurdan Generalized Polarization (GP), obtained by two-photon excitation microscopy, was used to compare PM lipid order. mRNA levels from *PDR18* or ergosterol biosynthetic genes were compared by real-time RT-PCR. Ergosterol content in cell membranes was quantified by GC-MS, after membrane isolation and lipid extraction.

Results & Conclusions

Pdr18 was demonstrated to be determinant for yeast tolerance to acetic acid. Consistent with its hypothesized role in PM ergosterol content, *pdr18*Δ mutant exhibited lower ergosterol levels than the wild-type, in the presence and absence of acetic acid. *PDR18* mRNA levels peak when cells resumed growth and the *ERG*-genes exhibited a coordinated transcriptional activation, consistent with a role for Pdr18 in ergosterol homeostasis.

During acetic acid stress cultivation, cell permeabilization peaks during the lag-phase and reduces to basal levels when cells resumed growth, *PDR18* expression partially counteracting this permeabilization. Exponentially-growing cells adapted to acetic acid have a higher PM electrochemical potential and a more ordered PM when *PDR18* is expressed.

Recent studies provide evidence supporting a physiological role for multidrug/multixenobiotic resistance (MDR/MXR) transporters other than extrusion of multiple toxic compounds functionally and structurally unrelated[3]. The role here proposed for Pdr18 in ergosterol non-vesicular transport and incorporation in yeast PM under stressing conditions, provides another example of an ABC transporter whose function is not consistent with the drug-pump model.

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This work was partially funded by FCT (ERA-IB-2/0003/2015,FAPESP/20107/2014,SAICTPAC/0019/2015). Funding received by iBB from FCT (UID/BIO/04565/2013) and PORLisboa2020 (N.007317) is also acknowledged. CPG and SNP are the recipients of FCT fellowships (SFRH/BD/92252/2013,SFRH/BPD/92409/2013) and FF is a FCT Researcher (IF/00386/2015).

Keywords: ergosterol homeostasis, acetic acid resistance, Saccharomyces cerevisiae, ABC transporters, Multidrug resistance transporters, membrane physical properties

O-29 - METABOLIC EQUILIBRIUM AND THE YEAST LONGEVITY

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Background

Diet has been shown to have a major impact on longevity regulation, and particularly calorie restriction (CR), that is restriction of calorie intake without malnutrition, is often considered the most robust non-genetic intervention to extend lifespan. Many studies demonstrated the beneficial effects of CR in life span extension of multiple organisms, as well as in the improvement of overall health in rodents. However, new evidences have emerged from studies in yeast and higher eukaryotes showing the importance of nutrient balance in dietary regimens and its effects on longevity regulation, challenging the notion that the indiscriminate reduction of caloric intake per se extends life span.

Method

Yeast CLS was evaluated by monitoring cell viability, defined as the ability of quiescent cell to re-enter the cell cycle upon return to nutrient-replete conditions. Cell cycle was assessed by flow cytometry.

Results & Conclusions

Here we show that ammonium induces shortening of CLS in the prototrophic strain *Saccharomyces cerevisiae* PYCC4072 and that these adverse effects depend on a fine balance between ammonium, glucose and Yeast Nitrogen Base. Maximum CLS extension could only be attained when nutrients of the YNB are not limiting glucose and ammonium consumption, and therefore ammonium is totally consumed before aging. The increase of CLS due to ammonium exhaustion in the culture medium, even in conditions where caloric restriction was not applied, is associated with cell cycle entrance in G0. Furthermore, ammonium can decrease yeast CLS independently of the metabolic process activated during aging as extended CLS was observed both for cells fermenting glucose or oxidizing ethanol in the several conditions of nutritional balance tested. Amino acids like glutamine can induce CLS shortening similarly to ammonium, but this effect was not observed with the poor nitrogen sources urea and proline.

In summary, results show that the equilibrium between carbon and nitrogen sources, regardless of the activated metabolic process acts as a determinant factor in the modulation of longevity, as long as there are no limitations of other nutrients. In line with these results, one can anticipate that nutritional balance can be an important target to consider for the definition of interventions to promote life span and healthy aging in humans.

References & Acknowledgments

This work was developed under the scope of NORTE-01-0145-FEDER-000013, supported by NORTE2020 through FEDER, by FEDER, through COMPETE, and by National funds, through FCT under the scope of the project POCI-01-0145-FEDER-007038 as well as through UID/BIA/04050/2013.

Júlia Santos holds a Post-Doc fellowship (SFRH/BPD/112108/2015) by FCT.

Keywords: Yeast longevity

O-30 - THE IMPACT OF PRIVATE USE OF THE 13-VALENT PNEUMOCOCCAL CONJUGATE VACCINE (PCV13) ON PNEUMOCOCCAL CARRIAGE AMONG PORTUGUESE CHILDREN

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Background
Streptococcus pneumoniae (pneumococcus) colonizes asymptomatically the human nasopharynx. Children attending day-care centers are major reservoirs of pneumococci contributing significantly to their transmission in the community. Pneumococcus is also a major cause of infectious diseases worldwide such as otitis media, pneumonia, bacteremia and meningitis. The major virulence factor of pneumococcus is its polysaccharide capsule, and at least 95 different capsular types (serotypes) are currently known. In Portugal, a seven-valent pneumococcal conjugate vaccine (PCV7, targeting serotypes 4, 6B, 9V, 14, 18C, 19F and 23F) became commercially available in 2001. In January 2010, PCV7 was replaced by a 13-valent PCV (PCV13, targeting PCV7-types plus serotypes 1, 3, 5, 6A, 7F and 19A). PCV13 was introduced in the national immunization plan in July 2015. We evaluated the impact of PCV13 private use on serotype distribution of *S. pneumoniae* carried by children attending day-care in an urban and a rural region of Portugal

Method
Three periods were studied: pre-PCV13 (2009-2010), early-PCV13 (2011-2012) and late-PCV13 (2015-2016). Pneumococci were isolated from nasopharyngeal samples of a total of 4,232 children up to 6 years old.

Results & Conclusions
Pneumococcal carriage remained stable in both regions (range: 59.5%-62.8%). Vaccination with PCVs was high in both regions (range: 76.8%-89.3%), having increased significantly in the late-PCV13 period ($p=0.016$ and $p=0.020$ in the urban and rural regions, respectively).

Carriage of PCV7-types decreased from 7.7% in the early-PCV13 to 4.3% in the late-PCV13 period in the urban region ($p=0.017$), but was maintained in the rural region at ca. 5% ($p=0.270$). Carriage of the additional PCV13-types decreased in both regions from 15% in the pre-PCV13 to 1.7% in the late-PCV13 period ($p<0.001$). These additional PCV13-types were mostly carried by unvaccinees in the early-PCV13 period ($p=0.004$).

Prevalence of serotype 19A was the most affected by PCV13, declining from 7.9% to 0.7% ($p<0.001$). The most prevalent PCV13-type in the late-PCV13 period was 19F (1.7% and 4.5% in the urban and rural region, respectively). The most prevalent non-PCV13-types in the late-PCV13 period were serotypes 15B/C (5.9%), 23B (5.7%) and 11D (5.6%) in the urban region, and serotypes 15A (6.4%), 23A (5.0%), and 35F (4.8%) in the rural region.

In conclusion, after 5-6 years of PCV13 use in the private market, PCV13-types have declined significantly among young children (average of both regions of 6.2%). As children are major reservoirs and sources of pneumococci, we anticipate these results should trigger a herd effect in other age groups, both in carriage and disease.

Keywords: Streptococcus pneumoniae, PCV13, Colonization, Disease, Serotype

O-31 - ADAPTIVE EVOLUTION OF NON-SACCHAROMYCES YEASTS TO PRODUCE WINES WITH LOW ETHANOL CONTENT

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Background

In recent years, alcohol level in wines produced around the world has increased as a consequence of global warming. Warmer climates led to grapes with higher glucidic content and affect their phenolic maturity and aromatic profile [1]. The arising concern about the health effects of the consumption of high alcoholic wines has promoted the search for ways to produce low alcohol wines. Although non-*Saccharomyces* yeasts do not ferment so vigorously, their use as starters is a promising alternative that may also have a beneficial influence on the organoleptic characteristics of wines [1]. In adaptive evolution (AE) yeast populations are exposed over time to a sub-lethal concentration of a stressing agent, until they acquire certain mutations that lead to adaptation. Since the exposition of yeast cells to KCl or furfural may lead to a metabolic shift with increase of the glycerol/ethanol ratio [2], an AE strategy based on these stress agents was applied aiming to select novel variants with lower ethanol production.

Method

Two non-*Saccharomyces* indigenous *y*east strains (134|MET and 483|LCH) and a *Saccharomyces* starter (771|SAC) were submitted to AE. To define the initial sub-lethal concentrations of each selective pressure, growth behavior and kinetics of each strain were characterized in the presence of increasing concentrations of KCl and furfural. Yeast cultures under AE were cultivated in synthetic must. At intervals of three/four days the medium was renewed and the number of generations calculated. Large scale fermentations in natural must were performed to assess the progress of AE process. The metabolic compounds were quantified by HPLC and the produced wines submitted to a sensory evaluation.

Results & Conclusions

Since the growth rate of the three yeasts was similarly affected by both selective pressures, with a severe effect at high concentrations, an identical initial concentration of each pressure was used for all strains. Although without significative changes in terms of ethanol and glycerol production, it is already notorious an improvement on the fermentative metabolism of the evolved strains. Moreover, during the sensory analysis, tasters expressed a preference for the wines produced with the evolved strains of each species. The wines of the evolved strains of 483|LCH were considered the best ones in terms of smell, taste, complexity and freshness.

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Keywords: Non-Saccharomyces yeasts, Adaptive evolution, Wine

O-32 - MARINE BACTERIA FROM AZORES AS POTENTIAL SOURCE OF TRANSAMINASES FOR DRUG DEVELOPMENT

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Background

Marine bacteria have been exposed to a variety of natural conditions that contributed to their evolution. The Azores archipelago, located in the middle of the Atlantic Ocean and presenting volcanic activity, is home to a vast microbial population which may harbor novel enzymes and compounds. [1]. However, cultivation of bacteria under laboratorial conditions is fundamental to the discovery and application of new biocatalysts [2]. Enzymes with new properties, such as a broad specify towards relevant industrial substrates, are needed to improve current processes and substitute classic chemical methods used in drug development. Transaminases or aminotransferases are a group of enzymes that transfer an amino group from a donor (amine) to an acceptor (ketone or aldehyde). The chiral amines produced are valuable molecules for the pharmaceutical industry as building blocks, and may lead to the development of new drugs.

Method

Marine samples were collected at São Miguel island. Several methods were used to grow bacteria in the laboratory, including: different media composition, oscillatory growth conditions, pre-treatment of samples and dilution to extinction. The isolates obtained were used for high throughput screening of transaminase activity using an amine donor and a ketone in 96 well plates. The positive isolates for transaminase activity were further tested with industrial relevant ketones and amines.

Results & Conclusions

The techniques of bacteria cultivation applied to Azorean samples resulted in a library with approximately 700 isolates. This microbial library is a living resource of biological material, with putative diversity of applications in biotechnology, as cells produce enzymes, antimicrobials and other secondary metabolites ready to be explored. The high throughput screening of the isolates, using 96 well plates, allowed the identification of bacteria with high production of transaminases. Besides, some isolates showed transaminase activity for different ketones, demonstrating their broad spectrum and promiscuity. These results show great potential for the application of these transaminases from the Azores natural resources in the pharmaceutical industry, thus contributing to the development of new drugs in the future.

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Keywords: Marine Bacteria, Biocatalysis, Enzymes, Transaminases

O-33 - COMPARISON OF ACTIVITY OF PSEUDOMONAS PUTIDA PPDYP PEROXIDASE AND OF ITS EVOLVED VARIANT 6E10 FOR 28 LIGNIN-RELATED PHENOLICS

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Background

Dye-decolorizing peroxidases (DyPs) are newly discovered microbial heme containing peroxidases that oxidize a wide range of different substrates from anthraquinone and azo dyes, β-carotene, aromatic sulfides and metal ions, to non-phenolic and phenolic lignin units, which put them in the front line for lignocellulose biorefineries. Recently, a new DyP enzyme, 6E10, was architected through directed evolution which was evolved towards 100-fold higher efficiency (k_{cat}/K_m) for 2,6-dimethoxyphenol (syringol) as compared to the native PpDyP enzyme from *Pseudomonas putida* MET94[1,2]. To verify if this new enzyme displayed potential for industrial applications, its enzymatic activity was determined for 28 different lignin-related phenolic substrates, and compared to the wild-type enzyme.

Method

The UV-visible absorption spectra of an array of syringyl, guaiacyl and hydroxybenzene lignin-related phenolic compounds were recorded before and after reaction with the enzymes. Next, the molar extinction coefficients of selected phenolic compounds were determined at their maximal wavelengths at different pH values. The Vmax of each reaction was measured in 96 well plates using a microtiter plate reader; reactions were started by the addition of 0.5 mM H_2O_2 to 5 mM of each substrate at pH 4 for reactions with wild-type PpDyP and pH 8 for evolved variant 6E10 and monitored at the maximal wavelengths for each substrate. Reaction mixtures were analysed by HPLC and substrate conversion yields were calculated.

Results & Conclusions

The 6E10 evolved enzyme by directed evolution was shown to have 3- to 700-fold higher enzymatic rates for the oxidation of 19 out of the 28 phenolic compounds tested than wild-type PpDyP. Moreover, the evolved enzyme was able to degrade 5 additional phenolic compounds not oxidized by the wild-type enzyme. The reaction products were analyzed by HPLC and conversion levels after 24 h of reaction were found near 100 % for some of the tested substrates. These results revealed that the 6E10 variant is a promising enzyme for biotechnological applications, not only due to higher activities presented when compared to the wild-type enzyme, but also, due to the higher promiscuity in the degradation of substrates.

References & Acknowledgments

This work was supported by Fundação para a Ciência e Tecnologia (FCT), Portugal (PTDC/BBB-EBB/0122/2014) and Research Unit GREEN-it "Bioresources for Sustainability" (UID/Multi/04551/2013).

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O-34 - AGED FREEZE-DRIED AMPOULES OF PRESERVED BIOTECHNOLOGICAL IMPORTANT FUNGI

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Background

The implementation of consistent fungal preservation techniques and appropriate quality assurance are key issues for an effective and efficient preservation. The cost and convenience of each method are important aspects to be taken into consideration such as the knowledge of all parameters capable of affecting the procedures [1]. Preservation methods currently used are highly empirical and in many instances, do not provide reliable genetic and phenotypic stability. Freeze-drying is commonly used to preserve fungal strains at room temperature, however, genetic and phenotypic alterations after long term-storage are yet unknown. Therefore, the main goal of the present experimental study is to evaluate the freeze-drying preservation method for the effective long-term preservation of strains belonging to *Aspergillus* section *Nigri*.

Method

Twenty-one strains representative of *Aspergillus* section *Nigri* were selected and preserved by freeze-drying. The strains were subjected to accelerated storage during 4 weeks at 37 °C. These samples were morphological, physiological and genotypical analysed. In order to detect macro and micro-morphological changes, growth for seven days at 25°C on Potato Dextrose Agar, Malt Extract Agar, Czapek Yeast Extract Agar and Czapek Dox Agar was performed. The physiological changes were monitored for the detection of ochratoxin A and fumonisin B2 as described elsewhere [2,3]. In order to identify genotypic changes, DNA fingerprinting techniques using the oligonucleotides M13 and $(GACA)_4$ were performed. All assays were evaluated at 3 points in time: before preservation (I), 2 (II) and 4 (III) weeks after preservation.

Results & Conclusions

For all the methodologies used to evaluate freeze-drying of fungi along time the major results are: 1) no significant changes were observed in the macro and micro-morphological analysis; 2) all strains maintained their mycotoxins production pattern, before and after ageing; 3) after ageing different DNA fingerprinting was observed.

In conclusion, freeze-drying can be considered a technique of excellence to be used on the maintenance of biodiversity within the filamentous fungi, and more accurately for *Aspergillus* section *Nigri*. However, it is recommended to consider possible genetic changes after long shelf-life periods.

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Keywords: Fungal Preservation, Genetic Stability, Phenotypic Stability

O-35 - ONE-STEP FRUCTO-OLIGOSACCHARIDES PRODUCTION AND PURIFICATION

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Background

Growing consumer awareness on high nutritional value ingredients and their impact in health has been raising the interest in functional food including prebiotics. Fructo-oligosaccharides (FOS) are prebiotic sugars that have been industrially produced via fermentation, by several microorganism enzymes, in two-step bioprocess. Due to enzyme activity inhibition by the high amount of glucose released during fermentation, the maximum theoretical yield obtained ranges between 55 to 60% of sucrose converted into FOS, with less than 60% of pure FOS [1]. To obtain high-content FOS we explored the use of a co-culture of *Aspergillus ibericus* MUM 0.349 as FOS producer strain, with *Saccharomyces cerevisiae* YIL162W (a yeast with the gene responsible for sucrose hydrolysis disrupted) as small saccharides removal.

Method

The whole-cell microorganisms were used in a one-step bioprocess. Fermentation conditions were optimized through a central composite design (CCD) in shaken-flask. Substrate used was 200 $g.L^{-1}$ of sucrose. Parameters such as initial yeast concentration, inoculation time, fermentative broth composition, temperature and pH were optimized. Sugars were analysed by HPLC. Finally, fermentation was scaled-up to a lab bioreactor size.

Results & Conclusions

Fermentations conducted in shaken-flasks using a single-culture of *A. ibericus* yielded 65 ± 5% of FOS with a purity of 60.4 ± 0.4% (w/w). The simultaneous inoculation of the co-culture reached the highest purity in FOS. The CCD model showed that the yeast extract concentration and the temperature were the most significant factors affecting FOS purity. Fermentations run under 30ºC, initial pH of 6.0 and 17 $g.L^{-1}$ yeast extract led to FOS mixtures with 97.4 ± 0.2% (w/w) purity. After model validation, fermentations were carried out in the bioreactor. The scale-up fermentations yielded 64 ± 2% of FOS, after 141 h, with a FOS content of 118 ± 5 $g.L^{-1}$ and a purity of 93.0 ± 0.5% (w/w). The one-step fermentation using the specific co-culture studied showed to be more efficient, economical and fast than the conventional two-step bioprocesses, thus avoiding the need of the conventional downstream treatments.

References & Acknowledgments

Nobre acknowledges the Portuguese Foundation for Science and Technology for the grant ref.SFRH/BPD/87498/2012, the strategic funding of UID/BIO/04469/2013 unit, COMPETE 2020 (POCI-01-0145-FEDER-006684), BioTecNorte operation (NORTE-01-0145-FEDER-000004) and the project MultiBiorefinery (POCI-01-0145-FEDER-016403) funded by European Regional Development Fund under the scope of Norte2020 - Programa Operacional Regional do Norte.

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Keywords: Fructo-oligosaccharides, Co-culture, Integrated Process, One-step fermentation, Aspergillus ibericus, Saccharomyces cerevisiae

O-36 - VALUATION OF THE ULTRAFILTRATION BY-PRODUCT FROM APPLE JUICE CONCENTRATE INDUSTRY TO PRODUCE APPLE CIDER

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Background

Resultant from clarification of concentrate apple juice industry, through the ultrafiltration process, is produced the by-product retentate. It is liquid to sludgy, with total solids content of 8%, usually discarded as an industrial waste. However, its origin and expected composition may allow to define it as a source of valuable compounds for food applications, such as the development of a cider. Using a simple biotechnological approach in a brewery plant, with the fermentation of apple juice concentrate together with retentate. Due to apple juice concentrate has deficiency in amino acids, retentate could generate a nutritional medium suitable for the yeast growth.

Method

The retentate suspensions were centrifuged to separate soluble and insoluble material. Then, the insoluble material was washed with water. The water insoluble material was extracted with *n*-hexane; ethanol with 1% (v/v) of acetic acid; acetone:water (6:4, v/v) with 1% acetic acid.

The cider was fermented using the apple juice concentrate, which was diluted to obtain 12ºBrix, and mixing it with the retentate in a proportion of 4:1 (v/v), reconstituting the original juice.

Results & Conclusions

The present work shows that retentate is very rich in carbohydrates, namely 45% fructose (dry weight basis), glucose (9%), oligosaccharides (5%), and polysaccharides (3%). The water soluble components represent 57% of total solids, easily recovered by centrifugation, mainly composed by free sugars. Retentate is also rich in protein (8%) and an available and inexpensive source of β-sitosterol (0.6%). It was the major free sterol identified, accounting for 22% of total lipophilic compounds recovered by *n*-hexane. From retentate it is also possible to recover 15% of insoluble compounds, mainly protein (36%) and polysaccharides (20%). The major amino acids present were valine (31.8 mg/g), alanine (26 mg/g), leucine (25 mg/g), and isoleucine (19.1 mg/g). Polysaccharides were composed mainly by xyloglucans.

The retentate suspension was successfully used as a nutritive source for production cider by a microbrewery. For the formulation of the nutritive medium a hydrolysis of proteins was promoted through the proteolytic enzymes present in the barley malt. The free amino acids released from retentate accounted for 0.39mg/mL, which are in the ideal range for the yeasts growth. A consumer sensorial evaluation revealed a good acceptance of the final product that have already been commercialised under the brand Vadia.

References & Acknowledgments

Project ProfitApple 38162, QREN I&DT in co-promotion, FEDER, COMPETE. FCT/MEC financial support for QOPNA (FCT UID/QUI/00062/2013), Elisabete Coelho (SFRH/BPD/70589/2010), through national funds, FEDER, PT2020.

Keywords: Retentate; Apple pomace sludge; Carbohydrates; Protein and amino acids composition; Cider; Lipophilic compounds; β-Sistosterol

O-37 - HIGH-RESOLUTION ANALYSIS OF MULTIDRUG RESISTANT KLEBSIELLA PNEUMONIAE: FROM KEY MOLECULAR DRIVERS TO CLONAL DELINEATION

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Background

Klebsiella pneumoniae (*Kp*) is nowadays recognized as one of the most challenging pathogens worldwide due to its ever-increasing implication in human infections, and association with a cumulative resistance to multiple clinically important antibiotics, including relevant and even last-resource therapeutic choices. This work pretends to elucidate the molecular basis for the expansion of multidrug resistant (MDR) *Kp* strains by a multidisciplinary approach including conventional genotypic, phenotypic, and molecular tools and some of the most cutting-edges high-throughput omics (genomics, metabolomics, proteomics) approaches.

Method

A collection of MDR *Kp* isolates from clinical settings [n=139; different hospitals and long-term care facilities (LTCFs); 2006-2017] recovered mostly in our region was deeply characterized using a comprehensive multilevel approach (clones, mobile genetic elements). Additionally, the latter and a representative international collection of the main MDR *Kp* clones was used to assess the potential of different high- throughput omics [whole genome sequencing (WGS), Fourier-transform infrared (FT- IR) spectroscopy, MALDI-TOF MS] as alternative tools for typing.

Results & Conclusions

This work provides a comprehensive overview of the molecular drivers for resistance to extended-spectrum cephalosporins (mostly CTX-M- 15 extended-spectrum beta-lactamase), carbapenems (mainly KPC-3 carbapenemase), and more recently, colistin (MCR-1), unveiling a selection of particular lineages (ST15, ST147, ST45) and species-specific plasmids (IncR/IncFII$_K$/IncX$_4$). In addition, our results included the development of an unmet quick and low cost bacterial typing tool based on FT-IR spectroscopy with potential for routine application and commercialization as a diagnostic tool, as evidenced by the application of this approach in a real-time context. Moreover, it was demonstrated a great relevance of surface bacterial components (and particularly the capsule) on the evolution, host adaptation and/or virulence of this pathogen that deserves to be further explored.

References & Acknowledgments

Work funded by FEDER (POCI/01/0145/FEDER/007728) and FCT/MEC (UID/MULTI/04378/201) funds. CR was supported by a fellowship from FCT/POCH (SFRH/BD/84341/2012) and a FEMS Research Grant (FEMS-RG-2014- 0089). AN was supported by a fellowship from FCT/POCH (SFRH/BPD/104927/2014).

Keywords: Klebsiella pneumoniae, antibiotic resistance, drivers, typing, high- throughput omics

Genomics and Systems Biology and Emerging Technologies

O-38 - SINGLE DOMAIN ANTIBODIES FOR BRAIN TARGETING AND DRUG DELIVERY

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Background

Neurological disorders are one of the major causes of mortality representing 12% of total global deaths each year. Despite the efforts effective brain drug delivery has systematically been challenged by the impenetrability of the blood brain barrier (BBB). This is especially true for drugs that address major unmet needs in the Central Nervous System (CNS) area such as Alzheimer's, Parkinson's, stroke, meningitis and brain cancers making the discovery or design of BBB penetrating drugs the main goal of neuroscience research. One approach is to target specific BBB transport systems and develop CNS drug delivery strategies that exploit these natural portals of entry into the brain. To achieve this there has been a strong interest in the development of monoclonal antibodies (mAbs) toward BBB receptors. Some of these mAbs transmigrate the BBB and have been tested as vectors to deliver drugs into the brain. However, these mAbs rely on broadly expressed receptors such as transferrin and insulin receptors possibly bringing unwanted side effects and resulting in a low fraction of the injected dose actually reaching the brain. Promising alternatives are single domain antibodies (sdAb). These smaller antibody molecules show improved tissue penetration reaching targets not easily accessible by conventional antibodies, higher stability, low immunogenicity and lower manufacturing costs. Moreover, since sdAbs lack the Fc domain of a full IgG antibody, the nonspecific uptake in tissues that highly express Fc receptors is low.

Method

We developed a rabbit-derived immune sdAb library as an antibody source of highly specific and improved BBB-transmigrating sdAbs. Specific BBB sdAbs were obtained by immunizing two New Zealand White rabbits with brain endothelial cells. Rabbits were then sacrificed and with the spleen and bone marrow we constructed a phage displayed-sdAbs library. Paralleled screens are currently being performed in both in vitro and in vivo models for selection and identification of sdAbs towards new BBB receptors with BBB penetration properties.

Results & Conclusions

A specific immune response towards brain endothelial cells was obtained for both rabbits. Both serums showed BBB crossing properties in the in vitro and in vivo models. sdAbs libraries against brain endothelial cells were efficiently constructed with a diversity with ~ 10^7. Phage display selections and functional screenings are currently being performed to identify potent BBB crossing sdAbs and novel BBB endogenous receptors that can provide a more selectively delivery into the brain.

References & Acknowledgments

Fundação para a Ciência e Tecnologia

Keywords: BBB, sdAbs, drug delivery, Brain targeting

O-39 - INNUENDO PLATFORM: INTEGRATING HIGH-THROUGHPUT SEQUENCING INTO BACTERIAL FOODBORNE PATHOGEN SURVEILLANCE

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Background

Pathogen surveillance and outbreak investigations are crucial tasks to prevent and control transmission of food and environmentally transmitted diseases. In this regard, molecular typing is frequently used in epidemiological investigations to establish relationships between different isolates. The continuously reducing costs of High-Throughput Sequencing (HTS) and availability of bench-top sequencers makes HTS applicable in routine surveillance and outbreak investigation, maximizing discriminatory power. However, lack of standardized and accessible bioinformatics infrastructure for data processing and integration, together with limited bioinformatics skills, continue to be the two major hurdles of HTS routine implementation. To overcome these limitations, we developed the INNUENDO platform, an infrastructure that provides not only a user-friendly interface but also the required framework for data analysis, from raw data quality assurance to integration of epidemiological data and visualization of the final analyses, democratizing the use of HTS techniques in everyday surveillance and outbreak investigation.

Method

It is critical to evaluate the quality of sequencing data and the potential existence of technical errors by setting up careful quality control (QC) measures on all algorithmic steps. The INNUENDO platform includes the INNUca pipeline for automatic QC evaluation from reads to draft genome assemblies, which ultimately aims at producing consistently high-quality and comparable genomic data. The curated genome assemblies are then analysed following a gene-by-gene typing based approach. The chewBBACA suite is used to perform the allele calling for whole genome MLST (wgMLST) profile definition. The wgMLST profiles generated for each isolate of interest can then be compared with profiles already stored in the platform's database for context. The wgMLST profiles of the isolates of interest, together with a selection of the ones closest in the database, are then filtered to produce a core genome MLST (cgMLST) and the data sent to PHYLOViZ Online for the construction of a minimum spanning tree annotated with the metadata, allowing an easier interpretation of possible epidemiological scenarios.

Results & Conclusions

Since the INNUENDO platform was developed with a modular design it allows the incorporation of different bioinformatic tools for the characterization of specific pathogens. It also aims to facilitate data sharing and communication between different institutions, promoting a multidisciplinary and multisectorial cooperation in surveillance and outbreak investigation, ultimately reaching the "One Health" concept. The use of open source tools and standardized protocols will allow a future accreditation of the INNUENDO platform.

References & Acknowledgments

More information on INNUENDO project (co-funded by EFSA) and the platform can be found in http://www.innuendoweb.org/

Keywords: genomics, epidemiology, web-based platform, high throughput sequencing, surveillance, outbreak detection

O-40 - SYSTEMS BIOLOGY APPROACHES IDENTIFY HOST MICRORNAS CONTROLLING INFECTION BY BACTERIAL PATHOGENS

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Background
MicroRNAs (miRNAs) are small RNA molecules that play crucial roles in post-transcriptional regulation of gene expression and have well-established functions in physiological and pathological processes. Accumulating evidence supports an important role for miRNAs in the interplay between bacterial pathogens and host cells (1), participating as host defense mechanisms, as well as exploited by bacteria to subvert host cellular functions.

Method
To comprehensively analyze miRNA function in the context of infection by bacterial pathogens, we apply integrated systems biology approaches involving image-based high-throughput functional screenings and RNA-sequencing.

Results & Conclusions
By performing screenings using genome-wide libraries of miRNA mimics (approx. 2,100 mature sequences), we identified host miRNAs that strongly determine the outcome of infection by the bacterial pathogens *Salmonella* Typhimurium and *Shigella flexneri*. Interestingly, we determined that infection by these two pathogens is regulated by distinct subsets of host miRNAs, and identified miRNAs that interfere specifically with different steps of infection. In parallel, RNA-seq analysis revealed that *Salmonella* induces major changes in host miRNA expression, whereas *Shigella* regulates a restricted number of miRNAs.

Among the strongest inhibitors of *Salmonella* infection we identified miRNAs that hinder G1/S cell cycle progression, uncovering a crucial role of the host cell cycle during infection (2). In particular, we demonstrated that miRNAs inducing G1 cell cycle phase arrest of host cells inhibit Salmonella intracellular replication. This occurs as a consequence of impaired maturation of the *Salmonella* containing vacuole in G1 cells.

In addition, we have identified and characterized miRNAs that concurrently regulate different steps of bacterial interaction with host cells. For example, we characterized the dual regulatory role of miR-29b-2-5p during *Shigella* infection, which results from the concomitant regulation of bacterial capture by host cells and of intracellular bacterial replication (3). We showed that miR-29b-2-5p, through the repression of its direct target UNC5C (a member of the UNC5 netrin-1 receptor family), induces the formation of filopodia that are essential for increased bacterial capture and internalization by the host cell. The increase of filopodia formation mediated by miR-29b-2-5p is dependent on RhoF and Cdc42 Rho-GTPases.

Overall, our work demonstrates the value of using unbiased genome-wide approaches to study miRNA function and highlights their potential to identify novel molecular players/pathways governing the complex interaction between host and bacterial pathogens.

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Keywords: microRNA, bacterial pathogens, image-based high-throughput functional screenings, RNA-sequencing

O-41 - GENOMIC ADAPTATION STRATEGIES OF STAPHYLOCOCCUS EPIDERMIDIS FROM COMMENSAL TO PATHOGEN

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Background
For several years, *Staphylococcus epidermidis* was considered merely as an inoffensive colonizer of the human skin. However, in the last decade, *S. epidermidis* has emerged as the leading cause of hospital infections associated to medical-indwelling devices. Humans can be colonized by diverse *S. epidermidis*, but infections are mainly caused by bacterial strains associated to a specific clonal lineage (CC2). However, the factors that enable *S. epidermidis* to change from a commensal to a pathogen are still not clear.

Method
In order to understand the genetic factors that contribute to the pathogenecity of *S. epidermidis*, the genomes of 83 clinical strains representative of hospital and community settings were sequenced using HiSeq. A reference pan-genome was assembled based on two reference strains and on the 83 isolates, by performing gene-by-gene alignment and whole-genome MLST using the BIGSdb. Genetic variation and prevalence of genes within the population was evaluated by comparing the genomes of the 83 strains against the pan-genome using the BIGSdb. To mimic a situation where a person colonized with *S. epidermidis* in the community (A) enters the hospital (B) and acquires a medical device associated infection caused by *S. epidermidis* (C), the strains were divided into three epidemiological groups: Community/Colonization (A); Hospital/Colonization (B); and Hospital/Infection (C) and were compared regarding their genomic content, following a rational of progression from colonization towards disease.

Results & Conclusions
The analysis revealed differences in genomic content of strains colonizing healthy persons in the community when compared to those colonizing patients in hospitals. Also, differences were observed between colonization and infection strains within the hospital. Following the progression of the genomic content of *S. epidermidis* from community to hospital and from colonization to infection, the acquisition and increase in frequency of genes involved in DNA recombination, resistance to β-lactams (12-fold), aminoglycosides (3-fold), tetracycline (7-fold), antiseptics (8-fold) and metals [cobalt, zinc, cadmium and copper (3-fold)] and mobile genetic elements (IS256, 3-fold), was apparent within the population. In addition, enrichment was observed in a group of 31 genes with unknown function. Enrichment could be either due to gene selection or acquisition of new genes. The pathogenic potential of *S. epidermidis* appears to be linked to the acquisition and selection of genes providing genetic flexibility and conferring resistance to antibiotics, antiseptics and metals. This might be an adaptation strategy in response to the different environmental cues *S. epidermidis* has to face during progression towards disease.

Keywords: S. epidermidis, Pathogen, Genomic profile

O-42 - THE TRANSCRIPTIONAL NETWORK REGULATED BY THE BIFUNCTIONAL ZYGOSACCHAROMYCES BAILII ZBHAA1 TRANSCRIPTION FACTOR IN RESPONSE TO ACETIC ACID- OR COPPER-INDUCED STRESSES

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Background

Zygosaccharomyces bailii is a spoilage yeast species and a promising cell factory due to its extraordinary capacity to tolerate acetic acid and other weak acid food preservatives [1,2]. The transcription factor Haa1 plays a major role in the regulation of *Saccharomyces cerevisiae* response to acetic acid due to its direct, or indirect, involvement in the regulation of 80% of the acetic acid-responsive genes, part of them acetic acid resistance determinants [3]. *Z. bailii* genome [4] encodes a functional homolog of *S. cerevisiae* Haa1 (ZbHaa1) required for adaptive response and tolerance to both acetic acid and copper stresses [5]. Phylogenetic analysis and other experimental evidences support the notion of the subfunctionalization of the single ancestral ZbHaa1 ortholog that originated the *S. cerevisiae* Haa1 and Cup2 paralogs after whole genome duplication [5]. This work aimed to get insights into genomic transcriptional regulation of *Z. bailii* under acetic acid or copper stresses mediated by ZbHaa1.

Method

The investigation of ZbHaa1-dependent genomic transcriptional alterations was assessed by mRNA-seq through the comparison of mRNA levels from *Z. bailii* IST302 parental and derived deletion mutant *zbhaa1* strains cultivated in the presence and absence of acetic acid (140 mM, pH 4.0) or $CuSO_4$ (0.08 mM). cDNA libraries were prepared using QuantSeq 3'mRNA-Seq Library Prep (Lexogen) and cDNA was sequenced using Illumina HISEQ2000. Reads were aligned to the reference *Z. bailii* IST302 genome [4] using STAR. Mapped reads were counted with HTseq. EdgeR was used for differential expression analysis.

Results & Conclusions

A total of 231 genes are down-regulated in *Z. bailii* IST302 upon acetic acid exposure and 28 are up-regulated. Among the 15 genes activated under the dependence of ZbHaa1 are *Z. bailii* homologs of *YGP1* and *HSP26* that in *S. cerevisiae* are also activated by Haa1 under acetic acid stress. Upon copper exposure, 208 genes were down-regulated and 66 genes up-regulated. Among the 9 genes activated under the dependence of ZbHaa1 upon copper stress is *CRS5* homolog that in *S. cerevisiae* is activated by copper under the dependence of Cup2. Results corroborate the subfunctionalization of ZbHaa1 ancestral.

References & Acknowledgments

This work was funded by FCT (PTDC/BBB-BEP/0385/2014, ERA-NET (ERA-IB-2/0003/2015 and UID/BIO/04565/2013) and PORLisboa2020 (N.007317).

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Keywords: Zygosaccharomyces bailii, ZbHaa1 transcription factor, Acetic acid tolerance, Copper tolerance, mRNA-seq

Bioprocess Engineering

P-001 - M13 BACTERIOPHAGE PURIFICATION USING POLY(IONIC LIQUIDS) AS NOVEL CHROMATOGRAPHIC MATRICES

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Background

M13 is a filamentous and non-lytic bacteriophage (phage) that infects E. coli via the F pilus. The outer layer is occupied by negatively charged N-terminal region of the major coat protein pVIII, which has a low isoelectric point. Currently, phage M13 has been used in phage display technology, and it is considered as a possible antibacterial therapeutic agent. Conventional phage purification involves 5 to 7 operational steps, with high operational costs and significant product loss (approximately 60%) [1]. Thus, there is a need to design a new large-scale process to achieve high yield, purity and minimize operation cost. Ion exchange chromatography is a common technique for the purification of different biomolecules. Although it has been used to purify some viral particles, the purification of M13 with anion exchange chromatography has only been reported twice [1, 2]. Ionic liquids (ILs) are salts composed of organic cations and organic/inorganic anions, which have melting points below the conventional 100 ºC. ILs availability is very broad since there are many known and potential new cations and anions combinations. Polymeric ILs (PILs) are a subclass of polyelectrolytes that comprise IL species connected through a polymeric back-bone to form a macromolecular framework. where the polymerizable group can be located either on the cation or the anion [3]. Currently, PILs are being studied for their affinity properties, in particular as separation and absorption tools for gas separation membranes, gas chromatography, capillary electrophoresis or solid-phase microextraction [3].

Method

In this work, we propose a scalable purification process for M13 bacteriophage using a novel stationary phase based on a PIL with a positively charged backbone structure. Poly (1–vinyl-3-ethyl imidazolium bis(trifluoromethylsulfonyl) imide) - poly(VEI-TFSI) predominantly acted as an anion exchanger under binding-elution mode.

Results & Conclusions

This revealed to be a rapid and simple method for the recovery of phage M13 with an overall separation yield of over 70% after a single downstream step. To the best of our knowledge, PILs have never been used as separation matrices for biological products and the results obtained, together with the large number of cations and anions available to prepare PILs, illustrate well the large potential of the proposed methodology.

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Keywords: M13 bacteriophage, Downstream processing, Poly(ionic liquids), Anion exchange, Batch adsorption

P-002 - DEVELOPMENT AND EVALUATION OF POTENTIAL YEAST PROTEIN EXTRACTS FOR RED WINES CLARIFICATION AND STABILIZATION

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Background

The wine industry is one of the most competitive sectors all over the world. Recently, new technologies have been combined in order to improve quality and sensorial diversity of the produced wines. Indeed, several stabilisation agents were developed to induce flocculation and sedimentation of particulate matter in wine, making its clarification and stabilization possible [1].

The most commonly used fining agents in wines are animal proteins, such as milk casein. However, its use is currently being related to food intolerance and to neurodegenerative diseases. To overcome this issue, suitable microorganisms must be selected for use in industrial processes [2]. In previous studies performed by our consortium, the potential of yeast protein extracts (YPE) in white wine clarification, stabilization, and curative processes was identified [1]. Therefore, the main objective of the present work is to select YPE with the potential to develop biological stabilization agents for red wines, without risk to the consumer. As a result, five YPE were selected from a diversified collection of oenological yeasts.

Method

Yeast protein extract selection was based on dry matter weight, protein content using BCA Assay kit and protein molecular weight profile of each protein fining agent by SDS-PAGE electrophoresis.

Results & Conclusions

SDS-PAGE electrophoresis showed that 50% of the total yeast protein is above 15 kDa of molecular weight, which is in accordance with the OIV demand (OIV-Oeno 494-2012). The denatured protein content was also determined by Differential Scanning Fluorimetry.

In conclusion, the selected YPE revealed promisor results and will be applied as fining agents in red wines. Furthermore, the effectiveness of these YPE will be evaluated by different oenological parameters such as turbidity, chromatic characteristics, lees volume and conductivity. Hence, we believe that this work will be an important contribution for the development of a new biological product which will improve the final quality of red wines.

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Keywords: Yeast, Wine clarification, Protein Extract

P-003 - INFLUENCE OF THE MEDIUM COMPOSITION ON THE B-GALACTOSIDASE SYNTHESIS BY KLYVEROMYCES MARXIANUS

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Background

β-galactosidase can be derived from various sources. Among yeasts, Kluyveromyces marxianus exhibits a high industrial potential for GRAS status, being considered safe, presents rapid growth and use of lactose as a carbon source (Zhou et al., 2013). The aim of this work was studied the influence of composition fermentation medium for the production of β-galactosidase using K. marxianus for the fermentation of lactose from whey permeate.

Method

K. marxianus ATCC 46537, was used in the submerged fermentations realized in a rotary incubator (150 rpm, 30°C, 24 h) with 1 g.L-1 initial cell concentration and medium composition (g.L-1): KH2PO4 (5.0), MgSO4.7H2O (0.6), yeast extract (1-12), (NH4)2SO4 (0-8) and lactose (10-70) from whey permeate. The study of the effects of pH (5-7) and medium composition in β-galactosidase production were performed by a fractional design of 24-1 trials plus 3 central points to obtain as the response specific activity (U.mgprotein-1) defined as μmol of glucose produced per minute per mg total protein. The β-galactosidase extraction was performed in mixer of type vortex using glass beads (Medeiros et al., 2008). The enzymatic activity was determined by initial rates of the hydrolysis in a 75 mL volume reactor of a 50 g.L-1 lactose, prepared in lactic buffer pH 6.5, at 30°C (Santiago et al. 2004). The biomass and total protein concentration were determined by spectrophotometry at 650 nm and by TOC-L analyzer, respectively.

Results & Conclusions

Specific activities varied from 8.537 to 35.535 U.mgprotein-1. The maximum specific activity was achieved at pH 7.0 and lactose, yeast extract and (NH4)2SO4 up to 70.0, 1.0 and 0.0 g.L-1, respectively. All variables had statistically significant effect at 95% confidence and this results show a significant influence of the lactose concentration in increased specific activity. β-galactosidase production was more significantly affected by lactose (effect of 10.64 U.mgprotein-1), followed by (NH4)2SO4 (-6.01 U.mgprotein-1), yeast extract (-5.86 U.mgprotein-1) and pH (4.49 U.mgprotein-1). In the fermentation at pH 6.0 and lactose, yeast extract and (NH4)2SO4 at 40.0, 6.5 and 4.0 g.L-1, respectively, the enzymatic activity of 16.889 U.mL-1 was achieved that implies an increase of 22% of the enzymatic activity when compared with Santiago et al. (2004)

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The authors wish to thank the UFU, CNPq, CAPES and FAPEMIG.

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Keywords: beta-galactosidase, Kluyveromyces marxianus, whey permeate, medium composition

P-004 - SCREENING OF THERMOTOLERANT YEAST STRAINS FOR MORE SUSTAINABLE, ECO-EFFICIENT AND COMPETITIVE INDUSTRIAL FERMENTATIONS

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Background

Yeast play a major role in the production of valuable compounds through microbial industrial fermentations. Yeast performance and adaptation to harsh industrial conditions are related to the productivity and cost-effectivity of industrial processes. Among these factors, temperature has a major influence on the activity of microorganisms, and the industry spends huge amounts of energy to cool or heat fermentations to optimize its processes. Thermotolerant yeast strains are utterly important to several industrial processes. In this work and under the scope of the ERA-IB project YeasTempTation, a collection of yeast strains comprising isolates from different industries was screened at wide range of temperature for evaluation of thermotolerant profiles.

Method

Yeast strains isolated from different industrial backgrounds (beer, bioethanol, 'cachaça' and winery processes) (22 total) were screened at a wide range of temperatures (from 12 to 42°C) in minimal medium[1]. Assays were carried out in a microplate incubator with agitation (28/40°C) and in orbital incubator (12/40/42°C). Yeast cell specific growth rate (μ_{max}) was measured by optical density at 600 nm. Glucose concentration from growth assays was determined by HPLC.

Results & Conclusions

Yeast strains growth profiles at optimal temperature (28°C) exhibited subtle variations presenting μ_{max} between 0.2-0.3h^{-1}. Nevertheless, at 40°C, differences between strains were amplified, where two strains managed to uphold a μ_{max} above 0.25h^{-1}. Yeast strains were also evaluated at 12°C, with all strains being able to grow, although the μ_{max} dropped to values below 0.1h^{-1}, where the control strain (CEN.PK113-7D) exhibited the highest μ_{max}. The six strains with the most promising performance in microplate assays at 40°C were also studied in Erlenmeyer flasks. Two of these strains (bioethanol strains) exhibited a μ_{max} of ca. 0.5h^{-1}, while CEN.PK113-7D showed the lowest μ_{max} (<0.3h^{-1}). The four *S. cerevisiae* strains with uppermost μ_{max} at 40°C were selected to evaluate at 42°C. At this temperature, major differences in glucose consumption were observed, with one strain being able to consume ca. 97% of glucose in the first 24h, with the highest μ_{max} (ca. 0.25h^{-1}). This study represents a first approach to a more in-depth research on thermotolerant strains adaptation and tolerance to high-temperature processes for industrial application.

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Financial support is aknowledged to Project ERA-IB "YeastTempTation" (ERA-IB-2-6/0001/2014), and FCT for the strategic funding of UID/BIO/04469/2013 unit and COMPETE 2020 (POCI-01-0145-FEDER-006684), RECI/BBB-EBI/0179/2012 (FCOMP-01-0124-FEDER-027462) and BioTecNorte operation (NORTE-01-0145-FEDER-000004). Lallemand Ibéria, SA and Unicer Bebidas, SA are acknowledged for the supply of yeast strains.

Keywords: Yeast, Thermotolerant, Fermentation, Sustainable

P-005 - CHARACTERIZATION OF POTENTIAL CLA-PRODUCING STRAINS ACCORDING TO LA TOLERANCE

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Background
Conjugated linoleic acid (CLA) isomers are naturally produced from dietary linoleic acid (LA) by ruminal bacteria. However, strains of lactobacilli, bifidobacteria and propionibacteria have also demonstrated the ability to produce those bioactive fatty acids. *In vitro* studies normally test CLA production at 0.5 mg/mL of LA, but possibly some strains can tolerate higher concentrations and if they are producers, CLA yields may probably be higher. This work aims to determine the maximum LA concentration that potential CLA-producing strains can tolerate in an *in vitro* production assay.

Method
Thirty five lactobacilli, 17 bifidobacteria, 1 propionibacterium and 1 lactococcus strains were submitted to a modified method described by Roméro-Pérez et al. [1]. Activated strains were inoculated in MRS agar plates containing 1, 2 or 5 mg/mL of LA for 48 h at 37 ºC. Strains from the agar plate at the highest LA tolerated concentration were then spiked into MRS broth with LA in a 96-well microplate and subsequently incubated at 37 ºC for 48 h; growth curves were recorded at 600, 620 and 660 nm in a microplate reader. Cultures without substrate were used as control. Agar plates experiment was carried out in duplicate and microplate experiment in triplicate.

Results & Conclusions
Among the strains tested, 18 (~33%) could not grow at the lowest LA concentration assayed, having considered their tolerance below 1 mg/mL. A group of 16 strains showed growth at 1 mg/mL of LA, 8 tolerated up to 2 mg/mL and 12 up to 5 mg/mL. At 5 mg/mL lactobacilli was the dominant group, whereas at 2 mg/mL was bifidobacteria. At <1 mg/mL and 1 mg/mL the distribution of lactobacilli and bifidobacteria was similar. The propionibacterium and the lactococcus strains could grow a 1 mg/mL. In conclusion, potential CLA-producing strains exhibit different LA tolerance degrees. This is a parameter to consider in future production tests.

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This work was financed by National funds via FCT – Fundação para a Ciência e a Tecnologia, under the project "Pro-TECh-CLnA - Microbial Production of Bioactive Conjugated Linolenic Acid Isomers to Obtain Functional Ingredients and Foods" reference PTDC/AGR-TEC/2125/2014. Financial support for the authors A.L. Fontes and L.L. Pimentel was provided by fellowships SFRH/BD/117721/2016 and SFRH/BPD/119785/2016, respectively, granted by the Portuguese government through FCT.

Keywords: LA tolerance, Probiotics, CLA, Lactic acid bacteria, Bifidobacterium

P-006 - NON-THERMAL SYNERGISTIC APPROACH TO LISTERIA MONOCYTOGENES INACTIVATION IN MILK: THE COMBINED EFFECT OF HIGH PRESSURE AND BACTERIOPHAGE P100

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Background

Regarding the increasing demand in the food market for reducing the use of chemical additives and environmentally harsh chemical sanitizers and disinfectants, bacterio(phages) could be considered a natural alternative that represents a renewed technology for food decontamination and preservation; it is an eco-friendly technology, which minimizes the impact on the nutritional and organoleptic properties and, at the same time, the endogenous and often beneficial microbiota is preserved. Despite the promising results obtained from phage application towards food decontamination, a noticeable bacterial regrowth has been observed during refrigerated storage of bio-treated foods, especially in studies targeting *Listeria monocytogenes*.

The objective of the present work was to evaluate the effect of synergistic process which combines mild high hydrostatic pressure – HHP and phage Listex™ P100 as a new non-thermal process for *L. monocytogenes* decontamination in milk.

Method

Two batches of UHT whole milk were inoculated with phage P100 with a final MOI of 10000 and 10 to 10^4 and 10^7 log (CFU/mL) of *L. monocytogenes*, respectively. One set of samples was subjected to mild HHP (300 MPa, 5min, 10 °C) and other set directly stored at atmospheric pressure (0.1 MPa) under refrigeration (4° C, non-pressure treated). Stability of P100 inoculated in milk was accessed at pre-set time intervals (0, 1.3 e 7 days) in non- and pressure-treated samples. Additionally, a third set was inoculated only with *L. monocytogenes* and submitted to a conventional heat treatment (pasteurization – 72 °C / 15s).

Results & Conclusions

Results from pressure treated samples with final MOI 10000 demonstrated cultural undetectable *L. monocytogenes* cells in the milk during all refrigerated storage (4 °C), being comparable to the results obtained from pasteurization. Otherwise, pressure treated samples with final MOI 10 resulted in a 1.08 ± 0.16 log cycles reduction followed by a bacteriostatic effect of up to the 7 days of storage. Phage particles were stable during all storage in milk and no significant differences were observed in the phage titers (P >0.05). In non-pressure treated samples inoculated with P100, *L. monocytogenes* regrowth was observed during the storage period. The pressure-phage system is a promising minimal food processing option, which offers freshness and unique properties to the raw food.

References & Acknowledgments

This work was supported by National Funds from FCT - Fundação para a Ciência e a Tecnologia through project UID/Multi/50016/2013

C. Maciel (SFRH/BD/104016/2014), S. Castro (SFRH/BPD/71723/2010) and V. Ferreira (SFRH/BPD/72617/2010) are recipients of FCT fellowships.

Keywords: Biocontrol, Bacteriophage, HHP, LIsteria monocytogenes

P-007 - HIGH HYDROSTATIC PRESSURE AND PEDIOCIN PA-1 AS A SYNERGISTIC SYSTEM TO LISTERIA MONOCYTOGENES INACTIVATION IN FERMENTED MEAT SAUSAGE

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Background

In the last two decades, the consumer's behaviour has experienced a global upsurge and on-going trend for healthier, fresh and natural foods. The growing demand for locally produced and minimally processed food, as well as the increased awareness of environmentally friendly technologies for food processing, turn out to be a relevant point in the consumer's buying decision. For this reason, the use of non-thermal technologies as high hydrotatic pressure (HHP) and biocontrol agents represents an interesting processing option to maintain food freshness and simultaneously reduce the microbial load. The aim of this work was to evaluate the combined effect of mild HHP and pediocin PA-1 as a potential *hurdle technology* to inactivate *Listeria monocytogenes* in Portuguese traditional fermented meat sausages.

Method

Pediococcus acidilactici HA-6111-2, isolated from a fermented meat sausage (10^7 CFU/g) or its semi-purified bacteriocin pediocin PA-1 (1280 AU/g) were added to the sausage paste (*ca.* 100 g) previously inoculated with *L. monocytogenes* (10^4 CFU/g). Two different strains were selected to perform separate assays, *L. monocytogenes* Scott A, a well described strain in HHP and *L. monocytogenes* 1942, isolated from Portuguese fermented meat sausage. Samples were pressurized at 300 MPa (10 °C), for 5 min and stored at atmospheric pressure (0.1 MPa) under refrigeration (4° C).

Results & Conclusions

For both *Listeria* strains, all treatments achieved enumeration below the detection limit of the enumeration technique, excluding samples inoculated just with *L. monocytogenes* cells (pressurized and non-pressurized). All treatments (*P. acidilactici* HA-6111-2 or its semi purified bacteriocin) reduced *L. monocytogenes* to undetectable levels by enrichment protocol throughout shelf life of fermented sausages at 4 °C. The synergistic effect of HHP and pediocin PA-1 reduced the *L. monocytogenes* initial load to undetectable levels immediately after HHP treatment, whereas combination of HPP and *P. acidilactici* HA-6111-2 resulted in the absence of the pathogen 72h after the treatment. In non-pressure treated samples inoculated with pediocin PA-1 or *P. acidilactici* HA-6112*, L. monocytogenes* was not detected only at 14 and 21 days of refrigerated storage, respectively.

The multi-hurdle technology herein presented constitutes a promising minimal food processing alternative, representing an eco-friendly technology for food decontamination and preservation.

References & Acknowledgments

This work was supported by National Funds from FCT - Fundação para a Ciência e a Tecnologia through project UID/Multi/50016/2013. C. Maciel (SFRH/BD/104016/2014), S. Castro (SFRH/BPD/71723/2010) and V. Ferreira (SFRH/BPD/72617/2010) are recipients of FCT fellowships.

Keywords: Biocontrol, HHP, Listeria monocytogenes, Pediocin PA-1

P-008 - PROTEIN ENRICHMENT OF MALT BAGASSE SUCH AS STRATEGY FOR NILE TILAPIA DIETS SUPPLEMENTATION

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Background

This study aimed the protein enrichment of brewery malt bagasse (MB) by solid state fermentation by *Rhizopus oligosporus* to be added in diets of Nile tilapia juveniles. The research was divided into three steps: i– preparation of the malt bagasse; ii– protein enrichment of the MB by *Rhizopus oligosporus*; and iii– biological assay on the juvenile Nile tilapia (*Oreochromis niloticus*).

Method

The MB was moistened with distilled water to 70% moisture content, and ammonium sulfate was added (1g of N per 100g of dry MB). About 300 g of wet MB were placed flasks covered with cotton plug and sterilized by autoclaving. The medium was inoculated with 2×10^6 spores g^{-1} of dry MB and the flasks were incubated at 25ºC/7days. After the state solid fermentation, the fermented MB was dehydrated in an oven at 65ºC/24h. A total of 120 sexually reverted juvenile Nile tilapia were used (68±5g), distributed at random into 24 experimental units (six treatments with four repetitions), represented with a total capacity for 130 L, equipped with individual flow at a rate of $100Lh^{-1}$ for the total exchange of the water. Therefore the juvenile tilapia was manually fed twice a day *ad libitum*, at 9 am and at 4 pm. The experiment was carried out according to the Ethics Norms for Research involving Animals (Protocol nº 081/14 of Federal University of Goiás). The data were analyzed by the ANOVA, and the means of each treatment compared using Tukey's test using the program *Assistat 7.7*, adopting a level of significance level of 5%.

Results & Conclusions

The juveniles were feeding for 67 days containing from 2 to 10% malt bagasse fermented and there was no significant difference in the parameters for the productive performance and hematological and biochemical parameters of the juveniles, however the fatty acid profile showed a significant increase in long-chain unsaturated fatty acids in the animals fed the diets containing high concentrations of the fermented bagasse, a result corroborated by the increase in omega 3 and omega 6 polyunsaturated fatty acids. The fermented malt bagasse by *R. oligosporus* allowed for its use as a protein supplement for juvenile tilapia, adding up to 10% without compromising the productive capacity and blood parameters of the species, improving the fatty acid composition and representing a useful destiny for this byproduct.

References & Acknowledgments

The authors wish to thank the National Council for Scientific and Technological Development (CNPq, Brazil) for financial support (Process number 444241/2014-0).

Keywords: Malt bagasse, State solid fermentation, Agroindustrial residue, Nile Tilapia

P-009 - IMPROVEMENT OF ETHANOL PRODUCTION USING SUGARCANE BROTH WITH FED-BATCH FERMENTATIONS: EFFECTS OF TEMPERATURE, INOCULUM AND SUBSTRATE CONCENTRATIONS UNDER VERY HIGH GRAVITY CONDITIONS

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Background

The fermentation of sugar cane must with a high concentration of sugar (VHG - Very High Gravity) can be used on an industrial scale for the production of fuel ethanol. This technology has the advantage of achieving higher levels of ethanol and reduction of production costs, although the challenges are to minimize the effects of osmotic and alcohol stress that the yeasts are subjected. This work studied ethanol from fermentation with high levels of sugar, from the VHG technology in order to produce wines with high alcohol levels and thereby decrease the amount of vinasse produced per liter of ethanol.

Method

This work studied ethanol production using a feed-batch bioreactor with a working volume of 1.5 L. The main goal of this research was to evaluate the temperature, substrate concentration and cellular concentration using a Central Composite Design (CCD) in the responses: yield in ethanol production, ethanol productivity and residual sugar. In the experiments were used sugar cane broth and molasses to obtain the composition defined by each experiment of the (CCD). An industrial Y-904 strain of *Saccharomyces cerevisiae* was used. All experiments had a fed-batch reactor filling time of 5 h and the responses were analysed considering a fermentation time of 10 h.

Results & Conclusions

The following operating conditions were selected: cell concentration in the reactor full 15% v/v, concentration of total reducing sugar (TRS) in full reactor of 300 g/L and temperature of 27 °C. Under these conditions the responses obtained were: ethanol concentration 150.86 g/L, yield 93.09%, productivity 5.02 $g_{ethanol}$/L.h and residual sugar 0.0 g/L.

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Financial support from Foundation for Research Support of the State of Minas Gerais – FAPEMIG, National Counsel of Technological and Scientific Development – CNPq, and Coordination for the Improvement of Higher Education Personnel (CAPES)-Brazil is gratefully acknowledged.

Keywords: ethanol, fermentation, productivity, yeast, yield

P-010 - AFFINITY DRIVEN AQUEOUS-TWO PHASE EXTRACTION OF ANTIBODIES: A MICROFLUIDIC APPROACH

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Background

Protein products such as antibodies, interferons and cytokines are biopharmaceuticals of critical importance which, in order to be safely administered to patients, have to be thoroughly purified in a cost effective and efficient manner, without compromising their structure and functionality. The use of aqueous two-phase extraction (ATPE) is a viable option for this purification due to its compatibility with biomolecules, but these systems are difficult to model and optimization procedures require lengthy and expensive screening processes.

Method

Here, a methodology for the rapid screening of antibody extraction conditions using a microfluidic channel-based toolbox, that dismisses the need for high-end equipment, is presented. A first microfluidic structure allows a simple negative-pressure driven rapid screening of up to 8 extraction conditions simultaneously, using less than 20 µL of each phase-forming solution per experiment, while a second microfluidic structure allows the integration of multi-step extraction protocols based on the results obtained with the first device.

Results & Conclusions

In this work, the microfluidic toolbox was used to demonstrate the potential of LYTAG fusion proteins used as affinity tags to optimize the partitioning of antibodies in ATPE processes, there was an increase of approx. 3.7 fold in the partition coefficient when compared with the same conditions without the affinity molecule. The toolbox was also used to perform extraction and back-extraction of the tagged antibodies in a continuous fashion, which is useful to increase the purity of the system. Overall, this miniaturized and versatile approach allowed the rapid optimization of molecule partition followed by a proof-of-concept demonstration of an integrated back extraction procedure, both of which are critical procedures towards obtaining high purity biopharmaceuticals using ATPE.

References & Acknowledgments

E.J.S. Brás and R.R.G. Soares acknowledge FCT for the PhD fellowships PD/BD/128167/2016 and SFRH/BD/97354/2013, respectively.
E.J.S. Brás also acknowledges IST-ID for the research grant BL176 under the research project RD0453-IBB2015/2017. A.M. Azevedo
also acknowledges FCT for the research contract under the program "Investigador FCT 2014" (IF/00048/2014/CP1214/CT0010).

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Keywords: Microfluidics, Aqueous Two-Phase Systems, Affinity Driven, Lytag

P-011 - CONTINUOUS CELL CONCENTRATION AND LYSIS USING INERTIAL MICROFLUIDICS

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Background

The optimization of upstream and downstream processes currently faces an unbalance, with great developments in the former not accompanied by improvements in the latter.

It is currently possible to easily produce large quantities of a product, while it is quite expensive and time consuming to optimize a large scale process for its purification.
The very low volumes and ability to test conditions in parallel presented by Microfluidics hold great potential in bridging this optimization process. However, to date, focus has been on the development of particular unitary processes, and no microfluidic system managed to integrate every stage of a purification process, such that the interactions between the different processes cannot be observed on the final product.

Method

This work focuses on the first stage of the downstream processing of cellular products, cell recovery and cell lysis.
Chemical lysis was performed in a long microfluidic channel (length: ~25cm, cross-section: 50x50 µm), injecting both E.coli cells and lysis solution, and compared with laboratory scale sonication.
By exploring inertial hydrofocusing effects at the microscale it is then possible to separate cells from other fermentation broth components inside a microchannel in a continuous fashion.
A narrow microchannel (length: 4cm, width: 9µm, height: 50µm) was used to concentrate intact E.coli cells from a fermentation sample, as well as to distinguish between different degrees of cell lysis (different times of sonication and chemical lysis agents).
GFP-Lytag was used as a model protein to study these effects.

Results & Conclusions

The method described can first be used to concentrate cells obtained from a continuous fermentation. These could then be exposed to different extraction conditions (i.e. different chemical lysis agents) to measure their efficacy and effects on the product protein. The same system was then re-used to separate the debris and protein content from any surviving cells in order to quantify the extraction yield.

References & Acknowledgments

Ricardo Fradique and Eduardo J.S. Bras acknowledge FCT for the PhD fellowships, PD/BD/113958/2015 and PD/BD/128167/2016 respectively.
INESC-MN and IBB acknowledge FCT for funding through the research units UID/NAN/50024/2013 and UID/BIO/04565/2013,respectively, and through the project PTDC/BBB-NAN/5927/2014. A.M. Azevedo also acknowledges FCT for the research contract under the program “Investigador FCT 2014” (IF/00048/2014/CP1214/CT0010).

Keywords: Cell lysis, Inertial microfluidics, E.coli

P-012 - THERMODYNAMIC ANALYSIS OF THE PHENOMENA BEHIND MABS ADSORPTION ON PHENYLBORONIC ACID AGAROSE-BASED SUPPORTS: AFFINITY VS MULTIMODAL CHROMATOGRAPHY

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Background

Monoclonal antibodies (mAbs) currently represent one of the leading families of biopharmaceutical industry in terms of therapeutic and market potential. The increasing market needs, the drastic improvements achieved namely at process upstream, and the emergence of biosimilars fostered the development of new downstream technologies and/or the improvement of existing ones. In this context, novel and cost-effective synthetic ligands that selectively capture mAbs from complex feedstocks are evolving [1,2] being important to fully characterize them as the interactions that occur with mAbs.

Method

In this work, the main goal was to investigate the complex phenomena involved in the adsorption of an anti-human IL-8 mAb from a clarified supernatant of Chinese Hamster Ovary cell cultures, towards phenylboronic acid ligands.
In order to understand the thermodynamics underneath the mAbs adsorption events, Flow Microcalorimetry (FMC) was exploited as a dynamic and on-line method considering instantaneous heat energy transfers, using the synthetic ligand *m*-aminophenylboronate (*m*-APB), under different pH (7.5, 8.5 and 9.0) and salt concentrations (0 and 150 mM NaCl).

Results & Conclusions

Results obtained show that anti-human IL-8 mAbs adsorption on P6XL resin is enthalpically driven ($\Delta H_{ads} < 0$), as expected for the reversible esterification reaction in which the affinity-based interaction between boronic acids, or boronate ligands, and molecules containing *cis*-diols relies.
At higher pH ranges and in presence of salt, a decrease on ΔH_{ads} of about 70% was observed as a decrease on ligand salt tolerance. This fact lead us to believe that the affinity-based *cis*-diol interactions still occur, although its contribution to the adsorption process is not exclusive nor the main one. Also, the thermodynamic profiling obtained is characteristic of ion exchange [3] corroborating that at these conditions, the ligand is in its tetrahedral boronate anion form and multimodal interactions such as electrostatic and hydrogen bonding can be promoted between the different ligand moieties and the mAb Fc portion.
Complementary experiments revealed that the presence of salt mitigate nonspecific adsorption towards the agarose-based matrix and hydrophobic groups present in the *m*-APB ligand.
Overall, we could conclude that the integration of the FMC system in chromatography could level up the comprehension beneath this complex science.

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Keywords: Monoclonal antibodies, Downstream processing, Chromatography, Phenylboronate ligands, Flow microcalorimetry

P-013 - LYTAG-DRIVEN PURIFICATION STRATEGIES AS A KEY TO INTEGRATE AND INTENSIFY THE DOWNSTREAM PROCESSING OF MONOCLONAL ANTIBODIES

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Background

Monoclonal antibodies (mAbs) are currently the most important class of recombinant protein therapeutics in the biotechnological and biopharmaceutical industry with more than 250 mAbs currently undergoing clinical trials. High titer producing cultures and complex mixtures containing high cell densities, together with an increasing growing demand for highly pure mAbs is making recovery and purification processes hot targets for improvement and opens important technological challenges in mAbs manufacturing platforms.

Method

This work explores the use of an affinity dual ligand based on a choline binding polypeptide tag (LYTAG) fused with the synthetic antibody Z domain (LYTAG-Z) as a tool to integrate and optimize the downstream processing of mAbs. Upon addition of this ligand to an animal cell culture broth, antibody-LYTAG-Z complexes are formed which can be easily captured and separated from host cell impurities by affinity chromatography, using three different strong anion exchange matrices charged with quaternary methyl amines (a choline analogue) – CIMmultus QA, HiTrap Q FF and gPore NW Q. Moreover, affinity partitioning in aqueous two-phase systems (ATPS) composed of polyethylene glycol (PEG - that have the ability to binding to the choline binding sites of LYTAG), followed by the phases' processing by chromatography was also investigated.

Results & Conclusions

A two-elution method was developed for the purification of mAbs and the performance of the different anion exchangers was tested and compared, with both CIMmultus Q and Q Sepharose allowing a recovery of more than 94% of mAbs from a CHO cell supernatant with a purity greater than 95%. Integration of clarification and primary mAbs recovery was successfully accomplished using a system composed of 7% PEG 3350 Da and 6% dextran 500,000 Da in which an extraction yield of 94.7±1.7% was achieved. IgG-rich phases were also further processed by chromatography, allowing a mAb recovery of 95.3±1.4% with a purity level of 91.4±13.0%. Thus, an integrated platform based on two purification steps – affinity extraction and affinity chromatography – results in an overall process yield of 90%, allowing the processing of mAbs directly from a non-clarified CHO cell culture.

References & Acknowledgments

This work was funded by the European Union 7th framework program under grant agreement no. 312004. IBB acknowledges Fundação para a Ciência e Tecnologia (FCT) for funding through the research unit (Ref. UID/BIO/04565/2013) and project PureMab (Ref. PTDC/QEQ-PRS/0286/2014). E.V. Capela and A.M. Azevedo also acknowledges FCT for the PhD grant SFRH/BD/126202/2016 and the research contract under the program "Investigador FCT 2014" (IF/00048/2014/CP1214/CT0010), respectively.

Keywords: Purification, Downstream processing, Monoclonal antibodies, Biopharmaceuticals, LYTAG, Chromatography

P-014 - EXTRACTION AND CHARACTERIZATION OF MICROALGAE PROTEINS FROM THE EXTREMOPHILE DUNALIELLA

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Background

Microalgae are mainly considered as a source of polyunsaturated fatty acids, pigments or biofuels. However, considerable amounts of other high value molecules can be extracted from microalgae for biotechnological applications [1]. Some of these are proteins like carbonic anhydrase, antioxidant proteins and enzymes from the glycerol cycle. Glycerol-3-phosphate dehydrogenase is an example of such proteins and may be used for clinical diagnostics (e.g., blood triglyceride levels) and industrial analysis of glycerol and glycerol-3-phosphate [2].

Method

To enhance the accumulation of these proteins, cultures of the microalgae *Dunaliella* sp. were subjected to three different physicochemical stress strategies – salt, pH and oxidative- in 1-L bubble column photobioreactors.

Results & Conclusions

After 24h of exposure to the salt or pH stress the protein content of the cells increases and is sustained until the end of the experiment.

Quantitative and qualitative characterization of protein extracts from the three stress conditions (total protein by BCA analysis and 2D gel electrophoresis) show that higher change in the protein expression profile is obtained exposing cells to 200g/L NaCl (salt stress). Among proteins overexpressed in this stress condition, two enzymes from the glycerol cycle stand out – glycerol-3-phosphate dehydrogenase (G3PDH) and glycerol-3-phosphate phosphatase.

Fractionation of protein extracts from the salt stress using a DEAE anion exchange chromatography leads to a preliminary purification of G3PDH. A fraction eluted at approximately 200mM NaCl contains the protein of interest. Two elution modes - step versus linear gradient are used to improve recovery yields and purification factors; around 42% and 18% (w/w) of the total protein fed to the column is recovered in the elution peak containing the enzyme of interest, respectively. Further downstream strategies to improve enzyme purity of recovered fractions and the quantification of their G3PDH activities are under way.

Heat-induced aggregation behaviour is observed in the pool of proteins from the flow-through fraction of the chromatography, indicating potential for proteins gelation ability.

These results make this microalgae a possible candidate to produce proteins with biotechnological applications.

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Keywords: protein expression under stress, Chromatographic recovery, Dunaliella sp.

P-015 - STIRRED CELL PURIFICATION OF MONOCLONAL ANTIBODIES BY ULTRAFILTRATION WITH POLYETHYLENEIMINE-MODIFIED POLYETHERSULFONE MEMBRANE

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Background

Monoclonal antibodies (mAbs) are used in the treatment of a wide range of diseases and are at the forefront of the new era of personalized therapy. Since the licensing of the monoclonal antibody for clinical use 30 years ago, the industry has expanded exponentially and, with the current approval rate, is expected to be worth 125 billions of dollars [1]. With this growing demand, the development of efficient and trustworthy purification processes is imperative, in order to remove all kinds of impurities with reduced yield loss.

In many mAbs purification platforms, packed-bed chromatography is the main process used as a polishing step to bind trace levels of process impurities and assure viral clearance. Even though these processes are able to meet purification requirements, they are expensive and have low throughput. In this context, several new membrane-based separations are being developed for the purification of mAbs, reducing the number of chromatographic steps without loss of final product purity [2].

Method

In this work a positively charged membrane was created. Commercially available ultrafiltration polyethersulfone/polyacrylonitrile membranes, with a MWCO of 500 kDa, were modified by sulfonation with sulfuric acid, followed by deposition of polyethyleneimine (PEI) and cross-linking with butanedioldiglycidylether. Two polymer PEI products with 2 kDa and 10 kDa were used to evaluate how polymer size and pH influence the fractionation of a CHO cell supernatant solution by ultrafiltration. The precursor and modified PES membranes were analyzed by X-ray photoelectron spectroscopy (XPS) to characterize the chemical composition of membranes surface.

Results & Conclusions

The XPS results confirmed the successful modification of PES membranes. The nitrogen analysis showed the presence of amine groups, which clearly shows the immobilization of PEI onto the membrane surface. Also, with the use of 10 kDa PEI, there is an increase on the intensity of the signal, which was to be expected when one uses a higher molecular weight polymer. The performance characteristics of the modified membranes were very satisfying, with membranes exhibiting selectivity according to pH. At the best operational condition, pH 9.0, the permeates exhibit very high values of antibody purity (96%), in a single step process.

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Keywords: Ultrafiltration membranes, Monoclonal antibodies, Positively charged membranes, Protein adsorption, Surface modification

P-016 - DEVELOPMENT OF A NOVEL IONIC-LIQUID-BASED PLATFORM FOR THE SEPARATION OF AROMATIC AND ALIPHATIC AMINO ACID MIXTURES

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Background

Amino acids are critical compounds in animal and human nutrition, being employed as food additives, feed supplements and artificial sweeteners. In particular, aromatic amino acids are amongst the most important for nutritional applications. The downstream processing of complex mixtures of produced amino acids comprises several stages that are rather difficult to be transposed to a large-scale and require a high investment, and thus the recovery and purification costs of amino acids can reach up to 80% of the final product cost. In this context, there is a crucial demand on the development of cost-effective processes for their fractionation and selective separation aiming at obtaining amino acids with high purity levels. It has been demonstrated that aliphatic amino acids can behave as salting-out agents when employed in ionic-liquid-based aqueous biphasic systems (IL-based ABS), allowing thus the replacement of the mostly used non-biocompatible inorganic salts.

Method

Based on this possibility, in the current work, we investigated the ability of forming ABS combining phosphonium-based ILs with aliphatic amino acids, and we then used these systems to separate mixtures of aromatic (L-Phe, L-Tyr and L-Trp) and aliphatic amino acids (L-Proline, L-Lysine and L-Lysine·HCl). To infer on the compositions required to form two-phase systems which could be used in separation processes, the liquid-liquid phase diagrams, tie-lines and tie-line lengths of the ABS investigated were firstly determined at 25ºC, and then used in the separation of aromatic and aliphatic amino acids.

Results & Conclusions

According to the gathered results, selective extraction efficiencies for opposite phases up to 85 % and up to 98% for aromatic and aliphatic amino acids, respectively, were attained in a single-step. The possibility of separating the aromatic amino acids from the IL-rich-phase was further evaluated, using a cation exchange column, achieving a recovery of 93% of the aromatic amino acids with 79% of removal of the IL. Based on these results, it was here demonstrated the remarkable ability of more benign IL-based ABS for the separation of amino acid mixtures, being their further recovery also addressed.

References & Acknowledgments

This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (FCT Ref. UID /CTM /50011/2013), financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. The research leading to reported results has received funding from the European Research Council under the European Union's Seventh Framework Programme (FP7/2007-2013)/ERC grant agreement no. 337753.

Keywords: Separation, Amino acids, Ionic liquids, Bioprocess development, Value-added biomolecules

P-017 - FUNGAL SCREENING FOR WINE DISTILLERY WASTEWATER BIOREMEDIATION

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Background

White-rot fungi (WRF), versatile lignin-degrading fungi with various extracellular enzymes, has been widely used for biological treatment. Elimination of pollutants and colour from wine distillery effluent (vinasse) is becoming increasingly important from environmental point of view. Due to the large volumes of effluent and presence of certain recalcitrant compounds, the treatment of this by-product is rather challenging.

Method

The potential of submerged fermentation (SmF) of vinasse was carried out by five white-rot fungi (*Irpex lacteus*, *Ganoderma resinaceum*, *Trametes versicolor*, *Plebia rufa* and *Bjerkandera adusta*), in order to determine the bioremediation performance and their ability to produce extracellular enzymes (laccase, versatile and manganese-dependent peroxidase).

Results & Conclusions

The results show that all strains were able to growth in SmF, although *P. rufa* had shown the best bioremediation performance since it removed 92.6%, 87.9% and 93.5% of colour, total organic carbon (TOC) and phenolic compounds, respectively. *P. rufa* was the only one that produced the three enzymes determined. Versatile and manganese-dependent peroxidades had shown the highest activity at the end of incubation period (0.04 U mL^{-1} and 0.09 U mL^{-1}, respectively), whereas the maximum laccase activity from *P. rufa* (0.17 U mL^{-1}) was on day 6. Thus, taking into account the criteria of decolourization efficiency, removal of phenolic compounds and enzymes production, the fungus *P. rufa* was selected for further vinasse biodegradation studies.

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This work is supported by the Innovation Platform of Vine and Wine, operation number NORTE-01-0145-FEDER-000038, in its research line entitled "WINEMAKING", co-financed by the European Regional Development Fund (FEDER) through the North 2020 (Regional Operational Program of North 2014/2020), and by the European Investment Funds by FEDER/COMPETE/POCI – Operational Competitiveness and Internationalization Programme, under Project POCI-01-0145-FEDER-006958; and National Funds by FCT - under the project UID/AGR/04033/2013.

Keywords: White-rot fungi, wine distillery wastewater, bioremediation

P-018 - REMOVAL OF POLLUTION AND TOXICITY FROM DOURO REGION VINASSE BY PHLEBIA RUFA

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Background

Vinasse is the aqueous effluent remaining after distillation of wine, wine lees, or fermented grape juice to extract ethanol or flavour compounds. This brown coloured and acid by-product presents high chemical oxygen demand thereby its treatment is a mandatory task.
White-rot fungi (WRF) have shown the ability to remediate wastewaters produced by the food, textile, and paper industry. This is due to the production of extracellular enzymes, which enable them to degrade lignin and phenolic compounds that are recalcitrant to conventional biological treatment systems.

Method

Vinasse was inoculated with *Plebia rufa* during 15 days at 27 ºC and 120 rpm to evaluate several parameters: colour, TOC, total phenols, pH, reducing sugars, laccase, versatile peroxidase and manganese-dependent peroxidase. Toxicity was evaluated by germination tests using seeds of garden cress (*Lepidium sativum)* and by microtox test using luminescent bacterium (*Vibrio fischeri*).

Results & Conclusions

The *P. rufa* had ability to decrease 82.7% of total organic carbon (TOC) and 97.3% of colour and phenolic compounds after 15 days of incubation. The maximum laccase activity (0.17 U mL^{-1}) was on day 6, whereas manganese-dependent peroxidade had shown the highest activity at the end of incubation period (0.06 U mL^{-1} on day 15). The ecotoxicity impact of fermented vinasse was evaluated by plant seeds and the marine bacterium *V. fischeri*. According to our results, vinasse fermentation by *P. rufa* had the ability to remove its toxicity, which resulted in 99% of seeds germination. These results suggest that *P. rufa* could be a potential alternative to conventional treatment processes.

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This work is supported by the Innovation Platform of Vine and Wine, operation number NORTE-01-0145-FEDER-000038, in its research line entitled "WINEMAKING", co-financed by the European Regional Development Fund (FEDER) through the North 2020 (Regional Operational Program of North 2014/2020), and by the European Investment Funds by FEDER/COMPETE/POCI – Operational Competitiveness and Internationalization Programme, under Project POCI-01-0145-FEDER-006958; and National Funds by FCT - under the project UID/AGR/04033/2013.

Keywords: Phlebia rufa, vinasse, toxicity, bioremediation

P-019 - PURIFYING MINICIRCLE DNA: A COMPARISON BETWEEN THE LYSINE AND CADAVERINE AFFINITY LIGANDS

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Background

Minicircle DNA (mcDNA) is a novel biomolecule which is nowadays trending in the field of non-viral DNA vectors. The induced recombination of the parental plasmid (PP) into this smaller circular DNA allows to excise the prokaryotic sequences necessary for biotechnological DNA production. Consequently, mcDNA does not present bacterial genes that may be harmful for the patient, such as the antibiotic resistance gene or the replication origin, providing this molecule with an upgrade status towards conventional plasmid DNA. However, given the novelty of this product, the purification of supercoiled (sc) mcDNA from host impurities, PP molecules and other mcDNA isoforms has not been much studied in the chromatography field. So far, the overall chromatographic strategies that have been reported rely on the PP backbone modification for affinity chromatography purification, which cannot be applied to all mcDNA vectors available on the market.

Method

Lysine and cadaverine ligands were immobilized onto epoxy monolithic disks. A comparison of the chromatographic behavior between the modified monoliths was performed at different pHs to understand the effect on the binding of a complex lysate sample. Afterwards, the ionic strength was manipulated to accomplish the separation of mcDNA from the remaining impurities. The mcDNA quality was assessed by determining the endotoxins, proteins and genomic DNA content, to guarantee that the final sc mcDNA complied with the regulatory agency requirements.

Results & Conclusions

The sc mcDNA isoform was successfully purified through both modified monoliths, by applying an increasing stepwise gradient of NaCl. Acidic pH conditions strengthened interactions established between nucleic acids and cadaverine monolith, in a higher extent than in lysine monolith, forcing sample elution to be carried out with higher salt concentration. In addition, it was observed a lower sc mcDNA recovery from lysine monolith, when compared to the cadaverine one. These data suggest that cadaverine ligands present higher selectivity towards the mcDNA molecule than lysine ligands. The final sc mcDNA sample presented reasonable host impurity levels, which fulfilled the regulatory agency specifications. Altogether, these results have certainly added value for the biotechnological application of this highly promising DNA delivery vector.

A.M. Almeida and A. Sousa acknowledge doctoral and post-doctoral fellowships (SFRH/BD/102284/2014 and SFRH/BPD/102716/2014, respectively) from FCT. The authors also acknowledge to BIA Separations for the monolithic supports.

Keywords: Affinity chromatography, lysine, cadaverine, monolith, Minicircle DNA

P-020 - THE POWER OF DESIGNED AFFINITY REAGENTS IN BIOENGINEERING

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Background
Molecular recognition through affinity interactions is ubiquitous in Nature and key for life. Affinity interactions consist in intricate networks of transient hydrogen bonds, hydrophobic, and electrostatic interactions established between affinity pairs target-receptor. Over the past 40 years monoclonal antibodies and derived structures became the standard binding proteins representing powerful tools in biotechnology and biosensing. Other synthetic and protein scaffolds, with the robustness and versatility required, are being explored by our group to enable the selective capture and oriented immobilization of biomolecules on surfaces. Still, the need to precisely control the production, characterization and stability of the affinity proteins, prompted the search for other alternatives (1).

Method
We employ biological and chemical combinatorial libraries supported by computational design tools to develop robust peptidomimetics based on different scaffold molecules. The scaffold molecules ranged from small synthetic ligands based on the triazine and Ugi reactions, to peptide-based β-hairpin engineered scaffolds and larger natural scaffolds.

Results & Conclusions
We studied the potential of these scaffold affinity reagents to find binding partners against several targets, tagged ecombinant proteins,
proteins with post-translational modifications, and to develop affinity-based purification processes (2,3). In particular, we will present examples of
small synthetic ligands based on one-pot combinatorial reactions and small protein domains to develop selective binders towards green fluorescent protein, tagged proteins, and human serum albumin. However, our approach can be employed to target any biomarker of interest. These affinity reagents were immobilized onto magnetic microparticles and employed as ligands for the selective capture and oriented immobilization of the target biomolecules from complex biological mixtures.

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This work was supported by the European Research Council through the grant reference SCENT-ERC-2014-STG-639123 (2015-
2020) and by the Unidade de Ciências Biomoleculares Aplicadas (UCIBIO), which is financed by national fun ds from Fundação para a Ciência e Tecnologia/
Ministério da Educação e Ciência (FCT/ME) (UID/Multi/04378/2013)
and cofinanced by the European Regional Development Fund under the PT2020 Partnership Agreement (POCI-01-0145-FEDER-007728).
The authors thank Fundação para a Ciência e a Tecnologia, Portugal, for the research fellowships PD/BD/1058 71/2014 for C. S. M. F., SFRH/BPD/97585/2013 for A.S.P., PD/BD/105753/2014 to RS, SFRH/BPD/112543/2015 to AJB.

Keywords: affinity reagents, bioseparation, biosensing, molecular modelling, protein and peptide design, biopharmaceuticals

P-021 - CHARACTERIZATION OF NANO-MICROENCAPSULATED PARTICLES OF GELATIN AND AVOCADO SEED. (P. AMERICANA)

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Background
Persea americana is known for its beneficial properties. Polyphenols extracted from avocado seeds are potential antioxidants, anti-bacterial, and anti-carcinogenic. These are sensitivity to various factors and it is therefore necessary to improve their stability. Gelatin is a polymer of animal origin, non-toxic, able to mask the taste and texture of some bioactive compounds, acting as an excellent encapsulating matrix. The objective of this work is to characterize the capsules of avocado seed extract with gelatin encapsulated in a nano spray dryer (Buchi-90) and in a Spray dryer (Buchi-290), to determine variation in size, morphology and antioxidant capacity

Method
The extracts of avocado seed were obtained with hydroalcoholic solvents, then the extract was concentrated and the ethanol removed. The sample was kept dry. This extract was encapsulated with gelatin as wall material by using two methods of spray drying, one being the Buchi-90 and the other, a Buchi-290.

Results & Conclusions
The resultant extracts were analysed to determine the variations of size and morphology by Scanning Electron Microscopy (SEM). The stability of the antioxidant capacity of the nano-microcapsules was determined in two solvents (0 and 50% ethanol), using the Oxygen Radical Absorption Capacity (ORAC) method, with Trolox as a standard. In the measurement of the antioxidant capacity the evaluated variables were pH (3 and 6) and agitation time (0, 0.5, 1 and 2 hours). In the results shown by SEM, a large variety of sizes is observed in the case of the nanocapsules, finding an average value of 1.78±1.99 µm. In addition to this, most nanocapsules were spherical in shape and in some cases, small cavities on their surface were observed. Microcapsules average size was 3.59±1.76 um, showing irregular spherical shape with cavities on their surface. As for the antioxidant capacity, better stabilities were observed in the samples analyzed in 0% ethanol compared to 50% ethanol, displaying low polarity. As for the time parameter, the antioxidant capacity of nanocapsules after an hour decreases, situation not observed in the microcapsules. In spite of the above mentioned, in both cases, this loss of AOC in the encapsulated material is lower than 50%, therefore, we conclude these nano-microcapsules could be used in food matrices, with potential antioxidants, amongst other benefits.

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Project: BiValBi:FP7-PEOPLE-2013-IRSES:PIRSES-GA-2013-611493. Scholarship: Doctorado Nacional CONICYT.

Keywords: Avocado, encapsulation, seed, Antioxidants

P-022 - EFFECT OF THE OPERATIONAL CONDITIONS OF AN ENZYMATIC TREATMENT ON THE PRODUCTION OF BIOACTIVE COMPOUNDS FROM OVERRIPE BANANA FLOUR

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Background

Bananas are one of the largest crops in the world. They are recognized for their high carbohydrate content, mainly starch, its content of minerals and vitamins, and other bioactive compounds. Countries like Chile does not produces banana, but imports them in amounts over 150000 tons per year; however, due to the rapid maturation process, the loss of that fruit can even reach up to 30%. Over-ripened bananas, those with a large number of brown spots in the peel and pulp are traded at very low cost or discarded, taking away the benefits of its consumption entails. An alternative of utilization is the production of a food ingredient with these discards; nevertheless, the high level of readily digested carbohydrates suggests improving the presence of bioactive compounds to counteract the effect of high sugars consumption. Because of this, the objective of this work is to establish the effect of an enzymatic treatment on the presence of resistant starch (RS) and compounds with antioxidant activity in a flour made with overripe whole banana (OWB).

Method

OWB were lyophilized, and then grind them. The enzymatic treatment was done at 50 and 70 °C with 0,5 or 5% of Viscozyme on a 25 g/100 g of OWB slurry made with phosphate buffer (pH 5.0). The RS content was determined by K-RSTAR enzymatic kit; the content of soluble phenolic compounds and the antioxidant activity were determined by Folin-Ciocalteu and ORAC methods respectively.

Results & Conclusions

Results shows that a 5% of enzyme does not produce differences in RS value; while when 0,5% of Viscozyme is applied, there is an increase of the RS content with the treatment time up to 210% of the original value (1.7% of RS). For phenolic compounds the best value, 1.97 gGAE / L, is obtained when 5% of enzyme and 50 °C is used. On the other hand, a decrease of the antioxidant activity with the processing time is observed, with values ranging from 37956,38 µmol TE / L (11386,91 µmol /100 g) in the control (time 0), to 24776,25 µmol TE / L (7432,87 µmol /100 g) for the assay at 50 °C, 5% enzyme, 24 hours. The differences between the behavior of CFT and antioxidant activity may be due to the release of other compounds that interfere with these measurements such as sugars, especially those with reducing end.

References & Acknowledgments

Acknowledgments: Project FONDECYT 1140909

Keywords: Overripe Banana, resistant starch, Antioxidants

P-023 - EPA AND DHA STABILITY AFTER PROCESSING IN CANNED TUNA WITH SOYA OR REFINED OILS

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Background

Metabolic disorders associated to nutritional patterns have currently reached a social and economic dimension: from noncommunicable diseases, cardiovascular disorders are the leading cause of death worldwide (17.5 millions deceases in 2012)[1]. Thus, EFSA recommends an intake of 0.25-0.50g EPA+DHA/day to assure the normal cardiac function, 2g for cholesterol reduction and 3g for safe blood pressure values [2]. Among the high EPA+DHA foods and for consumers, canned tuna is an attractive alternative to the perishable fresh fish, due to its long shelf life and sensory attributes. Furthermore, vegetable oils can be used as a preserving ingredient and help to promote sensory quality. However, little is known about the effects of formulation, processing and sterilization on the concentration of EPA and DHA of these high PUFA containing foods.

Method

Samples consisted in canned tuna in brine (TN) or oil (TS: with soya oil or TR: with refined olive oil), prior to (A) and after sterilization (B). Lipids were isolated according to the method described by Matyash et al.[3]. Samples were prepared according to Pimentel et al.[4] for the analysis of the fatty acids composition by GC-FID.

Results & Conclusions

All canned samples have a lipid content ten-fold higher than TN. The EPA+DHA content in TN-A and TN-B was 0.48-0.49 g/100 g product, 0.29-0.33 g/100 g product for TR-A and TR-B while for TS was 0.37 (A) and 0.41 (B). The obtained results showed that EPA+DHA in the canned samples was slightly lower by the presence of oil ($p<0.05$) and was not altered after thermal processing ($p<0.05$).Addition of oil slightly decreased the EPA+DHA in canned tuna. Thermal processing to obtain canned tuna with soya or refined olive oil did not affect the EPA+DHA contents.

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Keywords: Omega 3, Canned Tuna, Vegetable oils, FAME, GC-FID, Thermal Stability

P-024 - AEROBIC GRANULES SYNTHESIZED WITH EPS AND DEGRADING STRAIN RHODOCOCCUS FP1 FOR INDUSTRIAL WASTEWATER TREATMENT

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Background
Aerobic granular sludge-sequencing batch reactors (AGS-SBR) is a promising and innovative wastewater treatment system. AGS is composed of microorganisms embedded in a self-produced extracellular polymeric substances (EPS) matrix, forming spherical sludge aggregates [1,2]. Although AGS tolerance to toxicity, the indigenous microbial communities may not be effective in removing recalcitrant pollutants [3,4]. Bioaugmentation strategies (addition of specific microorganisms to the system) can be a solution to overcome the difficulty to eliminate certain compounds in wastewaters. However, it is still not a well-established strategy.

Method
EPS was extracted from aerobic granules from Nereda® wastewater treatment plants in Utrecht or Garmerwolde, Netherlands. The extraction procedure is as described by Felz et al., 2016 [5]. The synthetic granules were produced using the extrusion technique with CaCl2, by mixing the extracted EPS, a concentrated bacterial suspension of *Rhodococcus* sp FP1 (OD450 of 67.0), and substances 1 or 2. The produced synthetic granules were subjected to a shear stress test, 400 or 800 rpm in a closed vessel for 1 hour, in order to measure their strength.

Results & Conclusions
A specific mixture composed of EPS, bacterial suspension and substance 2 generated strong synthetic granules, similar to Utrecht granules used as a control in the shear stress test. Beads produce only with substance 2 and bacterial suspension showed to be weaker than the granules previously mentioned. Thus, the EPS can be considered a key component to increase the strength of the synthetic granules. However, some inconsistencies were observed for synthetic granules with higher concentration of EPS and substance 2, which could indicate that the composition and crosslinking potential of the EPS could be the limiting factor for the granules strength and not only the EPS concentration.

In summary, EPS composition and concentration can be important factors to be considered when synthesizing strong granules able to endure this shear stress test. In the future, the EPS biocompatibility and 2-fluorophenol biodegradation with these synthetic granules will be tested.

References & Acknowledgments
The authors wish to thank the company SIMTEJO for supplying the granules, and the financial support of European Social Fund, under Programa Operacional under the project NORTE-08-5369-FSE-000007. This work was also supported by Portuguese Funds from FCT - Fundação para a Ciência e a Tecnologia through the strategic funding of UID/Multi/50016/2013 and UID/BIO/04469/2013 units. C.L. Amorim wish to acknowledge the research grant from FCT (SFRH/ BPD/96481/2013).

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Keywords: bioaugmentation, extracellular polymeric substances, synthesized granules

P-025 - OPTIMIZATION OF LINOLEIC ACID EMULSION PREPARATION TO REDUCE SUBSTRATE LOSSES AFTER FILTER-STERILIZATION

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Background
Conjugated linoleic acid (CLA) isomers are bioactive fatty acids that can be produced microbiologically from linoleic acid (LA). *In vitro* studies normally test CLA production using a solution of pure LA at a specific concentration and an emulsifier, usually Tween 80, upon which the mixture is filter-sterilized [1]. However, preparation leads to LA losses requiring higher amounts of LA to achieve the intended concentration. Thus, the aim of this work was to optimize the LA emulsification strategy in order to obtain a more efficient and cost-effective procedure.

Method
Four different treatments were applied to LA solutions at 15 mg/mL with 2% (w/v) Tween 80: i) Filtration (0.45 µm-pore size membrane; F4); ii) Ultra-Turrax (90s [A] or 150s [B] at intervals of 30s) + Filtration (0.45 µm); iii) Sonicator (90s at intervals of 30s; 1s pulses [C]) + Filtration (0.45 µm). The application of a smaller pore sized membrane assuring microbiological sterility was further tested and two different treatments were applied: i) Filtration (0.20 µm-pore size membrane; F2); ii) Ultra-Turrax (150s at intervals of 30s [D]) + Filtration (0.20 µm). All experiments were carried out in duplicate. Aliquots of each solution were collected before and after treatment for LA concentration analysis by gas chromatography [2].

Results & Conclusions
Filtration of LA emulsion directly through a 0.45 µm-pore size membrane (F4) led to a 17.09% LA loss whereas the introduction of a blending step, either using a sonicator (C) or an Ultra-Turrax (A and B) led to lower losses of 13.01% and 7.17% and 7.40% ($p>0.05$), respectively. A smaller filter pore size membrane (0.20 µm), also contributed to lower losses; LA reductions were from 10.07% (F2) to 3.71% (D). In conclusion, a previous dispersion with Ultra-Turrax, independent of the filter pore size, demonstrated to be the best method to reduce substrate losses in filter-sterilization of LA emulsions.

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This work was financed by National funds via FCT – Fundação para a Ciência e a Tecnologia, under the project "Pro-TECh-CLnA - Microbial Production of Bioactive Conjugated Linolenic Acid Isomers to Obtain Functional Ingredients and Foods" reference PTDC/AGR-TEC/2125/2014. Financial support for the authors A.L. Fontes and L.L. Pimentel was provided by fellowships SFRH/BD/117721/2016 and SFRH/BPD/119785/2016, respectively, granted by the Portuguese government through FCT.

Keywords: Linoleic acid, Emulsions, Sterilization, CLA production substrate, Homogenization

P-026 - OPTIMIZATION OF PARA RESOLVASE UNTRANSLATED REGION – A STRATEGY TO BOOST MINICIRCLE DNA PRODUCTION.

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Background

Minicircles are plasmid-derived DNA molecules devoid of bacterial sequences, which can safely deliver a gene of interest into cells allowing for a higher expression when compared to conventional plasmids. Production of minicircles is performed in *Escherichia coli* by replicating a parental plasmid and promoting its recombination into miniplasmid and minicircle molecules, via induction of recombinase expression. Although the parA resolvase recombination system has been shown as highly efficient for minicircle production, the role of the secondary structure of messenger RNA on recombinase expression has been scarcely examined in this context. Evidence gathered in the literature indicates that gene expression in *E. coli* is inversely related to the stability of the secondary structure of the ribosome binding site. Here we present a strategy to improve resolvase translation and minicircle production by modifying the 5′ untranslated region (5′-UTR) of ParA resolvase.

Method

The original 5′-UTR of parA gene was optimized *in silico*, using an iterative thermodynamic model, to generate sequences with higher translation initiation rates (TIR) which should lead to more stable mRNA-rRNA complexes. The original 5′-UTR from P_{BAD}/araC-parA cassette was modified via SOEing PCR, generating 4 new cassettes (P_{BAD}/araC-par2A to P_{BAD}/araC-par5A) with increasing predicted TIRs. These cassettes were used to construct plasmids pMMBparA (original 5′-UTR) and pMMBpar2A to pMMBpar5A (improved 5′-UTRs). *E. coli* strains BW1P and BW2P were created by inserting one copy of the P_{BAD}/araC-parA or P_{BAD}/araC-par2A, respectively, into the bacterial chromosome. Expression of ParA resolvase was compared by analysing the production of *parA* mRNA via qRT-PCR and of ParA protein via densitometry and western blots analyses. To evaluate minicircle production, recombination efficiency was determined by densitometry analysis of agarose gels.

Results & Conclusions

In silico analysis of all 77-nucleotide-long mRNA subsequences comprising the parA 5′-UTRs predicted a TIR increase between 63 and 366-fold. Quantification of parA mRNA showed that expression from P_{BAD}/araC-par2A is higher than from the original cassette (2.5-fold and 1.6-fold for expression from the plasmids or chromosome, respectively). Also, although higher ParA mRNA and protein levels were obtained for expression from plasmids, a recombination efficiency over 98% was obtained with the BW2P strain, indicating that a single copy of an optimized parA expression cassette on the chromosome allows successful minicircle production. The remaining 5′-UTR sequences are currently at the final stage of evaluation to determine if a faster and more effective recombination system can be obtained.

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The authors acknowledge FCT for the doctoral grant (PD/BD/116842/2016) awarded to Cláudia Alves.

Keywords: Minicircle, parA resolvase, 5'-UTR, Ribosome Binding Site, mRNA secondary structure

Bioprocess Engineering

P-027 - EXTRACTION OF POLYPHENOLS FROM VINE PRUNING RESIDUES OPTIMIZATION

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Background

Vine pruning residue (VPR) is a by-product obtained after annual pruning of vines, abundant in Portugal, and a natural source of compounds with antioxidant activity. In a previous work, an integral valorization of this residue was proposed in which 13.7 kg of xylooligosaccharides, 13.1 kg of ethanol and 27 kg of lignin per 100 kg of VPR were extracted[1]. Here, we aim at optimizing antioxidants extraction using different fractions of organic solvent (ethanol /water). Therefore, the objective of this work was to attain an optimum extraction condition for polyphenolic compounds from grapevine pruning using a response surface methodology.

Method

For each assay the temperature (46-114°C), the extraction time (19-221 min) and ethanol concentration (30-70%) were determined by factorial design. The solids/solvent ratio was 40:1 (mL/g). The characterization and quantification of phenolic compounds was performed by UPLC. Total phenolic contents, expressed as gallic acid equivalents by absorbance following Folin-Ciocalteu method[2]. Antioxidant activity of VPR extracts was also determined following methods FRAP (ferric reducing antioxidant power), DPPH (radical scavenging activity assay) and ABTS (radical cation decolorization) expressed as Trolox equivalents[3-4] both were quantified by spectrophotometry. The percentage of inhibition was calculated as a function of the concentration of extracts and Trolox.

Results & Conclusions

The optimum extraction conditions were as follows: ethanol concentration, 45%; extraction time, 120 min; and temperature, 80°C. In these conditions the obtained extracts had 2.16 kg of phenolic compounds per 100 kg of VPR and thus higher antioxidant activity were obtained (FRAP = 3.81 kg Fe(II)/100 kg VPR, DPPH = 4.70 kg TE/100 kg VPR and ABTS = 16.48 kg TE/100kg VPR). In this context the VPR is a promising waste material for the generation of compounds with added value.

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Keywords: Vine pruning residue, Phenolic compounds, Antioxidants extraction

P-028 - INFLUENCE OF DEACETYLATED CHITIN NANOCRYSTALS CROSSLINKED WITH GENIPIN ON MECHANICAL, ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF STARCH-BASED FILMS

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Background

The search for active and biobased packages that efficiently contribute to reduce the environmental impact caused by the accumulation of agrofood and plastic waste on landfills is a priority. Herein, the use of starch has been exploited[1]. However, starch-based materials present poor mechanical and barrier properties and have no active function against food deterioration reactions, limiting their application. As an alternative, adding nanofillers with intrinsic antioxidant and antimicrobial activity to starch-based formulations helps to overcome these drawbacks[2].

In this work, the influence of chitin nanocrystals (ChitNC) and deacetylated chitin nanocrystals (dChitNC) on mechanical, antioxidant and antimicrobial properties of starch films, containing or not genipin, was studied. Aiming to develop a sustainable approach, starch recovered from potato industry byproducts was used.

Method

Starch was recovered from potato washing slurries. ChitNC were prepared by chitin acid hydrolysis and further partially deacetylated (dChitNC) in an alkaline medium.

Starch films containing 1 and 5% ChitNC and dChitNC, in the presence/absence of genipin, were produced by solvent casting. A starch film without any filler was used as control. The mechanical, antioxidant and antimicrobial properties of each produced film were determined.

