Safety and beneficial properties of Lactococcus and Leuconostoc isolated from conventional and organic artisanal cheeses from Serra da Canastra region, Minas Gerais state, Brazil.

Gabriela Zampieri Campos[1], Gustavo Augusto Lacorte[2], Ariane Flávia do Nascimento[3], Bernadette Dora Gombossy de Melo Franco[1], Svetoslav Dimitrov Todorov[1,4], Uelinton Manoel Pinto[1]

[1.] FoRC, Food Research Center, São Paulo, SP, 05508-000, Brazil ;
[2.] IFMG, Federal Institute of Minas Gerais, Bambuí, MG, Brazil;
[3.] IFMG, Federal Institute of Southern Minas Gerais, Muzambinho, MG, Brazil;
[4.] ProBacLab, University of Sao Paulo, São Paulo, SP, 05508-000, Brazil ;

Canastra Cheese is an artisanal product made from raw milk by small producers in Serra da Canastra region, in Brazil. The production process employs back-sloping inoculation driving fermentation by an endogenous culture called \"*pingo*", which is originated from the whey collected from the previous day's production. Cheeses are dairy products, where lactic acid bacteria (LAB), including those from the genera *Lactococcus* and *Leuconostoc*, play an essential role in fermentation processes as starter and/or adjunct cultures, and contribute as safeguard against pathogens due to their ability to produce numerous antimicrobial metabolites. Although several LAB are generally recognized as safe (GRAS), assessing their safety at the strain level is crucial for their use in the production of fermented foods, including dairy products. In the current study, we have evaluated the safety and beneficial properties for seven LAB strains of *Lactococcus lactis* (1B2, 1B3, 6D5 and 1E4), *Leuconostoc citrium* (10C2) and *Leuconostoc mesenteroides* (13B4 and 13A1) identified based on 16S rRNA partial gene sequence from dairy samples obtained from Serra da Canastra including *pingo*, conventional and organic artisanal cheeses with 1 and 14 days of ripening. As initial criteria for the selection of safe LAB, we evaluated the hemolytic properties, mucin degradation and antibiotic susceptibility/resistance according to EFSA recommendations. In the following step, the strains were evaluated by PCR for the presence of genes associated with vancomycin resistance (*vanA, vanB, vanC*), biogenic amines production (histidine decarboxylase, *hdc*; tyrosine decarboxylase, *tdc*; ornithine decarboxylase, *odc*) and virulence factors (enterococcal surface protein, *esp*; gelatinase, *gel*; aggregation substance, *asa*; cytolisin, *cyt*: hyaluronidase, *hyl*; endocarditis antigen, *efa*: and enterococcal pathogenicity island, *is16*). Desirable genes, related to the production of gamma-aminobutyric acid (GABA; *gad*) and nisin-encoding gene (*nis*), as well as the *in vitro* production of diacetyl were also evaluated. All analyzed strains exhibited gamma hemolytic activity, absence of mucin degradation, and sensitivity to antibiotics. None of the tested strains presented genes related to vancomycin resistance, biogenic amine production, or other virulence genes, except for the *Lc. lactis* strains 1E4 and 1B3, which harbored the *esp* gene and *is16*, respectively. One strain (*Lc. lactis* 6D5) exhibited the *gad* gene, while none of the strains showed the *nis* gene. Furthermore, the same strain (*Lc. lactis* 6D5) displayed low diacetyl production, while the others showed no detectable diacetyl production. Despite some undesirable observed traits, most of the strains of *Lactococcus* and *Leuconostoc* collected from conventional and organic systems were deemed safe, affirming their suitability for application in food fermentation processes.

References

FUGABAN, J. I. I. et al. Characterization of Partially Purified Bacteriocins Produced by Enterococcus faecium Strains Isolated from Soybean Paste Active Against Listeria spp. and Vancomycin-Resistant Enterococci. Microorganisms, v. 9, n. 5, p. 1085, 2021.
ABE, F. et al. Safety evaluation of probiotic bifidobacteria by analysis of mucin degradation activity and translocation ability. Anaerobe, v. 16, n. 2, p. 131–136, 2010.
CHARTERIS, W. P. et al. Antibiotic Susceptibility of Potentially Probiotic Lactobacillus Species. Journal of Food Protection, v. 61,

n. 12, p. 1636–1643, 1998.

Acknowledgements: FAPESP (2013/07914-8, 2023/05394-9) and CNPq (403661/2023-4).