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Bacteriocinogenic properties of Enterococcus faecium strains isolated from bats fecal samples

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Bats are unique mammalians, adapted to specific ecological. Even been mammalians, the specificity of the bats GIT microbiota is more like that of the avians, which is dominated by Proteobacteria, most probably related to their lifestyle and inhabitants. As an adaptation, bats have a rapid GIT transit time, which may reduce the stability of their microbiome, that may limit nutrient uptake, and influence pathogen exposure and the evolution of tolerance mechanisms and all this made their microbiome unique and can be regarded as source for differentiated microbial cultures.

The aim of this study was to screen for bacteriocins producers in the fecal samples of bats, with further objective to study development of potential probiotics with health promoting properties for bats, parts of the rehabilitation and conservation programs.

In the isolation process of LAB from the bats fecal samples applied triple layer approach was applied with objective to preselect bacterial cultures with antimicrobial properties. From more than 90 isolates presenting inhibitory properties observed in the preliminary screening, 6 cultures were selected for the following research, according to the preliminary bacteriocin test against *Listeria monocytogenes* 211, 603 and 620. Based on the preliminary Gram staining and catalase test, and test recommended by Burgey's Manual (de Vos et al., 2009), 6 cultures preselected as potential bacteriocinogenic LAB were differentiated by repPCR on total DNA, and two grouped into 2 clusters and further 16s rRNA identification showed to be part of the *Enterococcus faecium* species. It was interesting that same strains were obtained from fecal samples from different animals, pointing that animals from same groups can share their microbial populations.

The selected for further studies *E. faecium* TL01 and TL76 strains were producing antimicrobial peptides (bacteriocins) most probably different from already reported, since we were not able to record presence of tested Enterocin A, B, P, 50 genes. Produced bacteriocins were stable at large margins of pH and temperature, included at 121^{o}C for 15 min and presence of different chemicals applied in food processing processes and analytical procedures. Highest levels of bacteriocins produced by *E. faecium* TL01 and TL76 was recorded during stationary phase (12800 AU/ml and 25600 AU/ml, respectively) when cultured in MRS both at 37ºC. Moreover, the growth inhibition of *L. monocytogenes* 211, 603 and 620 was noted when bacteriocin containing CFS from *E. faecium* TL01 and TL76 was added. Samples collected at 10h from experimental and controls setups showed no presence and around log 8 CFU/ml viable counts, respectively, for *L. monocytogenes* strains. Bacteriocins produced by *E. faecium* TL01 and TL76 were showing very selective inhibitory activity against different strains of *L. monocytogenes* and only a few of other test microorganisms included in the examination panel were inhibited. *E. faecium* TL01 and TL76 can be considered as safe, as performed test showed negative results for the gelatin hydrolysis, proteolytic and hemolytic activity, mucin degradation tests and antibiotic resistance/sensitivity and further screening for presence of some virulence genes (recommended by Fugaban et al., 2021) regarding safety for enterococci.

References

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