Results & Conclusions

ChitNC presented a rod-like morphology with approximately 9.7 nm of width and 244 nm of length. When incorporated in starch formulations, ChitNC increased the films tensile strength and Young's modulus values, thus leading to more resistant and rigid films. dChitNC also promoted an increase of the starch films mechanical resistance. This effect was significantly pronounced when genipin was added. Moreover, starch films containing dChitNC crosslinked with genipin showed an increased antioxidant activity, when compared with all the films produced. All the produced starch-based films were innocuous from the microbial point of view.

As major conclusion, dChitNC-crosslinked with genipin revealed to be a promising filler for producing starch films with improved mechanical resistance and good antioxidant properties, extending their usage for active food packing applications.

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Thanks are due to FCT through national founds and FEDER, within the PT2020 Partnership Agreement, for funding QOPNA (FCT UID/QUI/00062/2013) and CICECO-Aveiro Institute of Materials (FCT UID/CTM/50011/2013; POCI-01-0145-FEDER-007679). I.G. (SFRH/BPD/104712/2014), C.N. (SFRH/BPD/100627/2014) and P.F. (IF/00300/2015) also thank the support of FCT. The authors also acknowledge to POTATOPLASTIC project (POCI-01-0247-FEDER- 017938), financed by FEDER trough POCI, "Isolago – Indústria de Plásticos, S. A.", project leader and to "A Saloinha, Lda." for providing potato byproducts.

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Keywords: starch films, deacetylated chitin nanocrystals, genipin, rigidity, antioxidant, antimicrobial

P-029 - NEW SOLUTIONS OF IMMOBILIZED YEASTS WITH OENOLOGICAL POTENTIAL

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Background

The wine sector is expanding worldwide, with more and more wine producers entering this sector, which leads to an increase in wine production. Thus, it is important that companies have access to new technologies, optimizing their production. The development of new methodologies, such as immobilized yeasts has great oenological potential, since they have several oenological applications and allow to improve the quality / price relation of the final product. This type of methodology nowadays begins to be used by the producers, especially in the production of sparkling wines, during the second alcoholic fermentation. In addition, they can also be used in alcoholic and malolactic fermentations, in the production of late harvest wines or in the correction of some problems, such as excessive volatile acidity or late fermentation. The aim of this work was the production of new solutions of immobilized yeasts with oenological potential.

Method

A set of yeasts strains isolated during different stages of grape musts fermentation were immobilized in two different inorganic porous supports: volcanic tuff and expanded clay. The immobilization success was accessed by scanning electron microscopy and to validate the potential of the immobilization, the immobilized solutions were tested in microvinification assays during 7 days. After this period the stability of the matrixes and the presence of yeast cells on the immobilized system were confirmed by SEM.

Results & Conclusions

The results proved that both supports with immobilized yeasts allowed the conversion of must sugars into ethanol, and can be reused during five batches with high efficiency, displaying the potential use of these matrices in oenological context.

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Acknowledgments: The authors would like to thank the HIT3CH project—HERCULES Interface for Technology Transfer and Teaming in Cultural Heritage (ALT20-03-0246-FEDER-000004) co-financed by the European Regional Development Fund (ERDF) and ALENTEJO 2020.

Keywords: Wine, must, Yeasts, immobilized cells, immobilization matrices, oenological processes

P-030 - MECHANICAL, ANTIMICROBIAL AND ANTIOXIDANT ASSESSMENT OF STARCH-BASED FILMS PRODUCED BY REUSING POTATO CHIPS BYPRODUCTS

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Background

The development of active biobased packaging materials is of major concern to food industry. Herein, starch, an abundant polysaccharide in nature, satisfies all the main features, making it a promising raw material for edible coatings/films [1,2]. However, starch leads to quite brittle and hydrophilic materials [3] without active properties and, therefore many efforts have been carried out to fulfill these features [4,5]. In this work, starch and phenolics compounds were recovered from potato chips industry byproducts, namely washing slurry and potato peels, respectively, and characterized. Further, these compounds were combined to produce an active starch-based film with improved mechanical, antioxidant and antimicrobial properties.

Method

The recovered starch size distribution, granular surface and calorimetry were determined. In addition, the total phenolic content and the antioxidant profile of the recovered phenolic extract were measured. The influence of phenolic extract concentration (0.1%, 0.5% and 1% w/w related to starch weight) on mechanical, antioxidant and antimicrobial properties of starch films. As previously described [6], both oil and waxes (2% w/w related to starch weight each) obtained from frying residues and potato peels were incorporated in the starch based formulations.

Results & Conclusions

For concentrations above 0.5%, the phenolic extract promoted a plasticizing effect as noted by the decrease of tensile strength and Young's modulus, and increase of elongation values. In addition, the antioxidant activity increase in about 35-55% and the microbial growth is inhibited, being this effect more pronounced for 1% of phenolic extract. These results revealed that potato chips industry byproducts possess biopolymers with potential for the development of active starch-based materials with improved mechanical, antioxidant and antimicrobial performance, opening applications on the food packaging field.

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Keywords: byproducts, packaging food, antioxidant, antimicrobial

P-031 - STRUCTURAL AND CYTOTOXIC CHARACTERIZATION OF SARGASSUM MUTICUM AND OSMUNDEA PINNATIFIDA ENZYMATIC EXTRACTS WITH BIOLOGICAL PROPERTIES

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Background

Seaweeds are an important source of healthy ingredients, including new biologically active molecules that may be exploited for food or nutraceutical applications. *Osmundea pinnatifida* and *Sargassum muticum* extracts have demonstrated important antioxidant, antidiabetic and prebiotic biological properties (1), yet their structural and cytotoxic characterization is still lacking.

Method

Enzymatic extracts of *O. pinnatifida* and *S. muticum* obtained with Viscozyme and Alcalase, respectively, were performed according to Rodrigues et al. (1). Structural characterization was based on FTIR-ATR and ^{1}H NMR analyses. The identification of functional groups in the NMR spectra was based on their chemical shift (δ_H) relative to the water 4.7 ppm).

Enzymatic extracts were evaluated for anti-hypertensive activity by the angiotensin-I converting enzyme (ACE) assay according to Tavares et al. (2). To determine the eventual cytotoxicity of the extracts, a mammalian cell line (L929) was used to assess effects on cellular metabolic activity.

Results & Conclusions

FTIR-ATR spectra of *S. muticum* and corresponding enzymatic extract obtained with Alcalase showed high similarity and practically only differences in absorption intensity were observable. In terms of *O. pinnatifida* seaweed and its Viscozyme enzymatic extract, the two spectra presented some qualitative differences in the region 1100 to 1600 cm^{-1}. The increment in the 1150 cm^{-1} band can be related with a possible role of the multi-enzyme carbohydrases complex on polysaccharides (agar) matrix and on cellulose, xylan and manan fibrils of the complex composite cell walls of red seaweeds. In what concerns the ^{1}H NMR spectra, the relative abundance of each type of protons is, in general, relatively similar for the two extracts except the percentages of protons belonging to the aliphatic H-C group directly bound to an oxygen atom (H-C-O) probably due to the presence of nonaromatic ring structures such as sugars; higher values (67%) were observed for the enzymatic extract of *O. pinnatifida* than for the *S. muticum* (43%) counterpart. The *O. pinnatifida* extract exhibited the strongest ACE inhibitory with an IC_{50} value of 111.2 ug/mL whereas for *S. muticum* activity was of little significance (444.4 mg/mL). No cytotoxic effects were observed on mammalian cells, which suggests that both enzymatic extracts can be considered non-toxic in the range of concentrations tested and be further tested for novel nutraceutical and functional food applications.

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Work supported by national funds through FCT/MEC (PIDDAC) reference project IF/00588/2015 and by FCT project UID/Multi/50016/2013.

Keywords: Seaweeds, Enzymatic Extracts, Biological properties, Structural characterization, Cytotoxicity

P-032 - MODULATION OF FATTY ACID PROFILE IN NANNOCHLOROPSIS OCULATA ADAPTATION TO TEMPERATURE STRESS

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Background

Microalgae are known as a potential source of specific lipids, namely polyunsaturated fatty acids (PUFA), important from both nutritional and functional standpoints (Matos *et al*., 2017). Those PUFA are usually produced under stress conditions, but such conditions concomitantly decrease cell yields (Chen *et al*., 2017). The aim of this research work was to modulate the stress provided to cultures of *Nannochloropsis oculata* in order to enhance PUFA production, while ensuring a steady increase in biomass. Temperature was the selected stress parameter, given its known effects on PUFA production: changes in lipid composition in response to temperature changes are a major cellular response to guarantee membrane fluidity adjustment (Renaud *et al.*, 1995).

Method

In order to assess the impact of temperature variations on lipid production and composition, an experiment was designed, where temperature cycles varied as follows: the culture was initially grown at 22.5 ºC for 3 days, upon which temperature was reduced to 10 ºC. After 3 more days, initial temperature was reestablished, for 4 days, and once again it was reduced to 5 ºC and left at such low temperature for 5 days. At the end of each temperature cycle, both growth (through optical density and cell counts) and lipid values (total content and fatty acid profile) were assessed using gravimetric and GC chromatography methods.

Results & Conclusions

Results showed that a gradual variation in temperature has no substantial negative impact on culture growth. Indeed, when temperature was decreased, cell growth was not affected and lipid values increased. Furthermore, the intracellular lipid content consistently increased within the temperature decreases along the growth cycle. Concerning the fatty acid profile, the content of the PUFA gamma linolenic acid and eicosapentaenoic acid was inversely related with temperature, whereas the ratio n6/n3 and the atherogenicity index (AI), which are related to the predisposition of lipids to cause cardiovascular diseases, were directly related with temperature.
From the abovementioned results, it can be concluded that a controlled temperature reduction throughout growth can be used to modulate lipid profiles and production in microalgal cultures.

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This work was supported by National Funds through project Norte-01-0145-FEDER-000011-RL1–QUALIFOOD, FCT (Fundação para a Ciência e a Tecnologia) through projects UID/Multi/50016/2013 and SFRH/BD/105304/2014 and by FCT/MEC (PIDDAC) project reference IF/00588/2015.
Keywords: Lipids, polyunsaturated fatty acids, microalgae, stress modulation

P-033 - OPTIMIZATION OF Γ-DECALACTONE PRODUCTION BY CO-CULTURES OF YARROWIA LIPOLYTICA MUTANT STRAINS

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Background

"Natural" labeled aromatic compounds are very sought worldwide for their variety of industrial applications since they involve cheaper processes and create greater incomes when compared to synthetic equivalents [1]. Among natural fragrances and flavors, lactones are a well-known family at industrial and biotechnological level with a production of hundreds of tons per year. Midst this family, γ-decalactone, a peach-like aroma, is the most important flavor and is commonly used in cosmetics and perfumes [2].

Method

The performance of mutant strains derived from Yarrowia lipolytica wild-type W29, MTLY40-2P strain overexpressing POX2 gene and JMY3010 that overexpresses LIP2 gene, was evaluated under different conditions of operation: cellular and castor oil concentration, operation mode (batch or step-wise fed-batch) and bioreactor type (STR or Air-lift). For the first time, a co-culture of both strains was used in order to improve γ-decalactone production from castor oil.

Results & Conclusions

STR batch experiments (20 g L-1 of glucose and 60 g L-1 of castor oil) showed that higher γ-decalactone concentration (1844 ± 46 mg L-1a) and productivity (90 ± 7 mg L-1h-1) were obtained with a co-culture, when compared to pure cultures of each strain. The main reasons were the decrease of the lag phase of the aroma production (observed in MTLY40-2P pure cultures) and the minimization of γ-decalactone consumption (observed in JMY310 pure cultures). In this study, the addition of castor oil pulses resulted in similar γ-decalactone titers and productivities, which suggested that fed-batch approaches did not improve this bioprocess and increased the overall production costs. In air-lift co-cultures (with the same glucose and castor oils concentrations), similar maximum γ-decalactone concentration were attained, however productivity was severely decreased (75 %). This was probably due to process limitation by oxygen deficit.

References & Acknowledgments

This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the project TUBITAK/0009/2014 ref:UMINHO/BI/75/2017, strategic funding of UID/BIO/04469/2013 unit and COMPETE 2020 (POCI-01-0145-FEDER-006684) and BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund under the scope of Norte2020 - Programa Operacional Regional do Norte.

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Keywords: γ-Decalactone, Y. lipolytica MTLY40-2P and Y. lipolytica JMY3010, Castor Oil, Batch and step- wise fed-batch cultures, Biotransformation

P-034 - PREDICTING BIOMOLECULE ADSORPTION ISOTHERMS ON 3RD GENERATION CHROMATOGRAPHIC ADSORBENTS

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Background

Biomolecule adsorption is determined by several types of interactions, including electrostatic, van der Waals, hydration and steric interactions, among others. Isotherm prediction has an undeniable value on reducing the experimental characterization time and cost of the biomolecule-adsorbent system and, also, providing important insights into the adsorption mechanism. Many models, empirical and mechanistic, have been employed to estimate the biomolecule adsorption isotherm. The Langmuir isotherm is widely used to describe the adsorption of macromolecules despite the unsuitability of its assumptions for these systems. Mass-action models are also often used to estimate protein isotherms. These models describe the process of protein adsorption as a stoichiometric exchange of surface counter-ions for one protein molecule, however they are often inadequate because they do not account for major non-ideal effects associated with protein adsorption, or do so in a thermodynamically inconsistent manner. Another protein isotherm model found in the literature uses the colloidal method. Here, the protein is treated as a sphere possessing a fixed potential at the surface. The potential of the adsorbent surface is also assumed to be constant for a given set of process conditions. Oberholzer and Lenhoff successfully calculated protein isotherms under linear and overloaded conditions with this approach. Furthermore, Thrash and Pinto further expanded the approach by incorporating the energy of interaction between adsorbed proteins and the contribution of water-release accompanying protein adsorption.

Method

In the present study, Thrash and Pinto methodology is applied to obtain biomolecule adsorption isotherms on 3rd generation chromatographic adsorbents. Lateral interactions between adsorbed proteins and water-release accompanying protein adsorption were experimentally measured with flow-microcalorimetry. Isotherms and flow-microcalorimetry experiments were conducted on GigaCap Q-650M resin at pH 9 and at ionic strengths of 0.05 and 0.1 M. BSA was used as a model protein.

Results & Conclusions

The resulted simulated isotherm was find to fit well with the experimental results at both salt concentrations. These findings highlighted the importance of microcalorimetric data on predicting the respective adsorption isotherm and, also, on interpreting biomolecule adsorption mechanism.

References & Acknowledgments

This work is supported by FEDER funds through the POCI - COMPETE 2020 (Project POCI-01-0145-FEDER-007491) and National Funds by FCT (Project UID/Multi /00709/2013).

Keywords: Modified colloidal model, Adsorption isotherms, 3rd generation ion-exchange chromatographic supports, Flow microcalorimetry

P-035 - TOOL-BOX OF ENGINEERED DYP-TYPE PEROXIDASES FOR LIGNIN DEGRADATION AND VALORISATION

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Background

Dye-decolorizing Peroxidases (DyP) are novel heme-peroxidases that are very attractive for biotechnological applications due to their ability to degrade lignin-related compounds. This, in association with their presence in the genome of several lignin-degrading organisms, support a putative physiological role in lignin biodegradation.[1,2]

Lignin is the most abundant aromatic polymer in Nature and a key renewable source of bulk, fine chemicals, materials and fuels. Lignin has a highly cross-linked, aromatic nature that is responsible for the inertness of lignin towards degradation which hampers its biotechnological potential. There is a line of investigation based in the search for enzymatic methods to selectively degrade lignin and therefore fully valorize plant biomass. [2,3]
In a previous work, a DyP from *Pseudomonas putida* MET94 (PpDyP) was characterized, with the kinetic data showing that PpDyP is able to oxidize with high efficiency anthraquinonic and azo dyes. Also oxidized phenolic lignin-related compounds, manganese and ferrous ions, at a lower specificity.[4] This enzyme has undergone direct evolution protocols, leading to a third generation variant (6E10) featuring a 100-fold enhanced catalytic efficiency for the lignin-related phenol 2,6-dimethoxyphenol (DMP).[2] In the present work, we are aiming to improve the stability of 6E10 through new rounds of random mutagenesis.

Method

Libraries of variants of 6E10 PpDyP were constructed by random mutagenesis using error-prone PCR. Through the variation of the Mn^{2+} concentrations, the mutation rate using the Taq polymerase was controlled. The variants were screened for improved thermal stability by measuring the remaining activity after an incubation at 40 °C. The best variants underwent kinetic, biochemical and structural characterization.

Results & Conclusions

Through this direct evolution approach, nine variants were initially found with improved stability and/or improved activity for syringol. From this nine variants, two were particularly interesting: the variant 29E4 that showed a 5-fold improved thermostability comparing to 6E10, and the variant 39G8 that featured a 3-fold increase in activity for DMP. The variants 27E4 and 35G5 had a small increase in the thermostability at 40 °C. The main course of this work at this moment is to apply the direct evolution protocol on 29E4 to further improve its stability and activity towards phenolic compounds.

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Keywords: Lignin, Dye-decolorizing Peroxidases, biocatalysis, directed evolution, biorefineries

P-036 - IN VITRO PRODUCTION OF BIOATIVE METABOLITICS AFTER ELICITATION WITH LIGHTS IN HYPTIS MARRUBIOIDES EPLING CALLUSES

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Background

Hyptis species (Lamiaceae) have several bioactive compounds of pharmacological applications and insecticidal action. *Hyptis marrubioides* Epling, commonly known as "hortelã do campo", is a species of plant found in the Brazilian Savana. Plant metabolic responses can be modulated by varying light quality, and the use of colored lamps as elicitor may be a promising strategy for an improved production of bioactive metabolites in plant and cell culture. In this study was evaluated the effect of the light type on the production of bioactive metabolites in *H. marrubioides* calluses.

Method

To evaluate the metabolic response after light elicitation, cultures were exposed for 20 days in different spectral ranges (white light), (blue light), (red and blue / red light) and in the dark light in 50% MS of the saline medium, containing 2 mg L^{-1} ANA and 1 mg L^{-1} BAP. After exposure in the presence of light, the calluses were subjected to drying at 35 °C. In the extraction, it was used 0,2 g of *H. marrubioides* in 5 mL of methanol in ultrasonic bath (30 min) and the analysis was performed in Shimadzu® CLAE, with SPD-M20A model DAD (λ = 254 nm) and LC18 column (25 cm x 4,6 mm, 5 µm, Supelcosil®). The mobile phase was composed of: (A) water / acetic acid (0,1%, v/v) and (B) MeOH, with a flow of 1,0 mL min^{-1}. Elution was 10 to 66% B (0-32 min), 66 to 10% B (32-35 min) and 10% B for 5 mL, with an injection volume of 20 µL. Three standards were used in the quantification of bioactive compounds: chlorogenic, ferulic and rosmarinic acid.

Results & Conclusions

The production of chlorogenic acid was higher under red light (0,24 mg g^{-1}) and dark (0,28 mg g^{-1}). The highest achieved concentration of ferulic acid was detected in the dark (0,02 mg g^{-1}). In the production of rosmarinic acid both in the dark (1,86 mg g^{-1}) and in the red + blue light (1,74 mg g^{-1}), both were stimulated and did not differ among each other, lower concentrations were found when submitted to light blue (0,59 mg g^{-1}), red (0,91 mg g^{-1}) and white (1,28 mg g^{-1}), with no difference between them.

References & Acknowledgments

The National Council for Scientific and Technological Development (CNPq), the Research Support Foundation of Goiás (FAPEG) and the Instituto Federal Goiano for their support.

Keywords: Elicitation, Ferulic acid, Light, Hortelã do campo

P-037 - A SIMPLIFIED STRATEGY TO MEASURE GLUCOSE RELEASE IN COOKED RICE SAMPLES

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Background
Many varieties of rice are considered a high glycemic index food. High glycemic index (GI) foods are characterized by fast-release of carbohydrates and higher blood glucose levels (Augustin et al., 2002). *In vitro* estimation of the amounts of glucose released during gastrointestinal digestion can be useful to identify or develop low GI foods for incorporation in a healthier diet. The aim of this work is to assess the possibility of using a simple and low cost method (dinitrosalicylic acid (DNS) assay) to estimate starch hydrolysis, and consequent glucose release, during the digestion of cooked rice along the gastrointestinal tract.

Method
Cooked white and integral rice samples were submitted to simulated gastrointestinal digestion using a standardized *in vitro* digestion protocol according to Minekus et al. (2014). Samples were taken in duplicate after oral, gastric and intestinal phases and were analyzed using the glucose oxidase/peroxidase method and the DNS method to determine glucose release/hydrolyzed starch. Total starch was determined using Megazyme's "Total Starch" (amyloglucosidase/ α-amylase method - AOAC method 996.11). Pearson´s correlation coefficient was used to assess correlations between D-Glucose kit and DNS method on glucose release from rice samples.

Results & Conclusions
A positive correlation was observed between results from D-Glucose kit and the DNS method for glucose release throughout simulated gastrointestinal digestion for white rice (r=0.96) and integral rice (r=0.99). Percentage glucose release increased steadily over time for both rice varieties, in both tests used. As expected, the results obtained with the D-glucose kit are lower than the results obtained with the DNS method, due to its specificity for free D-Glucose. Overall, results showed a 6-fold increase in glucose release between oral and intestinal phases in white rice compared to a 3-fold increase in brown rice. Furthermore, glucose values were 30-fold higher in order of magnitude in white rice versus brown rice. These differences are associated with the different biochemical and nutritional characteristics of the two rice varieties.

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This work was supported by Fundo Europeu de Desenvolvimento Regional FEDER, via Programa Operacional Competitividade e Internacionalização (POCI), through project reference POCI-01-0247-FEDER-017931. We would also like to thank the scientific collaboration of CBQF under the FCT project UID/Multi/50016/2013 and FCT/MEC (PIDDAC)-IF/00588/2015

Keywords: glucose release, DNS method, starch hydrolysis, glycemic index, rice

P-038 - EFFECT OF UV-C RADIATION ON SECONDARY METABOLISM OF CALLUS OF HYPTIS MARRUBIOIDES EPLING

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1 - Rede Pró Centro-Oeste; 2 - Instituto Federal Goiano

Background

Hyptis marrubioides Epling ("hortelã do campo") is an aromatic plant of the Brazilian Savana that can be a source of secondary metabolites, which are known to act as mechanisms of endogenous defense of the plant, being induced in response to biotic and abiotic stresses. Exposure to UV radiation in plants promotes defense response which modulates the biochemical composition and promotes the synthesis and accumulation of secondary metabolites, including phenolic compounds. The objective of this study was to evaluate the production of phenolic compounds in *H. marrubioides* calluses by exposure to UV-C radiation.

Method

H. marrubioides friable broths pre-established in MS culture medium with half of the original salt concentration, supplemented with 2 mg L^{-1} ANA, 1 mg L^{-1} BAP, 30 g L^{-1} of sucrose and 3,5 g L^{-1} of agar (Dinâmica®) were exposed to UV-C radiation in triplicate for 0, 30, 60, 120 and 240 seconds at 21 days of culture and the calluses were collected 24 and 48h after exposure to UV radiation. For the detection of the phenolic compounds the calluses were dried at 35 °C for 24 h and 0,2 g dry matter was added in 5 ml of methanol for 30 minutes in an ultrasonic bath and the chromatographic separation was performed in a Shimadzu® CLAE system with DAD detector (λ = 254nm) model SPD-M20A and LC18 column (25cm x 4,6mm, 5µm, Supelcosil®). The mobile phase was composed of: (A) water / acetic acid (0,1%, v / v) and (B) MeOH, with a flow of 1,0 mL min^{-1}. Elution was 10-66% B (0-32 min), 66-10% B (32-35 min) and 10% B for 5 min, with an injection volume of 20 µl.

Results & Conclusions

The chlorogenic and rosmarinic acid compounds were detected in *H. marrubioides* calluses, and as evidenced by Manaf et al. (2016) positively influenced by UV exposure. Chlorogenic acid synthesis was stimulated after 24 h (0,1085 µg mL^{-1}) exposure to UV-C radiation. In the detection of rosmarinic acid there was interaction between the variables tested, and the collection time 48 hours (0,9692 µg mL^{-1}) was superior to 24 h (0,4215 µg mL^{-1}), and that in both higher synthesis was detected at 30 seconds exposure to UV-C radiation (24 h: 0,7333 µg mL^{-1} and 48 h: 1,2274 µg mL^{-1}).

References & Acknowledgments

The National Council for Scientific and Technological Development (CNPq), the Research Support Foundation of Goiás (FAPEG) and the Instituto Federal Goiano for their support.

Keywords: Chlorogenic acid, In vitro culture, hortelã do campo, Rosmarinic acid

P-039 - EVALUATION OF MEMBRANE BIOPHYSICAL PROPERTIES OF MULTIDRUG-RESISTANT ISOLATES OF ESCHERICHIA COLI AND STAPHYLOCOCCUS AUREUS

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Abstract

Background: The ability of most compounds (nutrients and antibiotics) and ions to cross the bacterial cytoplasmic membrane by diffusion and active transport is highly dependent on cytoplasmic membrane fluidity [1]. It was our aim to study possible differences in the membrane fluidity i) of multidrug-resistant (MDR) isolates in comparison to a susceptible strain and ii) in absence and presence of antibiotics (towards which isolates were resistant), by measuring biophysical properties of their membranes, such as membrane anisotropy and membrane polarization, which were assessed through the use of fluorescent probes.

Methods: The membrane fluidity of *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, two MDR *E. coli* and two MDR *S. aureus* clinical isolates was determined from fluorescence anisotropy of DPH-labelled cells as well as from Laurdan Generalized Polarization (GP) measurements, at 24-h intervals up to 6 days. The same analyses of membrane fluidity were performed when the MDR isolates were grown in presence of sub-inhibitory concentrations of ciprofloxacin (CPX) or ceftazidime (CAZ).

Results & Conclusions

Results: The anisotropy values of all three *E. coli* strains studied were quite similar, suffering an equal similar trend throughout the 6 days. This outcome may indicate that the membrane phospholipid composition is very alike among *E. coli* strains, regardless the presence of resistance to multiple antimicrobials. Nonetheless, in the case of *S. aureus*, the anisotropy values were more increased in MDR isolates in comparison to the reference strain, meaning they have a less fluid membrane. The exposition of MDR isolates of both *E. coli* and *S. aureus* to sub-inhibitory concentrations of CPX or CAZ did not affect the anisotropy values, therefore not altering membrane fluidity. Laurdan GP values were calculated from excitation and emission spectra of Laurdan in *E. coli* membranes and *S. aureus*. Those values were very similar for all three *E. coli* strains and were indicative that two phases (liquid and solid) coexisted in the membranes studied. However, such values were statistically different among the three *S. aureus* strains and showed that the two MDR isolates have membranes in a gel-phase while the susceptible strain has coexisting phases. The presence of sub-inhibitory concentrations of antibiotics did not affect Laurdan GP values, neither for *E. coli* or *S. aureus* MDR isolates.

Conclusions: In summary, these preliminary studies on membrane properties of multidrug-resistant isolates indicate that the presence of sub-inhibitory concentrations of antibiotics does not alter membrane fluidity regardless the antimicrobial resistance profile of the isolates.

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This work received financial support from the European Union (FEDER funds through COMPETE) and National Funds (FCT, Fundação para a Ciência e Tecnologia), under the Partnership Agreement PT2020 through project UID/QUI/50006/2013-POCI/01/0145/FEDER/007265 (LAQV/REQUIMTE) and from Programa Operacional Regional do Norte (ON.2 - O Novo Norte), under the Quadro de Referência Estratégico Nacional (QREN) and funded by Fundo Europeu de Desenvolvimento Regional (Feder) through projects NORTE-01-0145-FEDER-000024.

Keywords: Membrane fluidity, Multidrug-resistant isolates, Escherichia coli, Staphylococcus aureus

P-040 - DEVELOPMENT OF AN IN VITRO VAGINAL EXUDATE ADHESION MODEL FOR BACTERIAL VAGINOSIS

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Background

Bacterial vaginosis (BV) is the worldwide leading vaginal disorder commonly recognized between menarche and menopause in women of all ethnicities. It is associated with serious health problems relating to both fertility and pregnancy. This dysbiosis is characterized by a reduction in lactic acid-producing bacteria, mainly *Lactobacillus* spp., accompanied by an overgrowth of strict or facultative anaerobic bacteria, predominantly *Gardnerella vaginalis*. However, *G. vaginalis* is also present in healthy women and its vaginal colonization does not always lead to BV. To better understand the complex interactions that occur between host and microorganisms, and as well as between microorganisms in the vaginal microenvironment, development of *in vitro* models that can simulate the *in vivo* conditions is required, since no adequate animal model exists.

Method

We developed a model that simulates the healthy vaginal mucosa, consisting of HeLa cells pre-coated with *Lactobacillus crispatus* and a chemically defined medium (CDM) known to mimic the female genital tract secretions. First, the ability of *L. crispatus* and *G. vaginalis* to grow in CDM was assessed and compared to growth in standard brain heart infusion medium. Then, in order to simulate BV development, an exclusion competitive initial adhesion assay was performed between *L. crispatus* and *G. vaginalis*. Additionally, the cytotoxic effect of *G. vaginalis* on the monolayer of HeLa cells, without the presence of *L. crispatus*, was also evaluated.

Results & Conclusions

L. crispatus and *G. vaginalis* were able to grow in the vaginal CDM. Importantly, a similar effect was observed in the known interference caused by *L. crispatus* in *G. vaginalis* adhesion to human epithelial cells. However, when *G. vaginalis* was added to the monolayer of epithelial cells without *L. crispatus*, it showed a great ability to adhere and induce cytotoxic changes in cell morphology of HeLa cells. This suggests that the tested vaginal CDM highlights known virulence factors in BV, confirming that using growth conditions more similar to the human vagina can help to identify specific mechanisms and factors that control bacterial populations within the female genital tract either in healthy or BV conditions.

References & Acknowledgments

This work was supported by national funds (FCT) by the strategic project of UID/BIO/04469/2013 and by BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by European Regional Development Fund under the scope of Norte2020. AR had an individual FCT fellowship (PD/BD/128037/2016). NC is an Investigator FCT.

Keywords: Bacterial vaginosis, Gardnerella vaginalis, lactobacilli, cytotoxicity

P-041 - LABORATORY EVALUATION OF CXCL13 CYTOKINE AS A BIOMARKER FOR LYME NEUROBORRELIOSIS AND/OR MULTIPLE SCLEROSIS

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Background

Lyme Borreliosis (LB) is a multisystemic disease caused by spirochetes, belonging to the *Borrelia burgdorferi* sensu lato complex, which includes different pathogenic species. The human transmission of spirochetes occurs through a vector (ticks of genus *Ixodes*) bite. In Portugal, LB is often under-diagnosed due to the clinical polymorphism. Diagnosis is mostly clinical and epidemiological. LB, when is not treated at an early stage, may be worsening, with a hematogenous dissemination of the agent, and may reach the skin, joints, heart, or to involve the nervous system. In this case, the disease is called Lyme neuroborreliosis (LNB). The spirochetes migrate of the bloodstream and cross the blood-brain barrier to the Central Nervous System (CNS), which may remain from months to years in the humans. Therefore, it is crucial to know a little more about the disease, especially NBL, and to develop new methods that support a more efficient diagnosis.

The goal of this work is to understand the role of CXCL13 as a biomarker for diagnosis of LNB *vs* Multiple Sclerosis (MS) in cerebrospinal fluid (CSF) samples from patients suspected or diagnosed of LB since it is known that human-specific cells (monocytes, macrophages and dendritic cells) produce this cytokine.

Method

An ELISA test was performed to evaluate the concentration of CXCL13 in CSF (cut-off 30pg/ml). So, we tested a total of (N=327) CSF samples, 304 of which suspected of LB, 25 of MS, two of Cerebral Vascular Accident, three of Guillain-Barré syndrome and other neurological disorders. Ten samples of Syphilis/HIV patients were included to evaluate the test specificity. All patients came from Lisbon, Evora and Coimbra regions. These samples were also assessed by immunological and molecular tests to detect anti-*B. burgdorferi* antibodies and molecular approaches to amplify *Borrelia* DNA, respectively.

Results & Conclusions

The results showed the following distribution: positive samples (n=88; >250pg/ml) from LNB patients, of which 82 (>30pg/ml) correspond to positive when the patients exhibit neurological symptoms; borderline (n=24; 20-30pg/ml) and negative (n=215; <30pg/ml). Our results revealed samples with high CXCL13 concentration correspond to neurological diseases, regardless of LNB or MS patients, but contrary to the expected syphilis/HIV patient samples, showed values of CXCL13 that we should not devalue.
This preliminary study points out this method for late LB. It is surely a promising tool for suspect cases of infectious illnesses with CNS involvement and not only for differential diagnosis of LNB or MS.

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Keywords: Borrelia burgdorferi sensu lato, Neuroborreliosis, CXCL13 cytokine, Cerebrospinal fluid, Multiple sclerosis

P-042 - RETROPEPSIN-LIKE ENZYMES IN BACTERIA: LESSONS FROM RICKETTSIAE

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Background

Many rickettsiae are pathogenic to humans causing severe infections including Mediterranean spotted fever (*Rickettsia conorii*) and Rocky Mountain spotted fever (*R. rickettsii*). The emergent and severe character of these diseases strengthen the importance of identifying new targets for the development of more efficacious therapies against rickettsioses. The relevance of proteolytic events for bacterial pathogenicity position proteases as relevant candidates for therapeutic intervention. Aspartic proteases of both pepsin and retropepsin-type have been extensively explored as therapeutic targets (e.g. HIV-1 protease). However, their presence in prokaryotes has been the matter of debate over the years; and, in consequence, their relevance in pathogenic bacteria life-cycles has been completely overlooked.

With this work, we demonstrate that a unique membrane embedded retropepsin is indeed present in Rickettsia, emerging as an intriguing target for therapeutic intervention against fatal rickettsioses.

Method

Using *R. conorii* gene homologue RC1339/APRc (for Aspartic Protease from *Rickettsia conorii*) as our model, the soluble domain of APRc was produced in *E. coli*, enzymatically characterized, and its crystal structure determined. Specificity preferences were determined by the high-throughput profiling approach PICS (Proteomics Identification of Cleavage Sites). Evaluation of rickettsial autotransporters/adhesins as potential APRc substrates was assessed by *in vitro* assays and expression of APRc in different ricketsial species determined by Western blot.

Results & Conclusions

Our results show that despite the low overall sequence similarity to retropepsins, the gene product of *rc1339*/APRc is indeed an active enzyme with features highly reminiscent of this family of aspartic proteases, such as autolytic activity impaired by mutation of the catalytic aspartate, accumulation in the dimeric form, optimal activity at pH 6, and inhibition by specific HIV-1 protease inhibitors. Specificity profiling confirmed common preferences between this novel rickettsial enzyme and other aspartic proteases (1). Structural analysis clearly show that the fold of APRc monomer resembles that of viral retropepsins, supporting the concepts that APRc may indeed represent a putative common ancestor of monomeric and dimeric aspartic proteases (2). Additionally, we have shown that APRc is expressed in *R. conorii* and *R. rickettsii* being integrated into the outer membrane of both species. Finally, we demonstrated that APRc is sufficient to catalyze the processing of two conserved autotransporter adhesin/invasion proteins, Sca5/OmpB and Sca0/OmpA, further pointing towards a key role of APRc in a proteolytic pathway relevant to the rickettsial life cycle.

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Keywords: Rickettsiae, Aspartic protease, Retropepsin, therapeutic target, Rickettsia conorii

P-043 - NEW INSIGHTS ON THE ROUTES OF ENTRY OF TWO SPOTTED FEVER GROUP RICKETTSIA IN MACROPHAGE-LIKE CELLS

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Background

Endothelial cells have long been considered the main target cells for rickettsiae. However, several studies have provided evidence of non-endothelial parasitism by rickettsial species with numerous intact bacteria being found within the cytoplasm of macrophages and neutrophils, both in tissues and blood circulation. This evidence has raised the debate about the biological role of this interaction during rickettsial pathogenesis. We have reported that two members of spotted fever group *Rickettsia* (*R. conorii* and *R. montanensis*) have completely distinct intracellular fates in human THP-1-derived macrophages (Curto P. et al. (2016) *Front. Cell. Infect. Microbiol*. 6:80). Although the interaction of rickettsiae with endothelial cells is a process relatively well studied, little is known about the interaction of rickettsial species with macrophages.

Method

In this work, we employed a pharmacological study to start understanding the host proteins involved in the rickettsial entry process into macrophages. PMA-differentiated THP-1 cells were pre-treated with each pharmacological inhibitor and then challenged with *R. conorii* and *R. montanensis* (MOI=10). Samples were then prepared for microscopy and Western blot analysis.

Results & Conclusions

We have identified a requirement of actin polymerization, receptor and non-receptor tyrosine kinase proteins and Pak1 for rickettsial entry into macrophages. Previous reports have demonstrated that PI3K is required for rickettsial invasion in Vero cells. However, our results suggest that PI3K is not required for rickettsial entry into macrophage cells, thereby suggesting that different pathways could be involved in rickettsial uptake in this cell type. Surprisingly, amiloride Na^+/H^+ exchangers inhibitors (DMA and EIPA) - which are known to be the main diagnostic test for macropinocytosis - were able to block rickettsial association with macrophage cells, thus suggesting a macropinocytosis-related pathway as a possible route of entry of rickettsiae in macrophages. These results, and our ongoing work involving high throughput transcriptomics and proteomics analysis of rickettsiae-macrophage interaction, will provide a deeper understanding on how differences in bacterial interactions with distinct cellular host factors contribute for species-specific patterns of rickettsial cellular tropism and pathogenicity.

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Pedro Curto is the recipient of FCT PhD grant SFRH/BD/96769/2013

Keywords: Rickettsia, Macrophages, Pathogenicity, Host-pathogen interactions

P-044 - DECIPHERING THE ROLE OF STAPHYLOCOCCUS AUREUS IN PSEUDOMONAS AERUGINOSA ADAPTATION TO CYSTIC FIBROSIS LUNGS: BYSTANDER OR PLAYMATE?

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Background

Pseudomonas aeruginosa is the major responsible for the high mortality rate of cystic fibrosis (CF) patients due to the development of chronic infections. To long persist, *P. aeruginosa* uses sophisticated mechanisms to achieve full-adaptation, mainly triggered by the harsh environmental conditions of CF lungs. The influence of *Staphylococcus aureus*, a primary colonizer of CF lungs, in increasing *P. aeruginosa* pathogenicity has also been reported. The main objective of this study was to decipher the impact of *S. aureus* on *P. aeruginosa* pathogenicity using *in vitro* conditions mimicking CF lungs.

Method

Antibiotic sensitive and resistant strains of *S. aureus* and *P. aeruginosa* were grown in artificial CF sputum medium and cultured at different timings to simulate a typical colonization of patient lungs. *S. aureus* was first cultured for 3 days, and then *P. aeruginosa* was co-cultured for another 4 days. In the last 4 days the co-cultures were exposed to aggressive ciprofloxacin treatment. Samples were collected every 24 h to analyze bacterial growth kinetics and phenotypic diversity.

Results & Conclusions

Results showed that growth kinetics of *P. aeruginosa* and its phenotypic diversity was not affected by the presence of *S. aureus*. Interestingly, the presence of *S. aureus* seemed to inhibit the emergence of *P. aeruginosa* small colony variants (SCV) that have emerged when the resistant strain was cultured alone. Likewise, *S. aureus* seemed not provide any resistant advantage to *P. aeruginosa* when facing ciprofloxacin treatments. These results do not substantiate the role of *S. aureus* on *P. aeruginosa* persistence pointed out by some studies that reported *S. aureus* extracellular factors increased *P. aeruginosa* growth activity and resistance towards antibiotics by inducing the emergence of SCV. Discrepancies may be explained by the different *in vitro* conditions used. The majority of the studies used standard laboratory medium that does not fully mimic the chemical and nutritional complex environment found in CF lungs. Furthermore, this study mimics a typical lung colonization timeline. In effect, *S. aureus* was cultured prior to *P. aeruginosa*, as happen *in vivo*, to allow its adaptation to CF environment. *P. aeruginosa* co-cultured with CF-adapted *S. aureus* may produce these different, but more reliable, outcomes. In conclusion, the development of *P. aeruginosa* chronic infections seems not to be driven by *S. aureus*.

References & Acknowledgments

This study was supported by the FCT (UID/BIO/04469/2013), COMPETE 2020 (POCI-01-0145-FEDER-006684) and BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund under the scope of Norte2020 - Programa Operacional Regional do Norte.

Keywords: Pseudomonas aeruginosa, Staphylococcus aureus, adaptation, Cystic Fibrosis

P-045 - STREPTOCOCCUS DYSGALACTIAE SUBSP. DYSGALACTIAE ISOLATED FROM MILK OF THE BOVINE UDDER AS EMERGING PATHOGENS: IN VITRO AND IN VIVO INFECTION OF HUMAN CELLS AND ZEBRAFISH (DANIO RERIO) AS BIOLOGICAL MODELS.

Cinthia Alves-Barroco[1]; Catarina Roma-Rodrigues[1]; Luís Raposo[1]; Catarina Brás[1]; Mário Diniz[2]; João Caço[1]; Pedro M. Costa[3]; Ilda Santos-Sanches[1]; Alexandra R. Fernandes[1]

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Background
Streptococcus dysgalactiae subsp. *dysgalactiae* (SDSD) is a major cause of bovine mastitis and has been regarded as an animal restricted pathogen, although rare infections have been described in humans. Previous studies revealed the presence of virulence genes encoded by phages of the human pathogen *Streptococcus pyogenes* (GAS) in SDSD isolated from bovine with mastitis [1]. Recently, we have reported the ability of SDSD isolates containing phage encoded GAS genes to adhere and internalize primary human keratinocytes [2]. To unravel the potential pathogenicity of bovine SDSD isolates harboring phage encoded GAS virulence genes, an *in vitro* and *in vivo* analysis was performed using human cell cultures from the respiratory system and zebrafish (*Danio rerio*), respectively.

Method
Six bovine SDSD strains, collected in two different time periods of isolation (2002-03 and 2011-13), were selected based on the presence or absence of GAS phage encoded virulence genes. One *S. pyogenes* invasive disease strain was used as infectious control. Humans cells from the respiratory system (Detroit 526: pharynx cell carcinoma line, BTEC: primary bronchial/tracheal epithelial cells and A549: lung adenocarcinoma cell line) and zebrafish (*Danio rerio*) was used for the *in vitro* and *in vivo* studies.

Results & Conclusions
Results showed that SDSD strains could interact with human respiratory cell lines and this capability seems to be isolate-specific and independent of the virulence gene content. All strains analyzed were able to cause invasive infections in zebrafish and therefore were an important cause of the fish morbidity and mortality. Although the data suggest that may have different host preferences, is possible to conclude that SDSD strains are able to infect different hosts and have potential zoonotic capability.

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This work was supported by UCIBIO which is financed by national funds from FCT/MEC (UID/Multi/04378/2013) and ERDF under the PT2020 Partnership Agreement (POCI-01-0145-FEDER-007728) and also by projects PTDC/CVT-EPI/4651/2012 and PTDC/CVT-EPI/6685/2014. FCT-MEC is also acknowledged for the funding for MARE through the strategic programme UID/MAR/04292/2013, plus the grant IF/00265/2015 to PC and the grant SFRH/BD/118350/2016 to CAB. The authors thank R. Bexiga and M. Oliveira for sample collection.

In memory of Ilda Santos-Sanches

Keywords: adhesion / bovine / host / internalization / Systemic infection/ Streptococcus dysgalactiae subsp. dysgalactiae/ zebrafish

P-046 - AN ECTOPICALLY EXPRESSED HUMAN CENTROSOMAL PROTEIN BINDS INCLUSION MEMBRANE PROTEIN OF CHLAMYDIA TRACHOMATIS AND IS RECRUITED TO THE VACUOLAR MEMBRANE

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Background

Chlamydia trachomatis uses a type III secretion system to translocate into the host cell about 60 inclusion membrane (Inc) proteins. These proteins possess a characteristic bilobed hydrophobic domain, responsible for their insertion into the inclusion membrane, and are good candidates to participate in the cross-talk between the host and the bacteria. Inc proteins have been described to participate in the manipulation of the host cytoskeleton (CT223/IPAM, CT813/InaC), vesicular and non-vesicular transport (CT115/IncD, CT116/IncE), and associated with the host cell centrosome (CT101, CT222, CT223/IPAM, CT288, CT232/IncB, CT850). However, the function of most of the Inc proteins is unknown.

Method

Results & Conclusions

To further our understanding of the biological role of Inc proteins, we first used a yeast-two hybrid (Y2H) screen of a mammalian cDNA library to identify candidate interacting partners of *C. trachomatis* Inc proteins. We then characterized an interaction between an Inc protein and a human protein of unknown function that localizes at the centrosome. By Y2H, we determined that the C-terminal region of both proteins are necessary for the interaction. The interaction between the Inc protein (deleted of its N-terminus and of the hydrophobic motifs that characterize Inc proteins) and the full-length centrosomal protein was further detected by co-purification or co-immunoprecipitation experiments after ectopic expression of the proteins in bacterial or mammalian cells. Furthermore, when ectopically expressed, the host centrosomal protein could interact with the full-length Inc protein, expressed by *C. trachomatis* during infection. Interestingly, the *C. trachomatis* recombinant strain overexpressing this Inc protein is slightly defective in its life-cycle. Immunofluorescence microscopy of mammalian cells infected by *C. trachomatis* showed that when ectopically expressed, the host centrosomal protein localizes at the inclusion membrane. Thus, our data reveals a new host cell target for an Inc protein, possibly involved in the previous described interaction between the inclusion and the host centrosome.

References & Acknowledgments

This work was supported by Fundação para a Ciência e a Tecnologia (FCT) through grant PTDC/IMI-MIC/1300/2014 and by Unidade de Ciências Biomoleculares Aplicadas (UCIBIO), which is financed by national funds from FCT (UID/Multi/04378/2013) and co-financed by the European Regional Development Fund (ERDF) under the PT2020 Partnership Agreement (POCI-01-0145-FEDER-007728). SVP holds a PhD fellowship (PD/BD/52210/2013) within the scope of the PhD program Molecular Biosciences (PD/00133/2012) funded by FCT.

Keywords: Host-pathogen interactions, Chlamydia trachomatis, Centrosome, Inclusion membrane proteins

P-047 - BORRELIA BURGDORFERI SENSU LATO IN ASYMPTOMATIC INDIVIDUALS: A PRELIMINARY STUDY

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Background

Lyme Borreliosis (LB) is an infectious, multisystemic and emerging disease caused by bacteria (spirochetes) of the *Borrelia burgdorferi* sensu lato (s.l.) complex. These agents are transmitted to humans and animals by hard tick's bite, mainly of the *Ixodes ricinus* species. In Portugal, LB is a notifiable disease since 1999. However, it is underdiagnosed because it shows signs and symptoms that can be confused with other illnesses as well as may evolve asymptomatically. It is known that there are individuals that even infected with *B. burgdorferi* s.l., do not develop clinical symptoms. On the other hand, these bacteria can survive in the blood components after the blood donation (1). So, the potential transmission of these infectious agents by hemotransfusions is currently a relevant issue.
The objective of this study was to evaluate the presence of antibodies against *B. burgdorferi* s.l., in asymptomatic individuals and to relate them to socio-demographic, clinical, travels and exposure to risk factors for this infection.

Method
For this investigation, it was obtained a total of 129 serum samples from volunteers and asymptomatic for LB, who were also asked to complete a clinical-epidemiological survey for additional information after informed consent. The sera were laboratory-assessed with a two-step approach, which included performing an indirect immunofluorescence assay (IFA) as a screening test, followed by a confirmatory Western blot (WB) analysis of the samples positive or borderline (titer ≥1/256 or 1/128), respectively.

Results & Conclusions
Samples (n=41; 31.8%) were reactive by IFA. Of these, the WB -IgM and -IgG evaluation revealed specific reactivity for LB in nine individuals (22%), with the following distribution: three positive samples (two for IgM and one for IgG) and six samples with borderline reactivity (two for IgM and four to IgG). On the other hand, the questionnaire data showed that although participants in the study had no contact with the vector tick, they were exposed to other risk factors.
Although more studies are needed these results showed that the participants in the study, at some point in their lives, might have contacted with *B. burgdorferi* s.l., as demonstrated by the antibodies against it, detected in the serological samples. These data are promising and in the future may be helpful to the blood donors.

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The authors thank all study participants without whom none of this work would have been possible.

Keywords: Lyme Borreliosis, Antibodies anti-B. burgdorferi s.l., Indirect Immunofluorescence

P-048 - A FUNCTIONAL SCREEN IN YEAST TO IDENTIFY CHLAMYDIA TRACHOMATIS VIRULENCE PROTEINS INTERFERING WITH EUKARYOTIC VESICULAR TRAFFICKING

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Background

Chlamydia trachomatis is the leading cause of sexually transmitted bacterial diseases and the causative agent of preventable infectious blindness. This obligate intracellular human pathogen thrives within a vacuolar compartment known as inclusion. During infection, *C. trachomatis* proteins containing a bilobed hydrophobic motif are delivered by a type III secretion system into the inclusion membrane. Among the bioinformatically predicted 60 *C. trachomatis* inclusion membrane (Inc) proteins, 35 have been shown to have this localization. Although the function of most Incs remains unknown, their localization suggests important roles in *Chlamydia*-host cell interactions.

Method

We aimed to identify Incs interfering with host cell vesicular trafficking by performing a functional screen in the eukaryotic model yeast *Saccharomyces cerevisiae*. We generated derivatives of the *S. cerevisiae* NSY01 strain encoding the predicted cytosolic parts of *C. trachomatis* Inc proteins fused to the green fluorescent protein (GFP) or to GFP and to the yeast SNARE protein Pep12p (to anchor the fragments to the cytosolic side of endosomes). These strains were used to screen for Incs causing vacuolar protein sorting (Vps) defects, detected by an assay based on the ability of the NSY01 reporter strain to produce a protein composed of carboxypeptidase Y and Invertase. This hybrid protein hydrolyzes sucrose into glucose and fructose at the cell surface when trafficking to the vacuole is disrupted. Normal or aberrant trafficking can be scored using an agar overlay solution indicating glucose production by formation of a brown precipitate.

Results & Conclusions

The production of Inc-GFP and Inc-GFP-Pep12p proteins in the constructed yeast strains was assessed by immunoblotting and their intracellular localization was analyzed by fluorescence microscopy. This revealed Inc fusion proteins with specific intracellular localizations. Using the Vps assay, we are in the process of identifying Incs capable of disrupting trafficking to the yeast vacuole and two positive hits have already been found. These and additional Incs emerging from this screen will be selected for further studies to understand their roles during *C. trachomatis* infection.

References & Acknowledgments

This work was supported by Fundação para a Ciência e a Tecnologia (FCT) through grant PTDC/IMI-MIC/1300/2014 and by Unidade de Ciências Biomoleculares Aplicadas (UCIBIO), which is financed by national funds from FCT (UID/Multi/04378/2013) and co-financed by the European Regional Development Fund (ERDF) under the PT2020 Partnership Agreement (POCI-01-0145-FEDER-007728). JNB holds a PhD fellowship (PD/BD/128214/2016) within the scope of the PhD program Molecular Biosciences (PD/00133/2012) funded by FCT.

Keywords: Bacterial pathogenesis, Chlamydia trachomatis, Type III secretion, Effectors

P-049 - DETERMINING VIRULENCE BY PROTECTING THE CELL FROM HOST INDUCED STRESSES: THE UNEXPECTED ROLE OF CANDIDA GLABRATA MULTIDRUG TRANSPORTERS

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Background

Candida species are opportunistic fungal pathogens known for their capacity to infect and persist in different niches within the human host. The successful prevalence of *Candida* spp. in immunocompromised patients is due to a rapid adaptation to different stress agents, orchestrated by transcriptional regulators that trigger molecular mechanisms responsible for antifungal resistance and virulence [1].

Method

Assessment of the importance of Major Facilitator Superfamily (MFS) transporters in *Candida glabrata* virulence was evaluated using the *Galleria mellonella* model of infection. Further characterization of the role of each transporter in *C. glabrata's* virulence was performed with *G. mellonella* hemocyte-yeast interaction assays and other methods according to the transporter's physiological function.

Results & Conclusions

The physiological role of the MFS multidrug transporters was found to confer resistance to stress agents, other then antifungal drugs, with relevance in the clinical setting. For instance, CgTpo3 confers resistance to polyamines, whose concentrations reach toxic levels in the urogenital tract [2], while CgAqr1 and CgDtr1 confer resistance to weak acids that accumulate to toxic concentrations in acidic niches, such as the vaginal tract or the phagolysosomes [3]. More recently, CgTpo1_1, CgTpo1_2 and CgDtr1 transporters were found to confer virulence in the *Galleria mellonella* model of infection [4], which prompted us to find the specific role of these transporters in *C. glabrata* virulence. Interestingly, CgTpo1_1 was found to confer resistance to antimicrobial peptides, while CgTpo1_2 and CgDtr1 were found to increase *C. glabrata* ability to proliferate inside *G. mellonella* hemocytes, in the case of CgDtr1 by conferring resistance to oxidative and acidic stresses found within the phagolysosome.

Overall, *C. glabrata* drug:H+ antiporters appear to be in the crossroad between antifungal resistance and virulence, thus constituting potential new targets for antifungal therapy.

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This work was supported by national funds through FCT – Fundação para a Ciência e a Tecnologia (contract PTDC/BBB-BIO/4004/2014 and BIOTECnico PhD grant PD/BD/116946/2016). Funding received by iBB-Institute for Bioengineering and Biosciences from FCT-Portuguese Foundation for Science and Technology (UID/BIO/04565/2013) and from Programa Operacional Regional de Lisboa 2020 (Project N. 007317) is acknowledged. The deletion mutants *Δcgtpo1_1, Δcgtpo1_2* and *Δcgdtr1* used in this work were kindly provided by Professor Hiroji Chibana, Chiba University, Japan.

Keywords: Candida glabrata, MFS transporters, Virulence, Antifungal Resistance, Galleria mellonella

P-050 - COMPARATIVE GENOMIC AND TRANSCRIPTOMIC ANALYSES UNVEIL NOVEL FEATURES OF AZOLE RESISTANCE AND ADAPTATION TO THE HUMAN HOST IN CANDIDA GLABRATA

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Background

Fungal infections, specially those caused by members of the *Candida* genus, are among the leading causes of hospital-acquired infections having associated high rates of morbidity and mortality. An alarming increase in the incidence of infections caused by *C. glabrata* has been reported in the last years this being mostly attributed to the increased resilience of this species to azoles, the frontline therapy used in prophylactic and active treatment of candidiasis.

Method

In this work we aimed at elucidating the molecular mechanisms underlying resistance to fluconazole and voriconazole in a resistant clinical isolate (FFUL887), an approach that is innovative as most knowledge in this field has not been obtained at a genome-wide scale. As such, the genome and transcriptome of strain FFUL887 were compared with those of the reference strain CBS138, found to be susceptible to azoles and having a publicly available genome sequence.

Results & Conclusions

The comparative genomics analyses performed led to the identification of prominent differences in FFUL887 genes documented to promote azole resistance in *C. glabrata*. Among these was the transcriptional regulator CgPdr1, with the CgPdr1 allele encoded by the FFUL887 strain including a K274Q modification not present in CBS138 nor in other azole-resistant strains. The significant increase in susceptibility to azoles of the FFUL887 strain upon deletion of the CgPDR1K274Q allele, along with results from transcriptomic profiling rendering evident the upregulation of 90 documented targets of CgPdr1 in the FFUL887 strain, support the idea that K274Q is a novel CgPdr1 variant that results in hyperactivity of this regulator and, consequently, on azole resistance. Notably, the FFUL887 strain exhibited a remarkably high susceptibility to organic acids at a low pH; a phenotype that was relieved upon deletion of the *CgPDR1* allele. This result suggests that the use of organic acids in combination with azoles could serve as a mean to sensitize azole-resistant strains. The analysis of the non-coding genome of the FFUL887 and of CBS138 strains also showed significant differences in the set of genes that could be under CgPdr1 regulation in the two strains supporting the idea that in the FFUL887 strain alterations of the CgPdr1-controlled regulatory network may have changed its architecture to improve the expression of azole-resistance genes.

References & Acknowledgments

Funding received by iBB-Institute for Bioengineering and Biosciences from FCT-Portuguese Foundation for Science and Technology (UID/BIO/04565/2013), from Programa Operacional Regional de Lisboa 2020 (Project N. 007317) and Ireland Science Foundation – 12IA1343 is acknowledged. Pfizer is acknowledged for providing voriconazole.

Comparative Genomic and Transcriptomic Analyses Unveil Novel Features of Azole Resistance and Adaptation to the Human Host in *Candida glabrata*

Keywords: Candida glabrata, azole resistance, CgPDR1, fungal infections

P-051 - GENETIC ADAPTIVE MECHANISMS MEDIATING RESPONSE AND TOLERANCE TO ACETIC ACID STRESS IN THE HUMAN PATHOGEN CANDIDA GLABRATA: ROLE OF THE CGHAA1-DEPENDENT SIGNALING PATHWAY

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Abstract

The increased resilience of *Candida glabrata* to azoles and the continuous emergence of strains resistant to other antifungals demands the development of new therapeutic approaches focused on non-conventional biological targets. Genes contributing to increase *C. glabrata* competitiveness in the different infection sites are an interesting and unexplored cohort of therapeutic targets. To thrive in the vaginal tract and avoid exclusion *C. glabrata* cells have evolved dedicated responses rendering them capable of tolerating multiple environmental challenges, including the presence of acetic and lactic acids produced by the commensal microbiota. In this work a cohort of vaginal clinical isolates were phenotyped for their tolerance to acetic acid stress at a low pH as well as for several traits that are known to influence sensitivity to this organic acid, including the structure of the cell envelope and the ability to consume the acid in the presence of glucose. The role played by the ORF CAGL0L09339g, an homologue of the ScHaa1, a critical regulator of acetic acid resistance in *S. cerevisiae*[1], in *C. glabrata* response and tolerance to acetic acid stress at pH 4 was also scrutinized using a transcriptomic analysis. The role of CgHaa1 as well as of several of its target genes in mediating virulence of *C. glabrata* against epithelial vaginal cells was also studied.

Results & Conclusions

Phenotyping of *C. glabrata* vaginal isolates demonstrated a clear increased resilience of these strains to acetic acid stress at a low pH, comparing with the tolerance exhibited by laboratory strains; consistent with the hypothesis that to adapt to the vaginal niche cells evolve responses that allow them to cope with the presence of organic acids at a low pH[2]. The higher tolerance of the vaginal strains was linked to a reduced permeability of the cell envelope to undissociated acetic acid molecules as well as to an ability to trigger the consumption of acetic acid in the presence of glucose. It was also shown that the CgHaa1 transcription factor (ORF CAGL0L09339g) controls an acetic acid-responsive system essential for tolerance of laboratory *C. glabrata*strains in presence of acetic acid at a low pH[3]. mRNA profiling showed that the genes up-regulated by CgHaa1 under acetic acid stress are involved in multiple physiological functions including membrane transport, metabolism of carbohydrates and amino acids, regulation of the activity of the plasma membrane H+-ATPase and adhesion. Consistently, under acetic acid stress CgHaa1 increased the activity and the expression of the CgPma1 proton pump and enhanced colonization of vaginal epithelial cells by *C. glabrata*. Comparison of the CgHaa1-dependent regulatory network active in *C. glabrata* with the corresponding *Saccharomyces cerevisiae*orthologue network revealed prominent differences, consistent with the idea that the two pathways have evolved divergently with the CgHaa1 pathway suffering a "functional expansion". The role of the CgHaa1-pathway in the extreme acetic acid-tolerance exhibited by vaginal C. glabrata isolates will also be discussed.

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Funding received by the Institute for Bioengineering and Biosciences from the Portuguese Foundation for Science and Technology (FCT) (UID/BIO/04565/2013) and from Programa Operacional Regional de Lisboa 2020 (project no. 007317) is acknowledged. FCT is also acknowledged for funding the Centre of Biological Engineering through contracts FCOMP-01-0124-FEDER-020243 and PTDC/EBB-EBI/120495/2010. Science Foundation Ireland and the Wellcome Trust are acknowledged for funding G.B.

Keywords: Candida glabrata, acetic acid, CgHAA1, ecological balance

P-052 - IDENTIFICATION AND ANALYSIS OF NEW TYPE IV SECRETION SYSTEM EFFECTORS FROM A LEGIONELLA PNEUMOPHILA STRAIN INVOLVED IN A RECENT LARGE OUTBREAK OF LEGIONNAIRES' DISEASE IN PORTUGAL

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Background

Legionella pneumophila is a facultative intracellular bacterium that is found in aquatic environments, where it parasitizes free-living protozoa. It is also an accidental human pathogen, as the inhalation of contaminated aerosols can lead to the infection of alveolar macrophages causing Legionnaires' disease, a frequently fatal type of pneumonia. The main virulence mechanism of *L. pneumophila* is a Type IVB Secretion System named Icm/Dot, which translocates bacterial effector proteins into the host cell. To date approximately 300 Icm/Dot substrates have been identified, making this system the most productive bacterial translocator, and *L. pneumophila* the organism with the most abundant effector pool.

The second largest *L. pneumophila* outbreak worldwide occurred in Vila Franca de Xira, Portugal, in 2014, and was caused by a novel strain, *L. pneumophila* Pt/VFX2014. Importantly, this strain caused the first reported case of probable person-to-person transmission of Legionnaires' Disease. Whole genome sequencing and bioinformatics analysis revealed that this strain belongs to serogroup 1 but phylogenetically segregates from all other strains previously sequenced, displaying a unique mosaic genetic backbone.

Results & Conclusions

Bioinformatics analysis of the genome of strain *L. pneumophila* Pt/VFX2014 was used to search for genes encoding potential unknown Icm/Dot substrates. The ability of the selected putative effectors to be translocated by the T4SS was confirmed using a reporter assay. To do this, *Legionella* strains were constructed in which these proteins were expressed fused to TEM-1 beta-lactamase, and the presence of these fusion proteins during infection was analysed by a FRET-based fluorescence assay. Three new Icm/Dot effectors were identified. Analysis of their function was initiated by studying their subcellular localization after ectopic expression of the corresponding GFP-tagged protein in mammalian CHO cells. Microscopy studies revealed specific distributions of two of the effectors, in the nucleus and in the subcortical region. Further studies are in progress and aim at understanding the role of these three novel *L. pneumophila* effectors during infection.

References & Acknowledgments

This work was supported by Unidade de Ciências Biomoleculares Aplicadas (UCIBIO), which is financed by national funds from Fundação para a Ciência e a Tecnologia (FCT; UID/Multi/04378/2013) and co-financed by the European Regional Development Fund (ERDF) under the PT2020 Partnership Agreement (POCI-01-0145-FEDER-007728), and by a Research Grant 2016 from the European Society of Clinical Microbiology and Infectious Diseases (ESCMID). I.S.F. is supported by a FCT post-doctoral fellowship (SFRH/BPD/102378/2014).

Keywords: Legionella pneumophila, Type IV secretion system, Effector protein, Outbreak strain

P-053 - EXPLOITING IMMUNOREACTIVE OUTER MEMBRANE PROTEINS FOR THE DEVELOPMENT OF NEW THERAPEUTIC STRATEGIES AGAINST BURKHOLDERIA CEPACIA COMPLEX INFECTIONS

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Background

Bacteria of the *Burkholderia cepacia* complex (Bcc) are multidrug-resistant and opportunistic pathogens capable of causing life-threatening lung infections among cystic fibrosis (CF), chronic granulomatous disease and immunocompromised hospitalized patients [1]. Chronic infections with Bcc bacteria remain associated with a faster decline of lung function and early death of CF patients. Effective therapeutic strategies to eradicate Bcc bacteria from patients are limited, as Bcc are inherently resistant to the majority of antimicrobials clinically available [1]. Therefore, strategies to protect patients from Bcc chronic lung infections are urgently required, being immunoprotective therapies an attractive approach to control these infections. Outer membrane proteins (OMPs) are the inter-phase of the bacterium-host interaction, being the primary antigen targets of the host immune system, being promising vaccine candidates. Particularly, OmpA-like proteins possess several characteristics desired for a vaccine candidate, such as high copy number in bacterial cell, high conservation intraspecies, direct contact with the host immune system and ability to stimulate strong antibody responses [2].

Method

To identify new Bcc antigens two experimental approaches were performed: i) *in silico* search for putative immunogenic OmpA proteins, and ii) immunoproteomics of the extracytoplasmatic proteome fraction of *B.cenocepacia* J2315 obtained from growth conditions that mimic the CF lung environment and using serum samples from CF patients with clinical history of Bcc infection.

Results & Conclusions

Bioinformatics searches within publicly available genomes of Bcc bacteria for genes encoding OmpA-like proteins revealed 10 genes, unevenly distributed among Bcc [3]. However, only 3 (BCAL2958, BCAL2645 and BCAL3204) were conserved in all Bcc genomes and potentially immunogenic [3]. Previously, we have shown the immunological characterization of BCAL2958 and antigen conservation among different Bcc species by western-blot [4]. In this work, we report the cloning, overexpression and immunological characterization of BCAL2645 encoding an OmpA-like protein from *B.cenocepacia* J2315. The immunoproteomic approach led to the identification of 31 immunoreactive proteins with CF serum samples from patients with a Bcc infection record. Seven of these proteins were found to be conserved in Bcc using bioinformatics tools and one was putatively located in the outer membrane (BCAS0764). Cloning, overexpression and preliminary results of the immunological characterization of BCAS0764 from *B.cenocepacia* J2315 will be also presented.

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Funding by FCT (Projects PTDC/BIA-MIC/1615/2014,UID/BIO/04565/2013) is acknowledged. SAS acknowledges a post-doc grant from FCT (SFRH/BPD/102006/2014). TP acknowledges a grant from BIOTECnico PhD program funded by FCT (PD/BD/135137/2017)

Keywords: Outer membrane proteins, Burkholderia cepacia complex, vaccine

P-054 - LISTERIA MONOCYTOGENES OUTBREAK STRAIN SHOWS ENHANCED VIRULENCE AFTER GROWTH UNDER COLD-OSMOTIC STRESS AND PASSAGE THROUGH AN IN-VITRO GASTROINTESTINAL MODEL

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Background

Exposure to a single or multiple sublethal stresses, as those impaired by food processing and food matrices, can enhance tolerance of *Listeria monocytogenes* to stresses and increase its survival and pathogenesis. This knowledge is needed for developing efficient control strategies to improve food safety. The aim of this study was to evaluate the effect of growth under stress conditions (osmotic and low temperature) on the subsequent survival of *L. monocytogenes* through the gastro-intestinal (GI) tract and on its virulence potential.

Method

Three *L. monocytogenes* were selected: Lm 2542, from an outbreak associated with cheese in Portugal; Lm 2594, from cheese; and, Scott A, from an outbreak related to pasteurized milk. Each strain was grown at three conditions: (i) BHI at 37ºC (control), cold stress (11 ºC), and cold-osmotic stress (6% (w/v) NaCl, 11 ºC). Subsequently, strains were inoculated into low fat UHT milk (24h, 11°C) and their survival through a simulated GI digestion was evaluated, followed by determination of invasion efficiencies in epithelial cell line Caco-2.

Results & Conclusions

Growth at 37ºC and subsequent passage through the GI tract lead to a 3.5 log reduction in Lm 2542 viable counts (> 1 log cycle reduction than observed for Lm 2594 and Scott A). Growth in cold stress resulted in similar reduction of cell numbers for Lm 2542 and Lm 2594 after the GI tract passage, when compared to growth at 37ºC, but Scott A presented a higher reduction (4 log). However, after growth in cold-osmotic stress, Lm 2542 survival was higher than the observed for Lm 2959 and Scoot A, and > 1 log in comparison to growth at 37ºC. This protective effect was particularly noticeable at the end of the gastric phase. This strain also presented a significantly higher invasion efficiency in Caco-2 cells after growth under cold-osmotic stress and subsequent GI tract passage when compared to growth at 37ºC or under cold stress; the other strains presented no differences in virulence when grown at 37ºC or under stress conditions.
The results obtained indicate that exposure to specific stress conditions may increase not only the resistance to the human GI tract, but also the infectious potential of some *L. monocytogenes* strains. More studies are necessary to a better understanding of the mechanisms that overlap between adaptation to stress and improved virulence related characteristics in these strains.

References & Acknowledgments

This work was supported by National Funds from Fundação para a Ciência e a Tecnologia through project 'UID/Multi/50016/2013'

Keywords: Listeria monocytogenes, stress, virulence, gastrointestinal tract

P-055 - CHARACTERISATION OF THE MORE PATHOGENIC SMALL COLONY VARIANTS (SCV) OF BURKHOLDERIA CEPACIA ISOLATED FROM A CYSTIC FIBROSIS (CF) PATIENT

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Background

Burkholderia cepacia is not a predominant *Burkholderia cepacia* complex species among the cystic fibrosis(CF) population characterized worldwide, but an exceptionally high incidence of *B.cepacia* and *B.contaminans* was registered at the major Portuguese CF Center at Hospital de Santa Maria(HSM), in Lisbon[1-3].The present study is dedicated to the characterization of two pairs of small and normal colony variants of *B.cepacia* isolated from a CF patient from the onset and a late stage of respiratory infection, expecting to get clues into the mechanisms underlying persistence, and pathogenicity of the small colony variant(SCV) sub-population of *B.cepacia,* a sporadic species in CF infections.

Method

Two pairs of small and normal colony variants of *B.cepacia* obtained at HSM from the same CF patient sputum were compared: concerning the stability of the SCV/normal morphotype, susceptibility against different classes of antimicrobials, cell motility, colony and cell morphology, growth rate, exopolysaccharide production, size of the biofilms formed and virulence potential in *Galleria mellonella* infection model.Electron transport chain(ETC) activity (based on tetrazolium salt(INT) reduction and NADH dehydrogenase activity) and ROS(reactive oxygen species) levels in absence or presence of antibiotics in these colony variants were also compared using the redox-sensing fluorescent probe DCFH-DA.

Results & Conclusions

The SCV phenotype (1/10 of the normal size) was found to revert to the normal size phenotype when a large number of cells were cultured on agar plates, although at a low frequency(~5%).The SCV phenotype was more unstable than the normal size counterpart phenotype.SCV variants displayed multiple phenotypic changes compared to the normal colony counterparts.SCVs isolates exhibited a defective activity of the ETC which limits ATP availability and consequently cell growth.SCVs are more resistant to all antibiotics tested than the normal morphotypes, a trait that may be related with the decreased uptake of antibiotics into the cell are likely to be membrane potential –and ATP-dependent.The ROS levels in the SCV under antibiotic stress were below the levels in the corresponding normal morphotypes, consistent with the multiresistance phenotype registered.The SCVs were also more virulent than the corresponding normal forms in the *G.mellonella* infection model.Collectively, these results point SCV as pathogenic forms of *B.cepacia* that may facilitate persistent and recurrent respiratory infections in CF patients.

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Funding received by iBB from PORLisboa2020(N.007317) and FCT(UID/BIO/04565/2013) is ackowledged as well as post-doctoral fellowships to CPC and DM-H.

Keywords: small colony variants (SCV), Burkholderia cepacia, cystic fibrosis, Virulence, Antibiotic Multiresistance, Electron transport chain activity, reactive oxygen species (ROS), Galleria mellonella

P-056 - EFFECTS OF AMINO ACID SUPPLEMENTATION ON A-SYNUCLEIN-MEDIATED TOXICITY – STUDIES ON THE YEAST MODEL FOR SYNUCLEINOPATHIES

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Abstract

α-Synuclein (aSyn) misfolding and aggregation is implicated in the pathogenesis of the neurodegenerative diseases known as synucleinopathies, as Parkinson's disease. The pathobiological mechanisms of aSyn are still not fully understood. Accumulating evidence suggests that dietary can play an important role in delaying the onset of halting the progression of neurodegeneration, through the regulation of several signal-transduction pathways. Recently, it was demonstrated that dietary supplementation with essential and/or branched chain amino acids exerts a variety of beneficial effects, including mitochondrial biogenesis and oxidative damage prevention. Nevertheless little is known about the impact of this intervention on the cellular pathways. Thus the aim of this work was to identify and explore the players and mechanisms associated with the effects promoted by amino acid supplementation, particularly of charged side chain amino acids (CSCA), in the yeast model for synucleinopathies. To tackle this challenge, chronological lifespan, autophagy flux (by the GFP-Atg8 processing assay), ROS accumulation as well as cell cycle were assessed.

Results & Conclusions

Notably, medium supplementation with CSCA was able to abolish the aSyn-mediated toxicity by increasing the CLS associated with a decrease in markers of oxidative stress, namely, lower accumulation of ROS and increased superoxide dismutase activity. Altogether, our findings envision CSCA supplementation as a possible novel mechanism to rescue cells from aSyn mediated toxicity and places this CSCA for future pharmacological research designed to improve synucleinopathies patients' prognosis.

References & Acknowledgments

This work was developed under the scope of the project NORTE-01-0145-FEDER-000013, supported by the Northern Portugal Regional Operational Programme (NORTE 2020), under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (FEDER), by FEDER, through the Competitiveness Factors Operational Programme (COMPETE), and by National funds, through the Foundation for Science and Technology (FCT), under the scope of the project POCI-01-0145-FEDER-007038. BSM is supported by the fellowship SFRH/BPD/90533/2012 funded by the Fundação para a Ciência e Tecnologia (FCT, Portugal).

Keywords: α-Synuclein, Saccharomyces cerevisiae, Synucleinopathies, charged side chain amino acids

P-057 - CALORIC RESTRICTION INCREASES LONGEVITY OF YEAST CELLS EXPRESSING ALPHA-SYNUCLEIN BY THE COORDINATED REGULATION OF PROTEOLYTIC SYSTEMS

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Abstract

Aging is a complex and multi-factorial process that results in the progressive accumulation of molecular alterations and disruption of different cellular functions. Several hallmarks of aging that represent age-common denominators in different model organisms have been proposed, including deregulated nutrient-sensing and loss of proteostasis. Autophagy and the ubiquitin-proteasome system (UPS) are crucial cellular processes for the maintenance of proteostasis mediating the complete degradation of damage and obsolete proteins and organelles, however these processes are, themselves, affected by aging. Caloric restriction (CR) is the only non-genetic intervention shown to promote lifespan extension, in several model organisms, linked to the modulation of the proteolytic systems. In the present work, using the yeast *Saccharomyces cerevisiae*, a simple and powerful model organism, we aimed to explore the contribution of the proteolytic systems for the beneficial effects promoted by CR intervention, during aging, in a proteotoxic stress context elicited by the heterologous expression of human α-synuclein (aSyn), a protein associated with Parkinson's disease. For that, chronological lifespan, representing the time that stationary yeast cells remain viable, was evaluated. Furthermore, autophagy flux, UPS activity and the RNA levels of important autophagy and UPS regulators, *ATG8, RNP4* and *UMP1,* were determined during CLS.

Results & Conclusions

The data gathered showed that aSyn expression leads to the decline of the UPS activity, nevertheless, CR intervention is able, not only to overpass that effect, but still to increase the activity of the UPS. Furthermore, CR balances the UPS and autophagy activities during aging, demonstrating that both systems are embedded in coordinated and compensatory process that results in the aSyn toxicity relief. This data open new avenues in the disclosure of potential therapeutic targets in the context of aSyn pathophysiology.

References & Acknowledgments

This work was developed under the scope of the project NORTE-01-0145-FEDER-000013, supported by the Northern Portugal Regional Operational Programme (NORTE 2020), under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (FEDER), by FEDER, through the Competitiveness Factors Operational Programme (COMPETE), and by National funds, through the Foundation for Science and Technology (FCT), under the scope of the project POCI-01-0145-FEDER-007038. HP is supported by the fellowship SFRH/133087/2017 funded by the Fundação para a Ciência e Tecnologia (FCT, Portugal). BSM is supported by the fellowship SFRH/BPD/90533/2012 funded by the Fundação para a Ciência e Tecnologia (FCT, Portugal).

Keywords: Aging and longevity, Saccharomyces cerevisiae, Proteolytic systems, Autophagy, Caloric restriction

P-058 - BIOFILM PHENOTYPE POTENTIATES VIRULENCE TRANSDUCTION IN ACINETOBACTER BAUMANNII

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Background

An alarming increase of bacterial resistance to antibiotics has occurred recently. Many factors contribute to this, mainly antibiotics misuse but also an intrinsic capacity of bacteria to trade genetic material. These exchanges are often prophage-mediated. Prophages are bacteriophages that integrate into the bacterial genome, being able to excise and enter other bacteria. They are found in many bacterial species, being particularly frequent in *Acinetobacter baumannii*, an important multidrug-resistant nosocomial pathogen. This species is known to produce biofilms; these protect bacteria against antibiotics and host defenses and are responsible for bacteria persistence in clinical environments. This work aimed at evaluating the contribution of biofilms for virulence spread among *A. baumannii* strains.

Method

Transduction was evaluated in both planktonic and biofilm cultures. A donor strain (ANC 4097) containing a prophage coding for a beta-lactam resistance gene, and a receptor strain (NIPH 146) susceptible to beta-lactams, were chosen for the assays. To distinguish strains, NIPH 146 was modified with an erythromycin-resistant plasmid, antibiotic to which the donor strain was susceptible. For transduction assays in both planktonic and biofilm cultures, strains were inoculated together or sequentially (receptor strain first), and transducing phenomena was evaluated at different time points of growth or biofilm formation; the effect of antibiotic pressure was evaluated using sub-minimal inhibitory concentrations of meropenem. Transduction was evaluated by plating cells in agar plates (to count all viable cells), and agar supplemented with meropenem (donor and transduced receptor strains), erythromycin (receptor strain), and meropenem+erythromycin (transduced receptor strain).

Results & Conclusions

Transduction occurred only in biofilms, being higher for cells subjected to antibiotic pressure (36% transduced cells compared to 14% in the absence of selective pressure). Furthermore, the transduced receptor cells displayed an increased resistance to meropenem and other beta-lactams, with levels similar to the donor strain, revealing an important effect of prophages in the bacterial phenotype. This work reveals that biofilms contribute to the spread of virulence via transduction among *A. baumannii*, probably by exerting a protective environment to the prophages that are exited upon exposure to antibiotics.

References & Acknowledgments

This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding UID/BIO/04469/2013 unit, COMPETE 2020 (POCI-01-0145-FEDER-006684) and the Project PTDC/BBB-BSS/6471/2014 (POCI-01-0145-FEDER-016678). This work was also supported by BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund under the scope of Norte2020 - Programa Operacional Regional do Norte. Ana Rita Costa acknowledges FCT for grant SFRH/BPD/94648/2013.

Keywords: Acinetobacter baumannii, Prophage, Transduction, Biofilm

P-059 - CANDIDA GLABRATA BIOFILM DEVELOPMENT ON MEDICAL DEVICES IS MODULATED BY THE PRESENCE OF ALTERNATIVE CARBON SOURCES

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Background

Candida species are the most common cause of fungal infection in humans. Their capacity to cause disease is dependent on the ability to grow within the human host environment and to assimilate the carbon sources available. Previous studies have shown that the presence of alternative carbon sources influence the behaviour of these fungal pathogens, suggesting that some carboxylic acid transporters have a crucial role in biofilm formation and resistance to antifungal drugs (Mota *et al*, 2015; Alves *et al*, 2017).

Method

Medical device-associated biofilms are clinically important due to their intrinsic and prevalent resistance to conventional antifungal drugs and immune system. Here we investigated the formation of *Candida glabrata* biofilms on medical devices, more specifically on polyurethane catheters, and the role of carboxylic acid transporters in this process, under distinct growth conditions and environmental stimuli.

Results & Conclusions

By clarifying the effect of host nutrients on biofilm formation and antifungal resistance, new and effective treatment strategies can be developed for catheter-related bloodstream infections sustained by *Candida glabrata*.

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This work is supported by the strategic programme UID/BIA/04050/2013 (POCI-01-0145-FEDER-007569) funded by FCT I.P. and by ERDF through COMPETE2020–Programa Operacional Competitividade e Internacionalização (POCI) and by project EcoAgriFood (NORTE-01-0145-FEDER-000009), supported by Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF). RA acknowledges DP_AEM and FCT for the PD/BD/113813/2015 grant.

Keywords: Candida glabrata, biofilm formation, alternative carbon sources, catheter

P-060 - COMPARISON OF THE PHYSICAL PROPERTIES OF BURKHOLDERIA CENOCEPACIA CLONAL VARIANTS ISOLATED DURING CYSTIC FIBROSIS CHRONIC LUNG INFECTION, STUDIED BY ATOMIC FORCE MICROSCOPY

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Background

Burkholderia cenocepacia, associated with worse prognosis and increased risk of death in cystic fibrosis (CF) patients, undergoes marked phenotypic and genomic changes during chronic lung infections. These modifications have been related with bacteria adaptive-evolution in the CF-lung and pathogenicity but information on physical properties is missing despite their crucial-role in cell growth, colonisation and biofilm formation[1]. Atomic force microscopy (AFM) allows the imaging of the bacteria surface with nanoscale-resolution together with the local-characterisation of the nanomechanics of living bacteria[1]. In this study, we compared the surface morphology and mechanical properties of three *B. cenocepacia* clonal variants isolated from the onset of chronic infection (IST439) until patient´s death after 3.5 years (IST4134) and the most antibiotic-resistant variant (IST4113), to identify eventual-alterations[2]. IST439-lipopolysaccharide (LPS) has the O-antigen-component (OAg) while the subsequent isolates do not[3]. Given that LPS-composition is an important factor in bacterial surface properties and host–bacterium interactions[3] and that the virulence potential[4] and ability to modulate dendritic-cell function[5], have been compared, this study may provide insights into their mechanics and impact in pathogenicity.

Method

The surface and mechanical-properties (adhesion, apparent-elasticity, and roughness) of *B. cenocepacia* variants were studied by AFM. Contact mode AFM-images and force-distance curves were obtained for single-cells that were fixed onto freshly-cleaved-mica and then acquired with an Agilent-Technologies 5100-series-AFM with a lever of nominal stiffness k=0.5N/m.

Results & Conclusions

The late-isolates were found to adhere more-effectively to the AFM-tip, compared with the first-isolate –the one expressing the OAg[3], suggesting the OAg-role in masking the electrostatic-interactions between lipid-A-core and AFM-tip. IST4113-isolate, with a higher-ability to produce exopolysaccharide and biofilms of larger-size[2], proved to be the most-adhesive. IST4134-cells have higher-apparent elasticity compared with the early-isolates. Interestingly, IST4134-variant was found to be more-invasive of epithelial-cells[5] and more-internalized by dendritic-cells, compared with IST439[6]. Late-variants topography showed distinct nanoscale-surface-architectures that were not seen in IST439. These are characterized by rod-like formations on the surface of the bacteria. IST4134-variant displays longer-rods that span up to its entire length while IST4113 exhibits shorter and more-disorganized structures. The presence of these structures may have a positive impact on mechanical and dispersive adhesion.

References & Acknowledgments

This work was supported by FCT-project-PTDC/FIS-NAN/6101/2014. Funding-received by iBB from PORLisboa2020-(N.007317) and by iBB-(UID/BIO/04565/2013) and BioISI-(UID/ MULTI/04046/2013) from FCT is acknowledged, as well as FCT-fellowships to AAH-(PD/BD/113629/2015)(BIOTECnico PhD-programme), MVV-(PD/BD/105975/2014), MSR-(SFRH/BPD/69201/2010).

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Keywords: Burkholderia cenocepacia, Atomic force microscopy, Cystic fibrosis chronic lung infection, Nanoscale surface architectures, Bacterial nanomechanics

P-061 - PROFILING THE IN VIVO TRANSCRIPTOME OF XANTHOMONAS CAMPESTRIS PV. CAMPESTRIS STRAINS WITH CONTRASTING VIRULENCE

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Background

Black rot disease caused by *Xanthomonas campestris* pv. *campestris* (*Xcc*) is the most important bacterial disease of Brassicas worldwide. Although several genomic approaches have been used to characterize the molecular mechanisms of host-pathogen interaction, little is known about *Xcc* regulation of virulence during pathogenesis.

Method

Virulence assessment of a set of 26 Portuguese *Xcc* isolates was carried out by measuring the Infected Leaf Area (%ILA), after inoculation on three *Brassica oleracea* cultivars (cv. 'Wirosa', cv. 'Beira' and cv. 'Bonanza'). The highest and lowest virulent *Xcc* strains (H-vir and L-vir, respectively) were selected, along with the most and least susceptible *B. oleracea* cultivars. Using a RNA-Seq approach, the *in vivo* transcriptome profiling of the two contrastingly virulent *Xcc* strains, inoculated on two contrastingly susceptible cultivars of *B. oleracea*, was established.

Results & Conclusions

Virulence assessment of *Xcc* strains showed very diverse virulence profiles, with no correlation with other features, such as race or host of origin. Strains CPBF213 and CPBF278, belonging to race 6, were the least and most virulent, respectively. Among the three tested hosts, cv. 'Bonanza' and cv. 'Wirosa' were the least and most susceptible, respectively.
RNA-Seq yielded a total of 530M 69bp reads, 1% of which was from *Xcc*. The established transcriptome of *Xcc* strains was independent of the host tested, suggesting that virulence is an inherent characteristic of the pathogen. In contrastingly virulent strains, a total of 154 differentially expressed genes (DEGs) were identified. While 27% of the identified DEGs were poorly characterized, the most represented functional categories were 'pathogenicity and adaptation' (PA), followed by 'signal transduction and regulation' and 'transport'. Among DEGs involved in PA were genes coding for type III effectors (T3Es), other secretion systems components, LPS biosynthesis, cell-wall degrading enzymes and detoxification enzymes. T3E coding genes *xopE2* and *xopD* were induced in L-vir strain, while *xopAC*, *xopX* and *xopR* were induced in H-vir strain. While *xopE2* was the most expressed DEG in L-vir strain, XCC3695, coding for a poorly characterized oxidoreductase, was the most expressed DEG in H-vir strain. Overall, low virulence appears to be the combined result of impaired sensory mechanisms, reduced detoxification of reactive oxygen species, decreased motility, higher production of pathogen-associated molecular patterns (PAMPs), associated to an overexpression of avirulence proteins and a repression of virulence proteins targeting the hosts' PAMP-triggered immune responses.

References & Acknowledgments

J.C. is a PhD grant holder of FCT (SFRH/BD/69787/2010). Support from Biosystems and Integrative Sciences Institute (BioISI, FCT/UID/ Multi/04046/2013) is also acknowledged.

P-062 - RECURRENT VULVOVAGINAL CANDIDOSIS (RVVC): EVIDENCE FOR ADAPTATION TO THE VAGINAL MUCOSA AND MICROEVOLUTION WITHIN THE HOST

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Background

Recurrent vulvovaginal candidosis (RVVC) is caused by *Candida albicans*, a commensal of healthy women's vagina. Recurrence is defined by the occurrence of four or more episodes per year, a condition that causes significant discomfort, pain and an overall life quality decrease in affected women. Despite the clinical importance of RVVC, little is known regarding the characteristics of the strains that are related with their persistence in the vaginal mucosa. By investigating these, we aim to unravel unique molecular and phenotypic cues of *C. albicans* that could be useful to design new strategies to control and treat RVVC.

Method

One hundred *Candida* spp. strains were obtained from 40 women attending the gynaecological consultation at Centro Hospitalar Cova da Beira, Covilhã, Portugal and a private clinic. Of these, 20 women were diagnosed with RVVC while other 20 women only had a single episode of infection (vulvovaginal candidosis, VVC). Species identification was performed by analysis of biochemical profiles with Vitek. In addition, antifungal susceptibility testing was performed to two antifungals (clotrimazole and fluconazole) by broth dilution. Finally, a subset of representative strains were molecular typed by analyzis of DNA macrorestrition patterns by pulsed-field gel electrophoresis (PFGE).

Results & Conclusions

We found that *C. albicans* was isolated from all VVC cases and RVVC cases; however we could also identify C. glabrata, C. lypolytica, C. tropicalis and Sacharomyces cerevisiae in RVVC cases. Seven out of 20 (35%) VVC isolates and 29/80 (36%) of RVVC isolates were susceptible to fluconazole and 12/20 (60%) VVC isolates and 39/80 (49%) were susceptible to clotrimazole. The resistant isolates had a minimum inhibitory concentration (MIC) of 8-512 µg/ml for fluconazole and 2-16 µg/ml for clotrimazole; and were distributed among all the species identified. In almost all RVVC cases, we observed a trend regarding the increase of the MIC along time; however preliminary PFGE results indicate that there is little variation in the genetic background of sequential strains. We conclude that *C. albicans* is still the major player in RVVC; nonetheless other *Candida* species can also contribute for pathogenesis. In addition, we found a high proportion of azole-resistant strains associated with persistence in the vaginal mucosa. Further investigation is needed to unravel the mechanisms of acquisition of azole resistance used by Candida spp. in the vaginal mucosa.

References & Acknowledgments

P.F.G. was supported by Instituto Nacional de Administração e Gestão de Bolsas de Estudo (INAGBE), Angola. J.R. was supported by Fundação para a Ciência e Tecnologia (SFRH/BPD/115145/2016), Portugal.

Keywords: Candida albicans, Persistence, Pathogenesis, Vulvovaginal infections

P-063 - NEW INSIGHTS INTO SALMONELLA TYPHIMURIUM VIRULENCE

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Abstract

The intracellular pathogen *Salmonella* Typhimurium emerged as a major cause of foodborne illness, representing a severe clinical and economical concern worldwide. The capacity of this pathogen to efficiently infect and survive inside the host depends on its ability to synchronize a complex network of virulence mechanisms. Therefore, the identification of new virulence determinants has become a hotspot issue in the search for new targets for drug development.

BolA-like proteins are widely conserved in all kingdoms of life. In *E. coli*, this transcription factor has a determinant regulatory role in several mechanisms that are tightly related with bacterial virulence. Therefore, in the present work we used the well-established infection model *Galleria mellonella* to evaluate the role of BolA protein in *S.* Typhimurium virulence.

Results & Conclusions

We have shown that BolA is an important player in *S.* Typhimurium pathogenesis. Specifically, the absence of BolA leads to a defective virulence capacity that is most likely related with the remarkable effect of this protein on *S.* Typhimurium evasion against cellular response. Furthermore, it was demonstrated that BolA has a critical role in bacterial survival under harsh conditions. Hence, we provide evidences that BolA is a determinant factor in the effectiveness of *Salmonella* to survive and overcome host defences mechanisms, which is as important progress to the understanding of the pathways underlying bacterial virulence.

References & Acknowledgments

This work was supported by grants from Fundação para a Ciência e a Tecnologia, Portugal ref:PD/BD/113983/2015. This work has also received funding from the FCT Project ref: PTDC/BIA-MIC/4046/2014 and the Project LISBOA-01-0145-FEDER-007660 (Microbiologia Molecular, Estrutural e Celular) funded by FEDER funds through COMPETE2020—Programa Operacional Competitividade e Internacionalização (POCI).

Keywords: Salmonella Typhimurium; BolA; virulence; Galleria mellonella

P-064 - PILOT-STUDY: LACTOBACILLUS CRISPATUS, A KEY PLAYER IN CYTOLYTIC VAGINOSIS?

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Background

Cytolytic Vaginosis (CV) is a vaginal disturbance of unknown etiology that is often confused with Vulvovaginal Candidosis (VVC). In fact, its prevalence is not yet clear and the mechanisms underlying its pathophysiology are also unknown. Nevertheless, the presence and overgrowth of *Lactobacillus* spp., often defined as vaginal probiotic microorganisms, may be related to this condition. This study aims to elucidate the relation between lactobacilli flora (mainly *L. crispatus*) and CV.

Method

15 clinical vaginal fluid samples were enrolled in this study (5 healthy women, 6 women with VVC, 3 women with CV and 1 woman co-diagnosed with CV and VVC). After DNA extraction, the bacterial 16S rRNA gene V1-V2 hypervariable regions were amplified in PCR reactions. Single end sequencing of V2 hypervariable region was performed on Illumina MiSeq platform. Finally, 16S metagenomics results were used to study the lactobacillary flora present in the different groups, including the dominant pattern.

Results & Conclusions

In the control group (healthy women), the prevalence and dominance of lactobacilli it is in accordance with previously described studies: *L. crispatus*, *L. gasseri*, *L. iners* and *L. jensenii* were the most prevalent species. The mean value of pH in this group was 4.14±0.17. Only one sample exhibited *L. crispatus* as dominant species (higher than 90%) and curiously it had a pH value similar to CV (pH=4.0). Regarding the VVC group, *L. iners* was the dominant and prevalent species (3/6 women > 90% and 1/6 woman > 70%); and a mean value of pH of 4.55±0.11 was observed. To date, the role of this species in the vaginal flora remains unknown. However, the two other cases of VVC showed a prevalence of *L. crispatus* (> 90%), presenting a mean value of pH of 5.50±0.50. In the CV group, all the samples showed prevalence and dominance of *L. crispatus (> 90%)*. In addition, all fluids had a pH value of 3.8. Considering experimental results and focusing in the role of *L. crispatus* it is possible to verify that it is present in the different experimental groups and that it seems to be dominant in extreme conditions, like pH values below and above the normal values; probably strains of these species could be more resistant to vaginal hostil conditions. Further studies should elucidate on *L. crispatus* probiotic efficacy and safety profile.

References & Acknowledgments

The authors would like to thank to Foundation for Science and Technology - FCT (SFRH/BDE/112920/2015) and Labfit-HPRD, Covilhã by funding this work.

Keywords: Lactobacillus crispatus, Cytolytic vaginosis, Vulvovaginal candisosis, Vaginal flora

P-065 - UNRAVELLING THE ANTIBIOFILM MECHANISM OF ACTION OF THE ANTIMICROBIAL PEPTIDE PEPR

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Background

Biofilm-related infections are particularly difficult to treat due to their reduced susceptibility to the currently used antibiotics. Thus, it is urgent to develop new antimicrobial agents active against bacterial biofilms. Antimicrobial peptides (AMPs) have been considered potential alternatives as antibiofilm agents [1]. However, more work is still needed specially to understand their mode of action at the molecular level, which will contribute to their optimization towards drug development. Here, we investigated the antibiofilm activity and mode of action of pepR, a peptide previously developed in our lab [2], against *S. aureus* biofilms.

Method

S. aureus ATCC 6538 was used in this work as a bacterial model to study the effect of pepR on both the planktonic and biofilms forms. The antibacterial activity of pepR against *S. aureus* in the planktonic form was evaluated using standard microdilution procedures to determine the minimum inhibitory concentration and the minimum bactericidal concentration. Two different assays were used to study the activity of the peptide on both *S. aureus* biofilm formation and pre-formed biofilms: the total biofilm mass was quantified using crystal violet staining, and the bacterial cells viability within the biofilm was tested using a resazurin reduction assay. The mechanism of action of pepR against both planktonic and biofilms forms of *S. aureus* was investigated through a combination of different assays such as colony count, flow cytometry and confocal microscopy imaging techniques.

Results & Conclusions

The results obtained show that pepR is able to act against both the planktonic and biofilm forms of *S. aureus*. The peptide exhibited a fast bactericidal activity against planktonic bacteria, which is related to its ability to disrupt the bacterial membrane integrity, and should be also involved in its ability to prevent staphylococcal biofilm formation. The peptide is also able to diffuse through a pre-formed biofilm and significantly reduce the bacterial cells viability in a dose-dependent manner, most likely due to the direct killing of embedded bacteria. Overall our results open up new possibilities to identify and screen new antimicrobial peptides that can effectively combat bacterial biofilms.

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This work was supported by FCT (PTDC/QEQ-MED/4412/2014, FAPESP/20107/2014, SAICTPAC/0019/2015) and Marie Skłodowska-Curie Research and Innovation Staff Exchange (RISE): call H2020-MSCA-RISE-2014, Grant agreement 644167, 2015–2019. SNP, SAD and DM-H are the recipients of fellowships from FCT (SFRH/BPD/92409/2013, PD/BD/114425/2016 and SFRH/BPD/91831/2012, respectively). FF is an FCT Researcher (IF/00386/2015).

Keywords: Bacterial biofilms, Antimicrobial peptides (AMPs), membrane permeabilization, Confocal microscopy imaging

P-066 - ARBUSCULAR MYCORRHIZAS IN SUGARCANE UNDER DIFFERENT PLANTING SYSTEMS AND SOURCES OF NITROGEN

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Background

This study aimed to evaluate the density of spores and mycorrhizal colonization in roots of sugarcane varieties (RB86-7515, CTC-4 and CTC-15), under planting systems, being the planting of stem portions and pre-sprouted seedlings (PSS) along with 3 sources of nitrogen: granular fertilizer, leaf application and inoculation with diazotrophic bacteria (*Azospirillum brasilense*).

Method

The experiment was deployed on the experimental campus of the Evangelical School of Goianésia, and was collected soil samples for spore count after the first cut. The experimental design was arranged in factorial 3x2x3 in randomized blocks with subdivided plots using 4 repetitions per treatment, where factor 1 was represented by varieties, factor 2 by planting systems and factor 3 by nitrogen sources.

Results & Conclusions

The planting of PSS associated with foliar fertilization and *A. brasiliense* presented better colonization that in PSS with granular fertilizer and stem portions with leaf fertilizer and via inoculant. The granular fertilizer showed increased amounts of spores over the foliar fertilization and inoculation of *Azospirillum brasilense,* the planting system of stem portions presented greater quantity of spores over the planting of PSS, it is worth mentioning that approximate results of spore density, mycorrhizal colonization and productivity without adding granular fertilizer are satisfactory for reducing the use of fertilizers.

References & Acknowledgments

Thanks to Fapeg - Foundation for Research Support in the state of Goias, that without financial assistance, participation in this event would not be possible.

Keywords: Pre-sprouted seedlings, biological nitrogen fixation, mycorrhizal fungi, Azospirillum brasilense.

P-067 - EVALUATION OF DIFFERENT SCREENING METHODOLOGIES FOR THE DETECTION OF MRSA FROM ENVIRONMENTAL SURFACES: SWABS, GAUZES, AND POLYWIPES

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Background

Methicillin-resistant *Staphylococcus aureus* (MRSA) can survive for long periods on inanimate objects, and therefore environmental surfaces are important reservoirs for dissemination. However, there is no standardized method for the detection of MRSA from environmental surfaces. The aim of the present study was to evaluate different screening methods to detect environmental MRSA contamination.

Method

A total of 447 samples were obtained from inanimate surfaces at a hospital in Luanda, Angola and a hospital in São Tomé and Príncipe, by three different methodologies: (i) sterile swabs moistened in saline solution; (ii) sterile cotton gauzes moistened in Tryptic Soy Broth; (iii) commercial premoistened sterile sponges (polywipes). After a broth enrichment step, all samples were plated onto Tryptic Soy Agar and chromogenic selective media for *S. aureus* and for MRSA. The *S. aureus* isolates were characterized by PFGE, *spa* typing, MLST, and SCC*mec* typing.

Results & Conclusions

Comparing the three screening methods, gauzes were the most effective (13 MRSA out of 98 samples; 13.3%), followed by polywipes (1/98; 1.0%) and swabs (2/251; 0.8%). Moistened gauzes were the most sensitive method ($p<0.00001$) while screening with swabs was the least efficient ($p=0.00002$). The majority of the MRSA isolates (75%) belonged to the main clonal types previously found among patients and healthcare workers in the same hospitals: ST5-IVa (n=7; 44%) and ST88-IVa (n=5; 31%).

The finding of MRSA on environmental surfaces is dependent on the screening methodology. Moistened gauzes followed by a broth enrichment step proved to be a very sensitive methodology.

Keywords: Staphylococcus aureus, Screening methodologies, Environmental surfaces, MRSA

P-068 - USE OF A MICROBIAL COMMUNITY FROM A MINE SITE FOR THE SUSTAINABLE RECOVERY OF METALS

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Background

As the world demand for base metals, such as Cu and Zn, and high-tech critical metals is currently growing, the processing of complex low-grade ores and mine tailings has begun to play a key role in the mineral economy. Bioleaching is a cost-effective and environment-friendly technology used for the recovery of metals through colonization of ores by adapted microorganisms. Thereby, it has emerged as a potential alternative to the conventional hydrometallurgical processes for extraction of metals from low-grade ores and mine waste. Portugal is one of the world's largest producers of W, a high-tech critical metal, and, therefore, has mine tailings with a significant amount of W that should be recovered.

Method

The main goal of this work was to bioleach W, Cu, and Zn from Panasqueira mine tailings. In addition, bioleaching was also used to remove polluting metals, such as As and Cd. A sample from Neves-Corvo Copper plant was collected and it was incubated with a mineral medium to obtain a microbial consortium, which was used subsequently in a bioleaching experiment. The assay was carried out in a 1 L Erlenmeyer flask, for 32 days, with 5% (w/v) of sterile Panasqueira mine tailings. The concentration of metals in leachate was periodically determined by Atomic Absorption Spectrophotometry. The microbial community of the sample from Neves-Corvo was characterized using 16S rRNA gene-based Illumina high-throughput sequencing.

Results & Conclusions

Although the bioleaching process enabled a recovery of 48.0% of Cu, 12.7% of Zn, 6.3% of As and 6.1% of Cd, the concentration of W in the leachate was always below the limit of detection (< 20 mg/L). The microbial community used in the assay was dominated by the phylum *Proteobacteria* (95.11%), followed by *Actinobacteria* (4.6%). At genus level, *Limnobacter* (48.5%), *Thermithiobacillus* (14.3%) and *Thiobacillus* (5.9%) were the most abundant genera. The identification and understanding of the microbial community used in the bioleaching processes will contribute to the improvement of bioleaching efficiency, since it is a crucial factor to determine the optimal operating conditions, namely, growth medium composition, temperature and pH.

References & Acknowledgments

This work was supported by National Funds through FCT (the Portuguese Science and Technology Foundation) via the projects: ERA-MIN/0004/2015 "Recognition of microbial functional communities and assessment of the mineralizing potential (bioleaching) for high-tech critical Metals – BioCriticalMetals" and PTDC/AAGREC/ 3839/2014 "Biotools for a sustainable supply of tungsten from biodetection to bioleaching and biorecovery – PTW".

