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# Diversity and Functional Characterization of Lactic Acid Bacteria in Alheira Sausage: Insights for Food Safety and Quality Control †

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**Abstract:** Alheira, a fermented sausage traditionally produced in the Portuguese region of Trás-os-Montes, undergoes a maturation process that reduces pH through the fermentative action of lactic acid bacteria (LAB). In this particular study, the aim was to explore the diversity of LAB in alheira. Twenty-eight alheira samples were collected from various regions of Portugal (including Vimioso, Mirandela, Vinhais, Mogadouro, Povoa de Lila, Bragança, and Valpaços), and LAB were isolated from MRS and M17 agar, and confirmed. After characterization of technological potential, including in-vitro antimicrobial activity against foodborne pathogens and acidification potential, a subset of 63 samples were selected for identification. By conducting genetic analysis of the 16S ribosomal region, the study revealed that *Enterococcus faecium* (27%) and *Leuconostoc mesenteroides* (19%) were the most frequently found LAB species. Furthermore, *Weissela viridescens*, *Staphylococcus epidermidis*, and *Latilactobacillus curvatus* were identified in only 1% of the total samples. Interestingly, the regions of Bragança, Valpaços, and Mirandela exhibited the highest diversity of LAB species. When comparing the LAB isolates with the technological features, it was found that *Pediococcus*, *Lactiplantibacillus*, and *Leuconostoc* demonstrated the strongest antagonistic activity against *Salmonella* Typhimurium, *Listeria monocytogenes*, and *Staphylococcus aureus*. Notably, *Enterococcus*, particularly the species *E. faecium* and *E. durans*, displayed a higher acidification potential. These findings emphasize the importance of a comprehensive LAB characterization for enhancing food safety and quality control measures associated with alheira production.

**Keywords:** fermentation; sausage; technological properties; acidification; biopreservation

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