

Characterization of bacteriocin production, safety and technological potential of two *Enterococcus faecium* strains isolated from Brazilian artisanal cheeses

Karina Maria Olbrich dos Santos¹, Antônio Diogo Silva Vieira^{1,2}, Jacqueline da Silva Oliveira¹, Cíntia Renata Costa Rocha³, Ana Catarina de Souza Lopes⁴, Laura Maria Bruno⁵, Maria de Fátima Borges⁵, Bernadette Dora Gombossy de Melo Franco², Svetoslav Dimitrov Todorov²

¹EMBRAPA, Caprinos e Ovinos, Sobral, CE, Brasil; ²Laboratório de Microbiologia de Alimentos, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, SP, Brasil; ³Departamento de Bioquímica, LIKA, Universidade Federal de Pernambuco, Recife, PE, Brasil; ⁴Departamento de Medicina Tropical, Universidade Federal de Pernambuco, Recife, PE, Brasil; ⁵EMBRAPA, Agroindústria Tropical, Fortaleza, CE, Brasil

Introduction: Many lactic acid bacteria produce bacteriocins with broad spectra of inhibition and may be applied in food preservation or as potential probiotic candidates.

Purpose: This study was on the characterization of bacteriocin/s produced by *Enterococcus faecium* EM485 and EM925, isolated from Coalho cheeses, and check for its safety and technological properties.

Methods: Isolates EM485 and EM925, differentiated by RAPD-PCR, have been tested for production of bacteriocin/s against food-borne pathogenic microorganisms and been investigated for their probiotic and technological potential including aggregation; hydrophobicity; deconjugation of taurocholic acid (TC), taurodeoxycholic acid (TDC), glycocholic acid (GC), and glycodeoxycholic acid (GDC); survival rates in the conditions simulating the GIT; resistance to antibiotics; and presence of virulence genes.

Results: Isolates EM485 and EM925 were selected based on their effective inhibition against *Listeria monocytogenes*, and classified as *E. faecium* based on 16s rDNA analysis. In MRS at 37°C, bacteriocins produced by both strains were detected as 3200AU/ml. These peptides were inactivated by proteolytic enzymes, but not by α -amylase, catalase and lipase. The two bacteriocins remained stable at pH from 2.0 to 10.0 and after exposure at 100°C for 120min and in presence of surfactants and salts. DNA from both strains generated positive PCR results for enterocin A and enterocin B genes. High levels of co-aggregation have been observed for both strains with *E. coli* (78.35 \pm 2.16% and 74.31 \pm 3.64%) or *Clostridium* spp. (81.13 \pm 1.92% and 84.26 \pm 3.44%). Both strains presented low levels of hydrophobicity (8.18% and 11.33%). *E. faecium* EM485 and EM925 were able to grow in presence of 0.5% of TC, TDC, GC and GDC, however, only being able to deconjugate GDC and TDC. Both strains showed good survival when exposed to the conditions simulating the GIT. When tested for presence of virulence genes, only tyrosine decarboxylase and vancomycin B generated positive PCR results.

Significance: This is the first report on detection and characterization of bacteriocinogenic *E. faecium* from Coalho cheeses with potential beneficial and technological properties with low virulence profile.