

Biosafety assessment of lactic acid bacteria isolated from raw milk, raw milk cheeses and dairy environment

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Lactic Acid Bacteria (LAB) have a long history of use in the food industry, particularly LAB from raw milk, raw milk cheeses and dairy environments. Autochthonous bacterial species are attractive because they can improve nutritional, technological and safety characteristics of several types of foods. Although LAB, especially lactobacilli group, has GRAS status, it is crucial to conduct studies to confirm safety at strain level. In this context, this study aimed to evaluate the production of gelatinase, haemolytic activity and antibiotic sensitivity of 12 LAB isolated from raw milk (n=4), raw milk cheeses (n=7) and dairy environment (n=1). The lactobacilli strains were cultured in 10 mL of MRS broth at 37°C under anaerobic conditions overnight. In addition, *Lactococcus lactis* and *Leuconostoc lactis* strains were cultured in 10 mL of LM17 broth at 37°C under aerobic conditions overnight. To assess gelatinase production, an overnight culture of each isolate was inoculated into tubes containing 90% of nutrient gelatine (5 g/L of peptone, 3.0 g/L of beef extract and 120 g/L) and 10% of MRS broth. *Bacillus thuringiensis* ATCC 10792 was used as a positive control. After 3 days of incubation, the tubes were immersed in an ice bath for 15 minutes. Gelatine hydrolysis was indicated by a liquid medium, even after exposure to cold temperatures. For haemolytic activity, the overnight culture of each bacterium was inoculated in TSA agar supplemented with 5% of horse blood under aseptic conditions. *Staphylococcus haemolyticus* DPC 6015 was used as a positive control for this test. The tests were conducted in triplicate with two repetitions. The minimal inhibitory concentration (MIC) of eight common antibiotic was determined using the broth micro dilution method, with concentrations ranging from 0.125 to 64 mg.L<sup>-1</sup>. Results showed that none of the isolates tested exhibited gelatinase activity; likewise, they were negative for  $\beta$ -haemolysis. All strains tested were susceptible to ampicillin (MIC 0.25 - 1 mg.L<sup>-1</sup>) and gentamicin (MIC 2 - 32 mg.L<sup>-1</sup>). However, one *Lb. paracasei* strain was considered resistant to erythromycin (> 64 mg.L<sup>-1</sup>) and chloramphenicol (MIC 8 mg.L<sup>-1</sup>). A strain of *Lb. plantarum* was considered resistant to chloramphenicol (MIC 16 mg.L<sup>-1</sup>). A strain of *Lc. lactis* was considered resistant to streptomycin (MIC 64 mg.L<sup>-1</sup>). Ten strains were considered resistant to tetracycline (MIC ranging from 16 mg.L<sup>-1</sup> to >64 mg.L<sup>-1</sup>). The MIC for vancomycin ranged from 0.25 to > 64 mg.L<sup>-1</sup>; for penicillin, from  $\leq$  0.125 mg.L<sup>-1</sup> to 2 mg.L<sup>-1</sup>. This study revealed three important biosafety characteristics of LAB isolated from dairy sources, providing valuable criteria for selecting strains for the food industry and further investigations into their probiotic potential.

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