Potential Probiotic Bacteria from Kefir Grains: Isolation, Identification by MALDI-TOF MS and In Vitro Gastrointestinal Resistance

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The isolation of new microorganisms is essential for biotechnological advances in the pharmaceutical, food, cosmetic and environmental sectors. Kefir, a beverage fermented by bacteria and yeasts, presents a promising microbial diversity for obtaining potential probiotic microorganisms adapted to various substrates. Evaluating gastrointestinal resistance in vitro is fundamental for selecting microorganisms that are beneficial to health, as they need to survive the acidic pH of the stomach, bile salts and intestinal enzymes in order to colonize the host, at least temporarily. The aim of this study was to isolate, identify and evaluate the gastrointestinal resistance of lactic acid bacteria from kefir grain microbiota. Homemade kefir grains, from Uberaba/MG and Araraquara/SP, were cultured daily in UHT milk (1:100 m/v) and incubated at 30°C until reaching pH 4.5 ± 0.2. For lactic acid bacteria (LAB) isolation, 1 g of each grain was macerated and diluted in 0.1% (m/v) peptone water, followed by serial dilutions. The samples were plated on MRS and M17 agar, then incubated at 37°C for 48 h under anaerobic conditions. Morphologically distinct colonies were isolated and subcultured to obtain pure cultures, followed by Gram staining and catalase testing for preliminary characterization. Bacterial identification was performed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). *In vitro* gastrointestinal resistance was evaluated using the static INFOGEST protocol, with assessment of bacterial viability after the initial, oral, gastric and intestinal phases. The data were evaluated by one-way ANOVA and Tukey\'s post-test ($p<0.05$). A total of 109 isolates were selected from the kefir microbiota, including 35 Gram-positive and catalase-negative bacteria. In Araraquara/SP grains, *Enterococcus durans* (6), *Enterococcus faecium* (4), and *Lentilactobacillus kefiri* (1) were identified. Belo Horizonte/MG grains contained *Enterococcus faecium* (22), *Lentilactobacillus kefiri* (1), and *Lactococcus lactis* (1). These microorganisms are commonly associated with food products. However, the *Enterococcus* spp. genus is controversial due to its potential production of virulence factors and antibiotic resistance. Only bacteria belonging to different genera were selected for evaluation of gastrointestinal resistance. The *L. lactis* strain had an initial population of 7.71 ± 0.16 log CFU/mL, but did not survive the gastric and intestinal phases. The other strains showed reductions of approximately 1 log compared to the initial population ($p<0.05$), with different sensibilitity to the oral, gastric and intestinal phases. *E. durans* decreased from 7.80 ± 0.16 to 6.87 ± 0.14; *E. faecium from* 7.59 ± 0.75 to 6.64 ± 0.47; *L. kefiri* from 7.53 ± 0.03 to 6.53 ± 0.08 log CFU/mL. The results show that the three strains selected from kefir grains are resistant to the simulated gastrointestinal environment, an important condition for providing systemic beneficial effects. The selected strains will be tested for antimicrobial activity, antibiotic susceptibility and cholesterol-lowering capacity in vitro, before being used for technological purposes.

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