Keywords: Illumina, Microbial community, Bioleaching, Critical metals, Mine tailings

P-069 - UNVEILING THE CONTRIBUTION OF ARS OPERON FOR ARSENIC TOLERANCE AMONG CLINICALLY-RELEVANT SALMONELLA SEROTYPES/CLONES

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Abstract

Arsenic environmental contamination by anthropogenic activities, including in animal-farming management (e.g. coccidiostatics/pesticides/waste), may represent a long-term selective pressure driving the selection of multidrug-resistant (MDR) emergent *Salmonella* serotypes/clones. Diverse arsenic tolerance (AsT) mechanisms were described, although dispersion and association with a tolerance phenotype remains unknown in *Salmonella*. Our objective was to study the occurrence of genes coding for arsenical efflux pumps and their implications in tolerance phenotypes in diverse *Salmonella* serotypes and clones, including emergent MDR and/or copper/silver tolerant ones. 283 *Salmonella* isolates (2000-2016; humans/foods/animal/environment) from 57 serotypes (including the most frequent: Enteritidis/n=16; Typhimurium/n=49; 4,[5],12:i:-/n=63; Rissen/n=15) were selected based in PFGE-types and/or STs obtained by MLST and different profiles of antibiotic-resistance or metal-tolerance (Cu/Ag/Hg/Te)[1,2]. Screening of arsenic tolerance genes, *arsB* and *acr3* (both coding for arsenical pump membrane proteins) was performed by PCR/sequencing. $MIC_{Na2HAsO4}$ were determined in aerobic and anaerobic atmospheres by the agar dilution method.

Results & Conclusions

A high frequency of AsT genes was found (149/283-53%) involving diverse serotypes (30/57-52%): *arsB* (24%-n=69; 8 serotypes) or *acr3* (29%-n=82; 24 serotypes). The *arsB* was almost restricted to the emergent pig-associated MDR "European clone" of *S*.4,[5],12:i:- (n=36/37-97%) and *S*.Typhimurium (n=25/25-100%). The *acr3* was highly dispersed, including in two emerging pig-associated serotypes *S*.Rissen (n=15/15-100%) and *S*.Derby (n=19/19-100%). Phenotypic assays showed higher $MIC_{Na2HAsO4}$ in isolates carrying *arsB* (MIC_{50}=>128mM) or *acr3* (MIC_{50}=8mM) than those without these genes (MIC_{50}=2mM) in both aerobic/anaerobic conditions. AsT isolates also carried frequently (n=106/149-71%; "European clone" and Rissen plus 9 serotypes) copper, silver ± mercury, tellurium tolerance genes. Occurrence of AsT, particularly among emergent MDR and copper/silver tolerant pig-associated *Salmonella* clones circulating in Europe, might contribute for their adaptation to food-animal farm environments contaminated with diverse metals and oxygen levels.

References & Acknowledgments

This work was supported by FCT/MEC through national funds and co-financed by FEDER, under the Partnership Agreement PT2020 [grant UID/MULTI/04378/2013 – POCI/01/0145/FERDER/007728]; the project NORTE-01-0145-FEDER-000011; Joana Mourão has a Post-doc fellowship supported by project NORTE-01-0145-FEDER-000024 and Andreia Rebelo a MSc fellowship supported by project UID/QUI/50006. The authors are greatly indebted to all of the financing sources.

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Keywords: Salmonella, Arsenic, Multidrug-resistance, Metal-tolerance, Pig-associated clones

P-070 - DISTRIBUTION OF ARSENIC TOLERANCE GENES (ARSA) AMONG ENTEROCOCCUS SPP. FROM DIFFERENT SOURCES, CONTINENTS AND TIMEFRAMES (<1906-2015)

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Abstract

Successful bacteria accumulate different genetic features shaping their evolution and fitness to diverse environments/hosts. Arsenic-compounds are widespread in nature and possibly contribute to selection of particular strains. The aim of this study was to evaluate the dispersion of *arsA* genes among *Enterococcus* from different sources, continents and timeframes.

The *arsA* genes (coding for arsenical-pump-driving-ATPases) were searched in GenBank *Enterococcus* genomes and used to construct a maximum-likelihood phylogenetic-tree. A PCR scheme+sequencing was developed to detect *arsA* alleles from all phylogenetic subgroups identified among 333 isolates (Portugal; human/animal/environment/food; 1996-2012)[1]. Na_2HAsO_4 susceptibility was evaluated by agar dilution (0,25 to 128mM; n=143 isolates).

Results & Conclusions

Two major phylogenetic groups (A; B) and 5 subgroups (55-70% nucleotide identity; AI-GenBank-EFU15692.1, AII-EEU88411.1, BI-EOT39237.1, BII-SET88118.1, BIII-EOH82892.1; n=42 isolates) were detected. In all phylogenetic-subgroups *arsA* was distributed in different sources and/or species: *arsA*_AI - n=13 (human/animal/environment/food; *E. faecalis*-10/ *E. faecium*-3; Europe/North-America/Asia; <1906-2015); *arsA*_AI + *arsA*_AII - n=13 (human/animal/feed; *E. faecalis*-13; Europe/North-America/Australia/Africa/Asia; 1951-2012); *asrA*_BI - n=5 (human/food; *E. dispar*-1/*E. malodoratus*-1/*E. avium*-1/*Enterococcus sp*-2; North-America; <1991-unknown); *arsA*_BII - n=6 (human; *E. faecium*-1/*E. raffinosus*-1/*Enterococcus sp*-4; Europe; unknown date); *arsA*_BIII - n=2 (animal; *E. villorum*-1/*E. hermanniensis*-1; Europe/North-America; 1981-unknown). PCR+sequencing identified *arsA*-AI/AII/BII in 7% (n=24/333) of Portuguese *Enterococcus* also from diverse sources and species. Variable Na_2HAsO_4 phenotypes were observed: *arsA*_AI+*arsA*_AII (MIC=32-64mM/n=7); *arsA*_AI (MIC=4-8mM/n=3; 32-128mM/n=5); *arsA*_AII (MIC=0,5-4mM/n=7); *arsA*_BII (MIC=4mM/n=1); no-genes (MIC=0,5-2mM/n=108, MIC=4-128mM/n=11 suggesting the occurrence of new genotypes).

The *arsA* genes have been spread in the last 100-years among *Enterococcus* from diverse origins. The A/B phylogenetic groups seem to reflect diverse evolutionary pathways, with A-group including *E. faecalis/E. faecium* and B-group mainly other species. These data potentially reflect diverse genetic exchanges in bacterial communities including *Enterococcus* and other *Firmicutes* also carrying these *arsA* variants.

References & Acknowledgments

This work was supported by FCT/MEC (Fundação para a Ciência e a Tecnologia / Ministério da Educação e Ciência) through national funds and co-financed by FEDER, under the Partnership Agreement PT2020 [grant number UID/MULTI/04378/2013 – POCI/01/0145/FERDER/007728]; the project NORTE-01-0145-FEDER-000011 – Qualidade e Segurança Alimentar — uma abordagem (nano) tecnológica; Joana Mourão was supported by a post-doc fellowship supported by project NORTE-01-0145-FEDER-000024, Ana Raquel Freitas by a post-doc fellowship supported by Fundação para a Ciência e a Tecnologia (SFRH/BPD/96148/2013) and Andreia Rebelo by a research fellowship supported by project UID/QUI/50006. The authors are greatly indebted to all of the financing sources.

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Keywords: Enterococcus, arsenic-compounds, metal-tolerance, phylogeny

P-071 - CONVENTIONAL FARMING DISRUPTS COOPERATION AMONG PHOSPHATE SOLUBILIZING BACTERIA ISOLATED FROM CARICA PAPAYA'S RHIZOSPHERE

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Background

The productivity of most systems, including agricultural systems, is limited by nutrient availability, namely of phosphorus (P). However, in conventional farming, much of the soluble inorganic phosphate (Pi) applied to soils as fertilizer is rapidly immobilized after application, turning it unavailable to plants. By contrast, organic farming replaces synthetic fertilizers and pesticides with biological inputs.Therefore, microbial processes are essential for productivity under organic farming, playing a crucial role in the sustainability. Even though cooperation between soil microbes occurs naturally, the large amount of chemical inputs currently used in conventional farming has had a great impact on soil productivity but also on its functionality. Our objective was to test the legacy of the farming system (conventional or organic) on the interactions among phosphate solubilizing bacteria (PSB) leading to Pi solubilisation.

Method

Using nine PSB isolated from *Carica papaya*'s rhizosphere (four from conventional and five from organic farming) we determined their *in vitro* Pi solubilisation and organic acids production when growing monoculture or in pairs (66 combinations). Quantification of Pi in monoculture and coculture: Bacterial strains were grown in liquids media (NBRIP) using inorganic phosphate (Tricalcium phosphate). (D'Angelo et al.2001; Nautiyal, 1999).

Results & Conclusions

When PSB grew monoculture, PSB isolated from conventional farming solubilised more Pi but when grown in pairs, these PSB solubilised the least Pi. Further, when the pairs included only PSB isolated from conventional farming, no Pi solubilising cooperation was observed, i.e. Pi solubilisation in pairs was smaller than monoculture. When PSB isolated from organic farming were present in the pairs, ~40% of the combinations resulted in Pi solubilising cooperation. Our work showed that there was a farming system legacy influencing the biotic interactions among PSB, and that the output of the interactions (cooperation or antagonism) was not phylogenetically determined. From the applied point of view, these results show the need to consider the ecology of the rhizosphere when isolating and identifying plant growth promoting rhizobacteria and to develop performance tests using soil.

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This study was supported by FAPERJ (#E-26 /110.081/2014; #E-26 /111.428/2014; #E-26 /111.458/2014), CNPq (#475436/2010-5; #312399/2013-8 grants) and FCT (projects PTDC/AGRO-PRO/115888/2009, PTDC/AGR-PRO/1852/2014 and UID/BIA/00329/2013 (2015-2017), and postdoc grants SFRH/BPD/85419/2012 to Teresa Dias and SFRH/BPD/109079/2015 to Margarida Santana and CAPES and FAPES (PhD fellowship to Juliana Melo).

Keywords: Carica papaya, Microbial cooperation, phosphate solubilisation, phosphorus solubilising bacteria, organic acids, farming system

P-072 - WATER FROM HYDROELECTRICAL RESERVOIRS USED FOR FISH FARMING: A POTENTIAL SOURCE OF TOXIC CYANOBACTERIA AND MICROCYSTINS

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Background

Cyanobacterial blooms have become a concern, due to a global increase in response to accelerated eutrophication and climate change. Aquaculture is one of the contributing factors for freshwater eutrophication. *Cyanobacteria* can produce cyanotoxins, the best-known is microcystin, which consists of more than 90 variants. The microcystin has been identified as strongly hepatotoxic and able to promote the formation of tumors. The present study aims to evaluate the microbiome of water from fish farms focusing on the C*yanobacteria*. In addition, the presence of toxic *Cyanobacteria* was evaluated through detection of the microcystin synthetize gene E (mcyE) and quantification of microcystin concentrations.

Method

Water samples were collected from inside the six fish farms and sampling area downstream and upstream of the farms, localized in hydroelectrical reservoirs of São Paulo state, Brazil. The microbiome was identified by PCR amplification of the V6 to V8 hypervariable region of the 16S rRNA gene using the primer pair CC-CD and sequenced on the Illumina NextSeq 500 platform. Real-time PCR was carried out in order to detect the mcyE gene. Quantification of microcystin concentrations (LR, YR, and RR) was performed by HPLC-DAD, after the extraction of the samples in 75% Metanol.

Results & Conclusions

Cyanobacteria included 27,7% of water samples microbiome, being the second most abundant phylum. The representation of *Cyanobacteria* was the most abundant and diverse at locations 4 and 5. In these areas, phyla *Actinobacteria* and *Proteobacteria* were also abundant. The dominant Cyanobacterial genera were *Synechococcus* and *Microcystis*. The others genera were *Cylindropermopsis*, *Chroococcidiopsis*, *Pseudanabaena* and *Carteria*. *Microcystis* and *Cylindrospermopsis* among others are the main genera involved in the production of toxins and are the most widespread toxic *Cyanobacteria* in Brazil. Some species of *Synechococcus* also synthesize microcystin, besides they synthesize 2-methylisoborneol (MIB) and geosmin that cause muddy and earthy taste and odor problems worldwide in drinking water and fish. The measured microcystin concentrations were the highest at locations 3 and 4, and appeared elevated at locations 2 and 5. At location 2, MC-YR showed the highest concentration of the measured microcystin types, while at most other locations MC-LR was the primary detected type. Microcystins and the mcyE gene were found in all sampling area, confirming the presence of toxic cyanobacterial species. Overall, it appears that a more diverse representation of *Cyanobacteria* is observed at locations where microcystin levels were elevated.

References & Acknowledgments

São Paulo Research Foundation (FAPESP), grant #2015/13025-7, grant #2014/13718-0 and grant #2013/50504-5

Keywords: Fish farm, Water, Cyanobacteria, Microcistin

P-073 - EXTENDED-SPECTRUM B-LACTAMASES-PRODUCING COLIFORMS IN SANDS FROM NORTH PORTUGAL

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Background

Environmental causes are nowadays an emerging concern for national and international authorities. Microbiological limits are currently imposed for recreational waters but compliance with the quality standards of water is not necessarily indicative of sand quality. WHO acknowledged the importance of microbiological contamination in sand but did not establish benchmarks for analysis. This causes public health problems as sands can act as reservoirs of antimicrobial resistant bacteria. The widespread use, and often abuse, of antimicrobial agents transformed antimicrobial resistance into a global challenge. WHO has already recognised this as a concern and published a list of global priorities for antimicrobial resistant bacteria. Production of β-lactamases is the main mechanism of resistance to β-lactam antimicrobials. Classic ESBL are encoded by the bla_{TEM} and bla_{SHV} genes but other enzymes have emerged over time, with CTX-M-type enzymes being the most prevalent, nowadays.

Method

This work aims to detect ESBL producing coliforms in recreational sands, like sea and river beaches and playground sandboxes, in North of Portugal. Sand samples from 35 locations were collected and analysed for the presence of Cefotaxime-resistant coliforms, bacterial identification and determination of resistance phenotype. The β-lactamase encoding genes were determined by DNA amplification via PCR and the results were analysed by 2% agarose gel electrophoresis.

Results & Conclusions

A total number of 65 bacterial isolates were analysed, from which 5 ESBL producers were found - 3 *Escherichia coli* and 2 *Klebsiella spp.*. Of these, 3 were found on river beaches and 2 on sea beaches. PCR results showed amplification of TEM, OXA, SHV, CTX-M-1, CTX-M-8 and CTX-M-9 β-lactamases type. The ESBL detected were CTX-M and SHV type. The need for a change of direction in antimicrobial resistance is becoming a matter of urgency, and it is therefore necessary to promote a rational and careful use of these drugs and enhance the involvement of patients.

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Keywords: Sands, Enterobacteriaceae, Antimicrobial, Resistance

P-074 - ESCHERICHIA COLI IS NOT A SUITABLE FECAL INDICATOR TO ASSESS WATER FECAL CONTAMINATION BY OTTERS

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Background

The detection of pathogenic microorganisms in aquatic environments is extremely relevant in terms of public health. As these laboratorial methodologies are usually difficult, expensive and time-consuming, they are frequently replaced by the assessment of fecal indicator bacteria, such as *Escherichia coli*. This fecal indicator bacterial species can be related with the microbiological quality of waters, since it is a commensal bacteria present in the gastrointestinal tract of warm-blooded animals, including humans, farm animals and wildlife. In fact, it has long been considered as the best indicator for the presence of *Salmonella*, a known pathogenic bacterial genus, frequently disseminated in the environment through fecal contamination. Although *E. coli* is a good fecal indicator, it has not yet been fully proven as suitable to assess water fecal contamination by otters.

Method

This study aimed to assess the presence of *E. coli* in fecal samples from Neotropical otters (*Lontra longicaudis* Olfers, 1818), to evaluate its potential as fecal indicator to be applied to the determination of water microbiological quality in areas where otters' populations are high. Twenty-six otter fecal samples, collected in Alto Paranapanema river basin, São Paulo State, Brazil, were analyzed for the presence of *E. coli*, using conventional bacteriological methods, namely inoculation onto Tryptone Bile X-glucuronide agar (TBX). Plates were incubated at 37°C for 24h. Isolates were characterized through their macro- and microscopic morphology, staining, and biochemical characteristics (catalase and oxidase reactions).

Results & Conclusions

Only 8 scat samples (30%) were *E. coli* positive, indicating that this microorganism is not a suitable fecal indicator to assess water fecal contamination by Neotropical otters, and should not be used to infer the presence of otter related pathogens in waters. Further research must be conducted in order to determine which is the best bacterial group to be considered as a good fecal indicator.

References & Acknowledgments

Work was supported by the Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon (Project UID/CVT/00276/2013). N. Pedroso was supported by grant #2014/08601-6, São Paulo Research Foundation (Fundação de Amparo à Pesquisa do Estado de São Paulo). Luciano M. Verdade holds a Productivity Scholarship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Proc. 309468/2011-6).

Keywords: Aquatic environments, Escherichia coli, Fecal indicator, Lontra longicaudis

P-075 - COLONIZATION AND BENEFICIAL EFFECTS ON ANNUAL RYEGRASS BY MIXED INOCULATION WITH PLANT GROWTH PROMOTING BACTERIA

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Background

Multi-strain inoculants have increased potential to accomplish a wider diversity of plant needs, mainly attributed to its multi-functionality. This work evaluated the ability of a mixture of three bacteria to colonize and induce a beneficial response on the pasture crop annual ryegrass. *Pseudomonas* G1Dc10 and *Paenibacillus* G3Ac9 were previously isolated from annual ryegrass and were selected for their ability to perform multiple functions related to plant growth promotion. *Sphingomonas azotifigens* DSMZ 18530^T was included due to nitrogen fixing ability.

Method

Plant growth promoting effects by the bacterial mixture were accessed in gnotobiotic plant inoculation assays using a defined cultivation medium. Plants were grown in a controlled-environment chamber and evaluated for dry weight and physiological parameters (leaf pigments, fatty acids content and electrolyte leakage). The colonization behaviour of the bacteria and their ability to coexist in plant tissues were assessed using fluorescence in situ hybridization and GFP-labelling, combined with confocal laser scanning microscopy and a cultivation-based approach for enumeration of plant colonizing bacteria.

Results & Conclusions

Plant inoculation significantly enhanced the growth of roots (37%) and shoots (19%). The leaf chlorophylls and carotenoids contents were increased by 61% and 68%, respectively, indicating improved photosynthetic performance. Electrolyte leakage was decreased by 39%, suggesting improved membrane integrity. Total fatty acids were increased by 75%, mainly due to the rise of linolenic acid, an omega-3 fatty acid with high dietary value. The three strains occupied the same colonization sites, localizing preferentially along root hairs and in stem epidermis. Endophytic colonization was observed as bacterial cells entered root and stem inner tissues (endodermis, cortical parenchyma and cortex). This study provides evidences for the potential of this mixture of strains for biofertilization.

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Financial support was provided by Project 54971 (ProDer – Programa de Desenvolvimento Rural, Portugal); Project PTDC/AGRAAM/100577/2008 (FCT, Portugal); and iNOVA4Health - UID/Multi/04462/2013 program financially supported by FCT, through national funds and co-funded by FEDER under the PT2020 Partnership Agreement. N. Castanheira was supported by a fellowship from FCT (SFRH/BD/69185/2010).

Keywords: Plant inoculation, Plant growth promoting bacteria, Annual ryegrass colonization, Fluorescence in situ hybridization

P-076 - A BACTERIAL COMMUNITY WITH HIGH SORPTION ABILITY: BIOREMOVAL OF ACENAPHTHENE AND PHENANTHRENE

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Background

Polycyclic aromatic hydrocarbons (PAHs) are micropollutants of special concern in petroleum refinery effluents. Acenaphthene (ACN) and phenanthrene (PHE) are particularly interesting given their classification as priority by the US Environmental Protection Agency. Increasingly stringent regulations are now worldwide imposed to encourage industries to treat wastewater. Although bioremediation is an eco-friendly and cost-effective treatment, the identification of PAH-removing bacteria is poorly described and needs further assessment for treatment optimization. Furthermore, biosorption as a bioremoval mechanism is generally overlooked and should be addressed. This work (Sanches *et al.*, 2017) aims to identify a microbial community from a petroleum refinery wastewater capable of removing acenaphthene and phenanthrene under anaerobic conditions as well as to elucidate the removal mechanisms.

Method

The enrichment of a microbial community from GALP refinery wastewater (Sines, Portugal) was conducted. Assays were conducted under simultaneous nitrate- and sulfate-reducing conditions at room temperature, in the presence of individual PAHs (100µg/L) and in the presence/absence of lactate. Microbial growth was followed by measuring optical density. PAHs and lactate were determined by High Performance Liquid Chromatography. Biodegradation and biosorption were assessed by conducting experiments with live and inactive biomass. Microbial communities growing in the presence of the PAHs and in the presence/absence of lactate were characterized by Next-generation Sequencing and quantitative Fluorescence *in situ* Hybridization.

Results & Conclusions

Microbial growth and bioremoval of both PAHs occurred only in the presence of lactate, suggesting microbial co-metabolism. Both PAHs were efficiently removed: 77% of acenaphthene was removed within 17h and phenanthrene wasn't detected beyond 15h. Although anaerobic biodegradation is often regarded as slow, the extremely high bioremoval rate constants determined for phenanthrene (0.156 h^{-1}) and acenaphthene (0.086 h^{-1}) show the outstanding bioremoval potential of this community compared with previously reported. Biosorption over inactive cells was considerably high (85-88%) and was probably related with their hydrophobicity. Besides biosorption, bioremoval of acenaphthene (52%) and phenanthrene (35%) by extracellular products also occurred, showing the community versatility. The PAH-removing consortium, which was mainly composed by bacteria affiliated to *Diaphorobacter* and *Paracoccus* genera, is promising for bioremediation of refinery wastewaters and other contaminated environments.

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Acknowledgments:
- FCT through post-doc fellowships SFRH/BPD/94303/2013, SFRH/BPD/97612/2013, SFRH/BPD/76707/2011, SFRH/BDP/95864/2013; - iNOVA4Health (UID/Multi/04462/2013) and GREEN-IT (UID/Multi/04551/2013), research units financially supported by FCT/Ministério da Educação e Ciência, through national funds and co-funded by FEDER under the PT2020 Partnership Agreement.

Keywords: Polycyclic aromatic hydrocarbons, refinery wastewater treatment, anaerobic bioremoval, biosorption

P-077 - IMPACT OF BACTERIA CR(VI)-BIOSENSOR ON PLANT GROWTH AND FUNCTIONALITY OF PLANT-BACTERIA SYSTEMS UNDER CONTAMINATION

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Background

If released to the environment, Cr(VI) toxicity affects plants and animals. In plants, chromium is absorbed by roots and inhibits growth. Therefore, plants used in phytoremediation need to cope with chromium toxicity. Metal biosensors are biotools able to detect/quantify certain bioavailable metals in water and soil samples, such as Cr(VI). This work aimed to evaluate the effect of chromate biosensors pCHRGFP2 *Ochrobactrum tritici* and pCHRGFP2 *Nitrospirillum amazonense* on plant growth under low Cr(VI)-contamination and also to demonstrate the biosensor functionality within plant tissues for detection of bioavailable metal, a concept that opens a range of possibilities for the mapping of contaminated soil.

Method

Functionality of biosensors pCHRGFP2 *O.tritici* and pCHRGFP2 *N amazonense* within tissues of inoculated plants was studied *in vitro* and in a controlled environment greenhouse. Systems were tested on Cr(VI)-contaminated soil and water. Three rice varieties (IAC_4440, BRS_6_CHUÍ, IRGA_425) and one maize variety (1060) were tested as hosts and subjected to Cr(VI) treatments. A toxicological study was performed, to assess tolerance of plant hosts to inoculum and doses of toxic metal. Biosensor plant colonization was observed by fluorescence and confocal microscopy. GFP signal from colonized tissue macerates was quantified by fluorimetry.

Results & Conclusions

Plants showed different sensitivities to Cr(VI). BRS_6_CHUÍ was the most resistant. Chromium affected development essentially in roots, which were totally inhibited in rice at 500 µM. Considering inoculum effect, *N.amazonense* was safe to plants under the range of inoculant dose tested, but *O.tritici* showed plant specificity and toxicity when inoculated at high numbers, inhibiting rice development but not maize. Inoculants improved growth of specific plant varieties at 1.25 ppm Cr(VI), which corresponds to weak soil contamination. Improvements were observed for both Cr(VI)-treated and untreated controls: benefits went beyond the neutralization of inhibition caused by Cr(VI). Plants inoculated with both Cr(VI)-biosensors, when exposed to Cr(VI), showed stable GFP expression within plant tissues. Biosensors showed to penetrate roots and later colonize shoots and leaves. Results indicated pCHRGFP2 *O.tritici* as better for maize variety 1060, while pCHRGFP2 *N.amazonense* showed more appropriate for rice. Both strains offered capacity to detect Cr(VI) contamination in soils by analyzing colonized tissues of rice and maize exposed to 25 mM Cr(VI). This concept of biosensor system provides samples easy to collect and handle, facilitating *in situ* monitoring of contaminated areas.

References & Acknowledgments

Work financed by Brazilian Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), process #400756/2012-9. R.M.F. and R.S.R.B. financed by fellowships (PDJ) #160619/2012-2 and (PVE) #304009/2012-1, respectively.

Keywords: biosensors, Ochrobactrum tritici, Nitrospirillum amazonence, maize, rice, chromium

P-078 - THE GORGONIAN CORAL EUNICELLA LABIATA HOSTS A DISTINCT PROKARYOTIC CONSORTIUM AMENABLE TO CULTIVATION

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Background

Metagenomic surveys of the microbiota associated with marine invertebrates have revealed an enormous diversity of symbiotic prokaryotes. Their functional features and genome architectures, however, remain elusive, mainly due to the lack of representative culture collections. Microbial communities inhabiting gorgonian corals are believed to benefit their hosts through nutrient provision and chemical defence; yet much remains to be learned about their phylogenetic uniqueness and cultivability.

Method

Here, we determined the prokaryotic community structure and distinctiveness in the gorgonian *Eunicella labiata* by Illumina sequencing of 16S rRNA genes from gorgonian and seawater metagenomic DNA. To address the cultivable fraction of the bacterial consortium associated with *E. labiata,* we used a carbon-reduced marine medium, lower incubation temperature (18°C) and longer incubation (4 weeks), to allow slow-growing bacterial symbionts to develop. In addition to the isolation of single colonies (N = 175), we employed a 'plate-wash' strategy (Hardoim, et al. 2014) to compare the phylogenetic diversity of the "total" gorgonian bacteriome and its "cultivatable" fraction.

Results & Conclusions

With 1016 operational taxonomic units (OTUs), prokaryotic richness was higher in seawater than in *E. labiata* where 603 OTUs were detected, 68 of which were host-specific. The taxonomic composition of the gorgonian bacteriome differed sharply from the bacterioplankton: while seawater samples were dominated by *Proteobacteria* of the clades SAR11, SAR68, SAR116 and *Rhodobacteraceae*, followed by *Actinobacteria* and *Bacteroidetes,* gorgonians were clearly dominated by the *Proteobacteria* orders *Oceanospirillales* and *Rhodobacterales*. One *Oceanospirillales* OTU, classified as *Endozoicomonas*, was particularly dominant, contributing to up to 59% of the gorgonian community, and closest relatives comprised exclusively uncultured clones from other gorgonians. We cultivated a remarkable 62% of the bacterial symbionts inhabiting *E. labiata*: *Ruegeria*, *Sphingorhabdus*, *Labrenzia*, other unclassified *Rhodobacteraceae* as well as *Vibrio* and *Shewanella* ranked among the ten most abundant genera in both the cultivation-independent and -dependent samples. In conclusion, the *Eunicella labiata* microbiome is diverse, distinct from seawater, and enriched in (gorgonian)-specific bacterial phylotypes. In contrast to current understanding, many dominant *E. labiata* symbionts can, indeed, be cultivated.

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Funding

- Portuguese Foundation for Science and Technology (FCT): project [EXPL/MAR-EST/1664/2013] and post-doctoral fellowship [SFRH/BPD/34282/2006] to AL-L.
- German Volkswagen (VW) foundation: post-doctoral fellowship to TK-C within the project [Az. 81 040-2].

Keywords: Host-microbe interactions, Symbiosis, Microbiome, Microbial cultivation, Next-generation sequencing

P-079 - VERSATILE NUTRIENT CYCLING AND DISTINCT ADAPTIVE FEATURES OF ALPHAPROTEOBACTERIAL SYMBINTS OF MARINE SPONGES

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Background

Sponges appeared about 600 million years ago. These early-branched metazoans are known to harbor dense and diverse microbial communities, and represent the emergence of animal-microbe relationships in the evolutionary history of our planet. It has been proposed that the marine sponge microbiome plays a role in nutrient provision, removal of metabolic by-products, chemical defense and shelter from disease. Despite the growing amount of information on sponge microbiomes, the role of the so far uncultivatable, yet very abundant alphaproteobacterial lineages that populate these sessile invertebrates remains unclear.

Method

In this study, we applied a sequence composition–dependent binning approach to recover genomes of uncultured *Alphaproteobaceria* from *Spongia officinalis* and seawater microbial metagenomes obtained via Illumina shotgun sequencing (Karimi et al., in review).

Results & Conclusions

Three genome bins were obtained, one from the sponge and two from the seawater metagenome. The sponge-derived genomic bin was found to belong to the *Rhodospirillaceae* family, and to represent an abundant, widespread and uncultivated sponge symbiont clade displaying a broad host range. High nucleotide (91.3%) and amino acid (95.2%) sequence conservation was observed between our reconstructed genome and one uncultured *Rhodospirillaceae* sp. binned from the Mediterranean host *Aplysina aerophoba*, suggesting functional equivalence among representatives of this clade across different host species and geographical locations. Functional genome mining further revealed the complete set of all proteins required for taurine-specific import and desulfonation in the symbiotic *Rhodospirillaceae* genomes. Taurine (2-aminoethanesulfonic acid) metabolism constitutes an important adaptive feature of sponge symbiotic *Rhodospirillaceae* spp.. Taurine-conjugated fatty acids and N-acyl taurines have been reported from diverse marine sponges, providing evidence for available organic sulfonated compounds that may be utilized by sponge symbionts. Moreover, the sponge-associated *Rhodospirillaceae* possess the capability of transforming both organic and inorganic forms of nitrate, suggesting that symbiotic *Rhodospirillaceae* spp. contribute to denitrification processes within the marine sponge holobiont. Besides, genes encoding for ABC transporters, dehydrogenases with different substrate specificities and glutathione lysis were frequent among sponge-associated *Rhodospirillaceae*. As ABC transporters are widely acknowledged for their roles in cell membrane rigidity and import/export of molecules, along with glutathione lysis, it could be postulated that regulation of osmotic pressure and detoxification capabilities are pivotal adaptive traits of sponge-associated *Rhodospirillaceae*.

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FCT(PTDC/BIA-MIC/3865/2012 and PTDC/MAR-BIO/1547/2014) and FEMS(FEMS-RG-2015-0115)

Keywords: Functional genomics, next-generation sequencing, Porifera, symbiosis, host-microbe interactions, ABC transporters, sulfur utilization, denitrification

P-080 - AMINOGLYCOSIDE RESISTANCE IN RALSTONIA PICKETTII

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Background

Ralstonia spp. are ubiquitous in water environments, including drinking water. The species *R. pickettii* is one of the most common in aquatic environments, including some environments classified as sterile or with extremely low microbial loads. This species has also been associated with nosocomial outbreaks. Aminoglycoside resistance is a variable phenotype in this species, suggesting that it can be acquired and not intrinsic as, for example, colistin resistance.

This study aims to: i) investigate the aminoglycoside resistance mechanisms in *R. pickettii* isolated from aquatic environments and assess a possible relationship with the type of water (mineral, tap, wastewater) from which they were isolated, and ii) searches for possible aminoglycoside resistance acquisition hints.

Method

Fifty five *R. pickettii* strains (including some evolved in the presence of gentamicin or UV radiation) were characterized for their antibiotics and metals tolerance. Based on the comparative genome sequencing analysis of two of the isolates, with distinct aminoglycoside resistance phenotype and isolated from the same habitat (hospital effluent), some genetic determinants were selected to analyse in all the strains under study. Those genetic elements were the ICEs (integrative conjugative elements), plasmids, genes encoding efflux pumps or associated with arsenic resistance.

Results & Conclusions

Besides to aminoglycosides, resistance to beta-lactams and colistin was frequent in *R. pickettii*, regardless the type of water. An association between aminoglycosides resistance and increased arsenite tolerance was observed. All the isolates resistant to aminoglycosides had minimal inhibitory concentrations (MIC) values of >256mg/mL to gentamicin and of 1.4mM to arsenite, while for the susceptible strains values of 6mg/mL and 0.05mM, respectively, were found. Accordingly, only the aminoglycoside resistant isolates had the genes *arsH* and *ACR3*, related to arsenite resistance. Also distinguishing the aminoglycoside resistant and susceptible strains was the presence of ICEs gene fragments, only in resistant strains, and the aminoacid sequence of the RND efflux pump. Although these findings suggested a common mechanism of resistance, such hypothesis was not supported by experimental evolution assays of aminoglycoside susceptible strains, in which aminoglycoside MIC values increased to resistance levels while arsenite tolerance did not vary. On the other hand, these data can suggest that besides a common aminoglycoside and arsenite resistance acquired eventually by horizontal gene transfer, mutation may lead to the acquisition of an alternative aminoglycoside resistance mechanism.

References & Acknowledgments

The authors acknowledge Fundação para a Ciência e Tecnologia for projects UID/Multi/50016/2013 and POCI-01-0145-FEDER-006939-LEPABE, and IVM grant (SFRH/BPD/87360/2012), and Erasmus Mundus Project SUD-UE for the PF grant (2013-2589).

Keywords: Ralstonia, aminoglycosides, gentamicin, arsenite, conjugative elements, water

P-081 - PROTECTIVE EFFECT OF NATIVE ARBUSCULAR MYCORRHIZAL FUNGI OF INDIGENOUS ORNITHOPUS COMPRESSUS AND LOLIUM RIGIDUM ON WHEAT GROWTH

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Background

The acidic properties of Alentejo soils in Portugal increase manganese (Mn) bioavailability, inducing plant toxicity and limiting crop production. Arbuscular mycorrhizal fungi (AMF) are obligatory symbiotic soil microbiota known to improve plant tolerance to heavy metal stress. In our study the protective effect of native AMF on wheat growth was investigated when extraradical mycelium (ERM) was previously developed in the soil by developer plants *Ornithopus compressus* (ORN) or *Lolium rigidum* (LOL). The repercussion of ORN and LOL treatments was also assessed through ICP-MS quantification of magnesium (Mg) and Mn on wheat tissues (leaves and meristems) compared to wheat grown in soil without a previous developer. Furthermore, the content of other several metals (e.g. Al, Fe, Zn) in plant tissues was also evaluated.

Method

Wheat growth was evaluated by determining shoot dry wheight. Dried samples of 3-week old plants grown in soil without and with previously developed native ERM of ORN (disturbed and undisturbed) and LOL[1] were digested with trace metal grade suprapur HNO_3 and H_2O_2. Elemental composition (e.g. Al, Ca, Fe, Mg, Mn, Na, P and Zn) was quantified through ICP-MS.

Results & Conclusions

Wheat shoot dry weight increased in the presence of ERM from previously grown indigenous species. ERM developed from ORN was the most successful in promoting wheat growth, reaching over 1.5 times the shoot weight obtained for LOL treatment. Wheat grown in the presence of ERM showed over 4 times less Mn concentrations in shoots (leaves or meristems) than wheat grown without. Meristems showed the lowest Mn concentrations given that they are actively growing sites for new leaves and due to the low phloem Mn remobilization. Disruption of the ERM from ORN resulted in a greater trace metals accumulation and lower wheat shoot phosphorus uptake, which can be a signal of poorly established AMF symbiosis. The present results point towards an improvement in crop production by combining previous establishment of indigenous tolerant AMF communities to a no-tillage system.

Preliminary results on AMF mode of action indicate that ERM-induced trace metal subcellular partitioning plays a role in Mn detoxification. Ongoing work will determine the influence of ORN and LOL treatments on wheat antioxidant enzyme activity and phyto-hormone balance[2].

References & Acknowledgments

This study was partially funded by FEDER, Programa Operacional Regional Alentejo 2020 under research contract ALT20-03-0145-FEDER-000039 and by National Funds through FCT under Project UID/AGR/00115/2013.

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Keywords: Arbuscular mycorrhizal fungi, Mn toxicity, Ornithopus compressus, Lolium rigidum, Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

P-082 - SPECIES-SPECIFIC RNA-FISH PROBES FOR YEAST IDENTIFICATION: EVALUATION OF THEIR SPECIFICITY AND PERFORMANCE

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Background

RNA-FISH is a powerful molecular technique that allows identification of individual microbial cells. The application of a unique protocol for microbial identification from the kingdom down to the species level simplifies the analytical procedure. Recently, our research group has developed an efficient RNA-FISH protocol for simultaneous analysis of fungi and bacteria using kingdom-specific probes. Thus, the aim of this work was to investigate the possibility of applying this protocol for specific detection of various yeast species using previously published species-specific probes. Their specificity and performance were analysed both *in silico* and experimentally.

Method

Suspensions of target (*Cryptococcus adeliensis*, *Dekkera bruxellensis and Zygosacharomyces bailii)* and non-target yeast isolates (*Lachancea thermotolerants, Pichia kudriavzevii, Rhodotorula sp., R. mucilaginosa, Saccharomyces cerevisiae, Hanseniaspora guillermondii* and *Torulaspora delbruekii*) were used. The autofluorescence of the cells was evaluated by epifluorescence microscopy using Cy3, FITC and Cy5 filter sets. The specificity and hybridization efficiency of the probes were evaluated *in silico* using NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and mathFISH (http://mathfish.cee.wisc.edu/). Seven different RNA-FISH assays were performed for each isolate following the protocol previously described by us [1]: (1) blank without probe addition; (2) negative control with EUB338; (3) positive control with EUK516; (4-7) test assays with *Z. bailii*, *D. bruxellensis*, *Brettanomyce*s and *C. adeliensis* previously published probes [2-4]. All probes were labelled with Alexa Fluor 647 (AF647) at the 5′-end.

Results & Conclusions

The microscopic observation of the target cells revealed that for avoiding false positives, associated to autofluorescence, a red-emitting dye could be used. Thus AF647-labelled RNA-FISH probes were applied in this study. The *in silico* analysis revealed that the probes tested possess not only a high specificity but also a high theoretical hybridization efficiency. This was confirmed by the experimental approach. The analysis of RNA-FISH treated yeast cells also showed that the FISH protocol, previously used for identification of fungi and bacteria, is suitable for specific detection of several yeast species. Therefore, the possibility of simultaneous identification of these microorganisms from the kingdom to the species level with a single protocol and with a good FISH performance looks promisor.

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Acknowledgements: This work was co-financed by FCT through PTDC/BBB-IMG/0046/2014 project and SFRH/BPD/100754/2014 grant and by ALT20-03-0246-FEDER-000004-ALENTEJO 2020 project.

Keywords: Fluorescence In Situ Hybridisation, Species-specific RNA-FISH probes

P-083 - CHROMOBACTERIUM SPP. RESISTOME AND ROLE AS PROGENITORS OF BLAKPC-LIKE GENES

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Background
Chromobacterium haemolyticum is increasingly reported to cause severe systemic infections in humans, especially after exposure to contaminated water. Furthermore, *Chromobacterium* species were suggested as the progenitors of KPC-like carbapenemases, due to the presence of class A carbapenemase encoding genes in their chromosome highly similar with bla_{KPC}[1].

Our goals were to perform an analysis of environmental *C. haemolyticum* antibiotic resistance features and analyze *Chromobacterium* spp. genomes to understand this genus role as progenitor of bla_{KPC}-like genes.

Method
Eight *C. haemolyticum* isolates were obtained in water from 3 rivers located in the Vouga River hydrographic basin, Portugal[2] and antibiotic resistance phenotypes were determined.

A PCR-based approach was used to inspect for bla_{KPC}-like genes. The putative class A beta-lactamase encoding genes and flanking regions reported in *Chromobacterium* spp.[1] were aligned to design PCR primers.

Chromobacterium genomes were inspected for the presence of putative class A beta-lactamase encoding genes. bla_{CRH-1} (JONK01000003), bla_{CRS-1} (CAEE01000676), bla_{CSP-1} (JTGE01000141) previously reported in *C. haemolyticum* DSM19808, *Chromobacterium* sp. C61 and *C. piscinae* ND17, respectively were used for similarity searches with BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) against the *Chromobacterium* genomes available. The deduced aminoacid sequences of all variants were aligned and inspected for motifs related to carbapenemase activity.

Results & Conclusions
All isolates exhibited resistance towards penicillins (amoxicillin and amoxicillin combined with clavulanic acid), extended-spectrum cephalosporins (cefotaxime and cefepime) and carbapenems (imipenem and ertapenem). Minimal inhibitory concentrations for carbapenems varied from 2 to over 32 µg/mL. The bla_{KPC}-like gene was amplified in all *C. haemolyticum* strains. Two new variants were identified: one encoded by bla_{CRH-2} (KY773614) detected in 7 strains and by bla_{CRH-3} (KY773615) in 1 strain. CRH-3 showed 97.93% similarity to CRH-2, 97.24% with CRH-1, 97.53% with CRS-1 and 68.62% with CRP-1.

The *in silico* analysis revealed that a class A beta-lactamase encoding gene, with over 50% similarity with bla_{KPC-2}, was present in 22 out of 39 available *Chromobacterium* genomes, and only in 5 out of 8 species with at least one genome sequenced. This suggests that the presence of a bla_{KPC}-like gene might be species related. Also, motifs related to carbapenemase activity were identified in all *Chromobacterium* spp putative class A beta-lactamase variants, including CRH-2 and CRH-3. Overall, results emphasized the *Chromobacterium* spp. role as progenitors of bla_{KPC}-like genetic determinants and evidence that the putative origin of several genetic determinants of resistance is linked to environmental bacteria.

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Keywords: Chromobacterium haemolyticum, KPC, Antibiotic Resistance, Opportunistic Pathogen, Environment, Beta-lactamases

P-084 - ENVIRONMENTAL SCREENING OF ANAMMOX BACTERIA IN NW PORTUGUESE COASTAL ENVIRONMENTS

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Background

Anthropogenic inorganic nitrogen inputs to aquatic systems are a major environmental concern in the EU and worldwide. The discovery of anaerobic ammonium oxidation (anammox) revealed the existence of a shortcut in the classic nitrogen cycle where ammonium is converted directly to dinitrogen gas, and removed from the system [1]. In recent years, the importance of this process has been demonstrated in both engineered and natural ecosystems [2, 3]. Anammox reaction is performed by a distinct group of bacteria belonging to the *Planctomycetes* phylum. Anammox bacteria have been reported in a variety of habitats, from marine anoxic basins to freshwater lakes. However, despite the widespread occurrence, low diversity of anammox bacteria has been reported with only five genera reported so far [3], the majority of them found in engineered systems. In this study, we aimed to examine the diversity and distribution of anammox bacteria in natural habitats along the NW Portuguese coast.

Method

Sediment samples were collected in different estuaries, coastal lagoons and saltmarshes. Total community DNA was extracted and anammox bacterial 16S rRNA gene sequences were amplified using specific set of primers. The amplicons were cloned and sequenced. Sequences were compared to reference sequences, aligned and submitted to phylogenetic analysis.

Results & Conclusions

Anammox-like sequences were recovered from sediment samples from different locations including Ave and Douro estuaries, and Ria de Aveiro. Ongoing phylogenetic analysis of the 16S rRNA genes has revealed already the presence of two known anammox bacterial gene, *Candidatus Scalindua* and *Brocadia*. Obtained results will enable the selection of samples for further characterization using next generation sequencing to characterize community composition and identify anammox lineages. The broad anammox bacterial distribution indicates that anammox can play an important role in removing fixed nitrogen from these systems.

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Ackowledgments: Project INNOVMAR/ECOSERVICES (NORTE-01-0145-FEDER-000035), supported by the Northern Portugal Regional Operational Programme (NORTE2020), through the European Regional Development Fund (ERDF). C. Teixeira acknowledges FCT for a postdoctoral grant (ref. SFRH/BPD/110730/2015) through POCH, cofunded by MCTES and the European Social Fund.

P-085 - CAN WASTEWATER ANTIBIOTIC RESISTANT BACTERIA SURVIVE A HEALTHY HUMAN GUT MICROBIOME?

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Background

Intended or unintended water reuse is becoming more common every day. The associated implications on the dissemination of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in the environment are recognized. However, how this contamination may threat human health is still a major question. The entry via food chain is a likely possibility, although it is arguable that the complex human gut microbiome may hamper the survival of environmental ARB and ARG. This was the major aim of this study in which was assessed the survival of known wastewater ARB in human fecal material.

Method

Feces-based microcosm assays were inoculated with i) wastewater ARB (*Escherichia coli* A2FCC14 and *Enterococcus faecalis* H1EV10) known to harbor the ARGs (bla_{TEM}, bla_{CTX}, bla_{OXA-A} and *van*A, respectively); ii) free-DNA extracted from the same bacteria and iii) the same ARB but in presence of sub-inhibitory concentrations of antibiotics (cefotaxime and vancomycin). Assays were conducted under aerobic and anaerobic conditions. ARB were monitored based on cultivation methods and ARGs based on quantitative PCR (qPCR).

Results & Conclusions

As major results, it was observed that strains survived in the presence of the faecal microbiota at least for a week and that their ARGs could be detected and quantified at least for one month. The gene *van*A was detected for a longer period than the genes bla_{TEM}, bla_{CTX} or bla_{OXA-A}, a trend that was also observed when cell-free DNA was the inoculant. With free-DNA the beta-lactamase genes decayed in 24 hours, while *van*A was detected and quantified until 7 days. Moreover, free-DNA do not implied an increase of antibiotic resistance. Comparing the microcosms conducted under aerobic or anaerobic conditions, it was observed that the strain *Enterococcus faecalis* H1EV10 behaved similarly, while the bacterial population of *E. coli* A2FCC14 has a sharper reduction anaerobically. Nevertheless, the ARGs bla_{CTX} and bla_{OXA-A}, harbored by the *E. coli*, were detected and showed only a slight reduction, and the gene *van*A displayed a constant abundance after seven days. The survival of ARB under specific conditions – e.g. during antibiotherapy, is ongoing. The main conclusion of this research so far, is that ARB with origin in treated wastewater are able to survive and are not outcompeted by the autochthonous microflora until a week and their ARGs can be quantified alike.

References & Acknowledgments

The work presented here is part of a project funded from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 675530.

Keywords: Wastewater, Antibiotic Resistant Becteria, Antibiotic Resistance Genes,

P-086 - THIRD GENERATION CEPHALOSPORIN-RESISTANT KLEBSIELLA PNEUMONIAE: DOES THE ORIGIN MATTER?

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Background
Third generation cephalosporins-resistant *Klebsiella pneumoniae* represent an important health-care associated infectious (HAI) agent, whose prevalence is increasing globally[1]. As many other HAI, *K. pneumoniae* end-up in urban wastewater treatment plants, often surviving the treatment process, being released to the environment. If these contaminants maintain the traits of clinical isolates was the question we aimed to answer in this study. With this aim, we obtained and characterized third generation cephalosporins-resistant *K. pneumoniae* isolates from clinical settings and from wastewater.

Method
A total of 52 isolates resistant to 3rd generation cephalosporins comprising 25 clinical isolates and 27 isolates from wastewater were selected for this study. Isolates were compared based on 1) antibiotic susceptibility to 5 classes of antibiotics (β-lactams, aminoglycosides, quinolones, sulfonamides and tetracyclines) by the disk diffusion method; 2) presence of genetic determinants potentially encoding extended-spectrum β-lactamases, carbapenemases or colistin resistance by PCR; and 3) the plasmids profile of each isolate based on PFGE.

Results & Conclusions
Multidrug resistance was observed in 74% (20/27) of the wastewater isolates and in 92% (23/25) of the clinical isolates. The genes bla_{SHV}, $bla_{CTX\text{-}M}$, bla_{TEM}, bla_{OXA} were highly prevalent in the clinical and wastewater cephalosporin resistant isolates - bla_{SHV} 96 *vs*. 89%, $bla_{CTX\text{-}M}$ 76 *vs*. 81%, bla_{TEM} 72 *vs*. 59% and bla_{OXA} 40 *vs*. 44%. However, the gene bla_{kpc} was detected only in clinical isolates (6%). The beta-lactam resistance genes bla_{VIM} and bla_{IMP} and the colistin resistance genes *mcr*-1 and *mcr*-2 were not detected. Curiously, in the wastewater isolates was observed a higher number of plasmids than in clinical isolates –22% *vs*. 44% of the isolates carried 1 plasmid, 48% *vs*. 36% carried 2 plasmids, and 26% *vs*. 16% carried 3 plasmids. Plasmids were not detected in 2% of the isolates and the presence of 4 was uncommon.

These results indicate that clinically relevant bacteria once released into the wastewater might retain clinical relevant traits, representing a threat for human health, either directly or indirectly through the spread of antibiotic resistance genes.

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The authors acknowledge the staff of the UWWTP for their support on samples collection and the partners from the Hospital for providing the clinical isolates for this study. This work was supported by FCT (Water JPI/0001/2013 STARE).

Keywords: *Klebsiella pneumoniae*, third generation cephalosporins, multidrug resistant

P-087 - FAECAL INDICATOR BACTERIA AND LISTERIA MONOCYTOGENES IN PONDS, NATURAL AND CONSTRUCTED WETLANDS: EVALUATING WATER QUALITY

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Background

Water contaminated with microbiological and chemical constituents can cause a variety of diseases. Wetlands may become contaminated by wild and domestic animal's faeces, agricultural runoff, or sewage, and are often overlooked as a reservoir and source of human infection by pathogenic microorganisms. The objective of this study was to examine natural (NW) and constructed (CW) wetlands and artificial ponds (AP), located in urban and rural areas of the north of Portugal, for the occurrence of faecal pathogens, indicator bacteria and *Listeria monocytogenes*.

Method

Water samples were collected from six sites at two sampling dates. In an urban area three AP (1- city garden; 2- city park; 3- 7th floor roof terrace at the city centre) were considered and in a rural area one AP (4- ecological pool), one CW (5- tourism house/Paço de Calheiros) and one NW (6- Bertiandos and S. Pedro of Arcos Lagoons; considered protected landscape) were considered. Microbial analysis were performed using the membrane filtration technique for the enumeration of total coliforms, *Escherichia coli* and *Enterococcus* spp., and detection of *L. monocytogenes* and *Salmonella* spp.

Results & Conclusions

Total coliform counts ranged between 100 to 1500 CFU/100 ml, *E. coli* between <1 to 570 CFU/100 ml, and *Enterococcus* between 9 to 610 CFU/100 ml. The city park AP was positive for *Salmonella* spp. in both sampling visits, while the NW (Bertiandos, rural area) was positive in one sampling visit. *Listeria monocytogenes* was present in samples collected from the urban AP of the city garden (once), the CW in the tourism house (both sampling visits), and the NW (once). These data show that natural and artificial wetlands and artificial ponds are a reservoir of faecal indicator bacteria, and enteric and foodborne pathogens.

References & Acknowledgments

This work was supported by National Funds from FCT - Fundação para a Ciência e a Tecnologia through project 'UID/Multi/50016/2013'

Keywords: wetlands, water, microbiological quality, Listeria monocytogenes

P-088 - E. COLI AND ARG ABUNDANCE IN AGRICULTURAL FARMS AFTER MANURE APPLICATION

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Background

Manure application on agricultural soils, which promotes the introduction of largely distinct bacterial communities in a previously established one, has been suggested as a major source of antibiotic resistance genes (ARG) in the Environment. The fate of ARG in the environment is complex, and some may even persist in the environment in the absence of selective pressures. Despite the number of publications on manure and soil resistomes, not many field studies have been performed with more than two dairy farms.

This work aimed to assess the impact of manure application on the abundance of viable fecal bacteria and ARG in cultivated soils (grassland) and adjacent water ditches (runoff) over time in field settings, in a range of dairy farms with different soil types.

Method

Samples of dairy manure, soil, and runoff surface water from 6 different farms were collected. CFU enumeration (*E. coli*), on selective culture media (TBX), as well as quantification of the 16S rRNA gene and resistance genes *tetW*, *sul1*, *ermB*, and *intI*, via qPCR was performed. Per farm, samples were collected at 6-time points: before manuring (control), and 1, 4, 7, 14, and 21 days after manuring. Enumeration of *E. coli* was performed after homogenization of 10 or 100 g of manure or soil, respectively. Water samples were filtered through 0.45 um-pore mixed cellulose-ester membranes (for CFU counts) and 0.22 um-pore polycarbonate membranes (for DNA extraction). Total DNA extracts were obtained using the QIAamp Fast DNA Stool Mini Kit, FastDNA SPIN Kit for Soil, and DNeasy PowerWater Kit for manure, soil, and water samples, respectively.

Results & Conclusions

Overall, results showed that both CFU and ARG abundances could increase after manure application in soils and ditch waters. The highest abundance was observed shortly after manuring, at different time points, however, even 21 days after manuring, *E. coli* was still detected in higher abundance than in control samples. these findings confirm that dairy manure application is a source of viable fecal bacteria and resistance genes in soils.

References & Acknowledgments

Keywords: Soil, Fate of Resistance, ARG, Manure

P-089 - ASSESSMENT OF TOTAL MESOPHILIC BACTERIA AND FUNGI IN FOOD ESTABLISHMENTS FROM NORTHERN PORTUGAL

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Background

Indoor air quality represents a concern to people since they spend an important part of their daily lives in closed spaces, research about airborne pathogens, inorganic pollutants or allergens have gained importance (Annesi-Maesano *et al*., 2013). To guarantee an adequate indoor air quality in food establishments, the application of some measures such as a proper maintenance of ventilation equipment's, air filtration or source control are fundamental (Wei *et al*., 2015). The objective of the present work was the evaluation of the indoor air quality of canteens and cafes from two universities in Portugal.

Method

Total mesophilic bacteria (TMB) and fungi were assessed by passive air sampling using settle plates. Determination of indoor air quality was carried out by the index of microbial air (IMA) contamination and policy. Total mesophilic bacteria (TMB) were cultured in plate count agar (VWR, Belgium) at 30°C for 72 hours. Fungal load was evaluated using rose bengal-chloramphenicol agar (HiMedia, India) at 22 °C for 5 days. Results were expressed in CFU/dm^2/h. Identification of filamentous fungi was carried out on lactophenol cotton blue staining (Hard Diagnostics).

Results & Conclusions

The study of the IMA values indicated that both canteens and cafes displayed a good indoor air quality. TMB counts observed in cafes were higher than the obtained in canteens, which can be associated to a better performance of cleaning and disinfection procedures, better ventilation, or the lower time that students spent in canteens. As contrary as observed for TMB, Fungi counts were higher in canteens than cafes, with higher counts in raw food and cooling areas. This can be associated to the presence of raw food products, high humidity or high temperatures due to cooking and preparation procedures which act as growth factor of molds. The most genus of fungi isolated in the present work was *Alternaria*, *Cladosporum*, *Aspergillus* or *Penicillium*, which have been associated to respiratory tract diseases. However, the satisfactory fungi levels observed in our work indicate the absence of a public health concern for students. Data of the present work could be used to improve the indoor air quality of food establishment at universities.

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Keywords: indoor air, fungi, bacteria, canteens, cafes

P-090 - BIODEGRADATION OF CARBAMAZEPINE BY THE BACTERIAL STRAIN LABRYS PORTUCALENSIS F11 – METABOLISM AND TOXICOLOGIC STUDIES

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Background

Occurrence of pharmaceuticals in the environment is a topic of concern. Most pharmaceuticals are not completely mineralized and are released on the sewage systems through excretion and by improper elimination and disposal(1). Municipal wastewater treatment plants (WWTPs) are not designed to remove them and they are released into the environment(2). They are classified as persistent microcontaminants due to their continuous release even if at low concentrations (3). Carbamazepine (CBZ) is an widely used anticonvulsant and has been suggested as a molecular marker of contamination in surface water and groundwater(4).

Method

Biodegradation of CBZ by the bacterial strain *Labrys portucalensis* F11 was tested as sole carbon and energy source (0.04 mM) and in the presence of acetate as primary carbon source. Transformation products (TPs) were detected and identified by UPLCQTOF/MS/MS. Ecotoxicologiacl effects of CBZ and the TPs resultant from biodegradation were evaluated at different trophic levels, i) zooplanckton (*Dapnhia magna*) and ii) plants (*Lipidium sativum*). The 24–48 h immobilization of *D. magna* bioassays were performed following the Standard Operational Procedures of Daphtoxkit F^{TM}. The toxicity was measured as the immobilization of *D. magna* according to the procedures OCED Guideline 202(5). The bioassay with *L. sativum* evaluated the potential toxicity considering the root elongation according to OECD Guideline 208(6).

Results & Conclusions

Strain F11 was able to degrade 95% of initial CBZ concentration during 30 days experiment. Supplementation with acetate increased degradation to 100% in 24 days. A group of 12 TPs formed in the microbial process were identified; CBZ degradation by strain F11 proceeds mainly by oxidation, hydroxilation and cleavage of the aromatic ring. The effect of whole biodegradation products on root elongation of *L. sativum* was practically neglectable; however the same exhibited toxicity to *D. magna*. Strain *Labrys portucalensis* F11 proved to be able to degrade CBZ and may be potentially useful for biotechnological applications.

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V.S. Bessa, I.S. Moreira and C. Piccirillo wish to acknowledge research grant from Fundação para a Ciência e Tecnologia (FCT), Portugal (Ref. SFRH/BD/90146/2012, SFRH/BPD/87251/2012 and SFRH/BPD/86483/2012 respectively) and Fundo Social Europeu (Programa Operacional Potencial Humano (POPH), Quadro de Referência Estratégico Nacional (QREN)). This work was supported by FCT through the projects PTDC/EBB-EBI/111699/2009, CEQUIMED-Pest-OE/SAU/UI4040/2014, and PEst-OE/EQB/LA0016/2013, and by a bilateral collaboration between CNR-Italy and Portugal (N. 0022729).

Keywords: Carbamazepine, Labrys portucalensis F11, Biodegradation, Degradation pathway, Toxicity

P-091 - BACTERIAL COMMUNITY AND AND ANTIBIOTIC RESISTANCE DYNAMICS IN A FULL-SCALE WASTEWATER TREATMENT PLANT WITH UV DISINFECTION

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Background
The removal of potentially hazardous microorganisms, as bacteria harbouring acquired antibiotic resistance genes (ARB&ARG), from wastewater is one of the major aims of the treatment. In wastewater treatment, dramatic variations on the bacterial community structure may occur and it is expected that these variations may have an important role on ARG selection.
An urban wastewater treatment plant (UWTP) using activated sludge as biological treatment and UV for disinfection was studied aiming at: i) assessing the dynamics of bacterial lineages during the whole process; ii) measuring of the variations on the abundance of ARGs after each treatment step; iii) inferring if population changes might be associated with ARGs distribution.

Method
Samples from the raw influent, secondary (activated sludge) and tertiary (UV disinfection) treated effluent were collected from an urban wastewater treatment plant, in three dates. Tertiary effluent was also analyzed after 3 days of incubation. Samples were analyzed for cultivable enterobacteria counts, 16S rRNA gene-based of microbial community and quantification of 8 ARGs using quantitative PCR.

Results & Conclusions
The stages of activated sludge and UV disinfection led to ~2 log-units reductions of enterobacteria CFUs. The bacterial community composition suffered the most intense rearrangements after secondary treatment, rather than after UV disinfection. In addition, quantitative PCR of ARGs showed a reduction of ~2 log-units after the secondary treatment and a negligible variation after UV disinfection. The effect of treatment was not identical for all ARGs examined, an observation that was consistent with the fact that different genes were most correlated with distinct bacterial populations. For instance, members of *Bacteroidaceae*, *Lachnospiraceae*, *Campylobacteraceae, Aeromonadaceae*, *Enterobacteriaceae* and *Moraxellaceae* were correlated with beta-lactamase and *qnrS* genes, while members of *Comamonadaceae*, *Neisseriaceae* and the classes *TM7-1* and *ZB2* were correlated with the gene *sul2*.

Keywords: antibiotic resistance, wastewater treatment, UV disinfection, antibiotic resistance genes, antibiotic resistant bacteria

P-092 - YEAST-BASED BIOCONTROL OF THE CACAO PLANT FUNGAL PATHOGEN CAUSING THE WITCHES' BROOM DISEASE

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Background
The cacao tree plant (*Theobroma cacao*) is severely affected by a devastating fungal disease (*Witches' Broom Disease*) caused by the basidiomycete *Moniliophthora perniciosa*, with a huge socio-economic impact on more than 25 million people in poor rural areas worldwide. Several methods to overcome this fungi lethal effect have been tested, though proven ineffective. Most of these methods are based on chemical treatments, which use is increasingly restricted, in particular due to the increasing advent of resistance, as well as the risks associated with cacao chemical contamination. Due to this scenario, new strategies to control the disease are required, including the use of biological control agents. One such group of organisms are the so-called killer yeasts, which cells secrete peptides (killer toxins) that are lethal to specific sensitive strains of other yeasts, as well as bacteria or fungi. Although this is a long-known phenotype, killer yeasts have seldom been applied by the industry. The antagonism of *M. perniciosa* by yeasts has also been reported[1]. The objective of the present work is to evaluate the potential of bioethanol industry-derived yeast strains as antagonists of *M. perniciosa*. The optimized strains and environmental conditions determined at laboratory scale, constitute an indispensable step for the development of an environmental friendly, sustainable biocidal-based process to control the spread of the disease and to treat infected cacao plants *in field*.

Method
Well-known killer strains as well as common yeast strains originating from Brazilian bioethanol and *cachaça* fermentation industry were used to test for biocidal activity against strains of *M. perniciosa* originating from infected fields from several producing countries. Antagonism was evaluated at different pH, in several rich and synthetic media, and in solid and liquid culture conditions.

Results & Conclusions
Results from these combinations revealed significant fungal growth inhibition by some of the yeast strains, very in particular the industrial ones.
These results are very promising as to select an economically interesting biocontrol agent for in field application against contaminated plants and/or prevention of the *Witches' Broom Disease*.

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This work is supported by the strategic programme UID/BIA/04050/2013 (POCI-01-0145-FEDER-007569) funded by national funds through the FCT I.P. and by the ERDF through COMPETE2020 – Programa Operacional Competitividade e Internacionalização (POCI) and by the project EcoAgriFood (NORTE-01-0145-FEDER-000009), supported by Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF).

PF acknowledges FCT for PD/BD/113814/2015 grant.

Keywords: Antagonism, Biocontrol, Fungi, Yeast, Cacao, M. perniciosa

P-093 - BIOCHEMICAL CHARACTERIZATION AND GENOMIC DECIPHERING OF SIDEROPHORES PRODUCED BY ENDOPHYTIC BACTERIA ISOLATED FROM PINUS PINASTER AFFECTED BY PINE WILT DISEASE

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Background

The massive use of chemicals has impacted farmer's economy and has also decreased environmental quality. Besides chemical compounds, the use of plant growth promoting bacteria (PGPB) has been suggested as an alternative to the classical strategies. *Pinus pinaster* trees affected by Pine Wilt Disease, caused by pinewood nematode (PWN), had an incredible endophytic diversity [1]. Endophytes may act as nematicidal and/or as PGPB by fixing atmospheric nitrogen, synthesizing phytohormones and enzymes, and sequestering iron from the soil.

Method

277 bacterial strains isolated as endophytes of *P. pinaster* trees from Portugal [1], 61 bacterial strains carried by PWN from Portugal [2] and 38 bacterial strains carried by PWN from USA [3] were evaluated for their production of siderophores on Iron-CAS agar plates [4]. The DNA of the best strains Arv20#4.1 and A41C3 was extracted and their genomes were sequenced by Illumina MiSeq, followed by assembly and annotation. Culture supernatants were assessed for siderophore activities by a modified CAS assay [5], to determine Fe, Ga, Cu, V and Al chelating compounds. Catecholate and hydroxamate siderophore production was performed [6,7]. Siderophores in supernatants were purified by HPLC. The resulting 30 fractions were characterized for metal chelation and siderophore type. The best candidates are under identification by LC-MS and MS/MS.

Results & Conclusions

The genomes of both strains showed genes involved in the production of siderophores as well as several genes that are involved in plant growth promotion. Siderophores production by both strains is dependent on days of growth. Strain Arv20#4.1 produced hydroxamate-type siderophores (25 µM) while strain A41C3 produced catecholate-type siderophores (70 µM) and showed more affinity to iron or copper, respectively. However, the best supernatant fraction of strain Arv20#4.1 (92 µM of NH_2OH equivalents) and of strain A41C3 (309 µM of DHBA equivalents) showed more affinity to chelation of Cu and Fe, respectively. That may suggest that strains produced different siderophores since other fractions showed different metal chelation ratios. Strains Arv20#4.1 and A41C3 showed siderophores with different metal chelation and different structures, hydroxamate and catecholate, respectively. Together with genomic characteristics, these strains need to be explored as PGPB and nematicidal potential towards the PWN.

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This work was supported by COST Action FP1305. D.N.P. was supported by FCT, postdoctoral fellowship SFRH/BPD/100721/2014.

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Keywords: Siderophores, Genome, Endophytes, Pine Wilt Disease

P-094 - BACTERIAL AND ARCHAEAL COMMUNITIES INHABIT THE SEDIMENTS FROM THE GULF OF MEXICO IN SIMILAR PROPORTIONS

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Background
Marine sediments are home to highly abundant and diverse microbial communities. However, due to its vastness, this environment still remains poorly studied. The Gulf of Mexico, an ocean basin characterized by both shallow waters and deep-water areas that can reach depths of up to 4,000 m, is no exception. Even though there are some studies describing the biological diversity of the Gulf of Mexico, very few of them involve microbial communities in the sediments, particularly from deep areas. In order to establish a baseline of the diversity of microorganisms living in sediments of the Southern Gulf of Mexico and to shed some light into the unknown microbial communities of the area, an oceanographic campaign (XIXIMI-04) was conducted during August of 2015.

Method
Several sediment cores were taken from 10 different locations and different depths, ranging from 1,500 to 3,500 m. In addition, the sediment cores were divided into three sub-samples: 0-5 cm, 5-10 cm and 30 cm o more. Total DNA was extracted and 16S rRNA metagenomic sequencing libraries were prepared and sequenced using the Illumina MiSeq technology and analyzed under QIIME pipeline.

Results & Conclusions
Preliminary results show slight differences across sampling sites at phylum level composition, in the first 10 cm. Noteworthy, microbial communities are clearly different from the samples taken from the deepest part of the sediment core. Interestingly, Archaea appears highly abundant, reaching 50% of the total prokaryotic community in some cases. Although these percentages are high at all locations and depths, different phyla were identified from the bottom of the sediment. Among them, the recently found candidate phylum *Lokiarcheota*, which represents a 20% of the total prokaryotes in deep sediments, was an interesting finding. Bacteria were mainly represented by *Proteobacteria* as the most abundant group (~70% in absolute values); followed by *Firmicutes* and *Actinobacteria* as the 2nd and 3rd most abundant phyla, respectively. Most changes among samples were observed at the class level, and some of the groups found suggested the presence of either methane seeps or hydrocarbon leakage at that point.

References & Acknowledgments

Research funded by the National Council of Science and Technology of Mexico - Mexican Ministry of Energy - Hydrocarbon Trust, project 201441. This is a contribution of the Gulf of Mexico Research Consortium (CIGoM).

Keywords: metagenomics, sediments, Lokiarchaeota

P-095 - ENDOPHYTIC BACTERIA FROM AROMATIC PLANTS AND THEIR POTENTIAL FOR BIOINOCULATION ON GREEN ROOFS VEGETATION

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Background

Green roofs are a type of multilayer construction that uses vegetation on top of buildings. In the last decade, their use has become more frequent due to the environmental advantages they offer in impervious urban areas, regarding stormwater retention, removal of atmospheric pollutants, attenuation of the urban heat island effect, among others. Selection of vegetation able to growth on the harsh environment of a rooftop is of major importance for the successful establishment of a green roof. The use of plant growth promoting bacteria (PGPB) on green roofs is a sustainable alternative to aid plant establishment and growth.

Method

Endophytic bacteria were isolated from *Lavandula dentata* L. plants collected at random from "Cantinho das Aromáticas". These isolates were characterized for their *in vitro* growth promoting traits. Two green roofs pilot systems were further established, comprising inoculation with a selected mixture of four isolated endophytic bacteria. Four different aromatic plant species (*Santolina chamaecyparissus*, *Santolina Lemon Queen*, *Armeria maritima*, *Festuca glauca*) and a succulent species (*Sempervivum tectorum*) were used for experiments. Plant development is being followed.

Results & Conclusions

A total of 56 culturable endophytic bacteria were isolated from the plant tissues of *L. dentata* plants corresponding to 38 different bacterial strains. All endophytic strains exhibit growth promoting traits and 21% were found to produce more than 40 mg/L of IAA. The strains *Pseudomonas graminis* (LR 1-9), *P. congelans* (LS 2-1) and *Bacillus aryabhattai* (LS 1-2) were amongst those that exhibited higher IAA levels and were selected for inoculation. *Paenibacillus kribbensis* (LR 2-11) was selected due to its antifungal activity. The vegetation used presented in general successful establishment and growth. The potential of bacterial endophytes as bioinoculants in green roofs vegetation is under analysis.

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Cristina M. Monteiro (SFRH/BPD/80885/2011) thank the grant from Fundação para a Ciência e Tecnologia (FCT, Portugal) and Fundo Social Europeu through the program POPH – QREN. S.I.A. Pereira received an individual research contract within the project "Biological tools for adding and defending value in key agro-food chains (bio – n2 – value)", nº NORTE-01-0145-FEDER-000030, funded by FEDER, under Programa Operacional Regional do Norte - Norte2020. This work was supported by National Funds from FCT-Fundação para a Ciência e Tecnologia through project UID/Multi/50016/2013. Authors thanks to SIRO and LANDLAB for the materials supplied.

Keywords: green roofs, aromatic plants, endophytic bacteria, plant growth promoting bacteria

P-096 - BIOPROSPECTING METAGENOME FROM PORCELLIO DILATATUS (CRUSTACEA,ISOPODA) GUTS FOR PLANT BIOMASS HYDROLYTIC ENZYMES

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Background

Second generation biofuels are renewable, more consensual, environmental friendly alternative and ideally cost-effective solutions of energy, since they are produced from plant-cellulosic-biomass (PCB) of wood and organic wastes components(1). However, due to the very complex and recalcitrant sugar polymers present in PCB, new and more economical strategies are needed (1). Terrestrial isopods *Porcellio* (*Crustacea*:*Isopoda*) plays a significant role on PCB decomposition in terrestrial ecosystems, and its hindgut are considered "hot-spots" for microbial-cellulolytic-degraders populations(2). The aim of present work was to identify novel and more efficient PCB-degrading enzymes namely cellulases, xylanases and chitinases, by culture-dependent (isolates and consortia) and -independent (metagenome library) methodologies.

Method

Porcellio dilatatus specimens were collected from different geographic sites of Portugal. Isopod hindguts were aseptically removed from the rest of the digestive tract. Samples were used for 1) Genomic DNA extraction for construction of clonal metagenomic library and for metagenomic 16S rDNA Illumina tags analyses; 2) Isolation of microbial consortia and bacteria with enhanced PCB-degrading activity.

Results & Conclusions

The metagenomic 16S rDNA Illumina tags analyses results provided evidences that the microbial diversity changes between isopods of different locations. From the 28.000 metagenomic clones screened, six clones shown cellulolytic activity and two clones had chitinolytic activity. Their inserts were sequenced and classified by BLASTx analysis. Blastx results showed similarity with cellulase C, endopeptidase; β-galactosidase, NADH dehydrogenase subunit 1 and hypothetical proteins. From 28 enriched cultures, 21 consortia demonstrated cellulolytic activity, 12 demonstrated xylanolytic activity and 4 consortia had chitinolytic activity. From a total of 157 colonies isolated from different enriched cultures, 14% of isolates showed cellulolytic activity, 12% of isolates showed xylanolytic activity, chitinolytic activity were not detected. Isolates were grouped by RAPD and representative strains were identified by 16S rRNA gene phylogeny. Isolates were related to strains of the genera *Pseudomonas, Flavobacterium and Roseomonas*. These results demonstrate that *Porcellio dilatatus* specimens can be a valuable source of PCB-degrading enzymes. The application of culture-dependent and -independent methodologies produced important outputs that can have applicability in different contexts.

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C.Coelho acknowledges a scholarship Project IN0756 -INV.EXPLORATORIA-IF/01061/2014. I.Tiago acknowledges an Investigator contract reference IF/01061/2014.

Keywords: Metagenome, Isopods, PCB-enzymes

P-097 - DEEP-BIOSPHERE METAGENOME MINING: FINDING BIOTECHNOLOGICAL SOLUTIONS FOR THE FUTURE IN CABEÇO DE VIDE DEEP-AQUIFER

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Background

Plant cellulosic biomass (PCB) constitute an important environmental friendly feedstock to produce second generation biofuel. However, PCB is composed by very complex recalcitrant sugar polymers, and that constitutes a barrier to its utilization. Nevertheless the use of recent molecular approaches has produced new promising PCB-degrading enzymes with considerable application potential.

The groundwater found in Cabeço de Vide deep-aquifer (CVA) has a distinct chemical composition and high pH value (11.4). Previous results shown that the major populations detected in CVA comprise uncultured microbial groups phylogenetically related to *Clostridia*, *Betaproteobacteria* and *Actinobacteria*. Most likely, the extreme conditions of CVA selected specially adapted microbial populations, that may be an interesting source of novel PCB-degrading enzymes. The aim of this work was to determine the potential of CVA as a source of novel PCB-degrading enzymes. The results of a recent metagenomic survey conducted in CVA - shotgun metagenome - was used as a database for screening for PCB-degrading enzymes.

Method

Six-hundred liters of CVA water were concentrated by filtration, total DNA was extracted and shotgun metagenome was obtained at Illumina platform (116.354.069bp). The de novo metagenomic assembly yielded 78734contigs, 16343 contigs larger than 1,500kb, N50 of 1,916bp, average 1.478bp, with 1.024 contigs longer than 10kb, the longest being 161,094bp. CVA database composed by 166379 translated protein sequences was used as database for local BLASTP with query files comprising proteins sequences of interest: namely laccases, peroxidases, cellulases, xylanases and chitinases.

Results & Conclusions

From 40771 hits, only protein sequences that showed e-value lower than 10^{-5}, start and stop codons and more than 1500bp were selected and classified by BlastP analysis. Of 2256 protein sequences analyzed around of 34% are putative laccases and peroxidases, 19% are putative cellulases, 2.35% are putative xylanases and 43% are putative chitinases. BlastP results showed similarity with glycoside hydrolases (GHs), catalase/peroxidase HPI, xylulose kinase, 1,4-beta-xylanase, endocellulases, chitin deacetylases and hypothetical proteins. The obtained results showed that this kind of environment may represent a hotspot for new PCB-degrading enzymes. Future work will include PCR amplification of selected genes with metagenomic DNA as template, and functional expression of the proteins to determine their enzymatic characteristics.

References & Acknowledgments

C. Coelho acknowledges a scholarship Project IN0756 -INV.EXPLORATORIA-IF/01061/2014. I. Tiago acknowledges an Investigator contract reference IF/01061/2014.

Keywords: Metagenome, PCB-degrading enzymes, Deep-biosphere

P-098 - FECAL CONTAMINATION INDICATORS IN DOURO ESTUARY

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Background

Estuaries are highly dynamic systems that face increased anthropogenic impacts due to human population growth and global climate changes. Consequence of unregulated urbanization, the contamination of the water resources by fecal material can become a potential severe human health hazard.

Method

In order to evaluate fecal contamination in a large metropolitan area, the lower, middle and upper areas of the Douro Estuary (NW Portugal) were seasonally sampled for fecal indicators (*Escherichia coli* and intestinal enterococci) between the spring of 2016 and the winter of 2017, using a selective culture medium approach (ChromoCult® Coliform agar and Slanetz & Bartley agar respectively). Also, spatial (along the water front) and temporal (monthly, weekly, daily, and hourly) scales of water quality were assessed, using the same indicators, at the estuarine beach of Zebreiros, during the 2016 bathing season.

Water column key environmental parameters (temperature, conductivity, turbidity, salinity, dissolved oxygen, and pH) were screened using a multiparameter YSI 6920 probe. Water column samples were retrieved at three depths (surface, middle and bottom) and analysed for total and organic carbon, total nitrogen, nutrient concentration (nitrates, nitrites, ion ammonium and phosphates), photosynthetic pigments (chl*a*), and direct microscopic count of microbial cells.

Results & Conclusions

Along the Douro estuary, water quality generally worsened downstream, with fecal contamination observed all year around. Higher values of both fecal indicators were found in the winter season, probably due to run-off, and/or over-flow of wastewater treatment plants, mainly located in the middle and lower estuary. Regarding Zebreiros beach, the microbiological descriptors followed the hourly tidal dynamics, with no daily, weekly, or monthly patterns. Fecal indicators deteriorated with tidal rising, probably due to tidal upstream currents carriage.
Our results emphasize the importance of understanding the dynamics of estuarine systems and the relevance of microbiological indicators monitoring. The obtained results can be used to draw policy recommendations to improve water quality assessment in estuarine environments and to reduce the potential hazard of estuarine beaches users.

References & Acknowledgments

This study was partially supported by the Project INNOVMAR (Reference NORTE-01-0145-FEDER-000035), Research Line ECOSERVICES, supported by the Northern Portugal Regional Operational Programme (NORTE2020), through the European Regional Development Fund (ERDFi); and the Strategic Funding UID/Multi/04423/2013 through national funds provided by FCT – Foundation for Science and Technology and European Regional Development Fund (ERDF), in the framework of the programme PT2020.

P-099 - ARBUSCULAR MYCORRHIZAL FUNGAL SPECIES DIFFER IN THEIR CAPACITY TO OVERRULE THE SOIL LEGACY OF MAIZE MONOCROPPING

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Background
Arbuscular mycorrhizal fungi (AMF) are promoted as biofertilizers for cleaner agricultural production. So far, most researchers have investigated the effects of AMF on plant growth under highly controlled conditions with sterilized soil. Therefore it is still poorly documented how the soil biotic context shapes AMF's impact on host plant performance.

Method
To focus on the impact of belowground interactions (plant-AMF-soil microbes) alone, we compared sterilized *versus* non-sterilized soil. We inoculated maize (*Zea mays* ssp. *mays*) seedlings with five commercial AMF inoculants (*Claroideoglomus claroideum*, *Funneliformis mosseae*, *Gigaspora* sp., *Rhizophagus irregularis* and *Scutellospora* sp.). Plants were pot-cultivated for nine weeks using soil which had been used for maize monocropping in the field.

Results & Conclusions
AMF inoculation was successful, despite an abundant native AMF community. As hypothesized: i) the soil biotic context interfered with AMF's benefits on maize growth; ii) AMF's benefits depended on the AMF species; and iii) *C. claroideum*, *F. mosseae* and *Gigaspora* sp. overruled the soil legacy of maize monocropping. We found little to no effects of AMF inoculation on maize growth and nutrients acquisition when plants were grown in sterilized soil. AMFs benefits to the host plants could not be explained by improved nutrition alone, because interaction with the remainder soil microbes also differed between inoculated AMF. Data show that the soil biotic context and AMF species should be taken into consideration when applying AMF inoculants in agriculture.

Keywords: AMF, maize, microbial community, nutrients, feedback

P-100 - THE ENDOPHYTIC MICROBIOME OF HALIMIONE PORTULACOIDES

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Abstract
Halimione portulacoides is a salt marsh plant that can participate in sediment remediation processes by sequestering contaminants into its tissues, and has been proposed as a biomonitor for mercury contamination (Válega et al., 2008). The holobiont perspective states that the entity composed by the plant host and its microbiome works together towards evolutionary fitness, revealing the importance of the plant microbiome (Vandenkoornhuyse et al., 2015). Endophytic bacteria colonize the inner tissues of the plant host without causing damage or eliciting a strong defense response (Quispel, 1992). Our aim was to achieve a deep understanding of the endophytic bacterial diversity present in aboveground and belowground tissues of *H. portulacoides* from Ria de Aveiro. To accomplish this, fifteen healthy specimens of *H. portulacoides* were collected from a salt marsh in Ria de Aveiro, surface-sterilized and analyzed for their taxonomic diversity of endophytic bacteria using Illumina technology for culture-independent 16S rRNA gene sequencing.

Results & Conclusions
In order to decrease interference of host DNA in the analysis, peptide nucleid acid (PNA) blockers were included in the 16S rRNA gene amplification reaction (Lundberg et al., 2013). The methodology used allowed for a high sequencing coverage for all samples (median 97.5 %) and revealed a high operational taxonomic units (OTUs) richness (median of 200 OTUs per sample). Results showed significant differences in the bacterial community structure according to sampling tissue ($p<0.05$). Belowground tissues presented higher OTU richness and diversity ($p<0.05$). Overall, the main phyla observed in the endosphere of the halophyte were Proteobacteria (62.5 % OTUs), Bacteroidetes (10.7 %), Planctomycetes (8.8 %), Actinobacteria (5.2 %) and Firmicutes (2.4 %). Core endophytic microbiome analyses performed using MetaCoMET (Wang et al., 2016) revealed that the aboveground tissues mainly comprised members of the family *Oceanospirillaceae*, and also included the families *Flammeovirgaceae*, *Enterobacteriaceae* and *Flavobacteriaceae*, while the belowground endophytic microbiome was dominated by *Enterobacteriaceae* and *Kiloniellaceae*. This work explored the diversity of the bacterial endophytic microbiome of *H. portulacoides* in depth and confirmed it as a bacterial hotspot. Putative ecological functions of the dominant taxa revealed a community that presented several plant growth promotion traits.

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This work was supported by European Funds (FEDER) through COMPETE and by National Funds through the Portuguese Foundation for Science and Technology (FCT) within project PhytoMarsh (PTDC/AAC-AMB/118873/2010 – FCOMP-01-0124-FEDER-019328). FCT is acknowledged for financing to CESAM (UID/AMB/50017/2013), Artur Alves (FCT Investigator Programme – IF/00835/2013), Isabel Henriques (FCT Investigator Programme – IF/00492/2013), Cátia Fidalgo (PhD grant – SFRH/BD/85423/2012), and Marta Alves (PhD grant – SFRH/BD/92999/2013).

Keywords: Endophytes, bacteria, halophytes, salt marsh, Illumina, PNA

P-101 - SULFIDE OXIDATION BY HETEROTROPHIC P. KOREENSIS A9 UNDER AEROBIC CONDITIONS.

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Background

Biological oxidation can be an efficient solution for bioremediation of sulfide contaminated gases and wastewater [1,2]. Microorganisms able to grow chemolithotropically on sulfide compounds are known. Knowledge on heterotrophic sulfur-oxidizing bacteria is scarcer. However, heterotrophs are more metabolically versatile [3]. Heterotrophic bacteria perform sulfide oxidation by the concerted action of three enzymes: sulphide: quinone oxidoreductase (SQR), persulfide dioxygenase (DOP) and rhodanese [1,2,3].

Method

Bacterial strain A9 was isolated from a sulfide enrichment inoculated with the filling of biofilter from a WWTP. After the isolation, the strain was tested for tolerance and degradation of sulfide. Crude extract-cells activity assays were also performed. Sulfide disappearance was monitored by direct potentiometry and sulfate production. The sulfide oxidase activity was measured based on sulfate production, as the major end-product [5].
Total genomic DNA was sequenced by 454-pyrosequencing. The genome was annotated using Rapid Annotation System Technology (RAST) [6], and functional annotation was performed using SEED, SWISS-PROT, COG and KEEG databases.

Results & Conclusions

Maximum sulfide removal (99,8%) and degradation rate (1,6 $mmol.h^{-1}$) was achieved in GY medium with 16 mM of sulfide. In activity tests using crude cellular extract and 10mM of sulfide, maximum sulfate concentration achieved was 0,858 mM, corresponding to enzyme activity of 6,435 $\mu mol\ h^{-1}$. The specific enzymatic activity ranged 1,828 to 2,927 U. mg^{-1} protein, which are very promising results when compared to bibliography [1, 5]. DNA sequencing of *P. koreensis* A9 and *de novo* assembly, generated the 6376154 bp draft genome with 60,1 % of G+C content. After assembly, 73 contigs with protein encoding genes were produced. RAST annotation disclose that this genome encodes 5738 predicted coding genes, 50% of the detected proteins were annotated in 542 sub-systems from SEED [6]. Functional screening analysis reveals that A9 possesses the genes required for sulfide oxidation to sulfate, with sulfite as the intermediate. In addition, rhodanese-like, glutathione:sulfur transferase, thiol peroxidase, among others, were identified which demonstrate the ability and versatility of A9 for sulfide bioremediation applications.

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Ana Novo and Irina S. Moreira wish to acknowledge the FCT (Fundação para a Ciência e Tecnologia, Portugal) funding's under the grants with references SFRH/BD/105089/2014 and SFRH/BPD/87251/2012 respectively.The work was supported by National Funds also through the projects HBioS (QREN-ADI 21592) and UID/Multi/50016/2013.

Keywords: Hydrogen sulfide, Heterotrophic sulfur oxidation, Pseudomonas koreensis A9, Genome analysis

P-102 - EFFECT OF THE INOCULATION OF PLANT GROWTH PROMOTING BACTERIA ON MAIZE PLANTS TO IMPROVE THEIR GROWTH UNDER DROUGHT STRESS

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Background

According to the United Nations, by 2050 the world population will reach approximately 9.6 billion people, which implies a major challenge for the agricultural sector to improve crop production and to ensure food availability in the near future. However, global warming and bad agricultural practices are impairing food productivity. Drought is one of the major limiting factors for plant growth, causing dehydration and nutrient deficiency. Therefore, the application of sustainable practices in agriculture is essential to maintain soil fertility and to increase crop productivity. It has been demonstrated that plant growth promoting bacteria (PGPB) have positive effects on plants growing under drought conditions.
The aim of this work is to evaluate the effect of two PGPB inocula size on maize growth and nutrient content under different water regimes (80, 60 and 40% of soil water holding capacity (WHC)).

Method

Thirteen PGPB were screened *in vitro* for osmotic tolerance and indole acetic acid (IAA) production under different osmotic potentials. Strains *Pseudomonas fluorescens* S3X and *Ralstonia eutropha* 1C2 were inoculated in maize under greenhouse conditions. Different bacterial treatments and two inocula sizes (2.5×10^6 and 3.3×10^3 cell. g^{-1} dry weight) were applied on plants growing at 80, 60 and 40% of soil WHC. Plants were harvested after 13 weeks. Dry biomass and the accumulation of N and P on roots and shoots were determined. The activity of soil enzymes, such as catalase, and the fluorescein diacetate (FDA) hydrolysis were also evaluated.

Results & Conclusions

Maize growth was affected by the water regimes applied, especially at 40% of WHC. Bacterial inoculation enhanced some biometric parameters at 80 and 60% of WHC, however, the effects observed were similar for both inocula size applied. It was observed a severe reduction of FDA activity at 40% WHC, which indicates that the microbial activity was negatively affected by low soil moisture.

References & Acknowledgments

This work was supported by National Funds through FCT under the project UID/Multi/50016/2013. S.I.A. Pereira received an individual research contract within the project "Biological tools for adding and defending value in key agro-food chains (bio – n2 – value)", nº NORTE-01-0145-FEDER-000030, funded by Fundo Europeu de Desenvolvimento Regional (FEDER), under Programa Operacional Regional do Norte - Norte2020. A. Vega had the support of PhytoSudoe grant SOE1/P5/E0189 funded by FEDER, under Programa INTERREG SUDOE. H. Moreira had the support of FCT grant SFRH/BPD/105152/2014. D. Abreu thanks to ADP Fertilizantes for finantial support.

Keywords: plant growth promoting bacteria, drought stress, maize

P-103 - INSIGHTS ON DIAZOTROPHIC BACTERIA AND WHEAT SYMBIOTIC INTERACTIONS BY FTICR-MS PROFILING

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Background

The world population is increasing rapidly and the need for food production will increase accordingly. The sustainability of food production can be accomplished with a reduction of the use of fertilizers and an increase of Nutrient Use Efficiency (NUE), which can be improved by using plant-growth-promoting microorganisms (PGPM). The interaction between plants and microorganisms is mediated through a chemical cross-talk poorly understood. Root exudates represent an important source of nutrients and signals that allow the plant to select the most suitable partners, which probably influence the root microbial assemblage. Hence, it is important to consider the complex communication between roots and bacteria during the first stages of interaction. During this interaction there are a large abundance of small compounds produced. In this work we aimed to characterize the chemical interactions between wheat roots and PGPM during the first stages of rhizosphere assemblage by FTICR-MS.

Method

We characterized the metabolites present in the growth media of 4 diazotrophic bacteria grown in pure culture or co-culture with roots of one of four wheat varieties. We tested two bacteria isolates of *Azospirillum brasilense* (one endophytic and other with associative lifestyle), one of *Herbaspirillum seropedicae* (endophytic), and one of *Herbaspirillum rubrisubalbicans* (associative), in interaction with wheat varieties including new and conservation cultivars of *Triticum aestivum* and *Triticum durum*. Seeds were surface sterilized and germinated in liquid medium. Media were collected after 7 days of growth, sterilized, and stored at -20° C until analysis. FTICR-MS (Fourier-transform ion cyclotron-resonance mass spectrometry) was used to analyze the metabolites present in exudates. A PCA was performed to compare the compounds profile of the various interactions.

Results & Conclusions

Around 280 compounds, with molar mass between 135 and 700 Da, were detected in the 24 experimental situations analyzed. Root exudates were more diverse than those of bacteria, although very affected by their presence, which highlights the importance of root exudates as substrates and signaling molecules in the crosstalk plant-bacteria. The PCA allowed the distinction between wheat varieties, bacteria strains and their interactions, emphasizing the interaction specificity. The analysis of metabolic pathways found active during the interaction evidenced those involved in organic acid metabolism, particularly the α-linolenic acid pathway. This work showed the potential of FTICR-MS as a powerful fingerprinting technique to distinguish bacteria/wheat interactions and shed light on important compounds.

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Keywords: Diazotrophic bacteria, Wheat varieties, Symbiotic interactions, FTICR-MS

P-104 - INTER-KINGDOM BIOFILM FORMATION BETWEEN BACTERIA AND FILAMENTOUS FUNGI ISOLATED FROM A DRINKING WATER DISTRIBUTION SYSTEM

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Background

The main challenge to the drinking water (DW) industry is to deliver a product that is microbiologically and chemically safe, aesthetically pleasing and adequate in quantity and delivery pressure. Biofilms constitute one of the major microbial problems in DW distribution systems (DWDS) that most contribute to the deterioration of water quality. Knowledge on DW biofilms has been mainly obtained from studies on bacterial biofilms even though, under natural conditions, they are usually viewed as complex communities where different organisms are present, including filamentous fungi (ff). Studies regarding ff biofilms are scarce despite their ability to form complex and multicellular biofilms [1, 2]. Diversity in microbial communities leads to a variety of complex relationships involving interspecies and intraspecies interactions that need to be understood. The aim of this study was to assess the dynamics of inter-kingdom biofilm formation between commonly detected ff and bacteria in DWDS.

Method

The ff *Penicillium expansum* and the bacteria *Acinetobacter calcoaceticus* and *Methylobacterium oryzae* [3] were used as model species. Biofilm formation was performed using microtiter plates with rotatory movements mimicking water flow behaviour in DWDS. Biofilms were analysed at different times in terms of biomass using crystal violet staining, metabolic activity was determined by the resazurin reduction assay and, morphology by epifluorescence, using calcofluor white M2R and DAPI, and bright field microscopies.

Results & Conclusions

The results confirmed that each individual species forms biofilms at 24, 48 and 72h with increasing biomass over time. Metabolic activity was higher at the 24h biofilms and then decreased overtime. Regarding mixed species biofilms, metabolic activity was higher when compared to single species biofilms at 24h and similar for the 48 and 72h biofilms. The results provided by microscopies allowed the understanding of the distribution of specific organisms in inter-kingdom biofilms.

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Afonso T.B. and Simões L.C. acknowledge the grants provided by the Portuguese Foundation for Science and Technology (FCT) under grants with the references PD/BD/128033/2016 and SFRH/BPD/81982/2011 respectively.

Keywords: Inter-kingdom biofilms, filamentous fungi, bacteria, drinking water distribution systems

P-105 - FLOW CYTOMETRY FOR THE ASSESSMENT OF MICROBIOLOGICAL CONTAMINATION OF OUTDOOR SCULPTURES

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Background
Urban outdoor public sculptures are some of the most vulnerable cultural objects due to their constant exposure to pollution and changing atmospheric conditions. The physicochemical changes undergone by these cultural objects over time and the variety of microorganisms growing on their surfaces make conservation treatments difficult and lead to the loss of the object's value [1, 2]. An assessment of the microbial contamination and biodeterioration status of outdoor sculptures must firstly be done in order to devise innovate solutions.

Method
In this work, we used flow cytometry as a quick and effective method to determine the total number of microorganisms present in samples collected from a granite sculpture from *Museu Internacional de Escultura Contemporânea de Santo Tirso* (MIEC), in Santo Tirso, Portugal. The samples were collected from five different sites of the sculpture using a swab as a non-invasive method, and suspended in peptone water with 1% Tween 80. A protocol adapted from [3] was chosen to eliminate debris and prepare the samples for analysis in a flow cytometer (BD Biosciences, USA). The samples were analyzed without adding any probes to determine the presence of autofluorescent microorganisms. Cell viability was also determined after addition of thiazole orange (TO) and propidium iodide (PI) (BD™ Cell Viability Kit, BD Biosciences, USA).

Results & Conclusions
Flow cytometry is an efficient technique for the determination of the presence of microorganisms on the surface of outdoor granite sculptures. Autofluorescence was detected when the samples were analyzed without the addition of probes, which could indicate the presence of microalgae and other autofluorescent microorganisms. Cell viability was determined after addition of TO and PI, which demonstrated that the percentage of live cells corresponded to a range of 3% to 37%, depending where the samples were collected from the sculpture. In conclusion, flow cytometry could be used in the future for a rapid quantification of the microorganisms present in stone sculptures and their cell viability, for application in studies concerning microbial contamination and biodegradation in Cultural Heritage.

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Aknowledgements: Financial support was provided by FCT-Fundação para a Ciência e Tecnologia through the project BIONANOSCULP (PTDC/EPH-PAT/6281/2014).

Keywords: preventive conservation, flow cytometry, biocontamination, sculpture

P-106 - HUNTING FOR SOIL NITRIFYING PROKARYOTES: FROM THE NIGHTMARE OF ISOLATION TO THE DREAM OF PHYSIOLOGICAL AND MOLECULAR CHARACTERIZATION

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Background
Nitrification, the aerobic oxidation of ammonia to nitrate through nitrite, is a chemolitotrophic energy acquiring mechanism performed by ammonia-oxidizing bacteria and archaea (AOM) and by nitrite-oxidizing bacteria (NOB) [1]. This process brings about serious ecological, agricultural and economic impacts. However, a lot about nitrifiers is still unknown, mainly due to their extremely slow growth and the difficulties associated with isolation [2].

Method
Enrichment and isolation of nitrifiers and NGS-based microbial profiling analysis was performed for 10 distinct Portuguese soils. Selective enrichment of AOM was achieved using $(NH_4)_2SO_4$ (0.2 mM to 20 mM) and urea, to cope with ammonia tolerance and urease production, and spectinomycin addition to enrich archaea. Medium with nitrate was used to select NOB. Enrichments were monitored by measuring ammonium, nitrite and nitrate and genes involved in N-metabolism were detected through PCR. After 175 days, enrichments without antibiotics were plated, morphologically different colonies were microscopically selected and diversity analysed by M13-PCR fingerprinting. FISH combined with flow cytometry (FISH-FCM) is being performed to search for betaproteobacterial AOM, NOB and Thaumarchaeota on enrichments after 240 days.

Results & Conclusions
A collection of 477 isolates of putative nitrifiers was obtained, encompassing 72 nitrite-oxidizers, 300 ammonia-oxidizers and 105 urea-oxidizers. Among ammonia-oxidizers, a decrease in number was observed with increasing concentration of ammonia, with only 25% of isolates able to grow at 20 mM. PCA of monitoring data revealed two major clusters among 10 NOB enrichments and five for 79 AOM ones. For NOB, cluster A enrichments had higher number of isolates and displayed faster nitrite consumption, whereas cluster B enrichments only responded upon medium renewal after 3 months. For AOM, clusters correlate with different media, with the highest NO_2^- production being initially observed in Cluster III, containing only urea-based enrichments, and after media renewal in Cluster II, with the highest number of isolates. Since the highest NO_3^- production was observed in clusters V and I, before and after medium renewal respectively, communities of these clusters should be performing both stages of nitrification, consuming produced nitrite. In fact, these soil samples correspond to NOB cluster A, the best nitrite oxidation performers. From soil microbial profiling analysis and FISH-FCM and targeted-gene PCR of enrichments, role of microbial communities contributing to N-cycle will be evaluated regarding soil type.

References & Acknowledgments
Support from FCT BioClub project (PTDC/AGRPRO/1852/2014) and BioISI (FCT/UID/Multi/04046/2013) is acknowledged.

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Keywords: FISH-FCM, Microbial profiling, Genomic fingerprinting, Nitrogen cycle, Ammonia-oxidizers, Nitrite oxidizers

P-107 - INTESTINAL COLONIZATION OF HEALTHY COMPANION ANIMALS WITH MULTI-DRUG RESISTANT (MDR) CTX-M-15-PRODUCING ESCHERICHIA COLI WITH TRANSFER ABILITY IN THE NORTH OF PORTUGAL

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Background

Companion animals share a common bacterial flora with Humans and nowadays there is a large overlap of antibiotic used in both human and veterinary clinic. These arguments validate the idea that companion animals could be a source of dissemination of resistant bacteria to humans, as well as enable horizontal transmission of resistance genes between bacteria. The ubiquitous character of *E.coli*, makes the resistant isolates a worldwide problem. This way, MDR *E.coli* encoding CTX-M-type extended-spectrum β-lactamases (ESBL), namely CTX-M-15-producers, are a raising concern as they represent an emerging problem in human and animal healthcare worldwide. The aim of this study was the detection of MDR ESBL-producing *E.coli* in intestinal colonization of healthy companion animals in Portugal.

Method

Fifty-six healthy companion animal fecal samples were enriched in TSB. Selection was performed in MacConkey agar with antibiotics. Antimicrobial susceptibility was determined by disk-diffusion-method by EUCAST. ESBL producers were detected by the double-disk-synergy-test. Presumptive identification was done by CHROMagar orientation. ESBL-producing *E.coli* (EC-ESBL) were selected for conjugation assays, screening for $bla_{\text{CTX-M}}$ groups (group1 positives were screened for $bla_{\text{CTX-M-15}}$), bla_{TEM}, bla_{OXA} and bla_{SHV} by PCR.

Results & Conclusions

A total of 79 *E.coli* were selected, showing high resistance profile: 91% resistant to amoxicillin, 24% to amoxicillin+clavulanic acid, 29% to cefotaxime, 30% to ciprofloxacin and 24% to sulphamethoxazole+trimethoprim. Thirty-nine (81%) isolates showed a MDR profile. A total of 24 isolates were MDR-EC-ESBL, of these, 15 carried the $bla_{\text{CTX-M-15}}$ gene, from those, 4 had conjugative ability of transferring the CTX-M 15 codifying gene.
The results substantiate the occurrence and ongoing spread of blaCTX-M-15 mainly associated with the high-risk *E.coli* sequence type 131 (ST131) clonal group, representing a threat to animal and human health. Moreover, helps to confirm the possibility of transfer of resistance genes between colonizing bacteria of animals and Human commensal bacteria. These findings highlight the idea that antibiotic resistance is a public health problem that needs to be managed in a one-health perspective.

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ROCHA-GRACIA, R. C., et al. Faecal Escherichia coli isolates from healthy dogs harbour CTX-M-15 and CMY-2 β-lactamases. *The Veterinary Journal*, 2015, 203.3: 315-319.

P-108 - EVALUATION OF DIFFERENT SAMPLING METHODS IN COMBINATION WITH RNA-FISH FOR DETECTING BIODETERIOGENIC MICROORGANISMS IN WOOD ARTWORKS

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Background

Artworks are inhabited by microorganisms that can cause biodeterioration. For developing proper safeguard strategies, it is fundamental to identify the microbial communities that are metabolically-active [1]. Depending on the materials used in the creation of artworks the various types of microorganisms adhere differently due to their different characteristics and to their state of conservation [2]. Thus, the selection of a proper sampling method as well as of an effective detection/identification technique, that allow reliable detection and identification of metabolically active microorganisms, is extremely important. RNA-FISH is one of the promising techniques that has been previously applied with this end [3]. Therefore, the aim of this work was to determine the effectiveness of various sampling methods for their combination with RNA-FISH to detect microbial cells involved in artworks biodeterioration. Our attention was specifically focused on wood artworks.

Method

Wood slabs were artificially inoculated with bacteria, yeasts and filamentous fungi usually found in artworks (*Bacillus* sp., *Rhodotorula* sp. and *Penicillium* sp. respectively). They were incubated for two weeks and then, various sampling methods were applied for collecting the cells: (i) a swabbing method (with cotton swab); (ii) three impression methods (with filter paper, nitrocellulose and nylon membranes); and (iii) a destructive method. The collected cells were fixed, recovered in suspension and counted. Four different RNA-FISH assays were performed with each sample following the protocol previously described by us [3]: a blank and three assays with addition of probes (of an equimolar mixture of EUB338-Cy3 and EUK516-6-FAM and of the individual probes separately). The analysis were carried out by epifluorescence microscopy.

