Anti-biofilm Activity of *Lactobacillus mucosae Extracellular Extracts against Staphylococcus aureus* from ovine mastitis

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**Background.** *Staphylococcus aureus* (S. aureus) is a major pathogen responsible for mastitis in dairy herds. The multidrug resistance and/or formation of *S. aureus* biofilm, considered as one of the virulent factor, may explain why mastitis is difficult to treat and persists in herd as chronic or recurrent disease. These factors often result in the frequent failure of antibiotic therapy and economic lost in dairy industry [1]. In this context, the development of new strategies, as alternatives or complements to antibiotic therapy for the management of mastitis, is particularly appealing. One sustainable alternative to treat or prevent mastitis with avoiding intramammary biofilm formation is the use of lactic acid bacteria (LAB) or their extracellular metabolites as mammary probiotics.

**Methods.** In present in vitro study, we evaluated the ability of CFCSs (Cell Free Culture Supernatants) from two ovine *Lactobacillus mucosae (L. mucosae)* isolates to prevent *S. aureus* (three ovine isolates from acute mastitis) biofilm formation using by molecular approaches as Fluorescence in situ Hybridization (FISH) and Flow Cytometry method combined with 16S rRNA fluorescent labelled probes (FISH-FCM) as well as spectrophotometric crystal violet assay (SCVA).

**Results.** The strongest biofilm forming ability determined was shown by *S. aureus* 91 (FISH: 8.37±0.19; FISH-FCM: 8.25±0.10; SCVA: 0.524±0.13, given as average values of absorbance measurement in 3–570 nm (A570± SD) in comparison with other two isolates 20 (FISH: 8.26±0.07; FISH-FCM: 8.32±0.03; SCVA: 0.381±0.03) and 203 (FISH: 8.21±0.10; FISH-FCM: 8.20±0.13; SCVA: 0.479±0.06). The CFCs of *L. mucosae* strains affected *S. aureus* biofilm formation in a strain-dependent manner. The most significant anti-biofilm effect was caused by *L. mucosae* 14K CFCS on *S. aureus* 203 (percentages of *S. aureus* biofilm formation after treatment ranged from 1.74 to 3.76%, detected by three methods). *L. mucosae* OV6 mediated approximately 10-fold diminishing of *S. aureus* biofilm formation, with exception of *S. aureus* 203 with higher discrepancy results achieved by FISH method and *S. aureus* 20 in results obtained by SCVA. Pearson’s R correlation test showed positive correlation of used methods with correlation coefficient (r) ranging within 0.719 - 0.923 and probability p=0.0001.

**Conclusions.** The ovine *L. mucosae* 14K CFCS appears to be an efficient alternative to the use of commonly prescribed antibiotics for the prevention of *S. aureus* biofilm formation, which may be responsible for troubles in treatment of infection mastitis. Antimicrobial metabolites from CFCS of *L. mucosae* 14K were effective in repressing the *S. aureus* biofilm. The future of our study will be concentrated on determination of antimicrobial compounds types secreted by *L. mucosae* 14K although seeing that, the *L. mucosae* isolates CFCS inhibited *S. aureus* biofilm formation without affecting bacteria growth, thus, the way in which these extracellular products influence bacterial-surface interaction seems to be more closely related to changes in surface tension and bacterial cell-wall charge.

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**Keywords:** *Staphylococcus aureus, Lactobacillus mucosae, biofilm formation*

**References**