TITLE: EVALUATION OF BENEFICIAL PROPERTIES OF *Lactiplantibacillus plantarum* (*Lactobacillus plantarum*) ST63HK AND ST66HK AS A POTENTIAL PROBIOTIC CANDIDATE FOR THE ORAL CAVITY

AUTHORS: Kim, H.; Fugaban, J.I.I.; 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:
In the oral cavity ecosystem different obstacles and mechanisms contribute to the effective reduction and control of different pathogenic "intruders". On the other side, specificity of the oral cavity conditions can be optimal for the colonization of different beneficial microorganisms including temperature, sufficient moisture, available nutrients, minerals, vitamins, and various surfaces that allow microbial attachment, and also including factors that will facilitate LAB to colonize. This study aimed to select beneficial strains from the oral cavity of healthy volunteers and to evaluate these as potential oral probiotic candidates. The selection process was based on the isolation, differentiation, identification and safety assessment of LAB strains, followed by series of experiments directing to the selection of appropriate candidates with beneficial properties.
In the preliminary screening two isolates from the oral cavity of a Caucasian volunteer were identified as *Lactiplantibacillus platarum* ST63HK and ST66HK based on 16S rRNA sequencing and differentiated based on repPCR analysis. Applied tests showed no hemolytic, proteinase, or gelatinase activities, neither production of biogenic amines. Both strains can be considered safe based on negative results for the detection of *efa*A, *cyt, IS*16, *esp, asa*1, and *hyl* virulence genes, and the absence of vancomycin resistance (*van*ABCDEG) genes. This strains were resistant to vancomycin, but not to other antibiotics applied in this study. Moreover, cell-to-cell antagonism indicated that strains ST63HK and ST66HK were able to inhibit the growth of *Bacillus cereus*, *B. pumilus*, *Enterococcus avium* and *E. faecalis*. *Lp. plantarum* ST63HK and ST66HK does not produce β-galactosidase and positive results were found for *eftu* and *gad* genes encoding adhesion and GABA production, respectively, but not for genes related to folate production. A cell surface hydrophobicity level of 39.04% and 8.63% was determined. The studied strain was able to survive in a wide range of pH values ranging from 4.0 to 8.0, and ox-bile concentrations, of up to 0.3%, as a single factor. In the simulated GIT passage model both strains showed a good survival rate after 3h, and also showed good survival in two artificial saliva models for 30 min. Interaction of both strains with drugs from different generic groups and oral cavity hygiene products was evaluated and the MIC for drugs showing an inhibitory effect on bacterial growth was determined to predict possible negative consequences for the combined application of evaluated drug/hygienic products and studied the oral cavity probiotic candidate. Overall, antagonistic properties, safety assessment, and high rates of survival in the GIT and oral cavity models, and even in the presence of commonly used drugs and oral hygiene products, indicate *Lp. plantarum* ST63HK and ST66HK to be a promising oral cavity probiotic candidates.

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

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