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THIRD INTERNATIONAL SYMPOSIUM ON NATURAL ANTIMICROBIALS:

Current status, challenges and perspectives





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DETECTION AND CHARACTERIZATION OF BACTERIOCIN-PRODUCING ENTEROCOCCUS STRAINS WITH ANTIMICROBIAL ACTIVITY AGAINST CLOSTRIDIUM PERFRINGENS

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Context and problematic: Necrotic enteritis (NE) caused by *Clostridium perfringens* is an emergence issue in poultry farming. New approaches, other than the use of antibiotics, are necessary to prevent the development of multidrug resistant bacteria and NE. Enterococci are commensal microorganisms that might produce antimicrobial peptides (enterocins) with activity against pathogens, being good candidates as probiotics.

Objective: To screen and characterize *Enterococcus* strains from poultry origin for their inhibitory activity against *C. perfringens* and to identify the bacteriocins they produce.

Methodology: 251 enterococci of poultry origin have been used in this study and five bacteriocinproducing enterococci from U. LAVAL collection as controls. First, all strains were screened for their inhibition activity against the indicator *C. perfringens* (X2967) by the "*spot on the lawn*" method. Then, the activity in their supernatants was studied by agar well diffusion and microtitration against a collection of 20 *C. perfringens* strains. Strains showing clear inhibitory activity against one or more indicator strains were further characterized: antibiotic resistance profile, gelatinase and beta-hemolysis activity. Genetic characterization was performed by whole genome sequencing (WGS) to study the genes encoding bacteriocins as well as the resistome, virulome, plasmidome and genetic lineages of bacteriocin-producer isolates.

Results: 12 enterococci showed clear inhibition activity against at least one of the 20 *C. perfringens*. Five of the 12 enterococci were susceptible to the nine antibiotics tested. The remaining strains presented at least resistance to one of the antibiotics being ciprofloxacin 50%, tetracycline 33% and erythromycin 33% the most frequent. The gelatinase activity was positive in one strain and no β -hemolysis was observed. Structural genes for enterocins were detected in the 12 enterococci strains by WGS encoding the following enterocins: Enterocin P, Enterocin L50 A/B, Enterocin A, Enterocin B, Enterocins NKR-5- 3A-D-Z, and Enterocin SE-K4; moreover, genes related to Staphylococcin CSSa/b type were also found. The resistome, virulome and plasmidome of these isolates are being analysed.

Conclusion: With current data, we can conclude that enterococci from poultry origin are enterocin producers and might have potential as probiotics; nevertheless, due to their role as opportunistic pathogens, a deep characterization is needed for determining their potential interest as probiotics.