

Screening for lactic acid bacteria with bacteriocinogenic potential from Brazilian artisanal and indigenous fermented food products

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Lactic acid bacteria (LAB) were historically recognized as important microorganisms used in the fermentation of dairy, meat, and vegetable products, playing a significant role in the development of flavors and textures, contributing to the safety and even can be considered as probiotics or postbiotics with health promoting properties for the consumers. As part of the metabolic properties, LAB can produce various antimicrobials, including bacteriocins, which provide a competitive advantage in interactions with other microorganisms. Bacteriocins are protein compounds that exhibit bactericidal or bacteriostatic activity against genetically related strains, including spoilage, foodborne and clinically relevant pathogens.

This study aimed to isolate bacteriocin-producing LAB strains from various artisanal and indigenous fermented foods products from Brazilian territories. For the isolation, the samples were homogenized in saline solution (0.85% NaCl), subjected to serial dilutions, and plated on Man, Rogosa, and Sharpe (MRS) agar, followed by incubation at 37 °C for 48 hours. The obtained colonies were cultivated in MRS broth at 37 °C for 24 - 48 hours and evaluated via biochemical and morphological tests for identification and classified as LAB based on catalase-negative and Gram-positive results (de Vos et al., 2009). The selected strains were evaluated for bacteriocin production against seven strains of *Listeria monocytogenes* and three strains of *Staphylococcus aureus*. For this, cell-free supernatants were collected by centrifugation (10.000 ×g for 10 min at 20°C) from previously grown cultures in MRS broth for 24h at 37°C. The supernatants were heat-inactivated (80°C for 10 min) and tested using the spot-on-the-lawn technique on BHI agar plates containing target strains. Bacteriocin activity was determined by the formation of inhibition halos around the treated supernatants (Fugaban et al., 2021).

Samples from numerous products were analyzed, including water kefir, milk kefir, sourdough starter, probiotic curd, fermented cassava flour, fermented cashew nut paste with olives, kombuchas of different flavors and brands, fresh Minas cheese, and fermented kefir and apple beverage. In total, 348 isolates were obtained, of which 123 were identified as LAB. Sourdough starter, probiotic curd, fermented paste, and fresh Minas cheese samples showed the highest proportion of LAB, while kefir, kombucha, and fermented beverage samples were dominated by yeasts, as observed based on physiological and biochemical tests. The antimicrobial activity was stronger when *L. monocytogenes* were applied as test microorganisms, compared to tested *S. aureus* cultures. This study highlights the microbial diversity in Brazilian artisanal fermented foods and the potential of LAB as sources of bacteriocins with antimicrobial activity. The findings suggest that specific LAB strains could be explored for the development of natural biopreservatives, especially for controlling foodborne pathogens such as *L. monocytogenes*. Future research should focus on the molecular characterization of the selected microbial cultures, their safety and expressed antimicrobials and additional beneficial metabolites and their application in various food matrices as potential beneficial cultures.

References

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