

Methods: Gluten-containing medium (GCM) was used to investigate the proteolytic activity of 120 lactic acid bacteria (LAB) isolated from two wheat (*Triticum aestivum*) and one sorghum (*Sorghum bicolor*) artisanal sourdoughs. Initially, LAB were screened by gram-positive staining and catalase test. The isolates were cultivated twice in MRS broth (24 h, 30°C), washed two times with NaCl (0.85% w/v) solution, and inoculated in the gluten citrate agar (GCA) surface (24 h, 37°C) to stimulate the production of proteolytic enzymes. Thereafter, microorganisms were harvested from the GCA and washed twice with sterile saline solution (Na Cl 0.85 w/v) supplemented with calcium chloride 10mM. Each inoculum (10% v/v) was added to GCM, and samples fermented (24h, 37°C) under agitation. The proteolytic activity was determined by SDS-PAGE and non-inoculated GCM was used as negative control. Specific primers for *Lactobacillus* sp. were used to identify gluten-degrading isolates at genus level by RT-qPCR.

Results: From 120 isolates, 23 were able to hydrolyze gluten when fermented GCM were compared with control. Besides, the results from RT-qPCR showed that all 23 proteolytic isolates were *Lactobacillus* sp.

Conclusions: Fermentation of gluten by selected lactobacilli is a potential tool to reduce gluten content in fermented gluten-based foods. These results are a preliminary effort to select gluten-degrading LAB to develop new foods with low content or absence of gluten for individuals with gluten allergy or intolerance. Additionally, more tests are necessary to verify the potential application of these lactobacilli as probiotics.

Investigating Potential Chemoprotective Role of Pomegranate Juice Through Analyzing Its Reciprocal Interactions with the Gut Microbiota

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Objective: While plants and their constituents have traditionally been used for preventing diseases, including cancer, the emerging concept of combination chemoprevention by multiple agents or “whole foods” is becoming increasingly attractive. This work aims to examine the chemopreventive role of polyphenol-rich pomegranate on the rat gut microbiota, as a step towards investigating its ability to neutralize polycyclic hydrocarbons-induced carcinogenesis.

Methods: Six seven-week-old male Sprague Dawley rats were randomly allocated to a control group, only fed a standard AIN76 diet, and a pomegranate group, fed the standard diet + 2.5 mL/kg/d standardized pomegranate juice. Pooled fecal samples from each group were collected twice weekly for eight weeks. DNA from 12 samples was extracted, quantified, and subjected to 16S rRNA amplicon sequencing by Illumina MiSeq. Sequencing data were analyzed by MG-RAST and MOTHUR. Additionally, GC/MS Metabolomic profiling was applied to pomegranate juice before and after its *in vitro* exposure to selected bacterial strains representing the gut microbiota.

Results: In pomegranate-fed rats, phylum Bacteroidetes (particularly genus *Prevotella*) and genera *Faecalibacterium* and *Blautia* were substantially more abundant, while *Eubacterium* and *Enterococcus*, among other Firmicutes, were less abundant. Overall, the Firmicutes-to-Bacteroidetes ratio increased by age and was also significantly lower in the pomegranate-fed rats. A significant decrease in amino acids and sugars was observed in the microbiota-treated juice, while organic acids significantly increased.

Conclusions: This pilot work serves as a standardization step for a model to test the potential chemoprotective effect of pomegranate

(and other natural polyphenols) against the carcinogenic effect of selected polycyclic hydrocarbons.

Akkermansia Muciniphila Robustness Towards Different Temperatures, Atmospheres and Gastrointestinal Conditions

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Objectives: Currently, *Akkermansia muciniphila* has been proposed as a next generation probiotic. However, detailed information addressing its viability under stressful conditions is scarce. Understanding a strain's resilience to potential stressful conditions is crucial for probiotic products manufacture. Herein, we evaluate the *A. muciniphila* susceptibility when exposed to environmental stresses including temperature, atmosphere and gastrointestinal passage (GIT).

Methods: For oxygen and temperature tolerance assays, *A. muciniphila* culture was incubated at different temperatures (4°C/22°C/37°C and 44°C), and under two atmospheres (aerobic and anaerobic) during 72 hours. Each 12 hours, pH values and *A. muciniphila* cultivable cell numbers were determined. To simulate GIT passage, an *in vitro* digestion method (1) was used.

Results: Overall, *A. muciniphila* exhibited a high oxygen tolerance with great stability in culturability (± 8.0 Log CFU/mL) detected after 72 hours exposure at 4°C and 22°C, 24 hours at 37°C and 12 hours at 44°C, as well when subjected to simulated GIT (7.8 ± 0.3 Log CFU/mL).

Conclusions: This work is the first to evaluate the resistant *A. muciniphila* culturability when subject to environmental stresses, suggesting that no strict technological contingencies are required when manufacturing probiotic products containing this bacterium as well its possible storage at household conditions or handling at higher temperatures.

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Reference

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Postbiotic Characterization in the Amensalistic Symbiosis and Correlation to the Resilience of Human Microbiota

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Objective: Lactic acid bacteria constitute a large group of Gram positive organisms belonging to the human microbiota. Among these the genus *Lactobacillus* is the most widely characterized.

Many studies in the literature showed that culture medium and abiotic factors influence the production of antimicrobial substances. Furthermore, recent studies highlight the potential of metabolomics to predict antimicrobial activity among different *Lactobacillus* species. The purpose of the study was focused on the production of postbiotics by two human origin strains of *L. fermentum*. Moreover, the evaluation of antimicrobial activity and the characterization of metabolites were performed.

Methods: In particular, the production of antimicrobial molecules by varying the culture medium for the greatest yield was achieved.