Title: ASSOCIATION OF *Candida albicans* AND *Streptococcus mutans* IN POLYMICROBIAL BIOFILM EXPOSED TO *Rosmarinus officinalis* (ROSEMARY) EXTRACT

Authors: Oliveira, J.R.[1], Jesus, D.[1], Martins, A.P.R.[1], Jorge, A.O.C.[1], Oliveira, L.D.[1]

Institution: [1]Departamento de Biociências e Diagnostico Bucal - Instituto de Ciência e Tecnologia (ICT) - Universidade Estadual Paulista (UNESP). Av. Eng. Francisco José Longo, 777 - Jardim São Dimas - 12245-000 - São José dos Campos, SP

Abstract:

C. albicans can form a complex microbial community with *S. mutans* in the oral cavity. They can form a biofilm on the tooth surface and develop caries. Thus, this study evaluated the association of *C. albicans* and *S. mutans* in polymicrobial biofilm, in addition, the effect of treatment with plant extract of rosemary. For this purpose, reference strains *C. albicans* (ATCC 18804) and *S. mutans* (ATCC 35688) were grown on solid medium (37°C/24 h) and then in liquid medium under the same conditions. Were used Sabouraud-dextrose (SD) agar and Yeast Nitrogen Base (YNB) broth for *C. albicans* and Brain Heart Infusion (BHI) agar and broth for *S. mutans*. After centrifugation (2000 rpm/10 min) the supernatant was discarded and the pellet suspended in saline (0.9% NaCl) twice in succession, and the solution of each microorganism was adjusted to 1 x 10^7 CFU/mL (forming units colony per milliliter) in a spectrophotometer. Then, in microtiter plate wells was added a part (100 µL) of *C. albicans* and a part (100 µL) of *S. mutans*. After preincubation (37°C/90 min) under stirring (75 rpm), the supernatant was discarded and added culture medium (BHI+YNB, 1:2). After 24 h incubation was replaced the medium and after 48 h the biofilm was subjected to treatment with glycolic extract of rosemary (200 mg/mL) for 5 min (n=10) and saline (n=10) was used as a control. After treatment, the biofilms were broken by sonication (25% power for 30 s), serial dilutions of suspensions were made and seeded on selective agars as SD agar with chloramphenicol (1%) for *C. albicans* and *Mitis salivarius* agar with sucrose (20%) and bacitracin (0.2 IU/mL) for *S. mutans*. After 48 h incubation, the CFU were counted and the data were statistically analyzed by ANOVA and Tukey Test ($p \leq 0.05$). Regarding the population of *C. albicans*, was observed a growth of 1.26 x 10^7 CFU/mL (± 0.08 x 10^7) in the control group and 0.15 x 10^7 CFU/mL (± 0.03 x 10^7) in the treated group and a reduction of 87.72% (± 2.06) ($p < 0.05$). Regarding the population of *S. mutans* there was a growth of 2.3 x 10^8 CFU/mL (± 0.43 x 10^8) in the control group and 0.87 x 10^8 CFU/mL (± 0.19 x 10^8) in the treated group, demonstrating a reduction of 61.85% (± 7.3) ($p<0.05$). Thus, it was demonstrated that in this polymicrobial biofilm the development of population of *C. albicans* was affected by the population of *S. mutans*, however the rosemary extract demonstrated antibiofilm effect on this polymicrobial community formed by *C. albicans* and *S. mutans*.

Keywords: *Candida albicans*; Polymicrobial biofilm; *Rosmarinus officinalis*; *Streptococcus mutans*;