Results & Conclusions

The results showed that independently of the sampling method applied it was possible to: i) recover the three types of microorganisms present in the samples, even though the majority of the cells recovered were filamentous fungi cells; and ii) observe fluorescent signals that allowed to identify each one after the application of the RNA-FISH technique. Among the sampling methods tested, the impression method with nylon membrane was the best in terms of number of cells recovered.

This work was co-financed by ALT20-03-0246-FEDER-000004-ALENTEJO 2020 project and by FCT through the project PTDC/BBB-IMG/0046/2014 and grants SFRH/BD/118028/2016 and SFRH/BPD/100754/2014.

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Keywords: sampling methods, RNA-FISH, biodeteriogenic, microorganisms, artworks

P-109 - DIVERGING FOR THE SAME GOAL: CHARACTERIZATION OF GEOBACTER EXTRACELLULAR ELECTRON TRANSFER PATHWAYS

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Abstract

Geobacter bacteria show a high versatility of electron donors and acceptors. The respiratory pathway of these microorganisms is designed in a way that allows the utilization of extracellular substrates, such as toxic metals and electrode surfaces. This particular feature has been inspiring the development of many biotechnological applications, particularly in the fields of bioenergy and bioremediation.[1] The most heavily studied *Geobacter* species have been *Geobacter sulfurreducens* (*Gs*) and *Geobacter metallireducens* (*Gm*). *Gm* displays some particular physiological aspects that are even more interesting compared to *Gs*, which include (i) their ability to reduce aromatic compounds, (ii) more efficient Fe(III) reduction rates and (iii) the capability to use nitrate as terminal electron acceptor.[2] Despite the enormous potential for practical applications, much less is known about the functional properties of *Gm* electron transfer components. In particular, the characterization of the periplasmic cytochromes, that bridge the electron transfer from the oxidation of intracellular organic compounds to extracellular electron acceptors, stands as a key point to understand the extracellular electron transfer mechanisms of these bacteria.[3] In this work, the periplasmic triheme cytochrome PpcA from *Gm* was heterogeneously expressed in *Escherichia coli* and studied in detail for the first time. Complementary biophysical techniques, including UV-visible, CD and NMR spectroscopy, were used to characterize this cytochrome. PpcA from *Gm* contains three low-spin *c*-type heme groups with His-His axial coordination, a feature also observed for its homologue in *Gs*. However, despite their very high sequence homology (80%), our studies revealed marked differences between the two cytochromes, including their functional redox potential ranges, redox-Bohr effects and relative orientation of the heme groups. Overall, this study provides for the first time biochemical and biophysical data of a periplasmic cytochrome from *Geobacter metallireducens*, particularly useful for the understanding of the mechanisms involved in the extracellular electron transfer processes of these bacteria.

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Acknowledgements

This work was supported by PTDC/BBB-BQB/3554/2014 (to CAS) and FCT/MEC (UID/Multi/04378/2013) and co-financed by the ERDF under the PT2020 Partnership Agreement (POCI-01-0145-FEDER-007728).

Keywords: Geobacter, Extracellular Electron Transfer, Bioremediation, Bioenergy

P-110 - NEW INSIGHTS ON MECHANISMS OF SULFONAMIDE BIO-TRANSFORMATION BY ENVIRONMENTAL BACTERIA

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Background

Since 1935, when sulfonamides were first introduced as antimicrobials, resistance to this class of drugs was observed in a variety of bacteria. In contrast to beta-lactam antibiotics that are hydrolyzed by resistant bacteria, bacterial sulfonamide resistance occurs through mutations in chromosomal DHPS gene (*fol*P) or acquisition of an alternative DHPS gene (*sul*) involved in the biosynthetic folate pathway. While partial mineralization or transformation of sulfonamides by bacteria have been recently reported, these processes seem by far more uncommon than sulfonamide-resistance, and eventually not related with resistance. This study aimed at investigating the capacity of aquatic-bacterial isolates to transform sulfonamides and assess if such capacity could be associated with resistance.

Method

Forty-seven Gram-negative isolates from wastewater (WW, n=4), surface-water (SW, n=7) and drinking-water (DW, n=36) able to grow in the presence of 50 mg/L SMX were included in this study. The presence of sulfonamide-resistance genes (*sul*1, *sul*2) was investigated. Biotransformation assays were conducted in mineral or in rich media supplemented with SMX(50 mg/L). Identification of the SMX-transformation product was carried out by LC-MS.

Results & Conclusions

SMX-tolerance could be explained by the presence of one or both of the analyzed *sul* genes in only 15 of the isolates, and only 14 were able to transform SMX. Interestingly, only 3 isolates able to transform SMX carried one of these antibiotic-resistance genes (*sul*1), and most (n=13) were recovered from drinking-water. None of these SMX-transforming isolates could use the antibiotic as the sole source of carbon and energy, being the biotransformation only possible through co-metabolism, with accumulation of acetylated SMX. Bacteria able to transform SMX were affiliated to *Pseudomonas* (n=12), *Brevundimonas* (n=1), and *Stenotrophomonas* (n=1). Among the transforming-strains, *Pseudomonas mandelii* McBPA4 converted up to 81% of the initial SMX concentration after 48 h incubation in a feed-batch reactor. *N*-Acetylation of SMX by these fresh-water bacteria might contribute to their environmental adaptation, especially to those tested negative for sulfonamide-resistance genes, but further studies are needed to rule out other mechanisms of sulfonamide-resistance.

References & Acknowledgments

This work was the result of the project: (i) POCI-01-0145-FEDER-006939 (Laboratory for Process Engineering, Environment, Biotechnology and Energy-UID/EQU/00511/2013) funded by the European Regional Development Fund (ERDF), through COMPETE2020-Programa Operacional Competitividade e Internacionalização (POCI) and by national funds, through FCT-Fundação para a Ciência e a Tecnologia; (ii) NORTE-01-0145-FEDER-000005-LEPABE-2-ECO-INNOVATION, supported by North Portugal Regional Operational Programme (NORTE 2020), under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (ERDF), and (iii) postdoctoral grant SFRH/BPD/80834/2011 funded by FCT.

Keywords: Antibiotics; Biotransformation; Sulfamethoxazole; Acetyl-sulfamethoxazole; Antibiotic resistance; Environmental bacteria

P-111 - MICROBIAL DIVERSITY OF ANAEROBIC SYNGAS-CONVERTING ENRICHMENTS FROM A MULTI-ORIFICE BAFFLED BIOREACTOR (MOBB)

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Background

Syngas fermentation can be used to produce fuels and chemicals from lignocellulosic biomass or other poorly biodegradable wastes (1). Bacteria can use CO and H_2 present in syngas via the reductive acetyl-CoA pathway, a key metabolic intermediate that can be further converted to a broad product spectrum (2). The aim of this study was to identify and characterize carboxydotrophic microorganisms in enrichments and evaluate their potential for syngas bioconversion.

Method

Anaerobic sludge that efficiently converted syngas (60% CO, 30% H_2, 10% CO_2) to methane, in a multi-orifice baffled bioreactor (MOBB), was used as inoculum to start enrichments with CO as carbon and energy source. Enrichments were started under a headspace containing 40% CO. Bottles amended with vancomycin and/or erythromycin were also inoculated to test the potential for enriching CO-converting methanogens. Cultures were monitored using liquid and gas chromatography, and microbial composition was analysed by DGGE and sequencing.

Results & Conclusions

Methane and acetate were produced in the enrichment, but no growth or methane production was detected in incubation with antibiotics. In the enrichment, organisms related to *Acetobacterium* and *Sporomusa* species were the predominant bacterial species and *Methanobacterium* and *Methanospirillum* were the dominant archaea. The enrichment was subcultured and pasteurized to select for spore-forming bacteria and to inactivate methanogens. A stable enrichment culture was obtained that converted up to 100% CO. This enrichment produced hydrogen and acetate. The pasteurized culture showed a low microbial diversity; more than 90% of the community was related to *Sporomusa ovata* (97% identity). The results suggest that methane production from CO in the MOBB is a combined activity of carboxydotrophic acetogenic bacteria and hydrogenotrophic methanogens. Interestingly, growth of *S. ovata* with high concentrations of CO was never shown before.

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FCT and European Social Fund (POPH-QREN): SFRH/BPD/104837/2014 and PD/BD/128030/2016.
ERC grant: project 323009.
Netherlands Ministry of Education, Culture and Science and the NOW and WIMEK: gravitation grant - project 024.002.002.

Keywords: Carbon monoxide, Carboxydotrophs, Methanogens, Acetobacterium, Sporomusa

P-112 - MICROBIAL COMMUNITY AND ANTIBIOTIC RESISTANCE GENES DISTRIBUTION IN DIFFERENT URBAN WASTEWATER TREATMENT PLANTS EFFLUENTS

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Background

Urban wastewater treatment plants (UWTPs) are major anthropogenic reservoirs of antibiotic-resistant bacteria (ARB), antibiotic resistance genes (ARG) and antibiotic residues. The information about the occurrence of ARBs and ARGs in UWTPs as well as the mechanisms of their dissemination and how they can represent a public health threat is still scarce. The hypothesis behind this study was that the profiles of ARGs released by different UWTPs can be correlated with the respective bacterial communities. With this aim, the bacterial community composition and ARGs profile were characterized in the effluents of three UWTPs located in the Oporto region.

Method

A total of 33 treated effluent samples were collected from three UWTPs (PT1, PT2 and PT3), over seven sampling campaigns. All the UWTPs use activated sludge (AS) as secondary treatment, and PT3 is complemented by UV disinfection. The bacterial community composition was analysed based on the 16S rRNA gene amplicon paired-end sequencing using Illumina MiSeq platform and the ARGs (bla$_{TEM}$, bla$_{SHV}$, bla$_{OXA-A,}$ bla$_{CTX,}$ *sul*1, *sul*2 and *qnr*S) and class 1 integrase *(intI*1) were measured using quantitative PCR (qPCR).

Results & Conclusions

Irrespective of the sampling dates, the effluents of the three UWTPs yielded distinct bacterial community compositions independent of sampling dates, albeit with some common traits at the class level. For all the samples *Betaproteobacteria* was the major class. When analysed at the order level it is clear a difference between PT1 and the other two UWTPs, with PT1 presenting a higher abundance of *Burkholderiales* and *Bacteroidales* than PT2 and PT3. Amongst the genes analysed, the same trend was observed for abundance (gene copy number/mL) and prevalence (gene copy number/gene copy number of 16S rRNA), with the ARGs being ranked as bla_{CTX}<bla_{SHV}<bla_{TEM}<*qnr*S<*sul*2<bla_{OXA}<*intI*1<*sul*1. Major differences between the three UWTP with regards to ARGs were the higher abundance and prevalence of $bla_{\text{OXA,}}$ and the higher abundance of *qnr*S, and bla_{TEM} in PT1 than in the other two UWTPs. In general, it was possible to infer that ARGs distribution may be correlated with the bacterial community composition, which in turn may depend on the quality of the inflow and/or the characteristics of the wastewater treatment.

Keywords: Antibiotic-resistant bacteria, Antibiotic resistance genes, Urban wastewater treatment plants

P-113 - THE EFFECT OF BACILLUS SUBTILIS IN THE INTERACTION PISOLITHUS TINCTORIUS-PINUS PINEA

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Background

The mycorrhizosphere involves several bacterial communities. However, studies on the functional significance of bacteria associated with ectomycorrhizae are scarce. Also, their effect on the level of phenolic compounds produced by ectomycorrhizal plants is almost unknown. Phenolic compounds are widely distributed in plant tissues (1; 2) being the main components of their antioxidant system. Given that their synthesis can be correlated with bacterial and / or fungal infections (3) research on this topic urge. The objective of this study was to analyse the effect of bacteria on plant mycorrhization.

Method

Microcosms were established with a mixture of vermiculite and peat (3:1), humidified with MMN medium and sterilized. The treatments were: Inoculation with Pisolithus tinctorius-Pis, inoculation with Bacillus subtilis-B1 and inoculation with the dual inoculum, B. subtilis plus P. tinctorius-PisB1. The fungal inoculum consisted of five plugs with a diameter of 14 mm of mycelium and the bacteria inoculum consisted of 4 ml (108 CFU/ml) suspended in saline solution (0.85% NaCl). The parameters analyzed were: phenolic compounds (catechin, chlorogenic and protocatechuic acid), biometrics (height, shoot and root dry mass) and nutritional content (nitrogen and phosphorus). The data obtained were analyzed by a two-way ANOVA and Duncan's multiple-variable test.

Results & Conclusions

The level of chlorogenic acid decreased with time. Protocatecuic acid increased over the 70 days. Catechin was overall superior in the bacteria-containing treatments, B1and PisB1. The seedlings inoculated with the inoculum duo (PisB1) had the higher dry mass, being 1.42- and 1.65-fold higher than the Pis treatment for shoot and root dry mass, respectively. The Pis and PisB1inoculated plants had higher efficient use of nutrients than plants inoculated with B1, by 1.18-fold PUE and 4.96-fold NUE for Pis and 1.10-fold PUE and 4.72-fold NUE for PisB1. B. subtilis had an impact in seedlings inoculated with P. tinctorius; it modified plant response at the level of phenolic compounds and it increased its biomass.

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1- DOI: 10.3390/ijms17020160
2- DOI: org/10.1007/s11947-011-0573-z
3- DOI: 10.1016/j.indcrop.2012.01.022.

Giovania Araujo wish to acknowledge the CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brasil) and Nadine Sousa wishes to acknowledge FCT (Fundação para a Ciência e Tecnologia, Portugal) for the grant SFRH/BPD/89112/2012.The work was also supported by National Funds, through UID/Multi/50016/2013.

Keywords: Bacteria, ectomicorrhizal, phenolic compounds

P-114 - INOCULATION OF HYPERACCUMULATING OCHROBACTRUM TRITICI STRAIN IN RICE PLANTS REDUCES ARSENIC UPTAKE.

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Background

Thousands of people around the world suffer from the adverse health effects of arsenic in their environment. This arsenic is taken up by crop plants and the uptake of metals and metalloids is not just regulated by the plant, but is also influenced by the endophytic bacterial community [1]. The facultative endophytic bacterial strain, *Ochrobactrum tritici* strain SCII24 was mutated. The genes *ArsB* and the gene *Acr3_1* were mutated, resulting in a double mutant strain with non-functional arsenite efflux pumps ArsB and Acr3p, resulting in a higher arsenite accumulating strain than the wild type [2]. It was expected that the double mutant strain would increase arsenite resistance, when endophytic.

Method

Oryza sativa seeds from Mondego variety were disinfected, germinated on water-agar plates and after five days transferred to Hoagland medium with ammonium nitrate as a nitrate source. The plants were assigned to five different groups: no bacteria with no arsenic (n=32), no bacteria with 20 µM sodium arsenite (n=32), double mutant strain with no arsenic (n=32), double mutant strain with 20 µM sodium arsenite (n=32) and double mutant strain with 33 µM sodium arsenite (n=25). After six days of growth some of the plants were inoculated with the mutated bacterial strain, washed and incubated for four days. All the plants were transferred to new medium with- or without arsenite. After a week of growth the plants were measured for different growth parameters, digested and arsenic was quantified by ICP-MS.

Results & Conclusions

Rice plants without bacteria took up more than 30% of the arsenic, while the inoculated rice plants did not take up arsenic. Arsenite impacted the plant growth negatively. With arsenite, bacterial inoculated plants had higher growth parameters compared to non-inoculated plants. In absence of arsenite, the presence of the bacterium had a negative effect on plant growth parameters. In conclusion, the arsenite resistance in rice plants due to the double mutated SCII24 strain opens up new research possibilities for the effect of bacteria on metal metabolism of plants and the results obtained could have applications in reducing arsenic contamination in important agricultural crops.

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Acknowledgements

This project was supported by project ERA-MIN/0002/2015. Merijn Moens was funded by a research grant from the project.

Keywords: phytoremediation, endophytic bacteria, Ochrobactrum tritici, arsenic resistance, rice plant

P-115 A NEW PLATFORM FOR THE CONTROL OF RABBIT VIRAL HEMORRHAGIC DISEASE, VARIANT 2

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The Portuguese Ministry of Agriculture, Forest and Rural Development (MAFDR) determined the constitution of a nine-member partnership, coordinated by INIAV IP and with the participation of first level Hunting Organizations (OSCs), to implement an integrative strategy to limit the cascading effects caused by the epizootic circulation of emergent rabbit hemorrhagic disease virus (RHDV2) among the wild rabbit population (dispatch 4757/2017, 31 May). This new virus emerged in France in 2010 and reached mainland Portugal in 2012, affecting wild rabbit, predators, and also the rabbit industry. Very recently, mortality of European hares due to RHDV2 was confirmed, which demonstrates the ability of this virus to jump across host species.

An Action Plan was proposed on 16th august. Twelve major measures were identified within three axis defined as: 1) Research Program, 2) Best Management Practices and 3) Sanitary Surveillance. Among the four research lines considered, the production of an oral vaccine, based on Viral Like-Particles produced in insect cells, aims to increase the wild populations' immunity to this virus, without the need to capture and manipulate free-ranging animals. At a time where no funding is yet made available, the third axis is already ongoing with the implementation of a National Surveillance Network monitoring wild rabbit mortality.

In a 3-month period, a network to preserve field samples collected by hunters and technicians was implemented, gathering 19 locations across the mainland territory. Samples are sent to INIAV to be tested for major rabbit pathogens (RHDV2, Myxoma virus and non-pathogenic caliciviruses). Cadavers are submitted to necropsy and differential diagnosis. Specific kits and protocols to guide the collection of biological samples from hunted lagomorphs and cadavers found in the field were distributed among the three OSCs.

A roadmap for theoretical and practical workshops has been set and a demonstration video was been produced to elucidate the practical details for appropriate collection of the different matrixes.

Although this project is still in its infancy, and the ultimate aim is to recover rabbit populations through the control of RHDV2 and implementation of best management practices, actions carried out so far have already positively impacted on the cooperation of the national scientific and technological system with field stakeholders. The main core of this partnership is the recognition of the need for strict proximity and interdependence between researchers, hunters and civil society, particularly when facing and controlling a complex disease whose dissemination is driven by insects, mammals, birds and man.

Keywords: Wild rabbit, rabbit hemorrhagic disease, RHDV2, surveillance, disease control

P-116 - NEUTRAL LIPID PRODUCTION FROM HYDROCARBON-CONTAMINATED CORK SORBENTS USING RHODOCOCCUS OPACUS B4

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Background

Several oil spillages have been reported both in land and aquatic systems, with tremendous negative impacts for human health and ecosystems [1,2]. Cork, used as oil biosorbent, can be a sustainable alternative to other conventional remediation techniques [3]. Management of the subsequent residue involves a significant cost associated to its treatment/disposal, since no economic valorization is currently performed. In this work, a biological, environmental friendly solution is proposed to valorize hydrocarbon-contaminated cork sorbents.

Method

After growth, *Rhodococcus opacus* B4 was cultivated in nitrogen-limiting conditions using two types of cork (natural and regranulated) previously impregnated in hexadecane (C16). Neutral lipids profile was evaluated by thin-layer chromatography (TLC). Fatty acid content and composition, C16 concentration and biochemical methane potential from *R. opacus* B4 lipid-rich biomass were determined using a gas chromatograph with a flame ionization detector.

Results & Conclusions

R. opacus B4 was able to degrade up to 96 % of the hexadecane impregnated in natural and regranulated cork sorbents after 48 h incubation. *R. opacus* B4 produced 0.59 ± 0.06 g of triacylglycerol (TAG) g^{-1} of C16 consumed (60 % TAG:cellular dry weight (CDW)) and 0.54 ± 0.05 g TAG g^{-1} of C16 consumed (77 % TAG:CDW), after growing on C16-contaminated natural and regranulated cork sorbents, respectively. TAG was mainly composed by fatty acids of 16 and 18 carbon chains, demonstrating the feasibility of using it as raw material for biodiesel production. Whole cell lipid-rich biomass, obtained from *R. opacus* B4 cultivated on C16-contaminated cork, was efficiently converted to methane at a yield of about 0.4 L g^{-1} (CDW).

The obtained results support a novel approach for the management of oil-spill contaminated cork sorbents, through its valorization by production of bacterial lipids that can be used as raw materials for biofuels production, minimizing economical costs and environmental impacts, when compared to conventional treatment technologies

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This work was financially supported by the Portuguese Science Foundation and European Social Fund through the grant SFRH/BD/64500/2009, the projects UID/BIO/04469/2013, COMPETE 2020 (POCI-01-0145-FEDER-006684); RECI/BBB-EBI/0179/2012 (FCOMP-01-0124-FEDER-027462) and BioTecNorte operation (NORTE-01-0145-FEDER-000004). The authors gratefully acknowledge Cortiçeira Amorim, S.A., Portugal, for providing the CORKSORB materials used in this work.

Keywords: Rhodococcus opacus B4, Neutral lipids, Cork sorbents, Hydrocarbons, Biomethane

P-117 - QUATERNIZED CASHEW GUM FOR AN ANTI-STAPHYLOCOCCAL AND BIOCOMPATIBLE CATIONIC POLYMER FOR BIOTECHNOLOGICAL APPLICATIONS

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Background

Cashew gum (CG) is a natural anionic heteropolysaccharide with potential use in the pharmaceutical and food industry, beyond promising application in (nano)biotechnology (Araruna et al., 2013). Chemical changes in this polymer structure have been carried out in order to obtain new properties, however until now there were no reports of modifications by introduction of functional groups to add it cationic character, which could change, for instance, antimicrobial and other pharmacological activities (Quelemes et al., 2013). This work aims to modify chemically the CG by using quaternization reaction, evaluate the quaternized cashew gum (QCG) effect on bacteria of the *Staphylococcus* genus, beyond to access its cellular biocompatibility.

Method

To a solution of CG, trimethylammonium chloride and sodium hydroxide, at three different molar ratios proportions, were added to produce QCG derivatives. After 14 h of reaction under heating in a water bath, the pH was adjusted and carried out the precipitation of each modified gum using acetone, followed up for dialysis and lyophilization. QCG-1, QCG-2 and QCG-3 were characterized by ATR-FTIR, elemental analysis, Zeta potential and 2D NMR ($^{1}H-^{13}C$). The QCG derivatives anti-staphylococcal activity was evaluated by determining minimum inhibitory and bactericidal concentrations (MIC and MBC) and by atomic force microscopy (AFM). Biocompatibility on erythrocytes, keratinocytes and fibroblasts was also accessed through hemolytic and cell toxicity assays.

Results & Conclusions

The characterization methods selected for this study have confirmed the CG chemical modification. The three QCG derivatives presented promising anti-staphylococcal activity, with potency related to the increasing degree of substitution of quaternary ammonium group introduced on the gum structure. It was highlighted, for instance, the QCG-3 derivative effect against a methicillin-resistant *S. aureus* strain, with MIC value of 31.25 µg/mL and MBC at 62.5 µg/mL. The QCG derivatives also presented biocompatibility on tested cells, thus suggesting that it could be applied as tool for the development of biomaterials with antiseptic action.

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Alexandra Plácido is gratefully to FCT by her grant SFRH/BD/97995/2013, financed by POPH–QREN–Tipologia 4.1–Formação Avançada, subsidized by Fundo Social Europeu and Ministério da Ciência, Tecnologia e Ensino Superior. Peter Eaton is funded by FCT via grant UID/MULTI/04378/2013.

Keywords: Cashew gum, Quaternization, Antibacterial, Staphylococcus spp., Biocompatibility

P-118 - IN SITU SYNTHESIS OF SILVER NANOPARTICLES IN A HYDROGEL OF CARBOXYMETHYL CELLULOSE WITH PHTHALATED-CASHEW GUM AS A PROMISING ANTIMICROBIAL AND HEALING AGENT

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Background

The potential of silver nanoparticles (AgNPs) associated with gels that have traditionally been used in the treatment of wounds has been investigated (Quelemes et al., 2013). For example, carboxymethyl cellulose (CMC) is a polymer that forms an amorphous and three-dimensional hydrogel that is better suited to produce nanoparticles than most aqueous systems (Araruna et al., 2013). This work describes the preparation of AgNPs *in situ* in CMC hydrogels containing natural cashew gum (NCG) or phthalated-cashew gum (PhCG) to evaluate antimicrobial and healing activities.

Method

The AgNPs were prepared *in situ* during the hidrogel's production containing CMC and glycerin, having sodium borohydride ($NaBH_4$) as a reducing agent, on the molar ratio of 1:6 in relation to the silver. The rheology study characterized the gels as non-newtonian and pseudoplastic. For the AgNPs characterization, a UV-vis spectroscopy was used in which the maximum band for NCG-AgNPs and PhCG-AgNPs was of 408 and 404 nm, respectively; Nanoparticle Tracing Analysis (NTA), Transmission Electron Microscopy (TEM) was used to determine the AgNPs morphology within the hydrogels and energy dispersive spectroscopy (EDS) which confirmed the presence of silver and the formation of nanoparticles.

Results & Conclusions

UV-Vis spectroscopy confirmed the formation of silver nanoparticles within the gels via the formation of a plasmon band at close to 406 nm in both cases. AgNPs exhibited an average size of 119.7 ± 5 nm for NCG-AgNPs and of 123.8 ± 8.9 nm for PhCG-AgNPs, by NTA. The morphology by TEM showed triangular formations, double triangles, and hexagons (NCG-AgNPs) and nano spherical, square, and aggregated irregular spheres (PhCG-AgNPs). Antimicrobial activity against *S. aureus* and *P. aeruginosa* was observed as inhibition halos, with low values of minimum inhibitory and bactericidal concentrations (PhCG-AgNPs, 0.84 $\mu gAg.mL^{1}$). The hydrogels were applied to wounds in rats, and PhCG-AgNPs showed a significant healing effect on the 4^{th}, 7^{th} and 14^{th} days, while NCG-AgNPs improved healing on the 4^{th} day when compared with the control group. AgNPs-containging hydrogels synthesized *in situ* showed excellent antibacterial activity and exhibited potential healing effects.

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Quelemes et al. (2013) Int. J. Mol. Sci., 14, 4969−4981.

Alexandra Plácido is gratefully to FCT by her grant SFRH/BD/97995/2013, financed by POPH–QREN–Tipologia 4.1–Formação Avançada, subsidized by Fundo Social Europeu and Ministério da Ciência, Tecnologia e Ensino Superior. Peter Eaton is funded by FCT via grant UID/MULTI/04378/2013.

Keywords: Silver nanoparticles, Hydrogel, Cashew gum, Antimicrobial activity, Healing

P-119 - IMPACT OF AN INCREASE IN RECIRCULATION RATE ON THE ECOPHYSIOLOGY OF A MOVING BED BIOFILM REACTOR

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Background
In the Aquaculture industry, water is saved by implementing recirculating aquaculture systems (RAS) which use complex treatment loops. Understanding how RAS biological filters (BFs) work under different scenarios is of utmost importance, since BFs are the main contributors to water purification (e.g. ammonia and nitrite removal). Moving bed biofilm reactors (MBBR) are one of the BF types used in RAS (1).

Method
In this line of thought, a study was undertaken in a laboratory RAS Trout system to evaluate the effect of a stress event (an increase of organic matter and nitrogen compounds due to increased recirculation) on the performance of a MBBR with Bioflow 9 carriers. Several water quality parameters were monitored daily during this period. The MBBR microbial community response was evaluated before, during and after the stress event, through batch tests using 1000 mL reactors and 300 MBBR carriers. Total ammonia nitrogen (TAN) removal efficiency, nitrification activity based on isotope analysis, and oxygen uptake rates were quantified. Additionally, carriers were collected for direct *enumeration* of total bacterial through 4(prm1),6-diamidino-2-phenylindole (DAPI) and for DNA extraction to screen the bacterial community structure under the different conditions (pre-stress, stress and post-stress).

Results & Conclusions
Results showed distinct responses of the MBBR for the different scenarios. Under the stress event, the biofilter performance was affected in terms of TAN removal and oxygen uptake rates in comparison with the other scenarios. Additionally, MMBR performance between pre- and post-stress events shows an increase of TAN removal from 15% to 43%, respectively. Oxygen uptake rates were 12% higher after the stress situation (2.79 mg L^{-1} h^{-1} versus 3.16 mg L^{-1} h^{-1}). Nitrification rates and bacterial community analysis are ongoing, and changes are to be expected due to the stress applied.

Our results show that stress events like the one tested might have a positive effect in nitrification activity of the MBBR, and consequently on its TAN removal capacity in RAS systems. Further research is required to assess the usefulness of this procedure, and how it might affect cultured fish.

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This research was supported by the Portuguese Science and Technology Foundation (FCT) through the ERA-NET COFASP project with the Ref. COFASP/0005/2015. The collaboration of CIIMAR-BOGA team is acknowledged.

Keywords: TAN removal, Bacterial structure, Recirculating aquaculture system, Moving bed biofilm reactor, Nitrification activity

P-120 - INNOVATIVE METHODOLOGY TO TRACK BIOLEACHING POTENTIAL

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Background

Bioleaching is a process used in metal extraction from ore (biohydrometallurgy), commonly driven by chemolithoautotrophic acidophilic bacteria, which require demanding conditions (anaerobiosis, very low pH). Bioleaching is caused by the sum of redox reactions, acid attack and action of metal ligands such as organic acids. This process is more environmental sustainable than conventional leaching, which usually produces toxic sludge that can contaminate water supplies.

Previous work revealed stainless steel degradation by heterotrophic bacteria from the CEMMPRE-Microbiology Culture Collection with leaching of Fe and Cr. Siderophores, high-affinity Fe biological ligands, were probably involved in the process.

These molecules are produced by bacteria in response to Fe-limitation, and are also able to coordinate 24 metals.

The present study aims to predict patterns of bacteria-metal interaction in order to track bioleaching potential, through a screening for siderophore-producing bacteria (SPB), in strains isolated from four different locations.

Method

Previous sampling campaigns resulted in the collection of a diversity of strains obtained by cultivation methods. These strains were typed, grouped and identified by sequencing 16S rRNA gene. Representative strains from different clonal groups were isolated from the following four distinct environments: Mine tailings (Panasqueira, Bassin1 and Bassin2), a lagoon covering mine tailings (Panasqueira), a mine gallery (Jales) and activated sludge from a wastewater treatment plant (Alcanena). These strains were screened for SPB using solid medium modified for multi-metal assays. SPs obtain from positive strains were chemical characterized by colorimetric assays. A multivariate analysis (Principal Component Analysis) was performed in order to compare results obtained using CANOCO version 4.5. The 16S rRNA gene sequences were aligned with CLUSTAL W program and phylogenetic dendrograms were constructed in order to establish a relationship between diversity, phylogeny and siderophore-mediated metal-interaction. A selection of SPB and negative controls was selected for bioleaching experiments.

Results & Conclusions

Isolates from each location, display different interactions with metals, pH preferences and siderophore class production, which reflects the diversity in the phylogeny. Bacterial community from Jales and Bassin 1 have a heterogeneous interaction with metals, as opposed to Bassin 2 and Alcanena's community. Regarding Panasqueira, isolates from Bassin1 produces more cathecol-type of siderophores, while isolates from Bassin2 produce more hydroxamate-type. The isolates from Alcanena produce exclusively hydroxamate-type of siderophores. Bioleaching experiments will allow to verify the bioleaching potential here detected.

References & Acknowledgments

Work financed by FCT (PTDC/AAG-REC/3839/2014) and European Commission (ERAMIN/0002/2015). Sara Pereira da Silva financed by a project fellowship (PTDC/AAG-REC/3839/2014). Romeu Francisco financed by FCT (SFRH/BPD/103241/2014).

Keywords: bioleaching, siderophores, siderophore-producing bacteria, metals

P-121 - EPS PRODUCTION BY BACTERIA FROM HIGH SALINITY WASTEWATER

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Background

Aerobic granular sludge (AGS) is a promising technology for treating industrial wastewater, possessing higher biomass retention and tolerance to toxic substrates than conventional activated sludge systems. The presence of extracellular polymeric substances (EPS) in the aerobic granules structure increases the bacterial protection towards stress conditions and the stability of granules. Several industrial wastewaters contain high salt concentrations in their composition, which can inhibit the bacteria responsible for nutrients removal in the AGS process [1]. A novel strategy to increase the robustness of the system to high salinity is to bioaugment the aerobic granules with halotolerant bacteria with ability to produce EPS. Using a robust AGS process, extra value can be obtained from the wastewater since the EPS from the biomass can be recovered and used as new biomaterials in different applications. The aim of the present study is to investigate the feasibility of EPS production by halotolerant bacteria isolated from high salinity wastewater collected in a fish canning industry.

Method

Bacterial isolates obtained from the saline water were grouped according to species similarity, based on RAPD profiles. Isolates displaying unique RAPD profiles were subsequently identified by 16S rRNA gene sequencing analysis. The potential for EPS production by isolates presenting a sticky growth on agar plates is currently being evaluated. The content in proteins, humic acids and carbohydrates of the extracted EPS is quantified using reference methods.

Results & Conclusions

The obtained isolates are closely related to bacterial strains from the *Acinetobacter*, *Psychrobacter*, *Arthrobacter*, *Bacillus*, *Exiguobacterium* and *Kocuria* genera. The isolated halotolerant bacteria present different ability to produce EPS. The most promising EPS producers will be used for bioaugmentation of an AGS process treating high salinity wastewater. Valorization of the wastewater through EPS recovery from the AGS is in line with the circular economy concept.

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C.L. Amorim acknowledges the research grant from Fundação para a Ciência e Tecnologia (FCT), Portugal (SFRH/BPD/96481/2013) and Fundo Social Europeu (Programa Operacional Potencial Humano (POPH), Quadro de Referência Estratégico Nacional (QREN)). This work was also supported by FCT through the project UID/Multi/50016/2013 and through the project "MultiBiorefinery" - Ref. POCI-01-0145-FEDER-016403.

Keywords: Halotolerant, Extracellular Polymeric Substances, Aerobic Granular Sludge, Wastewater, Salinity, Bacteria

P-122 - TRIMETHOPRIM REMOVAL BY WASTEWATER BACTERIA: ANTIBIOTIC BIODEGRADATION AND MICROBIAL COMMUNITY CHARACTERIZATION

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Background

Microbial populations in municipal wastewater treatment plants (WWTP) are continuously exposed to sub-inhibitory antibiotic concentrations. WWTPs are inefficient in eliminating such compounds, being found in the final effluent and discharged into the environment, impacting also in the environmental bacterial communities. Trimethoprim is an antibiotic commonly found in the treated wastewater effluent. Studies concerning trimethoprim reveal a scarce knowledge concerning 1) the characterization of bacteria populations accountable for its bioremoval and 2) the impact of trimethoprim constant presence at sub-inhibitory concentrations on the microbial community thriving in a WWTP environment. This study aimed at filling such knowledge gap on the trimethoprim research topic.

Method

Mixed cultures of activated sludge (AS), collected from a municipal WWTP were maintained in aerobic conditions in shaken flasks. Cultures were fed with synthetic wastewater with glucose and sodium acetate at a COD:N:P ratio of 100:5:1. Trimethoprim was spiked at a final concentration of 700 µg/L. Samples at different time points were taken to 1) evaluate biomass growth (gMLSS/L), 2) consumption of the carbon sources and 3) trimethoprim degradation (by HPLC). To the microbial characterization by Fluorescence *in situ* hybridization (FISH) samples before and after trimethoprim spikes were collected. Fluorescent probes for 16S rRNA were applied targeting large taxonomic groups of bacteria. Relative abundances were assessed by epifluorescence microscopy.

Results & Conclusions

Biomass was fed in a feast-and-famine strategy to obligate for trimethoprim consumption. Indeed only when the carbon sources started to be depleted, a significant decrease in the trimethoprim concentration occurred, suggesting that some bacteria populations consumed the antibiotic to fulfill their nutrient requirements. From FISH analysis it was possible to verify by microscopy substantial changes in the morphological structure of the community, suggesting that the presence of trimethoprim caused an additional stress to the biomass and that the microbial populations had a consequent dynamic behavior. By comparing the relative abundances of target bacterial populations it was possible to verify that variations were mostly detected in the *Beta-* and *Gamma- Proteobacteria* classes in the trimethoprim stressed community. Such results indicate a potential role of *Gamma-* and *Beta-Proteobacteria* in the biodegradation of trimethoprim.This study characterized for the first time an aerobic heterotrophic microbial community able to remove trimethoprim, and demonstrated that the presence of trimethoprim does impact in the microbial structure, although without compromising the overall metabolic capacity of such community.

References & Acknowledgments

Financial support from FCT (SFRH/BPD/97612/2013;SFRH/BPD/94303/2013 fellowships); iNOVA4Health research unit (LISBOA-01-0145-FEDER-007344) co-funded by FCT/MCES and by FEDER (PT2020 Partnership Agreement).

Keywords: wastewater, mixed microbial community, Antibiotic bioremoval

P-123 - FLUOROPHORE´S ROLE ON THE RELIABILITY OF MICROORGANISM DETECTION BY FLUORESCENCE IN SITU HYBRIDISATION (FISH)

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Abstract
FISH has been applied in many ecological and phylogenetic studies becoming the method of choice for the direct detection and identification of microorganisms in their natural environments [1]. For reliable results, it is crucial to minimise or avoid background fluorescence and cellular autofluorescence but also to maximise the specific FISH signals obtained. Red-emitting dyes have been usually used for eliminating the problems of non-specific fluorescence interference. Therefore, in this work we evaluated the role of three of these fluorophores (Alexa Fluor 647 (AF647), ATTO 647N and Cy5) on the reliability of the RNA-FISH results obtained both with a universal and a specie-specific probe.

Materials and Methods
RNA-FISH assays were performed using two strains of *Dekkera bruxellensis* (CBS 2797 and ISA 2101) following the protocol previously described by us [2]. Four assays were performed for each strain with the addition of different probes (1)-non-probe; (2) EUB338; (3) EUK516; (4) specific-RNA-FISH probe to *D. bruxellensis* (26S D. brux.5.1) previously described in the literature [3]. The probes used were: 5'-EUB338-, 5'-EUK516-, 5'-26S D. brux.5.1 labelled with AF647 and ATTO 647N as well as 5´-EUB338-Cy5 and 5´-EUK516-Cy5. Their performances were evaluated in terms of detectability and percentage/fluorescence intensity of the target cells, by epifluorescence microscopy and flow cytometry, respectively.

Results & Conclusions
As expected, no fluorescent signals were obtained without addition of probe and with EUB 338 labelled with the three fluorophores selected. The universal probe, EUK516, labelled with each of the fluorophores selected allowed the reliable detection of the yeast cells. Microscopic observations revealed that ATTO 647N-labelled probes conferred higher fluorescence stability to the hybridised cells than the other fluorophores tested (AF647 and Cy5). Likewise, by flow cytometry the best results were detected with EUK516 probe labelled with ATTO 647N. Nevertheless, cells stained with AF647 showed the lowest fluorescence intensities. Yeast cells stained with EUK 516 usually shows intense FISH signals. However, specie-specific probes can give weaker fluorescent intensities that can hinder the detection and identification of the target cells. Therefore, the fluorophores influence on the accurate detection and identification of the target cells was investigated using a specific RNA-FISH probe to *D. bruxellensis* (26S D. brux.5.1). Only, ATTO 647N and AF647 were selected as they showed the highest and lowest fluorescence with EUK 516 probe. The signals obtained by flow cytometry, even with low intensity, allowed to identify the cells hybridised with 5'-26S D. brux.5.1-AF647 as *D. bruxellensis*. However, no fluorescence was detected by epifluorescence microscopy. This indicates that this probe labelled with AF647 allowed the specific detection of *D. bruxellensis* by flow cytometry but not by epifluorescence microscopy. Conversely, intense fluorescent cells were detected when hybridised with the same RNA-FISH probe (5'-26S D. brux.5.1) but labelled with ATTO 647N both by epifluorescence microscopy and flow cytometry. Thus, this study highlighted that the selection of a fluorophore with high photostability and quantum yield, such as ATTO 647N, can improve FISH performance and contribute to avoid inaccurate identification of microorganisms by RNA-FISH technique independently of the method used for analysis.

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Acknowledgements: This work was co-financed by ALT20-03-0246-FEDER-000004-ALENTEJO 2020 project and by FCT through PTDC/BBB-IMG/0046/2014 project and SFRH/BPD/100754/2014 grant.

Keywords: Fluorescence In Situ Hybridisation, Fluorescent dyes

P-124 - SYNTHESIS AND EVALUATION OF THE POTENTIAL ANTIBACTERIAL ACTIVITY OF CHITOSAN DERIVATIVES

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Background

The biological growth and microbial activity in heritage artefacts, promotes and accelerates their deterioration, constituting a reason of great concern for its desirable preservation. This problem imposes ways of acting to avoid the deterioration. The application of biocides is one of the most used ways for the biodegradation prevention of Cultural Heritage artefacts and its application is aimed at preventing or controlling the microbial growth.[1-3] Chitosan and its derivatives have been used in the development of many products and on other application areas such as medicine, environment, nutrition, cosmetics, agriculture, photography, among others. This biopolymer has also been well studied due to its antimicrobial properties regarding fungi and bacteria (*Gram*-positive and *Gram*-negative).[4,5] In this work, a simple and efficient methodology was developed for the synthesis of chitosan derivatives. The final products were subsequently evaluated for their possible biocidal activity using several bacterial strains isolated from heritage context. The results of the biocidal activity allow us to conclude that the *N*-substituted chitosan derivatives of linear aliphatic chains are promising products for the short-term development of new biocides.

Method

Synthesis of chitosan derivatives using alkyl halides.
Identification and characterization by FTIR-ATR and ^{1}H-NMR.
Biocidal evaluation against biodeteriogenic bacteria isolated from patrimonial assets.

Results & Conclusions

Chitosan with the amine groups protonated was the derivative that showed major antibacterial activity. The product of derivatization with iodoethane showed similar activity when compared to the protonated chitosan. These results suggest that *N*-alkylation with ethyl group enhances the activity of chitosan. In relation to antimicrobial activity against *Pseudomonas aeruginosa*, the derivatization products with 1-bromooctane and 1-bromo-3-methylbutane revealed biocidal activity similar to protonated chitosan, suggesting that acyclic aliphatic hydrocarbons long-chain (C8) or branched, may influence the activity of these derivatives. In the case of the tests against *Bacillus sp*. it was found that all the acyclic aliphatic derivatives of chitosan affect the growth of these microorganisms. The results of the antimicrobial activity allow us to conclude that the *N*-substituted chitosan derivatives with linear aliphatic chains are promising products for the short-term development of new biocides.

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Project ALT20-03-0145-FEDER-00001 and grant SFRH/BD/128807/2017 (FCT)

Keywords: Chitosan, Biocide, Biocidal activity

P-125 - ADAPTIVE EVOLUTION AS A TOOL TO OBTAIN NOVEL STRAINS IMPROVED FOR BIOREMEDIATION

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Background

Bioremediation refers to pollutant elimination both in water and soil, mainly through microbial activity. Microbial effect can be enhanced by strains selection or modification. Even though genetic engineering strategies for bioremediation have proved successful, current regulatory requirements lead to costly legal processes to reach the EU approval. For strain selection, adaptive evolution (AE) strategies can be used to obtain enhanced strains from known species. By submitting a microbial population to a selective growth condition over repetitive cycles, small adaptive changes will accumulate during long term selection for the tested conditions [1]. This approach allows isolation of strains for new products without legal GMO concerns.

Method

AE experiments were performed in culture medium with pollutants as unique carbon source and selective pressure. Four different substrates were studied: anthracene and phenanthrene (polycyclic hydrocarbons), mineral oil (aliphatic hydrocarbons) and glyceryl tristearate (triglyceride). A WWTP inoculum was initially used and kept for over 50 cycles, each consisting of a 7-days incubation (continuous shaking) after 1% inoculum from previous cycle. This strategy keeps the dominant microbial strains, leading to wash-out of weaker ones. Total cell count was used to monitor microbial growth and microbial isolation was performed at intermediate cycles, using a crossed assay with the four selective and a general growth media. Bacterial and yeast strains were briefly characterized at morphotype level and genomic profiling was obtained by PCR-fingerprinting with 3 distinct primers. Molecular identification by 16S/26S rRNA gene sequencing was also conducted for selected isolates.

Results & Conclusions

A total of 320 isolates were obtained from initial inoculum (24) and the AE experiments with anthracen (68), phenanthrene (84), glyceryl tristearate (58) and mineral oil (86). Considering the crossed assay isolation, 46 isolates grew with anthracene, 50 with phenanthrene, 68 with glyceryl tristearate and 58 with mineral oil, while 98 were isolated from general growth medium. PCR-fingerprinting profiles allowed to establish clusters containing non-distinguishable isolates from different AE experiments, cycles or selective growth media. Since the initial inoculum was common to all AE experiments, these isolates were considered as members of the same strain and one of them was designated as strain representative. A sub-set of strain representatives presenting strong bioremediation potential were further selected for molecular identification and future characterization, based on ubiquity, persistency over time and metabolic diversity assessed from AE experiments.

References & Acknowledgments

P. Teixeira was financed by BioTask and FCT (SFRH/BDE/95903/2013). Support from BioISI (FCT, UID/Multi/04046/2013) is also acknowledged.

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Keywords: Adaptive Evolution, Bioremediation, PCR-Fingerprinting

Environmental Microbiology and Biotechnology

P-126 - VALORISATION OF MINING AREAS USING PHYTOTECHNOLOGIES – A FIELD EXPERIMENT

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Background

Mining operations are one of the major soil disruptors. The release of hazardous compounds, such heavy metals and metalloids (HM), pose serious risks to human health and contribute to the decline of soil's quality. The use of phytotechnologies (application of plants and microorganisms) in mine areas has the potential of reducing the amount or the toxicity of deleterious HM, promoting ecological restoration. Moreover, they can provide relevant economic revenues by using these brownfields to produce bioenergy crops.

The Borralha mine is a past producer of tungsten in Portugal, integrating soils with high HM concentrations (e.g. Cu and Cd) and can benefit from the application of phytotechnologies on its requalification. In this context, the main goals of the work are to evaluate the growth of a bioenergy crop (sunflower) assisted with arbuscular mycorrhizal fungi (AMF) in such soils, and assess its phytostabilization capacity.

Method

The field experiment was performed in Borralha mine (northern Portugal) and consisted on 9 m^2 plots sown with sunflower under 2 treatments (n=3): control and inoculated with *Rhizophagus irregular*is (AMF). Plants were harvested after 4 months and rhizosphere soil was collected to determine metal content. Plant biomass was determined after shoots and roots were oven dried. Plant tissues were then grinded for acid digestion. Metal content (Cu, Ni, Cd, Mn, Cr, Zn and As) and N and P levels in each plant section were assessed. Bioconcentration and translocation factors were calculated.

Results & Conclusions

Sunflower establishment was affected by AMF inoculation. The presence of AMF improved the phytostabilization capacity of sunflower contributing to the reduction of the spreading of HM. Overall results show that the production of sunflower in Borralha mine is an advantageous option to increase the value of this area to stakeholders, while mitigating the risk deriving from the soil contamination.

References & Acknowledgments

This work was supported by National Funds through FCT under the project UID/Multi/50016/2013 and by the project PhytoSudoe SOE1/P5/E0189, funded by FEDER - Fundo Europeu de Desenvolvimento Regional, under Programa INTERREG SUDOE. A. Vega had the support of Phytosudoe grant SOE1/P5/E0189. H. Moreira had the support of FCT grant SFRH/BPD/105152/2014. S.I.A. Pereira received an individual research contract in the framework of the project bio – n2 – value, nº NORTE-01-0145-FEDER-000030, funded by FEDER, under Programa Operacional Regional do Norte - Norte2020. The authors thank to EcoMuseu do Barroso, Junta Freguesia Salto e Câmara Municipal de Montalegre.

Keywords: phytotechnologies, bioinoculants, metal contamination, phytostabilization

P-127 - IMPACT OF SILVER NANOPARTICLES ON QUORUM SENSING DURING AEROBIC GRANULATION

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Background
The use of nanoparticles in the textile industry has been rapidly increasing, silver nanoparticles (AgNPs) being the most employed for their antimicrobial effects[1]. The release of AgNPs in wastewater is rising accordingly, but their impact on biological treatment is largely unknown[2]. Aerobic granular sludge is a novel wastewater treatment technology with a demonstrated potential for textile wastewater[3]. Aerobic granulation mechanisms are unclear, but quorum-sensing (QS) using N-acyl-L-homoserine lactones (AHLs) may play an important role[4,5]. This study analyzed the effect of AgNPs on AHL-based QS during aerobic granulation on synthetic textile wastewater.

Method
Two 1.5-L sequencing batch reactors (SBRs) were seeded with activated sludge flocs. The SBRs were run in 6-h cycles: 30-min static fill, 1.5-h anaerobic reaction, 3.5-h aeration, settling and 1-min drain. Aerobic granulation was induced by gradually decreasing the settling time from 60 to 5 min. Synthetic textile wastewater was fed to the SBRs[3] at 2.0-kg.m^{-3}.d^{-1} organic loading rate, as chemical oxygen demand (COD). SBR1 was supplemented with 10-mg L^{-1} AgNPs and SBR2 worked as AgNP-free control. Biomass morphology, sludge volume index (SVI), COD and color levels were analyzed[3]. AHLs were extracted from mixed liquor samples[4] and quantified using *Agrobacterium tumefaciens* NTL4(pZLR4) as reporter strain[5].

Results & Conclusions
Aerobic granules developed in both SBRs, becoming larger and denser along the operation time. SVI values decreased in both SBRs, indicating that aerobic granulation was attained, despite the presence of AgNPs. SBR1 even presented higher sludge settleability and biomass accumulation, possibly due to adsorption of AgNPs by the biomass consequently increasing its density. Treatment performance was not hampered by AgNPs, color and COD removal yields around 80% being achieved in both SBRs. The increase in AHL levels accompanying sludge granule development indicated that AHL-based QS plays a role in aerobic granulation. AHL levels were higher in AgNP-fed SBR1, suggesting that AHL-based QS may be also involved in cell protection against AgNPs.

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This work was financed by Fundação para a Ciência e a Tecnologia (FCT) through projects PTDC/EBB-EBI/120624/2010 and PTDC/AAG-TEC/4501/2014 and funding received by iBB-Institute for Bioengineering and Biosciences (UID/BIO/04565/2013). RDG Franca and ND Lourenço acknowledge FCT grants SFRH/BD/95415/2013 and SFRH/BPD/88095/2012, respectively.

Keywords: Aerobic granulation, Quorum sensing, Silver nanoparticles, Textile wastewater

Environmental Microbiology and Biotechnology

P-128 - A COMPARATIVE STUDY OF RARE EARTH METALS RECOVERY BY BACTERIA AND ALGAE.

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Background

This project aims the recycling of rare earth elements obtained from spent fluorescent lamps by the use of microorganisms in bioleaching and biosorption processes. Biosorption is the ability of some materials of biological origin to sequestrate or concentrate metals from aqueous solutions **(1)**. This mechanism presents some advantages over the chemical ones such as lower operation costs, reduction of chemicals and sludge to be deposit and higher efficiency in the detoxification of diluted effluents **(2)**. The specific aim of this work is to compare an algae and two bacteria in retaining different rare earth metals such as lanthanum, cerium, europium, terbium and yttrium.

Method

The metals entrapment is evaluated in batch assays using different bacteria as *Pseudomonas* and *Bacillus* strains, compared to the caption by the algae *Saccorhiza polyschides*. The toxicity effect of each metal and the biosorption capacity of the tested organisms are evaluated during their growth in previously established culture conditions. The algae samples were collected a year ago and very recently and dried before usage in metal solutions. Inductively Coupled Plasma, ICP technique was applied to measure the metal concentrations in all samples.

Results & Conclusions

The uptake capacity for each metal is established for different tested conditions. The bacteria are grown in the presence of increasing concentrations of each metal and in bi-metallic solutions, presenting specific reactions towards the toxicity of each element. Although the seaweed usually reveals higher entrapment ability towards heavy metals, the tested bacteria present a promising behavior towards each rare earth element. Kinetics and maximum uptake are determined.

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This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the research project PTDC/AAG-TEC/5269/2014, the strategic funding of UID/BIO/04469/2013 unit and COMPETE 2020 (POCI-01-0145-FEDER-006684) and BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund under the scope of Norte2020 - Programa Operacional Regional do Norte.

Keywords: Rare earth metals, Biosorption, Algae, Bacteria

P-129 - CORK TAINT: A NOVEL METHODOLOGY FOR THE ANALYSIS OF CORK DEFECTS

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Background

Cork is produced from the bark of *Quercus suber* L. (cork oak tree). Cork oak grows mainly in western Mediterranean countries, with Portugal being the world leader in cork production, with more than 720 thousand hectares of cork forest [1]. Sometimes these valuable material present defects that can damage the industrial performance, playing microorganisms an important role in this process. The so-called 'cork taint' represents one of the most unpleasant off-flavours spoiling bottled wines. The compounds associated with taint include microbial metabolites which can be produced by the mycobiota found in association with the bark and cork throughout the often lengthy processing, storage and cork transportation [2].

The aim of this work was to infer about microbial communities involved in several cork pathologies and its influence on the cork microstructure and its chemical composition, using methodologic strategies based on microscopic and spectroscopic techniques.

Method

Some identified pathologies were selected in raw corks planks in order to identify the fungal communities responsible for its defects. These samples were also analysed by Scanning Electron Microscopy (SEM) and X-ray diffraction.

Results & Conclusions

This microbial study showed a predominance of filamentous fungi such as *Talaromyces variabilis* and fungi of *Penicillium* genera. Cork with pathologies present a matrix and pores altered and degraded due to the significant presence of microorganisms, confirming the significant chemical and physical difference between the healthy samples and corks with signals of evident pathology. The methodological scheme adopted can constitute a very promising method to be applied in cork manufactured industry in order to analyse and prevent possible defects that can promote unpleasant tastes in wines.

References & Acknowledgments

The authors gratefully acknowledge the following funding sources "HIT3CH - HERCULES Interface for Technology Transfer and Teaming in Cultural Heritage", Ref: ALT20-03-0246-FEDER-000004, and "MEDUSA-Microrganisms Monitoring and Mitigation–Developing and Unlocking novel Sustainable Approaches" ref. ALT20-03-0145-FEDER-000015, co-financed by the European Union through the European Regional Development Fund ALENTEJO 2020 (Regional Operational Programme of the Alentejo)

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Keywords: Quercus suber, Cork defects, Cork taint, Microorganisms

P-130 - ENRICHMENT OF BACTERIAL STRAINS FOR THE BIODEGRADATION OF ENDOCRINE DISRUPTING COMPOUNDS FROM SEDIMENTS OF THE PEARL RIVER DELTA (PRD)

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Background

Endocrine disrupting chemicals (EDCs) are those which alter the homeostasis, reproduction, development and/or behaviour of organisms. Negative effects have been reported on aquatic species, wildlife, and humans due to exposure to very low (ng L−1) concentrations [1]. A large number of chemicals have been identified as EDCs. Wastewater treatment plants are not able to completly remove these chemicals, contributing to the contamination of receiving water bodies. The situation of The Pearl River Delta (PRD) is of particular concern due to the high industrialization and dense urbanization [2,3].

Method

Selective enrichments with bisphenol A (BPA), bisphenol S (BPS), 17β-estradiol (E2) and 17α-ethynilestradiol (EE2) were established with activated sludge collected from an aeration tank of a sewage treatment plant located in Coloane (Macao) and sediment samples from the discharge point of the same station. Bacterial strains were isolated from successful degrading consortia by plating on Nutrient Agar and identified by 16S rRNA sequencing. Degradation of the compounds by the isolates was tested first on minimal agar plates and then in liquid media, with EDCs as sole carbon source.

Results & Conclusions

After two months, the enriched consortia were able to completely degrade 10 mg L-1 of BPA in three days, while BPS was not degraded. For the hormones, degradation of E2 varied between 20 and 100%; degradation of EE2 varied between 36 and 77%, in 15 days. A total of 28 strains were isolated from the degrading consortia. The maximum extent of degradation obtained for the isolates was 67% for EE2 and 100% for E2, supplied at 4.5 mg L-1, and 23% for BPA and 34% for BPS, supplied at 9.5 mg L-1, during 28 days. The isolated strains represent valuable candidates for in situ bioremediation of contaminated environments. Promising results were obtained for strain identified as Castellaniella sp. ED55, able to degrade the four compounds at different extents. Further studies are ongoing to deepen the knowledge about the mechanisms of degradation by that strain, including genome sequencing and transcriptomic experiments.

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I. S. Moreira acknowledges a research grant from Fundação para a Ciência e Tecnologia (FCT), Portugal (Ref. SFRH/BPD/87251/2012) and Fundo Social Europeu (Programa Operacional Potencial Humano (POPH), Quadro de Referência Estratégico Nacional (QREN)). This work was supported by the Macao Science and Technology Development Fund (FDCT) through the project 011/2014/A1 and by National Funds through FCT under the project UID/Multi/50016/2013.

Keywords: Endocrine disrupting chemicals, Biodegradation, Macau, Enrichment, Castellaniella sp. ED55

P-131 - CHARACTERIZATION OF THE EFFECTS OF PHARMACEUTICALS IN ACTIVATED SLUDGE USING QUANTITATIVE IMAGE ANALYSIS

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Background

The extensive use of personal care products and pharmaceuticals for human consumption and veterinary usage led to the detection of these products in wastewater effluents and aqueous systems as rivers, surface waters and others. The ecotoxicology of pharmaceuticals was well studied by Backhaus [1]. The effect of these compounds on microorganisms is also an important study that should be taken into account when biological processes are used to remove these compounds from aqueous systems. Quantitative image analysis (QIA) is considered a useful technique for biological processes monitoring when combined to microscopy. The aim of this study was to analyze the effects of ibuprofen (IBU), paracetamol (PARA), and ethynylestradiol (EE2) on the biomass morphology using QIA.

Method

To analyze biomass morphological changes due to IBU, PARA, and EE2, four identical batch reactors were used during 24h. The reactors were inoculated with activated sludge and initial concentrations of pharmaceuticals of 1 and 10 mg L^{-1} were used. A control batch was also performed. Aggregated and filamentous biomass contents and structure were assessed by images acquired through bright-field microscopy. Aggregates were classified according to their size in two classes and for each studied class the aggregates area percentage was calculated. The aggregates total area per volume (TA/Vol) and filaments total length per volume (TL/Vol) were also determined [2].

Results & Conclusions

During the experiments with PARA, in spite of a slight variation, the results showed no significant effects in each class studied. The biomass structure was clearly affected by the addition of EE2. A deflocculation was observed which is corroborated by the sharp decrease on the intermediate aggregates area. With IBU, the effect of aggregates fragmentation is extensively higher than with EE2. It seems therefore clear that IBU presents substantial effect in bacterial biomass leading a deflocculating phenomenon with higher impact for 10 mg L^{-1}. QIA studies showed that IBU favors the growth of aggregated biomass (TA/Vol). For experiments with PARA and EE2, 10 mg L^{-1} just slightly favored the growth of filamentous bacteria (TL/Vol).

References & Acknowledgments

The authors thank the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UID/BIO/04469/2013 unit, COMPETE 2020 (POCI-01-0145-FEDER-006684) and BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund under the scope of Norte2020 - Programa Operacional Regional do Norte. The authors also acknowledge the financial support to Cristina Quintelas through the postdoctoral grant (SFRH/BPD/101338/2014) provided by FCT – Portugal.

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Keywords: Quantitative image analysis, Activated sludge, Pharmaeuticals

P-132 - GRAPEVINE GROWTH RESPONSE TO BIOINOCULANTS AND BIOCHAR APPLICATION

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Background

Grapevine is a perennial crop that is highly responsive to local environmental conditions and viticulture practices. Along with this, soil microorganisms should be taken into consideration since they provide important ecological services to the plant. Plant growth promoting rhizobacteria and arbuscular mycorrhizal fungi can establish symbiotic association with grapevine roots and due to their plant-beneficial traits they can enhance the growth and quality of the vineyard and the grape. Therefore, the use of such microorganisms as bioinoculants can benefit vegetative and productive parameters of grapevine. Soil amendments, such as biochar, can also improve plant performance and soil structure. Biochar is a product of pyrolysis of organic materials and its importance as an amendment has been recognized in the improvement of soil fertility and water retention.
This work aims to assess the effect of microbial inoculants and biochar on productive parameters of 10-year old grapevines and on vegetative parameters of new grapevines.

Method

The experiment was conducted at a 36-ha vineyard located in North Portugal inside the Vinho Verde appellation, on adult (10 year-old) and on new grapevine plants. In total, 6 treatments with three replicates each were applied in the experimental area, in a total of 18 plots (variety Alvarinho). The treatments applied comprised different combinations of PGPR, AMF, and biochar. Bioinoculants and biochar were applied at the time of the plantation in the new grapevines. In the 10-year old grapevines, bioinoculants were inoculated around plants and a soil scarification was done between lines to apply biochar. Plant performance will be followed for at least 3 growing seasons.

Results & Conclusions

The effect of bioinoculants and biochar application was evaluated at harvest. The number of grape bunches and total fruit yield were measured. Grape quality and nutrient content of 10-year old grapevines were assessed as well as biometric parameters of new grapevines.
This is a multi-year project where successive inoculations are planned to enhance plant performance over the years.

References & Acknowledgments

This work was supported by National Funds through FCT under the project UID/Multi/50016/2013 and by the project Biological tools for adding and defending value in key agro-food chains (bio– n2–value), nº NORTE-01-0145-FEDER-000030, funded by FEDER, under Programa Operacional Regional do Norte-Norte2020. S.I.A. Pereira received an individual research contract within the project bio–n2–value. A. Vega had the support of PhytoSudoe grant SOE1/P5/E0189 funded by FEDER, INTERREG SUDOE. H. Moreira had the support of FCT grant SFRH/BPD/105152/2014. The authors thank to IberomassaFlorestal the supply of biochar.

Keywords: grapevine, bioinoculants, grapevine biofertilization

P-133 - ACTIVITY OF ESSENTIAL OILS AGAINST MURAL PAINTING BIODETERIOGENIC FUNGI

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Background

Microorganisms play a considerable role in the deterioration of cultural heritage artefacts [1]. In the past, biocides based on chemical toxic compounds have been used to mitigate the bio- contamination. However, many of the most effective biocides have been banned due to their environmental and health hazards [2]. Therefore, developing proper remediation actions for contaminated historic materials based on environmentally safe solution is an urgent task. Essential oils (EOs) obtained from aromatic plants represent an alternative in the control of biodeterioration in cultural heritage context, without negative environmental and human impacts [3].

Method

The aim of this study was to evaluate the antimicrobial activity of six EOs of aromatic herbs against microorganisms attributed to biodeterioration of mural paintings. Six flavouring herbs of Alentejo were selected for this study: *Calamintha nepeta*, *Lavandula stoechas* subsp *luisieri*, *Mentha spicata*, *Rosmarinus officinalis*, *Thymus mastichina* and *Foeniculum vulgare*. EOs were extracted from aerial parts of plants by hydrodistillation and their chemical composition was evaluated by GC-FID. Antimicrobial activity was assessed by solid diffusion disk assays [4] against eleven fungal strains previously isolated from ancient Parish Church of Santo Aleixo mural paintings (Montemor-o-Novo, Portugal).

Results & Conclusions

Results composition showed some differences of chemical components of EOs, according to the different flavouring herb (either to diversity and proportion of its constituents). But, in general EOs tested showed high antifungal activity against *Aspergillus niger*, *Aspergillus* sp.1, *Epicoccum nigrum, Cladosporium* sp.1, *Penicillium brevicompactum, Penicillium* sp. 1, *Penicillium expansum* and *Fusarium* sp.1 strains. EOs of *L. luisieri* showed the highest antifungal spectra, with total inhibition of growth of all fungal strains in the concentration tested.

Results suggest the potential use of EOs in safeguard of cultural heritage, namely in mural paintings. This work is in progress to study the mode of application of EOs as fungal control.

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The authors gratefully acknowledge the following funding sources “HIT3CH-HERCULES Interface for Technology Transfer and Teaming in Cultural Heritage”, Ref:ALT20-03-0246-FEDER-000004, and "MEDUSA-Microrganisms Monitoring and Mitigation–Developing and Unlocking novel Sustainable Approaches" ref.ALT20-03-0145-FEDER-000015, co-financed by the European Union through the European Regional Development Fund ALENTEJO 2020 (Regional Operational Programme of the Alentejo)

Keywords: Essential oils, antifugal biodeterioration, Calamintha nepeta, Lavandula stoechas subsp luisieri, Mentha spicata, Rosmarinus officinalis, Thymus mastichina, Foeniculum vulgare

P-134 - BIOLEACHING POTENTIAL OF NON-ACIDOPHILIC BACTERIA FROM PANASQUEIRA MINE IN FUNDÃO-PORTUGAL

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Background

Increasing industrialization, and with it mining activity, can negatively affect the environment. This will lead to high concentrations of metals. Tungsten is one of the most important metals and it is mostly concentrated in Portugal and because of its scarcity in Europe it is considered by the EU a critical metal. Thus, it is important the development of sustainable technologies for the recovery of tungsten [1]. Siderophores are metal chelating agents produced and secreted by microorganisms, usually under Fe-limitation, that form Fe(III)-complexes or other metals that are captured selectively by the bacteria [2].

The focus of this work was to assess the structural diversity of the bacterial community from Panasqueira mine tailings,, look at the isolates functional strategies and to characterize them in terms of their ability to interact with tungsten.

Method

The number of organisms representing metabolic groups - heterotrophic, iron oxidizers, sulfur oxidizers/ reducers - were determined by Most Probable Number technique. The microorganisms were isolated in minimal medium and grouped by RAPD-PCR profiling. Minimum inhibitory concentration tests were performed in R2A medium with different concentrations of tungstate and evaluated after 5 days incubation (25°C). The production of siderophores was assessed by Chrome Azurol Sulfonate (CAS) assay.

Results & Conclusions

The two basins of mine tailings showed different biological activity, the old basin had a higher load of microorganisms and lower number of heterotrophic microorganisms than the recent basin. Total of 141 colonies were isolated and 83 populations were differentiated by their RAPD profile. Seventy-two strains presented high resistance to tungsten until concentrations well beyond the normal environmental concentration. Organisms able to produce siderophores were identified.

The microbial community include different metabolic groups of bacteria able to resist and interact with tungsten, with potential to be used in further bacteria-tungsten interaction studies.

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This work was supported by the project PTDC/AAGREC/3839/2014 from PO da Região_ Centro e PO da Região_Lisboa and by the project ERA-MIN/0002/2015 with public funds from FCT under the programme for International Cooperation ERA-NET, supported by ERA-MIN (2011-2015) funded under the EU 7th Framework Programme. The project ReNATURE_CENTRO-01-0145-FEDER-000007 also support the group's work. Carina Coimbra was funded by a grant from project ERA-MIN/0002/2015, Romeu Francisco from SFRH/BPD/103241/2014 and Rita Branco from SFRH/BPD/110807/2015.

Keywords: Resistance, Siderophores, W mining tailings

P-135 - PRELIMINARY EVALUATION OF DECOLOURISATION OF PROCION AND EVERDIRECT DYES BY YEASTS

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Background

The textile sector is a large and worldwide business and the most important in many countries [1]. This economic activity generates high volumes of effluents due to the large quantities of water used in fabric processing [2]. Furthermore, dyes are resistant to biodegradation and are responsible for toxicity and mutagenic effects to the aquatic life [3, 4]. The aim of this work was to evaluate the ability of selected yeast strains to decolourise specific dyes frequently used in the textile industry, namely reactive dyes.

Method

Previously selected yeasts were tested to their ability to decolourise dyes commonly used in textile industries. Procion Red, Procion Yellow, Procion Navy and Everdirect Yellow were selected for this work. Yeasts HOMOGS20, HOMOGST27A and LIIIS36 were cultivated in NDM medium supplemented with 100 mg/L of each dye, in a 24 well microplate that was incubated at 25 ºC for 48h and 100 rpm. Decolourisation was observed by naked eye. The yeast's ability to decolourise dyes in a solid medium was also tested using NSDM medium supplemented with 100 mg/L of dye and plates were incubated at 25 ºC for 48h. Decolourisation halos around colonies were observed as well as the colour of the colonies themselves.

Results & Conclusions

LIIIS36 was the most effective and versatile strain with regards to the ability to decolourise the dyes tested.
In these conditions, HOMOGST27A seems to be the only one that absorbed most of the dyes instead of performing true decolourisation.

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Financial support was provided by project "EcoTex – Desenvolvimento de soluções mais sustentáveis para coloração têxtil", co-financed by Fundo Europeu de Desenvolvimento Regional (FEDER) through Programa Operacional Regional do Norte (PONorte).

Keywords: Textile dyes, Dye decolourisation, Yeasts

P-136 - BIOREMEDIATION OF PETROLEUM-CONTAMINATED SOILS: MATHEMATICAL MODELLING AS A TOOL FOR THE SIMULATION OF ALTERNATIVE STRATEGIES

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Background

Soil contamination with petroleum-based fuels constitutes a severe environmental problem affecting numerous locations worldwide. *In situ* bioremediation is an alternative to the more aggressive physico-chemical methods. Current knowledge on hydrocarbons biodegradation in soils is still limited and needs further research. In this work, a mathematical model was developed to understand the processes/variables involved in the microbial decontamination of a soil after an oil spill. Different biostimulation strategies were simulated, specifically by adding different electron acceptors.

Method

A mathematical model was developed comprising the description of transport and transformation of petroleum in a saturated soil column. Hexadecane was chosen as a model compound, as alkanes are major constituents of petroleum fuels. The model was implemented in AQUASIM (Reichert, 1994), and addressed the adsorption/desorption of hexadecane, as well as aerobic and anaerobic biodegradation. Two different scenarios were considered: an organic soil column and a clay soil column. The influence of varying concentrations of electron acceptors was simulated (2.5 to 20 mg/L NO_3^-; 5 to 40 mg/L SO_4^{2-}; 10 mg/L O_2). Microbial degradation parameters were obtained from the literature, and the adsorption/desorption parameters were determined experimentally. For that, a series of batch assays was performed, in a range of hexadecane concentrations between 0 and 5 g/kg, for quantifying the partition of hexadecane in a soil/water mixture.

Results & Conclusions

The results from the batch experiments showed that hexadecane mostly adsorb on soil particles (>60%) for all the concentrations tested. From the mathematical modelling, it was observed that for an initial concentration of hexadecane of 60 mg/kg, hexadecane in the solid phase decreases to 0.7 mg/kg at 1 m depth after 2.5 years. At 2 m deep, the concentration was 1.6 mg/kg after 3.5 years. For both soil types, adsorption/desorption, as well as washout by the water flux, exert a greater influence on hexadecane removal than the biological processes. This is mainly due to the slow removal rate of the anaerobic reactions. This model is a useful tool for stakeholders in decision support systems. It will assist on directing bioremediation efforts towards more efficient management and restoration of contaminated sites.

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Acknowledgements: POCI-01-0145-FEDER-016575; ERC Grant n.º 323009; UID/BIO/04469/2013; POCI-01-0145-FEDER-006684; NORTE-01-0145-FEDER-000004; FCOMP-01-0124-FEDER-027462; SFRH/BPD/80528/2011.

Keywords: Bioremediation, Hexadecane, Mathematical modelling, Soil

P-137 - PRESSURIZED SYNGAS BIOCONVERSION: PHYSIOLOGICAL AND MICROBIAL CHARACTERIZATION

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Background

Syngas is mainly composed by CO, H_2 and CO_2 and represents one of the most inexpensive substrates for microbial fermentation. Bioconversion of syngas is a promising technology to recycle lignocellulosic biomass or carbon-based wastes, producing a variety of biofuels and biochemicals. However, a potential bottleneck in industrial implementation of syngas bioconversion technology is the gas-to-liquid mass transfer limitation, reducing the amount of gases available to be used as substrate by microorganisms. In this work, a pressurized bioreactor was used to improve syngas components solubility, assessing the influence of initial syngas pressure on methane and biochemicals production, on CO and H_2 consumption rate and on microbial communities' structure.

Method

Batch syngas bioconversion was performed in a pressurized bioreactor. Phosphate-buffered mineral salt medium was inoculated with anaerobic granular sludge. Bioreactor headspace was pressurized with syngas (60% CO, 30% H_2 and 10% CO_2) until the desired pressure (1.2×10^5 Pa, 3.0×10^5 Pa or 5.2×10^5 Pa). Throughout the bioconversion, CH_4, CO and H_2 in headspace were quantified by GC and liquid products by HPLC. Microbial communities' structure was assessed by 16S rRNA-based techniques (PCR-DGGE); RNA samples were sequenced (Illumina Miseq platform).

Results & Conclusions

The rise of syngas pressure led to a decrease on CO and H_2 consumption rates and CH_4 production rate. When methanogenesis was partially inhibited, propionate and butyrate were the main metabolites produced, showing a metabolic change with increasing syngas pressure. From the Illumina sequencing analysis, it was observed that the relative abundance of bacterial communities tend to decrease (72% to 46%), and archaeal communities increased (25% to 54%) by raising the pressure of syngas from 1.2×10^5 Pa to 5.2×10^5 Pa. In the inoculum and biomass incubated at 1.2×10^5 Pa syngas, 40% of total population were from *Deltraproteobacteria* class and their abundance was reduced 4-fold at 5.2×10^5 Pa. As a direct effect of high pressures of syngas, organisms belonging to *Firmicutes*, *Synergistetes* and *Thermotogae* phyla increased over 10-fold. The predominant phylotypes at highest pressures were related to *Methanobacterium* genus (archaea) and to *Eubacteriaceae*, *Synergistaceae* and *Syntrophobacteraceae* families (bacteria). These results showed a microbial population enrichment suggesting a high specialization for the substrate.

References & Acknowledgments

This work was financially supported by Portuguese Foundation for Science and Technology (FCT): strategic funding UID/BIO/04469/2013 unit and COMPETE 2020 (POCI-01-0145-FEDER-006684), Post-Doctoral and Doctoral grants (SFRH/BPD/101034/2014, SFRH/BPD/104837/2014, PD/BD/128030/2016); and by BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by European Regional Development Fund under the scope of Norte2020 – Programa Operacional Regional do Norte.

Keywords: Syngas, Pressurized bioreactor, Microbial communities

P-138 - MUCORALES STRAINS WITH BIOTECHNOLOGICAL USE: A POLYPHASIC APPROACH IDENTIFICATION

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Background

Mucorales are mostly terrestrial ubiquitous filamentous fungi with coenocytic hyphae and asexual reproduction based on specialized structures (sporangia). Most of these fungi formed zygospores as result of the sexual reproduction. The natural relationships of them are very poorly understood due to the lack of distinguishing morphological characters. Some Mucorales have an important role in nature as decomposers, namely the capacity to degrade xenobiotic or recalcitrant compounds, make them important candidates for biotechnological use in the bioremediation processes. In previous study, two Mucorales isolates, *Gongronella* sp. MUM 10.263 and *Rhizopus* sp. MUM 10.260, isolated from vineyard soils from Alentejo (Portugal), were submitted to a selective adaptation with metalaxyl, an acylalanine fungicide widely used against oomycetes causing downy mildews. Resulting adapted strains, Gongronella sp. MUM 10.262 and Rhizopus sp. MUM 10.261 showed high tolerance and capacity to degrade metalaxyl [1]. The aim of this study was to identify at species level the metalaxyl degrading strains using also several reference Mucorales strains from *Absidia, Circinella*, *Gongronella* and *Rhizopus* genera for a polyphasic approach.

Method

The approach combined classical morphology, molecular biology and Matrix Assisted Laser Desorption Ionization Time of Flight Intact Cell Mass Spectrometry (MALDI-TOF ICMS), as described elsewhere [2].

Results & Conclusions

The results of ITS phylogeny analysis showed that *Rhizopus* sp. is *Rhizopus oryzae*. The spectral analysis confirm that *Rhizopus oryzae*, grouping within other strains from that species. *Gongronella* sp. were grouped with the closely related species *G. butleri* and *G. lacrispora* but forming a distinct cluster. In addition, molecular data grouped *Gongronella* sp. in a separated cluster when compared with the newest specie *G. guangdongensis*, described by Liu et al (2015) [3]. Therefore, from the molecular and proteomic analyses the isolate MUM 10.263 seems to represent a putative new species of *Gongronella*.

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Keywords: Gongronella sp, Rhizopus oryzae, polyphasic approach identification, csM13-PCR, ITS-phylogeny, MALDI-TOF ICMS

P-139 - REVEALING THE DYNAMICS OF POLYMICROBIAL INFECTIONS: UPDATE ON THE QPCR AS A PROMISING TOOL FOR THE QUANTIFICATION OF BACTERIAL JUNGLES

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Abstract

P. aeruginosa and *S. aureus* are important opportunistic human pathogens in many polymicrobial infections. Interactions between them change the infection dynamics, resulting in increased potential for disease development. Determining the relative bacterial abundance through culture-dependent approaches is hindered by the presence of cells in a viable but poorly-cultivable state, or underestimated by the presence of cell-aggregates. The monitoring of bacterial pathogens by the use of culture-independent tools has led to fresh insights into the complex relationships between host and microbes, but often key experimental controls are lacking.
This study aimed at examining changes in microbial composition in *P. aeruginosa* (PA) and *S. aureus* (SA) communities by quantitative PCR (qPCR). Total RNA was extracted and normalized against the amount of an exogenous RNA control-molecule. The Cq value for each gene of interest was transformed into relative quantities taking the differences between the target and the calibrator exogenous mRNA.

Results & Conclusions

Despite its potential, several optimizations strategies had to be implemented in order to obtain reliable and meaningful quantifications of population dynamics by qPCR. Fluctuations in the Cq values for the *16S* rRNA from samples with the same amount of staring material highlight that the accuracy of mRNA quantification by qPCR is limited by mRNA losses during sample processing. Taking the mRNA losses detected, the gene expression of *PA* virulence-related genes was determined to assess if the inefficient mRNA recoveries could compromise the RNA functionality, providing an inaccurate transcription prolife. Results showed no significant differences in the gene expression of the selected genes.
Importantly, the use of the standardized exogenous mRNA was key to normalize mRNA losses across different samples. Without this exogenous control, the comparison between the expression level of the *16S* rRNA and the sample composition lead to misleading interpretations about the relative abundance of each species. Interestingly, we were able to demonstrate, as proof of concept, that using this normalization strategy, a good correlation ($P<0.05$) between the theoretical and the experimental PA/SA ratio was obtained.
Applying the qPCR-methodology coupled with the exogenous mRNA normalization strategy proved to be a reliable tool for the quantification of polymicrobial consortia.

References & Acknowledgments

Portuguese Foundation for Science and Technology (FCT), through the strategic funding of UID/BIO/04469/2013 and COMPETE 2020 (POCI-01-0145-FEDER-006684), and FCT and the European Community fund FEDER, through COMPETE and BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund under the scope of Norte2020. Grant of APM (SFRH/BD/132165/2017). NC is an Investigator FCT.

Keywords: Polymicrobial infections, qPCR, Quantification of mixed communities

P-140 - FROM GENOMIC AND TRANSCRIPTOMIC DATA TO THE IDENTIFICATION OF THE PUTATIVE ENZYMES INVOLVED IN MOLINATE DEGRADATION PATHWAY IN GULOSIBACTER MOLINATIVORAX ON4T

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Background
Molinate is a recalcitrant herbicide used in rice crop protection. So far, *Gulosibacter molinativorax* ON4^T is the only bacterial strain able to break molinate into azepane carboxylate (ACA) and ethanethiol through the activity of molinate hydrolase (MolA). While the sulphur compound is accumulated, ACA is mineralized, supporting ON4^T growth. Although the putative molinate degradation pathway has been proposed, only MolA was identified. The search for genes encoding the enzymes involved in molinate degradation and their genetic environment motivated this study.

Method
To achieve our goal, the genome of strain ON4^T was sequenced using an hybrid assembly of 454, Illumina and PacBio reads. In addition, the transcriptome of strain ON4^T grown in mineral medium supplemented with molinate or caprolactam was compared with that in Luria-Bertani broth. cDNA was produced from mRNA after rRNA-subtractive hybridization, and sequenced in the Ion Proton platform. Normalized data (TMM method) was used to compare the transcription profiles from each library, and to select the target genes to be quantified by qPCR for result validation.

Results & Conclusions
Genomic data revealed that *mol*A was not surrounded by other catabolic genes. This result motivated the use of a comparative transcriptomic approach to identify other genes encoding the potential enzymes involved on molinate degradation. Cytochrome-P450, and glutamine synthetase, among others, have been putatively identified as involved in the herbicide degradation. A qPCR approach is undergoing to validate the data.

References & Acknowledgments
This work was the result of the project: (i) POCI-01-0145-FEDER-006939 (Laboratory for Process Engineering, Environment, Biotechnology and Energy – UID/EQU/00511/2013) funded by the European Regional Development Fund (ERDF), through COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI) and by national funds, through FCT - Fundação para a Ciência e a Tecnologia; (ii) NORTE-01-0145-FEDER-000005 – LEPABE-2-ECO-INNOVATION, supported by North Portugal Regional Operational Programme (NORTE 2020), under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (ERDF); and (iii) postdoctoral grant SFRH/BPD/92894/2013 funded by FCT - Fundação para a Ciência e a Tecnologia.

From genomic and transcriptomic data to the identification of the putative enzymes involved in molinate degradation pathway in Gulosibacter molinativorax ON4T

Keywords: genome, transcriptome, Gulosibacter molinativorax, degradation, molinate

P-141 - ANTIBIOTIC-RESISTANT E. COLI IN AN UV-TREATED EFFLUENT: TROUBLED WATERS AHEAD?

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Background

Urban wastewater treatment plants (UWTP) are unable to effectively eliminate antibiotic resistance genes (ARGs) and antibiotic-resistant bacteria (ARB), thus important pathways for the dissemination of resistance into aquatic systems. Disinfection, e.g. UV irradiation, might be useful in containing such contaminants, though low amounts of ARGs and ARB still persist in the final effluent. Therefore, it is a priority to assess potential risks associated with the bacterial populations in UWTPs outflow.

In this work, we assessed antibiotic resistance and virulence features of cefotaxime-resistant *E. coli* strains that survived UV treatment.

Method

Non-clonal *E. coli* isolates (n=25) were obtained from the final effluent of a UWTP which included a UV-based tertiary treatment. Phylogroup determination and multilocus sequence typing (MLST) were conducted, followed by PCR screening of ARGs, virulence factors and characterization of integrons content. Plasmid DNA purification and replicon typing were performed, followed by mating assays. Illumina-based genome sequencing was performed for 6 selected isolates.

Results & Conclusions

Most of the 25 *E. coli* strains were multidrug resistant (80%) and harbored $bla_{\text{CTX-M-15}}$ (n=15), $bla_{\text{CTX-M-27}}$ (n=4), $bla_{\text{CTX-M-32}}$ (n=4) or $bla_{\text{CTX-M-1}}$ (n=2). PCR screening for other ARGs yielded positive results for $bla_{\text{OXA-1-like}}$ (n=8), *qnrS1* (n=10), *sul1* (n=15), *sul2* (n=15), *sul3* (n=2), *tet*(A) (n=14) and *tet*(B) (n=8). Plasmid DNA was purified from all isolates and restriction analysis resulted in 16 distinct plasmid profiles. *IncF* (n=22), *IncFIB* (n=17), *IncFIA* (n=14) and other six replicons (*IncI1*, *IncP*, *IncI2*, *IncN*, *IncB/O* and *IncX4*) were identified. Mating assays yielded transconjugants for 8 donor strains and co-transfer of tetracycline, gentamicin and sulfamethoxazole/trimethoprim resistance was observed. Strains were affiliated with phylogroups A (n=16), B2 (n=7) and C (n=2). MLST affiliated the strains to ST58 (n=1), ST131 (n=2), ST410 (n=1), ST453 (n=2), ST6897 (n=4), ST1284 (n=1) or unknown MLST types (n=14). *In silico* analyses of selected genomes (n=6) predicted all strains as potential human pathogens. and revealed 4 different serotypes as well as intrinsic and acquired resistance mechanisms previously unidentified by PCR. A variety of virulence factors such as *iss* (n=5), *gad* (n=4), *ipfA* (n=3) and many others (*sat*, *iroN*, *cma*, *iha*, *capU*, *astA*, *senB* and *mchF*) were also observed in these isolates.

UV-surviving strains were shown to possess determinants involved in pathogenicity, antimicrobial resistance and plasmid transfer, highlighting the need for improvements of wastewater disinfection to mitigate the risks associated with UWTP discharge.

Keywords: Urban wastewater treatment plants, UV-C radiation, Extended-spectrum beta-lactamases, Escherichia coli

P-142 - WHOLE-GENOME SEQUENCING OF A POTENTIALLY NEW PSEUDOMONAS SPECIES FROM A NON-CLINICAL NICHE IN ANGOLA UNCOVERS A WIDE ARRAY OF ANTIMICROBIAL RESISTANCE GENES AND MOBILE GENETIC ELEMENTS

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Background

The *Pseudomonas putida* phylogenetic group (PPPG) represents a heterogeneous cluster of environmental and opportunistic pathogens, such as *P. putida*, *Pseudomonas monteilii* and *Pseudomonas mosselii*.1 Due to their resemblance, species identification within PPPG is somehow challenging.1 In addition, very few reports have associated PPPG with antibiotic resistance genes (ARGs).2 We provide a snapshot of the ARGs, heavy-metal resistance genes (MRGs) and mobile genetic elements (MGEs) identified in an environmental isolate belonging to a potentially new *Pseudomonas* species.

Method

FFUP_PS_473 isolate was collected from a septic tank in Catumbela (Benguela, Angola). WGS was performed by Illumina HiSeq, *de novo* assembly by SPAdes and annotation by RAST. Species identification was initially assessed by MALDI-TOF/MS and later confirmed by multi-locus sequence analysis (MLSA, using *rpoD*, *gyrB*, *rpoB* and 16S rRNA genes) and average nucleotide identity (ANI) analysis. ARGs were searched by ResFinder, genomic islands (GIs) with IslandViewer 4 and bacteriophages by PHAST.

Results & Conclusions

FFUP_PS_473 genome size was ~5,3 Mb with 4871 coding sequences (CDS) and 59,8% of GC content. MALDI-TOF/MS identified FFUP_PS_473 as *Pseudomonas thermotolerans*, belonging to the PPPG. However, the genome of this isolate displayed ANI values ranging between 77-83% (below the 95% ANI cut-off for species delineation)1 when compared with *Pseudomonas* spp. type strains genomes. This was corroborated by MLSA values below the 97% MLSA cut-off,1 suggesting that FFUP_PS_473 indeed represents a new species, within the PPPG. The closest genome corresponded to *Pseudomonas alkylphenolia* type strain KL28 (83% similarity). Concerning ARGs, a new integron (designated In1453 by INTEGRALL) harboring *cmlA8*, $bla_{OXA-246}$, *qnrVC1* and *arr-2*, conferring resistance to chloramphenicol, beta-lactams, fluoroquinolones and rifampicin, respectively, was identified. A Tn*5393*-like transposon harboring resistance genes to streptomycin (*strA* and *strB*) was also identified. MRGs conferring resistance to arsenium, chromium, copper and cobalt-zinc-cadmium were also observed. 22 GIs were predicted, including a GI harboring In1453 and one housing the Tn*5393*-like transposon. Three intact elements with size ranging from 20 to 50 kb and a GC content of about 60%. Interestingly, *in silico* analysis revealed that related phages were infecting several environmental species of the *Pseudomonas fluorescens* and *koreensis* phylogenetic subgroups. The ANI and MLSA results suggest that the FFUP_PS_473 strain most likely represents a new species, belonging to PPPG. The simultaneous existence of ARGs and MRGs in non-clinical niches is particularly worrisome, due to the known co-selection of these genes exerted by widespread metal pressure in the environment.3

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Acknowledgments:
FEDER/POCI/01/0145/FEDER/007728, FCT/MEC, SFRH/BD/104095/2014, SFRH/BPD/95556/2013.

Keywords: Whole-genome sequencing, Pseudomonas putida phylogenetic group, antibiotic resistance genes, mobile genetic elements

P-143 - HIGH-RESOLUTION GENOMIC ANALYSIS OF THE GLOBALLY DISSEMINATED MULTIDRUG RESISTANCE KLEBSIELLA PNEUMONIAE CLONAL GROUPS 14 AND 15

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Abstract

Klebsiella pneumoniae (*Kp*) Clonal Groups (CG) 14 and 15 are overrepresented among multidrug resistant (MDR) *Kp* strains worldwide [1], however characterization of the different CG14 and CG15 lineages at the genome level is still missing. We performed a comparative high-resolution genomic analysis of *Kp* CG14 and CG15 in order to delineate these populations.

Results & Conclusions

WGS of nine representative Portuguese ST15 (4 *wzi*24-1 *wzi*93-1 *wzi*19-1 *wzi89*) and ST14 (1 *wzi*2-1 *wzi*16) isolates showing variable PFGE-types (2003-2013) were obtained using Illumina MiSeq (2x300bp pair-ended runs/coverage:100x). Genomic sequences of all CG15 (n=61; 2004-2015) and CG14 (n=29; 1986-2014) worldwide distributed isolates available on NCBI and BIGSdb databases (as from August 2016) were included. Assembly was performed using SPAdes and core genome (>80% similarity/coverage) was defined using home Perl scripts. Core genes were concatenated, aligned, and SNPs extracted to generate a maximum likelihood phylogenetic tree (100 bootstraps) in R (Phangorn package). The *cps* operon within and between lineages was analyzed using EasyFig and BRIG. Plasmid content of representative isolates determined by PLACNET (n=17) [2] and from complete genomes (n=5) was analysed by hierarchical clustering of plasmid proteomes (n=21 genomes/66 plasmids). Virulence, *cps* cluster genes, antibiotic resistance (ABR) and metal tolerance genes were searched using BIGSbd-Kp and/or ResFinder.

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Keywords: MDR Klebsiella pneumoniae, phylogenomics, cps locus, plasmidome

P-144 - GENOMIC INSIGHTS INTO THE MRSA CLONAL REPLACEMENT IN PORTUGUESE HOSPITALS

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Background

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the major nosocomial pathogens worldwide. In Portuguese hospitals, several clonal replacements were observed in the last two decades but the reasons behind those changes remains unclear. In this study, we aimed to identify the genetic factors associated to the rise of EMRSA-15 and the decay of the Brazilian clone, the most striking of the replacements that have occurred.

Method

The whole genome sequence of 12 isolates, representing the transition from the Brazilian (*n*=4) to the EMRSA-15 (*n*=8) clone, collected between 1995 and 2011, was determined. Libraries were generated with Nextera XT kit and sequenced using MiSeq (30x). Raw reads were *de novo* assembled and contigs analyzed using ResFinder v3.0[1] (identification of acquired antimicrobial resistance genes), VirulenceFinder v1.5[2] (identification of acquired virulence genes) and annotated with Prokka[3]. Additionally, genomes were compared by cgMLST[4] using chewBBACA[5], based on the schema available for *S. aureus*.

Results & Conclusions

Approximately 90% of the 1861 core gene alleles were different between EMRSA-15 and the Brazilian clone. Additionally, the two clones differed significantly in their accessory genome. Strains of the Brazilian clone had resistance genes for aminoglycoside, β-lactam, fluoroquinolones, macrolides, chloramphenicol, fosfomycin and tetracycline, while EMRSA-15 was resistant to β-lactams and macrolides only. Brazilian clone strains carried serine proteases (*splE, splB, splA*) and leukocidins (*lukDE*) encoding genes, whereas EMRSA-15 strains harbored several enterotoxin genes (*seg*, *sen*, *seu*, *sei*, *sem* and *seo*). Furthermore, the two clones differ in the content and nucleotide sequence of genes for cell wall anchored proteins (*sasA*, *sasC, sasD*, *sasF*, *sasG, sasK*, *sdrC*, *clfA*, *fnbA*). A total of four different phages were identified among the clones: two phages were specific for one of the two clones and the other two phages were shared between both clones. While the Brazilian clone strains carried a single plasmid harboring chloramphenicol resistance, EMRSA-15 strains carried three plasmids harboring penicillin resistance gene (*blaZ*), macrolide resistance (*ermC*) and heavy metal resistance genes (*cadD* and *ars*).

The Brazilian and EMRSA-15 clones differ significantly, both in the sequence of their core genes and in the content of accessory genome. Although carrying a lower number of antibiotic resistance genes than the Brazilian clone, EMRSA-15 carried a different set of virulence factors, cell wall anchored proteins, phages and harbored more plasmids. Altogether, these factors might have provided EMRSA-15 clone with a competitive advantage over the Brazilian clone.

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4- http://www.cgmlst.org/ncs
5- https://github.com/B-UMMI/chewBBACA/

Keywords: Staphylococcus aureus, MRSA, Whole genome sequencing, Clonal replacment

P-145 - MULTI-DRUG RESISTANT GRAM-NEGATIVE ISOLATES RECOVERED FROM AN AMAZONIAN MESOTROPHIC LAKE

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Background

Environmental resistance is one of the major concerns in public health today as we are facing an increase in the number of infections caused by multiresistant bacteria. Free-living micro-organisms play a key role as reservoirs of antibiotic resistance and also directly contribute to the spread of this resistance. Polluted aquatic systems have higher levels of contamination with multiresistant bacteria. In this context, we analyzed the cultivable resistome of an Amazonian mesotrophic lake that is used for water supply, irrigation and recreational activities and it is near to a densely populated region.

Method

Six sampling points were selected and one liter of water was collected in triplicate. Aliquots were taken for physical, chemical and microbiological analyzes. Gram-negative strains were isolated by culturing water samples in agar MacConkey supplemented with cefotaxime or imipenem. Genes encoding for beta-lactamase were screened by PCR. Antibiotic susceptibility testing was performed using standard disc diffusion method. The genomes of two isolates of public health interest (*E. coli* APC43A and *A. baumannii* APC25) were sequenced on the Ion Torrent PGM platform. Genomes were automatically annotated using RAST server and resistance genes were screened using the databases of CARD and ResFinder.

Results & Conclusions

A total of 33 strains were isolated. The main beta-lactamase gene detected by PCR was bla_{CTX} (13/33 – 39.4%). Thirty-one strains were classified as multi-drug resistant (93.9%). The sequencing and analysis of 16S rRNA gene revealed that the isolates were mainly affiliated to the genera *Escherichia*, *Acinetobacter*, *Klebsiella*, *Enterobacter*, and *Pseudomonas*. Genomic analysis showed that antibiotic resistance genes are located primarily in the chromosome and that mobile genetic elements are directly related to the presence of $bla_{CTX\text{-}M\text{-}15}$ genes. *E. coli* APC43A was identified as pathogenic specie and carries the virulence factors of Shiga Toxin-Producing *E. coli* (STEC). The genome of *E. coli* showed 25 pathogenicity islands and five resistance islands. *A. baumannii* APC25 showed 11 pathogenicity islands and 10 resistance islands. Beta-lactamase genes $bla_{OXA\text{-}208}$ and $bla_{ADC\text{-}25}$ were detected in *A. baumannii* genome by ResFinder but not by CARD. These results suggest a high dissemination of extended-spectrum beta-lactamase genes in Gram-negative species of Lake Água Preta. Although the lake does not present characteristics of an impacted environment, it contains multi-drug resistant pathogenic strains such as *E. coli* APC43A.

References & Acknowledgments

The authors would like to thank the financial support of the Brazilian and Portuguese agencies Coordenação de Aperfeiçoamento de Pessoal de Nível Superior and Fundação para a Ciência e Tecnologia.

Multi-Drug resistant Gram-negative isolates recovered from an Amazonian mesotrophic lake.

Keywords: Resistome, Environmental resistance, Escherichia coli, Acinetobacter baumannii, Genomics

P-146 - WHOLE-GENOME SEQUENCING OF LABRYS PORTUCALENSIS F11 AND RHODOCOCCUS SP. FP1 PROVIDES GENETIC INSIGHTS INTO THEIR XENOBIOTIC METABOLIC FEATURES

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Background

Bacteria with diverse xenobiotic-degrading capacities, harboring multiple catabolic pathways, are extremely promising for degradation of environmental pollutants. *Labrys portucalensis* F11 and *Rhodococcus* sp. FP1 were isolated from sediments collected at the industrial chemical complex of Estarreja, northern Portugal. Both strains have demonstrated ability to degrade several aromatic compounds under aerobic conditions, including pharmaceuticals (fluoroquinolones[1], fluoxetine[2], diclofenac and estradiols) and industrial and agro-chemicals (fluorobenzene[3], fluorophenol[4], fluoroanilines[5] and bisphenols). Understanding the metabolic potential of *Labrys portucalensis* F11 *and Rhodoccus* sp. FP1 could provide the basis for their application in bioremediation processes.

Method

Genomes were sequenced using Illumina HiSeq 2500 platform and FASTQ reads assembled using an algorithm based on de Bruijn graphs. Gene prediction and genome annotation was performed using Rapid Annotation Subsystems Technology (RAST) v2.0.

Results & Conclusions

The genome of *Labrys portucalensis* F11 comprises 7,952,755 bp with a GC content of 63.5% whilst the genome of *Rhodococcus* sp FP1 is composed of 9,630,728 bp with 67.2% of GC content. For F11, a total of 7406 coding DNA sequences (CDS) were predicted, of which 3224 (44% of CDS) were assigned to subsystems. For FP1, out of the total 9094 CDS, only 3258 (ca. 36% of the total CDS) were allocated in subsystems. On both cases, the most abundant subsystems were those involved in the metabolism of amino acid derivatives and carbohydrates. In addition, both genomes harbor genes involved in the catabolism of aromatic compounds, representing about 3.2 and 4.5% of the total protein-encoding genes of F11 and FP1, respectively. Metabolism of aromatics begins with ring hydroxylation and genes encoding enzymes for this step such as dioxygenases and monooxygenases were present on both genomes. In addition, genes predicted to encode different ring-cleaving dioxygenases that catalyze the dearomatization steps, namely, gentisate 1,2-dioxygenase, catechol 2,3-dioxygenase, protocatechuate 3,4-dioxygenase and catechol 1,2-dioxygenase were also present on both genomes. This work provides new insights into the genetic determinants that may help devising strategies for bioremediation of environments polluted with xenobiotics.

References & Acknowledgments

C.L. Amorim and I.S. Moreira acknowledge the research grants from Fundação para a Ciência e Tecnologia (FCT), Portugal (SFRH/BPD/96481/2013 and SFRH/BPD/87251/2012) and Fundo Social Europeu (Programa Operacional Potencial Humano (POPH), Quadro de Referência Estratégico Nacional (QREN)). I.S. Henriques was financed through the programme Investigador FCT (IF/00492/2013). This work was also supported by FCT through the projects UID/Multi/50016/2013 and UID/AMB/50017/2013.

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Keywords: Genome annotation; Xenobiotics; Metabolic pathways

P-147 - METAGENOMIC DETERMINATION OF STRUCTURAL MICROBIAL DIVERSITY FROM A BROWSE-FED GOAT DIGESTIVE TRACT

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Background

Second generation biofuels, mainly ethanol, produced from lignocellulosic biomass –LCB have been considered an important renewable energy alternative to fossil fuels[1]. However, due to LCB complex chemical structure, the improvement of LCB-deconstruction protocols (for further (bio)conversion into biofuels) is the main priority to make their production economically sustainable[1]. Goat digestive tract microbes are specialized in degradation of lignocellulosic plants, and may constitute a promising source for lignocellulolytic-degrading enzymes, nevertheless studies applying high-throughput sequencing techniques to determine the structural microbial diversity in goats' digestive tract are scarce[2]. This study aims to determine the structural microbial diversity along the digestive tract of a Portuguese browse-fed goat and was included in a more vast investigation concerning the study of the activity of microbial-lignocellulolytic-degrading enzymes present in the digestive tract of the goat.

Method

Total DNA was extracted from LCB content present in goat's rumen, omasum-abomasum, small intestine, cecum and large intestine. The structural microbial diversity was determined by metagenomic 16S rDNA Illumina tags analyses for *Bacteria* and *Archaea*. Quality control, phylogenetic analyses and classification of the sequences were performed in mothur package with SSU ARB-SILVA database.

Results & Conclusions

Populations related to 19 phyla of *Bacteria* were identified. However, only *Bacteroidetes*, *Cyanobacteria*, *Firmicutes*, *Proteobacteria* and *Tenericutes* were detected in all sections of goat digestive tract. Members of *Bacteroidetes* and *Firmicutes* constituted the predominant populations. The dominant populations in omasum-abomasum belonged to class *Bacteroidia* representing 38.1% of the total sequences, while in other digestive regions members of class *Clostridia* were predominant, achieving the highest value of 96.3% in small intestine. At genus level, 88.4% of the total *Bacteria* sequences constituted unclassified taxa, and phylum *Clostridia* encompassed 50% of the total unclassified sequences at genus level. Sequences of *Archaea* were detected in all sections of digestive tract except in the small intestine, and all sequences belonged to phylum *Euryarcheota*. In rumen and omasum-abomasum members of genera *Methanocorpusculum* represented ~98% of archaeal population, while in cecum and large intestine genus *Methanobrevibacter* represented ~99% of archaeal population. The results show the existence of dominant populations in the goat digestive tract belonging to *Bacteroidia* and *Clostridia* classes that are known to catalyzed biomass degradation in ruminants[2]. The high abundance and diversity of unclassified populations provides evidence that these populations can be a source of novel and uncharacterized microbial-lignocellulolytic-degrading enzymes.

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This research was supported by FCT, BLC3, CNC, CEF and ITQB.

Metagenomic determination of structural microbial diversity from a browse-fed goat digestive tract

Keywords: Goat, Metagenome, Illumina, Bacteria, Archaea

P-148 - GENOME SEQUENCE AND ANNOTATION OF THE NON-CONVENTIONAL YEAST HANSENIASPORA GUILLIERMONDII UTAD222

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Background

Species of the *Hanseniaspora* genus have several interesting metabolic and enzymatic properties, that are absent in *Saccharomyces cerevisiae*, and that contribute to the improvement of wines sensory profiles, even in co-inoculated fermentations. Recently, a transcriptomics-based approach was used to examine how *Hanseniaspora guilliermondii* UTAD222 affected *S. cerevisiae* during a wine fermentation. The presence of *H. guilliermondii* dramatically influenced the expression patterns of various flavour-active compounds associated genes in *S. cerevisiae*, which could be associated to the differences obtained on the aroma profiles of the wines (1). In this study we aim to shed light into the poorly studied biology and physiology of the *H. guilliermondii* strain, contributing to the better understanding of yeast–yeast interactions occurring during wine fermentation.

