

TITLE: BENEFICIAL PROPERTIES OF BACTERIOCINOGENIC *Enterococcus faecium*, EVALUATED IN GUT SIMULATED CHALLENGE MODEL

AUTHORS: Valledor, S.J.D.; Dioso, C.M.; Vazquez Bucheli, J.E.; Holzapfel, W.H.; Todorov, S.D.

INSTITUTION: ProBacLab, Department of Advanced Convergence, Handong Global University, Pohang, Gyeongbuk 37554, Republic of Korea

ABSTRACT:

Screening for bacteriocin producing LAB become a scientific trend, however, application of bacteriocinogenic strains and their efficacy in the model system *in situ* and *in vivo*, still merit more attention and deeper studies. As proteins by nature, bacteriocins can be affected by the presence of the proteolytic enzymes presence in the gut, but evidences showing that bacteriocinogenic strains can survive and express their antimicrobial peptides in the gut environments and effectively control the pathogens bacteria. The aim of this study was to isolate a safe bacteriocinogenic strain/s and after characterization of the produced antimicrobials to evaluate efficacy for control of *Listeria monocytogenes* in the model system mimicking gut environment.

The bacteriocin-producing strain *Enterococcus faecium* ST10Bz was isolated from boza, a Bulgarian cereal based fermented and identified by physiological, biochemical and bio-molecular techniques, including 16S rRNA sequencing. *E. faecium* ST10Bz produces a bacteriocin with strong activity against several *L. monocytogenes*, vancomycin-resistant *E. faecalis* strains and other *Enterococcus* species, but not against most of other tested LAB. Bacteriocin ST10Bz was produced in MRS broth at 25, 30 and 37°C, however, reaching a maximum production of 12800 AU/ml, 6400 AU/ml and 6400 AU/ml, respectively, as recorded against *Listeria monocytogenes*. Expressed bacteriocin/s by studied strain showed bactericidal mode of action against *L. monocytogenes* ATCC15313 and *E. faecalis* 200A. Based on PCR approach and appropriate sequencing of generated amplicons, *E. faecium* ST10Bz harbours genes encoding for enterocin A, B and P. In addition, strain ST10Bz was γ haemolytic, and tyramine-positive on biogenic amine production assay, which is a typical characteristic of *E. faecium* strains. Studied strain was susceptible to vancomycin, kanamycin, gentamycin, ampicillin, streptomycin, erythromycin, clindamycin, and tetracycline. And based on PCR analysis, DNA obtained from *E. faecium* ST10Bz generated positive results for presence of *map*, *mub* and *ef-tu* (adhesion genes), however, was negative for bacterial-binding/aggregation genes including *prgB*, *EF1249*, *EF2380* and *EF2662*. When cultured in the simulated gastrointestinal conditions, *E. faecium* ST10Bz showed high survival rate and reduced the number of *L. monocytogenes* on single and co-culture, respectively.

E. faecium ST10Bz can be considered as safe strain, producer of bacteriocins from enterocin A/B family, able effectively to inhibit *L. monocytogenes* in a GIT model system. Presence results will need to be validated in appropriate animal model and in addition to antimicrobial properties, related immunological marker will need to be monitored in order to suggest *E. faecium* ST10Bz as potential human probiotics with antimicrobial functions.

Keywords: probiotics, lactic acid bacteria, bacteriocins, safety, GIT model

Acknowledgments: NRF (South Korea) and Handong Global University (Pohang, South Korea) for supporting this project.