

Isolation and identification of probiotics – study of commercial products

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This study analyzed the viability and microbiological diversity of probiotic strains in commercial products, focusing on their biochemical, genetic and functional characteristics, in addition to evaluating antimicrobial safety. The original packaging of the products indicated CFU counts as: A, B, C, D and F with 5 billion CFU and E with 100 million CFU/capsule, corresponding to 9.70 log CFU/capsule for products with 5 billion CFU. Based on the comparative analysis of the experimental data and values described on the packaging, only products E and F maintained counts within the expected values. Biochemical tests indicated that all strains presented Gram-positive and catalase-negative characteristics, with a predominant morphology of short rods. In the gas production test, the strains of products C, D and E were characterized as producers, while product F did not show gas production activity, considering the microorganism as homofermentative. In product A, gas production varied between strains, suggesting the presence of different microorganisms. The analysis of genetic profiles by rep-PCR demonstrated homogeneity among the strains isolated from products E, F, C and D; in contrast, product A showed varied band patterns, evidencing microbiological heterogeneity. Molecular identification based on sequencing of the 16S rRNA gene allowed the identification of the strains present in the analyzed products. Strains of *Limosilactobacillus reuteri* were identified in products E, C and D, while *Lactobacillus rhamnosus* was predominant in product F. Product A, however, presented a mixed profile composed of *Limosilactobacillus reuteri*, *Lactiplantibacillus plantarum* and *Lactobacillus acidophilus*. Proteolytic activity analyses indicated that *Lbs. rhamnosus* IS01F, *Lpl. plantarum* IS01A and IS07A, and *Lbm. reuteri* IS05A demonstrated proteolytic capacity in skim milk. This activity may represent an advantageous or virulence factor, depending on its application.

In addition, the diacetyl production capacity was investigated, as it is considered a desirable property for probiotic cultures and fermentation, due to its antimicrobial properties and contribution to the typical butter aroma. In the present study, the strains *Lbs. rhamnosus* IS01F, *Lpl. plantarum* IS01A and IS07A, *Lab. acidophilus* IS12A, and *Lmb. reuteri* IS15A were identified as diacetyl producers. The agar diffusion method was used to assess antimicrobial resistance, in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines. All strains showed susceptibility to the antibiotics recommended by EFSA, except for vancomycin, which is considered intrinsic in lactobacilli. However, it was noted that *Lmb. reuteri* IS01D showed sensitivity to vancomycin. Furthermore, resistance to kanamycin was recorded in *Lpl. plantarum* IS01A and *Lmb. reuteri* IS05A. Antimicrobial resistance in probiotics raises questions about the safety of these microorganisms, since resistance genes can potentially be transferred to pathogenic microorganisms. The results obtained provide a detailed characterization of commercially available probiotic strains, evidencing both microbiological variability and the implications of safety and efficacy for food and therapeutic applications.

References

- Hill, C., Guarner, F., Reid, G. et al. 2014. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews of Gastroenterology and Hepatology*. 11, 506-514.
- Huys G, Botteldoorn N, Delvigne F, De Vuyst L, Heyndrickx M, Pot B, Dubois JJ, Daube G. 2013. Microbial characterization of probiotics--advisory report of the Working Group "Probiotics" of the Belgian Superior Health Council (SHC). *Molecules* 18(12):1911-1924.

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