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Biocontrol potential of Lactiplantibacillus plantarum 9A3 isolated from an innovative alheira: characterisation of its bacteriocin(s)

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Food safety is a global concern, particularly due to the presence of pathogenic microorganisms (European Food Safety Authority and European Centre for Disease Prevention and Control, 2024). The growing demand for natural alternatives to synthetic chemical preservatives has driven research into biocontrol strategies, such as the use of lactic acid bacteria (LAB) and their antimicrobial metabolites, including bacteriocins. These antimicrobial peptides can inhibit foodborne pathogens without compromising the quality of the food product (Azevedo, I., Barbosa, J., Albano, H., Nogueira, T., & Teixeira, P., 2024).

Alheira, a traditional Portuguese sausage-like product, has evolved through innovation, including alternative formulations to meet consumer preferences for healthier, tastier, and higher-quality products. The presence of LAB, such as *Lactiplantibacillus plantarum*, in this product can help inhibit undesirable microorganisms, thereby improving food safety. However, the effectiveness of biocontrol using native strains depends on a detailed characterization of the strain and its bacteriocins, assessing factors such as the spectrum of activity, stability, and mode of action.

This study aimed to evaluate the biocontrol potential of the *L. plantarum* 9A3 strain, isolated from an innovative vegetable- and mushroom-based alheira, by characterizing its bacteriocins. More specifically, the study examined:

i) The maximum bacteriocin production (Arbitrary Units (AU)/mL) during *L. plantarum* growth,

ii) The bacteriocins' mode of action and antimicrobial activity spectrum,

iii) The effects of enzymes, surfactants/detergents, pH, and temperature on bacteriocin activity,

iv) Bacteriocin adsorption and molecular size.

Additionally, Whole Genome Sequencing (WGS) was performed to identify genes related to bacteriocin production.

Genes encoding plantaricin E, plantaricin F, pediocin, and leucocin A were identified in *L. plantarum* 9A3. The produced bacteriocins exhibited a broad spectrum of activity against several strains of *Listeria monocytogenes*, *Clostridium* sporogenes, and *Clostridium perfringens*, with an estimated molecular size ranging from 37 to 52 kDa. The bacteriocins remained stable under various conditions, including temperatures from 4 to 100 ºC, pH levels from 2 to 8, and exposure to surfactants and detergents (Tween 20 and 80, SDS, EDTA at 0.1, 2, and 5 mM, urea, and sodium deoxycholate) as well as enzymes (papain and catalase). The highest activity (AU/mL =

12,800) against four *L. monocytogenes* strains was observed between 21 and 36 hours of *L. plantarum* 9A3 growth, indicating a bacteriostatic mode of action.

The studied strain appears to be a strong candidate for potential application as a protective culture in the food industry. Understanding the optimal conditions for bacteriocin production is essential to maximizing its applicability.

References

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