TITULO: PROFILES OF BIOFILM FORMATION AND PRODUCTION OF HYDROGEN PEROXIDE IN S. SANGUINIS STRAINS.

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Streptococcus sanguinis is a commensal species of the oral microbiome capable to initiate dental biofilms and to competitively inhibit the growth of pathogenic species through the production of H_2O_2. Strains of this species are also associated with cardiovascular infections. The aim of this study was to investigate diversity in biofilm formation and in production of hydrogen peroxide in S. sanguinis strains isolated from the oral cavity or from the bloodstream. To this goal, biofilm formation in microtiter plates and *in vitro* production of H_2O_2 were assessed in seven oral strains (SK36, SK49, SK72, SK115, SK160, SK330, SK353) and two blood isolates (SK678, SK1056). Briefly, 96-well polystyrene plates were inoculated with (1:100) dilutions of bacterial cultures at mid-log growth phase (A_{550nm} 0.3) in BHI with 1% sucrose. After 18h of incubation (37°C, aerobiosis), the formed biofilms were washed and stained with 1% crystal violet. Biofilms were then washed, incubated with ethanol and the absorbances (A_{575nm}) of ethanol eluates assessed as indirect measures of biofilm biomass. For H_2O_2 quantification, fresh (8h) BHI cultures (37^{o}C , aerobiosis) were centrifuged (16.000 x g, 5 min., 4 °C) and the culture supernatants transferred to microtiter plates (40µl/well) containing 160 µl/well of a solution of fresh sodium acetate (0.1 M; pH 5.0) with 0.1 µg of horseradish peroxidase and 10 µl of 1 mg/ml o-dianisidine solution. Then, plates were incubated (rt, 10 min.) protected from light, and the absorbances (A_{570nm}) of samples used for calculation of H_2O_2 concentrations based on a standard curve of H_2O_2. Significant variation was observed in biofilm biomass among the tested strains (mean: 2,57 ±1,33; range: 0,83- 3,90). Compared to the reference strain SK36, SK49 showed poor biofilm formation (53,7% lower than SK36), whereas the oral (SK115, SK160, SK330) and the blood strain SK1056 showed high biofilm formation (Kruskal Wallis; $p<0,05$). On the other hand, all the strains produced similar levels of H_2O_2, except for SK678 which showed reduced H_2O_2 production. These findings indicate diversity in biofilm formation among *S. sanguinis* strains in a fashion not associated with specific capacities to produce H_2O_2.

KeywordS: *Streptococcus sanguinis*, biofilm, hydrogen peroxide, phenotypic diversity.
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