Method

In this work the genome sequence of the *H. guilliermondii* UTAD222 strain was disclosed as well as a corresponding manually curated annotation. For this, a strategy coupling automatic gene detection (using gene-finder algorithms) combined with manual validation was applied.

Results & Conclusions

The analysis of the genome sequence of the UTAD222 strain will be presented and compared with those of other species of the *Hanseniaspora* genus and of *Saccharomyces cerevisiae* wine strains, in a comparative genomics perspective. Particular attention will be given to genes encoding enzymes involved in formation of volatile aroma compounds as well as genes encoding relevant enzymes or predicted to belong to nitrogen-sensing systems based on orthology with genes described to perform such function in *S. cerevisiae*.

Our results contribute not only for the understanding of the molecular basis of yeast dynamics during wine fermentation but also will boost the exploitation of *H. guilliermondii* as a microbial catalyst since it has several interesting phenotypic traits that turn it attractive for other industrial applications.

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This work was supported by FEDER through POCI-COMPETE 2020 and by Fundação Ciência e Tecnologia (BI/PTDC/AGR-TEC/3315/2014_2016, Project SMARTWINE-"Smarter wine fermentations: integrating omictools for development of novel mixed-starter cultures for tailoring wine production"). Support from Biosystems and Integrative Sciences Institute (BioISI; FCT/UID/Multi/04046/2013) is also acknowledged. Funding received by iBB-Institute for Bioengineering and Biosciences from FCT-Portuguese Foundation for Science and Technology (UID/BIO/04565/2013) and from Programa Operacional Regional de Lisboa 2020 (project no. 007317) is also acknowledged.

Genome sequence and annotation of the non-conventional yeast *Hanseniaspora guilliermondii* UTAD222

Keywords: H. guilliermondii, wine fermentation, comparative genomics, mixed-cultures

P-149 - THE RHIZOSPHERE MICROBIOME AND OAK HEALTH

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Background

Cork oak (*Quercus suber*) and English oak (*Q. robur*) are the most important and iconic tree species in Portugal and Britain. These species are commonly affected by chronic oak decline (COD), a syndrome characterized by progressive death of branches and extensive dieback over several years, leading to socioeconomic loss and environmental and ecological impacts. Acute oak decline (AOD) is a new decline-disease that threatens oak trees in the UK. It is characterized by necrotic stem lesions (caused by a polybacterial complex), *Agrillus* beetle galleries and dark and weeping patches on tree stems. In order to improve our understanding of *Quercus* species health, research has been carried out to study the factors that contribute to the decline of these trees. Here, we focus on the rhizosphere microbiome since these microbial communities interact with their host in a complex way and play a pivotal role in the tree functioning. They enhance tree disease resistance and nutrient uptake and therefore influence their growth, development and health. It is of the utmost importance to understand how these microbial communities are impacted by different factors and their role in the context of forest decline.

Method

This international project aims to characterize the bacterial and fungal communities of cork oak and English oak by high-throughput sequencing and correlate the rhizosphere microbiome with oak health status. Rhizosphere soil samples were collected from healthy and COD trees in both countries as well as from AOD trees in the UK. Bulk soil samples were used as rhizosphere effect control.

Results & Conclusions

The results showed that country/tree species (PT/*Q. suber* vs UK/*Q. robur*), forest location and soil compartment (rhizosphere vs bulk soil) are shaping the bacterial and fungal communities. Despite these structuring factors, the composition of rhizosphere microbiome is correlated with the tree health status of cork oak and English oak, but proof of cause and effect is required. Further studies will be needed to understand the ecological role of these bacterial and fungal consortia in tree health.

References & Acknowledgments

Diogo Pinho was supported by a FCT (PhD grant SFRH/BD/100665/2014) and COST Action BioLink FP1305 (STSM grant ECOST-STSM-FP1305-071116-080698)

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The rhizosphere microbiome and oak health

Keywords: Rhizosphere microbiome, Cork oak, English oak, Quercus, Oak decline, Tree health

P-150 - ACETIC ACID BACTERIA FROM VINEGAR: ISOLATION, GENOMIC PROFILING, AND WHOLE GENOME SEQUENCING

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Background

Acetic acid bacteria (AAB) are strictly aerobic *Alphaproteobacteria* known for their ability to oxidize ethanol into acetic acid, a process commonly referred to as vinegar production [1]. Currently, the vinegar industry is limited by the lack of adequate monitoring methodologies and the use of an undefined microbial community as inoculum, limiting the use of prophylactic or corrective measures in case of an acetification arrest [2]. The objective of this study was to identify and characterize the AAB producing vinegars at the industrial level.

Method

AAB were isolated from a variety of vinegars and were grouped based on REP-PCR and RAPD-PCR fingerprinting. Group representatives were selected for 16S rRNA gene sequencing and a phylogeny was reconstructed. DNA was extracted from five vinegar samples, corresponding to different stages of a red wine vinegar production cycle. Two regions of the 16S rRNA gene were amplified, sequenced by NGS and identified by homology search. Lastly, the genome of a cellulose-producing AAB was sequenced by 3rd generation sequencing (Oxford Nanopore MinION) and the whole genome was reconstructed.

Results & Conclusions

A dendrogram-based identification using type strains from different species of *Komagataeibacter* was applied to 22 AAB, purified from vinegar samples. AAB isolates were grouped into 5 strains based on genomic profiles and 16S rRNA gene phylogenetic reconstruction positioned all strains in genus *Komagataeibacter*. Since AAB exhibit highly conserved 16S rRNA sequences, identification at species level was not possible. The results of the microbial profiling demonstrate that *Komagataeibacter* spp. strains clearly dominate all phases of this industrial process. The acetification starts with a proportion of AAB of around 90% and this value progressively increases until 100% of AAB is reached. The whole genome sequencing of a cellulose-producing AAB resulted in a single contig with a size of 3.2 Mb and 5 plasmids (34-430 kb). Although this strain seemed phylogenetically related to *K. nataicola* and *K. sucrofermentans*, a genome similarity of 83% was found with a strain of *K. europaeus*, confirming the low applicability of 16S rRNA gene for AAB identification. Additional attempts to reconstruct multigenic phylogenies did not resolve the taxonomic affiliation of this strain. In agreement with the observed cellulose production, an atypical phenotype of *K. europaeus*, genome draft analysis revealed a complete cellulose synthase operon.

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Acetic acid bacteria from vinegar: isolation, genomic profiling, and whole genome sequencing

Keywords: acetic acid bacteria, genomic fingerprinting, microbial profiling, molecular identification, 3rd generation sequencing, Oxford Nanopore MinION

P-151 - THE GENOMIC ARCHITECTURE OF THE TWO COLD-ADAPTED GENERA EXIGUOBACTERIUM AND PSYCHROBACTER: EVIDENCE OF FUNCTIONAL REDUCTION IN EXIGUOBACTERIUM ANTARCTICUM B7 GENOME

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Background

Exiguobacterium and *Psychrobacter* are bacterial genera with several cold-adapted species. These extremophiles are commonly isolated from the same habitats in Earth's cryosphere and have great ecological and biotechnological relevance. *Psychrobacter* species have been isolated from the deep water of the sea, permafrosts, Antarctic glacial ice, and sediment, among other habitats. *Exiguobacterium* species are commonly isolated from glacial ice, hot springs, the rhizosphere of plants, permafrosts, and tropical and temperate soils. Our work presents the results of a comparative genomic analysis performed with the genera *Exiguobacterium* and *Psychrobacter*. The results obtained helped us understand and visualize the adaptive molecular diversity of these two phylogenetically distant but ecologically similar cold-adapted genera.

Method

We have carefully reviewed the seventy-nine genomes of both genera deposited in the GenBank and selected four genomes for each genus to perform the comparative analysis. The selection was based on two criteria: (i) the "habitat" and "growth temperature" information contained in the literature or BioSample data and (ii) the completeness of the genomes. Only psychrotrophic or psychrophilic species with genomes that were completely assembled were selected for analysis. The genome of *Exiguobacterium antarcticum* B7 was previously sequenced by our research group using a hybrid assembly methodology that used fragments and mate-paired libraries.

Results & Conclusions

The nucleotide identity between *Exiguobacterium* genomes was higher than 90%. Three genomic islands were identified in *E. antarcticum* B7 genome. These islands contained genes involved in flagella biosynthesis and chemotaxis, as well as enzymes for carotenoid biosynthesis. Clustering of cold shock proteins by Ka/Ks ratio suggests the occurrence of a positive selection over these genes leading to the formation of three distinct clades that are evolving independently. Neighbour-joining clustering of complete genomes showed that the species isolated at the poles were more closely related. A total of 92 genes were shared between *Exiguobacterium* and *Psychrobacter*. A reduction in the genomic content of *E. antarcticum* B7 was observed. It presented the smallest genome size of its genus and a lower number of genes because of the loss of many gene families compared to the other genomes. In our study, eight genomes of *Exiguobacterium* and *Psychrobacter* were compared and analysed. *Psychrobacter* showed higher genomic plasticity and *E. antarcticum* B7 presented a large decrease in genomic content without changing its ability to grow in cold environments.

References & Acknowledgments

The authors would like to thank Brazilian agencies CAPES and CNPq for its financial support.

Keywords: Psychrobacter, Exiguobacterium, cold adaptation, psychrophiles, Comparative genomics, Extremophiles

P-152 - DETECTION AND QUANTIFICATION BY REAL-TIME PCR OF ECTOMYCORRHIZAL FUNGI IN INOCULUM FORMULATIONS FOR URBAN TREES APPLICATION

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Background
Urban trees are key elements in mitigating the common environmental problems in urban areas, through provisioning crucial ecosystem services such as air quality improvement, decrease of water runoff and microclimate mitigation. Because of their well-known host tree benefits, Ectomycorrhizal Fungi (EcM) may play an important role in urban tree management, improving tree vigor, and thus the extent and resilience of ecosystem services delivered by urban trees under stress. Therefore it is important to develop dedicated EcM-inocula to improve urban tree health and its associated ecosystem service delivery. To support the study of the effect of inoculum application in urban context it is necessary to establish new biotechnology tools and test their efficacy in laboratory trials. The major aims of this work were (i) to isolate EcM species known to associate with the selected tree species chosen (*Tilia tomentosa*) and (ii) to develop and test (in situ & ex situ) a specific RT-qPCR assays for detection and quantification of ectomycorrhizal RNA. The latter will allow fast, quantitative monitoring of the selected target species over time, space or different environmental conditions and can be directly applied to RNA samples from *in-vitro* and field experiments.

Method
Two key-criteria were used in the fungi species selection: EcM species that are present on healthy urban trees, but absent on the unhealthy ones; and (ii) EcM species from forest/old park trees that are not found on the unhealthy street trees. Fungi were isolated from Northern Portugal and identified by molecular techniques (DNA extraction, PCR, sequencing). For each fungal species selected, a real-time reverse-transcription quantitative PCR (RT-qPCR) assay was developed based on the ITS sequences available in NCBI and UNITED data base enabling specific monitoring and quantification of viable or active EcM of interest during the experiments that will be conducted.

Results & Conclusions
Ten new fungal isolates were successfully isolated, comprising the genera *Russula*, *Pisolithus* and *Paxillus*. The standard curves obtained for mycelial quantification of the chosen EcM species satisfy the requirements for real-time PCR, showing high reaction efficiency. Under the conditions established in the present work, mycelium concentration was detectable to levels adequate for biomass in situ quantification.

References & Acknowledgments
This work was supported by National Funds through FCT under the project UID/Multi/50016/2013 and by the project URBANMYCOSERVE - Understanding and Managing Urban Ectomycorrhizal Fungi Communities to Increase the Health and Ecosystem Service Provisioning of Urban Trees, Transnational programme BiodivERsA3, funded by FCT. Miguel Ramos has the support of FCT grant SFRH/BD/111056/2015.

Detection and quantification by real-time PCR of ectomycorrhizal fungi in inoculum formulations for urban trees application

Keywords: real-time PCR

P-153 - SELECTION AND ACCLIMATIZATION OF STRAINS OF EDIBLE MYCORRHIZAL FUNGI FOR IMPROVED FIELD PERSISTENCE

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Background
The influence of environmental factors in the diversity of ectomycorrhizal (ECM) species has been shown however the response of ECM fungi to climate variation is poorly understood. Although it has been shown that some ECM fungi have the ability to acclimate to certain abiotic conditions, this may vary amongst strains due to different evolutionary histories. The use of native strains with strong adaptive skills to different abiotic and biotic scenarios could be determinant for the success of the plantations and therefore research on this topic urge. The present work will focus on screening high performance strains of the edible species *Lactarius deliciosus* (L. del.) and assess its ability to adapt to abiotic stresses to ensure a more sustainable choice of isolates when aiming at the production of edible mushrooms.

Method
The ability of L. del. to acclimate to cold and hot temperatures was studied by analyzing the effects of exposure on growth and amino acid metabolism. Fungal plugs were taken from previously grown cultures, placed in fresh medium, on top of a cellophane sheet. They were grown for three weeks at 25ºC and then subjected to different temperatures, namely 15ºC, 20ºC, 30ºC and 35ºC for 15 days. Progressive exposure was also tested for 15ºC where the temperature was lowered at a lower rate. The permanence at 25ºC was used as control. The expansion radius was measured every week for 6 weeks. The fungus was collected for metabolomic analysis.

Results & Conclusions
Among the different treatments, the highest growth was obtained at 25ºC, and 35ºC was lethal to the mycelium after 15 days of exposure. Temperatures of 15ºC and 30ºC were clearly stress temperatures to be considered in subsequent studies. L. del. isolate was shown not to be easily tamed regarding temperature. The fungus exposed to 15ºC and 30ºC for 15 days did not show any advantage when subcultured to that same temperature compared to those who had not been acclimated. These points will represent important and innovative contributions to the understanding of what triggers mycelium development and mushroom formation.

References & Acknowledgments
This work was supported by National Funds through FCT under the project UID/Multi/50016/2013 and by the project URBANMYCOSERVE - Understanding and Managing Urban Ectomycorrhizal Fungi Communities to Increase the Health and Ecosystem Service Provisioning of Urban Trees, Transnational programme BiodivERsA3, funded by FCT – Fundação para a Ciência e Tecnologia. Miguel Ramos has the support of FCT grant SFRH/BD/111056/2015.

Selection and acclimatization of strains of edible mycorrhizal fungi for improved field persistence

P-154 - TRANSCRIPTIONAL REGULATION OF PKS1 AND PKS15 IN PATHOGENIC MYCOBACTERIA

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Abstract

Mycobacteria are aerobic actinomycetes that contain a complex three-layer cell envelope. Amid the variety of lipids found in that structure are phthiocerol dimycocerosates (PDIMs) and their derivate phenolic glycolipids (PGLs). PGLs are known to be associated with virulence and defence mechanisms against stress[1]. The biosynthetic pathway responsible for synthesis of phenolphthiocerol moiety of the PGLs comprises *pks15/1* along with other genes encoding for type I polyketide synthases constituting PDIM + PGL locus[2]. The *pks15/1* locus was shown to be highly conserved among PGL-producers while it is disrupted by a few base pair deletion in non-producers[3]. The *pks1*, *pks15*, *fadD22* and *Rv2949c* genes are enclosed in the PDIM+PGL locus but it remains unknown whether they are co-transcribed as polycistronic transcription units. In this work, we aimed at identifying the regulatory pattern responsible for controlling *pks1* and *pks15* transcription through a genome-wide approach.
pks1 and *pks15* transcriptional regulation data was extracted from public databases. Differential expression analysis was based on 94 RNA-seq independent experiments from *Mycobacterium tuberculosis* and *Mycobacterium bovis* strains grown under stress conditions. Data was collected for all genes co-expressed with *pks1* and *pks15* (biclustered), for genes encoding polyketide synthases and for sigma factors.

Results & Conclusions

A regulation linkage between *pks1*, *pks15* and *fadD22* is consistently suggested from expression data collected from databases. RNA-seq data for *M. tuberculosis* reveals that *fadD22* and *pks1* are highly correlated, and also with the upstream gene *Rv2949c*, while in contrast *pks15* shows a minor correlation with the remaining gene set. For *M. bovis*, *pks15/1* and *fadD22* have the highest correlation, in parallel with *Rv2949c* (*Mb2973c*). Differential expression of *pks1*, *pks15*, and *fadD22* was revealed under hypoxia, iron and nutritional stress conditions. Coherent results in different ecotypes support the hypothesis that these four genes may constitute an operon. The RNA-seq analysis also revealed that *SigG* (expressed in SOS response and macrophage infection[4]) and *SigK* (unknown role[4]) are the σ factors with higher correlation with *pks1* and *fadD22*. Reconstruction of the regulatory network controlling *pks1* and *pks15* transcription includes cloning of putative promotors and construction of deletion mutants, which are currently underway.

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Keywords: transcriptional regulation, phenolglycolipid biosynthesis, pathogenic mycobacteria, RNAseq, pks1, pks15

P-155 - PROMOTERS INITIATION KINETICS AFFECT THE INFLUENCE OF COMPONENT GENES DYNAMICS ON GENE NETWORKS BEHAVIOR

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Abstract

Complex bioprocesses, such as cell growth and replication, are made possible by the temporally orderly execution of simpler semi-independent processes, such as time counting and decision making, that occur at various spatial and temporal scales. Understanding the information flow between the various scales is a fundamental goal of life sciences. It is well established that, in Escherichia coli, transcription is a multi-step process [1,2] whose regulation occurs mostly during the initial, rate-limiting steps via the action of activators, repressors and global regulatory molecules [3-5]. This results in a specific RNA production dynamics that defines also protein numbers. Recent studies [6-9] started exploring how the rate-limiting steps of individual genes affect genetic circuits. Here we present our recent findings on this subject, obtained from a comparison of the inner-dynamics of transcription when inducing the same promoter, Plac/ara-1 by different inducers (IPTG and arabinose) and different promoters (Plac/ara-1 and Plac). This comparison is based on data from a recent measurement technique of the duration of the steps prior and after initiation of the open complex [5][6] from measurements of time-intervals between consecutive RNA production events in single cells by live time-lapse microscopy and MS2-GFP RNA tagging. Next, using stochastic models of 2-genes switches with realistic multi-step initiation kinetics and empirically validated parameter values, we explore how rate-limiting steps in initiation can tune noise propagation and a circuit's responsiveness to external signals.

Results & Conclusions

First, we show that cell-to-cell variability in RNA numbers of individual genes due to extrinsic noise is promoter sequence-dependent, since promoters with relatively longer open complex formation filter the extrinsic noise more efficiently. Further, we show that, for 2-gene switches, there are ratios between the durations of the steps prior and after commitment to open complex that, by setting specific stability levels in the switch dynamics and the degree of response of each of gene to external signals, maximize the overall switch responsiveness to external signaling. We conclude that the rate-limiting steps kinetics determine the influence of global regulators (e.g. ・ factors [5]) and extrinsic noise (e.g. variability in RNA polymerases numbers [10]) on gene expression dynamics and that these phenomena extend to circuits. Thus, we argue that the sequence-dependent, and thus evolvable, kinetics of initiation of individual genes in E. coli may be a master regulator of information flow between genes and gene networks.

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Keywords: Transcription regulation, Open complex formation, Genetic Circuits, Information flow between genes and gene networks

P-156 - PHASE-VARIABLE TYPE I RESTRICTION-MODIFICATION SYSTEMS: A NEW CHARACTERIZATION METHODOLOGY FOR HIGH THROUGHPUT SEQUENCING DATA

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Background
Restriction-modification systems have long been considered a defence mechanism against foreign DNA. However, recent studies have uncovered a much wider variety of functions, from operon switching to hyper-variable shufflons. Type I systems (T1S) consist of three polypeptides: R (restriction, encoded by hsdR), M (modification, encoded by hsdM), and S (specificity, encoded by hsdS). T1Ss underlie population level changes in phenotype in a process called phase variation. The S subunit contains two variable target recognition domains (TRD), and is usually accompanied by inverted silent hsdS units, which allow for phase variation through recombination. T1S system with this function were reported across several bacterial genera, including Mycoplasma, Bacteroides, Streptococcus, Listeria, Enterococcus and Lactobacillus.

Method
We developed a software (ivrTyper) for the analysis of phase-variable type I restriction-modification systems based on short-read, paired-end, high throughput sequencing (HTS) data.
The integrity locus is evaluated. The presence of all open reading frames (ORF) is assessed via ORF coverage by mapping using ReMatCh.
As previously demonstrated, the hsdS allele being transcribed cannot be reliably determined using de novo assembly or standard mapping approaches due to the possible presence of multiple structural arrangement in an axenic culture. To overcome this limitation the conserved hsdS region, flanked by the hsdM gene, is used as target, allowing us to retrieve the read pair of the sequences that align to this region. By mapping these pairs to the various TRDs, the first variable region can be determined, and is then used as the new target region. Retrieving the pairs of the sequences that align to the identified TRD and mapping them to the second possible TRDs, allows the determination of the other variable hsdS region and identifying the dominant hsdS allele.

Results & Conclusions
Streptococcus pneumoniae contains two type I restriction-modification systems in its core genome, although only one, the ivr locus, with a six-phase variation, has been associated to events leading to phase variation (opacity). We used the ivrTyper for the automated detection of the dominant hsdS allele expressed by S. pneumoniae strains directly from paired-end raw data. By defining the appropriate target regions the tool can be adapted to any other bacterial species.

References & Acknowledgments ReMatCh and ivrTyper are available at https://github.com/B-UMMI/ReMatCh/ and https://github.com/cimendes/ivrTyper.

Keywords: High Throughput Sequencing, Phase Variation, Type I Restriction-Modification System, Streptococcus pneumoniae

P-157 - EXTRACELLULAR VESICLES AS EMERGENT NATURAL CARRIERS OF SMALL NUCLEIC ACIDS

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Background

Extracellular vesicles (EVs) are membrane-contained vesicles that are produced by all cells . They are important modulators of cell-to-cell communication delivering specific proteins, lipids and nucleic acids [1]. From the among species of nucleic acids, miRNAs are small non-coding RNAs with around 22 nucleotides in size that are able to regulate gene expression at the post-transcriptional level [2].
In the present work, our aim was to evaluate EVs as vehicles to deliver artificial nucleic acids to treat neurodegenerative disorders, namely Machado-Joseph Disease which is associated with an abnormal over-repetition of the CAG tract in ataxin 3 (ATXN3) gene, conferring toxic properties to ATXN3 protein [2] .

Method

Firstly, we designed 7 artificial miRNAs targeting mutant ataxin 3 (mutATXN3) mRNA. The silencing efficiency of each miRNA was then tested in a stable line of HEK293T encoding mutATXN3. Transfected mutATXN3 HEK293T cells were collected after 48h and 72h and mutATXN3 protein levels were evaluated by Western Blotting.
In the second part, HEK293T cells were transfected with each artificial miRNA and cellular medium was collected 48h later. A differential ultracentrifugation was then performed to isolate EVs. All EVs were characterized by Nanoparticle Tracking Analysis (NTA). The same amount of EVs was then placed in mutATXN3 HEK293T cells and the levels of mutATXN3 mRNA were subsequently evaluated by RT-PCR.

Results & Conclusions

Regarding to the first task we verified that mir-D presents the best silencing efficiency in both time points (42,2% at 48h and 51,4% at 72h). According to the results of RT-PCR, it has been confirmed that EVs can deliver artificial miRNAs.
In conclusion, this study supports that EVs are promising natural vehicles to deliver small nucleic acids.

References & Acknowledgments

This work was supported by funds from FEDER and the Competitive Factors Operational Program – COMPETE and by national funds through the Portuguese Foundation for Science and Technology.
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Extracellular vesicles as emergent natural carriers of small nucleic acids

Keywords: Extracellular Vesicles, Artificial miRNAs, Silencing

P-158 - PROFILING ANTIMICROBIAL TOLERANCE BY PLANKTONIC, BIOFILMS AND BIOFILM-RELEASED CELLS OF STAPHYLOCOCCUS EPIDERMIDIS

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Background

Worldwide, Staphylococcus *epidermidis* has been recognized as leading cause of several clinically relevant infections, especially in neonates and immunocompromised patients. Its ability to form biofilms, particularly in the surface of indwelling medical devices, is the primary cause of healthcare associated infections. On the last stage of biofilm lifecycle - disassembly, cells are released to the surrounding environment, being able to spread the infection and cause systemic diseases. These cells may be defined as biofilm-released cells (Brc). It is well known that planktonic cells (PLA) are more susceptible to antibiotics than biofilm cells (BF). So far, little is known regarding Brc tolerance to antibiotics.

The main goal of this work was to compare the susceptibility of Brc, PLA and BF of *S. epidermidis* clinical isolates, to 10 distinct antibiotics.

Method

Brc, PLA and BF cells were obtained using a previously developed method (França et al, 2016), with different *S. epidermidis* clinical isolates. The susceptibility of all populations of *S. epidermidis* 9142 to peak serum concentrations (PSC) of Dicloxacillin, Imipenem, Teicoplanin, Vancomycin, Ciprofloxacin, Rifampicin, Erythromycin, Gentamicin, Linezolid and Tetracycline was assessed after 2 hours of incubation, by CFU counting. Furthermore, 11 additional isolates were studied upon incubation with PSC of Vancomycin, to determine whether the results are transversal to distinct isolates among the same species.

Results & Conclusions

Our results demonstrated that Brc present a distinct tolerance profile when exposed to some antibiotics. While studying isolate 9142, Brc had a distinct tolerance phenotype with 6 out of 10 antibiotics. Regarding vancomycin assays, Brc presented an intermediate susceptibility to vancomycin when compared with other populations with 11 out of 12 isolates. Thus, this study outlines the impact of Brc on pathogenesis by demonstrating that the metabolic state and cell physiology of Brc present a distinct antibiotic tolerance profile, and might influence antimicrobial therapies against Staphylococcal infections.

References & Acknowledgments

This work was supported by national funds (FCT) by the strategic project of UID/BIO/04469/2013 and by BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by European Regional Development Fund under the scope of Norte2020. NC is an Investigador FCT.

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Keywords: Biofilm, biofilm-released cells, antibiotics

P-159 - SOCIAL INTERACTIONS BETWEEN LACTOBACILLUS INERS AND GARDNERELLA VAGINALIS BIOFILMS: AN UNEXPECTED FRIENDSHIP IN THE BACTERIAL VAGINOSIS ECOSYSTEM

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Background

Worldwide, bacterial vaginosis (BV) is the leading dysbiosis of the vaginal microbiome. BV is a complex polymicrobial condition characterized by a disruption of the vaginal econiche, normally resulting in a reduction of beneficial lactobacilli and an overgrowth of anaerobes. It is noteworthy that a hallmark feature of BV is the presence of a highly structured polymicrobial biofilm, primarily consisting of *Gardnerella vaginalis,* strongly adhered to the vaginal epithelium, and a variety of other bacteria.

There are some observational studies that described the rapid fluctuation over time of the vaginal microflora, showing that *Lactobacillus iners* is a dominant part of the vaginal flora in a transitional stage between abnormal and normal flora. Compared to other *Lactobacillus* species, *L. iners* has more complex nutritional requirements, a Gram-variable morphology and an unusually small genome, indicative of a symbiotic or parasitic lifestyle. However, till date, the role of *L. iners* in the development of a BV-associated biofilm remains unclear.

Method

This study aimed to unravel the interactions between *G. vaginalis* and *L. iners,* both isolated from BV cases, using an *in vitro* dual-species biofilm assembly. Bacterial coaggregation ability was determined for single- or between dual- species community. Furthermore, the total biofilm biomass was also determined by the crystal violet method. Next, we discriminated the dual-species populations in the biofilm by using Peptide Nucleic Acid Fluorescence *in situ* Hybridization method. Additionally, biofilm structure was evaluated using a confocal laser scanning microscopy analysis. Finally, the transcripts levels of *G. vaginalis* virulence genes, in a dual-species consortium, were determined by quantitative PCR.

Results & Conclusions

This study pointed out that *L. iners* seems to be well adapted to BV dysbiosis. We observed that *L. iners* was able to incorporate a pre-established *G. vaginalis* biofilm. Confocal microscopy analysis revealed that both species can live in close proximity, forming clusters from the bottom to the biofilm top layer. Curiously, *L. iners* did not affect *G. vaginalis* virulence, as determined by the transcription levels of key virulence genes. Remarkably, one could argue that *L. iners* is capable of surviving and adapting to a metabolic stress-related conditions found in BV.

References & Acknowledgments

This work was supported by national funds (FCT) by the strategic project of UID/BIO/04469/2013 and by BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by European Regional Development Fund under the scope of Norte2020. JC had an individual FCT fellowship (SFRH/BD/93963/2013). NC is an Investigador FCT.

Keywords: Bacterial vaginosis, Lactobacillus iners, Gardnerella vaginalis, PNA FISH, Biofilms

P-160 - INHIBITORY ACTIVITY OF TELEOST FISH MUCUS AGAINST BACTERIAL ISOLATES FROM FOOD AND CLINICAL ORIGIN

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Background

Epidermal mucus represents an important barrier of fishes towards the aquatic environment, playing a major role in their immunity. Among its functions, this protective layer has an antimicrobial potential, preventing bacterial colonisation of the skin. Fish mucus antimicrobial activity displays an interesting potential in biomedical and industry applications, namely in the treatment of infections caused by antibiotic resistant bacteria or in the control of food colonisers.

Method

In order to explore the antimicrobial activity of toadfish (Batrachoididae) and dragonet (Callionymidae) epidermal mucus, a range of bacterial strains from food and clinical (human and veterinary medicine) origin was selected. Bacteria, belonging to collections available at the Faculty of Veterinary Medicine, University of Lisbon, included two Gram negative (*Pseudomonas aeruginosa* from Diabetic Foot Infections, *Aeromonas hydrophila* A259 from human clinical origin) and four Gram positive isolates (*Staphylococcus aureus* from Diabetic Foot Infections, *Enterococcus faecalis* QSE123 from food origin, *E. faecalis* V583 from human clinical origin and *Enterococcus* sp. from veterinary clinical origin). Epidermal mucus was obtained by direct swabbing of the fishes' skin, collected by centrifugation, concentrated in an ultrafiltration apparatus with a membrane cut-off of 3000 Da and frozen at -20ºC until further processing. The antimicrobial potential of the mucus samples was evaluated using a spot-on-lawn approach. Briefly, isolates were inoculated in the surface of Brain Heart Infusion agar plates and 10 µl of mucus samples from each species were either directly placed on the medium surface or impregnated in a diffusion disk and placed in the medium. After incubation (24h, 37ºC), the mucus inhibitory activity was accessed by measuring the diameter of the inhibition zone on the inoculation site.

Results & Conclusions

Toadfish's mucus showed a strong inhibitory activity against *P. aeruginosa* from Diabetic Foot Infections when inoculated directly in the medium. Toadfish's and dragonet mucus was ineffective inhibiting the other isolates under study.
The present results highlight the potential of toadfish's mucus in inhibiting *P. aeruginosa* from Diabetic Foot Infections. Further investigations are required to unravel the optimal inhibitory concentration of the mucus against different bacterial strains, as well as its safety based on *in vivo* and *in vitro* cytotoxic activity.

References & Acknowledgments

Work was supported by the Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon (Project UID/CVT/00276/2013). Authors would also like to thank MARE, Marine and Environmental Sciences Centre - ISPA (UID/MAR/04292/2013), José Lino Costa and Pedro Félix (MARE-FCUL) and Fátima Gil (Aquário Vasco da Gama).

P-161 - ANTIMICROBIAL RESISTANCE AND BIOFILM PRODUCTION BY COMMENSAL AND CLINICAL ESCHERICHIA COLI ISOLATES FROM DOGS

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Background
Pyometra is a diestrual, chronic disease with acute manifestations in the adult, ovary-intact bitch, characterized by uterine bacterial infection with pus accumulation, with *Escherichia coli* being the bacteria most commonly associated with this disease. Its initial treatment involves empiric antibiotherapy, which suitability should be confirmed by antibiotic susceptibility testing. Resistance is a major health issue for veterinary medicine, rendering surveillance studies essential.

In this work, the susceptibility profile of commensal and clinical *E. coli* veterinary isolates was determined. Biofilm production was also evaluated, as this virulence factor is often associated to a tolerance increase to antibiotics.

Method
Antimicrobial susceptibility profile of 74 *Escherichia coli* isolates, including 41 commensal isolates and 33 from the uterine content of bitches with pyometra, was determined by disk diffusion (CLSI VET01-S2), using 6 antibiotics commonly used in veterinary medicine. Profiles were assessed by CLSI VET01-A4, CLSI M100-S23 and EUCAST guidelines. In order to detect β-lactamases-encoding genes, namely bla_{TEM}, bla_{SHV} and bla_{OXA} in those isolates resistant to β-lactams, a multiplex PCR was performed; positive and negative controls were used in all PCR reactions. Pellicle formation assays in Luria-Bertani medium were performed to study isolates' biofilm formation capacity. SPSS version 20.0 software was used for statistical analyses. Cohen´s kappa was used to measure the agreement between results.

Results & Conclusions
Resistance frequency determined with different guidelines was similar for ampicillin (59.5%), enrofloxacin (5.4%) and sulfamethoxazole/trimethoprim (10.8%); variations were observed for amoxicillin/clavulanic acid, cephalexin and cefotaxime (29.7-54.1%, 10.8-16.2% and 1.4-4.1%, respectively). Results varied slightly between clinical and commensal isolates, as well as their biofilm-forming ability (51.5%-57.1%). No significant correlation was found between pellicle formation and resistance profile. bla_{TEM}, bla_{SHV} and bla_{OXA} genes were detected in 25.5%, 11.8%, and 9.8% of isolates, respectively.

Results show the importance of determining the antimicrobial resistance profile of veterinary isolates and the need of using standardized and validated testing methods and harmonized interpretive criteria throughout European countries, to identify multidrug-resistant (MDR) bacteria, as they represent a clinical challenge for domestic animals practice. Being biofilm-forming ability related to bacterial virulence, its association with antimicrobial resistance determinants is of great concern, particularly in companion animals, due to close contact with humans.

References & Acknowledgments
This work was supported by CIISA, FMV/UL, Project UID/CVT/00276/2013.

P-162 - SURGICAL SITE INFECTION IN A HORSE BY AN EXTENDED SPECTRUM ß-LACTAMASE (ESBL) PRODUCING KLEBSIELLA PNEUMONIAE ISOLATE

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Background

Surgical site infections (SSI) are the most common cause of post-operatory infections[1]. In horses, this condition is associated with commensal and opportunist bacteria, being Gram-negative the most frequent type of bacteria isolated [2,3]. If these strains present a resistant profile, SSI may be difficult to control. In fact, the dissemination of multidrug-resistant (MDR) bacteria is a recognized worldwide problem for both human and veterinary medicine. Among MDR strains, ESBL-producing *Klebsiella pneumoniae* are frequently implicated in nosocomial infections[4], thus being considered by the World Health Organization as one of the most problematic bacterial species in the world[5].

Method

A swab sample was collected from a surgical site infection of a horse submitted to a colic surgery at the Equine Teaching Hospital of the Faculty of Veterinary Medicine, University of Lisbon. The sample was further processed using conventional culture methods, resulting in the isolation of a *K. pneumoniae*. Its antimicrobial resistance profile was determined by disk diffusion according to the Clinical Laboratory Standards Institute (CLSI) guidelines, regarding nine antimicrobial compounds commonly used in veterinary medicine.

Results & Conclusions

The isolate was resistant to most of the antibiotics tested, which included 3rd generation cephalosporins, fluoroquinolones and aminoglycosides. It was also positive for the production of ESBL, determined using a double-disc diffusion test also following CLSI guidelines.

This case report demonstrates the importance of identifying and controlling this type of bacterial strains, in both the human and veterinarian hospital settings, in order to avoid antimicrobial resistance dissemination.

References & Acknowledgments

This work was supported by the Centro de Investigação Interdisciplinar em Sanidade Animal (CIISA), Faculdade de Medicina Veterinária, Universidade de Lisboa (FMV/UL), (Project UID/CVT/00276/2013).

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Keywords: Klebsiella pneumoniae, ESBL, Horse, Surgical site infection

P-163 - IMPROVING A BACTERIAL ASP2OX FOR D-GLUCOSE OXIDATION USING O2 AS ELECTRON ACCEPTOR THROUGH DIRECTED EVOLUTION.

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Background

Pyranose 2-oxidase (P2Ox) are FAD-dependent enzymes that catalyze the oxidation of several aldopyranoses and disaccharides at the C2 position to yield the corresponding 2-keto-aldoses with concomitant reduction of O_2 to H_2O_2[1]. Currently, there is an increased interest in P2Ox due to the high range of substrates that they can use and the lack of anomer preference, making them perfect candidates to substitute glucose oxidases (GOx) in the construction of biosensors, as oxygen scavengers and biocatalyst for the production of rare sugars, fine chemicals and drugs[1]. In our laboratory it was for the first time characterized a bacterial P2Ox from *Arthrobacter siccitolerans* (AsP2Ox)[2] since bacteria for which molecular biology tools are well established have an utmost importance in the industrial biotechnology field. However, this enzyme showed a catalytic efficiency around six-orders of magnitude lower as compared to the fungal counterparts. Directed evolution is a powerful approach to improve enzyme efficiency and robustness employing methods for generating genetic diversity and identification of variants with desired properties.

Method

High-throughput protocols were optimized and validated at the level of cell growth, lysis and enzymatic assays for the application of directed evolution approaches targeting AsP2Ox. A library of 25,000 variants created by epPCR, was explored using high-throughput 'activity-on-plate' screenings assays. Biochemical analysis of the hit variant from the first generation of evolution and single variants constructed using site-directed mutagenesis, were performed.

Results & Conclusions

The first generation of evolution was set-up and the hit variant 2C9 was identified showing three mutations (A35T, F300V and Q343Q). In spite of its higher activity using crudes extracts, the purified variant was produced at lower yields as compared to the wild-type enzyme and was found to be inactivated by light. Therefore, single mutants (A35T and F300V) and one double mutant 2C9* (A35T/F300V) were constructed using site-directed mutagenesis, produced and characterized. We have concluded that the silent mutation (Q343Q) was destabilizing the enzyme and that both single mutations are important for the catalytic efficiency 3-fold higher of 2C9* as compared to wild-type. Consequently, 2C9* will be used as parent in the second generation of directed evolution.

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This work was supported by Fundação para a Ciência e Tecnologia (FCT), Portugal (PTDC/BBB-EBB/0122/2014) and Research Unit GREEN-it "Bioresources for Sustainability" (UID/Multi/04551/2013).

Keywords: AsP2Ox, bacteria, directed evolution, error-prone PCR, 'activity-on-plate', site-directed mutagenesis, catalytic efficiency

P-164 - DERMATOPHYTOSIS IN FARM ANIMALS IN PORTUGAL: A 16-YEAR STUDY

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Background

Dermatophytes are a group of keratinophilic fungi that affect the keratin substrate of domestic animals' skin, being responsible for severe skin, hair and nail infections known as dermatophytosis. The main fungi genera associated with these diseases are *Trichophyton*, *Microsporum* and *Epidermophyton*, which, due to their importance as zoonotic agents, represent a major public and veterinary health concern. The pathophysiology and epidemiology of these mycoses are quite heterogeneous and complex, being well described in pets. However, data available on the incidence of such diseases in farm animals are scarce.

Method

In this study, 62 farm animals (horses n=36; cattle n=10; donkeys n=4; goats n=10) with clinically suggestive ringworm lesions were investigated for the presence of dermatophytes. Samples were collected during a 16-year period (2001-2016), and included hairs and scales plucked from the periphery of the lesions. All samples were inoculated in Sabouraud dextrose agar supplemented with cycloheximide and chloramphenicol, incubated for 21 days at 28ºC and observed daily for the growth of dermatophytes. Identification of dermatophyte species was performed by micro and macroscopic examination of colonies.

Results & Conclusions

Out of the 62 animals included in this study, only 11 animals (17.7%) were proved to be positive for dermatophytes by microscopy and culture. As expected, species identified belonged to the genera *Trichophyton* (n=7, 63.6%) and *Microsporum* (n=4, 36.4%). Also, 33 animals (53.2%) were positive for other filamentous fungi species, while 3 animals (4.8%) presented yeast infections by *Candida* sp.
Results are in agreement with previous studies that refer to a low percentage of dermatophytosis in farm animals, with a higher prevalence of infections by *Trichophyton* species. Nevertheless, due to their major implications for animal and human health, further studies should be conducted in Portugal, aiming at contributing for managing programs and reducing zoonotic transmission to humans.

References & Acknowledgments

Work was supported by the Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon (Project UID/CVT/00276/2013).

Keywords: Dermatophytes, Farm animals, Zoonosis

P-165 - EFFICACY OF TWO PRE-SURGICAL SKIN ASEPSIS PROTOCOLS AS A PREVENTIVE MEASURE OF SURGICAL SITE INFECTIONS IN DOGS

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Background

According to the World Health Organization, surgical site infections (SSI) are considered a potential complication associated with any kind of surgical procedure, being the most preventable healthcare associated infections. Most SSI, which are associated with higher morbidity and mortality rates and increased costs in both human and veterinary medicine, are caused by commensal and pathogenic agents from the patient's microbiota, originated from the patients' skin, mucous membranes and hollow viscera of the gastrointestinal, genitourinary and respiratory tracts. Quantitatively, it has been shown that if a surgical site is contaminated with $>10^5$ CFU/gram of tissue, the risk of SSI is markedly increased.

Method

As the skin pre-surgical asepsis is one of the preventive measures for SSI development in humans and animals, but no guidelines are available for veterinary surgery, this study aimed to compare the efficacy of two antiseptic agents commonly used for pre-surgical skin preparation in dogs, namely an alcoholic solution of chlorhexidine gluconate (CHG) and an aqueous solution of povidone-iodine (PVP-I). Pre-asepsis and post-asepsis swab samples were collected from 46 dogs submitted to surgical interventions at a veterinary clinic in the Lisbon area, 23 of which were randomly assigned to be subjected to an asepsis protocol using 10% PVP-I and the other 23 to an asepsis protocol using an alcoholic solution of 2% CHG. Swabs were inoculated in Brain Heart Infusion agar plates and incubated at 37ºC for 24h for total bacteria quantification. Results analysis was performed through an ANOVA for repeated measures, using the statistic program R with the extension R commander.

Results & Conclusions

Quantification results for the pre-asepsis samples collected from animals allocated to the CHG protocol presented a mean value of $4.0x10^8$ CFUs/mL, while for animals allocated to the PVP-I protocol the value was of $8.03x10^7$ CFUs/mL. Regarding the post asepsis samples, results were negative for 16 dogs, while for one animal results were $>10^9$ CFUs/mL. Both protocols presented similar efficacy for surgical asepsis ($p>0.05$), since there were no statistically significant differences between the bacterial logarithmic reduction in the pre- and post-asepsis results from the two experimental groups.

Further evidence-based studies in veterinary surgery are mandatory, aiming at establishing guidelines for pre-surgical skin asepsis protocols, allowing to improve and standardize the techniques applied in veterinary surgery and to reduce the incidence of surgical site infections.

References & Acknowledgments

This work was supported by the Centre for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon (FMV/UL) (Project UID/CVT/00276/2013).

Keywords: surgical site infections, antiseptic agents, dogs

P-166 - INTESTINAL COLONIZATION WITH MULTIDRUG-RESISTANT GRAM-NEGATIVES OF RESIDENTS OF LONG-TERM CARE FACILITIES AND NURSING HOMES IN BRAGA AREA

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Background

Care of aging population has been a growing challenge to public health and health-care providers. There is a growing need for long-term care facilities (LTCF), nursing homes (NH) and home care (HC) due to disabilities of older people. This brings a new paradigm for the spread of bacteria showing multidrug-resistance (MDR) to antibiotics, with these extra-hospital health-care institutions becoming reservoirs of antibiotic resistance. The aim of our work was the detection of isolates producing extended-spectrum beta-lactamases (ESBL) and isolates with reduced susceptibility to carbapenems of *Enterobacteriaceae* and other gram-negative bacteria, in the intestinal flora of institutionalized residents in extra-hospital health-care institutions (LTCF and NH) in Braga region.

Method

Fecal samples of 27 residents of these institutions were collected (September-to-December, 2016) and analysed. One gram of each sample was suspended in 10mL of saline and 100mL of the suspension was spread on MacConkey agar with ampicillin (100mg/L)/ cefotaxime (2mg/L)/meropenem(1mg/L). Susceptibility to antibiotics was determined by disk-diffusion methods, according to Clinical and Laboratory Standards Institute (CLSI). ESBL-producers were detected by the double-disk-synergy-test and/or clavulanic-acid addition and PCR was performed for detection of bla_{TEM}, bla_{OXA-1}, $bla_{SHV,}$ $bla_{CTX-M-group-1}$, $bla_{CTX-M-group-2}$, $bla_{CTX-M-group-8}$, $bla_{CTX-M-group-9}$, $bla_{CTX-M-group-25}$, *tetA*, *tetB*, *aac(3)-II, sul1*, *aac(6)-Ib-cr* and *qnrB* genes.

Results & Conclusions

The study revealed 6 ESBL-producing *Enterobacteriaceae* colonizing 2 residents in LTCF (2-*Escherichia coli*/1- KESC group (*Klebsiella, Enterobacter, Serratia* and *Citrobacter* spp) and 3 residents in NH (2-*Escherichia coli*/1-KESCgroup). Isolates showed positive amplification for $bla_{CTX-M-group-1}$, $bla_{CTX-M-group-9}$, bla_{TEM}, bla_{SHV}, bla_{OXA-1}, *tetA, tetB, aac(3)-II, sul1* and *aac(6')-Ib-cr*. These isolates showed resistance to non-beta-lactam antibiotics, namely to tetracycline, ciprofloxacin, trimethoprim-sulfamethoxazole, gentamicin and amikacin. We detected 6 MDR-bacteria isolates and 1 isolate with reduced susceptibility to carbapenems. Our results indicate a dissemination of ESBL-producing *Enterobacteriaceae* in intestinal colonization of LTCF and NH patients, who may act as vehicles of MDR-bacteria within the health-care-facilities and healthy community.

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Keywords: Antibiotic Resistance

P-167 - DECIPHERING TRANSCRIPTION REGULATION OF ANTIFUNGAL DRUG RESISTANCE IN THE PATHOGENIC YEAST CANDIDA GLABRATA

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Background

Candida species are the fourth leading cause of nosocomial bloodstream infections in the US and constitute a relevant healthcare burden. *Candida glabrata* is the second most common cause of candidemia worldwide and its prevalence has continuously increased over the last decades. It presents high levels of intrinsic and acquired resistance to antifungals, which limits their clinical effectiveness. The understanding of resistance mechanisms and their regulatory networks is of great importance to develop better therapeutic approaches against fungal pathogens [1,2].

Method

Given the significant occurrence of antifungal resistance in *C. glabrata* isolates, the complexity of antifungal resistance mechanisms and their regulatory pathways was assessed. The role of the transcription factor CgPdr1 in the membrane proteome-wide response of *C. glabrata* against the antifungals clotrimazole and 5-flucytosine was evaluated using iTRAQ-MS. Additionally, a screening of 17 transcription factor (TF) encoding genes was performed to identify potential regulators of drug resistance. Functional characterization of the selected regulators is now underway, using RNA-sequencing.

Results & Conclusions

Two studies have determined the role of CgPdr1 in the membrane proteome-wide response of *C. glabrata* to the antifungals clotrimazole and 5-flucytosine [3,4]. Multidrug resistance transporters have a relevant role in both antifungal responses, however, distinct transporters are required for distinct antifungal resistance. Moreover, cell wall remodeling appears to be a general antifungal resistance mechanism. Additionally, ~50% of the responsive proteins were dependent on CgPdr1, highlighting the existence of additional targets of this major regulator of multidrug resistance in clinical isolates. By screening 17 TF mutant strains for a role in fluconazole resistance, 2 TF encoding genes were selected for further characterization. The absence of either regulator was shown to increase fluconazole susceptibility, translated by decreased fitness during growth in the presence of drug. In order to elucidate the specific role of each regulator in fluconazole resistance, transcriptomics analysis based on a RNA-seq approach is being used to enlighten the regulons of each TF and determine their specific roles in fluconazole resistance. This approach is shedding light in the complex interplay between regulatory features of resistance mechanisms; including mechanisms of activation, regulatory targets and phenotypic outcome. Such knowledge will contribute to find new targets for antifungal therapy.

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Deciphering transcription regulation of antifungal drug resistance in the pathogenic yeast *Candida glabrata*

Keywords: Antifungal drug resistance, Candida glabrata, CgPdr1, Transcription regulatory networks, Trasncription factor, RNA-sequencing

P-168 - IN VITRO EVALUATION OF AN ANTIMICROBIAL PEPTIDE DELIVERY SYSTEM TO BE USED IN THE CONTROL OF ENTEROCOCCAL PERIODONTAL DISEASE IN DOGS

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Background

Periodontal disease (PD) is one of the most frequent and widespread diseases in dogs. Its onset is due to the formation of a microbial biofilm in the teeth surface, known as dental plaque. This biofilm affects the periodontium leading to gingivitis and periodontitis. *Enterococcus* spp[1]. is frequently present in the canine oral cavity, being related with PD development in dogs. Considering the frequent multi-drug resistance (MDR) profile of these bacteria, new therapeutic strategies to control enterococcal PD in dogs are required[1]. A possible approach involves the administration of antimicrobial peptides such as nisin, a compound that exhibits antibacterial activity against Gram-positive bacteria. Search for delivery systems are essential for the development of nisin-based therapeutic protocols, aiming to control bacterial diseases. Recent studies describe guar gum gel as a promising topical delivery system, being a biodegradable, biocompatible, and non-toxic polysaccharide with several biomedical applications. We aimed to evaluate the potential of guar gum gel as a nisin topical delivery system to be used in the control of enterococcal PD in dogs[2].

Method

A collection of 43 enterococci isolates obtained from dogs diagnosed with PD and two human reference strains were used as bacterial models[1]. Nisin was incorporated in a guar gum gel (1.5% w/v), with final concentrations ranging from 0.05 to 4 mg/mL. *In vitro* susceptibility of enterococcal biofilms to nisin was assessed using a modification of the Calgary system, by determining the Minimum Inhibitory(MBIC) and Bactericidal Concentrations(MBEC)[2].

Results & Conclusions

MBIC values ranged from 0.1 to 1.5 mg/mL, with medium values of 0.51±0.21 mg/mL. MBEC ranged from 0.1 to > 4mg/mL, with medium values of 0.99±0.63 mg/mL.
Nisin incorporated in the guar gum gel showed an effective antimicrobial activity against all the enterococci tested, rendering this compound an appropriate candidate for dental plaque control. Since dogs are animal models for human PD, results may be extremely useful for human medicine. Furthermore, this innovative therapeutic strategy may allow decreasing antibiotics' administration, ultimately contributing to reduce the dissemination of MDR strains.

References & Acknowledgments

This work was supported by the Centre for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon (FMV/UL) (Project UID/CVT/00276/2013) and by Virbac Portugal.

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Keywords: Periodontal disease, nisin, guar gum

P-169 - EVALUATION OF THE HUMORAL IMMUNE RESPONSE INDUCED BY VACCINATION FOR CANINE DISTEMPER AND PARVOVIRUS

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Background

Canine distemper virus (CDV) and canine parvovirus (CPV) are two virus responsible for high mortality rates in canine populations (Greene & Decaro, 2012). These virus infect unvaccinated dogs or with incomplete vaccine protocols. Vaccines play a key role in preventing these diseases however; vaccination should be performed only with the frequency necessary to maintain protective immunity. On the other hand, there are several factors that may cause immunization failures, being the neutralization of the vaccine antigen by maternal antibodies the most common cause (Ford, 2013).

Method

In this study, the humoral protection for CDV and CPV was evaluated in 13 puppies during the 1st vaccination (Group A and Group B) and in 7 adult dogs that had not been vaccinated for at least 3 years (Group C). Group A included 5 puppies that initiated the vaccine protocol at 6 weeks and group B included 8 animals that started primary vaccination at 8 to 12 weeks. 3 blood samples were collected from each animal, coincident with the vaccination visits, at 3 to 4 weeks apart. The humoral response was evaluated using indirect ELISA for antibody detection.

Results & Conclusions

The response to CDV vaccination was earlier in comparison to the response for CPV. Regarding CPV, 80% of the puppies in group A were still unprotected after the administration of 2 doses of the vaccine. On the other hand, puppies of group B showed humoral protection for CPV after 2 vaccination administrations and 4 animals (50%) were soon protected after the first dose. In adult dogs, only 1 blood sample was taken that served as a serological screening to gauge the need for revaccination. The results also showed that during vaccination the response is individual and depends mainly on the initial titter of maternal antibodies acquired by the neonate. The variability found reinforces the need to determine individual humoral immunity levels. Humoral protection tests should be used to validate the vaccine efficacy induced by primary vaccination, assisting the veterinarian to establish individual vaccination protocols.

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This work was supported by the Centre for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon (FMV/UL) (Project UID/CVT/00276/2013).

Keywords: CPV, CDV, protective immunity, ELISA, maternal antibodies, vaccination protocols

P-170 - GENOTYPIC RELATIONSHIPS OF STREPTOCOCCUS DYSGALACTIAE SUBSP. EQUISIMILIS ISOLATES FROM HUMANS AND ANIMALS

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Background

Streptococcus dysgalactiae subsp. *equisimilis* (SDE) is a beta-hemolytic streptococcus being increasingly reported from human infections (1). SDE has also been documented in several animal species, but contradictory data on the taxonomy of these isolates has hampered the evaluation of their pathogenic potential for animals and humans (2). We aimed to determine the genotypic characteristics of these animal isolates, to compare with their human counterparts and to assess possible transmission between hosts.

Method

A collection of 64 *S. dysgalactiae* isolates recovered from diverse animal hosts were characterized by Lancefield group, *emm* typing and sequence analysis of 14 housekeeping genes. Sequences from the 14 loci were concatenated into a 6197 bp sequence for each isolate and a minimum evolution tree was constructed with MEGA 7 (http://megasoftware.net/). Our data was also compared with the *S. dysgalactiae* multilocus sequence typing (MLST) database (http://pubmlst.org/sdysgalactiae/) to allow a broader comparison with SDE human isolates. PHYLOViZ (http://www.phyloviz.net/) was used to establish relationships between sequence types (STs).

Results & Conclusions

Isolates belonged to Lancefield group C (n=53) and group L (n=11). Seven distinct *emm* types were found among the 23 *emm*-typeable beta-hemolytic isolates and these *emm*-types were different from those usually found among human SDE. Minimum evolution analysis showed that the type strains of SDE and *S. dysgalactiae* subsp. *dysgalactiae* (SDD) were two distinct monophyletic groups. While SDE isolates of human origin clustered with the SDE type strain, all beta-hemolytic isolates recovered from various animal hosts grouped into another monophyletic branch, closer to human SDE. Three SDE isolates recovered from human infections in Portugal presented *emm* types identical to those of animal isolates and grouped within the animal branch rather than in the branch grouping most human SDE. PHYLOVIZ confirmed the partitions observed in the ME tree and revealed that 9 out of 1107 SDE human isolates present in the MLST database had profiles typically found among SDE of animal origin. Our data suggests that beta-hemolytic SDE isolates usually isolated from animals can be differentiated from human SDE by their unique *emm* types and MLST profiles and that transmission of animal-associated SDE strains to humans occurs occasionally.

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Keywords: Streptococcus dysgalactiae

P-171 - TYPE OF DELIVERY AND ITS INFLUENCE IN ORAL YEAST COLONIZATION

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Background
Recent studies have shown the influence of the type of delivery on the composition and maturation of oral bacterioma, however, the influence of oral mycobioma is still unknown. Thus, our aim was to study the influence of the type of childbirth on oral yeast colonization in young adults.

Method
In 200 healthy students of the Faculty of Medicine of the University of Porto non-stimulated saliva was collected and plated in *Sabouraud Agar* supplemented with chloramphenicol for yeast isolation. *Candida* species were identified by *ChromAgar Candida* and molecular biology. Statistical analysis was performed using the chi-square test and t-test for independent samples.

Results & Conclusions
Students mean age was 21.61±1.86 years old, and total yeast prevalence was 37.5% for the applied methodology. The most isolated species was *Candida albicans* in 76.54% of colonized participants. In comparison to individuals born by caesarean section, the individuals born by normal delivery presented higher prevalence of oral yeasts colonization (41.6% vs. 25.8%, p = 0.035) and higher yeast counts (13.68±38.02 vs. 1.69±0.62 LogCFU/mL, p = 0.030).
In conclusion, our results suggest that birth by normal delivery promotes oral colonization by yeast, probably due to the higher transmission of yeasts at birth, which enhances its integration into the oral microbiome throughout life.

Keywords: Yeast, oral colonisation, birth delivery mode

P-172 - PEDIATRIC INVASIVE PNEUMOCOCCAL DISEASE IN PORTUGAL: CHANGING EPIDEMIOLOGY (2012-2016)

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Background

In Portugal, pneumococcal conjugate vaccines (PCVs) have been available in the private market since 2001 and since then we have reported significant changes in the serotypes responsible for invasive pneumococcal diseases (IPD) in the pediatric population[1,2]. In June 2015, PCV13 was included in the National Immunization Plan (NIP). We aimed to characterize isolates causing invasive pneumococcal disease (IPD) in 2012-2016.

Method

A total of 224 *Streptococcus pneumoniae* isolates recovered from children (<18 years) diagnosed with IPD in 61 hospital laboratories and pediatric departments located throughout Portugal between July 2012 and June 2016 were characterized by serotyping and antimicrobial susceptibility testing.

Results & Conclusions

The number of isolates recovered in each epidemiological year was constant and similar to that of 2011-12, except 2015-16 but this may be due to the fact that we are still receiving isolates from participating laboratories. Overall, 37 different capsular types, as well as non-typable isolates were detected. Although most isolates expressed capsular types included in PCVs, serotypes not included in any PCV formulation accounted for a significant fraction of the isolates (50%, n=111). Among these, serotypes 10A, 24F, 8, 15B, 12B were the most frequent, represented by at least 10 isolates each. Regarding PCV serotypes, isolates expressing serotype 14 (n=25), serotype 1 (n=18), serotypes 3 (n=14) serotype 7F (n=13), 6B (n=12) and 19A (n=10) were the most frequently found. Overall, 23% of the isolates were penicillin non-susceptible. Resistance to erythromycin was expressed by 22% of the isolates and simultaneous expression of erythromycin resistance and penicillin non-susceptibility was found in 14% of the isolates.

PCV13 serotypes are still expressed by a considerable proportion of isolates responsible for IPD. The data presented here emphasizes the potential role of PCV13 in the NIP in further diminishing pediatric IPD.

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Keywords: Streptococcus pneumoniae, Pediatric invasive disease, Vaccines

P-173 - DISTRIBUTION OF PUTATIVE VIRULENCE MARKERS IN ENTEROCOCCUS FAECIUM: TOWARDS A SAFETY PROFILE REVIEW

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Background

The criteria for identification of *Enterococcus faecium* (Efm) with ability to cause human infections are under debate within the European Food Safety Authority (EFSA). Strains expressing ampicillin resistance ≤2mg/L and lacking IS*16/esp/hyl* genes should be regarded as safe for animal nutrition (1), despite the poor knowledge about putative virulence markers (PVM) distribution in community-Efm (2,3). We analyzed the distribution of major PVM and ampicillin phenotypes in large Efm collections to further discuss the safety of strains under a Public Health perspective.

Method

Thirty-three PVM were assessed by PCR/sequencing among clonally disparate Efm (n=328; 31 countries of the five continents; 1986-2015) from different sources. We analyzed ampicillin-susceptibility (Etest/broth microdilution) according EUCAST guidelines, clonal relationship (MLST) and genomic location of PVM (S1-PFGE/hybridization) as described (4).

Results & Conclusions

Infection-derived Efm were the more enriched in PVM (Figure-1) and the increase in ampicillin-MIC was positively correlated with an enrichment in different PVM. PVM coding for surface (*esp/sgrA/ecbA/*complete-*acm*) and pili proteins, or others enhancing colonization (*hyl/ptsD/orf1481*) or plasticity (IS*16),* were strongly associated with clinical-Efm (mostly clade A1), but also observed in clades A2/B at different rates. *ptsD* was a good marker of ampicillin-resistant Efm. *ptsD*, IS*16, orf1481, sgrA,* and hospital variants of complete pili-gene-clusters are proposed for safety assessment of *E. faecium* strains. Our study expands on the distribution of PVM in diverse Efm lineages and demonstrates the enrichment in infection-derived strains of PVM not previously included in the list of Efm safety criteria of EFSA. These data will be helpful to support efforts in the containment of human infections caused by *E. faecium*, a bacterial species particularly prone to exhibit multidrug resistance, and can greatly impact the risk assessment of Efm strains under different Public Health contexts, namely clinical diagnosis and food safety.

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Acknowledgements:

This work received financial support from FEDER funds (POCI/01/0145/FEDER/007728) and National Funds (FCT/MEC) under the Partnership Agreement PT2020 (UID/MULTI/04378/2013) and project NORTE-01-0145-FEDER-000011 [QREN – Qualidade e Segurança Alimentar – uma abordagem (nano)tecnológica], and was co-funded by a ESCMID Research Grant 2013. ARF was supported by a fellowship [grant SFRH/BPD/96148/2013] from FCT.

Keywords: Public Health, food safety, molecular markers, virulence, human infections, risk, Enterococcus faecium

P-174 - TRICHOMONIASIS AND ITS DIAGNOSIS: COMPARISON BETWEEN MICROSCOPY AND PCR

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Background

Trichomonas vaginalis (TV) is the protozoan responsible for Trichomoniasis, which is a worldwide sexually transmitted disease, with an incidence of 180 million new cases per year. Nowadays, the gold standard for its diagnosis is based on microscopic observation of live parasites on vaginal swabs, or *in vivo* culture of TV, which is very time-consuming. As microscopy for TV requires a very experienced observer, results may vary from laboratory to laboratory, and sensitivities may not be as expected. Some advances have been made towards developing new and more sensitive diagnostic tests for TV, such as *Polymerase Chain Reaction* (PCR), which can also be very specific, depending on the *primers* used for the technique. However, PCR is used only as a confirmatory test in some clinical cases. The goal of this study relies on comparing the results obtained with microscopy as the classic laboratorial test for trichomoniasis, and a specific PCR for TV.

Method

We used 151 samples of vaginal swabs from a population of women from Lisbon, associated with risk behaviours. Microscopic observation of all samples was performed, and nucleic acids were extracted. For this PCR, we used specific primers that codify for beta-tubulin in TV, as described by Hardick and Simpson.

Results & Conclusions

The results obtained by an experienced observer with microscopy revealed 4 positive cases, which corresponds to a prevalence of 2,56%. Using our technique, there were 8 positive results obtained, corresponding to a prevalence of 5,3%. Some similar studies from other countries have shown a variety of results, although the sensitivity is very frequently higher with PCR. The existence of false positive results on this study is unlikely due to the high specificity of the target sequence. Negative controls were performed in every assay, ensuring no contaminations. Therefore, we conclude that using a PCR technique with specific primers for TV doubles the rate of detection of trichomoniasis, when compared with microscopy, the actual gold standard for TV detection.

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Keywords: Trichomonas vaginalis, Microscopy, PCR

P-175 - CARBAPENEM-RESISTANT KLEBSIELLA PNEUMONIAE CLINICAL ISOLATES HARBOURING SIMILAR CONJUGATIVE PLASMIDS

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Background

Klebsiella pneumoniae is a major cause of health care associated infections, with high incidence in urinary tract infections, pneumonia, septicaemia and soft tissue infections. The occurrence of multidrug-resistance in this pathogen seriously increases the severity of the associated infections and the increasing occurrence of carbapenem resistance has become evident in the last years. Often, this phenotype is associated with the carbapenemase encoding, plasmid borne bla_{KPC} gene. The comparison of plasmids among isolates is a common approach to infer the mechanisms of spread of antibiotic resistance genes, herein used to infer bla_{KPC} dissemination in clinical isolates.

Method

Carbapenem-resistant *K. pneumoniae* isolates from clinical samples were characterized in terms of phenotypic and genotypic characteristics and number of plasmids. Plasmids further characterized were those that could be transferred by conjugation to a selected receptor. Conjugation assays were carried using clinical carbapenem-resistant *K. pneumoniae* isolates as donors and an environmental *K. pneumoniae* as recipient. Transconjugants were selected in the presence of tetracycline (16 mg/L) and meropenem (0.25 mg/L) and further characterized for resistance phenotypes, specific genotypes and plasmid profiles. The number and sizes of plasmids were compared based on pulsed field gel electrophoresis and optical DNA mapping (ODM). ODM was also used for plasmid profiling.

Results & Conclusions

The clinical carbapenem-resistant *K. pneumoniae* isolates were resistant to different classes of antibiotics (beta-lactams, aminoglycosides, quinolones, cephalosporins, sulphonamides and carbapenems) and susceptible to tetracycline. Among the 2-4 plasmids observed in a single isolate, in general, only one could be transferred by conjugation. These plasmids had sizes ranging from 50 kbp to 140 kbp and all contained the gene bla_{KPC}. In transconjugants, this gene was observed to be associated with the replicon types FIA and N, and to the acquisition of resistance to meropenem, cephalothin and ceftazidime. Ciprofloxacin resistance was not observed to be transferred. Based on ODM analysis it was possible to confirm a high similarity between the conjugative plasmids from different clinical isolates.

The combination of ODM and conjugation assays gave evidence for the spread of bla_{KPC} via horizontal gene transfer among clinical isolates and the potential of dissemination also for environmental strains. The association of specific replicon types in *K. pneumoniae* to conjugative plasmids harbouring the bla_{KPC} gene is of interest for predicting how the spread of carbapenem resistance occurs.

References & Acknowledgments

This work was financially supported by the national funds from FCT—Fundação para a Ciência e Tecnologia and the Swedish Research Council through the project NanoDiaBac ENMed/0001/2014 and UID/Multi/50016/2013.

Keywords: Klebsiella pneumoniae, Optical DNA Mapping, Carbapenem resistance, Conjugative plasmids

P-176 -

P-177 - MARINE ACTINOMYCETE-DERIVED POLYMERIC COMPOSITES WITH ANTIMICROBIAL ACTIVITY AGAINST MRSA HIGHLY RESISTANT STRAINS

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Background

Currently, silver and iodine associated to a secondary dressing as physical support are marketed for bacterial wound infections. These show several disadvantages, such as limited periods of use due to toxicity and the need to remove the dressing, which may result in injury/trauma to the skin. Among Ocean´s biodiversity, the marine bacteria are an important and unexplored resource for pharmaceutical applications. Selected marine actinomycetes MAR4 strains[1] belonging to Streptomyces lineage that were isolated from marine sediments collected along the Madeira archipelago revealing antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) were used as source of natural antimicrobial compounds.[2] Hydroxypropylcellulose (HPC), a cellulose derivative that is water-soluble, biocompatible and biodegradable, was used as physical support.

Method

We have used a multidisciplinary biotechnological approach focused on the bio-prospection of non-toxic antimicrobial natural products (NPs) for the development of a novel generation of wound dressings.[3] The purified active compound was mixed with HPC in ethanol yielding a final compound concentration of 30 mg/ml. Thin films were produced by dye cast technique. The biologic activity of the composite disks obtained was tested against four different clinical MRSA strains, by growth inhibition halos in TSA solid medium and the respective MIC (minimal inhibitory concentration) was determined by serial dilution in 96-well plates. The mechanical properties of composite films were characterized and scanning electron microscopy (SEM) was performed.

Results & Conclusions

The composite HPC films dissolved readily, in solid as in liquid medium and presented an MIC value of 0.98 mg/ml against all MRSA strains tested. SEM analysis showed that the association of HPC and the pure compound results in a smoother and porous interconnected structure, that may promote the diffusion of nutrients and oxygen into the bulk of the matrix. Marine-derived actinomycetes were successfully used to develop antimicrobial, biodegradable, biocompatible, water-soluble wound dressings for treatment/prophylaxis of wound infections, a potential alternative to the use of silver and iodine band-aids.

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Dedicated to the memory of Prof. Ilda Santos-Sanches.

Keywords: marine-derived actinomycetes, natural products, polymeric composites

P-178 - CONTROL OF PORPHYROMONAS GINGIVALIS USING A BACTERIAL-DERIVED PEPTIDOGLYCAN HYDROLASE

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Background

The human periodontium health is commonly compromised by inflammatory conditions, which are designated by periodontal diseases. *Porphyromonas gingivalis* is considered a keystone periodontal pathogen, capable of breaking the homeostatic relationship with the host and initiating an inflammatory disease. It is thought that the dysbiotic changes caused by this pathogen could be reversed by its specific removal, being an excellent target candidate for therapy. Due to limitations of the current therapies that often leads to the recurrence of the disease and the increasing bacterial resistance to antimicrobials, there is a need for novel approaches to control periodontal disease. Peptidoglycan hydrolases (PGHs) are lytic enzymes that cleave bonds in the bacterial peptidoglycan (PG). In contrast to the well-known bacteriophage-encoded PGHs, such as endolysins, the exogenous use of bacterial-derived PGHs to control pathogenic bacteria remains poorly explored. In this work we explored the use of a *P. gingivalis*-encoded PGH against itself.

Method

An *in silico* analysis was carried out on the genome of *P. gingivalis* 2561 (GenBank AP009380.1) for the identification of putative PGHs. A probable N-acetylmuramoyl-L-alanine amidase, denominated by PgPALys, was cloned in the pET-28a expression vector and its expression in *E. coli* BL21 (DE3) and subsequent purification was optimized. The antibacterial activity of the recombinant protein (alone or in combination with the outer membrane permeabilizer EDTA) was assessed against *P. gingivalis* 2561 and *P. gingivalis* HG66 after incubation at 37 °C in the anaerobic chamber for up to 24 hours.

Results & Conclusions

Antibacterial assays demonstrated that PgPALys is active against *P. gingivalis*, reducing 1.08 ± 0.38 and 1.45 ± 0.37 logarithm units of *P. gingivalis* 2561 and *P. gingivalis* HG66 cells after 24 hours of incubation, respectively. The well-known chelator EDTA was used in combination with PgPALys to increase the permeability of *P. gingivalis* outer membrane (OM) and thus enhance the antibacterial activity. Overall, the antibacterial activity of PgPALys/EDTA improved with the time, resulting in an inactivation of 2.03 ± 0.58 and 2.53 ± 0.71 logarithm units of 2561 and HG66 cells after 24 hours of contact.
This work shows the potential of a *P. gingivalis*-encoded enzyme for the control of the aforementioned periodontal pathogen, providing new insights into the use of a bacterial-derived PGH for the control of bacterial diseases.

References & Acknowledgments

This study was supported by FCT by strategic funding of UID/BIO/04469/2013 unit, COMPETE 2020 (POCI-01-0145-FEDER-006684), BioTecNorte operation (NORTE-01-0145-FEDER-000004, Project RECI/BBB-EBI/0179/2012 (FCOMP-01-0124-FEDER-027462), and by TecMinho through the project 045/TT/2014. SS is an Investigator FCT (IF/01413/2013).

Keywords: periodontal disease, peptidoglycan hydrolase, lysin

P-179 - IMPACT OF AN ANTHOCYANIN RICH BLUEBERRY EXTRACT UPON LACTOBACILLUS AND BIFIDOBACTERIUM GROWTH AND SUBSEQUENT IMPACT UPON CACO-2 VIABILITY

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Background

Blueberries have been associated with several potentially beneficial properties, including antioxidant and anti-inflammatory activity, and most recently have been associated with the modulation of the intestinal microbiota. Previous works have shown that an anthocyanin rich blueberry extract, while capable of inhibiting potential pathogens had no significant impact upon the growth of probiotics, even though it did stimulate organic acid production. As such, this work aimed to assess whether this extract, alone or when fermented by different probiotic bacteria, had any negative impact upon CaCo-2 cells' viability.

Method

The blueberry extract was fermented by 12 and 24 h by *Bifidobacterium lactis* Bo, *Bifidobcaterium lactis* BB12, *Lactobacillus plantarum*, *Lactobacillus rhamnosus* and a mix (1:1:1:1) of all the bacteria. The extract and the fermented supernatants' biocompatibility was assessed using Caco-2 intestinal cells and the XTT colorimetric method. The total phenolic and anthocyanin content was determined through HPLC-DAD, while organic acid production and sugar consumption (during the fermentation) were evaluated using an HPLC-RI system.

Results & Conclusions

Results demonstrated that the extract did not exert any inhibition of the cellular metabolism appearing to, in fact, stimulate it. Additionally, when comparing the results observed for the fermented extract with those of the corresponding negative controls, it can be seen that the presence of extract led to lower inhibition percentages, (which ranged from -20 to 40%). The microorganism which showcased the most interesting activity was *Bifidobacterium animalis* Bo. This bacteria was the only among the considered microorganisms that caused no loss of cell viability. In fact, the presence of the extract alone, it appeared to induce an increase of the overall metabolic rate.

References & Acknowledgments

The authors would like to acknowledge the funding provided by Fundação para a Ciência e Tecnologia (FCT, Lisboa, Portugal) through the project UID/Multi/50016/2013.

Keywords: Blueberry extract, Probiotics, Cell viability, Anthocyanins

P-180 - POLYPHASIC IDENTIFICATION AND TYPING TRICHOPHYTUM RUBRUM STRAINS OF CLINICAL ORIGIN

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Background

The dermatophyte *Trychophytum rubrum* is the most frequent aetiological agent of dermatophytosis in humans around the world representing a major public health problem, not just for European countries but also for tropical countries where climate conditions allow major propagation for this ascomycete. For instance, in Portugal, *T. rubrum* was the dermatophyte most frequently isolated (83.3%) in a toenail onychomycosis geriatric population survey [1]. The identification, pathogenicity, biology, and epidemiology of *T. rubrum*, is of interest for both dermatologists and medical mycologists [1,2]. Currently, in many countries and clinical laboratories, *T. rubrum* strains isolated from lesions are primarily identified by conventional culture-based methods, including colony morphology and slide culture only. This approach does not provide evidence of intraspecific variations with a lack of information to track infections, determine common sources of infections and recurrence or reinfection after treatment, and analyse their virulence and drug resistance [3]. The aim of this work is to use a polyphasic approach to study *T. rubrum* from different geographic origins in order to identify intraspecific characteristics with clinical interest.

Method

About 40 European and South American *T. rubrum* and reference strains were used. Macro and micro morphological techniques, urease assay, dermatophyte milk agar test and hair perforation test (HPT) where combined with molecular biology techniques, such as the analysis of internal transcribed spacer (ITS) region, Trubrum specific primers for differentiation among closely related species, mating type MAT1-1 a-box characterisation and DNA fingerprinting (e.g., $(GACA)_4$).

Results & Conclusions

Culturally *T. rubrum* strains showed white and cottony colonies on the obverse and blood-red pigment on the reverse. *T. rubrum* strains were urease negative and inhibited in dermatophyte milk agar. In the HPT, which is useful to differentiate *T. rubrum* from *T. interdigitale*, any strain was able to perforate the hair despite normal growth being observed. The analysis of ITS region confirmed all the strains as a *T. rubrum* species as well as the Trubrum primers generate a typical amplicon of 200 bp. The DNA fingerprinting is now explored in order to find the best approach to differentiate intraspecific variations and/or geographic differences. In conclusion, there are several techniques that can be applied to identify and characterise *T. rubrum* from different origins depending of the technologies available in each clinical laboratory or country.

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Keywords: Dermatophytes;, Geographic variability

P-181 - RELIABILITY OF A MULTIPLEX ALLELE-SPECIFIC POLYMERASE CHAIN REACTION (MAS-PCR) FOR THE DETECTION OF MULTIDRUG-RESISTANT MYCOBACTERIUM TUBERCULOSIS CLINICAL ISOLATES FROM BRAZIL

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Background

The World Health Organization has estimated 10.4 million new tuberculosis (TB) cases and 1.4 million TB deaths in 2015. Globally there were an estimated 480 000 new multidrug resistant (MDR) TB cases with an average proportion of extensively drug resistant (XDR) TB of 9.5%. These numbers had made the rapid detection of MDR and XDR-TB a top priority to allow early initiation of appropriate therapy and to prevent the dissemination of drug resistant strains. However, the implementation of the currently available commercial molecular methods for the rapid detection of MDRTB is hindered by their high cost and requirement for specialized personal, infrastructures, and equipment.

Method

Here we evaluated the reliability of a multiplex allele-specific polymerase chain reaction (MAS-PCR) for the detection of the most common mutations associated with isoniazid (INH) and rifampicin (RIF) resistance in a collection of 108 *Mycobacterium tuberculosis* clinical isolates. These included 49 MDR isolates, 12 INH^R, 1 RIF^R and 46 susceptible to both drugs. The reference strain H37Rv was included as control. All isolates were identified by IS6110 and RD typing. Drug susceptibility testing and the line probe assay Genotype MTBDRplus were used as phenotypic and genotypic gold standards, respectively.

Results & Conclusions

All isolates were identified as *M. tuberculosis* using IS6110 and RD typing. From the 49 MDR, 40 (81%) were correctly detected as MDR; from the 12 INH^R, 3 were correctly detected as INH^R (25%); the only RIF^R was correctly detected (100%). From the 46 susceptible isolates, MAS-PCR correctly detected 35 isolates (76%). Full concordance with Genotype MTBDR*plus* was obtained. The discrepant results were analyzed by DNA sequencing. It was found silent mutations, point mutations not associated with resistance and other mutations not contemplated by MAS-PCR or by the Genotype MTBDR*plus*.
This study shows that the MAS-PCR is a rapid, technical feasible, inexpensive and reliable method for the detection of MDR *M. tuberculosis* clinical isolates. As for other molecular methods, it should be wisely used to rule out or to assign MDRTB and should be complemented with data obtained with the phenotypic drug susceptibility testing. This study also shows that the frequency of individual drug-associated gene mutations in each setting needs to be carefully evaluated before the implementation of any molecular method for the diagnosis of MDRTB.

References & Acknowledgments

Funding: Fundação para a Ciência e a Tecnologia, Portugal (UID/Multi/04413/20139 and SFRH/BPD/100688/2014) and CAPES/MEC, Brazil (88881.064961/2014-01).

Keywords: Tuberculosis, Resistance, Mutations, MAS-PCR

P-182 - CORRELATION BETWEEN CARBAPENEM RESISTANCE, B-LACTAMASES, PORINS AND EFFLUX PUMPS IN ACINETOBACTER BAUMANNII CLINICAL ISOLATES

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Background

Acinetobacter baumannii is recognized as one of the most challenging nosocomial pathogens, whose infections are often associated with epidemic spread of multidrug-resistant strains. A. baumannii strains resistant to carbapenems (CARB) are an emergent threat as they limit the range of therapeutic alternatives and pose a considerable threat to clinical patient care and public health.

Method

Here, we investigated the potential mechanisms contributing to CARB resistance in 16 clinical isolates circulating in Lisbon. Strains were typed by ERIC-PCR. Drug susceptibility was assessed by disc diffusion. All isolates were screened for genes encoding IMP, VIM, SIM, GES, KPC, NDM, TEM, SHV, OXA-23, OXA-24, OXA-51, OXA-58, and OXA-143 β-lactamases and ISAba1. The contribution of efflux mechanisms to CARB resistance was also studied by synergism assays with efflux inhibitors (EIs), ethidium bromide real-time efflux activity evaluation and the expression analysis of efflux pump genes and porins in response to imipenem.

Results & Conclusions

Strain typing revealed clonal dissemination among isolates. All strains presented a multidrug resistant phenotype characterized by resistance to CARBs, fluoroquinolones and aminoglycosides. The β-lactamases OXA-23 were detected in 3 strains and OXA-24 in 13. OXA-51 were detected in all but ISAba1 was not detected upstream this oxacillinase excluding its contribution to CARB resistance. The results showed the existence of synergistic interactions between EIs and CARBs and ethidium bromide extrusion. Efflux assays demonstrated that these strains have increased efflux activity which could be inhibited in the presence of EIs. The EPs adeB, adeJ, adeG, craA, amvA, abeS and abeM were found overexpressed in response to CARBs. We could not demonstrate an association between CARB resistance and the expression of the porins ompA, carO or oprD.

This study demonstrated the contribution of efflux mechanisms to CARB resistance in A. baumannii clinical strains. Clinical CARB resistance is a combination between increased efflux activity and β-lactamases production such as OXA-23 or OXA-24. Overexpression of EPs may impact the clinical outcome of A. baumannii infections and treatment should consider alternative therapeutic combinations such as the use of efflux inhibitors.

References & Acknowledgments

Fundação para a Ciência e a Tecnologia, grants UID/Multi/04413/2013 and SFRH/BPD/100688/2014.

Keywords: Acinetobacter baumannii, Carbapenems, B-lactamases, Efflux pumps, Efflux inhibitors

P-183 - SHAPING A STAPHYLOCOCCUS AUREUS AUTOLYSIN FOR USE IN AN EARLY INFECTION BIOSENSOR

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Background

The presence of bacterial products in sterile body fluids can be the reflection of an infection and may be used as a diagnostic biomarker. A powerful indicator of bacterial infection is the presence of free parts of the bacterial cell wall, as these molecules are unique to bacteria and released during bacterial growth or by action of host lytic enzymes. Likewise, increased levels of circulating free DNA in blood can also be a surrogate marker for infection.

We are developing a non-invasive bifunctional biosensor for identifying bacterial infections at early stage, by detecting both the presence of peptidoglycan molecules and DNA in body fluids, using the binding capacities of the major autolysin Atl of the pathogen *Staphylococcus aureus*. This enzyme is a bifunctional protein with two independent catalytic domains, an amidase (AM) and a glucosaminidase (GL). Atl is secreted and extracellularly cleaved into AM and GL, that hydrolyze the peptidoglycan. AM is natively implicated in cell wall cleavage and homologues exist in many bacterial species, showing that it recognizes and associates with different peptidoglycan molecules. Furthermore, recent studies in our lab showed that the GL domain binds to DNA of unspecific sequence, a characteristic never reported for a cell wall lytic enzyme (1).

Method

The two domains of Atl will be independently overexpressed, purified and immobilized at the sensor surface to sense fragments of bacterial products in body fluids, as surrogate markers of the presence of infectious agents: GL will be immobilized at the sensor surface to detect DNA, while AM will be immobilized to detect muropeptides. Specific amino acid substitutions in AM will increase its binding affinity to muropeptides and abolish its hydrolytic capacity, in accordance with its structure (2). Likewise, GL protein will be mutagenized to increase its binding capability to DNA.

Results & Conclusions

Recombinant GL and AM proteins were overexpressed and purified, and their DNA and muropeptide-binding abilities, as well as hydrolytic activities have been tested; while their immobilization to different sensor surfaces is ongoing.

The development of this biosensor, a non-invasive method effective in detecting early infection, would be a huge breakthrough in the clinical diagnostic field.

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This work was funded by FEDER funds through the COMPETE 2020 Program and National Funds through FCT- Portuguese Foundation for Science and Technology under Projects Number POCI-01-0145-FEDER- 007688, Reference UID/CTM/50025; POCI-01-0145-FEDER-007728, FCT/MEC/UID/Multi/04378/2013 and PTDC/FIS-NAN/0117/2014.

Keywords: Staphylococcus aureus, Biosensor, Infection, Autolysin

P-184 - THE CONTRIBUTION OF EFFLUX TO B-LACTAMS RESISTANCE IN NON-ESBL-PRODUCING ESCHERICHIA COLI CLINICAL ISOLATES

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Background

β-lactam resistance is an emergent problem in the clinical setting and one of the antimicrobial resistance (AMR) hotspots. *E. coli* develops resistance to β-lactams mainly by the deeply studied β-lactamase production. The contribution of efflux mechanisms to this resistance phenotype has been less explored.

Method

Here, we have investigated the interplay between of efflux mechanisms and β-lactamase activity among 11 *E. coli* clinical isolates resistant to β-lactams. Clinical strains were identified using API galleries and 16S rDNA sequencing. Strains were typed by ERIC-PCR and the phylogenetic groups determined by multiplex PCR using the Clermont method. Drug susceptibility testing was assessed by disc diffusion. All isolates were screened by PCR for genes encoding the β-lactamases OXA-1, TEM and SHV and plasmid-mediated AmpC β-lactamases. The presence of AmpC promoter mutations was screened by DNA sequencing. The contribution of efflux mechanisms to β-lactam resistance was studied by synergism assays with efflux inhibitors (EIs) and ethidium bromide (EtBr) real-time efflux activity evaluation.

Results & Conclusions

Strain typing showed 8 ERIC patterns. Two strains belong to group A (commensal), 8 belong to group B2 (virulent extra-intestinal) and one strain was nontypable. These strains were resistant to ampicillin and amoxicillin/clavulanic acid but susceptible to extended-spectrum cephalosporin's. Concomitant resistance to quinolones and chloramphenicol was observed. β-lactamase production was detected in all strains and included the presence of TEM in combination with OXA-1, SHV, or AmpC overexpression and/or plasmid-mediated AmpC production. The results showed the existence of synergistic interactions between EIs and β-lactams and EtBr efflux in these strains. Efflux assays demonstrated increased efflux activity in these strains which could be inhibited by EIs. These results demonstrated that efflux activity play a role in β-lactam resistance despite the production of β-lactamases in non-ESBL-producing *E. coli* clinical isolates and the use of EIs can be considered for the development of new adjuvant therapies to tackle β-lactam resistance.

References & Acknowledgments

Fundação para a Ciência e a Tecnologia, grants UID/Multi/04413/2013 and SFRH/BPD/100688/2014.

Keywords: Escherichia coli, B-lactams, B-lactamases, Efflux pumps, Efflux inhibitors

P-185 - SCREENING OF SPOROTHRIX SCHENCKII COMPLEX SPECIES AND FIRST REPORT OF AN AUTOCHTHONOUS FELINE SPOROTRICHOSIS CASE BY S. MEXICANA IN PORTUGAL

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Background

Sporotrichosis is one of the main subcutaneous mycoses reported worldwide, being particularly endemic in Latin America, South Africa, India and Japan. The etiological agents are a complex of closely-related dimorphic species known as *Sporothrix schenckii* complex. These organisms are found in natural habitats such as living and decaying plant materials, excrements and soil. The disease is usually acquired by traumatic inoculation of spores of the fungi. Sporotrichosis is considered a rare disease in Europe but several cases have been reported, namely in Italy, France and Spain. Recent cases of human sporotrichosis have also been reported in Portugal, one of which had an autochthonous origin in a patient from Lisbon. This work aimed to develop a PCR-based assay to detect and identify *S. schenckii* complex members in environmental samples, and to perform an environmental screening of these species in several regions of Portugal.

Method

A PCR assay was implemented, which specifically amplify a fragment of the Internal Transcribed Spacer (ITS) of *S. schenckii* complex members. The assay was optimised using DNA extracted from pure cultures of several fungi, including pathogenic and environmental *Sporothrix* species, and artificially contaminated samples, such as soil and bat guano. The PCR assay was used to assess the presence of *S. schenckii* complex in 95 environmental samples (soil, plant material, bat guano), and in 7 clinical samples from cats suspected of sporotrichosis. Samples were collected in Lisbon area, Ribatejo, and Alentejo. Amplified DNA fragments were sequenced using standard methods, allowing the differentiation of *S. schenckii* complex species based on polymorphisms existent in ITS region.

Results & Conclusions

A proven positive PCR amplification result was obtained from a clinical sample of a cat suspected of developing sporotrichosis. The ITS amplicon generated was sequenced and the nucleotide sequence was identical to the species *Sporothrix mexicana*, a rare agent of infection. The cat was collected from an abandoned colony in Lisbon area. The animal's handler, who suffered a scratch from the same animal, stated she previously developed a skin lesion which was diagnosed as sporotrichosis. The impact of the presence of autochthonous *S. mexicana* in Portugal, and its potential zoonotic transmission, will be discussed.

References & Acknowledgments

The authors are grateful to clinicians and clinics who collaborated in this study: Conceição Peleteiro and Madalena Pinto Coelho from VetPat; Cláudio Cabral from Pontovet; Animais e Cãopanhia and AndiCat.

Keywords: Sporotrichosis, Environmental screening, molecular identification, Sporothrix schenckii complex, ITS region

P-186 - INHIBITORY EFFECT OF NATURAL COMPOUNDS ON CANDIDA PLANKTONIC CULTURES AND BIOFILMS

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Background

The incidence and prevalence of invasive fungal infections has been increasing, especially in immunocompromised and hospitalized patients with serious underlying diseases. Several species of *Candida* are the main etiological agents of these types of infection, particularly *Candida albicans*. Pathogenicity of *Candida* species is attributed to several virulence factors including the capability to form biofilms, which show higher levels of antifungal resistance. The use of natural products, such as extracts of plant's essential oils, have been explored as alternative antifungal therapeutic strategies. However, these naturally occurring compounds have not yet found any established role in the formulation of conventional pharmaceutical antifungal products. In addition, most published works assessed the antifungal activity of these compounds in planktonic yeast cells, existing a knowledge gap on the potential action these compounds may have against yeast biofilms.This work aimed to assess the antifungal activity of extracts of essential oils, and the eventual synergy these compounds may have with conventional antifungal drugs, against isolates of *Candida* growing as planktonic cultures and as biofilms.

Method

The Minimum Inhibitory Concentration (MIC) of several natural products, such as carvacrol, eugenol and thymol, against isolates of *Candida albicans* and of other clinically relevant *Candida* species was assessed using a standard microdilution method. Natural products were tested alone and as a combination with fluconazole and voriconazole. The antifungal activity of a selection of natural products, alone and in combination with fluconazole, was also tested against biofilm's models of the same *Candida* isolates by a semiquantitative colorimetric assay based on the reduction of tetrazolium salt (XTT).

Results & Conclusions

Of the natural compounds tested, the ones which showed the most evident antifungal activity, against most *Candida*cultures tested, were carvacrol, eugenol and thymol. Antifungal synergy between carvacrol and eugenol and the conventional antifungals fluconazole and voriconazole was also noticed against most planktonic *Candida* cultures. Antifungal synergy between carvacrol and fluconazole was also noticed when combinations of these compounds were tested against a resistant strain of *Candida albicans* growing as biofilm. Our results revealed an interesting antifungal activity of some natural compounds, alone and in combination with conventional antifungal drugs, including against *Candida* biofilms.The potential impact of these results will be discussed in the context of the search for novel alternative antifungal therapies.

Keywords: Candida albicans, biofilms, natural compounds

P-187 - EFFLUX ACTIVITY DIFFERENTIALLY MODULATES THE LEVELS OF ISONIAZID AND RIFAMPICIN RESISTANCE AMONG MULTIDRUG RESISTANT AND MONORESISTANT MYCOBACTERIUM TUBERCULOSIS STRAINS

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Background

With the growing body of knowledge on the contribution of efflux activity to *Mycobacterium tuberculosis* (Mtb) drug resistance, increased attention has been given to the use of efflux inhibitors (EIs), as adjuvants of tuberculosis (TB) therapy. Here, we have investigated the effect of the EIs verapamil and thioridazine on the efflux levels of isoniazid (INH) and rifampicin (RIF) monoresistant, and multi- and extensively drug resistant (MDR/XDR) Mtb clinical strains to describe how efflux activity modulates the levels of resistance in strains presenting different drug-resistance associated mutations.

Method

Strains were characterized by drug susceptibility testing (DST) plus quantitative DST using the MGIT960/TB eXIST system, in presence and absence of verapamil or thioridazine. Genotypic characterization focused on the analysis of the genes involved in INH and RIF resistance with the aid of the line probe assays Genotype MTBDR*plus* and MTBDR*sl* and DNA sequencing. Isolates were genotyped by spoligotyping and MIRU-VNTR. Efflux activity was quantified by real-time fluorometry following the accumulation and extrusion of ethidium bromide, a broad-range efflux substrate. The growth rates in the presence and absence of efflux inhibitors were evaluated by the analysis of growth curves using the MGIT960/TB eXIST system.

Results & Conclusions

The Mtb strains studied included three pan-susceptible, two INH resistant, three RIF resistant, and six MDR, of which two were XDR strains. Strains sharing the same mutation for antibiotic resistance displayed different MICs. Ethidium bromide efflux assays showed that the MDR strains have increased efflux activity when compared with the monoresistant strains and the susceptible reference strain. The EIs were more effective in reducing the resistance levels of the MDR strains presenting increased efflux activity than in monoresistant strains. The results also showed that growth rates of the Mtb strains vary according to their resistance pattern and associated mutations. The MDR strains showed increased growth rates, indicating a higher fitness for MDR strains compared to the monoresistant or the susceptible strains.

The results demonstrated that efflux activity differentially modulates the levels of INH and RIF resistance in *M. tuberculosis* clinical isolates presenting different drug resistance associated mutations and drug resistance profiles. Understanding how the genetic background influences the efflux activity and consequently the effect of efflux inhibitors will aid in the design of more effective and efficient therapeutic measures for TB therapy.

References & Acknowledgments

Fundação para a Ciência e a Tecnologia, grants UID/Multi/04413/2013, SFRH/BPD/100688/2014 and SFRH/BPD/95406/2013.

Keywords: Tuberculosis, Resistance levels, Mutations, Efflux activity, Efflux inhibitors

P-188 - COMBATING ANTIMICROBIAL RESISTANCE: A ONE HEALTH APPROACH AT THE NATIONAL REFERENCE LABORATORY OF ANTIBIOTIC RESISTANCES AND HEALTHCARE ASSOCIATED INFECTIONS

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Background

Antibiotics are powerful tools for fighting and preventing infections. Nevertheless, widespread use of antibiotics has resulted in an alarming increase in antibiotic-resistant infections, with therapeutic options becoming increasingly limited, expensive and often more toxic. One of the main aim of the National Reference Laboratory of Antibiotic Resistances and Healthcare Associated Infections (NRL-AR/HAI), at National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal, is to contribute to the knowledge of the spread of antibiotic resistance (AR) among humans, and to suggest actions according to the flow of AR genes related to animals and environment in view to the actual concept of "One Health".

Method

The NRL-AR/HAI has been developing work in several priority areas, with action at prevention level carried through the training, dissemination of results from its team, and the coordination and participation in projects aiming the clarification of the national AR situation, namely in a European and International context. The laboratory deals with human isolates that are from three different networks: Norma 004/2013 from DGS/Ministry of Health, EARS-Net (European Antimicrobial Resistance Surveillance Network) and ARSIP (Antimicrobial Resistance Surveillance Program in Portugal). In the area of AR in veterinary and environment, NRL-AR/HAI has also performed several studies through research collaborations with universities and other specialized laboratories. The NRL-AR/HAI is applying a multidisciplinary approach combining the most advanced "omics" techniques with classic phenotypic and epidemiologic tools to study bacterial AR mechanisms.

Results & Conclusions

The results obtained so far among human isolates show that, in Portugal, nowadays, the dissemination of carbapenemases (mostly KPC) among *Enterobacteriaceae* is the main AR problem, both in nosocomial and in community-acquired infections. More bad news are the emergent problem of colistin resistance mediated by plasmids. Good news are the decreasing of MRSA percentages in invasive isolates in Portugal, however still very high when comparing with other European countries. Along with the One Health concept, the NRL-AR/HAI integrates information from human, veterinary and environmental bacteria in order to the analysis of resistance dissemination. Therefore, it contributes to the clarification of critical hot spots, which may be crucial to identify new potential effective antimicrobials that can circumvent therapeutic failures.

References & Acknowledgments

V. Manageiro was supported by FCT fellowship (grant SFRH/BPD/77486/2011), financed by COMPETE-FEDER and national funds (POPH-QREN).

Keywords: One Health, Antibiotic Resistance

P-189 - ELECTROCHEMICAL IMMUNOSENSOR FOR HUMAN CYTOMEGALOVIRUS DETECTION

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Background

Electrochemical biosensors are widely used for the determination of several substances with different properties and for the continuous monitoring of biological processes. Moreover, immunosensors with electrochemical detection offer enhanced specificity and sensitivity, thanks to the specific antigen–antibody complexation, higher simplicity, through the simpler instrumentation, and reduced costs.

It is known that traditional analysis methods for viral infections are expensive and time consuming. In this way, we have been working in an easy and cheaper test to be used as an alternative that overcomes these drawbacks. This test aims to detect human cytomegalovirus (HCMV) in urine samples by using as analyte its dominant antigen, glycoprotein B (gB-HCMV).

HCMV is a herpes virus that does not represent a serious problem in immunocompetent individuals. However, in immunosuppressed individuals and those with immature immune system, the reinfection can be severe or even fatal. Additionally, HCMV infection is the most frequent cause of embryonic and fetal pathology induced by a virus and the majority of the infected children does not manifest any symptom at birth, making essential to develop a screening test to detect this virus at early stage in order to minimize permanent damages.

Method

This immunosensor assay scheme is based on gB-HCMV sandwiched between a primary antibody, previously immobilized onto magnetic beads functionalized with protein G (MBs-PrG), and a secondary anti-gB antibody labelled with *Horseradish peroxidase* (HRP) to obtain an electrochemical signal.

Results & Conclusions

The immunoassay analytical performance was tested. The use of MBs-PrG as solid support for primary antibody immobilization allowed the orientated antibody immobilization in order to expose antigen binding sites. Furthermore, the use of magnetic beads induce faster incubations compared to antibodies immobilized on solid surfaces, improves separation steps of the immunoassay and avoids matrix interferences.

References & Acknowledgments

This work is supported by FEDER funds through the POCI - COMPETE 2020 (Project POCI-01-0145-FEDER-007491) and National Funds by FCT (Project UID/Multi /00709/2013).

Keywords: Magnetic beads immunoassay, Electrochemical detection, Human cytomegalovirus, Glycoprotein B

P-190 - COMBINED TREATMENT WITH BACTERIOPHAGES AND ANTIBIOTICS AS A STRATEGY TO CONTROL BIOFILM ASSOCIATED INFECTIONS

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Background

Bacterial biofilms are sessile microbial aggregates with unique community properties, showing a high degree of tolerance/resistance to disinfection by chemicals, antibiotics, and to the human immune system. The opportunistic pathogen *Pseudomonas aeruginosa* is one of the most frequent causes of biofilm-associated infections, causing infections extremely difficult to treat. Currently, bacteriophages (phages) that are specific for pathogenic bacteria are becoming a potential solution for the treat such infections.

Method

In this study, *P.aeruginosa* biofilms were formed and subjected to treatment by *P.aeruginosa* phage vB_PaM_EPA1 (EPA1) alone or in combination with antibiotics (gentamicin, ciprofloxacin, meropenem) of different classes. EPA1 was isolated from the effluent from a wastewater treatment plant. TEM images show that this phage belongs to the *Myoviridae* family. Its genome has a 91,3 kb, a GC content of 49.2% and encodes 178 putative genes, from which 147 have no predicted function. Phage and antibiotics with different multiplicity of infection (MOI) and minimal inhibitory concentration (MIC), respectively, were simultaneously or sequentially (phage suspension was added first then antibiotic were added with a delay of 6 hours) added to 48 hours old-biofilms. After 24-hour treatment, bacterial survival was measured by colony forming unit (CFU) counting method.

Results & Conclusions

Results showed that in individual treatments of phage (at MOI1) and antibiotics (at MIC) generally had significant reductions on the number of viable cells ranging from 0.5 to 3,7 logs. However, when they were sequentially added to the biofilms, a synergistic effect (>8 logs) was detected, namely with phage-gentamicin and phage-ciprofloxacin combinations. In opposition, an antagonistic effect was detected when phage and ciprofloxacin were simultaneously added. Overall, our results show that combination of phages and antibiotics are very effective against *P.aeruginosa* biofilms particularly when they are applied sequentially and this constitutes a good strategy to control biofilm-associated infections.

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Keywords: Bacteriophage, biofilm, antibiotic, treatment

P-191 - EVOLUTIONARY DYNAMICS OF PKS1 AND PKS15 GENES INVOLVED IN PHENOLIC GLYCOLIPID BIOSYNTHESIS AMONG MYCOBACTERIUM TUBERCULOSIS COMPLEX BACTERIA

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Background

Mycobacterium genus exhibits a hydrophobic surface composed of arabinogalactan and mycolic acids. In a limited number of species, mostly pathogenic to man, among exposed lipids on the cell wall, we find phenolic glycolipids (PGLs). Most of the genes necessary for PGL biosynthesis are located at the PDIM + PGL locus, which include *pks15* and *pks1*. The *pks15-1* product is crucial for PGL production, since in its absence there is no PGL production. The *pks1* and *pks15* genes encode the polyketide synthetase Pks15-1 which possesses six functional domains: KS (ketoacylsynthase), produced from *pks15*; KR (ketoreductase) and other four domains, produced from *pks1*. Since cell wall is the interface of the bacterium with the host cell, modifications in PGL biosynthetic pathway are expected to interfere with PGL display at the cell wall, thus exerting downstream effects during infection.
In this work, we analysed *in silico* the nucleotide diversity of the *pks15-1* locus in clinical *M. tuberculosis* and *M. bovis* from all over the world and explored the underlying structural and functional consequences.

Method

Mycobacterium tuberculosis H37Rv nucleotide and protein sequences were used as reference and aligned using BLAST against complete *Mycobacterium* genus sequences available in NCBI. Nucleotide variations (SNPs, deletions and insertions) and amino acid substitutions were annotated. 3D protein models were predicted for Pks1-15 in most dissimilar isolates. The microevolutionary history of *pks15* and *pks1* in *M. tuberculosis* and *M. bovis* strains was reconstructed based on nucleotide variation and neighbour-joining.

