TITLE: Antimicrobial activity of extract and fractions of *Streptococcus mutans* on *Candida albicans* in *Galleria mellonella* model

AUTHORS: SANTOS, J.D.[1]; BENTUBO K.L.[1]; FUGISAKI L.R.O.[1]; MEDINA R.P.[2]; SILVA D.H.S.[2]; FUCHS B.B.[3]; MYLONAKIS E.[3]; JORGE A.O.C.[1]; JUNQUEIRA, J.C.[1]

INSTITUTION: [1]Institute of Science and Technology, UNESP - Univ. Estadual Paulista, São José dos Campos, SP, Brazil. [2]Chemistry Institute of Unesp, Campus of Araraquara, SP, Brazil. [3]Brown University, Providence, Rhode Island, USA.

ABSTRACT: *In vitro* studies have shown that *Streptococcus mutans* can produce metabolites capable of inhibiting *Candida albicans*, becomming interesting the identification and development of new substances for the treatment of oral candidiasis. In this study, we evaluated the antimicrobial and immunomodulatory effects of the metabolites produced by *Streptococcus mutans* (UA159) on experimental candidiasis in *Galleria mellonella* model. For the preparation of *S. mutans* supernatant, the strain was incubated in BHI broth at 37°C with 5% of CO_2 for 24 h, and then a standardized suspension of *S. mutans* (10^7 cells/ml) was cultured in BHI broth for 4 h (37°C in 5% of CO_2). The supernatant metabolites of *S. mutans* culture were filtered using 0.22 μm porosity membrane and extracted with ethyl acetate (3 times). After that, it was concentrated on a rotary evaporator and lyophilized to provide the crude extract. The extract was fractioned in a C-18 derivatized silica column (150 g, Φ = 3.5 cm) using different solutions of MeOH:H_2O (36:64, 49:51, 60:40, 76:24, 100:0) as eluent and yielded 5 fractions (245 mL each) at the Chemistry Institute of Unesp, Campus of Araraquara (NuBBe). The prophylactic and therapeutic effects of the crude extract and their fractions were evaluated on experimental candidiasis in *Galleria mellonella* by survival curve test, as well as by the quantitation of *C. albicans* cells in the hemolymph of *G. mellonella* larvae. In the analysis of survival rate, 100% of the larvae died within 48 h after inoculation of *C. albicans.* The larvae treated with the crude extract and F2 fraction after *Candida* infections (therapeutic study) showed an increase of 25% in survival rate (p=0.0039 and p=0.0004 respectively). However, the group with prophylactic treatment did not exhibit improvement in survival rate, demonstrating that the extract was not able to stimulate the immune system. These findings were confirmed by the CFU/mL counting, in which it was observed a reduction in the *C. albicans* number for the crude extract and F2 fraction compared to the control group. The results of this study suggest that *S. mutans* can produce metabolites capable of inhibiting *C. albicans* with potential to be used as a therapeutic agent for *Candida* infections.

KEYWORDS: *Streptococcus mutans*, *Candida albicans*, Experimental candidiasis, *Galleria mellonella*.

FINANCIAL SUPPORT: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), (2016/03395-4)