Health & Wellbeing

Lactic Acid Bacteria isolated from “innovative” alheiras as potential biocontrol agents

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Introduction

Nutrition and health concerns are having an increasingly significant influence on consumers' food choices. In response, the food industry has begun to offer a wider variety of products that reflect changes in consumer tastes and preferences. Other combinations of a*lheiras* ("innovative") made from codfish, veal, lamb, mushrooms, tofu, soy, and vegetables were lunched in the market in an attempt to reach those consumers with "healthier and cleaner" lifestyles without losing tradition (**1**).

Also, consumers concern have been on the rise specially related to chemical preservatives use which led to a demand for more "natural" and "minimally processed" food. As a result, there has been a great interest in naturally produced antimicrobial agents. The use of bacteriocinogenic lactic acid bacteria (LAB) have demonstrated a biocontrol effect without a negative impact on sensorial characteristics. Being important part of the beneficial bacteria located inside human gut, the use of LAB and/or their metabolites as natural antimicrobials has been a hot topic due to their capacity to enhance food safety and shelf-life extension (**2**).

Main Objectives

- Characterization of three LAB isolated from "innovative" *alheiras* regarding their safety and beneficial features
- An additional verification of antimicrobial activity of each strain against several foodborne pathogens was performed
- Investigation of virulence factors such as gelatinase, DNAse, haemolytic activity, and biogenic amines production as well as antibiotic susceptibility and presence of genes encoding virulence factors

Methodology

For all tests performed, LAB cultures were grown for 24h in 10ml of Man Rogosa and Sharpe broth at 37 ºC and then:

- Gelatinase activity was assayed using Modified Luria-Bertani broth supplemented with 50.0 g/l of gelatin and tubes were incubated at 30 ºC for 7 days. Then tubes were placed into the refrigerator for approximately 30 minutes. The production of sufficient gelatinase turned the medium liquid even when placed in the refrigerator, indicating a positive result
- DNase activity was tested using DNase agar and incubated for 48h at 37 ºC. A clear halo around the colonies indicated a positive result
- Bover-Cid and Holzapfel (1999) method was used for the detection of amino acid decarboxylase-positive microorganisms in order to identify their potential to produce the biogenic amines tyramine, histamine, putrescine and cadaverine
- Production of haemolysin was determined by streaking isolates onto Columbia Blood Agar plates, incubated at 37 ºC for 24 hours after which plates were examined for haemolysis activity
- Sixteen virulence genes were investigated, namely surface adhesin genes (*esp*, *ace*, *efaAfs* and *efaAfm*), aggregation protein gene (*agg*), extracellular metallo-endopeptidase gene (*gelE*), cytolysin genes (*cylA*, *cylB cylM, cylLL* and *cylLS*), hyaluronidase gene (*hyl*), aggregation substance precursor (*asa1*) and genes related to biogenic amines (*hdc1*, *tdc* and *odc*)
- The susceptibility of antibiotics ampicillin, chloramphenicol, clindamycin, erythromycin, gentamicin, kanamycin, streptomycin, tetracycline and vancomycin were evaluated by determining their minimum inhibitory concentrations (MICs, µg/ml) using the broth microdilution method
- Spectrum of antimicrobial activity was achieved using several different strains of *Clostridium perfringens*, *Clostridium sporogenes*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* Enteritidis, *Staphylococcus aureus* and *Campylobacter jejuni*
- Titration was performed using Phosphate Buffer pH 6.5 as diluent in microplates and bacteriocin activity was tested against *Listeria monocytogenes* strains NCTC 1194, CECT 911, CECT 936 and CEP 104794

Figure 1 – Antibiotic susceptibility preparation

Results & Discussion

- All strains were negative for gelatinase, DNAse and biogenic amine production
- All tested strains were considered γ-haemolytic due to the absence of clear zones around the colonies which indicates the absence of haemolytic activity

Table 1 – Virulence genes present for each strain

	agg (1553bp)	*gelE* (419bp)	*efaAfm* (735bp)	*efaAfs* (705bp)	*cylM* (742bp)	*cylLL* (253bp)	*ace* (1008bp)	*asa1* (375bp)
***Leuconostoc mesenteroides* (4-8)**	+	+	+	+	+	+	+	+
***Lactobacillus curvatus* (9A3)**	+	+	+	+			+	+
***Pediococcus acidilactici* (10A2)**	+	+		+	+	+		+

Table 2 Antibiotic susceptibility for each strain. * All strains are intrinsically resistant to vancomycin

	AMP	CHL	CLI	ERY	GEN	KAN	STREP	TET	VAN*
***Leuconostoc mesenteroides* (4-8)**	R	R	S	R	R	R	R	R	R
***Lactobacillus curvatus* (9A3)**	S	S	S	S	S	S	S	S	R
***Pediococcus acidilactici* (10A2)**	R	R	S	R	S	R	R	R	R

- The virulence genes present do not necessarily mean that the strain is virulent

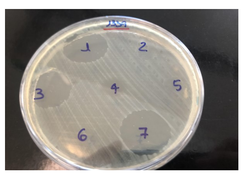

Figure 2 – Halo inhibition in *L. monocytogenes* strain

- Antimicrobial activity against *Clostridium perfringens*, *Clostridium sporogenes* and *Listeria monocytogenes* was observed
- The higher bacteriocin activity (12800 AU/ml) was observed for *Lact. curvatus* 9A3, while *Leuc.mesenteroides* 4-8 and *P. acidilactici* 10A2 produced bacteriocins with lower activities (100 AU/ml) against the *L. monocytogenes* tested strains
- Related to antibiotic susceptibility, only *Lact. curvatus* 9A3 was sensitive to all antibiotics tested

Conclusions

The studied LAB showed essential attributes, such as the absence of important virulence factors and genes, and the production of proteinaceous compounds with antimicrobial activity against the foodborne pathogen *L. monocytogenes*. This indicates that "innovative" *alheiras* are also a source of LAB with appealing characteristics for the food industry. Further tests are required, but the LAB studied, and the strain *Lact. curvatus* 9A3, seem to be promising candidates to be used in future biocontrol approaches.

References

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