TITLE: MOLECULAR AND GENETIC CHARACTERIZATION OF BLIS (*BACTERIOCIN--LIKE INHIBITORY SUBSTANCE*) E86 PRODUCED BY *ENTEROCOCCUS FAECIUM* E86 WITH BIOTECHNOLOGICAL APPLICATIONS

AUTHORS: DE FARIAS, F.M.[1]; BASTOS, M.C.F.[1]; ALBANO, R.M.[2]; TEIXEIRA, L.M.[1]; SILVA, D.C.V.[3]; BONELLI, R.R.[1]; MIGUEL, M.A.L.[1]

INSTITUTION: INSTITUTO DE MICROBIOLOGIA PAULO DE GÓES, UFRJ (AV. CARLOS CHAGAS FILHO, 373, CIDADE UNIVERSITÁRIA, ILHA DO FUNDÃO, RIO DE JANEIRO)[1]; INSTITUTO DE BIOLOGIA ROBERTO DE ALCÂNTARA GOMES, UERJ (BOULEVARD 28 DE SETEMBRO, 87, VILA ISABEL, RIO DE JANEIRO)[2]; INSTITUTO OSWALDO CRUZ, FIOCRUZ (AV. BRASIL, 4365, MANGUINHOS, RIO DE JANEIRO)[3]

ABSTRACT:

The demand for new antimicrobial compounds, both in the food industry and in the medical field, has been the focus of the scientific area in recent decades. Therefore, the study of bacteriocins and bacteriocin-like inhibitory substances (BLIS) has grown, and promising results have emerged using them as a tool against deteriorating or pathogenic microorganisms. These antimicrobial compounds are peptides or proteins ribosomally synthesized by prokaryotes, which have inhibitory activity against other prokaryotes. Some bacteriocins produced by *Enterococcus* spp., referred to as enterocins, have the capacity to inhibit various human and animal pathogens [*Clostridioides difficile*, vancomycin resistant enterococci (VRE) and *Listeria monocytogenes*, among others]. In 2008, Miguel and collaborators isolated, from meat pie, a strain capable of producing a BLIS, identified as *Enterococcus faecium* E86. Considering the great potential of this BLIS as a biopreservative, the present project aims to characterize genetically and molecularly the strain *E. faecium* E86. Then, sequencing and assembly of the *E. faecium* E86 genome were performed. *In silico* analyzes were carried out to identify the genetic clusters involved in the enterocin TW-21 (*entTW21entiTW21*) and enterocin P (*entPentiP*) biosynthesis. Through RT-PCR, it was verified that all 4 genes responsible for the biosynthesis of both enterocins are transcribed. Based on the *DomTHREADER* platform, it has been observed that the secondary structure of the hypothetical immunity protein EntiTW21 is very similar to the structure of the immunity protein of carnobacteriocin B2 and probably shares similar biological functions. To our knowledge, this is the first report of the hypothetical immunity protein of the enterocin TW-21 and its bacteriocinogenic cluster. After purification from the culture medium and the mass spectrometric analyzes, it was observed that only enterocin P was responsible for the antimicrobial activity of the strain. The bacteriocin in question was able to inhibit all strains of *Listeria* spp. and *Enterococcus* spp. (being VRE or not) evaluated in the spectrum of action test. In the majority of the cases, enterocin P caused an intense bacteriolytic activity, with the exception of the strain *L. monocytogenes* 3236, against which enterocin P exhibited a bacteriostatic activity. At the end of the 24 h analysis, the count of viable cells of *L. monocytogenes* 3236 in relation to the initial count was the same.

Keywords: Genome sequencing, bacteriocin, enterocin, *Enterococcus faecium*, biotechnological application

Development Agencies: CNPq and FAPERJ