Results & Conclusions

Comparative analysis of 142 *M. tuberculosis* and *M. bovis* genomes evidenced the accumulation of polymorphisms at the various domains of *pks1* and *pks15*, with greater accumulation within the KR domain, while mutations at KS were more frequently shared among studied strains. Mutations were also frequent in the intergenic region, leading to frameshifts and preventing transcription of *pks1* in clinical isolates that are currently in circulation. Some of the detected variations result in semi- or non-conservative aminoacyl substitutions leading to truncated proteins wherein several structural domains are absent. The microevolutionary history of both genes differs between the two ecotypes of *Mycobacterium tuberculosis* complex, depicting greater genomic and aminoacyl conservation in *M. bovis* strains, contrary to *M. tuberculosis*, in which accumulation of several disrupting mutations potentially lead to non-functional Pks1-15. Although a pseudogenization scenario is hypothesized for *M. tuberculosis,* we cannot discard the possibility of these gene products being important riboregulators involved in the arms race against the host.

Keywords: pks1, pks15, pseudogeneization, Mycobacterium tuberculosis complex, phenolic glycolipid biosynthesis

P-192 - CEFOTAXIME, IMIPENEM AND COLISTIN RESISTANCE IN KLEBSIELLA PNEUMONIAE FROM PETS IN PORTUGAL

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Background

The dissemination of multi-resistant microorganisms has been considered a public health concern in medicine and agriculture[1,2]. The prevalence of *Klebsiella pneumoniae* producing extended-spectrum β-lactamases (ESBLs) or carbapenemases, as well as those colistin-resistant strains, is increasing worldwide[3].

In this work, we want to analyze the susceptibility of antibiotics and to detect imipenem or colistin resistance among *Klebsiella pneumoniae* isolates recovered from pets in Portugal.

Method

A total of 500 faecal samples were recovered from dogs and cats between January to April 2017, and were seeded on MacConkey agar supplemented with meropenem (0.8 mcg/ml). The MALDI-TOF MS method was applicable in this study to confirm the bacterial species identification. *Klebsiella pneumoniae* isolates were kept and further studied (one per animal). Antimicrobial susceptibility was performed by modified Kirby–Bauer sensitivity testing methods. Susceptibility testing and phenotypic detection tests were used in this study, according to the norms of the Clinical Laboratory Standards Institute (CLSI)[4].

Results & Conclusions

In this study, *K. pneumoniae* isolates were recovered in MacConkey agar supplemented with meropenem from 17 of the 500 faecal samples tested (3.4%). Isolates were identified by biochemical tests and data was confirmed by MALDI-TOF. All of the isolates were resistant to third generation cephalosporins (cefotaxime and/or ceftazidime), aztreonam and ciprofloxacin (100%), and high prevalence of resistance was detected for tetracycline (88%) and ertapenem (82%). It's interesting to remark that two isolates showed reduced susceptibility for imipenem, one of them was resistant to imipenem and five additional isolates exhibited colistin resistance.

The therapeutic options for infections that could be caused by this type of strains are limited not only in case of resistance to cephalosporins and carbapenems, but also to other antibiotics.

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Keywords: Antibiotic resistance, Klebsiella pneumoniae, Maldi-TOF, Public health

P-193 - ALTERNATIVES TO CONVENTIONAL ANTIBIOTICS: CHLORHEXIDINE AND NISIN INHIBITORY ACTIVITIES AGAINST DIABETIC FOOT ULCER STAPHYLOCOCCI

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Background

Diabetes *mellitus* (DM) is a chronic disease that affects more than 422 million people worldwide, with 15 to 25% of patients developing diabetic foot ulcers (DFU) in their lifetime. Around half of these ulcers become clinically infected, usually by opportunistic pathogens, being *Staphylococcus aureus* the most frequent [1]. The presence of antibiotic resistant *S. aureus* strains is a major problem in DFU treatment. Therefore, it is utterly important to define new strategies to control these infections, based on antimicrobial compounds that represent an alternative to conventional antibiotics, such as chlorhexidine and nisin.

Chlorhexidine is a broad-spectrum antiseptic, active against bacteria, fungi and some enveloped viruses. Despite its potential, the increasing use in hand hygiene and patient washing raises concern regarding development of acquired bacterial resistance. Nisin is an antimicrobial peptide produced by *Lactococcus lactis* that is mainly active against Gram-positive bacteria. Nisin has been used for pathogen control in food products and differs from conventional antibiotics regarding its synthesis, toxicity, resistance mechanisms and mode of action. As the exposure to sub-lethal antimicrobial concentrations may enhance resistance towards these biocidal compounds, it is crucial to determine their minimum inhibitory (MIC) and bactericidal concentration (MBC) values against selected pathogens.

Method

This work aimed to evaluate chlorhexidine and nisin antibacterial activity against 23 *S. aureus* strains isolated at Lisbon medical centres from infected foot ulcers of DM patients, including both multidrug-resistant and MRSA strains (22 and 35%, respectively)[2]. Isolates *in vitro* susceptibility to chlorhexidine and nisin was assessed using standard microdilution assays.

Results & Conclusions

All strains, including those with relevant antibiotic resistance profiles, presented susceptibility to these compounds. Mean MIC values were 6±2 and 90.0±22.8 µg/mL, and mean MBC values were 15±16 and 495.2±149.9 µg/mL, for chlorhexidine and nisin, respectively. Results support the potential use of these compounds in clinically infected DFU. They also provide a valuable contribution for the establishment of effective antimicrobial protocols, as the application of these inhibitory compounds may ultimately contribute to the reduction of conventional antibiotic administration to these patients and to the dissemination of resistant strains.

References & Acknowledgments

This work was supported by the Centro de Investigação Interdisciplinar em Sanidade Animal(CIISA), Faculdade de Medicina Veterinária, Universidade de Lisboa(FMV/UL),(Project UID/CVT/00276/2013).

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Keywords: Chlorhexidine, Diabetic Foot Ulcer, Nisin, Staphylococcus aureus

P-194 - IN SILICO SELECTION OF AN SSDNA APTAMER AGAINST HER2-POSITIVE BREAST CANCER CELLS USING COMPUTATIONAL DOCKING SIMULATION

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Background

Human epidermal growth factor receptor type 2 (HER2/ErbB2) is a breast cancer associated protein overexpressed in 20% of breast cancers, being involved in cell growth regulation, survival and differentiation.[1,2] The location of HER2 on the cell surface has contributed to its appeal as a tumour-targeted therapy.[3] Aptamers, generated from Systematic Evolution of Ligands by EXponential Enrichment (SELEX), emerged as potential tool for application in target cancer therapy due to their three-dimensional structures that recognize cell surface receptors.[4]

Method

In this study, HER2-aptamers were screened and identified using SELEX technology. After cloning and sequencing, aptamers were modelled through m-fold software and posteriorly, the docking simulation was predicted using ZDOCK server.[5,6] These *in silico* predictions measured the aptamer-HER2 interactions through a combination of shape complementarity and statistical potential terms for scoring.

Results & Conclusions

Based on *the interaction score*, a candidate ssDNA-aptamer (HER2-31; 5´-CACGTGCAGGGTGGATAGCAATCTATCCGGTCCCACTGTTCGGTGGTCGC -3') was selected. Targeted-specificity of the selected HER2-31 was validated through cytometry and fluorescence microscopy assays in HER2-positve breast cancer cells. Our results indicate that SELEX technology is an efficient method to screen specific protein-bound ssDNA, and HER2-31 could be used as an agent in HER2-based diagnosis and targeted therapy. Also, the results provide valuable guidelines for the application of docking simulations for the prediction of aptamer-ligand structures, as well as for the design of novel features of ligand-aptamer complexes.

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In silico selection of an ssDNA aptamer against HER2-positive breast cancer cells using computational docking simulation

Keywords: aptamers, SELEX, breast cancer, HER 2

P-195 - IN VITRO AND IN VIVO ANTITUMOR PROPERTIES OF HISTONE DEACETYLASE INHIBITORS IN CANINE DIFFUSE LARGE B-CELL LYMPHOMA

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Background
Non-Hodgkin lymphoma (NHL) represents 90% of all lymphomas is one of the leading causes of cancer-related death. Therapeutic options for lymphoma have improved remarkably, however regardless of the elected therapy the majority of patients succumb to this disease. Therefore, there is a continuing need to develop novel therapeutic strategies. Canine lymphoma and human NHL share many histopathological, molecular, genetic and clinical features, providing clinically realistic opportunities to explore therapeutic protocols that may translate to human clinical trials. HDACi, molecules that inhibit histone deacetylase, have emerged as a powerful new class of anti-cancer drugs. HDACs catalyse the deacetylation from histones, leading to chromatin condensation and transcriptional repression. By inhibiting deacetylating enzymes activity, HDACi regulate aberrant deacetylation and modify gene expression in cancer cells, culminating in cytotoxicity. To date, three HDACi have been approved for cancer therapy by the FDA for T-cell lymphoma treatment. Therefore, we explored HDACi for treatment of canine lymphoma.

Method
A library of HDACi compounds were screened on a CLBL-1, a canine lymphoma cell line. CLBL-1 cells were subjected to increasing drug concentrations and cell viability/proliferation were assessed. In order to elucidate mechanisms of action underlying the cytotoxic effect, histone acetylation status were assessed using immunoblotting studies. Furthermore, levels of caspase 3/7 activation and Annexin V/7-AAD analysis were performed to clarify the nature of cell death induced. Finally, the anti-tumor effect of panobinostat on canine NHL cells was further tested in a murine tumor xenograft model. CLBL-1 cells were injected subcutaneously in SCID mice to establish tumors. After tumor induction, mice were randomly assigned into 3 groups (n=5): vehicle, 10 mg/kg and 20 mg/kg. Treatment consisted of intraperitoneal injections 5 days/ week for 2 weeks.

Results & Conclusions
Results showed that HDACi compounds presented cytotoxic effect on CLBL-1 and a clear histone deacetylation status of CLBL-1 after HDACi treatment. Considering the strong cytotoxic activity, panobinostat was selected as the most promising molecule for further investigation. Panobinostat treatment induced high levels of caspase activation in a dose-dependent manner. Furthermore, a higher percentage of apoptotic cell death was determined. Regarding in vivo studies, panobinostat treatment at 10 mg/kg and 20 mg/kg strongly inhibited tumor growth when compared to vehicle treated mice.
In summary, herein we investigate for the first time *in vitro* and *in vivo* cytotoxic effects of panobinostat on canine B-cell lymphoma. This study thus provides new data validating HDACi as a novel cancer therapy for veterinary applications and translation for human therapeutic applications.

Keywords: Non-Hodgkin Lymphoma, Animal model, HDACi

P-196 - RECOMBINANT P53-MINICIRCULAR DNA: FROM THE DESIGN TO THE IN VITRO P53 EXPRESSION

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Background

Human Papillomavirus (HPV) infection is nowadays a major threat for women worldwide, especially considering its association with more than 99% cervical cancer cases. As a matter of fact, HPV E6 oncoprotein is responsible for the progression of this tumor due to the inactivation of the p53 tumor suppressor. Thus, novel therapeutic approaches for cervical cancer are currently under thorough exploration. One of such consists on gene therapy for the delivery of genetic material containing p53-encoding sequence to cancer cells. Although plasmid DNA has been the main non-viral vector used in DNA therapeutics in the past years, the possibility of using a DNA vector with reduced size, decreased toxicity and increased transfection efficiency takes minicircle DNA (mcDNA) to the vanguard of gene therapy.

Method

Firstly, p53-encoding sequence was cloned into the parental plasmid (PP), followed by transformation of *E. coli* ZYCY10P3S2T by heat shock. Production parameters were adjusted to optimize the recombination of PP into mcDNA. Afterwards, given the importance of mcDNA purity, a new chromatographic column was studied to simultaneously explore the affinity character of two ligands (arginine and lysine) immobilized in the same functional group (triazine). For this matter, hydrophobic binding/elution conditions were studied to purify the supercoiled (sc) mcDNA-p53. Finally, immunocytochemistry, RT-PCR and western blot techniques were carried out to assess the transfection efficiency and gene expression of mcDNA and PP in *in vitro* cervical cancer cell models.

Results & Conclusions

Higher mcDNA-p53/PP-p53 ratio was achieved by inducing recombination for 2h with 0.01% of L-arabinose. Although sacrificing sc mcDNA recovery, column selectivity was favored when binding the sample at lower salt concentration, retrieving sc mcDNA-p53 with lower impurity content. On the other hand, pH manipulation allowed to enhance sc mcDNA-p53 recovery yield, while eluting some impurities. HeLa cells were successfully transfected with PP and mcDNA vectors, being more efficient with the last one. p53 transcripts were detected by RT-PCR in transfected cells and p53 protein expression was confirmed by western blot. Taking all these data into account, mcDNA-p53 seems to have been successfully engineered, being promising to proceed in *in vitro* and *in vivo* studies.

References & Acknowledgments

A.M. Almeida and A. Sousa acknowledge doctoral and post-doctoral fellowships (SFRH/BD/102284/2014 and SFRH/BPD/102716/2014, respectively) from FCT.

Keywords: P53 tumor suppressor, Human Papillomavirus, Gene therapy, Biotechnological Platform, Minicircle DNA

P-197 - EXPLOITING SILVER-CAMPHOR DERIVATIVE COMPLEXES AS NOVEL ANTIMICROBIALS

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Background

WHO has recognized antimicrobial resistance as one of the greatest threats to human health. Because of the rapid dissemination of drug resistance in pathogens, many of the compounds that were highly effective became obsolete during the past decades. Aiming at contribute to find new compounds with antibacterial activity, several silver complexes ($[Ag(NO_3)(L)_2]$, $[Ag(NO_3)(L)]_n$)[1,2] with camphor derived ligands were synthesized and their antibacterial activity was evaluated.

Method

Complexes were characterized using spectroscopic techniques (FTIR, NMR and Elemental analysis). The antibacterial activity of the complexes was quantified by assessing the MICs, using standard procedures. The bacteriostatic or bactericidal activity of compounds and the emergence of resistance were also assessed.

Results & Conclusions

MIC values were determined by microdilution methods[1,2] for the silver complexes and $AgNO_3$. Results are displayed in table 1.

Table 1	**MIC (µg/mL)**			
Complex	***E. coli* ATCC25922**	***P. aeruginosa* 477**	***B. contaminans* IST408**	***S. aureus* Newman**
$AgNO_3$	47	39	74	73
$[Ag(NO_3)(OC_{10}H_{14}NNH_2)]$	22	27	27	56
$[Ag(NO_3)(OC_{10}H_{14}NNMe_2)_2]$	58	54	41	134
$[Ag(NO_3)(OC_{10}H_{14}NOH)_2]$	51	52	59	119
$[Ag(NO_3)(OC_{10}H_{14}NC_6H_5)_2]$	59	55	91	54
$[Ag(NO_3)(OC_{10}H_{14}NC_6H_4CH_3)_2]$	37	53	66	55

$[Ag(NO_3)(OC_{10}H_{14}NC_6H_3(CH_3)_2)_2]$	40	52	105	47
$[Ag(NO_3)(OC_{10}H_{14}NC_6H_4OH\text{-}m)_2]$	52	53	75	113
$[Ag(NO_3)(OC_{10}H_{14}NC_6H_4CF_3)_2]$	52	60	55	105
$[Ag(NO_3)(OC_{10}H_{14}NC_6H_4F)_2]$	59	53	77	93
$[Ag(NO_3)(OC_{10}H_{14}NC_6H_4Cl\text{-}p)_2]$	52	55	57	151
$[Ag(NO_3)(OC_{10}H_{14}NC_6H_4Cl\text{-}m)_2]$	53	53	44	110
$[Ag(NO_3)(O_2SNC_{10}H_{13}NNH_2)_2]$	52	36	38	114
$[Ag(NO_3)(O_2SNC_{10}H_{13}NN(CH_3)_2)_2]$	107	108	56	257

Results show a higher inhibitory effect of some camphor imine and camphor sulphonylimine complexes compared to silver nitrate in both Gram-negative and Gram-positive bacteria, indicating that the compounds are promising alternatives to existing drugs. The antibacterial activity of complexes was found to be dependent on the characteristics of the camphor ligand. Complex **$[Ag(NO_3)(OC_{10}H_{14}NNH_2)]$** exhibited the highest antibacterial activity towards *E. coli*, *P. aeruginosa* and *B. contaminans* (22, 27 and 27 µg/mL, respectively), while complex **$[Ag(NO_3)(OC_{10}H_{14}NC_6H_3(CH_3)_2)_2]$** displayed the highest activity against *S. aureus* (47 µg/mL).

All compounds under study display a bactericidal effect when used in concentrations equal or above the MIC.

A resistance emergence frequency of *ca.* $4x10^{-10}$ was detected both for *P. aeruginosa* 477 towards complex **$[Ag(NO_3)(OC_{10}H_{14}NC_6H_4F)_2]$** and for *E. coli* ATCC25922 towards complex **$[Ag(NO_3)(OC_{10}H_{14}NNH_2)]$**. In all other combinations of bacterial strain /compound, the frequency of emergence of resistance was $<4x10^{-10}$. Future studies will focus on the elucidation of mechanisms underlying the antibacterial activity and the potential use of camphorimine and camphor sulphonylimine Ag(I) complexes as effective infection treatment agents.

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Keywords: Silver complexes, Camphor, Antibacterial activity, Emergence of resistance

P-198 - STRUCTURAL AND FUNCTIONAL STABILIZATION OF SILK SERICIN: BIO-ORIGAMI FOR SKIN REGENERATION.

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Background

Development and optimization of a bioorigami film with impregnated silk sericin was pursued, for skin regeneration applications. The selected bioorigami exhibited a homogeneous and translucid appearance, and devoid of any fractures or cracks. Several formulations were produced, with varying integrated sericin contents, viz. 0, 1, 2, 5, 10, 20 and 50 $mg_{sericin}/mL_{bioorigami}$. The optimized bioorigami presented antioxidant activity, as expected, indicating a potential utilization in skin regeneration with prolonged release of the bioactive protein.

Method

The selected bioorigami exhibited a homogeneous and translucid appearance, and devoid of any fractures or cracks. Several formulations were produced, with varying integrated sericin contents, viz. 0, 1, 2, 5, 10, 20 and 50 $mg_{sericin}/mL_{bioorigami}$. The optimized bioorigami presented antioxidant activity, as expected, indicating a potential utilization in skin regeneration with prolonged release of the bioactive protein. The infrared spectra of the bio-origami films integrating silk sericin indicated that the protein did not engage in any bonds with the polymeric matrix, which otherwise could have reduced its antioxidant activity.

Results & Conclusions

The physico-chemical characteristics of the several bioorigami films produced were evaluated in detail, via FTIR, XRD, XRF, XRT, TGA, DSC, transdermal protein permeation, kinetics of protein release from the bioorigami films, and free radical scavenging activities. The results gathered clearly suggest that the optimized bioorigami films integrating crude sericin extract had both obvious radical scavenging effects with the 2.2-diphenyl-1-picryl-hydrazil (DPPH) assay, and exhibited prolonged release of the bioactive protein, further suggesting potential biopharmaceutical applications such as skin regeneration.

References & Acknowledgments

Project funding by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, São Paulo, Brazil; Refs. No. **2016/08884-3 (Project PneumoPhageColor) and 2016/12234-4 (Project TransAppIL)**), is hereby gratefully acknowledged. Funding by Fundação de Amparo à Pesquisa do Estado de São Paulo **(FAPESP Ref. No. 2016/16641-3)** in the form of a M.Sc. fellowship granted to Liliam Katsue Harada is hereby gratefully acknowledged. This work also received support from CNPq, National Council for Scientific and Technological Development – Brazil, in the form of a Research Productivity (PQ) fellowship granted to Victor M. Balcão **(Ref. No. 306113/2014-7)**. The authors have no conflicts of interest whatsoever to declare.

Keywords: Sericin from Bombyx mori, Bioorigami film, Antioxidant activity, Transdermal permeation, Kinetics of protein release, Skin regeneration

P-199 - CHITOSAN AS AN EFFECTIVE INHIBITOR OF MULTIDRUG RESISTANT ACINETOBACTER BAUMANNII

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Background

Acinetobacter baumannii is a multidrug resistant pathogen often associated with nosocomial outbreaks, with an innate ability to evade nearly all traditional antibiotics and a capacity to adhere and colonize medical devices.

Chitosan is a biocompatible and non-toxic polymer which has been shown to have a strong antimicrobial activity against antibiotic resistant microorganisms, with potential to become an alternative to traditional antimicrobials.

Method

Chitosan's biological activity was assessed upon two *A. baumannii* strains (one clinical multidrug resistant strain (MDR) and one reference strain CCUG 61012) in planktonic and sessile environments. From a planktonic standpoint minimal inhibitory concentrations (MIC) and the minimal bactericidal concentrations (MBC) were determined while from a sessile perspective minimal biofilm inhibitory concentrations (MBIC), adhesion and biofilm formation were assayed through biomass and metabolic activity inhibition.

Results & Conclusions

Results showed that the chitosan molecular weights tested were effective in inhibiting *A. baumannii*'s planktonic and sessile growth. For the first MICs and MBCs were obtained at relatively low concentrations (0.5–2 mg/mL). For the latter, MBICs varied between 1 and 8 mg/mL and chitosan effectively inhibited both *A. baumannii*'s adhesion and biofilm formation, with this inhibitory activity being more pronounced from a biomass formation standpoint. Analyzing the differences observed between the reference and the MDR *A. baumannii*'s strains, the MDR strain was, in general, as susceptible as the reference strain to chitosan's activity.

Overall, chitosan showed high potential as a possible natural alternative to the treatment of multidrug resistant *A. baumannii* infections with the high antibiotic resistance profile of this microorganism not being an impediment to chitosan's activity both in planktonic and sessile settings.

References & Acknowledgments

The present work was supported by National Funds from Fundação para a Ciência e Tecnologia (FCT, Lisboa, Portugal), through project UID/Multi/50016/2013, and from QREN-ANI, through project EcoTex (17819). Additionally, the author E.M. Costa would like to acknowledge FCT and Aquitex S.A. for his Ph.D. grant SFRH/BDE/103957/2014. Moreover, the authors would like to acknowledge CHTMAD–Hospital Centre of Trás-os-Montes e Alto Douro for kindly providing the clinical isolate used in this work.

Keywords: Chitosan, Antimicrobial, Antibiofilm, Multidrug resistant strain

P-200 - IN VITRO EVALUATION OF TP53 PURE SUPERCOILED PDNA BIOLOGICAL PERFORMANCE IN DIFFERENT CANCER MODELS

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Background

The tumour suppressor p53 remains one of the most interesting therapeutic targets in cancer gene therapy due to its consistent mutation in numerous cancers. To date most pre-clinical and clinical strategies involving this biological target have been based on the use of viral vectors for p53 transgene delivery, posing significant safety issues that possibly be overcome with their non-viral plasmid-based counterparts.

Method

In this study, we have taken advantage of a highly robust affinity chromatography platform based on an L-methionine agarose matrix to purify the supercoiled topoisoform of plasmid DNA non-viral biopharmaceutics encoding p53 (p53-pDNA). The purified biopharmaceuticals were complexed with liposomes to comprehensively analyze theirs *in vitro* biological performance and therapeutic potential in different cancers including those of lung, and cervix.

Results & Conclusions

The results indicate that L-methionine affinity matrix provided a suitable platform to completely purify sc p53-pDNA under precise chromatographic conditions. *In vitro* transfection with pure sc p53-pDNA attained a higher production of the tumour suppressor protein in cancer cells when compared to native pDNA samples containing both oc and sc topoisoforms. Also, p53 production following transfection was significantly higher in HeLa cervix cancer cells in comparison to that obtained in A549 lung cancer cells. HeLa cells presented the higher apoptotic behavior, demonstrating that p53-based transgene therapy may be particularly effective in this cancer. Overall, our findings emphasize the potential of sc pDNA non-viral gene-based therapy and provide important insights into the therapeutic potential of this approach.

References & Acknowledgments

This work was supported by FEDER funds through the POCI - COMPETE 2020 - Operational Programme Competitiveness and Internationalization in Axis I - Strengthening research, technological development and innovation (Project POCI-01-0145-FEDER-007491) and National Funds by FCT - Foundation for Science and Technology (Project UID/Multi /00709/2013). J.F.A. Valente and A. Sousa also acknowledge PhD and Postdoctoral fellowships (Ref SFRH/BD/96809/2013 and Ref SFRH/BPD/102716/2014, respectively).

Keywords: Gene Therapy, L-methionine affinity chromatography, Supercoiled plasmid DNA, Tumor suppressor p53

P-201 - ANTIADHESIVE AND ANTIBIOFILM EFFECT OF MALVIDIN -3-GLUCOSIDE AND MALVIDIN-3-GLUCOSIDE/NEOCHLOROGENIC ACID MIXTURES UPON STAPHYLOCOCCUS

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Background
Anthocyanins are water soluble pigments that have been frequently associated with a vast array of potential biological properties like immunomodulatory effects, antioxidant capacity or as an antimicrobial agent. While there are several studies demonstrating the antimicrobial effect of anthocyanin rich extracts, few works can be found reporting on the antimicrobial activity of pure anthocyanins. Moreover, works regarding these compounds focus on planktonic state cells disregarding the effects upon biofilms (the most common form for bacteria to be found in nature) and bacterial adhesion (an essential step for infection to occur). Therefore, the present work aimed to determine the effects of malvidin-3-glucoside, a major component of a previously reported extract [1] and the impact of its association with neochlorogenic acid (the only non-anthocyanin phenolic present in said extract), upon Staphylococcus.

Method
A group comprised of 6 different Staphylococcus with varying resistance profiles was used to screen for the antimicrobial activity of malvidin-3-glucoside and a malvidin-3-glucoside/neochlorogenic acid mixture.

Results & Conclusions
While no significant inhibition of staphylococcal growth was observed, both malvidin-3-glucoside and malvidin-3-glucoside/neocholorgenic acid possessed an interesting antibiofilm activity (with reductions of biofilm entrapped cells up to 2.5 log cycles, metabolic inhibition rates up to 81% and up to 51% of biomass inhibition). When considering the bacteria's capacity to adhere to plain polystyrene surfaces the inhibition ranges were considerably lower than those observed for polystyrene surfaces coated with plasmatic proteins was considerably higher (45% for adhesion in the presence of extract and 39% for adhesion after the surface was exposed to extract). Overall, the tested compounds, while relatively ineffective in inhibiting the growth of staphylococci were capable of hampering both biofilm formation and bacterial adhesion.

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Acknowledgments:
This work was supported by the National Funds from FCT - Fundação para a Ciência e Tecnologia through project UID/Multi/50016/2013 and through the PhD grants of Sara Silva (

UID/Multi/50016/2013) and Eduardo Costa (SFRH/BDE/103957/2014).

Keywords: Staphylococcus, MRSA, MRSE, VRSA, Antibiofilm, Antiadhesive, Neochlorogenic acid, Malvidin-3-glucoside

P-202 - MECHANISMS OF ANTIMICROBIAL AND ANTI-INFLAMMATORY PROPERTIES OF ESSENTIAL OILS OF MENTHA SPP.

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Background

Flavouring herbs of *Mentha* species are widely used in culinary and traditional medicine. The notorious properties attributed to these aromatic plants are related with their chemical composition. Essential oils (EOs) are compounds of several secondary metabolites, characterized by their antimicrobial, antioxidant and anti-inflammatory properties, often used as alternative therapy.

The aim of this work was to evaluate the antimicrobial properties of EOs of *M. spicata* and *M. pulegium* wild grown in Alentejo (Portugal) against pathogenic or saprophyte opportunist bacteria as well as access to their i*n vitro* antioxidant activity and anti-inflammatory potential.

Method

EOs were obtained by hydrodistillation and chemical composition was analysed by gas chromatography. Antimicrobial activity was evaluated using the solid medium diffusion method and the determination of the minimum inhibitory concentration (MIC) [1]. *In vitro* antioxidant and anti-inflammatory properties were evaluated by inhibitions of catalase and lipoxygenase enzyme activities [2]. Toxicological activity of essential oils was evaluated by determination of LD_{50} in *Swiss* mice [3].

Results & Conclusions

EOs are rich in oxygenated monoterpenes and the major components of *M. pulegium* were pulegone (80%) and isomentone (5%) while carvone (57%), 1,8-cineol (12%), limonene (7%), and 4-terpineol (7%) were the main components of *M. spicata* EO.

Both EOs revealed a broad spectrum of antibacterial action, inhibiting the growth of all strains. Antimicrobial effect of *Mentha* EOs is strongly associated with their major components and high content of oxygenated monoterpenes. Mixtures of these EOs are able of reduce MIC values of all strains, revealing synergetic effects of some components.

EOs presented high potential to inhibit catalase activity, high anti-inflammatory potential in anti-lipoxygenase activity. The observed high antimicrobial, antioxidant and anti-inflammatory potential of these EOs suggest their potential use in food and /or pharmaceutical industries, however, other studies are required for determine safety doses.

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The authors thanks to Laboratorio Flaviano Gusmão, Lda (Évora, Portugal) for providing the bacterial tested strains.

This work is funded by "HIT3CH - HERCULES Interface for Technology Transfer and Teaming in Cultural Heritage", ALT20-03-0246-FEDER-000004, and "MEDUSA-Microorganisms Monitoring and Mitigation–Developing and Unlocking novel Sustainable Approaches", ALT20-03-0145-FEDER-000015, co-financed by European Regional Development Fund ALENTEJO 2020.

Keywords: Mentha spicata, Mentha pulegium, Essential oils, Antimicrobial, Anti-inflammatory properties

P-203 - SCREENING OF ANTIMICROBIAL ACTIVITY OF THE EXOPOLYSACCHARIDE OF PORPHYRIDIUM CRUENTUM

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Background

Porphyridium sp. is a microalgae that produces and excretes a sulphated polysaccharide, the exopolysaccharide (EPS), into the culture medium. It is known that polysaccharides may have biological activity as anti-inflammatory, antibacterial and antiviral.

The aim of this work was to evaluate the biological activity of EPS. The antimicrobial activity of EPS was tested against 14 microbial strains. Several tests were carried out, namely bacterial antibacterial, antifungal and antibiofilm capacity. [1, 2].

Method

EPS was tested at concentrations from 0.5% to 4%, in order to evaluate the anti-bacterial activity against several microorganisms, namely *Escherichia coli, Bacillus cereus, Salmonella enteritidis, Listeria innocua, Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Staphylococcus aureus* methylcyllin-resistant (MRSA), *Staphylococcus aureus* (MSSA), *Candida albicans Malassezia furfur, Malassezia inova* and *Malassezia sympodialis*.

To test the antifungal activity, *Microsporum canis* and *Tricophyton rubrum* were used. These microorganisms were cultured in a medium selective for dermatophytes for 14 days at 30 º C. In order to test the anti-bacterial and antifungal activity of EPS, the plaque microdilution method was used at minimum inhibitory concentrations (MIC).

Results & Conclusions

The results obtained suggest that the EPS of *Porphyridium cruentum* has potential to be used as a natural bacteriostatic. However, further microbiological studies will be needed to understand the mechanism of action.

It was observed that at the concentration of 4%, EPS exerted a greater inhibitory activity in the 24 hours of incubation, although not very important for the majority of the microorganisms, reaching a maximum of 11% for MRSA, followed by two Gram negative, *S. enteritidis* (9%) and *E. coli* (7%) and fungus *C. albicans* (6%).

Pseudomonas aeruginosa did not show any inhibition in any medium in the presence of EPS. It is important to note that as the concentration of EPS decreases the percentage of inhibition of *S. aureus* increases. 0.5% EPS poorly inhibited S.enteritidis, E.coli and B.cereus.

The results obtained suggest that the EPS of *Porphyridium cruentum* has potential to be used as a natural bacteriostatic.

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Keywords: P. cruentum, EPS, antimicrobial, bioactivity

P-204 - IN VITRO ANTIMICROBIAL ACTIVITY OF PLANTS EXTRACT OBTAINED BY COLD MACERATION AND DECOCTION AGAINST STAPHYLOCOCCUS AUREUS ISOLATED BOVINE MASTITIS

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Background

Staphylococcus aureus is one of the main pathogens causing bovine mastitis. Usually this intramammary infection is treated with conventional antimicrobials, however, due to the increasing number of multi-resistant microorganisms worldwide, therapeutic failures have been reported frequently. In contrast, natural substances such as extracts obtained from plants are being increasingly studied as alternative in the treatment of these i*nfections*. Thus, the aim of the present study was to evaluate the activity of natural extracts against *S. aureus* isolated from animals with bovine mastitis.

Method

Extract activities were evaluated by the disk diffusion test and agar-well diffusion test, according to CLSI guidelines. Aqueous extracts of *Dysphania ambrosioides*, *Pachyrrhizus tuberosus*, *Piper aduncun* and *Piper medium and Dieffenbachia pictada* were obtained by cold maceration and by decoction. S. *aureus* analyzed were recovered from the animals with bovine mastitis.

Results & Conclusions

Two strains *S. aureus* were evaluated. In relation to the first group, according to the well diffusion test performed with the extract obtained by cold maceration, there is no halo formation of inhibition of growth for any extract evaluated. However, evaluating the extract obtained by decoction, it was possible to observe that the *Piper medium* showed a good inhibitory activity against this microorganism, with inhibition halo ranging from 18 to 21mm. Regarding the diffusion disk test, all the extracts obtained by decoction showed regular inhibitory activity. Regarding the second group evaluated, by diffusion disk test, *Dysphania ambrosioides* and *Pachyrrhizus tuberosus* presented antimicrobial activity in both extractions; however, *Piper aduncum* was effective only when the extraction by decoction. Thus, although more studies on these plants are necessary, it can be concluded that the extract obtained by decoction presented greater antimicrobial activity than the extract obtained by cold maceration, and that the plants of the family *Piperaceae* had good antimicrobial activity and could be used in the treatment of bovine mastitis caused by *S. aureus*.

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Keywords: natural extract, antimicrobial activity, Staphylococcus aureus, bovine mastitis

P-205 - EXPLORING GENETIC AND PHENOTYPIC BIODIVERSITY OF NON-SACCHAROMYCES YEASTS FROM PORT WINE

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Background

Non-Saccharomyces (NSAC) yeasts, particularly abundant at first phase of alcoholic fermentation, have assumed increasing importance in oenology due to their potential to increase organoleptic characteristics of wine enhancing aroma profile. NSAC yeasts are particularly relevant for the production of Port wine since in this type of wine fermentation is stopped prematurely through the process of must fortification. This work aimed to isolate, identify and characterize the population of NSAC yeasts present in spontaneous fermentations of Port wine. Our final goal is to identify NSAC yeasts with biotechnological potential to be used in the vinification of Port wine.

Method

The population of NSAC yeasts isolated from different industrial-scale spontaneous fermentations of Port wine, cryopreserved from 2012-2016, was identified by PCR analysis of 5.8S-ITS region[1] followed by confirmation of specie by PCR analysis with primers designed specifically for each specie. RAPD-PCR[2,3] enabled to distinguish strains within species. NSAC yeast selected from most representative strain groups were submitted to a phenotypic screening for relevant oenological conditions associated with stress factors in the must, namely, resistance to different concentrations of ethanol, pH, SO_2, osmotic stress, different nitrogen and carbon sources and temperatures. Acetic acid production was also quantified for the selected strains.

Results & Conclusions

Of the ~500 NSAC yeasts isolated based on their morphology, from the spontaneous fermentations of Port wine, were identified 8 different species (*Hanseniaspora uvarum, Metschnikowia pulcherrima, Kluyveromyces thermotolerans, Issatchenkia orientalis, Torulaspora delbrueckii, Rhodotorula mucilaginosa, Issatchenkia occidentalis e Hanseniaspora osmophila*). Interestingly, the clonal characterization of strains evidenced a wide diversity of strains within each specie. Phenotypic screening reveled that strains within the same specie showed different levels of tolerance to the stress factors tested. These results able the selection of native NSAC yeasts with higher potential to be tested in spontaneous fermentations of Port wine. Successful selection and application of NSAC yeasts in future vinifications of Port wine will simultaneously highlight the potential of native NSAC yeasts as key factors for preserving Port wine terroir and ensure a better control of the fermentation process in the cellars.

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This study was financially supported by EU in the frame of the Portugal 2020-(SI I&DT) programme. POCI-01-0247-FEDER-017736

Keywords: Non-Saccharomyces yeasts, Port Wine, Genetic characterization, Phenotypic screening

P-206 - GENOTYPIC AND PHENOTYPIC CHARACTERIZATION OF PIG CARCASS SALMONELLA FROM A PORTUGUESE SLAUGHTERHOUSE

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Background

Salmonella is a major pathogen worldwide, accountable for 94,625 confirmed cases of illness in the European Union (EU) in 2015[1]. The majority of human salmonellosis cases are foodborne, associated with animal or human fecal contamination of foodstuffs.

Pork and thereof products have been identified as a relevant source of human salmonellosis cases in strong-evidence outbreaks in the EU [1]. The slaughtering process can represent the last step for decreasing *Salmonella* contamination in fresh pork.

As this genus may be an important vehicle of virulence and resistance determinants, in this work we characterized the presence of these traits in *Salmonella* isolates obtained from surface swabs of pig carcasses in a Portuguese slaughterhouse.

Method

Forty-four *Salmonella* isolates were obtained and serotyped. Detection of virulence genes associated with plasmid (*spv*C), invasion (*inv*A, *inv*H, *sop*B), enterotoxin (*stn*), cytolysin (*sly*A), survival within macrophages (*pho*P, *pho*Q) and fimbriae formation (*agf*A, *pef*A) was carried out by PCR as previously described[2]. *Salmonella enterica* subsp. *enterica* CECT443 and CECT722 were used as positive controls. Antimicrobial resistance to compounds including quinolones, cephalosporins, beta-lactams, tetracyclines, sulphonamides and amynoglicosides, commonly used in veterinary and/or human medicine, was determined by the disk diffusion method, following CLSI guidelines for veterinary susceptibility testing.

Results & Conclusions

The *agfA*, *invA*, *invH*, *phoP*, *phoQ*, *slyA*, *sopB* and *stn* genes were detected in all (44/44) the *Salmonella* isolates. Oppositely, none (0/44) of the isolates harbored *pefA* or *spvC* genes.

No resistances were recorded for Cephalexin or Cephotaxime, and the majority of isolates were susceptible to Amoxicillin/clavulanic acid, Chloramphenicol, Gentamicin and Nalidixic acid. Resistance to Penicillin was observed in all isolates (44/44), followed by Tetracycline (36/44) and Streptomycin (34/44). Almost every (41/44) isolates were resistant to 2 or more antimicrobials, frequently concurrently to Ampicillin, Penicillin, Streptomycin, Trimethoprim/sulfamethoxazole and Tetracycline (23/41). The results point out the high frequency of virulence genes and antimicrobial resistance profiles in *Salmonella* isolates from pig carcasses, emphasizing the role of pork in foodborne cases of human salmonellosis.

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This work was supported by the Centre for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon (FMV/UL) (Project UID/CVT/00276/2013).

Keywords: Salmonella, Virulence, Resistance, Pig carcass

P-207 - PREVALENCE OF VIBRIO PARAHAEMOLYTICUS IN MARKETED SHELLFISH IN PORTUGAL

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Background

Vibrio parahaemolyticus is a marine bacterium recognized as human pathogen, and frequently associated with foodborne diseases worldwide, which are on the rise due to the increasing demand for shellfish products. Portugal, a coastal country has a tradition of shellfish consumption eaten raw or lighted cook. The aim of this study was to evaluate the prevalence of *V. parahaemolyticus* in live bivalve mollusks available for consumption on Portuguese retail outlets, to assess the potential pathogenic risk for consumers.

Method

Vibrio occurrence and abundance were examined in different marketed bivalve species, using the most probable number-polymerase chain reaction (MPN-PCR) approach.

Results & Conclusions

Vibrio sp. were successfully detected in all the studied samples (n = 45), and 65% were positive for *V. parahaemolyticus* presence (*ToxR*), with abundance ranging from > 3 to up 10^6 MPN g^{-1}. Moreover, pathogenicity associated genes, thermostable direct hemolysin (*tdh*), and thermostable direct hemolysin-related hemolysin (*trh*), were observed in 20% and 13% of the samples, respectively. Higher *V. parahaemolyticus* abundances were found in *Cerastoderme edulis* from aquaculture origin. In addition, characterization of vibrio isolates, with special attention to genotypes associate with pathogenicity and antibiotic resistance, was performed. The resistance patterns to 16 antibiotics exhibited a wide variability, with isolates from the same Vibrio species showing distinct resistance profiles. Resistance to at least two antibiotics was detected in all the isolates, with additional intermediate resistances. Resistance to 3 or more antibiotics was detected in several isolates. This is the first report on *V. parahaemolyticus* prevalence in marketed shellfish in Portugal, and our results highlight the need to include in the routine shellfish examination the surveillance of this bacterium that can carry a potential health risk to consumers.

References & Acknowledgments

This research was partially supported by the Project INNOVMAR (Reference NORTE-01-0145-FEDER-000035), Research Line INSEAFOOD, supported by the Northern Portugal Regional Operational Programme (NORTE2020), through the European Regional Development Fund (ERDF). and the Strategic Funding UID/Multi/04423/2013 through national funds provided by FCT and ERDF, in the framework of the programme PT2020.

Keywords: Vibrio parahaemolyticus, Shellfish, Food Safety

P-208 - ASSESSMENT OF FT-IR RESOLUTION TO DISCRIMINATE L. MONOCYTOGENES CELLS EXPOSED TO TEMPERATURES LINKED TO FOOD PROCESSING CHAIN

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Background

Fourier Transform Infrared (FT-IR) spectroscopy is a high-throughput technique, providing a fingerprint of the biochemical composition (nucleic acids/polysaccharides/proteins/fatty acids) of a bacterial cell [1-2]. It offers a wide range of applications, including monitoring changes occurring in response to food-related stress conditions [1-2]. Very few studies using a reduced number of conditions analyzed temperature effect on cellular components of important food-borne pathogens, such as *Listeria monocytogenes,* by FT-IR [1-3]. This study used FT-IR and chemometrics to assess changes in *L. monocytogenes* exposed to a range of cold and high temperatures/different times used in the food processing chain.

Method

Two *L. monocytogenes* strains belonging to the clinically-relevant serotypes 4b (herring with spices/Romania/2013) and 1/2a (hot dog/Portugal/2013) were tested. Isolates were grown on TSBYE (30^{0}C/150 rpm/20h) to reach stationary phase. Aliquots (1ml) of three independent cultures were tested at different heat temperatures/time combinations (100°C-15minutes; 60°C-43minutes; 72°C-30seconds) and cold temperatures/time combinations (4°C-24/48hours; 10°C-60minutes) to mimic environmental conditions during food processing (e.g. pasteurization, cold-storage). Broth cultures of treated/non-treated cells were centrifuged (5min/5000rpm) and washed (0,9% saline solution-3x). The homogenized pellet with residual liquid (3ul) was transferred to the ATR crystal and air-dried. Spectra were acquired from 4000-400cm^{-1} (6 replicates/resolution of 4cm^{-1}/32 scan co-additions), pre-processed (standard normal variate/Savitzky-Golay filter/mean centred) and analysed by unsupervised (Principal Component Analysis-PCA) and supervised methods (Partial Least Square Discriminant Analysis-PLSDA) using Matlab version 6.5 release 13 and the PLS Toolbox version 3.5 for Matlab.

Results & Conclusions

In both strains, a PCA and a PLSDA model including all spectra clearly distinguished heat from non-treated *L. monocytogenes* cells. The cells treated at the three different heat temperatures/times tested were further discriminated in a second PLSDA model, linked to specific combinations of variable bands in amide I and II of proteins (1500-1700cm^{-1}) and the mixed region of fatty acid/proteins/phosphates (1200-1500cm^{-1}), suggesting the involvement of different metabolic pathways. On the other hand, no differences were encountered between cells exposed or not to cold temperatures/times, indicating minor changes in *L. monocytogenes* strains. This study provided evidences that FT-IR is a fast and reliable method to distinguish cells submitted to different heat stresses. Its application to evaluate bacteria behaviour under other food processing methods, including recent new ones, could also be of interest.

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This work was supported by FCT/MCTES (ID:ERA-IB-16-014)

Keywords: FT-IR, spectroscopy, temperature, Listeria monocytogenes

P-209 - STUDIES WITH VIBRIO ALGINOLYTICUS OMPK TOWARDS THE DEVELOPMENT OF A VACCINE FOR SOLEA SENEGALENSIS

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Background

Vibriosis is one of the most prevalent fish diseases caused by bacteria belonging to the genus *Vibrio*. *Vibrio alginolyticus*, a Gram-negative bacterium, is one of the *Vibrio* pathogens common to human and marine animals. Bacterial fish infections affect the productivity of the aquaculture farms and force the use of antibiotics that can later be present in the fish product. Recombinant vaccines produced in bacteria would allow a higher productivity at a lower cost than the use of antibiotics. The outer membrane proteins (OMPs) from *Vibrio* species can induce protective immunity. OmpK of *V. alginolyticus* was shown to contain a high number of conserved regions in its sequence and high similarity with OmpK from other *Vibrio* species. OmpK characteristics make it a viable target for fish vaccination.

Method

For the production of OmpK, two strategies were designed: production of a recombinant protein without both the signal peptide and a his-tag (OmpK1); production of a protein bearing both its native signal peptide and a C-terminal his-tag (OmpK2). These strategies led to the genetic construction of the recombinant plasmids pet21a-Ompk1 and pet23a+-OmpK2. The recombinant plasmids were used to transform *E. coli* BL21 and recombinant proteins were produced after IPTG induction. Both proteins were found to be overproduced as inclusion bodies (IBs) whose recovery relied on centrifugation and washing.

Results & Conclusions

The native signal peptide included in pet23a+-OmpK2 did not enable protein translocation to the periplasm of *E. coli*. The r-OmpK2 was recovered from IBs, followed by solubilisation and purification/renaturation by metal-ion affinity chromatography (IMAC) using Ni-NTA resins and subsequent dialysis.

As r-OmpK1 was designed to lack his-tag and be produced as IBs, after IB recovery and protein solubilisation, renaturation and purification was based on dialysis. In the case of the OmpK1, the lack of the affinity tag impaired its complete purification and fully refolding. In spite of not being pure the amount of OmpK1 recovered is much higher than the amount of impurities allowing its use in ELISA methods. The semi-pure protein yield is 0.11 $g_{OmpK1}/g_{dry\ weight}$. In the case of OmpK2, the protein purity was close to 100% (SDS-PAGE analysis) but still with sub-optimal refolding efficiency leading to a final yield of 0.90 $mg_{OmpK2}/g_{dry\ weight}$.

Due to its high purity, OmpK2 will be used for fish vaccination trials. Currently, a strategy to obtain pure and refolded OmpK2 is being optimized to increase the process yield.

Keywords: aquaculture, fish vaccine, recombinant proteins, immunological analyses, protein purification

P-210 - IMPACT OF STORAGE TEMPERATURE ABUSE ON THE MICROBIAL QUALITY AND SAFETY OF FRESH-CUT VEGETABLES AND FRUITS

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Background
Pre-packed fresh-cut fruits and vegetables satisfy the growing consumers demand for more convenient food. However, these products are highly perishable with short storage life under refrigeration temperatures below 5 ºC. Several foodborne outbreaks linked to fruits and vegetables had been reported annually in the EU [1]. The impact of storage temperature abuse on the microbial quality and safety of fresh-cut fruits and vegetables was evaluated.

Method
Packages of pre-packed fresh-cut vegetables (shredded carrot and cress) and three pre-packed fresh-cut fruits (melon, melon-coconut, and pineapple-mango salads) from the same production lot were collected from retail. One package was immediately analysed, while three packages were stored until the last day of the expiration date at 4, 8 or 12 ºC. For the shredded carrot and cress samples, three additional packages were open and closed by folding the package, to mimic what a consumer could do at home, and stored until the last day of the expiration date at 4, 8 or 12 ºC. Microbial analysis were performed according to the procedures of International Organization for Standardization (ISO) [2] for enumeration of mesophilic aerobic counts (TVC), *Enterobacteriaceae*, *Escherichia coli*, coagulase-positive staphylococci, Lactic Acid Bacteria (LAB), and yeast and molds), and detection of *Salmonella* spp. and *Listeria monocytogenes*. pH values were measured for each sample.

Results & Conclusions
Samples analysed immediately after purchase presented TVC levels between 6.9 – 8.9 Log CFU/g, *Enterobacteriaceae* between 4.3 – 6.3 Log CFU/g, LAB between 3 - 9 Log CFU/g, yeasts between 3.5 – 5.3 Log CFU/g, molds between < 1.0 – 4.3 Log CFU/g. *Escherichia coli* and coagulase-positive staphylococci were absent (< $1.0x10^1$CFU/g) in all samples. Fresh-cut melon was positive for *Salmonella* spp., and the pineapple-mango salad was positive for *L. monocytogenes*. In general, stored packages at 4, 8 and 12 ºC until de expiration date presented higher counts of *Enterobacteriaceae*. Cress sample (closed packaged) stored at 12 ºC was positive for *L. monocytogenes* after 3 days of storage. Otherwise no differences were found among storage at different temperatures.

The presence of pathogens is worrisome and overall results indicate the need of implementing rules in the production and cold chains of these products to ensure food safety and quality.

References & Acknowledgments
This work was supported by National Funds from FCT - Fundação para a Ciência e a Tecnologia through project 'UID/Multi/50016/2013' and SafeConsumE – European Union Horizon2020 Grant Agreement No 727580.

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Keywords: Pre-packed, fresh-cut, fruits, salads, Food safety, consumer, pathogens

P-211 - MICROBIOLOGICAL QUALITY OF READY-TO-EAT FRUITS AND VEGETABLES PURCHASED AT RETAIL IN PORTUGAL

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Background
Global production, distribution and consumption of fresh produce has increased since consumers today are more aware of its importance on providing micronutrients and fiber. Ready-to-eat (RTE) fruits and vegetables are widely available at retail, and are eaten raw, usually without additional washing procedures (1). Thus, pathogen contamination may represent a serious risk to consumers. The microbiological quality and safety of RTE fruits (n=10) and vegetables (15) collected at four supermarkets from the city of Porto was evaluated in this study.

Method
For each sample, the microbiological parameters were tested accordingly to ISO standards, including enumeration of mesophilic bacteria (ISO 4833-1:2013), total coliforms (ISO 4832:2006), moulds and yeasts (ISO 21527-1:2008), *Escherichia coli* (ISO 16649-2:2001) and coagulase-positive *Staphylococcus* (ISO 6888-2:1999). The detection of foodborne pathogens *Listeria monocytogenes* (ISO 11290-1:1996) and *Salmonella* (ISO 6579:2002) was also tested.

Results & Conclusions
Accordingly to the guidelines applied in Portugal (2), several samples showed poor microbiological quality (mesophilic bacteria – 76% unsatisfactory; 20% borderline; yeasts – 44% unsatisfactory; 52% borderline; moulds – 32% unsatisfactory; 56% borderline) and hygiene indicators microorganisms also exhibited high counts (coliforms – 68% unsatisfactory; 8% borderline; *E. coli* – 8% unsatisfactory). High counts of *E. coli* were obtained in two RTE salad samples. Additionally, *L. monocytogenes* was found in one package of water cress. The screened samples showed no positive results for presence of *Salmonella*. *Staphylococcus* coagulase positive counts were classified as satisfactory in all 25 samples.

The high results obtained in quality indicator organisms tend to limit the product's shelf-life once it may accelerate spoilage due to acid and gas production (3,4). From the 25 samples, only one was found satisfactory for all the microbiological parameters analysed. Detection of *L. monocytogenes* represents a safety concern.

References & Acknowledgments
This work was supported by National Funds from FCT - Fundação para a Ciência e a Tecnologia through project 'UID/Multi/50016/2013'and by SafeConsumE – European Union Horizon2020 Grant Agreement No 727580.

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Keywords: Food Safety, Consumer, Ready-to-Eat, Microbiological Quality

P-212 - THERMAL RESISTANCE OF SALMONELLA TYPHIMURIUM INOCULATED IN A SWEET PASTRY FOOD MATRIX

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Background

Salmonella Typhimurium is one of the major *Salmonella* serotypes associated with food-borne outbreaks. The microorganism adaptability to adverse conditions motivates the carrying out of studies for its control (Doyle & Buchanan, 2012).

The aims tof this study is to evaluate the thermal resistance of *Salmonella* Typhimurium by determining the D and z values in a sweet pastry food matrix, "Cavacas de Resende" liquid mass, consisting of 8 whole eggs, 7 egg yolks, 280g of flour and 250g of sugar.

Method

Salmonella Typhimurium ATCC 14028 (Oxoid C6000L) was inoculated into "Cavacas de Resende" liquid mass at concentration of 1.33×10^8 cfu/g. The determination of D and z values were carried using 5±0.1g (triplicate) of liquid mass vacuum-packed, applying a water bath, following different heat treatments: 52°C (45, 135, 180 and 225 min.); 55°C (15, 30, 45, 60, 70 and 80 min.); 58°C (5, 10, 15, 20, 25 and 30 min.); 61°C (1, 2, 3, 4 and 5 min). The D value was calculated at each temperature by the absolute value of the inverse slope regression line between the log10 surviving microorganisms and the heat treatment time. The z value was calculated by the absolute value of the inverse slope regression line between the log10 D values and the heat treatment time.

Results & Conclusions

The a_w of the food matrix prior to inoculation was 0.94 with pH of 7.26. The D values obtained were 53.19min, 20.45min, 6.95min and 1.60min, at 52°C, 55°C, 58°C and 61°C, respectively. From the calculated D values, the corresponding z value was 5.96°C. Channaiah *et al*. (2017) obtained for a cocktail of *Salmonella* Newport, *Salmonella* Typhimurium and *Salmonella* Senftenberg in Muffins, D values of 62.16±2.99 min, 40.09±0.88 min, 16.46±1.71 min, for 55, 58 and 61ºC respectively with z value of 10.40±0.63ºC, higher than values obtained in this study. It is expected that the reduction of *Salmonella* is being effective in baked products, however the different food matrices may hinder the inactivation of *Salmonella*, since the temperatures and adequate times are not reached, especially in the product thermal center, emphasizing the necessity to implement safety measures, especially in products whose thermal treatment is not sufficient for the inactivation of the microorganism.

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Keywords: Salmonella, Food safety, D value, z value

P-213 - GENOME-WIDE IDENTIFICATION OF SACCHAROMYCES CEREVISIAE GENES INVOLVED IN THE MODULATION OF TOLERANCE TO THE CONJUGATED EFFECT OF ETHANOL AND ACETIC ACID AT LOW PH AND HIGH TEMPERATURE

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Background

The understanding of the complex molecular mechanisms underlying *Saccharomyces cerevisiae* tolerance to multiple-stresses occurring during bioethanol production from agricultural and forest residues is essential to improve the robustness of industrial yeast strains. Fermentation arrest caused by acetic acid and ethanol accumulation at low pH and supra-optimal temperatures has a negative impact in ethanol productivity and bioprocess economic viability. Previous chemogenomic studies have identified genes implicated in *S. cerevisiae* resistance to single stresses(e.g. acetic acid[1],ethanol[2] or high temperatures[3])based on the susceptibility of single deletion mutants. It is likely that the analysis of the conjugated effect of multiple stresses may reveal differences from what can be anticipated based on the datasets emerging from the action of single stresses. Also, it became recently clear that there are genes that, despite being strong determinants, for example, of tolerance to acetic acid, can be deleterious when expressed under formic acid stress[4]. The present study was performed to get insights into the genes/signalling pathways involved in the modulation of tolerance to multiple-stresses in different media.

Method

The identification of non-essential genes whose deletion is responsible for increased sensitivity/resistance to the simultaneous presence of acetic acid(15-25mM) and ethanol(4.5-5.5%(v/v)) at pH4.5 and 35°C in MM4 and YPD agar media, respectively, with/without an additional K+ concentration(20mM), was performed using the Euroscarf haploid deletion mutant collection. Mutants that exhibited deficient or absent cell growth/ improved growth in agar plates supplemented with acetic acid and ethanol while having a similar wild type growth in control plates were considered as susceptibility/resistance phenotypes.

Results & Conclusions

This chemogenomic analysis allowed the identification of 507 mutants more susceptible and 26 more tolerant than the parental strain to the simultaneous presence of acetic acid and ethanol at pH4.5 and 35ºC. Clustering of genes required for maximum tolerance to these stresses, based on their biological function, indicates an enrichment of those involved in carbohydrate metabolism, chromatin remodelling, protein modification(acetylation/deacetylation), vacuolar/lysosomal transport, and proton homeostasis. The few susceptibility genes identified are related with lipid/fatty acid metabolism and transcriptional control. The set of tolerance genes related with mitochondrial morphogenesis and replication/maintenance of the mitochondrial genome and lipid metabolism that did not emerge from previous single stress chemogenomic datasets, deserves further attention as potential genetic determinants of robustness against multi-stresses of industrial relevance.

References & Acknowledgments

This work was funded by FCT project ERA-IB-2/0003/2015,POR Lisboa(Project N. 007317) and FCT(UID/BIO/04565/2013). LMM is a FCT Doctoral program AEM student(PD/BD/128035/2016).

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Keywords: Chemogenomic analysis, S. cerevisiae, Acetic acid, Ethanol

P-214 - ANTIFUNGAL EFFECT OF ORGANIC ACIDS ON ASPERGILLUS FLAVUS AND PENICILLIUM NORDICUM

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Background
The control of fungal contaminations is of great importance, since moulds are responsible for the loss of approx. 10% of the world's food production. Additionally, certain fungi produce highly toxic compounds known as mycotoxins.

Biological methods are being considered to combat fungal contamination, meeting consumers' demands for healthier and safer food. Lactic acid bacteria (LAB) are interesting for biopreservation because of their combined antifungal and probiotic properties. Antifungal ability of LAB is mainly associated to the production of organic acids (OA). Concerning mycotoxins, there are insufficient works demonstrating the influence of these acids in their production.

In this perspective, several OA produced by LAB were tested against two major mycotoxin-producing fungi: *Aspergillus flavus* and *Penicillium nordicum*, respectively producers of aflatoxins (AFs) and ochratoxin A (OTA).

Method
Lactic (LA), acetic (AA), propionic (PA), butyric (BA), phenyllactic (PLA), hydroxyphenyllactic (OH-PLA) and indole lactic acids (ILA) were tested by incorporation in MRS medium (0.1-8.0 mg/mL). Petri plates were centre inoculated with a spore suspension and incubated at 25 ºC for 7 days. Fungal diameters were measured daily and mycotoxins analyzed by HPLC at the end. All experiments were conducted in triplicate. Inhibitory concentrations (IC) were calculated.

Results & Conclusions
All tested OA were able to inhibit to some extent the fungal growth and the production of mycotoxins, with strongest effects observed on mycotoxins. Fungal growth was specially affected by BA, PA and AA, with 2 g/L being sufficient to suppress totally the growth of both fungi. However, AA evidenced a reduced effect on mycotoxins inhibition comparing to BA and PA. The least efficient of all acids was LA. Mycotoxin inhibition by BA, PA and AA resulted mainly from its capacity to impair fungal growth, while PLA, ILA and OH-PLA inhibited the mycotoxins production without affecting significantly fungal growth. Overall, higher IC values were obtained for *P. nordicum* than for *A. flavus*. IC_{90} (g/L) for AFs inhibition were: BA(0.25) < PA(0.38) < PLA(0.87) < AA(1.17) < ILA(1.47) < OH-PLA(1.8) < LA(3.92). IC_{90} (g/L) for OTA inhibition were: BA(0.95) < PA(1.53) < AA(1.66) < ILA(3.15) < PLA(4.5) < OH-PLA(13.09) < LA(45.9).

These properties of OA can make LAB a promising solution to reduce mycotoxin levels in food systems. The use of LAB with well-studied anti-mycotoxin properties and high production of most active OA can be an important advantage for food products.

References & Acknowledgments
Ana Guimarães and Luís Abrunhosa received support through grant SFRH/BD/103245/2014 (FCT), and UMINHO/BPD/51/2015 from project UID/BIO/04469/2013 (FCT/MEC), respectively.

Keywords: mycotoxins, organic acids, Aspergillus flavus, Penicillium nordicum

P-215 - BACTERIAL CONSORTIUM AS A TOOL FOR INCREASING EFFICIENCY IN TELLURIUM BIOLEACHING

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Background

The European industry has a demand for high-value metals and this is forcing an increase in mining activity. The issues associated with this reality are leading to a European objective of a sustainable industrial approach based in a circular economy [1]. Tellurium is ever more important for its uses in industries like photovoltaics and medical and its sources are thin and localized. Bioleaching of tellurium from residues (mine or industrial) can be used to complement the tellurium obtained by mining and, at the same time to gap the problems associated with mining runoffs and leachates.This work aims to evaluate the ability of bacterial strains and bacteria consortia to leachate complex ores, with high or low content of tellurium.

Method

Bacterial strains were recovered by inoculating media with biologically active mine tailings and enrichment with tellurite [Te(IV)]. Tellurite resistance on recovered strains was tested by minimal inhibitory concentration (MIC) and the ability to reduce Te(IV) to metallic tellurium [Te(0)] was observed on plates containing up to 3mM of Te(IV).The leaching of a high tellurium containing ore and of a mine tailing (low concentrated ore) was performed with individual strains and with a bacterial consortium under aerobic conditions for 30 days. The leached metal was quantified by ICP-MS.

Results & Conclusions

Three strains, *Bacillus subtilis* ALJ98a, *Paenibacillus taichunguensis* ALJ98b and *Paenibacillus tundrae* ALJ109b, were able to grow in concentrations of up to 10mM of Te(IV), and were able to reduce 60 to 85% of soluble Te(IV) to metallic Te(0).

Leaching tellurium from a mine tailing resulted in solubilisation of 3 to 23% of Te(IV) from the sediment to the leachate. The combined effect of the bacterial consortium was incremental in solubilising telluride from the tailing, increasing solubilisation from 2.42%, the action of the biologically active sediment alone, to 18.57%.

The results indicate that tellurium can be recovered to a high extent from discarded mine tailings by using a sustainable methodology of microbial bioleaching. An efficient strategy should consider, a specific bacterial consortium to be selected depending on the composition of the mine tailing.

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Keywords: Tellurite reduction, Bioleaching

P-216 - SURVIVAL AND ACTIVITY OF BRETTANOMYCES/DEKKERA IN MONOVARIETAL WINES

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Background

Volatile phenols in wines are responsible for unpleasant aromas (horse sweat, leather, clove, barnyard), which affect the wine quality. These compounds are produced from the degradation of hydroxycinnamic acids mainly by the yeasts *Brettanomyces/Dekkea* (Kheir et al., 2013). The aim of this work was to evaluate the survival and activity of *Brettanomyces/Dekkera* in monovarietal wines and to estimate the influence of the grape variety.

Method

Fifteen wines, five of each of the grape varieties Touriga Nacional (TN), Cabernet Sauvignon (CS) and Syrah (S), were inoculated with one strain of *Brettanomyces/Dekkera* previously selected from a group of 18 wine isolated strains. The pH and ethanol concentration of all wines were adjusted to 3.6 and 13% v/v before inoculation. Yeast growth and survival were monitored by viable counting in solid media and by flow cytometry (BD Accuri™ C6) using the fluorescent dyes propidium iodide and Syto™ 9 for discrimination of live and dead cells. The volatile phenols were analyzed by GC-FID.

Results & Conclusions

Yeast populations of 10^7 CFU/mL were dramatically reduced to undetectable numbers (<3000 CFU/mL) in 24h in all wines as revealed by the plate count method. Plate viable counts of 10^4-10^6 CFU/mL were, however, detected after 48h in 4 TN and 3 CS wines. Cell densities of almost 10^7 CFU/mL were reached in the subsequent sampling times until the end of the experiment (432h). CFU counts were generally lower in the Syrah wines, with only two wines showing plate counts of around 10^6 CFU/mL from 96 and 144h onwards. Viability measurement by flow cytometry showed the presence of viable cells at levels of 10^6 cells/mL in populations not detected by the plate count method, suggesting that a significant part of the populations is in a viable-but-not-culturable state (VBNC). Yeasts were able to attain the culturable state in most of the wines but the time required for the recovery was highly dependent on the wine, being longer in the Syrah wines. The metabolic activity (production of volatile phenols) of VNBC cells seemed to be lower than culturable cells, especially in Syrah wines. The VBNC physiological state has been described in wine yeast populations in response to stress factors such as ethanol and SO_2 (Du Toit et al., 2005).

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Acknowledge: CNPq (Brasil) for the financial support

Keywords: Brettanomyces/Dekkera; Wines

P-217 - ARE INDEED MEATS SOLD IN PORTUGAL WITHOUT CLOSTRIDIUM DIFFICILE?

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Background

The incidence and severity of diarrhea associated with *Clostridium difficile* have been increasing exponentially[1]. The *C. difficile* infections (CDI), which were believed to be almost exclusively nosocomial and occurring mainly in immunocompromised and elderly patients, are now becoming increasingly common among low-risk individuals[2]. In 2014, an outbreak with the hypervirulent ribotype 027 strain[3] was firstly reported in Portugal and, among others, this ribotype have been largely isolated from animals and food. Given the bacteria spores nature and their presence in the intestinal tract of animals, it would be expected to found *C. difficile* in several foods. This study aimed to detect and quantify *C. difficile* from different meats sold in traditional commerce and supermarkets in *Porto* and *Lisboa*.

Method

Quantification of *C. difficile* was performed using direct enumeration in *Clostridium difficile* Agar (CLO) and detection was performed using a pre-enrichment of each sample in *Clostridium difficile* Moxalactam Norfloxacin (CDMN) broth and a pre-treatment with ethanol before inoculation on the recovery culture media CDMN agar.

Results & Conclusions

No *C. difficile* was found in any of the 143 samples analyzed. These results should not be ignored, since it is not possible to be sure if the meats analyzed, in fact, did not have *C. difficile* or if the methods used were not sensitive enough to detect its presence. In the absence of standardized methodologies, further studies using other detection/quantification methods of *C. difficile* should be performed not only on meats, but also on other types of foods, including ready-to-eat foods. Despite no confirmed cases of foodborne diseases caused by *C. difficile*, the increased CDI incidence suggest that contaminated foods could/may be contributing to community- and hospital-associated CDIs.

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This work was supported by National Funds from the *Fundação para a Ciência e a Tecnologia* (FCT) through project UID/Multi/50016/2013. Financial support for author J. Barbosa was provided by a post-doctoral fellowship SFRH/BPD/113303/2015 (FCT).

Keywords: Clostridium difficile, detection, enumeration, meat

P-218 - SCREENING OF BACTERIOCINOGENIC LACTIC ACID BACTERIA CULTURES AND THEIR CHARACTERIZATION AS POTENTIAL PROBIOTICS

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Background

Ingestion of probiotic lactic acid bacteria (LAB) has been pointed out to confer a range of health benefits, including the immune system stimulation, reduction of infectious illness, among others. Food products containing probiotics should follow the guidelines established by FAO/WHO (2001). In general, probiotics have to be harmless to the host, they cannot be pathogenic, mutagenic or carcinogenic, allergenic or provoke any type of adverse reactions. The aim of this study was to select different bacteriocinogenic LAB isolated from different food products and study some of their probiotic characteristics in terms of safety, functional and physiological properties.

Method

Two hundred and eighty LAB isolates were screened for the antimicrobial activity against several microorganisms. The bacteriocinogenic LAB cultures were identified by 16 sRNA. The presence of some virulence factors, antibiotic resistances, resistance to simulated gastrointestinal tract conditions and ability to adhere to human colon adenocarcinoma cell lines Caco-2 were investigated.

Results & Conclusions

From the screening of antimicrobial activity, only seven out of the 280 LAB isolates were selected due to bacteriocin production. Six *Pediococcus pentosaceus* and one *Lactobacillus plantarum* were identified by 16S rRNA gene sequence analysis. None of the isolates showed the presence of virulence factors and none antibiotic resistances were detected. With the exception of *L. plantarum* R23, all the isolates were not inhibited through the simulated gastrointestinal tract conditions. After the simulated gastrointestinal tract passage, only *P. pentosaceus* CFF4 was able to adhere to Caco-2 cells. Even though additional studies should be performed, *P. pentosaceus* CFF4 seems to be a potential probiotic candidate.

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Keywords: Antimicrobial activity, Lactobacillus plantarum, Pediococcus pentosaceus, probiotic

P-219 - A NEW SPECIES OF PENICILLIUM SECTION RAMOSA FROM TUNISIAN APPLES

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Background

One of the limiting factors that influence the economic value of fruit is the short shelf-life caused by fungal deterioration. The symptoms of infection may remain dormant until post-harvest. In a mycotoxin contamination survey of apples from markets in Tunisia, 54 *Penicillium* strains were isolated. However, two isolates could not be assigned to any described species based on morphological and molecular phylogenetic analyses. The aim of this study was the characterisation and description of this new putative species.

Method

For morphological analyses, strains MUM 17.62 and MUM 17.80 were inoculated in triplicate in CYA, YES, G25N, CSN and MEA media and incubated in the dark at 25 ºC for 7 days. CYA plates were also incubated at 30ºC and 37ºC. Colony size was measured and for microscopy analysis, fungi grown in MEA was used. Multilocus sequence analysis (MLSA) was performed through comparison of partial β-tubulin (*benA*), calmodulin (*cmd*) and nuclear ribosomal internal transcribed spacer (*ITS*) region with sequences available in GenBank derived from type strains of *Penicillium* species. All the sequences were aligned and phylogenetic trees were assembled using MEGA.

Results & Conclusions

For MUM 17.62 and MUM 17.80, the colonies growth was very restricted in the different media. No growth was observed on CYA at 30 ºC and 37 ºC. The strains showed slight differences in green colour. Both presented velutionous, sulcate and irregular colonies in MEA. Microscopically, the conidiophores were biverticillate and the conidia ellipsoidal. MLSA revealed that the two strains belonged to *Penicillium* section *Ramosa*. Fingerprinting using the M13 microsatellite showed that the two strains are not clones and analysis of the isoepoxydon dehydrogenase (*idh*) gene revealed that they are not patulin producers. In summary, in terms of multigene phylogeny the two strains are closely related to *P. lenticrescens, P. chroogomphum* and *P. soppii* of the section *Ramosa*. However they well-circumscribe a novel fungal species, *Penicillium tunisinus* sp. nov.

References & Acknowledgments

Keywords: Polyphasic approach, MLSA

P-220 - LACTOBACILLUS PLANTARUM SURVIVES DURING OSMOTIC DEHYDRATION AND STORAGE OF PROBIOTIC CUT APPLE

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Background

Osmotic dehydrated cut apple containing probiotics would be suitable for the growing market of fresh-cut fruits. The application of these intermediate moisture products into other products brings new possibilities to the food industry. The objective of the present study was to investigate if *Lactobacillus plantarum* 299v could be incorporated in apple cubes during osmotic dehydration (OD). In addition, the impact of sucrose and sorbitol as osmotic agents on the incorporation was also studied. The viability of *L. plantarum* in the apple cubes during storage was studied and the viability of the probiotic was also evaluated after a quick simulation of the digestion of probiotic apple cubes through the gastro-intestinal tract.

Method

The effects of 40 and 60 °Brix osmotic solutions of sucrose or sorbitol on the viability of *L. plantarum* 299v during the OD at 37 °C and 1013 or 150 mbar was evaluated; storage at 4 °C and a quick simulation (2 h) of the digestion of the osmotically dehydrated probiotic apple cubes through the gastro-intestinal tract were also performed and the viability of the probiotic was evaluated (Emser et al., 2017).

Results & Conclusions

Lactobacillus plantarum 299v (10^7 – 10^8 cfu/g) was successfully incorporated in apple cubes during the OD process (24 h) at 37 °C and normal atmosphere, as well as in vacuum, using 40 and 60 °Brix sucrose or sorbitol solutions. Osmotic solutions with lower soluble solids content seemed more adequate for the incorporation. Both sucrose and sorbitol proved to be suitable as osmotic agents.

Lactobacillus plantarum, incorporated in apple cubes, survived over a storage period of 6 days at 4 °C maintaining constant values of 10^7 cfu g^{-1}. In addition, the viability of *L. plantarum* did not decrease during a quick simulation of the passage of the apple cubes through the gastro-intestinal tract (2 h), which is essential for the beneficial effect of a probiotic.

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This work was supported by National Funds from FCT – Fundação para a Ciência e Tecnologia through project UID/Multi/50016/2013 and by the European M.Sc. in Food Science, Technology and Business (BiFTec). J. Barbosa acknowledges the support provided by the post-doctoral fellowship SFRH/BPD/113303/2015 (FCT). The authors also acknowledge Campotec for graciously supplying the apples for this study.

Keywords: Osmotic dehydration, probiotic, Lactobacillus plantarum, sorbitol, prebiotic

P-221 - EFFECT OF SALINITY ON THE PRODUCTION OF SULFATED POLYSACCHARIDES BY THE MICROALGAE PORPHYRIDIUM CRUENTUM

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Background

Marine microalgae are unicellular photosynthetic microorganisms that have gained commercial interest since they are easily grown and contain bioactive compounds. *Porphyridium cruentum* is a marine microalga with a great interest due to the production of high quantity of extracellular sulfated polysaccharides, which have several biological properties, such as immunomodulating, anti-inflammatory, and antioxidant [1]. These properties have been associated to the existence of characteristic structural features, including the presence of sulfate esters [1,2]. As the polysaccharides content and composition may change according to the environmental conditions of growth, the aim of this study was to evaluate the polysaccharides produced by *P. cruentum* grown in different salinities.

Method

Three different salinities (18, 32, 50 g NaCl/L) were used to grow *P. cruentum* (culture collection of Necton, S.A). The polysaccharides were obtained by centrifugation followed by ultrafiltration (cut-off of 10 kDa). Neutral sugars were determined by gas chromatography (GC) as alditol acetates and glycosidic linkages were determined by methylation analysis [3].

Results & Conclusions

The extracellular media of the microalgae grown in 18 and 32 g NaCl/L showed higher content of polymeric material (116-154 mg/L) and, accordingly, also extracellular polysaccharides (60-70 %). The overall sugars composition of *P. cruentum* was similar in all samples, being the main sugars Xyl, Gal, uronic acids, and Glc. Also, the content of sulfate esters was identical in the three samples (11-15%, w/w). The analysis of the glycosidic substitution revealed the presence of (1→3) e (1→4)-Xyl, (1→3,4)-Gal, (1→2,3,4)-Gal, (1→3)Glc, and (1→3,6)-Glc, the typical linkages described for the exopolysaccharides from *P. cruentum* [3].

The scale-up of the *P. cruentum* production revealed a similar content and composition in sulfated polysaccharides. Then, this study showed the potentiality of *P. cruentum* as a source of these bioactive compounds, which is independent of the main variations of the medium salinity, being the lowest concentration the most profitable.

References & Acknowledgments

Thanks are due to FCT through national founds and FEDER, within the PT2020 Partnership Agreement, for funding QOPNA (FCT UID/QUI/00062/2013) and CICECO-Aveiro Institute of Materials (FCT UID/CTM/50011/2013; POCI-01-0145-FEDER-007679). C.N. (SFRH/BPD/100627/2014) thanks the FCT support. The authors acknowledge Necton for providing the samples.

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Keywords: exopolysaccharides, salinity, microalgae

P-222 - CONFIRMATION OF SALMONELLA SPP., CRONOBACTER SPECIES, CAMPYLOBACTER SPECIES BY THE MALDI BIOTYPER SYSTEM

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1 - Bruker Daltonics

Background
There is increasing demand for regulation of pathogenic bacteria in foods. Producers, manufacturers and distributors require rapid, accurate identification and confirmation of potential food borne contaminants. The MALDI-Biotyper method combines straightforward sample preparation with Matrix Assisted Laser Desorption/Ionization Time of Flight (MALDI-TOF) to rapidly confirm the presence of pathogenic bacteria. A direct smear of an isolated colony, from a wide range of common selective (traditional agars and chromogenic agars) and non-selective agars are acceptable for use with the technology.

Method
The performance of the MALDI-Biotyper as a confirmation method of ISO reference and proprietary methods in the scope of food microbiology was verified. The validation analyzed 3 sets of inclusivity and exclusivity panels; one for *Salmonella*, *Cronobacter*, and *Campylobacter*. Following ISO 16140-6 (DIS, 2017) and AOAC guidelines, each set of inclusivity and exclusivity panels consisted of 150 target isolates and 100 non-target isolates. The following selective culture media were tested: XLD, BGA, Rapid'Salmonella (Bio-Rad), Brilliance Salmonella (ThermoFisher) and ASAP (bioMerieux) for *Salmonella* spp inclusivity and exclusivity panels, ESIA and CCI for the *Cronobacter* spp inclusivity and exclusivity panels, mCCDA, CampyCefex, Rapid'Campyloabcter (Bio-Rad), CampyFood Agar (bioMerieux) in the *Campylobacter* spp inclusivity and exclusivity panels.

Results & Conclusions
All inclusivity isolates were correctly identified and all exclusivity isolates were correctly excluded. The MALDI-Biotyper also correctly identified all isolates to a genus level for *Salmonella* and *Cronobacter*. For all *Campylobacter* cultures, all isolates were correctly identified to a species level identification.
This new method is an efficient, rapid and reliable alternative to the traditional confirmation methods for the Gram-negative bacteria. The method is able to provide confirmation of presumptive positive results from select agars in seconds rather than hours or days.

References & Acknowledgments
Acknowledgments to Q-Laboratories (OH), USA, the independant laboratory that has generated more than 50% of the data.

Keywords: MALDI-TOF MS, Identification, Confirmation, Salmonella, Cronobacter, Campylobacter

P-223 - FAST AND RELIABLE LISTERIA SPECIES IDENTIFICATION WITH MALDI-TOF MASS SPECTROMETRY

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1 - Bruker Daltonics; 2 - BrukerDaltonics

Background

Selective media are widely used to detect or enumerate *Listeria* spp and *L. monocytogenes,* and a confirmation procedure is required to discriminate the closely related *Listeria* species. Using the same workflow for all micro-organism, MALDI-TOF MS is an attractive alternative for fast and reliable pathogen identification.

Method

The ability of MALDI-TOF MS to confirm *Listeria* spp and *L. monocytogenes* isolates directly from non selective and selective media was evaluated.

210 relevant target and 100 non-target strains, i.e. 100 *L. monocytogenes*, 110 *L. grayi*, 11 *L. innocua*, 12 *L. ivanovii*, 14 *L. seeligeri*, 11 *L. welshimeri* strains, and 100 strains belonging to *Bacillus, Enterococcus, Lactococcus, Leuconoctoc, Lysinibacillus, Staphyloccous, Streptococcus* genus, were isolated on TSYEA, Ottaviani & Agosti Agar, Oxford, MOX, Palcam and Rapid L.mono. Isolates were transferred onto disposable targets and covered with matrix to extract the proteins. Spectra were acquired and analyzed with the MALDI Biotyper, based on MALDI-TOF MS combined with Software, Sub-typing Module and a comprehensive reference library

Results & Conclusions

All the isolates were correctly identified, for all the culture media. No bias was observed. The MALDI Biotyper enables fast and reliable identification of the mostly encountered *Listeria* species.The MALDI Biotyper enables the differentiation of the mostly encountered *Listeria* species in food and environmental samples. There is no influence of the culture media on the identification results, and sub-culture on a non-selective agar is required. Handling time for 95 isolates is approximately 20 min, the time-to-result is less than 45 min.

References & Acknowledgments

Acknowledgments to Q-Laboratories (OH), USA, the independent laboratory that has generated more than 50% of the data

P-224 - EXTENDED SPECTRA DATABASE FOR QUALITY INDICATORS AND OTHER SPOILERS IDENTIFICATION BY MALDI-TOF: A NEVER-ENDING STORY…

Markus Timke[1]; Daniele Sohier[1]; Rui Rocha[1]; Stephanie Richter[1]; Markus Kostrzewa[1]

1 - Bruker Daltonics

Background

Indicator organisms are employed to reflect the microbiological quality of foods and beverages. They are most often used as a measure of the hygienic or sanitary conditions, but they are as well used to describe an organism whose presence or level indicates the potential for future spoilage or as a "surrogate" organism; for example, those organisms that model the behavior of pathogens under certain conditions. In addition, some "indicators" are not specific organisms but assays for groups of organisms, such as Enterobacteriaceae (EB).

Method

Opening lines to develop the database are to focus on groups of micro-organisms, one after another. This helps in creating a road map, starting with the most commonly encountered then also moving to spoiler species linked to more specific industrial processes or formulations.
In that step-by-step database implementation program, isolates from dairy, meat and malt fermentation processes and strains collections were characterized using both 16S rDNA sequencing and MALDI-TOF Mass Spectrometry. A minimum of 20 Mass Spectra were obtained for each isolates to set up a Reference Spectrum. Ideally, whenever possible, three different isolates or more were analyzed per species.

Results & Conclusions

The global database contains currently 104 species of lactic acid bacteria, species of 155 Enterobacteriaceae, 396 species of the main spore-forming bacteria spoilers including Alicyclobacilliaceae, Bacilliaceae, Paenibacilliaceae and Clostridia, and as well 193 yeasts. Blind isolates were analyzed and were accurately identified and distinguished from over 7311 other microorganisms present in the overall database.

The integration of MALDI-TOF MS combined to a fit-for-purpose database into existing food and beverage production processes helps to improve quality assurance practices, providing accurate identification and short-time to result. This high-throughput routine platform is as well a promising technology to identify quickly the origins of product spoilage, or to develop a strategy for quality indicators profile's and other spoiler's surveillance.

Keywords: MALDI-TOF, Identification, Listeria, Listeria monocytogenes

P-225 - INTERSPECIES INTERACTIONS AND BACTERIOPHAGE CONTROL IN S. ENTERITIDIS AND E. COLI MIXED BIOFILMS

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Background

Salmonella Enteritidis and *Escherichia coli* are important foodborne pathogens, commonly related to outbreaks[1].Their ability to form biofilms contributes to their virulence and enables their survival on different food contact surfaces[2].In mixed biofilms, interspecies interactions can occur, resulting in positive,negative or neutral outcomes to each species[3,4].As these microbial communities present great resistance to antimicrobials used in food industries (disinfectants,biocide,antimicrobials), bacteriophages, bacterial viruses, can be regarded as good and safe candidates for biofilm biocontrol[5].This study aimed at characterizing the interactions established between two *S.*Enteritidis and two *E.coli* strains, previously reported to be strong or weak biofilm producers, respectively, in mixed biofilms.Moreover,the efficacy of a bacteriophage cocktail as a biofilm control agent was assessed.

Method

24h old mono and dual species biofilms, in all possible combinations of weak and strong biofilm producers of *S.*Enteritidis and *E.coli*, were grown under dynamic conditions (120rpm), at 37°C, in 96-well plates.PVP-SE2 and vB_EcoM_CEB1 bacteriophages, which are specific for *S.*Enteritidis and *E.coli*, respectively, were used in the control of these biofilms.For the infection assays, 4, 8 and 24h of treatment were used, and at each time-point, biofilms were disrupted by sonication and the number of viable cells was determined.

Results & Conclusions

Our results showed that the number of viable cells in mono-species biofilms was higher than in dual-species biofilms.Also, there was a tendency for weaker biofilm producers to form stronger biofilms when in the presence of strong biofilm producers, and weaker biofilms when in the presence of weak biofilm producers.Furthermore, the presence of two strong biofilm producers does not benefit any of the species.Regarding the antibiofilm efficacy of the bacteriophage cocktail, the most efficient time of action for both bacteriophages was 8 hours.Overall, higher reductions in bacterial cells numbers were obtained for the *S.*Enteritidis than *E.coli* due to bacteriophage growth characteristics.We suggest that unknown determinants present in *S.*Enteritidis and *E.coli* biofilms formed by strong biofilm producers have a positive influence in the formation of biofilms by weak biofilm producers.Moreover, the bacteriophage cocktail can be used in the control of *S.*Enteritidis and *E.coli* mixed biofilms.

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Financial support is acknowledged to FCT for the strategic funding of UID/BIO/04469/2013 unit and COMPETE 2020(POCI-01-0145-FEDER-006684), RECI/BBB-EBI/0179/2012(FCOMP-01-0124- FEDER-027462) and BioTecNorte operation (NORTE-01-0145-FEDER-000004).Catarina Milho is supported by the FCT fellowship SFRH/BD/94434/2013.

Keywords: Biofilms, Bacteriophage, Biocontrol, Foodborne pathogens

P-226 - DISEASE RESISTANCE INCREASE POTENTIAL AND SUGARCANE CROSS-COLONIZATION BY ENTEROBACTER SP. BAC323

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Background

Plant growth-promoting bacteria assist plant development by different mechanisms, such as biological nitrogen fixation, phosphate solubilization, siderophores production, phytormonium synthesis and biological control of pathogens. *Enterobacter* sp., strain bac323, is an endophytic bacterium, previously isolated from *Anacardium othonianum* Rizz, characterized with potential for phytonutrient synthesis. The aim of this work was to evaluate the disease resistance increase potential of *Enterobacter* sp. bac323 and cross-colonization of sugarcane

Method

Firstly, the antagonism test was performed with combined culture between Enterobacter sp. bac323 and fungi Fusarium sp. Bacteria tension was placed at four cross-shaped equidistant points, 1.0 cm from the edge of the petri dish, containing PDA medium and a 9 mm fungal mycelium disc in the center of the plate. The assay was incubated at 28 ° C ± 2 for 5 days and antagonist activity was determined by measuring fungal growth with the bacterium and comparison with the control containing only the pathogen at the center of the plaque, resulting in a percent inhibition. The interaction between bacterium and sugarcane was tested by root adhesion, in which 2 cm of fragments of roots of Saccharum sp. were disinfected and immersed in bacteria solution (OD: 1.0) for 15 minutes, followed by washing in saline solution (0.85%) for 15 seconds to count the colony forming units (CFU) of weakly adhered cells and minutes for strong adhesion. All experiments were repeated three times in totally casual design.

Results & Conclusions

The results showed that *Enterobacter* sp. bac323 is able to inhibit the growth of *Fusarium* sp., the causal agent of sugarcane pokkah boeng disease, in 54.1% as radial growth inhibition percentage. Also, the adhesion root test had more C.F.U. found with strongly adhesion, that showed a potential to use the *Enterobacter* sp. bac323 as inoculant for sugarcane growth-promoting. Further studies will test *in vitro* bacterial inoculation in sugarcane seedlings to observe the effect in plant growth.

References & Acknowledgments

The authors would like to thank the Goiás Federal Institute of Science Education and Technology Rio Verde Campus – Goiás, Brazil, for the infrastructure and the experimental material. The authors would also like to thank the Goiás Research Foundation (Fundação de Amparo à Pesquisa do Estado de Goiás –FAPEG) The National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico CNPq).

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Keywords: antagonism test, Plant growth-promoting bacteria, interaction between bacterium and sugarcane

P-227 - COMBINED TREATMENT WITH BACTERIOPHAGES AND ANTIBIOTICS AS A STRATEGY TO CONTROL BIOFILM ASSOCIATED INFECTIONS

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Background

Bacterial biofilms are sessile microbial aggregates with unique community properties, showing a high degree of tolerance/resistance to disinfection by chemicals, antibiotics, and to the human immune system. The opportunistic pathogen *Pseudomonas aeruginosa* is one of the most frequent causes of biofilm-associated infections, causing infections extremely difficult to treat. Currently, bacteriophages (phages) that are specific for pathogenic bacteria are becoming a potential solution for the treat such infections.

Method

In this study, *P. aeruginosa* biofilms were formed and subjected to treatment by *P. aeruginosa* phage vB_PaM_EPA1 (EPA1) alone or in combination with antibiotics (gentamicin, ciprofloxacin, meropenem) of different classes. EPA1 was isolated from a wastewater treatment plant effluent. TEM images show that this phage belongs to the *Myoviridae* family. Its genome has a 91.3 kb, a GC content of 49.2% and encodes 178 putative genes from which 147 have no predicted function. Phage and antibiotics with different multiplicity of infection (MOI) and minimal inhibitory concentration (MIC), respectively, were simultaneously or sequentially (phage suspension was added first then antibiotic were added with a delay of 6 hours) added to 48 hours old-biofilms. After 24-hour treatment, bacterial survival was measured by colony forming unit (CFU) counting method.

Results & Conclusions

Results showed that in individual treatments of phage (at MOI 1) and antibiotics (at MIC) generally had significant reductions on the number of viable cells ranging from 0.5 to 3.7 logs. However, when they were sequentially added to the biofilms, a synergistic effect (>8 logs) was detected, namely with phage-gentamicin and phage-ciprofloxacin combinations. In opposition, an antagonistic effect was detected when phage and ciprofloxacin were simultaneously added. Overall, our results show that combination of phages and antibiotics are very effective against *P. aeruginosa* biofilms particularly when they are applied sequentially and this constitutes a good strategy to control biofilm-associated infections.

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Keywords: Bacteriophage, Antibiotic, Biofilm, Pseudomonas aeruginosa, Cocktail, Therapy

P-228 - WINEMETRICS: A NEW APPROACH TO UNVEIL THE "WINE-LIKE AROMA" CHEMICAL FEATURE.

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Background

"The Human being has an excellent ability to detect and discriminate odors but typically has great difficulty in identifying specific odorants". Furthermore, "from a cognitive point of view the mechanism used to judge wines is closer to pattern recognition than descriptive analysis." Therefore, when one wants to reveal the volatile "wine-like feature" pattern recognition techniques are required. Sensomics is one of the most recent "omics", i.e. a holistic perspective of a complex system, which deals with the description of substances originated from microorganism metabolism that are "active" to human senses. Depicting the relevant volatile fraction in wines has been an ongoing task in recent decades to which several research groups have allocated important resources. The most common strategy has been the "target approach" in order to identify the key odorants. That process produced an extensive list of substances that play, at least individually, a role on the perceived quality of the wine. However, the combined effect of volatiles responsible for triggering the mechanism of wine-like perception is less explored. While accepting that chemical reconstruction of the volatile 'sensometabolome' is an important branch of research in this area, our vision is that the reconstruction work should be transferred to "those who know better" i.e. the yeast. The absence of the impression substances feature description constitute an obstacle to define the role of the "aroma quality drivers" on a global market perspective, therefore we will attempt to reconstruct the chemical feature "driven" by the yeast.

Method

The objective of the present work was to perform comparative sensorial and metabolomics analysis with four yeast strains from different origins and/or technological applications (cachaça, wine and laboratory), during a fermentative process, in order to characterize their aroma profile and the ability to produce the "wine-like" aroma. Fermentations were analyzed daily by HS-SPME-GC-MS and submitted to sensory analysis. Multivariate tools such as PCA and PLS-R were used in order to extract the compounds related with the "wine-like" aroma, by fusion of chemical with sensory data.

Results & Conclusions

This approach demonstrates that acetaldehyde; ethyl esters of fatty acids were related with "wine-like" aroma. With PLS-R we were able to develop a model capable to predict "wine-like" with a correlation of 0.8. With this methodology we were capable to create a pipeline that can be used in the future for strains selection which regards the ability to produce compounds related with the "wine-like" aroma.

Keywords: Fermentation, Metabolomics, Flavour

P-229 - CHEESE WHEY AS SUBSTRATE FOR OBTAINING PIGMENTS PRODUCED BY MONASCUS RUBER IN SOLID FERMENTATION

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Background

Monascus species can produce yellow, orange, and red pigments, depending on the employed cultivation conditions. They are classified as natural pigments and can be applied for coloration of meat, fishes, cheese, beer, and pates, besides their use in inks for and dyes for textile, cosmetic, and pharmaceutical industries. The aim of this study was the producing pigments by *Monascus ruber* CCT 3802, using the cheese whey as substrate.

Method

The whey cheese was added to PDA and to measure radial growth rates we inoculated the center of each plate with 0.01ml of spore suspension containing 0.2% (w/v) bacteriological agar. The plates were incubated at 30ºC and the area covered by the fungus measured with a paquimeter every 24h until the plates were covered with fungal growth. The radial growth velocity was determined from the slope of the linear regression using the equation $r(t)=a+vcr.t$, where r is the radius of the colony, a is the linear regression constant, vcr is the radial growth rate (mm.h-1) and t is the cultivation. The *Monascus ruber* colonies were scrapped of agar surface and the pigments were extracted with ethanol 95°GL. The pigments were submitted to the scanning on spectrophotometer and color parameters determination on colorimeter ColorQuest.

Results & Conclusions

There was growth of the microorganism for all concentrations of whey applied and there was a significant difference between the growth rates of the fungus obtained for each medium when compared to the control medium (without cheese whey). When analyzed under different pH values, fungus growth rates were higher at pH 6.0 in culture medium containing $20gL^{-1}$ of whey powder, corresponding to a concentration of $6.4gL^{-1}$ lactose as the carbon source. At extreme pH values, such as pH 2.0 and 8.0, the growth of the fungus presented inhibition and the visual aspect differed, presenting yellow and red coloration, respectively. The growth rate at pH 3.0, 4.0 and 5.0 did not differ significantly ($p \leq 0.05$), however, it was observed that in different pH values, orange and red pigments were produced, which shows the clear influence of pH on the production of pigments produced by *Monascus ruber*.

References & Acknowledgments

Cheese whey is, therefore, shown as a viable, cheap and profitable alternative to the production of biopigments produced by *Monascus ruber*, given the high nutrient content of this by-product and the high growth of the fungus in this medium when under appropriate conditions of pH and concentration.

Keywords: Microbial pigments, Solid cultivation, Radial growth, Agroindustrial residue

P-230 - A SYNTHETIC CARDOSIN B-DERIVED RENNET FOR SHEEP AND GOAT CHEESE MANUFACTURE

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Background

Different sheep and goat cheeses with world-renowned excellence are produced using aqueous extracts of *Cynara cardunculus* flowers as coagulants. However, the use of this vegetable rennet is mostly limited to artisanal scale production, and no effective solutions to large-scale industrial applications have been reported so far. In this sense, the development of a synthetic rennet based on the most abundant cardoon milk-clotting enzymes (cardosins) would emerge as a solution for scalability of production and for application of these proteases as alternative rennets in dairy industry. In this work, we report the development of a new cardosin B-derived rennet produced in the generally regarded as safe (GRAS) yeast *Kluyveromyces lactis*, as well as its functional and structural characterization.

Method

Using a stepwise optimization strategy - consisting of culture media screening, complemented with a protein engineering approach with removal of the plant-specific domain, and a codon optimization step—cardosin B production in *K. lactis* was optimized. A thorough analysis of the specificity requirements of synthetic cardosin B, as well as its biochemical and structural properties were also determined.

Results & Conclusions

We successfully improved cardosin B production yield (35×) in *K. lactis*. Our results demonstrated that the secreted enzyme displays similar proteolytic properties, such as casein digestion profiles as well as optimum pH and temperature, with those of native cardosin B. From this optimization process resulted the rennet preparation VRen, requiring no downstream protein purification steps. The effectiveness of VRen in cheese production was demonstrated by manufacturing sheep, goat, and cow cheeses. Interestingly, the use of VRen resulted in a higher cheese yield for all three types of cheese when compared with synthetic chymosin (1). Kinetic characterization and specificity profiling results with the isolated recombinant protease clearly suggest that synthetic cardosin B displays lower catalytic efficiency and is more sequence selective than native cardosin B. Elucidation of the structure of synthetic cardosin B confirms the canonical fold of an aspartic protease with the presence of two high mannose-type, N-linked glycan structures. These subtle variations in catalytic properties and the more stringent substrate specificity of synthetic cardosin B help to explain the observed suitability of this rennet for cheese production (2).

Altogether, these results clearly position synthetic cardosin B (VRen) as an alternative/innovative coagulant for the cheese-making industry.

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Keywords: synthetic rennet, cheese, cardosin B, Kluyveromyces lactis, aspartic proteases

P-231 - FILTER MEDIA COMPARISON FOR WINE BRETTANOMYCES REMOVAL

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Background

The presence of *Brettanomyces bruxellensis* is of great concern for wine producers due to off-flavor production associated with the compounds 4-ethylphenol and 4-ethyl guaiacol, as well as tetrahydropyridines, acetic acid, ethyl acetate and isovaleric acid. Filtration is one of the available tools for eliminating this contaminant from wine. Several authors have studied the removal of *Brettanomyces* cells from wine by filtration, but this process is far from being fully understood (Renouf et al. 2007, Umiker et al. 2013).

Method

In the present work, the efficacy of several filters, commercially available as pleated cartridges for the wine industry, was compared. Filters differing in composition and micron rating, namely depth filters made of polypropylene (PP, 0.6 and 1.0 µm), borosilicate glass microfiber (GF, X, and V), and polyethersulfone membrane filters (PES, 0.45, 0.65, and 1.0 µm) were tested. Red wine inoculated with *B. bruxellensis* was used. The U.S. Food and Drug Administration (FDA) guidelines for filter validation for aseptic processing, aimed at the pharmaceutical industry, were followed, thus providing a margin of safety well beyond that expected in wine production. Filtrated wine was analyzed by plate counting (Duarte et al. 2017).

Results & Conclusions

No yeast cells were detected in wines filtered through PES filters in all micron ratings tested (0.45, 0.65 and 1.0 µm), corresponding to a logarithmic reduction value (LRV) higher than 8.2, showing their high efficacy in removing *B. bruxellensis*. Similar efficacy was achieved for X grade GF filters. On the opposite, low retention was obtained with PP filters (0.6 and 1.0 µm; LRV lower than 3.5) and V grade GF filters (LRV lower than 5.1).

This work showed that different filter compositions with similar micron ratings gave different retention of *B. bruxellensis*, highlighting the relevance of the filter media in the removal mechanisms. Additionally, it points towards the importance of a complete characterization of the filters media and not just pore size, either in scientific works or technological operations regarding *Brettanomyces* removal by filtration.

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Keywords: Brettanomyces bruxellensis, filter characteristics, wine, filtration

P-232 - ASSESSMENT OF THE IMPACT SYNTHETIC FE(III)-CHELATES AMENDMENT ON SOIL MICROBIAL COMMUNITY DYNAMICS

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Background

Iron has a crucial role in plant nutrition, being an essential element for plant growth. However, one-third of the Earth soil is iron deficient, resulting in iron deficiency chlorosis (IDC) growth of several crops worldwide, including staple foods. The use of synthetic Fe(III)-chelates is one of the most effective measures to correct IDC in plants, but their environmental impact must be mastered. Therefore, the search for more effective Fe-chelates remains an important issue. Previously [1], a 3,4-HPO Fe-chelate was proposed as a novel-fertilizing agent. Since the increase of nutrients availability in soil is thought to have an impact on the microbial composition, this question needs to be addressed. Therefore, the aim of the study was to investigate the effect of iron complexes of the 3,4-HPO class of ligands on soil bacterial dynamics to better understand their pathways.

Method

Laboratory scale soil columns (LSSC) were set up, with different soils origin and characteristics (Agricultural, Forestry and Urban), and exposed to two iron-chelates using rain simulations. The structure and abundance of the bacterial community was evaluated by automated ribosomal intergenic spacer analysis (ARISA) and qPCR (*rpoB*) approaches.

Results & Conclusions

Cluster analysis of ARISA profiles revealed that the soil characteristics were the major driving selection for the microbial community composition, with the samples from the same soil type clustering together, disregarding the amendment performed. Also, it emerged that the microbial community of forestry and agricultural soils were more similar (46%) than the one present in urban soil, as expected due to the plant influence. Considering each soil type individually, it was possible to observe a clear response to Fe(III) amendment on the microbial assemblage. Additionally, it seems that one of the compounds, the Fe-chelate derived from the ligand Deferiprone, induced greater and/or faster changes, and that the forestry soil was more prone to the microbial shift. These changes can underline a selection for bacteria that can use Fe(III) in its metabolism, or more tolerant to its presence, that needs to be better understood.

References & Acknowledgments

R. B. R. Mesquita and L. S. Mesquita thank to Fundação para a Ciência e a Tecnologia (FCT) and POCH of FSE for the grants SFRH/BPD/112032/2015 and PTDC/AAG-MAA/5887/2014_BI_2, respectively. The authors also thank the support from National Funds from FCT - Fundação para a Ciência e a Tecnologia, Portugal, through project PTDC/AAG-MAA/5887/2014 and scientific collaboration from FCT project UID/Multi/50016/2013 and UID/Multi/04423/2013.

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Keywords: Soil, Iron-chelates, Microbial diversity

P-233 - SIMULTANEOUS SYNTHESIS OF PREBIOTIC MIXTURES CONTAINING GOS AND FOS

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Background

Prebiotics are short-chain oligosaccharides able to promote specific changes in the composition and/or activity of the gastrointestinal microflora [1]. Examples include galacto-oligosaccharides (GOS) and fructo-oligosaccharides (FOS), and several benefits associated with their consumption have been described [2;3]. GOS and FOS can be obtained by enzymatic synthesis through hydrolysis and transglycosylation reactions using lactose and sucrose, respectively, as substrates. In this work, we report for the first time the simultaneous production of FOS and GOS using a crude extract with dual enzymatic activity obtained by *Penicillium brevicompactum* fermentation.

Method

The crude enzymatic extract from *P. brevicompactum* was obtained by submerged fermentation at 28 ºC,150 rpm, 20 days, using a culture medium containing (g/L): lactose (20), peptone (4), yeast extract (4) and salts. β-Galactosidase and β-fructofuranosidase activities were determined using ONPG and sucrose, respectively, as substrate. The enzymatic synthesis of prebiotics was performed at 37 °C mixing 5 mL of crude enzyme with 5 mL of a sugar solution (200 g/L lactose + 200 g/L sucrose) at pH 4.5 [4]. Samples were taken at different time points and analyzed by HPLC.

Results & Conclusions

P. brevicompactum produced a crude extract presenting both β-galactosidase (75 ± 3.2 U/L) and FFase (10326 ± 88 U/L) activities. The crude extract hydrolyzed both substrates (lactose and sucrose), and the simultaneous formation of GOS (trisaccharide) and three FOS (GF2, GF3 and GF4) was found. The prebiotic mixture obtained after 30 hours was composed of 5.8 ± 0.2 g/L of GOS (trisaccharide) and approximately 30 g/L of FOS. The total FOS concentration corresponds to 12.4 ± 0.6 g/L of GF2, 11.8 ± 0.4 g/L of GF3 and 5.5 ± 0.3 g/L of GF4. The potential of crude extracts with dual enzymatic activity for the simultaneous synthesis of two different types of prebiotics, namely lactose-based and sucrose-based oligosaccharides, is demonstrated. This strategy of prebiotic production was never explored and could represent an interesting approach to obtain prebiotic mixtures with enhanced biological effect.

