Production of bioactive peptides through fermentation

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Objectives: Reducing antibiotic use in livestock is a global priority to mitigate antimicrobial resistance and protect human, animal, and environmental health. Bioactive peptides generated through microbial fermentation are emerging as sustainable alternatives, offering antibacterial and immunomodulatory effects aligned with the One Health approach. This study aimed to characterize a novel fermentative hydrolysis using a *Bacillaceae* species (identity withheld for confidentiality), that produces peptides with antibacterial and anti-inflammatory activities, as well as its impact on the gut microbiome.

Materials and methods: Fermentation was carried out using the selected *Bacillus* strain in the presence of milk for 7 days in different substrates (milk, whey and BHI) and pH levels, bacterial growth and proteolysis were evaluated through that time. After fermentation, coagulated proteins were removed by filtration, and peptides were isolated from the soluble fraction by ultrafiltration. Antibacterial activity was tested against *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella enterica*, *Bacillus cereus* using broth microdilution assays, and minimum inhibitory concentrations (MICs) were calculated.

Results: The novel bacterial strain was able to ferment and induce proteolysis throughout the 7 day-incubation in both milk and whey substrates, but not in BHI. Proteolysis was concomitant to antibacterial activity development against all referred strains. Interestingly, the highest and fastest activities were obtained with whey as a substrate. A standardized 2-day fermentation and ultrafiltration protocol was developed for industrial up-scaling. Isolated fermentation-derived peptides using this protocol showed a dose-dependent antibacterial activity, with MICs of 110 µg/mL against *Escherichia coli* O157, 90 µg/mL against *Staphylococcus aureus*, 150 µg/mL against *Listeria* monocytogenes, 120 µg/mL against *Salmonella enterica*, 180 µg/mL against *Bacillus cereus*.

Conclusions: Peptides obtained by fermentation show high promise as antimicrobial agents, offering a sustainable and effective alternative to conventional antibiotics and disinfectants used in the food industry. These results reinforce the global efforts within the One Health approach to combat antimicrobial resistance, contributing to innovative solutions that promote food safety and the protection of public health. In this work we developed a standardized fermentation protocol, easily adaptable to industrial scale, which enables the application of these peptides in food safety and animal production contexts.

Acknowledgments/Funding: This work has been funded by a grants from Lusófona University (SmartPep and Fazer + (Fermenta)).