TÍTULO: Production of amyloids by *Streptotoccus sanguinis* at different growth conditions

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ABSTRACT:

Streptococcus sanguinis is a pioneer species of dental biofilms with affinity to salivary and host components adsorbed to enamel surfaces. Production of extracellular DNA (eDNA) was shown to be required for initial steps of *S. sanguinis* biofilm maturation but the roles of other exopolymers, such as amyloid fibers (AF), in biofilm maturation remain to be investigated. This study aimed to characterize the AF production in *S. sanguinis* strains and to investigate roles of AF in biofilm formation. To that goal, AF production were assessed in nine *S. sanguinis* strains (SK36, SK49, SK72, SK115, SK160, SK330, SK353, SK678 and SK1056). Bacterial colonies from agar plates stained with Congo Red were analyzed by polarized light microscopy. Protein extracts of concentrated culture supernatants of 18h-cultures (37ºC, 10% CO_2) in chemically defined medium (CDM) w/ 1% glucose were stained with thioflavin T (ThT), and fluorescent intensities measured in a 96-well plate fluorometer. Biofilms formed in BHI with 1% sucrose supplemented or not with an inhibitor of AF formation (EGCG; 1mM; 0,482mg/mL) (18 h; 37ºC) were stained with crystal violet for comparisons of biofilm biomasses. AF were detected in culture supernatants of all strains, but significant differences in relative amounts of AF were observed between strains (Mann Whitney; $p<0,05$). Unexpectedly, EGCG promoted significant increases ($p <0.05$) in biofilm biomass in several strains, although it did not affect the biofilm formation by strains SK36, SK678 and SK1056. These findings indicate that *S. sanguinis* strains differ in the production of AF, although no association between production of AF and biofilm formation could be detected. Further studies to identify proteins involved in AF formation will be necessary to better understand the roles of these exopolymers in *S. sanguinis* biology. This study was partially supported by FAPESP (proc. 2018/02054-4), CAPES (Finance Code 001) and (CNPq) (proc.148479/2018).

Keywords: *Streptococcus sanguinis*. Biofilm. Amyloids. Extracellular DNA. Congo Red. THioflavin T. Polyphenols. Extracellular matrix.