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This study was supported by FCT (UID/BIO/04469/2013 unit); COMPETE 2020 (POCI-01-0145-FEDER-006684); project BioTecNorte (NORTE-01-0145-FEDER-000004) and project MultiBiorefinery (POCI-01-0145-FEDER-016403) funded by the European Regional Development Fund under the scope of Norte2020. SCS acknowledges her grant (SFRH/BPD/88584/2012) from FCT.

Keywords: galacto-oligosaccharides, fructo-oligosaccharides, enzymatic synthesis, prebiotics

P-234 - MICROBIAL AND CHEMICAL EFFECTS OF THE ADDITION OF PHENOLICS ON A RED WINE DURING THE MLF AND STORAGE

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Background

Depending on their structure and concentration, wine phenolic compounds can activate or inhibit microbial growth[1] and metabolisms[2], therefore, affecting the final quality of the wine. The pre-fermentation steps, mostly maceration, regulates the initial phenolics´ concentrations of a wine. Most of the actual studies have been done in culture medium and at phenolics´ concentrations.

Method

In this study, different groups of phenolics (flavan-3-ols, flavonols, hydroxycinnamic acids - HCA and stilbenes) were added, at three times their initial concentrations (previously determined), in a 2016 Douro red wine (Portugal) collected before malolactic fermentation (MLF). Two batches of samples were used for the addition of catechin (flavan-3-ols), the concentrations added varying of ±10 mg/L. Chemical and microbial characteristics of the wines were followed along spontaneous MLF and a short storage period. HPLC and GC methods were used for the analysis of the metabolites composition of the samples. All the wine samples were replicated and compared to a control. Microbial growth was measured by plating using the drop-count technique.

Results & Conclusions

The strongest effects were observed during MLF, and more precisely during the initial stage of MLF. With 35mg/L of added initial catechin, bacterial growth and malic metabolism were enhanced. The opposite phenomenon was observed with 45mg/L. Moreover, yeasts count, total anthocyanins and tartaric acid concentrations were higher in the samples implemented with 35mg/L of catechin than in the control. As for the ones with 45mg/L of catechin added, a decrease of acetic acid and an increase of ethanol were observed, suggesting a possible shift in the heterofermentative pathway towards ethanol production. HCA and flavonols also inhibited bacterial growth and delayed the degradation of malic acid, as well as the production of lactic and acetic acid. Resveratrol (stilbene) had a similar effect for bacteria and lactic acid, but at lower scale. The initial addition of ferulic and coumaric acids (HCA) caused, at the end of MLF, an increase of free caffeic acid. Kaempferol (Flavonol) and resveratrol were metabolized, almost entirely at the early MLF stage. 3 months after the initiation of MLF, the samples with an initial raise of HCA and resveratrol, can be differentiated from the control by a lower pH, anthocyanins, and yeasts concentrations. After five months' storage, the samples with high initial concentration of flavan-3-ols added are characterized by lower esters concentrations and higher ethyl phenols concentrations.

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Keywords: phenolics, wine, microorganisms

P-235 - EVALUATION OF THE IMPACT OF FERTILIZERS ON SOIL AND SOIL LEACHATES - CHEMICAL COMPOSITION AND MICROBIAL COMMUNITY ASSEMBLAGE

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Background

The excessive use of fertilizers may have an important impact on surface and ground water quality, essentially due to run-off and leaching of nutrients and contaminants, with clear social and economic consequences and, ultimately affecting human health and wellbeing. The increase of nutrients availability in the soil, mail also affect their microbial assemblages. Field crops are traditionally burned in many parts of the World, but little is known how the procedure affects nutrients and microbes.

Method

Laboratory scale soil columns (LSSC) were set up to study the impact of commercial inorganic fertilizers in the chemical composition and on the microbial community present in unburnt and burnt soil, and respective soil leachates. Traditional culturable methods were used together with a molecular microbiology approach to evaluate the impact on the bacterial abundance and diversity. The structure and abundance of the bacterial community was evaluated by automated ribosomal intergenic spacer analysis (ARISA) and qPCR (*rpoB*) approaches. Furthermore, a sequential injection method, capable of real-time monitoring of the soil leaching processes was developed using spectrophotometric detection to attain a multiparametric determination of calcium, magnesium, and iron(III) content.

Results & Conclusions

The results demonstrate that the use of fire in agriculture initially reduces de number of soil bacteria (one order of magnitude), although the subsequent use of fertilizers can restore bacterial abundance to unburn soil levels. This increase can be consequence of the essential micronutrient (calcium and magnesium) amendment, that fostered a positive correlation ($p < 0.05$) with bacterial abundance in burned soil, after fertilizer application. As expected, since soil particles can act as bacterial reservoirs, the number of bacteria in soil leachates (10^2) was substantially smaller than in the soil itself (10^7). Moreover, the addition of fertilizers seems to have no impact on the microbial abundance present in the leachates. Cluster analysis of ARISA profiles revealed that soil burning changes the soil microbial composition, and that the use of fertilizers seems to be a driving force in the shape of the soil bacterial community succession. Also, the results showed that the bacterial isolates from soil and soil leachates are typically microorganisms present in the soil environment.

References & Acknowledgments

R.B.R. Mesquita thanks to Fundação para a Ciência e a Tecnologia (FCT) and POCH of FSE for the grants SFRH/BPD/112032/2015. The authors also thank the support from National Funds from FCT - Fundação para a Ciência e a Tecnologia, Portugal, through project PTDC/AAG-MAA/5887/2014 and scientific collaboration from FCT project UID/Multi/50016/2013 and UID/Multi/04423/2013.

Keywords: Agricultural soil, Soil leachates, Fertilizers impact, Microbial diversity, Chemical composition monitoring

P-236 - GENETIC DIVERSITY, MICROBIOLOGICAL AND PHYSICO-CHEMICAL CHARACTERIZATION OF ARTISANAL PORTUGUESE PDO CHEESES

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Background

Portugal manufactures a wide variety of artisanal cheeses produced through spontaneous fermentation of unpasteurized milk. Azeitão, Nisa and Rabaçal cheeses are among the products that have been recognized by the European Community with the attribution of the Protected Denomination Origin -PDO- status. These unique cheeses receive a great deal of attention from consumers around the world but their microbiological richness is rather unexploited.
The present study analyzed PDO cheeses from the aforementioned geographical regions regarding genetic diversity, microbiological and physico-chemical features.

Method

PDO cheeses collected at the regions under investigation were characterized using conventional microbiology for the presence of lactic acid bacteria, *Enterococcus* spp., mesophiles at 30°C, coliforms, *E. coli*, molds and yeasts, *Listeria monocytogenes*, *Salmonella* spp. and coagulase-positive *Staphylococcus aureus*. Physical-chemical parameters like water activity, pH, humidity, chlorides, ash, total protein, total acidity, fat and water-soluble nitrogen were also assessed. Genetic diversity was evaluated after total DNA isolation using two distinct approaches, a commercial kit and an *in-house* method, followed by PCR-amplification using the random primers M13 and OPC-15.

Results & Conclusions

In what concerns physico-chemical parameters, no major differences were observed among the cheese samples. As for conventional microbiology, none of cheeses harbored *L. monocytogenes* nor *Salmonella*; Azeitão showed the highest microbial counts, followed by Rabaçal, Nisa cheese revealed the lowest numbers. These microbiological differences were further confirmed after genetic diversity assessment using PCR-fingerprinting. Rabaçal cheese, produced from a mixture of ewe and goat milk using animal rennet, showed rather distinct profiles (below 70% similarity) when compared to Azeitão and Nisa cheeses, both produced from ewe's milk using *Cynara cardunculus* rennet. It is also important to highlight that DNA isolation methodologies lead to distinct amplification patterns, demonstrating the importance of using diverse approaches to obtain a more complete picture of the underlying genetic diversity.

Overall results showed distinct features among the samples under analysis, pointing towards the microbiological specificity of the PDO cheeses from these regions, an issue to be further explored in future investigations.

References & Acknowledgments

Experimental work was supported by Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon (Project UID/CVT/00276/2013). Teresa Semedo-Lemsaddek is financially supported by FCT (SFRH/BPD/108123/2015). Authors also thank PDO cheese producers for supplying the samples.

Keywords: ARTISANAL CHEESE, PDO, GENETIC DIVERSITY, MICROBIOLOGICAL CHARACTERIZATION, PHYSICO-CHEMICAL CHARACTERIZATION

P-237 - ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS AGAINST FOOD-RELATED BACTERIA

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Background

To reduce, or even eliminate, microbial contamination in food products and food contact surfaces, synthetic or natural compounds can be supplemented directly to foods, incorporated in their packages, or used for cleaning and disinfection. Essential oils (EOs), complex mixtures of volatile compounds produced by plants, have long been regarded as good antimicrobials and antioxidants, being used to extend the shelf-life of food products. Their broad activity constituting a valuable weapon against foodborne multidrug-resistant microorganisms.

Method

The present study evaluated the antimicrobial activity of twenty essential oils commercially available from two distinct suppliers. Bacteria used, belonging to collections available at the Faculty of Veterinary Medicine, University of Lisbon, included Gram-negative (*e.g., Aeromonas hydrophila, Pseudomonas aeruginosa*) and Gram-positive isolates (*e.g., Enterococcus faecalis, Listeria monocytogenes*).

Antimicrobial activity was assessed using the agar diffusion assay as outlined in the Clinical and Laboratory Standard Institute's guidelines. Briefly, bacterial suspensions (turbidity equivalent to 0.5 McFarland) were prepared using semi-solid medium and poured over BHI plates, after solidification 10 microliters aliquots of each essential oil (1:2 and 1:10 dilutions) were either directly placed on the medium surface or impregnated in a diffusion disk and placed in the medium. After incubation (24h, 37ºC), diameters of inhibition zones were measured with a ruler and recorded in mm.

Results & Conclusions

A wide variation in the inhibitory properties of the tested EOs was observed, even between the same essential oil commercialized by the two brands under analysis. Nevertheless, all EOs inhibited at least one of the microorganisms under study, with the majority showing antimicrobial effects against more than half of the bacterial targets. Despite observed differences, three of the EOs showed higher inhibitory potential, namely thyme, oregano and clove. These OEs presented antimicrobial activity against both Gram-positive and Gram-negative bacteria, with diameters of inhibition zones attainment up to 37 mm.

Overall, the present study highlights the putative applicability of EOs, with special emphasis on thyme, oregano and clove, as antimicrobial agents against foodborne bacteria. Additionally, since EOs are environment-friendly compounds and present low mammalian toxicity, they should be considered as good food preservatives and food contact surfaces disinfectants.

References & Acknowledgments

Experimental work was supported by Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon (Project UID/CVT/ 00276/2013). Teresa Semedo-Lemsaddek is financially supported by FCT (SFRH/BPD/108123/2015). Authors also thank Soria and New Directions Aromatics for supplying the essential oils.

P-238 - PECTIC POLYSACCHARIDES: A BISCUITS ACTIVE INGREDIENT FOR ACRYLAMIDE MITIGATION

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Background

Both colour and flavour compounds are generated during thermal processing, along with toxic compounds such as acrylamide (AA) [1], classified as probably carcinogenic to humans (class 2A) [2].

The main route for AA formation is the reaction between reducing sugars and asparagine. However, this reaction is minimized at low pH. Sugar acids, such as galacturonic acid (GalA), although contributing to AA formation, have also pH-lowering properties. Our hypothesis is that pectic polysaccharides, composed by (α1→4)-GalA repeating units, having higher ratio of carboxylic groups in relation to only one reducing end sugar, can provide medium acidity to minimize AA formation. Although relevant for all diets, this property is even more important for fructose-rich foods, which include the biscuits for diabetics, where AA content is usually high.

Method

Biscuits were prepared by addition to the dough of GalA in monomeric, oligomeric, and polymeric forms. Control biscuits were prepared in according with AACC Method No.10-54, characterized for dough pH and water activity, biscuits appearance, texture, and colour [3]. Acrylamide was extracted and quantified by LC/ESI-MS^n.

Results & Conclusions

The biscuits prepared with addition of 1% GalA in relation to the flour showed 95% increase in AA content when compared with control biscuits. This shows that the influence of reducing ends is higher than that of the carboxylic acids for acrylamide mitigation. When the biscuits were prepared with oligomeric GalA, due to the decrease of the ratio of reducing ends in relation to carboxylic acids amount, no additional AA was formed. The same trend occurred when adding 1% and 5% commercial pectin (CP, 50% methylesterification), the later allowing a decrease of AA content of 72%. When using biscuits formulations with fructose, addition of 2% of GalA-rich deesterified polysaccharides to the dough decreased AA from 256 $\mu g/kg_{biscuits}$, which is a value higher than 150 µg/kg, the benchmark maximum value recommended for AA, to 116 µg/kg.

In conclusion, addition of pectic polysaccharides to the dough is a reliable acrylamide mitigation baking strategy that also opens new opportunities for AA mitigation in fructose-rich formulations for diabetics.

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Keywords: Acrylamide, galacturonic acid, pectic polysaccharides, tartaric acid, pectate, mitigation

P-239 - USE OF THE FRINGS ACETATOR FOR THE DEVELOPMENT OF ACETIC FERMENTATION OF CASHEW OF THE CERRADO GOIANO

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Background

The objective of this work was to produce cashew vinegar through submerged fermentation using Frings. A cashew alcoholic fermentation containing 11.6% (m / v) ethanol was submitted to *Acetobacter* sp acceptance in a process submerged in Frings. The Acetator (Frings, Germany), used for cashew vinegar production in submerged fermentation at the pilot scale, was obtained from Frings of Brazil. The total volume of the acetator is 8.0 L, with working volume of 6.0 L, consisting of a transparent reaction vessel with cooling coils and turbine aeration, a foam rupture disc, probes for temperature measurement and manometry, and valves to control loading, unloading and cooling. The equipment has a height two times greater than its diameter. Inside the reactor, there is a cooling coil, whose function is to dissipate the heat generated by the exothermic reaction of fermentation and heat transfer from the motion of the aerator motor. The cooling water of the reactor is controlled by instrumentation to maintain a temperature oscillation of ±0.3°C.

Method

The physicochemical parameters evaluated for acetic fermentation: total acidity was determined by titration with 0.1 M sodium hydroxide and an alcoholic solution of phenolphthalein as the indicator. The total dry extract quantified by the mass difference of the stainless steel Petri dishes before and after the evaporation of the sample in a water bath at 100 ° C. The relative density 20 ° C / 20 ° C and alcohol content (% v/v) were determined using a digital densimeter (Anton Paar, Austria, model DMA4500M).

Results & Conclusions

The titratable acidity, of the product was 4.2% (m / v), determines the amount of the free acidic functions present in the vinegar, being within the limits established by the Brazilian legislation, minimum 4%. The total dry extract and density presented, respectively, values of 51.96 g / L and 0.9985 g / L, both according to the literature. The alcohol content had 1.0% (v / v), it does not present a minimum parameter in Brazilian legislation, only the maximum value, of 1.0% by volume at 20 ° C. According to the results it was possible to obtain vinegar from cashew alcoholic fermentation, using the Frings acetator, corroborating with current Brazilian legislation.

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Keywords: pilot, ethanol and acetobacter

P-240 - PRODUCTION OF AN ANTILISTERIAL MEDIUM TO BE USED IN THE INDUSTRY OF PORTUGUESE TRADITIONAL MEAT PRODUCTS

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Background

Listeria monocytogenes is a great concern in the industry of Portuguese traditional meat products. Bacteriocins, produced by lactic acid bacteria (LAB) are of great importance in order to face this concern. The reason for that is because some bacteriocins have demonstrated antimicrobial activity against *L. monocytogenes* and do not alter the organoleptic characteristics of the products.

Although MRS broth can fill some fastidious growth requirements of many LAB, it cannot be used in the food industry, not only because it is very expensive, when used for large-scale commercial applications, but also, it contains constituents not approved in food production.

The objective of this study was to seek for a food grade medium that could promote growth of an autochthonous strain of *Lactobacillus* and production of bacteriocin active against *L. monocytogenes*.

Method

From previous studies with different LAB, isolated from traditional meat products, an autochthonous *Lactobacillus* strain was selected since it demonstrated antimicrobial activity against *L. monocytogenes* and do not alter the organoleptic characteristics of the tested products.

The maximum antilisterial activity (five strains) was when the selected *Lactobacillus* strain was grown in MRS broth, in reconstituted skimmed milk (RSM, 11% w/v) and in an inexpensive food grade medium with high protein content, for 39 h at 37 °C.

Results & Conclusions

For the three media, results showed that the maximum antilisterial activity was dependent on the target strains of *L. monocytogenes*.

When grown in MRS, the maximum antimicrobial activity was about 12800 Au/mL and it was reached after 16-39 hours of growth. In RSM the maximum antimicrobial activity (6400 Au/mL) was observed between 16 and 30 hours of growth. In the food grade medium the maximum antimicrobial activity was 6400 Au/mL and it was achieved after 16 and 39 hours of growth.

This medium demonstrated to be a potential alternative to grow bacteriocinogenic Lactobacillus at industrial scale.

References & Acknowledgments

This work was co-funded by Fundo Europeu de Desenvolvimento Regional (FEDER), through Programa Operacional Regional do Norte (PO Norte), in the scope of the project DEM@BIOFUMADOS .: Demonstrador do Biofumados - Tradição vs Qualidade - Produção de Enchidos e Fumados Tradicionais Portugueses (NORTE-01-0247-FEDER-006373) by National Funds from FCT - Fundação para a Ciência e a Tecnologia through project 'UID/Multi/50016/2013'

Keywords: Lactic Acid Bacteria, Bacteriocins, Listeria monocytogenes

P-241 - DOES THE PRESENCE OF LINOLEATE ISOMERASE GENE IMPLIES CONJUGATED LINOLEIC ACID PRODUCTION?

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Background

Conjugated Linoleic Acid (CLA), geometrical isomers of Linoleic Acid (LA), are of great interest for their potential health-promoting properties. Linoleate isomerase (LAI) is one of the microbial enzymes responsible of the transformation of LA to CLA and is found in several bacterial species including lactobacilli and bifidobacteria. Free LA inhibits the growth of many microorganisms, and it has been suggested that microbial conversion of LA into CLA might function as a detoxification mechanism [1].

Method

Twenty-three *Lactobacillus* and sixteen *Bifidobacterium* strains from the Food Technology department of the INIA (Madrid, Spain) collection, were activated in MRS supplemented with L-cystein, and in RCM respectively, under anaerobic conditions at 37ºC. Cultures were growth in the appropriate medium and incubated for 7 hours; then a solution of LA in TWEEN80 was added to the cultures to a final concentration of 1mg/ml, and incubated overnight. Afterwards, CLA was measured at 234 nm according to Rodríguez-Alcalá [2]. LAI gene presence was detected using primers designed for LAI genes of *Lactobacillus* and *Bifidobacterium*. PCR amplification products was subjected to electrophoresis in an agarose-TBE gel, and visualized to detect the gene presence.

Results & Conclusions

LAI gene presence was positive for all assayed *Bifidobacterium* strains. However, only 3 strains from this group showed absorbance variation above 0.500.Concerning *Lactobacillus* strains, more than 15 bacteria were LAI positive but in general absorbance variation was below 0.300. Interestingly, some positive LAI gene *Lactobacillus* did not show absorbance increment at 234 nm. In conclusion, the presence of LAI gene does not guarantee CLA production. This may be related to the fact that microorganism tolerance level to free LA is a key factor in CLA production.

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This work was supported by project RTA2013-00029-00-00 from the Spanish Ministry of Economy, Industry and Competitiveness and FCT – Fundação para a Ciência e a Tecnologia, under the project Pro-TECh-CLnA - Microbial Production of Bioactive Conjugated Linolenic Acid Isomers to Obtain Functional Ingredients and Foods reference PTDC/AGR-TEC/2125/2014. Financial support for the authors A.L. Fontes and L.L Pimentel was provided by fellowships SFRH/BD/117721/2016 and SFRH/BPD/119785/2016, respectively, granted by the Portuguese government through FCT. Authors also like to thank the scientific collaboration of CBQF under the FCT project UID/Multi/50016/2013.

Keywords: Lactic acid bacteria, Conjugated Linoleic Acid, Bifidobacterium

P-242 - ANTIMICROBIAL PROPERTIES OF OREGANO OIL (ORIGANUM VULGARE) AGAINST SALMONELLA ENTERITIDIS, STAPHYLOCOCCUS AUREUS AND LISTERIA MONOCYTOGENES IN "ALHEIRA"

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Background

"Alheiras" are traditional, slightly smoked, naturally fermented meat sausages typical of the Northern regions (Trás-os-Montes) in Portugal and have become very popular in urban centers. Essential oils (EOs) show good antimicrobial properties, becoming a good natural alternative to the use of chemical preservatives. Oregano EO already demonstrated, *in vitro*, antimicrobial activity, against *Salmonella* Enteritidis, *Staphylococcus aureus* and *Listeria monocytogenes*. The aim of this study was to investigate the antimicrobial effect of oregano EO against *Salmonella* spp., *L. monocytogenes* and *St. aureus* in paste of "alheira" during storage.

Method

The experimental conditions were: i) not inoculated paste as control; ii) paste inoculated with cocktail of *L. monocytogenes* (c*Lm*); iii) paste inoculated with c*Lm* with 4% EO; iv) paste inoculated with c*Lm* with 1.5% EO; v) paste inoculated with c*Lm* with 0.5% EO; vi) paste inoculated with c*Lm* with 0.195% EO; vii) paste inoculated with c*Lm* with 0.0975% EO. The same was done for *S.* Enteritidis and *St. aureus*. Each trial was performed in triplicate. Microbiological analyzes and determination of water activity and pH values were performed after 4h, 3, 7, 15 and 21 days of storage at 4 ºC.

Results & Conclusions

For *S*. Enteritidis and *St. aureus* at 4%, ocurred a reduction of ~ 3 log at the beginning of the storage time and, after 7 days, *L. monocytogenes* were not detected. At 1.5% only *S.* Enteritidis was not detected after 15 days. At 0.5%, 0.195% and 0.0975%, the reduction (~1-2 log) was only observed at the end storage, for *S.* Enteritidis and *St. aureus*. *L. monocytogenes* decreased slowly over time and a 2-3 log reduction was observed for all concentrations investigated after 15 days and, for the lowest concentrations used, there was ~5 log reduction after 21 days. Counts of lactic acid bacteria were ~10^9 CFU/ml for all samples and no significant differences in the values of pH and a_w were detected. Utilization of EOs could be used as a natural technique to improve the safety of meat and fermented meat products. These results could be useful for the meat industry as a food safety tool; however, the concentration required to achieve an antimicrobial effect in foods might be incompatible with their organoleptic acceptance. This needs to be further investigated.

References & Acknowledgments

This work was supported by FCT - through project 'UID/Multi/50016/2013' and financial support for H. Albano was provided by the project bio – n2 – value, nº NORTE-01-0145-FEDER-000030, funded by FEDER, under Norte2020.

Keywords: Fermented meat, alheiras, essential oils

P-243 - ANTIMICROBIAL POTENTIAL OF DIFFERENT PROPOLIS EXTRACTS

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Background
Propolis is a mixture of different types of beeswax and resins collected from plants by bees (*Apis mellifera*). Propolis is known for having antibacterial activity conferred by the presence of flavonoids, aromatic acids and esters in their composition[1]. Also, it has bactericidal action, due to the presence of ferulic and caffeic acids, antifungal, antiviral, anti-inflammatory, anti-tumor and antioxidant activity[2]. The objective of this study was to evaluate the chemical characteristics and antimicrobial activity of different propolis extracts using different solvents (propylene glycol, ethanol, water at pH 8.0, sunflower oil and olive oil) at two temperatures (room temperature and 50 ºC).

Method
Four samples of propolis, from different sources, were extracted using five different solvents at two temperatures. Each propolis extract was characterized chemically by determination of pH value, color, total antioxidant activity and phenolic compounds by both spectrophotometry and HPLC methods. Antimicrobial activity of each propolis extract was performed by the disk diffusion method against 32 target microorganisms. For the extracts inhibiting growth, their minimum inhibitory concentrations were determined.

Results & Conclusions
Propolis extracted with ethanol and propylene glycol (PG) allowed better extractions of the compounds presented in propolis and higher antimicrobial activity against several microorganisms. The concentration of phenolic compounds of PG extracts was similar to those of ethanolic extracts. The antioxidant activities of all extracts were similar and their composition was identical, varying only in the content of flavonoid compounds. Caffeic acid, coumaric acid, ferulic acid, quercetin and kaempferol were identified. Although further tests are required, propolis extracted with PG, a compound that according to the United States FDA is Generally Recognized as Safe (GRAS), could be a promising product to be used in food industry to, for example, reduce the microbial loads of raw meats and avoid cross-contaminations.

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This work was supported by National Funds from the *Fundação para a Ciência e a Tecnologia* (FCT) through project UID/Multi/50016/2013. Financial support for author J. Barbosa was provided by a post-doctoral fellowship SFRH/BPD/113303/2015 (FCT) and for H. Albano was provided by the project bio – n2 – value, nº NORTE-01-0145-FEDER-000030, funded by FEDER, under Norte2020.

Keywords: Antimicrobial activity, Extraction, Propolis, Phenolic compounds

P-244 - EXPLORING BARREL AGED BEER HETEROGENEITY USING VOLATILE FINGERPRINTING

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Background
Several beer styles involve barrel ageing, where transformations occur involving extraction of wood compounds, oxidative reactions and metabolism of wild microorganisms populating the barrel. Thus, there is high variability and heterogeneity in chemical composition and sensory profile of the final product. This work explores global volatile fingerprinting as a tool to assess heterogeneity in different beers aged through different methods. The potential of this tool was explored in the characterization and establishment of correlations between ageing methodology and final product composition.

Method
Six beers were aged in Port wine barrels. Three beers were fermented in inox VATs and aged in barrel, one during 16 months and two other during 11 months. The other three beers were fermented and aged in the barrel during 3 months, two were pitched with S. cerevisiae and the other fermented by wild barrel microbes. For comparison one unaged control was also analyzed. For volatile fingerprinting minor volatiles in beer samples were retrieved by dicloromethane microextraction and analyzed by GC-MS. PCA was performed accompanied by cluster analysis of variable loadings regarding to the main components extracted, which led to the final volatile fingerprints and correlations.

Results & Conclusions
A scatterplot with 3 dimensions correlating samples and volatiles was outlined. Control unaged beer was mainly correlated with isoamyl acetate and was near the cluster of both beers fermented in barrel with S. cerevisiae inoculum, which correlated with z-2-penten-1-ol content. Beers fermented in inox VATs and aged during 11 months were found in another cluster, correlating with ethyl octanoate, ethyl butyrate and 2-phenylethyl acetate, as well as whiskey lactone. Mainly correlating with typical Brettanomyces metabolites 4-ethylphenol and 4-ethylguaiacol, beer fermented by wild barrel microbes comprised another cluster, correlating also with 2-ethyl-1-hexanol and ethyl 2-methylbutyrate. Finally, beer fermented in inox VAT and aged during the longest time period was the one correlating with a higher number of volatiles, implying a higher sensory complexity. In its cluster compounds such as tyrosol, ethyl lactate and 4-methyl-1-pentanol could be found. Overall, volatile fingerprinting of beers was a suitable tool to assess beer characteristics, similar beer were found in similar clusters and correlations with volatiles were coherent with the production methods.

References & Acknowledgments
Authors would like to acknowledge the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UID/BIO/04469/2013 unit and COMPETE 2020 (POCI-01-0145-FEDER-006684) and under the scope of the Project RECI/BBB-EBI/0179/2012 (FCOMP-01-0124-FEDER-027462).

P-245 - BIOETHANOL PRODUCTION FROM WHOLE SLURRY OF HYDROTHERMALLY TREATED BREWER´S SPENT GRAIN AT HIGH SOLID LOADINGS

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Background

Brewer's spent grain (BSG) represents about 85% (w/w) of brewing by-products, being a potential low-cost feedstock for ethanol production. In works aiming at converting this residue to ethanol, up to 22 g/L of ethanol were produced, with yields varying between 23 and 81% [1]. However, such values are still far from satisfactory for biofuels production. In order to make bioethanol production economically feasible, ethanol titers must surpass the 40 50 g/L barrier, to reduce distillation costs to acceptable values [2]. To attain this, ethanol production solely from BSG must explore the whole fractions of sugar rich BSG, resort to high solid loadings and maintain high process efficiencies throughout the unit operations.

Method

BSG from craft beer industry was mixed with water (25 % of solids) and submitted to autohydrolysis treatment at 160 ºC for 5 min in a pressurized stainless reactor. Whole slurry (liquid and solid phases) from autohydrolysis was employed as substrate for ethanol production by enzymatic saccharification using Cellic Ctec2 (Novozymes) at 50°C and 200 rpm followed by fermentation in Erlenmeyers fitted with air locks at 30°C and 150 rpm.

Results & Conclusions

BSG had 32.1 ± 1.9 g of glucan/ g of raw material, which where fractioned during pre-treatment. 60% of glucose in BSG was solubilized to the liquid phase during pre-treatment and 40% remained in the solid, from which about 70% were retrieved through enzymatic saccharification. Overall, with pretreatment and saccharification it was possible to obtain 57.7 g/L of glucose, representing 70.6% glucose yield. Fermentation of the whole fraction of pretreated BSG allowed a final ethanol titer of 42.27 ± 0.03 g/L at 94.0 ± 0.6% yield. Therefore, ethanol production from BSG was attained at high ethanol titers without compromising process efficiency, representing an attractive and economically feasible solution for bioethanol production.

References & Acknowledgments

Authors acknowledge the Portuguese Foundation for Science and Technology (FCT) under the scope of UID/BIO/04469/2013 unit and COMPETE 2020 (POCI-01-0145-FEDER-006684), the Project RECI/BBB-EBI/0179/2012 (FCOMP-01-0124-FEDER-027462) and BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by European Regional Development Fund under the scope of Norte2020 - Programa Operacional Regional do Norte. Authors acknowledge Fermentum-Engenharia das Fermentações Lda. for providing the raw materials used.

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P-246 - STUDY OF THE INTERACTION OF HYDROXYCINNAMIC ACIDS WITH DIFFERENT PROTEINS

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Background

Wine is a product that has intrinsically high added value due to its sensory quality, nevertheless, some flaws can appear during winemaking resulting in a poor-quality product, thus reducing its commercial value. One of these flaws known as "Brett character", results from the contamination by *Brettanomyces bruxellensis* producing volatile phenols [1]. These phenols are produced from the metabolism of certain precursor phenolic compounds. The understanding of how these compounds, phenolic acids and their conjugates, are metabolized by the wine microbiota, has been a key issue of study [2]. The aim of this work is to contribute to the development of strategies to remove the precursor substrates from the wine preventing the formation of volatile phenols.

Method

Knowing that proteins can bind to phenolic compounds [3], and that these are normally present or added to wine, this interaction could be a solution for the reduction of the content of the precursors of volatile phenolics. Three types of proteins were used, bovine serum albumin (BSA), casein (CAS) and ovalbumin (OVA), to bind to hydroxycinnamic acids (HCAs), p-coumaric acid, ferulic acid and caffeic acid. The analytical technique chosen to study the interaction of hydroxycinnamic acids (HCA) with proteins was the fluorescence quenching approach. With this technique, the fluorescence of the analyte (protein) is measured with and without the presence of the quencher (HCA).

Results & Conclusions

The results showed that the interaction of the HCAs with CAS presented higher values for the thermodynamic binding constant (Ka) and for the number of binding sites per phenols/protein (n). An HPLC-DAD method was also carried out and the obtained results were in good agreement with the ones obtained by fluorescence quenching.

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Acknowledgements: Susana Vidigal acknowledges the financial support in the framework of the project "Biological tools for adding and defending value in key agro-food chains (bio – n2 – value)", nº NORTE-01-0145-FEDER-000030, funded by Fundo Europeu de Desenvolvimento Regional (FEDER), under Programa Operacional Regional do Norte - Norte2020.

The authors would also like to thank the scientific collaboration of FCT through the project UID/Multi/50016/2013.

Keywords: Protein-phenolic interaction, Caffeic acid, p-Coumaric acid, Ferulic acid, Bovine serum albumin, Ovalbumin, Casein

P-247 - ELECTRIC FIELDS EFFECTS ON CAROTENOIDS FROM TOMATO BY-PRODUCTS

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Background

Ohmic heating, also called electro-heating is an innovative thermal technology. Heat is generated inside the food and it is proportional to the square of the electric field strength and the electrical conductivity of the matrix. This technique may cause an electrochemical degradation due to number of reactions, including heat, electrode reactions and electrolysis of the solution. This study aimed to study the effects of electric fields on polyphenols and carotenoids from tomato by-products.

Method

Industrial tomato by-products were used in this study.
Different times (0, 15 and 30 min), temperatures (40, 55 and 70 ºC), electrode fields (4, 6 and 11 V/cm) and percentage of ethanol:water ratio (0, 35 and 70 %) were applied.
Extracts were obtained with ethanol:water and after centrifugation. Total antioxidant, total phenolics and carotenoids content were assessed by the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), Folin Ciocalteu's and spectrophotometric methods, respectively. Individual carotenoids were analyzed by High-Performance Liquid Chromatography with Diode-Array Detection (HPLC-DAD).

Results & Conclusions

The results showed that total polyphenols content were of 151.48 ug/gFW at the optimum extraction conditions – i.e. 15 min, 70 ºC and 70% of ethanol at 6 V/cm. Nevertheless, the same was not shown to the carotenoids, since their optimum extraction conditions were 30 min, 55 ºC and 35% of ethanol at 6 V/cm. The individual carotenoids identified in tomato by-products were lutein, lycopene and β-carotene. β-carotene was not extracted through the application of ohmic heating, as opposed to the organic solvents extraction. However, lycopene was found in samples submitted to fast heating treatment (few seconds until it reaches 70 ºC) coupled with moderated electric fields of 6 V/cm and 4 V/cm with a concentration of 5.0 and 3.8 µg/mL, respectively.
Results suggested that depending on combination of temperature and electric field carotenoids and polyphenols can be selectively extracted. Higher temperatures (70 ºC) at 4 V/cm are good to extract polyphenols but degrade carotenoids. Further studies are necessary to understand the influence of electrical variables on extraction profile of bioactive compounds.

References & Acknowledgments

The authors would like to thank to the projects Co-promoção nº 016403, NORTE-01-0145-FEDER-000004 and OCI-01-0145-FEDER-006684. Furthermore, the authors would also acknowledge the FCT projects, SFRH/BD/111884/2015, SFRH/BPD/81887/2011, UID/BIO/04469/2013 and the scientific collaboration of CBQF under the FCT project UID/Multi/50016/2013.

Keywords: Ohmic Heating, Electric Fields, carotenoids, Lycopersicon esculentum, ABTS

P-248 - EVALUATION OF ANTIOXIDANT, ANTIMICROBIAL AND PREBIOTIC ACTIVITIES OF A XYLOOLIGOSACCHARIDE-RICH GRAPE POMACE EXTRACT

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Background

Grapes are one of the most cultivated fruit crops worldwide, from which more than 70% is intended to wine industry that generates up to 20% of wasted biomass in the form of grape skin, seeds, stems and residual pulp, known as grape pomace (Spanghero *et al.*, 2009; Corbin *et al.*, 2015). Recently, extraction of xylooligosaccharides from lignocellulosic feedstocks has become very common, as these molecules have a potential impact on gastrointestinal health, mainly due to their selectively stimulation of gut microbiota – mainly via prebiotic and antimicrobial activities - but also for their antioxidant activity. The objective of this work was to evaluate the biological properties of a xylooligosaccharide-rich grape pomace extract.

Method

Grape pomace extract was obtained through enzymatic extraction using 100 IU/g of an enzymatic cocktail produced by *Aspergillus niger* 3T5B8, containing xylanase activity. Extraction was performed using citrate buffer with pH 5 as solvent, and extraction was performed at 40 °C for 4 hours, under agitation. The extract was filtered under vacuum and lyophilized after adding 2% (w/w) of maltodextrin. Antioxidant, antimicrobial and prebiotic activities were evaluated.

Results & Conclusions

The xylooligosaccharides-rich grape pomace extract presented low concentration of total phenolic compounds but high antioxidant activity (ABTS and DPPH methods). The extract presented antimicrobial activity against methicillin-resistant *Staphylococcus aureus* at concentration of 3% (w/w), and bacteriostatic activity against *Escherichia coli* and *Salmonella enteritidis* at concentration of 2% (w/w). Prebiotic activity was also evaluated using *Lactobacillus* and *Bifidobacterium* spp and results showed a relevant activity mainly upon *Bifidobacterium* spp.
In conclusion, xylooligosaccharide-rich grape pomace extract presented relevant antioxidant, antimicrobial and prebiotic activities.

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Acnowledgments: This work was supported by National Funds from FCT - Fundação para a Ciência e a Tecnologia through project UID/Multi/50016/2013.

Keywords: xylooligosaccharides, grape pomace, antioxidant, prebiotic, antimicrobial

P-249 - DETERMINATION OF MINERALS IN ACETIC FERMENTED CASHEW OF THE CERRADO(ANACARDIUM OTHONIANUM RIZZ)

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Background

Minerals are inorganic compounds with different functions in the body, but are considered essential nutrients because they are not produced by the body and therefore must be obtained through diet. Minerals regulate the metabolism of various enzymes, acid-base balance, osmotic pressure, muscular and nervous activity, facilitates the transfer of essential compounds through the membranes and, in some cases, are part of the constituent elements of the body's tissues.The objective of this work was to determine the content of minerals present in acetic fermented cashew of the Cerrado obtained through submerged fermentation using Frings.

Method

The determination of minerals in acetic fermented samples was quantified by spectrophotometry with relative precision, macro and micromineral analysis were performed by nitroperchloric digestion followed by reading in Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

Results & Conclusions

They identified two macronutrients and two micronutrients, respectively: Nitrogen (N), potassium (K), iron (Fe) and manganese (Mn). Nitrogen was the mineral found in most of the macronutrients, 15.65 g.Kg -1, followed by potassium 2.70 g.Kg -1, the micronutrients obtained 6.45 mg.kg -1 for iron and 2.79 mg.kg -1 for manganese. Some minerals, such as nitrogen, potassium and iron, may have been used by yeast *S. cerevisiae* as a source of micronutrients, or it was still a constituent of solids retained during the filtration of the assay, just as manganese is one of the important minerals in respiratory activity and in the yeast growth rate in the alcoholic fermentation stage for the production of acetic fermented. The use of ICP-OES instrumental technique and nitroperchloric digestion of the sample proved to be adequate for the simultaneous determination of mineral elements in acetic fermentation

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Keywords: macronutrients, micronutrients, vinegar and submerged fermentation.

P-250 - SCREENING OF ANTIOXIDANT ACTIVITY IN DIFFERENT COFFEE BLENDS

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Background

Coffee beverages, prepared from roasted coffee beans, are widely consumed throughout the world for their physiological effects and attractive aroma and taste.Two species are of significant economic importance Coffea arabica (Arabica) providing 75% of the world production and Coffea canephora (Robusta) wich provides 25% of the world production[1]. Several studies have indicated that a strong antioxidant activity (AA) of coffee is generally associated with its content of phenolic compounds.

Chlorogenic acids (CGAs), which include many different isomeric forms, are the predominant phenolic compounds in coffee beans.The aim of this study was evaluated the antioxidant activity of the different blends with different proportions Arabica/Robusta extracted with a single-dose capsule system, for a medium roasting.

Method

Twelve blends supplied by Delta Cafés were analyzed. Total Phenolic compounds (TPC) were analysed using the method Folin-Ciocalteu [mg gallic acid equivalent (GAE)/coffee] and AA using two methods: ABTS [mg ascorbic acid equivalent (EAA)/coffee] and DPPH [mg Trolox equivalent (ET)/coffee]. The phenolic compounds profile was determinated by LC-MS.

Results & Conclusions

Based on TPC and AA results it was possible to observe that exist inter-lot variability (p<0,05), although that fact only occurs in some blends. High positive correlation between TPC and AA (by ABTS method) was observed, which means that the antioxidant activity is mainly related to the presence of the phenolic compounds in the blends. Similar tendency was observed for DPPH results, although lipophilic antioxidants were evaluated in this method. In general the increase of Robusta coffee in blends led to an increase of the TPC and the AA (no linear relationship) as observed for the best five blends. These blends showed similar phenolic compounds profile, including as main compounds: neochlorogenic acid (5-CQA), chlorogenic acid (3-CQA), cryptochlorogenic acid (4-CQA), feruloylquinic acid, 4,5-Dicaffeoylquinic acid, 3,5-Dicaffeoylquinic acid and 3,4- Dicaffeoylquinic acid. The results demonstrated the high antioxidant potential of these blends related with the high CGA's concentration, proving the importance coffee consumption as natural source of antioxidants compounds.

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Keywords: antioxidant activity, chlorogenic acids, coffee blends

P-251 - ANTI-INFLAMMATORY AND ANTIOXIDANT POTENTIAL OF AQUEOUS EXTRACT OF AROMATIC PLANTS FROM ALENTEJO

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Background

Alentejo region is rich in medicinal aromatic plants (MAPs) that are used in some Portuguese traditional food as well as in traditional medicine. MAPs are rich in phenolic compounds with some important biological properties, such as antioxidant, anti-inflammatory and anti-tumour activities. Previous studies of essential oils and aqueous extracts of some flavouring herbs of Alentejo revealed important antioxidant properties and capacity to inhibit anti-cholinesterase enzymes, often associated with oxidative stress and neurodegenerative diseases [1].

The aim of this study was to evaluate antioxidant and anti-inflammatory potential of four aqueous extracts of *Calamintha nepeta* subs *nepeta, Coriadrum sativum*, *Mentha spicata* and *Mentha pulegium* wild grow from Alentejo.

Method

Aqueous extracts were obtained by lyophilization of decoction waters of plant and their chemical composition was performed by total phenols and flavonoids quantification.

Screening of antioxidant activity was performed with evaluation of potential of extracts to inhibit lipid peroxidation by β-carotene/linoleic acid method and quantification of some enzymes related with antioxidant response, such as catalases (CAT), glutathione-*S*-transferases (GST) and glutathione reductases (GR) in hepatic mice homogenates. *In vitro* anti-inflammatory potential was evaluated measuring inhibition of albumin denaturation and anti-lipoxygenases activity (LOX) [2].

Toxicological proprieties were evaluated with lethality test in *Artemia saline* [3].

Results & Conclusions

Aqueous extracts presented high contents of phenolic and flavonoid compounds.

The screening of antioxidant activity showed that extracts presented high ability to inhibit lipid peroxidation (50 µg/mL) and catalase activity (80 µg/mL) with inhibition values higher than 50%.

A high inhibition of glutathione-*S*-transferase activity was observed, suggesting an antioxidant protection in glutathione oxidation/reduction cycle. Extracts showed high anti-inflammatory potential with ability to inhibit albumin denaturation and LOX. Moreover, extracts showed very low toxicity to *Artemia salina* lethality test (2300 $<LC_{50}<$4000 µg / mL).

The high antioxidant and anti-inflammatory properties of these extracts suggest their potential application in food and / or pharmaceutical industries.

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This work is funded by "HIT3CH - HERCULES Interface for Technology Transfer and Teaming in Cultural Heritage", Ref: ALT20-03-0246-FEDER-000004, co-financed by the European Union through the European Regional Development Fund ALENTEJO 2020 (Regional Operational Programme of the Alentejo)

Keywords: Aromatic plants, Aqueous extracts, Anti-inflammatory, Antioxidant

P-252 - BREWER'S SPENT GRAIN AS A POTENTIAL SOURCE OF ANTIOXIDANTS: CHARACTERIZATION OF EXTRACTS

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Background

Brewer's spent grain (BSG) is one of the most abundant by-product of the brewing industry. While currently used as animal feed, BSG has some components with nutritional and functional (e.g. dietary fiber, protein and phenolics) potential which makes it an interesting source for added-value compounds/products to be incorporated into foodstuffs or cosmetic products.

Antioxidant compounds can help maintain the oxidative homeostasis and therefore they are thought to play an important role in the prevention of degenerative diseases. Moreover, from a technological standpoint they may aid preventing product oxidation and, consequently, extend the shelf life of products.

Considering the above made arguments, the present work aims to produce BSG extract's and characterize their antioxidant capacity.

Method

BSG was extracted using hydroethanolic extracts (Solid Liquid Extraction) and the resulting extracts antioxidant capacity was measured through ABTS and DPPH radical's inhibition as well as through DNA agarose gel electrophoresis.

Results & Conclusions

The results showed that the different extracts were capable of inhibiting ABTS and DPPH radicals. Moreover, of the different water:ethanol ratios, 60 % (v/v) ethanol appeared to be the fraction with the highest antioxidant capacity while the extractions performed using only water or ethanol exhibited significantly lower values.

Interestingly, when considering the capacity to protect DNA from H_2O_2 induced damage, all of the extracts exhibited a strong activity, with concentrations as low as 20 µL of extract/mL, being able to fully prevent DNA cleavage. Similar results were observed when considering an H_2O_2 iron cation system. However, in this case, 100% ethanol and 100% water extracts were not always 100% effective in inhibiting DNA degradation. In fact, for both, concentrations above 200 µL of extract/mL appeared to loose their effectiveness registering up to ca. 40% DNA degradation for ethanol and 15% DNA degradation for water. Overall, none of the extracts (at the tested concentrations) led to the cleavage of the DNA molecule, regardless of the presence of iron cations, and therefore had no pro-oxidant activity.

In sum, BSG extracts' possess an interesting antioxidant activity that appears to possess some biological relevance and represents an interesting source of antioxidant compounds to be exploited.

References & Acknowledgments

The authors would like to thank to the projects: Co-promoção, NORTE-01-0145-FEDER-000004 and OCI-01-0145-FEDER-006684. Furthermore, the authors would also acknowledge the FCT project UID/BIO/04469/2013 and would also like to thank the scientific collaboration of CBQF under the FCT project UID/Multi/50016/2013. The author T. Bonifácio-Lopes would like to acknowledge the grant UMINHO/BD/11/2016.

Keywords: DNA oxidation, Brewer's Spent Grain, ABTS, DPPH, Antioxidant Capacity

P-253 - ANTIOXIDANT AND ANTICHOLINESTERASE PROPERTIES OF ESSENTIAL OILS OF LAVANDULA SPP.

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Background

Flavouring herbs and spices are often used in Mediterranean cuisine for cooking, beverages, confectionary and foods production, not only to improve or modify the flavour, but also to avoid its deterioration [1]. Aromatic plants and their essential oils (EOs) are an important source of bioactive products with application in pharmaceutical, agronomical and food industries. Moreover, herbs and spices and their EOs have an important role in the oxidative stress protection in different pathologies, such as atherosclerosis, diabetes and neurodegenerative diseases.

A large amount of aromatic plants are endemic of the south of Portugal and used by local population as condiments in food preparations as flavours and food preservatives. For this study, were selected EOs of three *Lavandula* species, widespread in the South of Portugal, in order to evaluate their antioxidant potential and their ability to inhibit cholinesterase activities.

Method

EOs of *Lavandula stoechas* L. subsp. *luisieri* Roseira and *Lavandula pedunculata* (Mill) Cav. (Alentejo) and *L. viridis* L'Hér (Algarve) were extracted from the aerial parts of plants by hydrodistillation and chemical composition was evaluated by GC-FID [2]. Antioxidant activity was determined by three methods: DPPH radical, β -carotene/linoleic acid and reducing power assay [2]. The inhibitory activity of Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) were evaluated by UV-Vis spectrometry [3] .

Results & Conclusions

EOs showed antioxidant capacity by the three different mechanisms studded, presenting high potential to protect the oxidation of lipid substrate and a high ability to inhibit *in vito* AChE and BChE activities. These results suggest the potential use of these essential oils as nutraceutical or pharmaceutical preparations in the prevention of the oxidative stress and neurodegenerative pathologies, related to cholinesterase activity.

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Keywords: Essential oils, Lavandula stoechas L. subsp. luisieri Roseira, Lavandula pedunculata (Mill) Cav., L. viridis L'Hér, antioxidant properties

P-254 - CO-CULTURE FERMENTATION OF AFRICAN NATIVE SORGHUM AND MILLETS WHOLE GRAINS - TECHNOLOGICAL AND BIOCHEMICAL POTENTIAL OF PROBIOTIC AND INDIGENOUS STRAIN COMBINATION

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Background

Probiotic strains co-cultured with microbial strains isolated from indigenous cereal-based foods can be used as starter cultures for the production of potentially probiotic beverages. This combined culture might contribute to improve texture, by *in situ* production of exopolysaccharides (EPS), and nutritional properties but may also exert beneficial health effects. Millets and sorghum, with high nutritional value and richness in phytochemicals, are promising substrates for the development of new fermented cereal-based products. Hence, this study aimed to evaluate the technological/biochemical potential of single and co-cultures on the fermentation of dry-milled *Sorghum bicolor* (Sorghum), *Eleusine coracana* (Finger Millet) and *Pennisetum glaucum* (Pearl Millet) cereals, obtained locally in Kenya and Burkina Faso, respectively.

Method

Lactobacillus plantarum 299v was used as the probiotic strain.The three indigenous strains (C2, 32LABPT05 and 2LABPT05), were previously isolated from traditional Kenyan and Burkinabé cereal-based products and identified as *Weissella* strains producers of EPS. Both strain types were inoculated at 1% (1:1 ratio) and fermentation was performed at 30 °C/200 rpm in an orbital incubator. Samples were taken at different fermentation time points (in the presence of 10% sucrose) until pH≈4.5-5.0 was reached and monitored for microbial growth (lactic acid bacteria and contaminant bacteria), pH, sugars/organic acids concentration (HPLC) and total phenolics concentration (spectrophotometry).

Results & Conclusions

In general, acidification and growth capacities were more clearly observed during each millet's fermentation than during sorghum's. EPS production (based on visual assessment) was only observed during millet's fermentation. The single culture with indigenous strains reported higher acidification rate, and higher EPS production when compared with the co-culture. The growth of C2/32LABPT05/2LABPT05 appeared to be unaltered by the addition of the probiotic strain. On the other hand, the growth of *L. plantarum* 299v was affected by the presence of the indigenous strain during the eight-hour fermentation; on average, viable cell numbers of *L. plantarum* 299v were 0.65 fold lower than the indigenous strain. In terms of fermentation capacity, glucose was the most consumed sugar, while lactic acid was the mostly produced acid. Moreover, increased levels of total phenolic compounds were observed in fermented sorghum-based products. Overall, the best co-culture behaviour was observed in the corresponding cereal from which they had been originally isolated. Studied co-cultures presented good potential for development of new probiotic beverages.

References & Acknowledgments

This work was supported by the Portuguese Foundation for Science and Technology (FCT) in the framework of the project ERA-AFR/0002/2013 and the doctoral grant SFRH/BD/133084/2017.

Keywords: native whole grains, indigenous strains, probiotic strains, fermentation, nutritional value, exopolysaccharides

P-255 - MICROWAVE ASSISTED DEHYDRATION OF BROCCOLI BY-PRODUCTS AND SIMULTANEOUS EXTRACTION OF BIOACTIVE COMPOUNDS

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Background

Broccoli by-products from frozen-food industry account for 45% of the initial broccoli heads, sharing their nutritional value and bioactive compounds of commercial broccoli heads [1]. However, due to their high moisture content, the valorisation of compounds, including carbohydrates, requires stabilization to inhibit enzymes, prevent microbial growth, and degradation of the product [2].

Method

Therefore, in this study, a new technology based on the application of microwave hydrodiffusion and gravity (MHG) was used to dehydrate broccoli by-products and simultaneously recover the water-soluble diffused compounds for food ingredients use. Moreover, MHG impact on carbohydrates extraction was evaluated by six boiling water sequential extractions of 1 h each.

Results & Conclusions

The hydrodiffusion allowed to obtain a dried material with 12% moisture in 43 min when 550 g of broccoli by-products were used. Diffused water contained up to 317 µg/mL gallic acid equivalents of phenolic compounds, 11 mg/mL free sugars, 9 mg/mL amino acids, and 356 µg/mL glucosinolates, depending on the type of by-product used. Further carbohydrate extraction of dBB reached higher yields in comparison with initial broccoli by-products (iBB). Beyond free glucose and fructose, a total of 76% of dBB pectic polysaccharides were extracted, whereas only 60% of pectic polysaccharides were extracted from iBB.

This work shows the potential of MHG for dehydration of by-products, allowing their stabilization while recovering water-soluble compounds by diffusion. Additionally, the MHG dehydration works as a pre-treatment that improves the extraction of polysaccharides. The water-soluble material recovered by diffusion and boiling water extraction, rich in free sugars and pectic polysaccharides, respectively, may be valuable as ingredients in food applications. Overall, these results indicate that MHG technology has potential to be used in industrial by-products valorisation [3].

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Acknowledgements: Thanks are due to "Fundação para a Ciência e a Tecnologia" (FCT)/MEC for the financial support to the research unit QOPNA (FCT UID/QUI/00062/2013) and to the research unit CI&DETS of Viseu under the project UID/Multi/04016/2016 through national funds and where applicable co-financed by the FEDER, within the PT2020 Partnership Agreement. Furthermore, we would like to thank the Polytechnic Institute of Viseu and CI&DETS for their support, Monliz SA for providing broccoli by-products, and Unicam and Milestone srl (Italy) for the scientific collaboration. Sónia Ferreira (SFRH/BD/103003/2014) and Cláudia Passos (SFRH/BDP/107881/2015) were supported by FCT grants.

Keywords: microwave hydrodiffusion and gravity, Brassica, polysaccharides, protein, phenolic compounds, free sugars, amino acids, glucosinolates

P-256 - NONENZYMATIC PRODUCTION OF HONEY-LIKE OLIGOSACCHARIDES

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Background

Honey is essentially a concentrated aqueous solution of fructose and glucose, but it also contains a very complex mixture of other carbohydrates and substances, such as enzymes, amino and organic acids, vitamins, minerals, pigments, aroma substances [1]. Some of the carbohydrates present in honey, such as palatinose, the fructooligosaccharides (FOS) 1-kestose, 6-kestose, and neokestose [2], panose [3] and raffinose [4] are reported to have prebiotic effect. The presence of FOS in honey may be attributed to the transfructosylation activity of enzymes from microorganisms found in honey [5]. The present work hypothesizes that nonenzymatic reactions could occur in honey promoting the formation of oligosaccharides. This can be supported by the fact that honey maturation conditions, such as high sugar concentrations in acidic media, induce condensation of carbohydrates [6].

Method

To validate the previous hypothesis, six aqueous model solutions (moisture content of 20%) containing sucrose plus glucose, and sucrose plus fructose were prepared using water and diluted citric acid at pH 4.0, pH 2.0. The model solutions were kept in an oven at 35 ^{0}C, which is the normal temperature inside beehives with brood production.

Results & Conclusions

Electrospray ionization mass spectrometry (ESI-MS) analysis revealed the occurrence of non-enzymatic oligosaccharide synthesis with a degree of polymerization (DP) up to 6 after 5 months. Ligand-exchange/size-exclusion chromatography (LEX-SEC) separation of the oligosaccharides formed and methylation analysis allowed to observe that the produced oligosaccharides had a glycosidic linkage composition similar to that reported for honey oligosaccharides [8]. The structure and identity of the oligosaccharides were further elucidated by gas-chromatography coupled to mass-spectrometry (GC-MS) after derivatization to the alditol acetates derivatives. The FOS 1-kestose was identified in all model solutions, except in the solution prepared with sucrose plus glucose with diluted citric acid at pH 2.0.
In conclusion, honey-like oligosaccharides, including those with reported prebiotic activity, may be produced in solutions highly concentrated in carbohydrates, submitted to moderate temperatures, without the intervention of enzymes.

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Keywords: oligosaccharides, prebiotics, nonenzymatic reactions

P-257 - EDIBLE MICROALGAE CHLORELLA VULGARIS: A NON-CONVENTIONAL SOURCE OF STARCH

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Background

Microalgae comprise an extremely diverse collection of photosynthetic and unicellular microorganisms which have an extraordinary potential due to the ability to produce a wide range of commercially biomolecules. *Chlorella vulgaris* is a green microalga and is one of the only two microalgae approved for human nutrition. This microalga is a rich source of starch, representing its main polysaccharide. However, there are not many studies concerning the characterization of *C. vulgaris* starch in order to evaluate its feasibility as a source of starch. Therefore, the aim of this study was the extraction, purification and physico-chemical characterization of the *C. vulgaris* starch from a commercial microalgae production.

Method

A hot water extraction was performed using deffated biomass. The extract was dialyzed against distilled water at 4ºC. The insoluble starch precipitated during dialysis was separated by centrifugation.

Results & Conclusions

C. vulgaris starch obtained was composed by 32% amylose and the amylopectin contains 2% of branching points, a composition similar to the starch found in cereals such as corn and wheat [1]. The particle size distribution revealed a mean of 20 µm and the SEM showed a granule size between 1 and 2.5µm mostly with a spherical shape, which is smaller than potato starch (<110µm) or wheat (30µm) starch [2]. The starch obtained presented a thermal behaviour and a FTIR spectrum similar to plant starch. The X-ray diffraction showed an amorphous pattern, which may be related with the harsh conditions used for the extraction, losing the described semi-crystalline pattern [3]. *C. vulgaris* starch showed a hydrolysis rate performed by α-amylase very similar to potato commercial starch. Moreover, *C. vulgaris* biomass revealed to be a low glycemic index food and, therefore, very favourable for human nutrition.

The results suggest that the starch produced by the microalga *C. vulgaris* is similar to plant starch, and therefore, they have a great potential as a non-conventional source of starch that can be applied in diverse fields, such as in the food sector.

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Keywords: Microalgae, Chlorella vulgaris, Starch

P-258 - CARRIAGE OF STAPHYLOCOCCUS AUREUS AMONG PORTUGUESE NURSING STUDENTS: A LONGITUDINAL COHORT STUDY OVER FOUR YEARS OF EDUCATION

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Abstract

Staphylococcus aureus is a major human pathogen that can colonize healthy people mainly in the anterior nares. The aim of the present study was to evaluate *S. aureus*, including methicillin-resistant *S. aureus* (MRSA), nasal colonization over time among Portuguese nursing students.

In this longitudinal cohort study, we collected 280 nasal swabs from nursing students at 14 time points over four years of schooling (2012-2016). The isolates were characterized by pulsed-field gel electrophoresis (PFGE), *spa* typing, multilocus sequence typing (MLST), and SCC*mec* typing for MRSA.

Among 47 students, 20 (43%) carried methicillin-susceptible *S. aureus* (MSSA) at admission, but none was colonized with MRSA. A total of 19 students (40%) became colonized after exposure during the nursing training, out of which five carried MRSA. Overall, 39 students (83%) had *S. aureus* detected at least once during the study period. Among the 97 MSSA isolates, most (65%) belonged to four clones: PFGE A-ST30 (21%), B-ST72 (20%), C-ST508 (13%), and D-ST398 (11%). Three of the five MRSA carriers were colonized with the predominant clone circulating in Portuguese hospitals (ST22-IVh) and two with ST3162-II. Colonization of nursing students was highly dynamic with continuous appearance of strains with distinct PFGE types in the same individual.

A considerable proportion of students became colonized by *S. aureus*, including MRSA, during the nursing education, evidencing this population represents an important reservoir of *S. aureus*. Therefore, education on infection control measures in nursing schools is of major importance.

Keywords: Staphylococcus aureus, MRSA, Nasal cariage, Nursing students

P-259 - METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS ISOLATED FROM CHILDREN HOSPITALIZED IN LUANDA, THEIR MOTHERS AND THE ENVIRONMENT: PREDOMINANCE OF ST5-IVA AND ST88-IVA

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Abstract

The nosocomial prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in a pediatric hospital in Luanda has been previously estimated as 82.4%. The aim of the present study was to evaluate potential MRSA reservoirs in this hospital.

In January 2017, 79 hospitalized children and their respective guardians (78 mothers and 6 fathers) were nasally swabbed for MRSA carriage. Additionally, 49 inanimate surfaces were screened. All isolates were tested for the presence of the *mecA* and Panton Valentine leukocidine (PVL) genes, and characterized by pulsed-field gel electrophoresis (PFGE) and SCC*mec* typing. Selected isolates were analyzed by *spa* typing and multilocus sequence typing (MLST).

A total of 15 children (19%) and nine guardians (10.7%) were MRSA nasal carriers and 12 inanimate surfaces (24.5%) were contaminated with MRSA. Two lineages comprised 80% of the MRSA, including isolates from children, mothers and the environment: PFGE A-ST5-SCC*mec*IVa (61%) associated to *spa* types t105/t311/t11657/t14047, related to the international Pediatric clone (ST5/IV or VI), and PFGE B-ST88-IVa (19%), associated to *spa* types t186/t325/t786, corresponding to a major African clone. The remaining seven MRSA isolates belonged to five minor lineages: PFGE D-t3092-ST72-V (n=1), PFGE E-t148-ST72-V (n=2), PFGE X-t6278-ST30-V (n=1), PFGE AQ-t1476-ST8-V (n=2), and PFGE AF-t957-ST601-IVg (n=1). Five mother/children pairs were colonized with identical MRSA strains. PVL was detected in the single MRSA ST30-V.

Hospitalized children, their guardians and inanimate surfaces represent MRSA reservoirs and constitute likely transmission routes in the hospital setting. Infection control measures should focus on guardians and disinfection of surfaces to avoid the MRSA spread within the hospital and to the community.

Keywords: MRSA, Luanda

P-260 - PORTUGUESE PIGS AS RESERVOIRS OF MCR-1-PRODUCING ENTEROBACTERIACEAE

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Abstract

The *mcr-1* gene encoding a phosphoethanolamine transferase has been recently identified as a source of acquired resistance to polymyxins in *Escherichia coli*. It has been occasionally identified in other species such as *Enterobacter cloacae* and *Klebsiella pneumoniae*. It is now admitted that the *mcr-1* gene has very likely emerged first in animals due to a high selective pressure with colistin in veterinary medicine since the mid 1960's. Our study aimed to prospectively analyze the prevalence and the occurrence of the *mcr-1* gene among Enterobacteriaceae recovered from two pig farms in Portugal.

One-hundred fecal samples from two different Portuguese pig farms were screened for polymyxin-resistant Enterobacteriaceae using the Superpolymyxin® selective plates. Isolates were confirmed to be resistant to colistin by using the biochemical Rapid Polymyxin NP test. Susceptibility testing was evaluated by broth microdilution for colistin and by disk diffusion for other antibiotics.

Screening of the *mcr-1* gene and other resistant determinants was performed by PCR amplification followed by sequencing. PCR-based replicon typing (PBRT) was realized with the PBRT kit (Diatheva®). Clonality and phylogeny assays were determined by PFGE analysis, by MLST, and by the Clermont method identifying *E. coli* phylogroups.

Ninety-four isolates (18 *K. pneumoniae* and 76 *E. coli*) being resistant to colistin were recovered. Noteworthy, they all carried the *mcr-1* gene. All the isolates presented an MIC to colistin ranged from 4 to 64 µg/ml. Among the *E. coli mcr-1* positive strains, 7 co-produced an extended-spectrum ß-lactamase. All *K. pneumoniae* isolates belonged to Sequence Type ST45 and all *E. coli* belonged to the B1, A and F phylogenetic groups. Twenty-nine different *E. coli* clones were identified belonging to twenty-six different ST mostly detected as new STs. PBRT showed that the *mcr-1* gene was carried on a diversity of plasmid including IncP, IncHI2, IncX4 and IncY plasmids. Insertion sequence IS*Apl1* was identified upstream and/or downstream of the *mcr-1* gene in IncHI2-carrying *mcr-1* isolates.

This study showed a wide dissemination of MCR-1 in two different pig farms in Portugal, further highlighting the wide diffusion of that colistin resistance determinant in veterinary medicine. Furthermore, we showed here the worrying dissemination of *mcr-1* not only in *E. coli* but also significantly in *K. pneumoniae*.

Keywords: MCR-1, colistin, pigs, Portugal, SuperPolymyxin medium

P-261 - EXTENDED-SPECTRUM-B-LACTAMASES PRODUCING ESCHERICHIA COLI AS INTESTINAL COLONIZER OF STUDENTS FROM UNIVERSITY OF PORTO

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Abstract

Dissemination of multidrug-resistant bacteria represents a preoccupation in terms of public health with enormous implications for the future. In hospitals and other healthcare institutions this problem has been documented, however, in community, antibiotic resistance is also emerging[1]. Due to the actual extended-spectrum-β-lactamase (ESBL) producers endemicity and carbapenemase producers emergence, we aimed to detect intestinal colonization with these antibiotic resistance mechanisms producers in healthy young adults, particularly students from University of Porto (UP).

Results & Conclusions

Faecal samples of 30 university students from UP (ICBAS and FFUP) were analyzed. Isolates were selected on MacConkey agar with cefotaxime, ceftazidime and meropenem (2 mg/L). Susceptibility to antibiotics was achieved by disc diffusion methods according to the CLSI and identification of the isolates was performed by CHROMagar orientation, API20E and ID32GN. ESBL producers were detected and/or confirmed by the double-disk-synergy-test and clavulanic acid addition. In selected isolates PCR was performed for detection of bla_{TEM}, bla_{OXA}, bla_{SHV}, $bla_{CTX\text{-}M\text{-}group\text{-}1}$, $bla_{CTX\text{-}M\text{-}group\text{-}2}$, $bla_{CTX\text{-}M\text{-}group\text{-}8}$, $bla_{CTX\text{-}M\text{-}group\text{-}9}$, $bla_{CTX\text{-}M\text{-}group\text{-}25}$, *tetA, tetB* genes, and for 32 virulence factors and pathogenicity-associated-island-PAI. Additionally, phylogenetic groups were characterized and the presence of O25b-ST131 clonal group was evaluated, by PCR. Conjugation assay using *E. coli* K802N as recipient strain was performed to study the transferability of resistance and virulence genes. After 5 months students colonized with ESBL producers were followed up.

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We are grateful to the students of FFUP and ICBAS who agreed in participate in this study and to Professor Niza Ribeiro for collaboration through ICBAS students contact.

Keywords: Escherichia coli, ESBL, community, healthy students

P-262 - EXTENDED-SPECTRUM CEPHALOSPORIN-RESISTANT CMY-2-PRODUCING SALMONELLA HEIDELBERG AND S. MINNESOTA IN POULTRY MEAT IMPORTED INTO THE EUROPEAN UNION

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Abstract

Extended-spectrum cephalosporin-resistant (ESC-R) *Salmonella* has been described at low level in EU, including in Portugal. Nevertheless, the increasing poultry meat trade involving countries with different animal production practices could be an important source of epidemic clones carrying ESC-R genes. In this study, phenotypic and genotypic characterization of resistance to ESC was carried out, as well as the clonal relatedness analysis of *Salmonella* obtained from poultry meat samples imported into Portugal in the scope of official border control between 2014 and 2015. Antibiotic susceptibility tests (ampicillin-A/chloramphenicol/ciprofloxacin-Cip/gentamicin/kanamycin/meropenem/nalidixic acid-Na/pefloxacin-P/streptomycin/sulfamethoxazole-Su/tetracycline-T/trimethoprim) and detection of β-lactamase production (amoxicillin-clavulanic acid-Amc/cefepime/ceftazidime-Cz/cefotaxime-Cx/cefoxitin-Fx) were performed by disk diffusion and/or microdilution methods (CLSI/EUCAST). Detection of genes encoding for qAmpC (bla_{CMY}/bla_{MOX}/bla_{FOX}/bla_{LAT}/bla_{ACT}/bla_{MIR}/bla_{DHA}/bla_{ACC}) and extended-spectrum β-lactamases (bla_{TEM}/bla_{SHV}/bla_{CTX-M}), plasmid-mediated quinolone resistance [*qnrA/qnrB/qnrC/qnrD/qnrS/qepA/aac(6')-Ib-cr/oqxAB*] and other antibiotic resistance genes were performed by PCR/sequencing. Plasmid characterization was assessed by conjugation assays, replicon typing (PCR-PBRT/pMLST) and hybridization experiments (I-*Ceu*I/S1-PFGE nuclease). Clonal analysis was performed by *Xba*I-PFGE/MLST.

Results & Conclusions

Seven (n=7/31; 23%) frozen gizzards samples imported from Brazil were positive for *Salmonella*. These isolates belonged to *S*. Heidelberg (n=6; ST15/eBG26) and *S*. Minnesota (n=1; ST548/eBG77) serotypes and presented multidrug-resistant profiles (mostly AAmcCzCxFxCipPNaSuT), including to ESC (MIC_{Cx}=8-16mg/L/MIC_{FX}=32->256mg/L) and/or fluoroquinolones (MIC_{Cip}=0.25-0.5mg/L). All but one carried bla_{CMY-2} gene, located on two epidemic plasmids, IncA/C (ST2-n=5) or a transferable IncI1 (ST12-n=1). *S*. Heidelberg was associated with 5 PFGE-types, including one similar to an American epidemic clone. This study reveals the presence of uncommon and/or invasive ESC resistant *Salmonella* strains in imported poultry products. The emergence of those clinically-relevant poultry-related serotypes associated with international trade should be taken into account in the monitoring of antibiotic resistance trends and in re-evaluation of European regulation specifically Regulation (EC) 2073/2005 that stipulates for fresh poultry meat a food safety criterion that considers the absence of *S*. Enteritidis and *S*. Typhimurium in all the 5 units comprising the sample and does not take into account other *Salmonella* serotypes.

References & Acknowledgments

The authors would like to thank to Apostolos Liakopoulos (Department of Bacteriology and Epidemiology, Central Veterinary Institute, Wageningen University & Research, The Netherlands) for providing the PFGE-profiles of the *Salmonella* Heidelberg isolates from The Netherlands. This work was supported by FCT/MEC through national funds and co-financed by FEDER, under the Partnership Agreement PT2020 [grant number UID/MULTI/04378/2013 – POCI/01/0145/FERDER/007728]; the project NORTE-01-0145-FEDER-000011; Joana Campos is supported by a Ph.D. fellowship from FCT [grant number SFRH/BD/93091/2013] and Joana Mourão is supported by a Post-doc fellowship from project NORTE-01-0145-FEDER-000024. The authors are greatly indebted to all of the financing sources.

Keywords: Salmonella, Antimicrobial resistance, Poultry meat, Clones, CMY-2

P-263 - ANALYSIS OF THE INFLUENCE OF DRINKING WATER ON MOUSE GUT MICROBIOTA THROUGH 16S RRNA GENE DEEP SEQUENCING

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Abstract
The intestinal tract microbiota has been shown to influence many important physiological functions[1]. Nevertheless, external factors that affect these microorganisms are not fully understood. The effects of drinking water, particularly, are still poorly studied[2]. In this way, the aim of this work was to investigate the effects of different types of drinking water on mouse gut bacterial community. For that purpose, BALB/c J Unib female mice were randomly distributed in four groups, which received disinfected water from a drinking water treatment plant, a commercial bottled mineral water, tap water, and autoclaved tap water (control), respectively. Water samples were filtered through 0.22 micrometer membranes, which were stored at -20°C for posterior analysis. Each group received sterilized diet and the assigned water for nine days, followed by five days in which all groups received autoclaved tap water. Finally, the original condition was reestablished for other nine days, comprising three experimental stages. At the beginning of each stage, feces were sampled and freezed at -20°C. Additionally, at the last experimental day, mucosa-adhered (from the initial to the final portion of the gut) microbes were collected. The experiment was performed according to the Conselho Nacional de Controle de Experimentação Animal, and the study was approved by the Comissão de Ética no Uso de Animais from the UFMG. After DNA extraction, 16S rRNA gene amplicons[3] (hypervariable region V4) were generated and sequenced at MiSeq (Illumina), according to the manufacturer instructions[4]. Sequences were processed with Mothur software[5]. Diversity and statistical analysis were conducted with R packages Phyloseq[6] and Vegan[7].

Results & Conclusions
Significant changes in bacterial communities of different groups were detected, principally in the control compared to the others. An increase in the relative frequency of clinically important taxa, such as Acinetobacter and Staphylococcus, was observed in samples of the groups that received tap and disinfected water. Interestingly, the control samples showed little intra-group variation at the end of the experiment, and also the largest distance compared to the other groups. Differences between groups were significantly associated with water type and time. The results indicate that drinking water can possibly affect intestinal tract bacteria, and act as a link for antibiotic resistant taxa from different environments.

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Our acknowledgments to the Companhia de Saneamento de Minas Gerais (COPASA-MG/Brazil) for providing the water sample from Rio das Velhas DWTP and to the Laboratory of Microbiology (DESA/UFMG) for the support with water samples processing.
Keywords: Mouse gut microbiota, 16S rRNA gene, Drinking water

P-264 - DEVELOPING AN AFFORDABLE BUT RELIABLE HUMAN BLOOD EX VIVO MODEL TO ANALYSE GENE EXPRESSION BY STAPHYLOCOCCUS EPIDERIMIDIS

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Background

Staphylococcus epidermidis is a commensal inhabitant of healthy human skin and mucosae. However, when external barriers, such as the skin, are damaged, the bacterium gains accesses into the bloodstream and emerges as an opportunistic pathogen. *S. epidermidis* can originate important infections such as medical device-associated bloodstream infections. This is mainly due to its ability to attach and form biofilms on the surface of vascular catheters. Hence, due to the clinical relevance of *S. epidermidis* medical device-associated bloodstream infections, human blood is frequently used as an *ex vivo* model, to mimic the environment encountered by the bacterium and study its behavior. An important limitation in the use of human blood is the availability of donors and the considerable quantity of blood necessary. As any other biological resource, the use of blood shall be reduced to a minimum and, thus, our goal was to test the influence of different volumes of human blood on the stability of *S. epidermidis* gene expression and on bacterial culturability.

Method

For this study, planktonic cells of *S. epidermidis* PT12003 (isolated from a patient after a stomach surgery) and SECOM005A (isolated from healthy human skin) were used. Different volumes of human blood (600, 500 and 200 µl) were tested using a multiplicity of infection of 1 neutrophil to 100 bacteria, and compared with the results obtained with a previously optimized *ex vivo* model (1 mL of blood, França et al., 2016). After 2 hours of incubation at 37ºC and slight agitation, the transcription levels of three genes was determined using quantitative PCR (qPCR). The number of culturable bacteria was determined by CFU counting.

Results & Conclusions

The results demonstrated that smaller volumes of human blood (up to 200 µl) did not significantly affect the transcription levels of the studied genes nor the culturability of bacterial cells, suggesting that lower quantity of blood can be used in *ex vivo* studies addressing gene expression studies. Furthermore, specific differences found between isolates were constant in all volumes.

References & Acknowledgments

This work was supported by national funds (FCT) by the strategic project of UID/BIO/04469/2013 and by BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by European Regional Development Fund under the scope of Norte2020. SB had an individual fellowship (UMINHO/BD/15/2016) supported by the Doctoral Program in Chemical and Biological Engineering (NORTE-08-5369-FSE-000052 Operation) by NORTE 2020 Program and co-funded by European Social Fund.NC is an Investigador FCT.

Keywords: Staphylococcus epidermidis, gene expression, human blood, ex vivo, model

P-265 - HIGH RATE OF 16S RRNA METHYLASES ASSOCIATED TO CARBAPENEMASES AMONG ENTEROBACTERIAL ISOLATES RECOVERED FROM HOSPITALIZED CHILDREN, ANGOLA

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Abstract

Aminoglycosides (AG) play an important role in antimicrobial therapy in severe infections, usually in combination with β-lactam agents. AG resistance usually arises from enzymatic modification of the drug, however another mechanism corresponding to the production of 16S rRNA methylases (RMT) that are mostly plasmid-encoded is currently emerging. We have recently developed the first culture medium for selecting AG pan-resistant bacteria, mainly RMT producers. Our objective was to perform a prospective screening of RMT producers using this novel screening medium.

Rectal swabs were collected from hospitalized children in a hospital in Angola during a one-week period, January 2017. After an overnight pre-culture in broth, samples were screened for pan-AG Enterobacteriaceae using the selective SuperAminoglycoside medium, supplemented by gentamicin 30 µg/ml and amikacin 30 µg/ml. PCR experiments were performed using primers specific for RMT genes. Genotyping was performed by PFGE analysis and by MLST.

A total of 36 samples were collected from children being 3 months to 13 years-old. A total of 22 pan-AG-resistant Gram-negative isolates were recovered from 20 patients. A total of 16 isolates harboured the *rmtB* gene (9 *Escherichia coli*, 5 *Klebsiella pneumoniae*, 1 *Enterobacter cloacae*, and 1 *Enterobacter aerogenes*), five harboured the *armA* gene (2 *K. pneumoniae*, 1 *E. aerogenes*, 1 *E. cloacae*, and 1 *Citrobacter freundii*), and a single *K. pneumoniae* harboured the *rmtC* gene. All the isolates were resistant to broad-spectrum cephalosporins by production of either CTX-M type enzyme (CTX-M-15 in 15/22 isolates, CTX-M-55 in 2 isolates) or NDM-5 carbapenemase (10 isolates). The *rmtB* gene was carried in most of cases by a conjugative 125-kb IncFIB plasmid that also carried the $bla_{\text{TEM-1}}$ penicillinase gene (10/16 isolates). The other identified plasmids belonged to IncL/M, IncN and IncY incompatibility groups and respectively carried $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-55}}$ and $bla_{\text{NDM-5}}$ in association with *rmtB*. The *armA* gene was carried by a conjugative IncA/C plasmid, being often associated with the $bla_{\text{NDM-5}}$ carbapenemase gene (3/5 isolates). MLST showed a high diversity of genetic backgrounds, either for *E. coli* and *K. pneumoniae* isolates. However, ST448 was the most frequent RmtB-producing *E. coli* clone identified (5/9).

We report here a high rate of RMT producers in this population of hospitalized children in Angola, colonizing 55% of the patients. Worringly, co-association between carbapenemases and RMT enzymes was often identified, leading to pandrug resistance. We believe that the global prevalence of RMT producers is largely underestimated, raising questions concerning the future of AG.

Keywords: Angola, Aminoglycosides, rRNA methylases, Enterobacteriaceae, SuperAminoglycoside medium

P-266 - PANDRUG-RESISTANT ENTEROBACTERIAL ISOLATES RECOVERED FROM HOSPITALIZED PATIENTS, SÃO TOMÉ AND PRÍNCIPE

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Abstract

Multidrug resistance (MDR) in Gram negatives is increasingly reported nowadays. Worryingly, a series of plasmid-borne determinants conferring resistance to the last-resort antibiotics carbapenems, aminoglycosides, and colistin may be associated in some given isolates. Very few epidemiological data are available regarding the occurrence of those acquired resistance determinants in African countries, and no data at all in some countries such as São Tomé and Príncipe. We therefore initiated a prospective study in the central hospital of that country in order to evaluate the occurrence of MDR bacteria and identify the corresponding mechanisms of resistance.

Rectal swabs were collected from hospitalized patients (children and adults) during a one-week screening period, March 2017. After an overnight pre-culture in broth, samples were screened for pan-aminoglycoside-, carbapenem-, or polymyxin-resistant Enterobacteriaceae using the respective selective media SuperAminoglycoside, SuperCarba, and SuperPolymyxin. PCR experiments were further performed using primers specific for corresponding resistance genes. Genotyping was performed by pulsed-field gel electrophoresis.

A total of 35 samples were collected among which 27 carbapenemase producers were identified, all producing OXA-181, including 23 *Escherichia coli* (representing three different clonal lineages) and 4 *Klebsiella pneumoniae* (all clonally-unrelated). Seven OXA-181-producing *E. coli* isolates corresponding to a single clone co-produced the 16S rRNA methylase RmtB conferring high-level resistance to all aminoglycosides. A single *K. pneumoniae* isolate produced RmtB. Most of those isolates (90%) were additionally producing the CTX-M-15 extended-spectrum β-lactamase. Finally, only a single isolate was found to exhibit acquired resistance to colistin, being an *E. coli* strain exhibiting a wild-type β-lactam and aminoglycoside susceptible phenotype, but producing the plasmid-encoded MCR-1 determinant.

We report here a very high rate of patients colonized by MDR Enterobacteriaceae, including producers of the ultimate antibiotic resistance mechanisms, namely the OXA-181 carbapenemase, the 16S rRNA methylase RmtB, and the phosphoethanolamine transferase MCR-1. Noticeably, co-occurrence of those resistance mechanisms was often identified. Also of note is the wide dissemination of OXA-181 producers, a phenomenon recently observed in Angola, another Portuguese-speaking African country. This high rate of colonization by MDR bacteria in São Tomé and Príncipe, a low-income country with limited access to antibiotics, is particularly worrisome.

Keywords: São Tomé, pan-aminoglycoside-, carbapenem-, and polymyxin-resistance, Enterobacteriaceae, SuperAminoglycoside medium, SuperCarba medium, SuperPolymyxin medium

P-267 - IDENTIFICATION OF A NOVEL CLOSTRIDIUM DIFFICILE REGULATORY PROTEIN THROUGH THE ANALYSIS OF A GUT SIGNATURE FOR SPORULATION IN THE HUMAN GASTRO-INTESTINAL TRACT

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Background

Bacteria that reside in the gastrointestinal tract of healthy humans are essential for our health, sustenance and wellbeing. About 50 to 60% of those bacteria have the ability to produce resilient spores, specialized for host-to-host transmission. Recently a genomic signature of sporulation within the human intestinal microbiome was identified. This signature includes 66 genes and is dominated by genes with an established function in sporulation. Among these are the products of the *spoIIIA* operon which forms a channel by which the mother cell feeds the forespore and is a universal feature of endosporeformers, or the genes coding for the RNA polymerase sigma factors that control gene expression during sporulation. This list also includes genes that code for global transcriptional regulators, such as *spo0A*, which is essential for sporulation. Importantly, approximately 30% of the signature genes have no known function in sporulation and code for products with no similarity to known proteins.

Method

We use genetic and cell biology tools recent developed for the intestinal pathogen *Clostridium difficile*. We established the pattern of expression of the genes under study in conditions that promote sporulation using transcriptional fusions to the *SNAP* fluorescent reporter gene. We constructed a mutant for a signature gene (*CD2589*) by allelic-coupled exchange (ACE). This system allows the construction of in-frame deletion mutants in any location in the genome using a heterologous *pyrE* gene, as a negative selection marker. The mutant is then complemented upon restoration of the *pyrE* gene.

Results & Conclusions

We examined the expression of the signature genes during sporulation in *C. difficile*. Of the genes with unknown function, 12 out of 19 show cell-type-specific expression: 3 are mother cell-specific genes and 9 are forespore-specific genes. We also analysed the role of one of the signature genes with unknown function during sporulation. We show that deletion of *CD2589* resulted in increased sporulation. In agreement with this result we observed increased expression of Spo0A-dependent genes in the *CD2589* mutant. Mutation in this gene do not alter the expression of the genes coding for the toxins or genes involved in motility. These results indicate that CD2589 is a negative regulator of sporulation.

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Keywords: Clostridium difficile, Sporulation

P-268 - CANINE MODEL FOR SEPSIS: A CONTRIBUTION TO THE CLASSIFICATION AND STRATIFICATION OF SEPTIC PATIENTS

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Abstract

Sepsis is a severe condition that can appear following many other diseases. It affects many human beings, as well as animals. Given its high prevalence and mortality rates, the study of this condition is paramount. Canine Parvovirus is a disease that can be a predisposing cause of sepsis, regarding gut bacterial translocation. Studies involving naturally CPV infected dogs can be useful regarding the investigation of sepsis mechanisms, not only in animals but also in human beings (Remick & Ward, 2005).

The main purpose of this work was to propose and implement a sepsis patient scoring and stratification system based on the PIRO model. In parallel, the differences between different SIRS diagnostic criteria were evaluated. Another goal was to study inflammatory mediators, as well as the presence of bacterial DNA in dogs prone to sepsis, confronting those results with the clinical scores.

Results & Conclusions

The 31 animals that composed the experimental group were scored according to several parameters regarding Predisposition, Response and Organ dysfunction (all the subjects were attributed an equal Infection score). Data was retrieved from clinical records of the Infectious Diseases Isolation Unit of the Veterinary Teaching Hospital of ULIsbon Veterinary Faculty. The animals were also subjected to slightly different SIRS diagnostic criteria. CRP concentration as well as IL-6 and TNF-α quantity were measured in 13 and 12 animals, respectively. *E. coli* and *Staphylococcus* spp. bacterial DNA presence in blood samples was also investigated in 13 animals of the experimental group.

Blood CRP measurements were significantly higher in CPV infected dogs when compared to dogs in the control group ($p<0.001$). Based on approved Veterinary Medicine SIRS criteria (AUC=0.85A) a CRP threshold value for the presumptive diagnosis of SIRS was found (83.8 mg/L). The differences between using different SIRS diagnostic criteria, on hospital admission as well as after 48h, were significant ($p<0,01$). The differences between scores obtained on hospital admission and after 48h were not statistically significant ($p=0,187$). A correlation between the increase in CRP concentration and the increase in the PIRO score was not found ($r_s=0,187$; $p=0,540$). IL-6 and TNF-α expression was detected in 5 and 6 animals, respectively and bacterial DNA was detected in 3 of the 12 animals that composed the experimental group.

This work represents a contribution towards the development of a classification system for septic canine patients. It is urgent to implement new biomarkers, capable of enabling a more objective monitoring of both human and animal septic patients.

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This work was supported by the Centre for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon (FMV/UL) (Project UID/CVT/00276/2013).

Keywords: Parvovirus, Sepsis, CRP, PIRO, bDNA

P-269 - BIOCIDE AND MUPIROCIN RESISTANCE AMONG MRSA IN PORTUGUESE HOSPITALS: A REASON FOR CONCERN?

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Abstract

The current approach for methicillin-resistant *Staphylococcus aureus* (MRSA) decolonization combines the use of chlorhexidine gluconate (CHG) and mupirocin (MUP). The aim of the present study was to evaluate the resistance to biocides and MUP in Portugal.

We assessed the prevalence of six biocide resistance genes among 185 MRSA, representatives of the major clonal lineages circulating in Portuguese hospitals between 1985 and 2011. Biocide resistance genes, including the chromosomal genes *norA*, *lmrS*, *mepA*, *sepA* and the plasmidic genes *qacAB* and *smr*, were detected by PCR. Antibiotic resistance testing, including MUP, was carried out by the disc diffusion method. Minimum inhibitory concentration (MIC) of CHG was determined by broth microdilution.

Overall, the prevalence of biocide resistance genes was very high for *sepA* (99.5%), *mepA* (96.2%), and *norA* (83.8%), intermediate for *lmrS* (64.3%) and *qacAB* (23.8%), and low for *smr* (1.1%). *qacAB* and *norA* genes were associated to the Iberian clone (ST247-IA) (84.4%, $p<0.001$ and 95.6%, $p=0.026$, respectively), while *smr* was associated to the Portuguese clone (ST239-IIIA) ($p=0.004$). *lmrS* gene showed a higher predominance in 1992-93 (84%, $p=0.035$) and among both the Iberian (77.8%, $p=0.021$) and NY/Japan related (ST105-II) (88.9%, $p=0.037$) clones. A significant association between biocide resistance genes and antibiotic resistance was observed. Low-level resistance to MUP was detected in a single isolate recovered in Azores in 2007, with a MIC value of 32 mg/L. High non-susceptibility to MUP (80%) was associated to EMRSA-15 (ST22-IVh) clone ($p=0.020$). The MIC for CHG ranged between 0.125 and 4 mg/L, and a cut-off of ≥ 1 mg/L for CHG non-susceptibility was defined for the present collection. CHG non-susceptibility was correlated to the presence of *qacAB* gene (93.2%, $p<0.001$), the 1996-97 period (79.3%, $p=0.050$), and the Iberian clone (86.7%, $p<0.001$).

There is an overall high prevalence of chromosomal biocide resistance genes in Portugal and the use of biocides might be selecting for antibiotic-resistant isolates. Although CHG non-susceptibility is still low, a relation between MUP non-susceptibility and the dominant MRSA clonal lineage (EMRSA-15) in Portuguese hospitals is of concern.

Keywords: MRSA, Mupirocin resistance, Biocide resistance

P-270 - ESTABLISHMENT OF GALACTOSE UTILIZATION IN SACCHAROMYCES CEREVISIAE PE-2 THROUGH EXPRESSION OF THE CEN.PK113-5D GAL2 GALACTOSE PERMEASE

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Background

Saccharomyces cerevisiae PE-2 is one of the most robust yeast chassis for use in second-generation bioprocesses [1-2]. Unfortunately, we noticed that it is incapable of utilizing galactose, which is abundant in diverse agro-industrial derived substrates. In this study we analysed the galactose utilization pathway (Leloir pathway) of this strain and identified the Gal2 galactose permease as the limiting step.

Method

The putative amino acid (aa) sequences of the *S. cerevisiae* JAY291 (haploid derivate of PE-2) proteins involved in galactose utilization (Gal2, Gal1, Gal7, Gal10, Gal5, Gal4, Gal80 and Gal3) were aligned (Clustal Omega) with the corresponding proteins of other industrial and laboratorial strains, revealing several point mutations in the Gal2 permease. PE-2 was then transformed with a 2 micron plasmid containing the CEN.PK113-5D *GAL2* under the regulation of the *TDH3* promoter and *PGI1* terminator, and the resulting transformants were physiologically characterized in liquid YP containing 2% galactose plus 150 µg/mL G418.

Results & Conclusions

Homology-based analysis of the *S. cerevisiae* PE-2 Leloir pathway allowed the identification of 12 aa substitutions in the Gal2 sequence that are not conserved across other industrial and laboratorial strains. Three of these point mutations were found in the transmembrane domain 7 (TM7), a region important for substrate recognition [3]. Among these, the most significant includes the substitution F336L, as the loss of aromatic aa in TM7 is reported to be critical for galactose transport activity [3]. These results suggested that the galactose permease of *S. cerevisiae* PE-2 might lack galactose transport activity, which was further supported by the fact that expression of the CEN.PK113-5D *GAL2* in PE-2 established its galactose utilization capacity. In fact, with this modification PE-2 was faster than CEN.PK113-5D in consuming 2% galactose (12h vs 14h, respectively). The high galactose utilization efficiency of this newly constructed PE-2 strain opens new perspectives and opportunities for the valorisation of galactose-containing second-generation substrates.

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Study supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UID/BIO/04469/2013 unit and COMPETE 2020 (POCI-01-0145-FEDER-006684), Project MultiBiorefinery (POCI-01-0145-FEDER-016403), RECI/BBB-EBI/0179/2012 (FCOMP-01-0124-FEDER-027462) and BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund under the scope of Norte2020 - Programa Operacional Regional do Norte.

Keywords: Industrial Saccharomyces cerevisiae PE-2, Galactose utilization, Leloir pathway, Gal2 galactose permease

P-271 - MOLECULAR ANALYSIS OF 3RD GENERATION CEPHALOSPORIN-RESISTANT ENTEROBACTERIACEAE IN CENTRO HOSPITALAR DO BAIXO VOUGA, AVEIRO

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Background

3rd generation cephalosporins (3rdGC) are one of the primary choices to treat human infections caused by Gram-negative bacteria. The prevalence of clinical isolates displaying resistance to these antibiotics, and producing extended-spectrum β-lactamases (ESBLs) and/or plasmid-encoded AmpC cephalosporinases has been increasing [1]. Moreover, the co-occurrence of ESBLs genetic determinants and genes conferring resistance to other antibiotic classes is frequently reported [2]. This results in limited therapeutic options and/or unsuccessful therapy. This work focused on the characterization of 3rdGC resistant clinical isolates in order to contribute to the development of effective strategies to reduce resistance prevalence

Method

Forty-six 3rdGC-resistant Enterobacteriaceae isolates were obtained in Hospital D. Pedro, Aveiro, and were characterized in terms of genetic diversity, antibiotic resistance genes and mobile genetic elements.

Genotypic diversity was evaluated using BOX-, ERIC and REP-PCR. Clinically relevant genes conferring resistance to β-lactams, fluoroquinolones, sulfonamides and aminoglycosides were searched by PCR, as well as sequences related with gene mobility, like integrase genes and insertion sequences.

Plasmid conjugation assays were performed for $bla_{\text{CTX-M}}^{+}$ and bla_{AmpC}^{+} strains using *Escherichia coli* CV601 as recipient strain. Transconjugants were confirmed by BOX-PCR and detection of $bla_{\text{CTX-M}}$ and bla_{CMY} genes. Plasmid DNA was extracted from donors and transconjugants and replicon typing [3] was performed.

Results & Conclusions

Molecular typing results showed high intra-species variability suggesting a non-clonal dissemination of the ESBL-producers and bla_{AmpC}-carriers.

β-lactamase genes were detected in 45 isolates. bla_{TEM} was the most prevalent gene (98.7%) while bla_{SHV} was the least prevalent (6.5%). bla_{CMY} was the most frequent pAmpC gene, detected in 11 isolates. $bla_{\text{CTX-M-15}}$ was present in 17 isolates and was associated with insertion sequence IS*Ecp1*. Aminoglycosides, trimethoprim, chloramphenicol and streptothricin resistance determinants were identified in the variable regions of class 1 and 2 integrons, found in 34 and 1 isolates, respectively. Conjugative plasmids belonging to groups IncF (n=19 isolates), IncK (n=4), IncB/O (n=2) and IncI1 (n=5), were identified. Mating assays originated 2 and 3 cefotaxime resistant transconjugants carrying $bla_{\text{CTX-M}}$ and bla_{CMY} genes, respectively.

In conclusion, dissemination of resistance to 3rdGC in this hospital was related to the transfer of mobile genetic elements, namely IncB/O, K, I1 and F replicons. Future strategies to control the dissemination of resistance should consider this information.

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Keywords: Antibiotic resistance;, clinical isolates, Enterobacteriaceae, 3rd generation cephalosporins, ESBLs, AmpC, mobile genetic elements, plasmids, integrons

P-272 - CHARACTERIZATION OF ANTIMICROBIAL RESISTANCE IN STAPHYLOCOCCUS EPIDERMIDIS COLONIZING VETERINARY STAFF AND STUDENTS

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Abstract

Staphylococcus epidermidis is one of the main components of the skin microflora of animals and humans. Yet, it is also recognized as an important opportunistic pathogen in human and veterinary medicine. Humans in close contact with animals, particularly veterinary staff, have an increased risk for colonization with animal-associated staphylococci. Nevertheless, information is scarce concerning colonization of this risk group by *S. epidermidis* as well as the genotypes and phenotypes of these bacteria. The aim of this work was to characterize a collection of 112 *S. epidermidis* isolates from nasal colonization in veterinary staff and students.

The entire collection of *S. epidermidis* isolated from nasal swabs of veterinarian doctors, nurses, technicians and students was typed by *Sma*I-PFGE. Antibiotic susceptibility was determined by disk diffusion and biocide susceptibility evaluated by determination of minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs). Efflux activity was detected by determination of ethidium bromide (EtBr) MICs and the EtBr-cartwheel method. Antibiotic resistance determinants and plasmid-encoded efflux pump genes associated with reduced susceptibility to biocides were screened by PCR.

Several *Sma*I-PFGE types and sub-types were observed revealing a fairly heterogeneous population. Amongst the 112 *S. epidermidis* isolates, 60.7 % were methicillin resistant (*mecA*$^{+}$, MRSE) and 58.4 % were multidrug resistant (MDR). We detected high frequencies of resistance to macrolides (50.9%), fusidic acid (45.4%), lincosamides (33.9%), aminoglycosides (26.8%), fluoroquinolones (25.9%) and tetracycline (23.2%). Resistance to rifampicin (4.5%), trimethoprim-sulphamethoxazole (2.7%), chloramphenicol (1.8%) and mupirocin (0.9%) was also detected. A diverse array of resistance determinants was detected, including *blaZ*, *mph(C)*, *erm* genes, *aadD*, *aacA-aphD*, *aph(3')-IIIa*, cat_{pC221}, *fus* genes and *mupA*. MIC/MBC distributions enabled also the detection of significant non-wild-type (NWT) populations of *S. epidermidis* towards several biocides, namely benzalkonium chloride, cetrimide, chlorhexidine, pentamidine, tetraphenylphosphonium, triclosan and the heavy-metals cadmium and arsenate, as well as to the efflux marker EtBr. These NWT populations were correlated with carriage of the plasmid-encoded efflux pump genes *qacA/B* and/or *smr* (38.4%) or with the presence of other efflux unrelated resistance determinants, such as the triclosan resistance determinant *sh-fabI*. Overall, carriage of plasmid-encoded efflux pump genes was statistically associated with MDR phenotypes.

This study reveals a high prevalence of biocide and antibiotic resistant *S. epidermidis* colonizing humans in close contact with animals, highlighting the need of incorporating these commensal bacteria in antimicrobial resistance surveillance, as they can act as vehicles for dissemination of antimicrobial resistant strains from human-to-animal-to-human.

Keywords: Staphylococcus epidermidis, colonization, resistance, antibiotics, biocides

P-273 - CHARACTERIZATION OF PLASMID ENCODED EFFLUX DETERMINANTS FROM STAPHYLOCOCCUS EPIDERMIDIS

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Background
Staphylococcus epidermidis colonizes the skin and mucous membranes of both Man and animals and is also considered an important opportunistic pathogen in human and veterinary medicine. Several antimicrobial resistance determinants described for this bacterium are carried in plasmids. Their localization in mobile genetic elements may facilitate the dissemination of these determinants intra- or inter-species. The aim of this work was to characterize plasmid profiles of *S. epidermidis*, correlating them with the presence of antimicrobial resistance determinants, with emphasis on those coding for efflux pumps (EP).

Method
The study collection consisted of 112 *S. epidermidis* isolates from nasal colonization in veterinary staff and students. Plasmid DNA was isolated from all isolates by a modified alkaline lysis method. Plasmid profiles were determined by digestion with the restriction enzyme *Eco*RI and electrophoretic pattern analysis. PCR was used to screen for plasmid-encoded EP genes associated with resistance to antibiotics (*msrA*, *tetK*, *vga*(A) and *vga*(C)), biocides (*qacG*, *qacJ*) and to the heavy metal cadmium (*cadA*, *cadD)*. The presence of these determinants was correlated with previously established resistance profiles.

Results & Conclusions
All *S. epidermidis* isolates carried plasmids, the majority (74/112, 66%) harboring more than one plasmid. The great diversity of *Eco*RI-patterns encountered revealed a heterogeneous plasmid population, where large plasmids (^{3} 23 kb) were predominant (99/112, 88.4%). PCR screening of EP genes identified the *msrA* gene in 35/57 (61.4%) erythromycin resistant isolates and the *tet*(K) gene in 25/26 (96.2%) tetracycline resistant isolates. The *vga*(A) and *vga*(C) genes were detected in 13/19 (68.4%) and 15/19 (78.9%) isolates with constitutive resistance to clindamycin, and in 3/19 (15.8%) and 15/19 (78.9%) isolates with inducible resistance to clindamycin, respectively. The *cadA* gene was detected in only 3/112 (2.7%) isolates, all displaying high cadmium minimum inhibitory concentrations. The remaining genes screened, *cadD, qacJ* and *qacG,* were not detected.

This work highlights the high diversity of plasmids circulating in commensal *S. epidermidis* and the importance of these elements, particularly of plasmid-encoded EP genes, in antimicrobial resistance phenotypes. It also emphasizes the need to better understand the dissemination pathways of these resistance elements, exploring the link between the human and the animal perspectives.

Keywords: Staphylococcus epidermidis, efflux, antimicrobial resistance, commensal

P-274 - PREVALENCE OF BIOFILM PRODUCTION IN STAPHYLOCOCCUS EPIDERMIDIS COLONIZING VETERINARY STAFF AND STUDENTS

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Background

Staphylococcus epidermidis is a commensal and opportunistic pathogen of humans and animals. Biofilm production is a major virulence factor in this species, frequently associated with resistance to antimicrobials. Albeit the relevance of biofilm in clinical isolates is extensively studied, its importance in commensal isolates is less explored. This work aimed to characterize the biofilm production capacity of 112 S. epidermidis isolates collected from nasal swabs of Veterinary staff and students.

Method

All isolates were characterized regarding their ability to produce biofilm by the Congo Red Agar (CRA) plate method, using a colorimetric scale and by an adhesion assay based on the Crystal Violet (CV) method, applying photometric parameters. For application of the CRA method, several parameters were optimized, namely media, inoculation method, incubation time and a colorimetric scale for categorization of biofilm production. The CV method was performed in flat-bottom 96-well polystyrene plates and biofilm production categorized according to ranges of OD570nm values. The biofilm-associated genes icaAD and aap were screened by PCR.

Results & Conclusions

The following parameters were defined for optimized application of the CRA method: brain heart infusion (BHI) media supplemented with 5% sucrose, 1.5% NaCl, 2% glucose and 0.04% Congo Red; spot inoculation and an incubation time of 24h. Isolates producing red colonies were defined as non-biofilm producers whereas those forming black colonies were classified as biofilm-producers. The screening of biofilm production by CRA identified 26/112 (23.2%) biofilm-producers whereas the CV method enabled the identification of 66/112 (58.9%) biofilm-producers, which were further classified as strong-producers (14 isolates); moderate-producers (20) or weak-producers (32). Overall, the CV method presented an enhanced performance in identifying biofilm-producers.

The icaAD genes were present in 34/112 (30.4%) of the isolates while the aap gene was detected in 82/112 (73.2%). Twenty eight isolates (25%) carried both genes. The CRA method detected biofilm production associated with the genotypes icaAD+aap- and icaAD+aap+ while the CV method detected either combination of both genes. A relationship between genotype icaAD+aap+ and strong biofilm production was observed.

References & Acknowledgments

The results obtained demonstrate that biofilm production is frequent in commensal S. epidermidis and the importance of biofilm-associated genes other than ica in the biofilm production phenotype.

