

TITLE: TERPINEN-4-OL: ANTIMICROBIAL ACTIVITY AND GENE EXPRESSION *slpA* INVOLVED IN THE ADHESION OF *LACTOBACILLUS ACIDOPHILUS* IN BIOFILM

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ABSTRACT:

Lactobacillus acidophilus is frequently found in high numbers in both superficial and deep carious lesions. The use of natural antimicrobial agents that do not cause side effects, bacterial resistance and that is effective in controlling the biofilm, has been the object of great interest. In this study, we investigated the antimicrobial effect of Terpinen-4-ol on *L. acidophilus* biofilm grown on bovine enamel and dentin and also in gene expression *slpA* involved in biofilm adhesion. Enamel specimens were placed in microtiter plate wells and covered with sterile human saliva for 1 h 30 min. Then, 1 ml of *L. acidophilus* (10^7 CFU ml⁻¹) was distributed in the wells and the plate was incubated for 24 h. Dentin specimens were stabilized within falcon tube (50 mL), 500 µL of *L. acidophilus* were added to the tubes and centrifuged (3000, 4000 and 5000 rpm). Then, the specimens were treated with Terpinen-4-ol (0.24% and 0.95%) and chlorhexidine (CHX 0.12%) for 60 seconds. Cell viability of the resulting biofilm was assessed using Confocal Laser Scanning Microscopy (CLSM). The *slpA* gene expression was investigated by real time PCR after exposure *L. acidophilus* to Terpinen-4-ol and CHX for 15 and 30 minutes. Terpinen-4-ol showed antimicrobial activity against *L. acidophilus* biofilm developed in enamel, with action similar to CHX. In biofilm on dentin Terpinen-4-ol showed a statistically significant difference ($p < 0.05$) with the control. There was a reduction in *slpA* expression when in contact with Terpinen-4-ol for 15 minutes, however the gene overexpression occurred after 30 minutes of exposure. These findings demonstrate that Terpinen-4-ol shows a significant antimicrobial activity and the ability of modulating *slpA* gene expression involved in the adhesion of *L. acidophilus* in biofilm.

Keywords: *Lactobacillus acidophilus*, biofilm, real time PCR, Terpinen-4-ol

Financial support: FAPESP 2014/23241-6; 2015/08742-1