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Biofilm Formation by ST17 and ST19 Strains of *Streptococcus agalactiae*

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Abstract

Bacterial biofilms are an important virulence factor with a vital role in evasion from the host immune system, colonization and infection. The aim of the present study was to evaluate in vitro the effects of three environmental factors (H⁺, glucose and human plasma) in biofilm formation, by carrier and invasive *S. agalactiae* strains of ST17 and ST19 sequence types, including DNase producers and non-producers. Bacteria ability to assemble biofilms was classified based on crystal violet assay. Biofilm formation was also monitored by scanning electron microscopy. Depending on the growth medium used, each bacterial isolate could fit in different biofilm production categories. Our data showed that optimal conditions for *S. agalactiae* biofilm assembly were reached after 48 h incubation at pH 7.6 in the presence of glucose and inactivated human plasma. In the presence of inactivated human plasma, the biofilm biomass of ST19 strains experienced a higher increase than ST17 strains. The composition of the extracellular polymeric matrix of the three strongest biofilm producers (all from ST17) was accessed by enzymatic digestion of mature biofilms and proteins were shown to be the predominant component. The detailed identification of the extracellular protein components should contribute to the development of new therapeutic strategies to fight *S. agalactiae* infections. Acknowledgments The authors wish to thank Barbara Spellerberg for the kind gift of *S. agalactiae* strains from collection of the Institute of Medical Microbiology and Hygiene (IMMH), Ulm University, Germany. We are grateful to Joao Paulo Gomes and Alexandra Nunes (Instituto Nacional de Saúde Doutor Ricardo Jorge, INSA, IP) for providing bN-acetylglucosaminidase enzyme and 96-well flat-bottomed polystyrene cell culture plates, respectively. We thank Rita Sobral (Nova School of Science and Technology, FCT/UNL) for the critical review of the manuscript and Hemovida® for IHP donation.

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