Keywords: Staphylococcus epidermidis, Biofilm, Commensal, Methods

P-275 - TOWARDS THE IDENTIFICATION OF THE VIRULENCE-ASSOCIATED SRNAS FROM THE BURKHOLDERIA CEPACIA COMPLEX

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Background

Small non-coding RNAs emerged in the last years as key regulators of post-transcriptional gene expression in bacteria. Gene expression regulation by sRNAs is exerted at the post transcription level, affecting mRNAs or proteins. Regulation exerted at the level of mRNAs is often carried out with the aid of the protein chaperone Hfq. Regulation can be achieved by blocking the access of ribosomes and/or directing them to degradation, or by enhanced translation of key stress-regulated transcriptional factors [1]. Cases of gene expression activation are also known, resulting from the exposure of the mRNA ribosome binding site upon interaction with sRNA/Hfq [1]. Despite the identification of hundreds of bacterial sRNAs mainly due to high throughput techniques such as RNASeq [2], their roles on bacteria physiology and virulence remain largely unknown. This is the case of bacteria of the *Burkholderia cepacia* complex (Bcc), a group of opportunistic pathogens capable of causing lethal lung infections among cystic fibrosis patients. The aim of the present work is to unveil sRNAs expressed by bacteria of the Bcc when infecting a host.

Method

The nematode *Caenorhabditis elegans* was used as an infection model. A strategy was designed and optimized to recover bacterial RNA from nematodes at the L2 stage of development, infected with *B. cenocepacia* K56-2. After infection of nematodes for a pre-determined period, the nematodes were processed and the infecting bacteria recovered.

Results & Conclusions

Total bacterial RNA was extracted and analyzed concerning their concentration and integrity. Total bacterial RNA was processed to remove ribosomal RNA (rRNA). Samples of bacterial RNA depleted in rRNA were analyzed by RNASeq. Results and bottlenecks of the strategy used to obtain bacterial RNA from infecting bacteria will be presented and discussed.

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Acknowledgements: Funding by FCT-Fundação para a Ciência e a Tecnologia (projects PTDC/BIA-MIC/1615/2014 and UID/BIO/04565/2013) is gratefuly acknowledged. TP acknowledges a grant from the BIOTECnico PhD program funded by FCT (PD/BD/135137/2017). SAS acknowledges a post-doc grant from FCT (SFRH/BPD/102006/2014).

Keywords: small non-coding regulatory RNAs

P-276 - ANTIBIOTIC AND METAL(LOID) TOLERANCE OF PEDOBACTER LUSITANUS AND CLOSELY-RELATED SPECIES

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Background
Pedobacter lusitanus NL19 was isolated from a sludge sample collected from a deactivated uranium mine, Quinta do Bispo (Viseu, Portugal). The sampling area has high levels of metals and radionuclides. Genome analysis of NL19 disclosed several antibiotic resistance-related genes (β-lactamase, efflux pumps, etc) and also genetic determinants related with the resistance to metal(loid)s (arsenic, zinc, cobalt and cadmium).

Method
The main objective of this study was to investigate the antibiotic and metal(loid)s tolerance of *P. lusitanus* NL19 and its four closely-related species *P. himalayensis* MTCC6384^T, *P. hartonius* DSM19033^T, *P. cryoconitis* DSM14825^T and *P. westerhofensis* DSM19036^T as well as the genus type strain *P. heparinus* DSM2366^T.

Results & Conclusions
The results of disk-diffusion susceptibility testing and MIC determination demonstrated that all species are resistant to amoxicillin/clavulanic acid, ampicillin, apramycin sulfate, aztreonam, cefepime, cefoxitin, ceftazidime, cefuroxime, penicillin G, piperacillin, piperacillin/tazobactam, amikacin, gentamicin, netilmicin, streptomycin, tobramycin, ciprofloxacin and colistin. All the species are resistant to ertapenem and the majority were sensible to meropenem and imipenem. The exceptions included resistance to meropenem for *P. westerhofensis* and decreased susceptibility to imipenem for *P. himalayensis*.

The tolerance to metal(loid)s was tested through the determination of the MIC. *P. lusitanus* exhibited higher MICs than the other strains for copper and cadmium and lower MICs for arsenic and cobalt. *P. lusitanus*, *P. himalayensis* and *P. hartonius* were the most tolerant to uranium, zinc and nickel.

Two genes encoding putative β-lactamases were identified in the genome of *P. lusitanus* NL19. The proteins encoded by these genes were considered novel members of class A β-lactamases (LUS-1) and subclass B3 metallo-β-lactamases (PLN-1) due to their low scores with other β-lactamases. In order to understand if these two enzymes had the ability to hydrolyse β-lactams, the genes $bla_{LUS\text{-}1}$ and $bla_{PLN\text{-}1}$ were expressed in *E. coli* (fused to an *E. coli* signal peptide) and MIC to β-lactams were determined. Results indicate that PLN-1 is able to degrade carbapenems.

Our study shows that the multiresistance phenotype identified in *P. lusitanus* NL19 is also observed for its closely-related species. Therefore, strains from *Pedobacter* genus have an unexplored pool of antibiotic resistance genes as well as putative novel antibiotic resistance mechanisms. The tolerance to metal(loid)s was more strain-specific and, therefore, it can be more related to their original environmental niches.

References & Acknowledgments
Thanks to: CESAM support (UID/AMB/50017-POCI-01-0145-FEDER-007638), FCT/MEC through national funds, co-funding by FEDER, within the PT2020 and Compete2020. CC (SFRH/BD/98446/2013) and TC (SFRH/BPD/77900/2011) were supported by FCT.

Keywords: Pedobacter, Antibiotic resistance, Metal(loid)s tolerance, Beta-lactamases

P-277 - EPIDEMIOLOGICAL LINKS OF STAPHYLOCOCCUS SAPROPHYTICUS ALONG THE MEAT PRODUCTION CHAIN

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Background
Staphylococcus saprophyticus is associated with 10-20% of urinary tract infections (UTI) in sexually active women worldwide. It is also found as a colonizer of animals and contaminant of food products. However, it is unknown if infections in hospitals are linked to consumption of contaminated food. We aimed to understand the population structure and the source of *S. saprophyticus* that are pathogenic to human.

Method
We analyzed 207 *S. saprophyticus* isolates from UTI (n=128), slaughterhouse (n=61) and food (n=18) by a combination of phenotypic (antimicrobial susceptibility and biofilm formation) and molecular techniques (Pulsed-field gel electrophoresis (PFGE)). Methicillin-resistant isolates were screened for the presence of *mecA* and *mecC* by PCR.

Results & Conclusions
The 207 *S. saprophyticus* isolates clustered into 18 clonal types and 157 subtypes by PFGE suggesting high genetic diversity. The most prevalent PFGE type was type A comprising 59% of the isolates (n= 123). The same PFGE types were found in isolates from UTI, slaughterhouse environment and food suggesting dissemination among these settings.

Overall, *S. saprophyticus* isolates were highly susceptible to antibiotics. Only seven methicillin-resistant isolates that carried *mecA* were identified in the collection. While 38% of the isolates from UTI were resistant to erythromycin, 50% of the slaughterhouse isolates were resistant to tetracycline. Moreover, *S. saprophyticus* from food and slaughterhouse, when compared to clinical isolates, showed a decreased frequency of resistance to fusidic acid and fosfomycin – considered genetic markers of the species. At least 50% of the isolates in the collection were strong biofilm producers.

This study provides evidence for the dissemination of *S. saprophyticus* within clinical, slaughterhouse and food settings. While animals and/or colonized workers could be the source of contamination in the slaughterhouse, manipulation of contaminated food may also increase the risk of UTI by *S. saprophyticus*. Antimicrobial resistance is low in *S. saprophyticus*, but resistance to methicillin, erythromycin, and tetracycline are emerging.

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Keywords: Population structure, Colonization, Food, Antimicrobial resistance, mecA, Biofilm

P-278 - SCREENING FOR HERPESVIRUS AND CHLAMYDIALES IN MEDITERRANEAN LOGGERHEAD SEA TURTLES

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Abstract

Sea turtles are important components of marine ecosystems and valid indicators of environmentally challenged habitats and ecosystem health [1]. Sea turtles are vulnerable to viral, bacterial and fungal diseases. Herpesviruses are the most studied and were associated with distinct syndromes [2]. Loggerhead turtles are reported to be infected worldwide even if no screenings, nor disease cases, were reported in the Mediterranean. Nevertheless, viral DNA was recently detected from tissue samples of healthy Mediterranean loggerhead turtles [3]. Chlamydiales include pathogens for humans and other animals. They are responsible for different infections in reptiles, yet few reports of naturally occurring chlamydiosis are documented. In sea turtles, only one case was reported as cause of death of hundreds of juvenile green turtles [4]. Thus, this study was aimed at performing a screening for Herpesviruses and Chlamydiales on clinically healthy live *Caretta caretta* from Italy.

Results & Conclusions

Twenty *Caretta caretta,* rehabilitated at the Marine Turtle Research Centre in Portici (SW Italy), were sampled. Two nasal, two oropharyngeal and two conjunctival swabs were collected for each animal. After total DNA extraction, samples were submitted to a pan-Herpesvirus conventional nested PCR method [5] and a pan-Chlamydiales quantitative PCR method [6]. The screening for herpesviruses yielded all negative results. Possible explanations for this finding are: lower susceptibility of Mediterranean loggerhead turtles to herpesviruses; lower viral load, accordingly the viral pathogenesis [3]; lower sensitivity of the test, although it detected different herpesvirus in several animals, because of degenerated primers. Contrarily, Chlamydiales were present in all samples. This finding supports the hypothesis that Chlamydiales could be part of the turtle microbiome and confirms the role of reptiles as natural reservoir for this order [7]. This study improves the characterization of the microbiome of sea turtles, crucial to understand and manage the pathologies of these endangered species.

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This work was supported by CIISA (UID/CVT/00276/2013) FMV/ULisboa, Portugal and by a PhD fellowship shared by the University Federico II and the Stazione Zoologica Anton Dohrn of Naples, Italy.

Keywords: Caretta caretta; Herpesvirus; Chlamydiales; Mediterranean

P-279 - LANTHIPEPTIDES OF ARCHAEA: THE CASE STUDY OF HALOFERAX MEDITERRANEI

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Background

Lanthipeptides are ribosomally synthesized and post-translationally modified peptides (RiPPs). Their modifications include the dehydration of Ser and Thr residues immediately followed by a cyclization reaction between the dehydrated amino acids and Cys to form their characteristic lanthionine (Lan) and/or methyllanthionine (MeLan) amino acids. The catalysis of these reactions is performed by different types of enzymes (LanB, LanM, LanKC and LanL) that define the classes of lanthipeptides. These proteins are encoded in a wide range of bacterial species and have homologues also in Eukarya (LanCL).

Method

The objectives of our work were: i) identify lanthipeptide clusters in the publicly available archaeal genomes, ii) characterize the archaeal lanthipeptide gene clusters and iii) characterize the dehydration and cyclization of archaeal lanthipeptides.

Results & Conclusions

In Archaea, 21 enzymes were identified, all of them belonging to the LanM family (PF05147). However, in archaeal genomes, these *lanM* genes are confined to the class Halobacteria. *Haloferax mediterranei* ATCC 33500 was selected for further characterization since it has three *lanM* genes: one on the chromosome and the other two on a plasmid. At least one gene encoding a putative lanthipeptides (*lanA*) was detected in the genetic neighbourhood of each *lanM*. Other *lanM*-neighbouring genes encode ABC-like transporters, but none are the SunT-type (LanT) that are classically involved in the processing of LanM-modified lanthipeptides. Three expression vectors were constructed, each containing the *lanM* and *lanA* genes of the same cluster. The *lanA* gene was cloned in order to produce a His_6 –LanA peptide. Each plasmid was used to produce the lanthipeptides in *E. coli*. After IMAC purification and MALDI analysis, only non-dehydrated peptides were identified, indicating that archaeal LanM enzymes are not functional in *E. coli.* Most probably, archaeal LanM enzymes require high levels of salt due to the halophilic nature of *Haloferax mediterranei.* To overcome this hurdle, three novel plasmids were constructed to allow the expression of *lanM* and *lanA* genes in the model archaeon *Haloferax volcanii*.

In conclusion, Archaea, in particular halobacteria, encode lanthipeptide enzymes that are homologous to those found in Bacteria and Eukarya. In Bacteria, these peptides commonly have antibacterial activity. If archaeal lanthipeptides are archaeocins, these peptides will be a valuable tool for the development of novel selective markers for Archaea. This will be highly relevant to improve the tools involved in their genetic manipulation procedures.

References & Acknowledgments

Thanks to: CESAM support (UID/AMB/50017-POCI-01-0145-FEDER-007638), FCT/MEC through national funds, co-funding by FEDER, within the PT2020 and Compete2020. TC(SFRH/BPD/77900/2011) was supported by FCT.

P-280 - GROWTH MEDIA EFFECT ON THE PRODUCTION OF PEDOPEPTINS BY THE NEWLY DESCRIBED SPECIES PEDOBACTER LUSITANUS NL19

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Background

NL19, the type strain of the recently described species *Pedobacter lusitanus*, exhibits significant antibacterial activity against several relevant pathogenic bacteria, including methicillin resistant *Staphylococcus aureus* (MRSA). This bioactivity was first identified in tryptic soy agar (TSA). When grown in its equivalent broth (TSB100%), no activity was identified. However, antibacterial activity is detected when cells are grown in 4 fold diluted TSB (TSB25%).

Method

The main goals of this study were: i) to identify which component of TSB100% medium was responsible for inhibiting the production of antibacterial compounds, ii) identify the peptides responsible for the antibacterial activity, iii) identify the genes involved in their biosynthesis, and iv) understand if NL19 closely-related strains also exhibit antibacterial activity.

After testing the effect of the dilution of each of the TSB components on the antibacterial activity, we concluded that repression of activity was induced by peptone from casein (PC). Therefore, the constituents of *P. lusitanus* NL19 supernatant grown in TSB containing only 25% of PC (PC25%) were separated by HPLC. LC-ESI-MS analysis of HPLC fractions revealed that antibacterial activity was due to pedopeptins, which are a mixture of three structurally identical peptides produced by a *Pedobacter* spp. that are lipopolysaccharide inhibitors. The nonribosomal peptide synthetase genes putatively involved in pedopeptins biosynthesis were identified in the genome of *P. lusitanus* NL19 (*ped_nrps1* and *ped_nrps2*). Results show that the production of pedopeptins is affected by the concentration of PC. Therefore, we investigated the expression levels of the genes *ped_nrps1* and *ped_nrps2* in TSB100% and in PC25% media through absolute quantification by real time PCR. The results confirm that in TSB100% these genes are down-regulated. The production of pedopeptins by *P. lusitanus* NL19 closely-related species *P. himalayensis* HHS22^T, *P. cryoconitis* DSM14825^T, *P. hartonius*WB3.3-3^T, *P. westerhofensis* WB3.3-22^T and by the genus type strain *P. heparinus* DSM2366^T was also evaluated. However, no antibacterial activity was observed.

Results & Conclusions

Our study confirms that pedopeptins are nonribosomal antibacterial peptides which are not produced by all *Pedobacter* spp. strains. In addition, we show that the production of antibacterial compounds can depend on the type of cultivation (liquid vs solid), even using the same proportions of carbon and nitrogen sources. Pedopeptin production can be inhibited due to transcription repression of *ped_nrps* genes, caused by high concentration of PC.

References & Acknowledgments

Thanks are due, to FCT/MEC through national funds, for the financial support of CESAM (UID/AMB/50017–POCI-01-0145-FEDER-007638). The authors acknowledge FCT financial support to TC(SFRH/BPD/77900/2011), CC(SFRH/BD/98446/2013), AA(IF/00835/2013) and AE(BPD/102572/2014).

P-281 - TWO NEW SPECIES OF RHODOBACTER ISOLATED FROM WATER OF A FORMER URANIUM MINE

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Background

During an evaluation of the microbial community structure of a former uranium mine, a large number of bacterial strains were isolated from water samples of the mine. Phylogenetic characterization based on the 16S rRNA gene sequences showed that among the isolates, three white-pale pigmented strains, designated 7B-409, 7A-375 and 7A-384, were closely related to the members of the genus *Rhodobacter*. Further genomic, physiological and chemotaxonomic characterization showed that these isolates represented two new species within the genus *Rhodobacter*.

Method

Growth in different temperature (4-45°C) and NaCl concentration (0, 1, 3, 5, and 10%, w/v) ranges were tested using R_2A for 7 days. The pH range for growth was examined at 25°C over a pH range from 5 to 10. Strains ability to use different carbon sources and enzyme activities were determined with the Biolog GN2 system, API 20NE and API ZYM galleries according to the manufacturer's instructions. Analysis of polar lipids and lipoquinones were performed as described previously [1]. Fatty acid methyl esters were separated, identified and quantified with the standard MIS Library Generation Software [1]. The 16S rRNA gene was amplified by PCR and sequenced and the phylogenetic analysis was performed using Mega 7 software.

Results & Conclusions

The phylogenetic analysis of the 16S rRNA gene sequences shows that strains 7B-409 and 7A-375 cluster together and share a similarity of 100% in the 16SrRNA sequences. Strain 7A-384 cluster together with *Rhodobacter blasticus* and share 97% of 16SrRNA sequence similarity. These three Urgeiriça strains are also closely related sharing about 96.8% of sequence similarity. However, several characteristics distinguish these two groups of strains from each other and from the most closely related species. In the fatty acid composition of strains 7B-409 and 7A-375, the relative amounts of the fatty acid 16:0 is higher (±13%) when compared to strain 7A-384 (±1.5%) and the fatty acid 18:1 w7c 11-methyl is present only in the former strains. The presence of a unknown glycolipid in the lipid profile of strains 7B-409 and 7A-375 and an unknown aminolipid in strain 7A-384 are also features that distinguish these isolates. Based on these chemotaxonomic differences, on the 16S rRNA gene sequence analysis and also in carbon source assimilations, we propose two new species of the genus *Rhodobacter*, *Rhodobacter mucilaginosus* for strains 7B-409^T and 7A-375 and *Rhodobacter albus* for strain 7A-384^T.

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Project ReNATURE (Centro-01-0145-FEDER-000007); project ERA-MIN/0002/2015 FCT. R.B was funded by SFRH/BPD/110807/2015.

Keywords: Rhodobacter sp., Taxonomy, New species

P-282 - CARBAPENEM-RESISTANT ACINETOBACTER BAUMANNII IN PORTUGAL - CLONAL DYNAMICS EXPLAINED BY WGS

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Background

Carbapenem-resistant *Acinetobacter baumannii* (*Ab*) strains have become endemic in many European countries, including Portugal, where particular clones have dominated over the years (e.g.OXA-40-producer ST98 until 2006, and then the worldwide disseminated OXA-23-producer ST208). Recent data (ECDC,2016) confirms a high prevalence (>50%) of carbapenem-resistant *Ab*-infections in Portugal, although we lack information regarding recent clones. This study aims to characterize clonality of recent carbapenem-resistant *Ab* isolates from Portuguese nosocomial and community settings and to explore the antimicrobial resistance and virulence features contributing for the success of particular lineages.

Method

A total of 177 *Ab* isolates (2010-2015) from two hospitals (H, CB), a Long-Term Care facility (LTCF) and a community clinical laboratory (CT) were studied. Antibiograms and Blue-Carba (search for carbapenemases) were performed. Clonality was assessed by FTIR-spectroscopy coupled with multivariate analysis-PCA and representative isolates of epidemic (8) and sporadic clones (4) were submitted to WGS (Illumina HiSeq 2000). Assembly was performed using SPAdes and annotation by RAST/Geneious. Antibiotic resistance genes and virulence factors were searched using Resfinder/VFDB.

Results & Conclusions

FTIR revealed two well-delimited clusters: cluster I (ST218), and cluster II (ST208), while sporadic STs were presented as dispersed and unrelated. ST218 (single locus variant of ST208) was the most disseminated clone (115/177), being found in two hospitals and in the community, while ST208 was only observed in hospital-H with a decreasing trend over the years (44/177). WGS revealed genomes with a medium size of 3.9Kb and 38.9% GC content. No plasmids carrying antibiotic-resistance genes were found among ST208/ST218. All ST218 isolates and all but one isolate belonging to ST208 were XDR, OXA-23-producers (Tn*2006*; chromosomal location) and Blue-Carba positive. ST218 revealed higher levels of aminoglycoside resistance (confirmed by the detection of additional genes such as *aacA4*, *aadA1* and *armA*), and enriched virulence content (e.g. presence of *hemO* gene in ST218). These findings may justify the exchange of ST208 by ST218 observed in these clinical settings. Sporadic STs (18/177; e.g.ST234/ST552/ST1557/ST1558) were more susceptible to antibiotics (MDR=11%), with only one OXA-24-producer isolate from LTCF [plasmid-location (pMMCU3-99%Ident)]. Besides, they lacked several putative virulence operons present in ST208 and ST218.

This work provided new data regarding the molecular evolution of *Ab* clones in Portugal, unveiling the possible reasons for the replacement of ST208 by ST218 (higher antibiotic resistance and an enlarged virulence potential). Moreover, the data presented here, by unveiling features contributing for the circulation and persistence of these clones may aid in future prevention and/or treatment strategies.

References & Acknowledgments

Acknowledgments: SFRH/BD/88028/2012,SFRH/BPD/95556/2013,SFRH/BD/132497

Keywords: Acinetobacter baumannii, Clones, Virulence

P-283 - MOLECULAR CHARACTERIZATION OF BOVINE MASTITIS ENTEROBACTEREACEAE ISOLATES FROM ENTRE-DOURO E MINHO, PORTUGAL, DAIRY FARMS AND SCREENING OF ANTIBIOTIC RESISTANCE GENES

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Background

Bovine mastitis are usually caused by bacteria and origin a negative economic impact on milk production [1]. Therefore, a good identification and characterization of the microorganisms in its origin and a selection of the antibiotics for their treatment are essential. The objective of this study was to characterize phenotypic and genotypically 29 isolates suspected to be *Klebsiella* spp. (23 dairy farms, Entre-Douro and Minho region, Portugal, years 2008, 2009 and 2017); evaluate the antimicrobial susceptibility against selected antibiotics and characterize molecularly antibiotic resistance genes in these isolates. An *Klebsiella pneumonia*-ATCC 13883 was also included in the study.

Method

Phenotypic characterization was performed through the description of grown colonies on MacConkey Agar plates, Gram and capsule staining. Mobility, indole production and the hydrogen sulphide formation tests were also performed. Molecular characterization was performed for the 16S rRNA region by PCR [2], followed by the sequencing of the amplified amplicons. Antimicrobial susceptibility tests were performed by the disk diffusion method and detection of the bla_{TEM}, bla_{CTX-M} and bla_{KPC-15} antibiotic resistance genes by PCR [3-5].

Results & Conclusions

Two *Klebsiella pneumoniae*, 1 *Klebsiella oxytoca*, 18 *Escherichia coli*, 1 *Cronobacter sakazakii*, 2 *Serratia marcescsens*, 2 *Enterobacter cloacae*, 1 *Enterobacter hormaechei*, 1 *Enterobacter* sp. and 1 *Citrobacter koseri* strains were identified. A phenotypic resistance of 23%-ampicillin, 19%-ticarcillin, 16%-cefazolin, 16%-kanamycin, 16%-amoxicillin+clavulanic acid, 16%-trimethoprim-sulfamethoxazole, 10%-tetracyclin, 3%- tobramycin and 3%-chloramphenicol was shown. The bla_{TEM} gene was detected in 4 *Escherichia coli* and in 1 *Serratia marcescens* strains. The bla_{CTX-M} gene was detected in 1 *Escherichia* coli strain. The bla_{KPC-15} gene was not detected. We conclude that, for a more accurate identification of the microorganisms present in bovine mastitis milk, the complementarity between phenotypic and molecular characterization is advisable, since alone they did not allow us to obtain conclusive results. Comparing the oldest and the most recent isolates, the number of the antimicrobial resistances increased, probably due to the excessive use of antibiotics in mastitis treatment.

References & Acknowledgments

The authors acknowledge to Dr. Joana Correia, CAVC, Vila do Conde, Portugal, for providing recent mastitis milk isolates and to Dr. Helena Ramos, Hospital Santo António-CHP, Porto, Portugal, for providing the *K. pneumonia* ATCC 13883.

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Keywords: Klebsiella spp., Enterobacteriaceae, bovine mastitis, antimicrobial resistance, 16S rRNA, blaTEM, blaCTX-M, blaKPC-15

P-284 - REGULATING THE TERMINATOR: ROLE OF SALMONELLA SRAL SRNA IN THE EXPRESSION OF RHO MRNA

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Abstract

RNAs are important effectors in the process of gene expression. In bacteria, the levels of the transcripts must be rapidly adjusted in response to constantly changing environmental demands. Several factors modulate RNA degradation, namely small non-coding RNAs (sRNAs) that are crucial regulators of gene expression, and can directly modulate the expression and/or stability of their targets.

SraL is a sRNA whose expression is directly controlled by σ^S (RpoS) and it is induced in several stress conditions namely stationary phase, anaerobic shock and SPI-2 (*Salmonella* Pathogenicity Islands) inducing conditions (Silva IJ et al 2013). The chaperone Trigger Factor was described as its first biological target in *Salmonella* Typhimurium. SraL was shown to interact with the 5′-UTR of the target mRNA down-regulating its expression (Silva IJ et al 2013).

Results & Conclusions

In the current work, we have used MAPS technology (MS2-affinity purification coupled with RNA sequencing) to uncover other targets of SraL sRNA. Contrarily to what happens with trigger factor, SraL was shown to up-regulate the mRNA expression of a crucial protein for transcription termination. Using bioinformatic tools and mutagenesis experiments, we have shown that SraL directly interacts with the 5′-UTR of this mRNA target. Moreover, we have elucidated the mechanism of action of SraL sRNA to control this important target.

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This work was financially supported by: Project LISBOA-01-0145-FEDER-007660 (Microbiologia Molecular, Estrutural e Celular) funded by FEDER funds through COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI) and by national funds through FCT - Fundação para a Ciência e a Tecnologia"; Project PTDC/BIA-MIC/1399/2014 also through FCT; European Union's Horizon 2020 Research and Innovation Programme [grant agreement no. 635536]. I. J. Silva [SFRH/BPD/84086/2012] was recipient of a FCT Post-Doctoral Fellowship.

Keywords: RyjA, small RNA, premature transcription termination, Rho factor

P-285 - BOLA IS A TRANSCRIPTIONAL SWITCH THAT TURNS OFF MOTILITY AND TURNS ON BIOFILM DEVELOPMENT

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Abstract
Microbes are capable of an extraordinary range of adaptations to challenging environments, making them extremely versatile organisms. In face of stress many bacteria commonly turn off flagella formation and switch to a sessile state of growth activating the production of fimbriae and extracellular polysaccharides. This process frequently leads to the formation of matrix-encapsulated communities of microorganisms named biofilms. BolA is a morphogen involved in several cellular processes, namely in the establishment of such microbial communities.

Results & Conclusions
Using High-throughput technologies we have shown that BolA protein is a new bacterial transcription factor, involved in the negative and positive regulation of several important genes. Interestingly, this protein plays a key role in the switch between motile and sessile lifestyles. BolA represses flagellar biosynthesis with severe consequences for bacterial swimming capacity, and enhances biofilm development, through fimbriae-like adhesins and curli production. Moreover, our results in *Escherichia coli* indicate that BolA modulates biofilm development through a complex network involving c-di-GMP, one of the most important players in the regulation of this pathway. We are also investigating the role of BolA in bacterial persistence and its role in virulence. We want to further decipher the role of BolA, this important transcription factor specifically involved in the transition between the planktonic and biofilm life-style.

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This work was supported by: Project LISBOA-01-0145-FEDER-007660 (Microbiologia Molecular, Estrutural e Celular) funded by FEDER funds through COMPETE2020 – Programa operacional Competitividade e Internacionalização (POCI). This work has also received funding from the FCT Project ref: PTDC/BIA-MIC/4046/2014.

Keywords: BolA, Biofilm, Flagella, Transcriptional factor

P-286 - INSIGHT INTO THE IMPACT OF BOLA IN THE METABOLIC NETWORK OF SALMONELLA TYPHIMURIUM THROUGH NMR METABOLOMICS

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Background

BolA is a global transcription factor with both activator and repressor functions. Initially found in *Escherichia coli,* the *bolA* gene is induced at the start of the stationary phase and in response to a variety of stress agents. Its expression results in significant physiological changes, such as acquisition of a spherical shape by cells, modulation of membrane permeability, and promotion of biofilm development, all of which are important for adaptation to stressful environmental conditions [1]. Despite considerable progress towards understanding the role of BolA as a major cell regulator, further research effort is needed to uncover the molecular interactions underlying its mode of action. Preliminary results of our group indicate that BolA is involved in the pathogenesis of enteric bacteria. In this study, we want to assess the impact of different levels of expression of the *bolA* gene in the metabolic network of *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium), an important human pathogen.

Method

An NMR-based metabolomics approach is used here. Three strains of *S.* Typhimurium were studied: wild-type (wt); a *bolA* knockout mutant (Δ*bolA*); and an overexpressing strain of *bolA* (pWSK29-*bolA* designated as *bolA*+). These strains were grown in minimal, virulence-inducing medium, as well as in rich medium [2]. Cell sampling was performed at the early and the late stationary phases; metabolites were extracted with a mixture of methanol:chloroform:water and analyzed by NMR. A multivariate analysis of the ^{1}H-NMR data was conducted to identify the unique metabolic features brought about by BolA.

Results & Conclusions

Interestingly, the three strains showed different growth profiles in the virulence-inducing medium. Specifically, *bolA*+ reached the highest optical density, followed by the wild-type and the Δ*bolA* strains. This result denotes a beneficial effect of BolA on cell physiology when conditions become harsher. Furthermore, multivariate analysis of metabolomics data was able to discriminate between the metabolic profiles of these strains. The metabolic pathways that are modulated by BolA will be presented and the contribution of our results to clarify the role of BolA in virulence will be discussed.

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GGL was supported with a grant in the framework of Project LISBOA-01-0145-FEDER-007660

Insight into the impact of BolA in the metabolic network of Salmonella Typhimurium through NMR Metabolomics

Keywords: BolA, Salmonella, NMR, Metabolomics

P-287 - EVIDENCE FOR THE TRANSMISSION OF ST398 METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) FROM PIGS TO HUMANS ALONG THE FOOD PRODUCTION CHAIN

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Background

MRSA is one of the most important antibiotic-resistant pathogens in the hospital and community and can colonize and cause infections in animals in veterinary settings. Pigs have been described as an important reservoir of ST398 MRSA. Although until recently, ST398 MRSA was strictly confined to veterinary settings, cases of human ST398 MRSA infection have been increasingly reported. Pork is the most consumed meat in Portugal and worldwide, but it remains to be clarified if ST398 MRSA can be transmitted through the food processing chain. We aimed to understand if MRSA colonizing pigs could be transmitted to meat and humans during pig processing in slaughterhouses.

Method

In total, 75 samples were collected in 2016 from a slaughterhouse in Lisbon metropolitan area at different working days and different sites including pigs (n=30), meat (n=26), equipment (n=8) and operators (n=11). MRSA were isolated on CHROMagar MRSA and screened for beta-lactam resistance (*mecA* and *mecC*) and biocide resistance genes by PCR. Species identification was confirmed by PCR amplification of the *nuc* gene and antimicrobial susceptibility was tested by disk diffusion method against 17 antibiotics. Isolates were characterized by PFGE, *spa* and SCC*mec* typing. Virulence factors were screened by PCR, including ACME, leucocidins, haemolysins, super-antigenic toxins, toxic shock syndrome toxin, enterotoxins and IEC.

Results & Conclusions

A high prevalence (60%) of MRSA was found in live pigs. MRSA was also found in meat (23%), clean and washed hands of operators (40%), clean conveyor and dirty cutting table (38%). The majority of strains belonged to *spa* type t011 (58%) - associated to ST398 - and carried SCC*mec* type V (55%). High prevalence of resistance to tetracycline and clindamycin (100% each), erythromycin (76%), chloramphenicol (65%) and gentamicin (61%) was observed and biocide resistance genes were detected: *mepA* (87%), *sepA* (71%), *norA* and *lmrS* (61% each), and *qacAB* (52%). Almost all strains carried virulence genes *hlb*, *hlg* and *sel* (65% each), but did not have ACME, PVL and IEC. MRSA strains were grouped in a major PFGE cluster (with 14 subtypes), but the same MRSA ST398 strain, as defined by PFGE subtype, was identified in animals, meat samples, equipment and workers.
The results suggest that multidrug resistant ST398 MRSA with potential virulence is frequently transmitted from pigs to pork meat, slaughterhouse environment and humans by cross contamination. Resistance to biocides particularly disinfectants in use, might be the cause of MRSA persistence in the slaughterhouse environment.

Keywords: MRSA, ST398, Transmission, Food chain production, Slaughterhouse

P-288 - UNDERSTANDING THE METABOLIC PROCESSES THAT SHAPE THE ADAPTATION OF ESCHERICHIA COLI TO THE MOUSE GUT

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Background

The mammalian gastrointestinal tract is colonised by many microorganisms, namely bacteria. Interactions established among these organisms, their host and the nutritional environment of the gut form what has been termed the "lost organ". Renewed interest in understanding forces shaping this "organ" and enormous genetic and metabolic flexibility bacteria possess, have brought about the drive and potential to engineer bacterial communities to promote human health and improve industrial or biotechnological processes. However, in such environments structuring artificial communities in a stable and predictable way is still underdeveloped. This study aims to identify genetic targets and physiological mechanisms for colonisation and evolutionary adaptation to mammalian gut in a prevalent microbiota member, *Escherichia coli*. The goal of this study is understanding how these processes are shaped by the metabolic environment/microbiota complexity.

Method

Previously, we studied adaptation of *Escherichia coli* K-12, a human intestinal strain, as single species in Germ-free mice (mono-colonisation) and in polymicrobial community (complex microbiota). Whole Genome Sequencing identified potential adaptive targets. Here, we established new phenotypic assays to characterise the effects of key mutations and metabolomics was performed with 1H-NMR of intestinal contents.

Results & Conclusions

We previously identified several beneficial adaptive targets of *E. coli* to the mouse gut in the presence of a complex microbiota (Barroso-Batista et al, 2014). Here, we identified mutations selected during mono-colonisation to determine the process of adaptation in the absence of microbiota. Ability to metabolise a sugar alcohol was the only target common to both mouse models, providing evidence for remarkable specificity. Facing complex microbiota *E.coli* adapted for use of sugars (*srlR, kdgR*) and anaerobic respiration (*dcuB, focA*) (Barroso-Batista et al, 2014), whereas in mono-colonisation mutations instead pointed to increased ability to use protein/aminoacids (*lrp, dtpB, frlR*). The beneficial effect of those strains correlated with metabolomics data: our working hypothesis is that oxygen depletion and breakdown of complex sugars by other members of the microbiota limit *E.coli* to metabolise simple by-product carbon sources through anaerobic respiration. In the opposing scenario, absence of microbiota-derived by-products and excess of protein/aminoacids are favoured as colonisation factors. Through experimental evolution we gained insight on how to shape the metabolic traits of *E.coli* through genetic engineering to better colonise specific host environments. This work also highlights the versatility of *E.coli* and its potential as a biotic sensor.

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Keywords: Microbiota Evolution Metabolism

P-289 - UNVEILING THE ROLE OF MULTICOPPER OXIDASES IN PATHOGENIC GRAM POSITIVE BACTERIA

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Background

Copper is an essential trace element required by all organisms, as a structural and/or catalytic cofactor in several enzymes. However, free copper ions are highly toxic due to their ability to generate reactive oxygen species, via Fenton-type reactions, which damage lipids, DNA and proteins [1]. Thus, it is crucial for any organism to tightly control the intracellular concentrations of copper ions, a role played by dedicated homeostasis systems, including copper-transporting P-type ATPases, copper chaperone and, more recently identified, the multicopper oxidases (MCO) that are present in several microorganisms, mainly Gram-negative [2,3,4]. Copper derivatives are commonly used as feed supplements for livestock and as bactericidal agents. Furthermore, copper is also used by the host immune system to prevent infection, present at higher levels in the brain and lungs, organs that are infection sites of *S. aureus* (meningitis and pneumonia).The emergence and transfer of multidrug resistance genetic elements that also carry heavy metal resistance genes is increasing and is particularly relevant for multiresistance development in *Staphylococcus aureus*.

The aim of this study is to clarify the role of multicopper oxidases in copper resistance of different strains of *S. aureus* and *Enterococcus faecium*.

Method

The multicopper oxidase genes were cloned for heterologous expression in *Escherichia coli* and purified to homogeneity. The biochemical and kinetic properties are currently being determined. In parallel, the minimal inhibitory concentration (MIC) to copper and other heavy metals are being estimated for the different *S. aureus* strains, for aerobic, microaerobic and anaerobic growth conditions.

Results & Conclusions

The *S. aureus* and *E. faecium* MCO heterologously produced present copper oxidase activity. The copper MIC is strain-dependent in *S. aureus* and the presence and localization (chromosome/plasmid) of the operon that contains the MCO is associated with the copper resistance level. The impact of copper in bacterial growth is higher under anaerobic conditions.

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Acknowledgments: Financial support from UCIBIO/REQUIMTE Lisbon-Oporto project UID/Multi/04378/2013 (PI Sofia Pauleta).

Keywords: Staphylococcus aureus, Enterococcus faecium, Multicopper oxidase, Copper resistance

P-290 - STRUCTURE OF THE FECAL ESCHERICHIA COLI POPULATION AMONG HEALTHY BITCHES IN ESTRUS

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Abstract

Pyometra is a common diestrous disease of bitches. *Escherichia coli* is isolated from the uterus of up to 90% of bitches with pyometra, being mainly assigned to phylogenetic group (PG) B2, and characterized by a high number of uropathogenic *E. coli* virulence factor (VF) genes (1). This may be seen in the light of the "*special pathogenicity theory*". However, as these *E. coli* isolates derive from the host's fecal and perineal microbiota (2), the "*prevalence theory*", needs further attention.

The main objective of this study was to characterize fecal *E. coli* population for clonal identity and phylogenetic background.

Rectal swabs were collected from 30 bitches (mean age 3.7 ± 0.33 years) in estrus. Up to 10 colonies per sample of suspected *E. coli* were randomly picked, and confirmed by PCR screening for the presence of *E. coli* 16S rRNA. A total of 285 *E. coli* fecal isolates were obtained and analyzed. Phylogenetic group and clonal relationships among *E. coli* isolates were assessed by PCR and REP-PCR, respectively as described by (3) and (4). Results were analyzed by Z test.

Results & Conclusions

The 285 isolates were discriminated in 103 *E. coli* clones, which were assigned to PG B2 (33%), B1 (26%), A (23%), and D (17%). A total of 26 dominant clones were found, from which 46.2% were from PG B2. The majority (75%) of B2 dominant clones were β-hemolytic ($p < 0.05$). PG B2 was significantly more prevalent in pauciclonal (65%) than multiclonal (25%) samples ($p < 0.01$).

Results suggest that the prevalence and the special-pathogenicity hypothesis can be complementary. Besides host susceptibility, the structure of the intestinal bacterial population may play an important role as a trigger of an *E. coli* infection episode and the development of pyometra.

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Funding: UID/CVT/00276/2013

Keywords: Escherichia coli, Canine, Phylogenetic group, Clonal analysis

P-291 - WITHIN-HOUSEHOLD SHARING OF FECAL AND VAGINAL ESCHERICHIA COLI BETWEEN HEALTHY FEMALE DOGS

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Abstract

In the bitch, *Escherichia coli* strains isolated from the vagina and uterus derives from the host's fecal and perineal microbiota (1). These isolates are mainly assigned to phylogenetic group (PG) B2 and characterized by a high number of uropathogenic *E. coli* virulence factor genes (2). PG B2 *E. coli* seems to be better adapted to vagina than *E. coli* from other PG. We hypothesized that PG B2 *E. coli* would be more likely to be shared between dogs from the same kennels than other PG commensal *E. coli*.

The objective of this study was to determine the prevalence of within-household sharing of fecal and vaginal *Escherichia coli* clones between healthy female dogs.

Rectal and vaginal swabs were collected from 18 female dogs belonging to 8 different dog kennels (K). A total of 4 samples per dog were obtained, one vaginal and fecal sample at two sampling time points (estrus and diestrus). Up to 10 colonies per sample of suspected *E. coli* were randomly picked, and confirmed by PCR screening for the presence of *E. coli* 16S rRNA. Phylogenetic group and clonal relationships among *E. coli* isolates were assessed by PCR and REP-PCR, respectively as described by (3) and (4).

Results & Conclusions

Within-household sharing of *E. coli* occurred in 7 kennels comprising 16 bitches. A total of 429 isolates were discriminated in 125 different clones with only 15 clones shared between dogs (1-4 clones/kennel). The identified shared clones represent a total of 197 isolates, exhibited diverse phylogenetic backgrounds. In 2 kennels (K1, K3), only one clone was shared between 2 hosts. In 3 kennels, two clones were shared between 2-3 hosts. In two (K2, K7) of these kennels, the shared clones were both from PG B2. In the remaining 2 kennels with more than one shared clone, isolates were assigned to different phylogenetic groups. In K4 and K5, 4 and 3 clones were shared between two hosts; respectively. Only PG B2 clones (4 in 7) were shared within fecal and vaginal samples at the two time points (K2, K3, K4) thus representing 59.8% of shared isolates (109/182).

Within-household sharing of *E. coli* detected in this study suggests that both direct contact and environmental reservoirs may be routes of sharing of bacteria. The fact that only E. coli from PG B2 were shared between bitches and within the 4 sampling points reflects the higher virulence potential and the strong colonization capacity associated with this PG.

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Funding: UID/CVT/00276/2013

Keywords: Escherichia coli, Within-household sharing, Female dog

P-292 - A NEW STAPHYLOCCOCAL PROTEIN SYSTEM INVOLVED IN SURVIVAL TO CELL WALL DAMAGE

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Background

The *murF* gene product catalyzes an essential step of peptidoglycan biosynthesis. However, we obtained a *murF* insertional mutant that, although harboring the lethal damage, is able to survive (1). Transcriptional analysis of this *murF* mutant showed an altered expression profile with the up-regulation of two small consecutive ORFs, coding for hypothetical proteins, exclusively conserved in staphylococcal species. Downstream of these two proteins is encoded one protein with 9 transmembrane domains.

We hypothesize that these 3 proteins may form a new three-component system and have an important role in *Staphylococcus* survival and cell integrity. These proteins were named ScwdA, ScwdB and ScwdC for Staphylococcal factor involved in cell wall damage response.

Method

To functionally address the proteins, we characterized ScwdA-B interaction through bacterial two-hybrid system and co-elution strategies; protein-DNA interactions are being addressed through EMSA assays. *S. aureus* overexpression and knock-out mutants for *scwdA-B* genes were characterized regarding the impact on growth and on antibiotic resistance. Structural characterization of ScwdB is underway by NMR spectroscopy.

Results & Conclusions

Results showed that the two proteins do not interact and that ScwdA is able to bind to DNA. This interaction is being further characterized.

The overexpression of *scwdA-B* genes resulted in a growth rate decrease and in a lag phase extension, suggesting a thoroughly regulation of the system. However, overexpression of the *scwdA* gene seems to confer an advantage to bacteria grown in the presence of bacitracin, an inhibitor of the peptidoglycan lipid carrier. These results suggest that ScwdC could sense a damage in the cell wall or the membrane and activate a signal cascade that starts by the activation of ScwdB which, in turn, activates ScwdA that can act as a response regulator, mediating transcription. This hypothesis is reinforced by the identification of an amphipatic helix in ScwdB. Amphipathic helices show a distribution of hydrophilic and hydrophobic aminoacids that is well suited for membrane binding.

The ^{1}H-^{15}N HSQC spectra showed that ScwdB is unstructured in solution and the spectra is affected in the presence of the membrane mimetics SDS and TFE. We are currently addressing the interaction of ScwdB with the membrane by acquiring HSQC spectra in the presence of membrane fractions extracted from *S. aureus* cells.

References & Acknowledgments

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This work was funded by FEDER funds through the COMPETE 2020 Program and National funds through FCT under Projects nº POCI-01-0145-FEDER-007688, Reference UID/CTM/50025; POCI-01-0145-FEDER-07728,FCT/MEC/UID/Multi/04378/2013 and PTDC/FIS-NAN/0117/2014.

P-293 - EMERGENCE OF MCR-1 AMONG CARBAPENEMASE-PRODUCING ENTEROBACTERIACEAE IN THE CLINICAL SETTING

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Abstract

Background:
The use of polymyxin as a last line of defence against infections caused by multi-drug resistant Gram-negative pathogens is being seriously challenged by the emergence of plasmid-borne colistin resistance genes (*mcr*). Herein, we report the emergence of *mcr-1* among carbapenemase-producing *Enterobacteriaceae* from asymptomatic carriers and infection sites in hospitalized patients in Portugal.

Method:
Carbapenemase production was detected by selective screening media (Brilliance CRE, Oxoid) and real time PCR (Xpert Carba-R, Cepheid). Presence of *mcr-1*, $bla_{CTX\text{-}M\text{-}I}$ and bla_{KPC} was screened by PCR and sequencing. Antibiotic susceptibility profiles of *mcr-1* positive isolates were determined by disk-diffusion or the reference broth microdilution method (for colistin) according to EUCAST guidelines (EUCAST x2). Clonal relatedness was evaluated by Fourier transform infrared (FT-IR) spectroscopy, multilocus sequence typing (MLST) and *wzi* capsular typing for *Kp* isolates. Whole genome sequence of representative ST45 *K. pneumoniae* was performed by Illumina HiSeq (2x150bp paired-ended reads/coverage 100x), and genomes assembled using SPAdes and further annotated by Prokka. Plasmid replicon content was evaluated by PCR, as described.

Results & Conclusions
A total of twenty-five *mcr-1* carrying isolates (24 *K. pneumoniae*, 1 *E. coli*) were identified as colonizers (n=18, 72%) or causing infections (n=7, 28%) among 16 hospitalized patients between September 2016 and February 2017. They represent 6.8% of all carbapenemase producers identified among hospitalized patients in our geographic region. Isolates carried *mcr-1.1*, were resistant to colistin (MIC between 4 and 8 mg/L), and all except the *E. coli* isolate produced KPC-3 and most CTX-M-15. All *K. pneumoniae* isolate belonged to ST45 and carried *wzi*101/K24, except one (ST1112). WGS showed additionally that *mcr-1* (absence of IS*Apl1*) was located within a 33.546bp IncX4 plasmid, identical to that previously reported in Portugal. Additional resistance genes encoding resistance to aminoglycosides (*aac(6')Ib-cr*, *aac(3)-IIa*), beta-lactams ($bla_{SHV\text{-}1}$, $bla_{OXA\text{-}1}$) and fluoroquinolones (*qnrB66*, *aac(6')Ib-cr*) *oqxAB*, *catA1*, *tet(A)* were identified. Other replicons present were N, FIIk and FIA. The *E. coli* isolate belonged to a new combination of alleles.

This study raised the alarm for an unexpectedly high occurrence of *mcr-1* and colistin resistance among multidrug resistant *K. pneumoniae* isolates, which may constitute a breakdown of the paradigm of *mcr-1* distribution (mostly in non-human sources by far). Furthermore, our results stress the need for appropriate and close monitoring of colistin-resistance and *mcr-1* in different settings in our country.

References & Acknowledgments
Work funded by FEDER (POCI/01/0145/FEDER/007728) and FCT/MEC (UID/MULTI/04378/201) funds. CR and AN were supported by fellowships from FCT/POCH (SFRH/BD/84341/2012 and SFRH/BPD/104927/2014, respectively).

Keywords: mcr-1, Enterobacteriaceae, clinical, infection, carriers

P-294 - ROLE OF A RND-TYPE EFFLUX PUMP IN ANTIMICROBIAL RESISTANCE IN ARCOBACTER BUTZLERI

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Background

Arcobacter butzleri is a heterogeneous species vastly distributed in different environments. In humans, it has been associated with enteritis or bacteraemia, with outbreaks suggesting and strengthening food and water consumption as the most probable route of transmission. Beyond its association with disease and wide distribution and prevalence, *A. butzleri* presents resistance to several classes of antimicrobial agents, being considered as having multidrug resistance; however, its resistance mechanisms are still poorly addressed. Considering that efflux pumps (EP), namely of the resistance-nodulation-cell division (RND)-type, play a role in antimicrobial resistance, we aimed to evaluate the presence of these EPs in twenty genomes of *A. butzleri* previously sequenced and annotated by us, associate with antibiotic resistance, and perform its functional characterization.

Method

We started to evaluate the overall role of EPs in antimicrobial resistance of *A. butzleri* by determining the minimum inhibitory concentration (MIC) of several antimicrobials in the presence and absence of efflux pumps inhibitors (EPI). Then, mutants of the putative inner membrane efflux transporter (cmeB) and of the putative transcriptional regulator *(*tetR) of the RND-type EP were constructed by insertional inactivation, or natural transformation using a mutated-tetR gene, respectively. For both parent and mutant strains, MICs were compared for the same group of antimicrobials.

Results & Conclusions

One of the RND-type EP found in the genome of 15 of 20 strains analysed, was composed of three genes (cmeA, cmeB, cmeC) organized in an operon, closely resembling the CmeABC efflux pump of *Campylobacter jejuni*. The putative transcriptional regulator open reading frame (*tetR* gene) was located immediately upstream of the operon on the opposite strand. Overall, the results of MIC determination in the presence and absence of EPI indicate that EPs contribute to the tolerance/resistance of *A. butzleri* to various antimicrobials. Then, we observed that cmeB inactivation led to a reduction of the MIC to ciprofloxacin, β-lactams and erythromycin, highlighting the contribution of this system to antibiotic tolerance/resistance. A correlation between tetR truncation and erythromycin resistance was established and validated by the construction of a mutant with a truncated-*tetR*. The transformation of two different *A. butzleri* strains with a truncated *tetR* gene resulted in an increase of at least 8-fold in the MIC of erythromycin of the transformants, when compared with the wild-type strains, suggesting that the mutation is functional and supporting the role of this gene on the regulation of the efflux pump. Together, these findings establish the functionality of this RND-type efflux pump, contributing to the intrinsic resistance of *A. butzleri*.

Keywords: Arcobacter butzleri, Efflux pump, Antimicrobial resistance

P-295 - IMPACT OF PEPTIDOGLYCAN AMIDATION IN CELL ENVELOPE NET CHARGE, CELLULAR AGGREGATION AND BIOFILM FORMATION IN STAPHYLOCOCCUS AUREUS

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Background
Peptidoglycan (PG) is a highly dynamic, surface-exposed macromolecule that undergoes several secondary modifications during its biosynthesis. The MurT-GatD enzymatic complex is necessary for the amidation of glutamate of the stem peptide of *Staphylococcus aureus* PG. This secondary modification influences critical processes, such as growth rate, beta-lactam and lysozyme resistance, being implicated in antibiotic resistance and in pathogenesis (1).

However, the impact of amidation on these phenotypes varies depending on the genetic background of the strain, suggesting the existence of different strategies of resistance to beta-lactam and lysozyme, dependent on PG amidation (2).

Method
Several *S. aureus* strains from different genetic lineages, and their respective *murT-gatD* conditional mutants, grown with and without inducer, were tested for: a) surface charge alteration, through a cytochrome C association based method; b) bacterial aggregation capacity, by growing cultures in rich media and allowing cells to sediment for a few minutes; c) biofilm formation capacity, using the crystal violet assay; d) slime production was observed by growing the strains in Congo Red Agar (BHI + 5% sucrose + 0.08% Congo Red).

Results & Conclusions
In the absence of inducer, the net surface charge of the *murT-gatD* mutants was consistently lower than that of the respective parental strain, indicating that PG amidation is responsible for a more positive surface charge.

Such a strong impact on the surface net charge could be related to altered cell aggregation and surface-adhesion properties. Overall, non-amidated mutants from the different strains showed either higher capacity to form biofilm or increased cellular aggregation capacity. We observed that these two distinct phenotypes were associated with the biofilm matrix composition. In fact, amidation mutants of slime-positive strains showed higher aggregation capacity, whereas amidation mutants of slime-negative strains showed higher biofilm forming capacity.

Our results show that PG amidation in *S. aureus* has a drastic effect on the net surface charge of the cell and results in altered cellular aggregation or biofilm formation patterns, depending on the composition of the biofilm matrix.

References & Acknowledgments
This work was funded by FEDER funds through the COMPETE 2020 Program and National Funds through FCT- Portuguese Foundation for Science and Technology under Projects Number POCI-01-0145-FEDER- 007688, Reference UID/CTM/50025; POCI-01-0145-FEDER-007728, FCT/MEC/UID/Multi/04378/2013 and PTDC/FIS-NAN/0117/2014. Funding was also provided by projects PTDC/BIA-MIC/3195/2012 and ESCMID 2015 grant awarded to R. Sobral.

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Keywords: Staphylococcus aureus, Biofilm, Peptidoglycan amidation

P-296 - ASSESSMENT OF THE BIOTIC EFFECTS OF CEPHALEXIN ON CLINICAL STRAINS OF STAPHYLOCOCCUS AUREUS AND ENTEROCOCCUS FAECALIS ISOLATED FROM A POLYMICROBIAL INFECTION

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Abstract

Even though polymicrobial infections are very common, their study is still at an early stage. In these infections, particularly when biofilms are formed, microorganisms establish a complex network of interactions that play an important role in the severity and progression of disease, with consequences in virulence, in the immune response and in the effectiveness of antimicrobial therapy. In an era in which resistance to antimicrobials is an alarming and rapidly expanding scenario, the choice of appropriate treatment is paramount, with consequences in preventing the development and spreading of resistances.

The biotic interactions between strains of *Staphylococcus aureus* and *Enterococcus faecalis* in the presence of cephalexin were studied, both in the planktonic state and in biofilms. The strains were selected from a collection of veterinary clinical isolates. The studies were conducted for single-species or dual-species combinations and at two time points: 6h and 20 h. The viability of cells in the planktonic phase was determined by culture-dependent methods and the cellular viability of biofilms was evaluated through metabolic activity quantification and Live/Dead staining. The biofilm-forming capacity of the tested strains was quantified, as well as their matrix production.

Results & Conclusions

We showed that co-culturing the two strains in the presence of cephalexin benefits the most sensitive (*S. aureus*) in a greater extent than the resistant one (*E. faecalis*). In fact *S. aureus* were able to survive and were culturable when co-exposed to concentrations of cephalexin higher than their minimum inhibitory concentration. The biofilm-forming capacity of the most sensitive strain is affected in the same manner, however at an early stage of biofilm formation both strains benefit from being in co-culture. Exposure to cephalexin caused changes in the morphology of the strains.

References & Acknowledgments

This work was partially supported by the project INNOVMAR - Innovation and Sustainability in the Management and Exploitation of Marine Resources (reference NORTE-01-0145-FEDER-000035, within Research Lines NOVELMAR and INSEAFOOD), supported by North Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF).

Keywords: Polymicrobial infections, Biofilms, Antimicrobial resistance, Cephalexin, Staphylococcus aureus, Enterococcus faecalis

P-297 - YEAST AS A TOOL TO UNDERSTAND PROTEASOME-MEDIATED SENSITIVITY TO THE CHEMOTHERAPEUTIC AGENT CISPLATIN

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Background

Cisplatin is a highly effective chemotherapeutic drug, as a DNA-damaging agent that induces apoptosis of rapidly proliferating cells. Unfortunately, cellular resistance occurs often. A large fraction of tumor cells harbor mutations in p53, contributing to defects in apoptotic pathways and drug resistance. To uncover new strategies to eliminate tumors that have defects in the p53 pathway, we established a simplified model devoid of a p53 ortholog to study cisplatin-induced regulated cell death, using the yeast *Saccharomyces cerevisiae*. We previously showed that cisplatin induces an active form of cell death accompanied by DNA condensation and fragmentation/degradation, but no significant mitochondrial dysfunction. We further demonstrated that proteasome inhibition with MG132 or genetically increased resistance to cisplatin.

Method

In this study, we sought to determine how proteasome inhibition is important for cisplatin resistance by analyzing how it affects several phenotypes associated with the DNA damage response.

Results & Conclusions

We found central DNA damage response modulators are not required for cisplatin resistance imparted by MG132. Moreover, MG132 does not seem to increase damage tolerance or affect the activation of the DNA damage response. These results suggest the proteasome is involved in modulation of cisplatin toxicity downstream of DNA damage. Proteasome inhibitors can sensitize tumor cells to cisplatin, but protect others from cisplatin-induced cell death. Elucidation of this mechanism will therefore aid in the development of new strategies to increase the efficacy of chemotherapy.

References & Acknowledgments

This work was supported by FCT I.P. through the strategic funding UID/BIA/04050/2013 and FCT-ANR/BEX-BCM/0175/2012, as well as a Post-PhD fellowship to S. Chaves (SFRH/BPD/89980/2012) and a PhD fellowship to A. Rego (SFRH/BD/79523/2011).

Keywords: Cisplatin

P-298 - MOLECULAR CHARACTERIZATION OF THE NEW KPC-21 VARIANT DETECTED AMONG A CARBAPENEMASE-PRODUCING ENTEROBACTERIACEAE SURVEY IN PORTUGAL

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Abstract

In Portugal, the epidemiological stage for the spread of carbapenemase-producing *Enterobacteriaceae* (CPE), increased from sporadic isolates or single hospital clones, in 2013, to hospital outbreaks, in 2015. Here we report data from a six-month study performed under the European Survey on Carbapenemase-Producing *Enterobacteriaceae* (EuSCAPE) with the collaboration of 10 Portuguese Laboratories.

Results & Conclusions

This study included 94 *Klebsiella pneumoniae* and 10 *Escherichia coli* collected in 2013-2014. Antimicrobial susceptibility was performed according to EUCAST guidelines. Detection and characterization of oxyimino-β-lactam, and carbapenem resistance-encoding genes were performed by molecular approaches. KPC-21-producing *E. coli* was characterized by whole-genome sequencing. Genetic relatedness of isolates was investigated by PFGE and multilocus sequence typing (MLST). Subgroups of *E. coli* STs were analysed based on sequence variation of the *E. coli* fimbrial adhesin gene *fimH*. During the study period, 67 isolates (61 *K. pneumoniae* and 6 *E. coli*) non-susceptible to carbapenems were identified in participant hospital laboratories. We identified 37 $bla_{\text{KPC-type}}$ (including one new variant: $bla_{\text{KPC-21}}$), 1 $bla_{\text{GES-5}}$, and 1 $bla_{\text{GES-6}}$ plus $bla_{\text{KPC-3}}$, alone or in combination with other *bla* genes. The remaining 29 isolates were non-susceptible to carbapenems due to association of PMAβ (CMY-2 and DHA-1) and/or ESBL (mainly CTX-M-15) beta-lactamases with porin deficiency. Bioinformatics analysis of the ST131-*fimH30* KPC-21-producing *E. coli* identified the new variant $bla_{\text{KPC-21}}$ in a 12,748 bp length plasmid, with a mean coverage of 580-fold and GC content of 58.5%. The $bla_{\text{KPC-21}}$ gene was harboured on a non-Tn*4401* element, presenting a partial IS*Kpn6* (ΔIS*Kpn6/*Δ*traN*) with the related left IR (IR_L) upstream and with a truncated Tn3 transposon downstream. PFGE and MLST analysis showed an important diversity, as isolates belonged to distinct PFGE and STs profiles.
Portugal was one of the EuSCAPE participating countries that presented higher proportions of KPC-positive *K. pneumoniae*. Although the percentage of carbapenem non-susceptible *K. pneumoniae* is still low in invasive infections (3.7%, reported by EARS-Net 2015), with unrelated hospital outbreaks detected, the number of inter-institutional transmission is increasing.

References & Acknowledgments

V. Manageiro was supported by FCT fellowship (grant SFRH/BPD/77486/2011), financed by the European Social Funds (COMPETE-FEDER) and national funds of the Portuguese Ministry of Education and Science (POPH-QREN). We thank to the 10 Laboratories participating in the Network EuSCAPE-Portugal for sending bacterial isolates to the National Reference Laboratory of Antibiotic Resistances and Healthcare Associated Infections, National Institute of Health Dr Ricardo Jorge.

Keywords: KPC-21, WGS, Euscape, Carbapenemase

P-299 - DEEP SEQUENCING ANALYSIS OF BACTERIAL COMMUNITY AND ANTIMICROBIAL RESISTANCE GENES EMBEDDED IN INTEGRON-CASSETTE ARRAYS IN AN AQUATIC ENVIRONMENT

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Background

Aquatic environments act as antimicrobial resistance genes reservoirs, establishing an important link between clinical and environmental contexts. Dissemination of resistance genes is strongly associated with mobile genetic elements, among which integrons have great importance[1]. Since traditional strategies used to explore these elements have a very limited scope[1,2], our goal was to investigate the diversity of resistance genes contained in integron-cassette arrays and the bacterial taxonomical profile through deep sequencing in a freshwater environment.

Method

Three water samples were collected at das Velhas river (MG/Brazil), and filtered through 0.22 µm membranes. After DNA extraction, libraries for V4 16S rRNA gene[3] and integron-cassette[4] arrays (KAPA LongRange HotStart PCR Kit and KAPA HyperPlus kit, Roche) were constructed and sequenced with MiSeq (Illumina). 16S sequences were trimmed and processed with AfterQC[5] and QIIME[6]. Additional analyses were performed with Phyloseq[7] and PiCrust[8]. Integron-cassette sequences were trimmed[4], assembled[9] and are currently being annotated[10] for further comparison to ARDB, CARD and INTEGRALL databases.

Results & Conclusions

A total of 5421 OTUs were observed, with a similar taxonomic profile among samples. On average, Proteobacteria (Alpha, 7.9% and Betaproteobacteria classes, 36.8%), Actinobacteria (Actinobacteria, 15.6%), Bacteroidetes (Cytophagia, 6.8% and Saprospirae, 4.1%), and Firmicutes (Bacilli, 5.6% and Clostridia, 1.4%) prevailed. PiCrust indicated a high diversity of genes with average frequency <4%, including genes possibly related to multidrug resistance and homologous recombination. The main OTUs associated with these genes belonged to *Hydrogenophaga* and *Ramlibacter* (Betaproteobacteria). Results suggest the presence of antimicrobial resistance elements possibly associated with integrons. Analysis of integron-cassette arrays will clarify the importance of these elements in resistance dissemination.

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Our acknowledgments to COPASA(MG/Brazil) for das Velhas river samples.

Keywords: Antimicrobial resistance, Integron-cassette, 16S rRNA gene, Deep sequencing, Freshwater

P-300 - WHOLE GENOME SEQUENCING AND ITS APPLICATIONS IN ANTIBIOTIC RESISTANCE

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Abstract

Antibiotic resistance is an emerging problem, becoming a serious threat to global public health. The causes of its spread are complex, as are the strategies to combat this threat. Whole-genome sequencing (WGS) has been shown to provide the required power of discrimination to track transmission networks of antimicrobial resistance. Indeed, is of highest importance to look for innovative ways to deal with antibiotic resistance superbugs.

Results & Conclusions

In the scope of the analysis of nonsusceptibility of Gram-negative isolates recovered from human, veterinary and environment samples, we identified the presence of a high diversity of resistance mechanisms, with emphasis in the recently described plasmid-mediated *mcr* gene, conferring resistance to colistin. To understand the genetic background of those resistance mechanisms, which included oxyimino-β-lactam, fluoroquinolone and colistin resistance-encoding genes, we performed whole-genome and plasmid-sequencing using a MiSeq (Illumina) sequencing strategy. A set of bioinformatic web tools were used to estimate the presence of pathogenicity determinants, antibiotic resistance genes, and clinically relevant mobile genetic elements. Indeed, the efficient gene capture and spread of resistance determinants by mobile genetic elements are factors to be taken into account, due to their contribution for the co-selection of multidrug resistant strains in the different settings and environment. Furthermore, WGS might be used with great benefit in combination with phenotypic methods for surveillance purposes.

References & Acknowledgments
VM is supported by the FCT, financed by the European Social Funds (COMPETE-FEDER) and national funds of the Portuguese Ministry of Education and Science (POPH-QREN) fellowship SFRH/BPD/77486/2011.

Keywords: WGS, Antibiotic Resistance, One Health

P-301 - EVOLUTION AND ADAPTATION OF STREPTOCOCCUS PNEUMONIAE POPULATION IN THE ERA OF CONJUGATE VACCINES

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Background

Streptococcus pneumoniae (pneumococcus) colonizes asymptomatically the human nasopharynx. Children attending day-care centers are major reservoirs of pneumococci contributing significantly to their transmission in the community. Pneumococcus is also a major worldwide cause of infectious diseases such as otitis media, pneumonia, bacteremia and meningitis. The major virulence factor of pneumococci is its polysaccharide capsule, and at least 95 different capsular types (serotypes) are currently known. In Portugal, a seven-valent pneumococcal conjugate vaccine (PCV7, targeting serotypes 4, 6B, 9V, 14, 18C, 19F and 23F) became commercially available in 2001. In January 2010, PCV7 was replaced by a 13-valent PCV (PCV13, targeting PCV7-types plus serotypes 1, 3, 5, 6A, 7F and 19A). PCV13 was introduced in the national immunization plan in July 2015.

Method

We evaluated the impact of PCV13 private use on clonal distribution of *Streptococcus pneumoniae* carried by children attending day-care centers in an urban and a rural region of Portugal. Three periods were studied: pre-PCV13 (2009-2010), early-PCV13 (2011-2012) and late-PCV13 (2015-2016). We selected a total of 657 isolates from this collection to be typed by Multilocus Sequence Typing (MLST), the state of the art technique for molecular typing of *S. pneumoniae*.

Results & Conclusions

We identified 171 sequence types (STs), of which 39 were new allelic combinations, and 5 new alleles. The most prevalent STs were ST439 (n=32, 4.9%), ST30 (n=29, 4.4%) and ST180 (n=28, 4.3%), associated with serotypes 23A/B, 16F and 3, respectively. For most serotypes covered by the vaccine, antibiotic resistant clones were selected overtime. The multiresistant ST179 was associated with vaccine type 19F in the late-PCV13 period. Serotype 19A was the most diverse serotype in the pre-PCV13 period with 13 STs; however, it was associated with only 3 STs in the late-PCV13 period. Regarding the serotypes not covered by the vaccine, in the late-PCV13 period, novel, fully susceptible clones were detected. These were of ST4083, ST5139, ST473, ST39, ST9976 and ST445 associated with serotypes 34, 15A (both ST5139 and ST473), non-typeable, 16F and 22F respectively.

In conclusion, following use of PCV13, we observed a decrease in the number of lineages associated with vaccine types and the emergence of clones, not detected in the early periods, associated with non-vaccine types.

Keywords: Streptococcus pneumoniae, MLST, PCV13

P-302 - SPOROTHRIX BRASILIENSIS: PLOIDY AND GENETIC TRANSFORMATION

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Abstract

Thermodimorphic fungi of the *Sporothrix schenckii* complex are the causative agents of sporotrichosis, a chronic subcutaneous infection that affects humans and other mammals. Among the clinically relevant species of the complex are *S. schenckii sensu stricto, S. brasiliensis, S. pallida, S. globosa* and *S. mexicana*. Particularly, infections caused by *S. brasiliensis* are a major health problem, mostly due to the highly aggressive phenotype of the disease, difficulties in its treatment and its zoonotic transmission.

Infection and virulent factors of *S. brasiliensis* are far from being uncover. However, molecular manipulation methodologies for these fungi are scarce. Our aim is to contribute for the development of molecular tools that allow the production of null mutant strains.

Results & Conclusions

Previous analysis of *S. schenckii sensu stricto* ploidy by conventional methods has pointed it as a diploid organism [1]. Nevertheless, our results from FACS analysis of several clinical isolates has suggested a haploid profile for these fungi. Moreover, we have set together a transformation method for *S. brasiliensis* using an *Agrobacterium tumefaciens*-mediated transformation (ATMT) system. Our data show that ATMT is applicable in *S. brasiliensis*, with high transformation efficiency.

Overall, our results point to advances on strategies for molecular manipulation of *S. brasiliensis* that will allow characterization of putative virulence factors.

References & Acknowledgments

This work was developed under the scope of the project NORTE-01-0145-FEDER-000013, supported by the Northern Portugal Regional Operational Programme (NORTE 2020), under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (FEDER).

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P-303 - A TOOL FOR THE EXTRACTION OF RELEVANT AND REPRESENTATIVE HOMOLOGUES OF A GIVEN GENE: THE EXAMPLE OF A TRANSMEMBRANE PROTEIN FROM EUKARYOTIC AND PROKARYOTIC GENOMES

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Abstract
A major challenge in the evolutionary analysis of a gene is the selection of a reliable dataset from public databases, even from heavily curated ones. Difficulties arise from the existence of very unbalanced databases with many species extremely overrepresented– an issue not solved with the use of representative genomes, a term inconsistently referring to species or strains - and problems in defining within-species homologues as belonging to independent specimens or homologues with a single genome. Also, databases on conserved motifs of protein families often contain dubious inclusions and the same redundancy issues. We aimed to design a simple tool from retrieving data where homology searches were conducted on a smaller but more informative database: a) that contains a single representative genome/proteome on the species level; b) where multiple matches within a species directly reflects homologues within the same genome, and c) e-values from BLAST searches are statistically more reliable. We employed this approach on the study of the evolution/residue conservation of members of the AceTr family (TCDB 2.A.96) present in Eukaryotic and Prokaryotic genomes.

Method
Over 11000 complete genomes were downloaded from NCBI's Assembly platform as individual FASTA files. Redundancies at the species level (such as multiple strains) were removed, bringing the dataset down to 3545 organisms, spanning the full range of the tree of life. A custom database was compiled from the remaining files using the BLAST command-line application. Protein BLAST searches using queries Satp[1] (*Escherichia coli*), Ma4008 (*Methanosarcina acetivorans*) and ADY2[2] (*Saccharomyces cerevisiae*) were performed (using the cut-off e-value 10^{-5}). Retrieved proteins were aligned with the PROMALS3D software[3] (whose algorithm includes secondary structure prediction) and protein conservation logos were displayed using WebLogo (http://weblogo.berkeley.edu).

Results & Conclusions
A total of 821 hits were obtained for the three searches. Homologues were mostly present in bacteria, fungi and archaea but instances of presence in Eukaryotic Euglenids and green algae were also detected. The alignment of all the retrieved proteins reveals a set of highly conserved residues across all taxa. Some residues have already been tested for their functional importance in the protein (unpublished results). The use of an unambiguous database for the selection of homologues allowed to maximize the spectrum of conservation of the protein (as overrepresentation of species thins the ratio conserved/non-conserved residues). The tool developed can prove highly relevant in evolutionary and functional studies.

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Work supported by the strategic programme UID/BIA/04050/2013 (POCI-01-0145-FEDER-007569) and PTDC/BIAMIC/5184/2014 funded by national funds through the FCT-IP and by the ERDF through the COMPETE 2020-POCI; and the project EcoAgriFood (operação NORTE-01-0145-FEDER-000009), supported by NORTE-2020, under the PORTUGAL 2020 Partnership Agreement, through the ERDF. DR acknowledges FCT (SFRH/BD/96166/2013).

***both authors contributed equally to this work**

P-304 - THE INTERPLAY OF NITROGEN AND CARBON SOURCES WITH OTHER MACRONUTRIENTS AND ITS IMPLICATION ON SURVIVAL OF WINE YEAST

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Background

The composition and nutrient balance of the medium is of extreme importance for a successful fermentation to occur. One of the major nutrients that can influence a fermentation performance by yeast is assimilable nitrogen (YAN). During a grape-must fermentation, suboptimal concentrations of YAN can lead to sluggish or stuck fermentations, with great economic impact. On the other hand, the addition of excess ammonium can also be detrimental. In fact, ammonium toxicity and its effects on the chronological life span (CLS) of Saccharomyces cerevisiae have been described, with ammonium being capable of shorting longevity of both auxotrophic and prototrophic yeast strains [1, 2], an effect mediated by major nutrient-signalling pathways, TOR-SCH9 and RAS-PKA (2, 3). The present work aimed to gain new insights on the regulatory mechanisms responsible for the nutrient modulation of yeast CLS also extending the studies to wine strains.

Method

In the present work, we manipulated different nutrients in the culture medium and assessed nutritional signalling pathways under extended- or shortened- CLS conditions. We further looked into the effects of ammonium in yeast metabolism by evaluating the production/consumption of several fermentation metabolites.

Results & Conclusions

Results showed that the two wine yeast strains tested displayed similar response to the ammonium effects on CLS indicating that this is a conserved trait. The results also showed that in both strains the effect of ammonium was largely dependent on the balance between carbon and nitrogen sources with other macronutrients and independent of the glucose metabolism (fermentation or respiration). Our results with the yeast model are in line with several studies in other model organisms showing a major link between macronutrient balance and longevity regulation.

References & Acknowledgments

This work was developed under the scope of the project NORTE-01-0145-FEDER-000013, supported by the Northern Portugal Regional Operational Programme (NORTE 2020), under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (FEDER), by FEDER, through the Competitiveness Factors Operational Programme (COMPETE), and by National funds, through the Foundation for Science and Technology (FCT), under the scope of the project POCI-01-0145-FEDER-007038 as well as through the strategic funding UID/BIA/04050/2013. Júlia Santos holds a Post-Doc fellowship (SFRH/BPD/112108/2015) funded by FCT.

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P-305 - ORAL MICROBIOME IN A SAMPLE OF ELDERLY DETERMINED BY QPCR

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Abstract
The oral microbiome is associated with oral health and dysbiosis in this microbiome is the cause of conditions such as caries and periodontal disease. Furthermore, there is mounting evidence that the oral microbiome may be related to systemic health and determined by demographic and host related factors such as diet. One of the factors studied as being related to systemic health is the ratio between Firmicutes and Bacteroidetes which also seems to change with age.

Firmicutes and *Bacteroidetes* phyla are present in the gastrointestinal tract in higher quantity as opposed to the oral microbiome where the main phylum is *Bacteroidetes*, followed by *Firmicutes*. For both microbiomes, it is true that the balance is changed through aging (Greenhalgh et al, 2016). Also, microbial diversity decreases with disease status generating a dysbiotic microbiome (Kilian et al 2016). We evaluated total bacterial load and Firmicutes to Bacteroidetes ratio in saliva samples from people over 65 years old. DNA was obtained from 10 mL mouthwash samples using the InstaGeneMatrix®. In order to quantify the bacterial load, the *Bacteroidetes* and *Firmicutes* phyla the SsoFast™ EvaGreen®Supermix (Bio-Rad®) was used in conjugation with a set of primers for each group. The amplification and detection of DNA by real-time PCR was performed with the CFXConnect™ Real-TimeSystem(Bio-Rad®).

Results & Conclusions
Our results show that females seem to have a lower total bacteria load and a lower F/B ratio when compared to males. The age group under 65 had a mean total bacteria load higher than the other age groups and showed a lower F/B ratio. Regarding individuals with a Mediterranean diet type they present a higher mean total bacteria load and a lower F/B ratio than individuals with a non-Mediterranean diet. However, none of the statistics are significantly different. Our results are in agreement with previous studies, since there is a higher number of *Firmicutes* than *Bacteroidetes* (Greenhalgh et al, 2016) in the saliva. Nevertheless, *Firmicutes* and *Bacteroidetes* are in lower amount compared to a healthy population from Zaura *etal.* (2009). The mean ratio *Firmicutes*/*Bacteroidetes* was higher than the ratios found by Walter *et al.* (2014) in the gut microbiome.

Our sample were only 40 individuals which is substantially lower than what is suggested to see an effect for most microbiome studies (Odamaki etal, 2016). Therefore, these results have to be considered as preliminary and supplemented by analysis of further samples.

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SalivaTec was funded by +Centro - Programa Operacional Regional do Centro (Quadro de Referência Estratégico Nacional) and through the Fundo Europeu de Desenvolvimento Regional. CIIS through funds from the Portuguese National Funding Agency for Science, Research and Technology granted to Unit 4279 - UID/MULTI/4279/2016.

Keywords: Oral Microbiome, elderly, Bacteroidetes, Firmicutes

P-306 - THE ALTERNATIVE SIGMA FACTOR F (SIGF) REGULATES SECRETION IN SYNECHOCYSTIS SP. PCC 6803: THE PARTICULAR CASE OF EXTRACELLULAR POLYMERIC SUBSTANCES (EPS) PRODUCTION

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Background

Cyanobacteria are prolific sources of compounds with biotechnological potential. Since some strains can be easily genetically modified, understanding the cyanobacterial secretion mechanisms is an emergent research field in order to engineer these pathways and implement profitable industrial systems. For instance, despite the increasing interest in cyanobacterial extracellular polymeric substances (EPS) due to their biomedical and industrial potential (1, 2), the knowledge on the regulatory factors involved in their production and export is still limited, hindering the implementation at industrial scale (3).

Method

An extensive approach was used to study the physiological impact of the knockout of the gene encoding the transcriptional regulator group 3 alternative sigma factor F (SigF, Slr1564) in the unicellular cyanobacterial model *Synechocystis* PCC 6803 (4). Δ*sigF* and the respective wild-type strain were characterized in terms of ultrastructure, growth, lipopolysaccharidic profile, content in total carbohydrates, released and capsular polysaccharides. Other aspects of Δ*sigF* and wild-type secretion capacity were investigated by elucidating the protein content, pigments and membrane vesicles present in extracellular media. A quantitative proteomic analysis (iTraq) and *in silico* studies of SigF consensus binding motifs revealed possible (direct or indirect) targets of SigF. Several validation studies were performed (oxidative stress defenses, photosynthesis/respiration, photosynthetic pigments, motility, etc.).

Results & Conclusions

In this work, we show that SigF is important for regulation of different secretion systems in *Synechocystis*, particularly in the case of EPS production. The results obtained with the Δ*sigF* mutant indicate that, although growth is significantly impaired, the EPS production strongly increases compared to wild-type. Differences in secretion of proteins, vesicles and pili were also observed. Δ*sigF* is non-motile and presents morphological alterations in the cell envelope. iTraq analysis showed alterations in protein levels of Δ*sigF* related to the maintenance of cell envelope and membrane transporters, and mechanisms of ATP production, motility and carbon metabolism being affected. Remarkably, a large number of chaperones and proteins involved in protein modification/degradation are in higher amounts in Δ*sigF*, suggesting that cells are under stress, probably due to their low carotenoid content (oxidative stress protectors). Overall, these results provide new evidences about the role of SigF on *Synechocystis* physiology and its importance for environmental adaptation. SigF is also the first regulatory element associated to EPS production in *Synechocystis*. Δ*sigF* represents a promising platform to study/manipulate EPS production and to obtain higher yields of polymer from *Synechocystis*.

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Keywords: secretion, cyanobacteria, extracellular polymeric substances (EPS), sigma factor

P-307 - SUBSTRATE INHIBITION OF URACIL PHOSPHORIBOSYLTRANSFERASE (UPRT) IN DISTINCT MICROBIAL SPECIES AND ITS IMPLICATIONS IN THE PHENOTYPE OF URACIL AUXOTROPHS

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Background
Unlike the established practice, the supply of exogenous uracil cannot fully rescue uracil auxotrophy in Ura- mutants of some species of industrial and/or medical significance (e.g. *Ashbya gossypii*, *Candida guilliermondii*, *C. albicans*, *Leishmania donovani*), which require uridine for proper growth [1-4]. Here we reveal the molecular mechanism underlying this phenotype in *A. gossypii* and disclose common features with protozoan parasites and other flavinogenic/pathogenic fungi.

Method
Analysis of the *A. gossypii Agura3* (Ura- mutant) radial growth on minimal medium containing distinct pyrimidine supplements allowed the detection of a bottleneck in the *A. gossypii* pyrimidine salvage pathway at the Uracil Phosphoribosyltransferase (AgUPRT) level. This enzyme catalyzes the production of uridine monophosphate (UMP) from uracil and phosphoribosyl pyrophosphate (PRPP), being encoded by *AgFUR1*. Recombinant AgUPRT was produced and purified from *Escherichia coli*, and its activity characterized spectrophotometrically [4].

Results & Conclusions
Characterization of recombinant AgUPRT revealed that it is susceptible to substrate inhibition by uracil, thus explaining the hypersensitivity of *A. gossypii Agura3* to uracil and its requirement for uridine [1]. This UPRT substrate inhibition mechanism, together with similar Ura- phenotypes, is also present in medically relevant protozoan parasites [4] and phenotypic evidences indicate that it likely exists in other flavinogenic fungi [2]. Substrate inhibition of AgUPRT favours the preservation of PRPP for use in purine synthesis/salvage. While in *A. gossypii* and other flavinogenic fungi (e.g., *C. guilliermondii*) purine synthesis is crucial to support the highly active biosynthesis of riboflavin [1-2], in human pathogens such as *C. albicans* and *L. donovani* the intracellular availability of purines is essential during infection [4-5].

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Study supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UID/BIO/04469/2013 unit and COMPETE 2020 (POCI-01-0145-FEDER-006684) (Post-Doc grant to T. Q. Aguiar), BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund under the scope of Norte2020 - Programa Operacional Regional do Norte, Project RECI/BBB-EBI/0179/2012 (FCOMP-01-0124-FEDER-027462), Post-Doc grant SFRH/BPD/110640/2015 to C. Oliveira and PhD grant PD/BD/113812/2015 to R. Silva.

Keywords: Uracil/uridine auxotrophy, Pyrimidine metabolism, Uracil phosphoribosyltransferase, Substrate inhibition, Ashbya gossypii

P-308 - EXPLORING A NOVEL EFFECT OF LACTOFERRIN ON THE PLASMA MEMBRANE TOWARDS THE ELUCIDATION OF THE MECHANISMS OF ACTION: FROM YEAST TO HUMAN CELLS

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Background

Lactoferrin (Lf) is an iron-binding glycoprotein normally present in several biological fluids. It exhibits a broad range of interesting biological activities, from which its anticancer and antifungal activities stand out. Our group has been studying the mechanisms and targets underlying Lf anticancer/antifungal activities in order to improve its therapeutic efficacy and rational application. Indeed, we previously demonstrated that Lf triggers a mitochondrial and caspase-dependent regulated cell death in *Saccharomyces cerevisiae* (1). Moreover, we found that Lf selectively induces apoptosis in highly metastatic cell lines displaying the proton pump V-ATPase at the plasma membrane (2).

Results & Conclusions

Here, we show how studies with yeast unveiled a novel effect of Lf on the interplay between proton pump ATPases and the plasma membrane, which were then validated in human cell lines. Results will be discussed in an integrated manner regarding their contribution towards understanding the molecular basis of Lf anticancer activity and impact on a potential expanded clinical application.

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Keywords: lactoferrin, H+-ATPases, anticancer, plasma membrane

P-309 - FIGHTING BACTERIAL INFECTIONS WITH AVIAN IGY ANTIBODIES

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Abstract

Antibody therapy against antibiotic-resistant bacterial pathogens is a promising alternative to traditional drug therapies. The current state-of-the-art and emerging strategies on antibody engineering allow the development of highly effective multifunctional therapeutic antibodies. However the bottleneck of immunotherapies directed against bacterial infectious is the identification of ideal antibody targets. Avian antibodies have been successfully used in therapeutic strategies against microbial and viral pathogens. Moreover the molecular cloning of phage-display libraries from avian sources is simplified over mammalian systems, thus favoring screening and multiple engineering strategies.

Results & Conclusions

We have been combining the use of avian model hosts for antibody generation with structural and functional rationalization of potential bacterial therapeutic targets and vaccine antigens. We implemented an avian antibody platform for the development of target-specific immunological libraries in *Coturnix japonica* model hosts. We developed proprietary housing systems for egg-laying quails, meeting the requirements of Directive 2010/63/EU on animal experimentation and the 3Rs principles. These allow the parallel development of multiple bird immunization protocols with antibody titer monitoring performed on laid eggs. We are particularly focused on targeting surface-exposed virulence factors in Gram-negative bacteria, including membrane channels and transporters, but also cell adhesion factors and enzymes. Here we present the case study of *E.coli* TolC bacterial channel, responsible for extrusion of cytotoxic agents including antibiotics and critical in multi-drug resistance phenomena. We developed avian antibodies directed against *E.coli* TolC and demonstrated their ability to modulate the extrusion activity of this channel. The assays were performed with purified polyclonal IgY from hyperimmune egg-yolks, demonstrating the versatility of the approach for functional read-outs and generation of optimal immune repertoires for downstream monoclonal selection. Ultimately these antibodies are likely to complement the activity of existing antibiotics.

Our setup and methodology will definitely contribute to the development and refinement of new antimicrobial strategies based on avian antibodies.

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This work was supported by FEDER funds through the Operational Program Competitiveness Factors – COMPETE, by national funds through the Portuguese Foundation for Science and Technology (FCT), FCT Investigator IF/00123/2013, Strategic Project Ref. COMPETE POCI-01-0145-FEDER-007440 and sponsored by HBT - Saúde e Biotecnologia Lda.

Keywords: Antimicrobials, IgY antibodies, Microbial antigens, Biopharmaceuticals, Immunotherapies, Immunodiagnostics, Antibiotics, Bacterial membrane channels

P-310 - PKH1P-YPK1P AND PKH1P-SCH9P PATHWAY ACTIVATION UNDERLIES MITOCHONDRIAL-MEMBRANE PERMEABILIZATION AND CYTOCHROME C RELEASE IN REGULATED CELL DEATH INDUCED BY ACETIC ACID

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Background

The yeast *Saccharomyces cerevisiae* undergoes a mitochondrial-dependent regulated cell death (RCD) exhibiting typical markers of mammalian apoptosis. Changes in sphingolipid metabolism have been linked to modulation of cell fate in both yeast and mammalian cells. We previously assessed the role of sphingolipids in cell death regulation using a well characterized yeast model of acetic acid-induced regulated cell death, finding that Isc1p, inositol phosphosphingolipid phospholipase C, plays a pro-death role in this process. Indeed, *isc1*Δ mutants exhibited a higher resistance to acetic acid associated with reduced mitochondrial alterations.

Method

Results & Conclusions

In this study, the role of several signaling pathways in acetic acid-induced RCD was assessed. Our results show that Pkh1p through Ypk1p and Sch9p regulate acetic acid-induced RCD, since single mutants are resistant to acetic acid. We also found that acetic acid exposure leads a Pkh1p dependent-phosphorylation of both Sch9p and Ypk1p and that Isc1p is regulated by Sch9p under acetic acid stress. Both single and double mutants lacking Isc1p or/and Sch9p have the same resistant phenotype, and *SCH9* deletion impairs the translocation of Isc1p to mitochondria upon acetic acid exposure. We also found that the higher resistance of all mutants correlates with higher levels of endogenous mitochondrial phosphorylated long chain bases (LCBPs), suggesting that changing the sphingolipid balance in favour of LCBPs in mitochondria results in increased survival to acetic acid. In addition, our results suggest that Pkh1p, in contrast to Ypk1p, is necessary for *isc1*Δ resistance to acetic acid-induced RCD. Indeed, while double deletion of *ISC1* and *PKH1* has a drastic effect on cell survival, which is associated with increased ROS accumulation and release of cytochrome *c,* double deletion of *ISC1* and *YPK1* has no effect on cell survival. Overall, our results suggest that Pkh1p-Ypk1p and Pkh1p-Sch9p pathways contribute to RCD induced by acetic acid, and that Sch9p pathways modulate acetic acid-induced cell death, through the regulation of Isc1p cellular distribution, thus affecting the sphingolipid balance that regulates cell fate.

References & Acknowledgments

This project was financially supported by Fundação para a Ciência e Tecnologia (FCT) by means of a fellowship to A.R (SFRH/BD/79523/2011) and to S.R.C. (SFRH/BPD/89980/2012), and by projects Pest-C/BIA/UI4050/2011, FCOMP-01-0124-FEDER-027652, FCOMP-01-0124-FEDER-028210, Pest-C/SAU/LA0002/2011, FCOMP-01-0124-FEDER-022718, PTDC/BBB-BQB/1850/2012, FCT-ANR/BEXBCM/0175/2012, and UID/BIM/04293/2013. KFC and YAH are funded by National Institutes of Health Grant numbers GM113196 and GM118128, respectively.

Keywords: Yeast, Acetic Acid, Sphingolipids, Cell Death

P-311 - STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF ACETR TRANSPORTER FAMILY

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Background
The AceTr transporter family members are carboxylic acid transporters with six predicted transmembrane segments (TC 2.A.96). This family has members in fungi, bacteria, archaea and protozoa. Our team functionally characterized several members of AceTr family: AcpA from *Aspergillus nidulans*, an acetate transporter expressed in germinating conidia; Ady2 from *Saccharomyces cerevisiae,* an acetate/propionate/formate transporter; and SatP from *Escherichia coli*, a succinate/acetate transporter.

Method
The Ma4008 gene from *Methanosarcina acetivorans* was recently characterized as an acetate-proton symporter, by expression of a codon optimized version in the *E. coli actP*Δ *lldP*Δ *satP*Δ strain. In this work, we analysed the role of the conserved signature motif "NP(A/V)P(L/F)GL(M/S)" of the AceTr family by site directed mutagenesis analysis, demonstrating their crucial role for the acetate uptake. Ady2 mutant alleles were fused with a GFP tag to get insights in protein membrane targeting.

Results & Conclusions
None of the residues of this signature motif were found to be critical for the correct membrane targeting under the conditions tested. In a predicted SatP 3D model, obtained by modelling with the YetJ crystal structure, a molecular docking analysis was done to unveil putative binding sites and ligand interactions. This analysis allowed to identify three putative binding sites for acetate near the protein pore entrance facing the cytoplasm, involving residues of the signature motif.

References & Acknowledgments
Work supported by the strategic programme UID/BIA/04050/2013 (POCI-01-0145-FEDER-007569) and PTDC/BIAMIC/5184/2014 funded by national funds through the FCT-IP and by the ERDF through the COMPETE 2020-POCI; and the project EcoAgriFood (operação NORTE-01-0145-FEDER-000009), supported by NORTE-2020, under the PORTUGAL 2020 Partnership Agreement, through the ERDF. DR acknowledges FCT (SFRH/BD/96166/2013).

Keywords: Transporter

P-312 - STUDY OF THE HUMAN COPPER TRANSPORTERS HCTR1 AND HCTR2 USING A YEAST HOST

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Abstract

Copper (Cu) is an essential trace element for both eukaryotic and prokaryotic organisms. It plays a crucial role as co-factor of metalloenzymes that participate in important cellular processes, such as growth, development and physiology (Lutsenko, 2016). Although its importance in maintaining cell health, high level of this ion is extremely toxic (Wang et al., 2011). Therefore, cells possess tight regulated systems to conserve copper homeostasis. One of these mechanisms includes the endocytosis of the Copper Transporter 1 (Ctr1) at high Cu levels, a process already verified in yeast and human cells (Liu et al., 2007; Maryon et al., 2013). Besides these new advances, the molecular mechanisms that are behind the intracellular trafficking of the human Ctr1 protein are still poor understood. So, to get new insights into this mechanism, an heterologous expression system was created using the yeast *Saccharomyces cerevisiae* as host. Human *CTR1* and *CTR2* genes tagged with YFP were cloned into p416-GPD plasmid and transformed into a *S. cerevisiae* strain disrupted for copper transporters.

Results & Conclusions

Importantly, phenotypic assays demonstrated that human Ctr1 complemented the yeast *ctr*-mutant strain for the ability to grow in a medium containing non-fermentable carbon sources. Moreover, hCtr2 was clearly localized at the plasma membrane. Data will be discussed regarding a new strategy to improve the expression of h*CTR* genes in the yeast *S. cerevisiae*.

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Keywords: Copper Transporters, Yeast, Endocytosis, Posttranscriptional regulation

P-313 - SELECTION OF YEAST STRAINS ISOLATED DURING ALENTEJO WINES PRODUCTION TO BE USED AS STARTERS

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Background

The knowledge of new species of yeasts potentialities with fermentative characteristics and/or with specific sensorial properties can be an important tool for the development of oenological processes with higher microbial complexity in a controlled system, conferring a higher wine quality and promoting innovative organoleptic characteristics to the final product.
Identification and characterization of yeasts with certain oenological properties is very important to explore their fermentation potential and improve wine production.
The aim of this study was the identification and characterization of yeast strains with oenological potential, isolated from different grape musts fermentations from Alentejo region.

Method
Initial screening allowed a selection of 16 different yeast strains from grape must from Touriga Nacional, *Cabernet Sauvignon*, Antão Vaz and Síria, at various stages of the fermentation process, to be used in immobilization systems, on the development of new oenological solutions. Batch cultures of these microorganisms were performed in order to characterize kinetic growth, carbohydrate consumption and ethanol production. Selected strains were characterized by their macroscopic and microscopic characteristics. DNA were extracted and amplified for strains identification and characterization [1].

Results & Conclusions
The identification was done by the sequencing of PCR products obtained after amplification of the D1/D2 domain of the large subunit ribosomal RNA gene, and it was possible to identify *Saccharomyces cerevisiae*, *Torulaspora delbrueckii*, *zygoascus meyerae, Pichia kudriavzevii, Rhodotorula mucilaginosa, Saccharomyces sp. and Hanseniaspora sp.*. strains.
Molecular analysis by M13-PCR allowed to characterize the genetic profiles for all the isolated yeast strains, showing differences on molecular profiles that allowed grouping according to phylogenetic proximity, both between different species and between strains of the same species.
This approach allows better microbiologic knowledge about the grape must flora and to improve the selection of the most appropriate consortia to be used in the creation of new starters for immobilization solutions.

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Acknowledgments: The authors would like to thank the HIT3CH project—HERCULES Interface for Technology Transfer and Teaming in Cultural Heritage (ALT20-03-0246-FEDER-000004) co-financed by the European Regional Development Fund (ERDF) and ALENTEJO 2020.

Keywords: Yeasts, Wine, DNA extraction, PCR, Sequencing, Phylogenetic analysis

P-314 - THE ROLE OF METABOLISM IN THE SENSITIVITY OF S. CEREVISIAE TO THE ANTI-CANCER AGENT CISPLATIN

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Background

Cisplatin is an antineoplastic, cytotoxic and effective agent that has DNA as the main target. It is used extensively in the treatment of various tumors, inducing cell death by apoptosis in cells with increased proliferative capacity. Unfortunately, cellular resistance occurs often. During the last years, several studies were conducted to further understand the pathways that are responsible for cisplatin resistance and/or sensitivity. The majority of the research was performed using monolayer cell cultures. However, studies in Saccharomyces cerevisiae have also advanced this field, since yeast cells share metabolic similarities with cancer cells and can be easily manipulated. This Crabtree-positive yeast has the ability to adjust the metabolism according to environmental conditions: in a rich glucose background, the main metabolic pathway for energy is fermentation; however, when low glucose concentrations or non-fermentable carbon sources are available, oxidative phosphorylation is the main energy pathway. Evidence suggests that cisplatin cytotoxicity depends on the glucose metabolism of tumor cells.

Method

We analyzed how cisplatin resistance is affected by the carbon source present in the medium (including no carbon source, glucose, galactose or lactate), in both wild-type cells and select mutants.

Results & Conclusions

We show that yeast cells may be used to further dissect this mechanism, as metabolism also affects their resistance to cisplatin. Indeed, when yeast cells are exposed to cisplatin in glucose-containing media cytotoxicity was increased. We further analyzed whether respiratory capacity or proteins normally involved in the regulation of metabolic pathways are responsible for this phenotype. Understanding this mechanism will aid in the development of strategies to modulate this pathway to sensitize cells to this chemotherapeutic agent.

References & Acknowledgments

This work was supported by FCT I.P. through the strategic funding UID/BIA/04050/2013 and FCT-ANR/BEX-BCM/0175/2012, as well as a Post-PhD fellowship to S. Chaves (SFRH/BPD/89980/2012).

Keywords: Cisplatin

P-315 - DEVELOPMENT OF THERAPEUTIC IGY ANTIBODIES AGAINST VIBRIO ANGUILLARUM

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Background

Aquaculture has received remarkable attention in recent years as an alternative to traditional fishing activities, currently limited due to the restriction of fishing quotas. New techniques have therefore been developed in order to increase production and profit of aquaculture activities. However, over-exploitation, pollution, appearance of infectious diseases and antimicrobial resistance, have emerged as concerning consequences of such development [1]. *Vibrio anguillarum* is a Gram-negative bacterium causing fish infections in aquaculture systems and leading to significant economic losses [2]. This infection is usually treated with antibiotics; however, the recurrent bacteria resistance to such drugs urges the development of alternative therapeutic strategies for the problem. The use of antibodies, namely avian Immunoglobulin Y (IgY) purified from hen egg yolks, is a promising approach for the control of *V. anguillarum* infections in aquaculture [3]. This IgY-based therapy allows the development of a microbe-specific biological drug at reduced cost, thus ideal for aquaculture industry.

Results & Conclusions

Here we present data on the production, purification and characterization of chicken IgY antibodies against *V. anguillarum* whole-cell extracts. We successfully generated hyperimmune chicken hosts, presenting a robust and prolonged titer against the target microbe. We further purified the IgY fraction (>95% purity) from selected hen eggs and proceeded to characterize the antimicrobial potential of anti-whole-cell *V. anguillarum* antibodies, using bacterial growth assays. These revealed a promising bacteriostatic effect of anti-*V. anguillarum* antibodies, that promoted a 50% bacterial growth inhibition when supplemented to microbial cultures. The results obtained support the use of anti-*V. anguillarum* IgY antibodies as alternative antimicrobial agents to prevent and combat infections by *V. anguillarum* in aquaculture systems and set up the basis for further downstream refinement of the approach.

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Keywords: Aquaculture, Vibrio anguillarum, antibiotic resistance, virulence determinants, antibodies, immunoglobulin Y (IgY).

P-316 - SIGNALS AND MECHANISMS OF ENDOCYTOSIS OF EUKARYOTIC LACTATE TRANSPORTERS

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Abstract

Eukaryotic cells internalize or recycle their plasma membrane proteins by endocytosis in response to physiological or stress signals imposed by a changing environment. Defects in membrane cargo trafficking lead to protein mislocalization and activation of turnover pathways, which can result into cellular stress and is often related to human diseases. The α-arrestins connect signalling pathways triggered by external stimuli to the endocytosis of specific plasma membrane transporters or receptors. The *Saccharomyces cerevisiae* lactate transporter Jen1p has been used as a model membrane cargo protein for elucidating aspects of the mechanisms that control the endocytic turnover of specific transporters in response to the presence of glucose.

Results & Conclusions

Here, we discover a novel pathway of Jen1p endocytosis mediated by the α-arrestin Bul1p in response to the presence of cycloheximide or rapamycin, or prolonged growth in lactate. While cycloheximide and rapamycin affect pleiotropically cell homeostasis and have been previously shown to provoke endocytosis of several transporters, the response to prolonged growth in lactate proved to be related to moderate alkalinization of the growth medium. This work supports an emerging concept of dual role of the substrate in transporter endocytosis by eliciting conformational transitions of transporters and triggering the activation of α-arrestins.

References & Acknowledgments

Acknowledgements

This work was supported by: the strategic programme UID/BIA/04050/2013 (POCI-01-0145-FEDER-007569) and the project PTDC/BIAMIC/5184/2014 funded by national funds through the FCT I.P. and by the ERDF through the COMPETE 2020 - Programa Operacional Competitividade e Internacionalização (POCI); This work is a result of the project EcoAgriFood: Innovative green products and processes to promote Agri-Food BioEconomy" (operação NORTE-01-0145-FEDER-000009), supported by Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF). GT acknowledges FCT for the SFRH/BD/86221/2012 PhD grant.

Keywords: Jen1p, Endocytosis, Bul1p, Conformational transitions, Alkalinization, α-arrestins

P-317 (sup) - CHITOSAN AND CODFISH HYDROXYAPATITE FORMULATION TO BE USED AS COATING MATERIAL TO CIRCUMVENT PERIPROSTHETIC JOINT INFECTIONS

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Periprosthetic joint infection (PJI) is a common complication after orthopedic surgery and increasing as the number of primary arthroplasties is also growing. Consequently, there is a great need for an effective strategy that can simultaneously eradicate infection and promote new bone formation.
Chitosan is a potential valuable biomaterial for biomedical applications with special interest for this application due to its antimicrobial [1] and osteogenic properties [2].
Hydroxyapatite (HAp) can be extracted from natural sources such as fish bones, which are byproducts of fish industry [4]. Recent work showed that it was possible to extract HAp based materials from Atlantic codfish bones with similar biocompatibility to commercial products [3].

Chitosan medical grade and hydroxyapatite (HAp) obtained from Atlantic codfish bones in the form of paste was produced to be further used in titanium prosthetic joints, combining the antimicrobial properties of chitosan and osteogenic ability of HAp. The formulations were tested with to evaluate their antimicrobial potential upon relevant pathogenic microorganisms, considered to be the main infectious agents of PJI. Briefly, an optimized paste of 3% of chitosan and 5% of HAp was tested with Gram-positive *Staphylococcus aureus* (*S. aureus*) and methicillin-resistant *Staphylococcus aureus* (MRSA) as well as Gram-negative *Escherichia coli* (*E. coli*). Controls of chitosan and HAp alone, as well as acetic acid 1% (solution in which chitosan was dissolved) were also evaluated. The number of viable bacteria was counted after 0, 90, 180 and 360 minutes, time after which no more growth was observed for all tested conditions (previously verified).

Medical grade chitosan (DD of 87.6 - 92.5% and MW 200 - 400 kDa) and codfish derived HAp (extracted from codfish bones, kindly provided from Pascoal company) were used to develop the paste. The HAp was subjected to high energy ball milling process to decrease the particles size and to sonication process to homogenize the HAp suspension. The required formulation was produced through an optimization process until a homogeneous paste was created containing 3% of chitosan solution and 5% of HAp.
The produced paste showed to present noticeable antimicrobial potential against both Gram-positive bacteria, more pronounced for *S. aureus*. The *E. coli* tested showed to be unaffected by the presence of the paste.

[1] Tavaria et al., Curr Bio Comp, 12:114 (2016)
[2] Costa-Pinto et al., JTERM, 6: 21 (2012)
[3] Piccirillo et al., Mat Sci Eng, 51:123 (2015)
Work supported by national funds through Biotherapies-Bioengineered Therapies for Infectious Diseases and Tissue Regeneration", funded by Norte2020.

Keywords: Chitosan, Codfish hydroxyapatite, Paste