Biofilm formation by ST17 and ST19 strains of *Streptococcus agalactiae*

Inês Silvestre[1, 2*], Maria José Borrego[2], Luísa Jordão[2]

[1] Nova School of Science and Technology
[2] National Institute of Health Doutor Ricardo Jorge
*Corresponding author: inesilvestre@gmail.com; i.silvestre@campus.fct.unl.pt

5º CCQL-PLP
Congresso de Controle da Qualidade Laboratorial para Países de Língua Portuguesa

INTRODUCTION

Streptococcus agalactiae is an important human pathogen that colonizes the gastrointestinal and genitourinary tracts, and a prevailing cause of neonatal morbidity and mortality worldwide. Bacterial biofilms are an important virulence factor with a vital role in evasion from the host immune system, colonization and infection, that could enhance *S. agalactiae* pathogenicity and virulence. Considering the dispersed and often conflicting results concerning biofilm production by *S. agalactiae*, the aim of the present study was to evaluate biofilm formation under standardized experimental conditions.

OBJECTIVES

Evaluate 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, DNase producers and non-producers. Access the composition of the extracellular polymeric matrix for ST17 strains with the strongest biofilm production.

METHODOLOGY

Thirty-eight clinical *S. agalactiae* strains of human origin, from colonization and infection, DNase producers and non-producers, belonging to ST17 (n=18) and ST19 (n=20) lineages, were evaluated for biofilm formation on an abiotic surface (polystyrene) at different pH values, 4.5, 7.0 and 7.6; and the presence or absence of glucose and plasma [1]. Bacteria ability to assemble biofilms was classified based on crystal violet assay [2]. Scanning electron microscopy (SEM) technique was used to evaluate the biofilm phenotype and three enzymes, DNase, proteinase K and b-N-acetylglucosaminidase, were used to disperse mature biofilms to investigate the composition of the EPS.

RESULTS

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 (Table 2).

Table 2. Effect of IHP on biofilm formation by *S. agalactiae* ST17 and ST19 strains. *S. agalactiae* biofilm assembly was followed for 48 h. Biofilms were monitored in RPMI-glucose medium only (control) or supplemented with 15% IHP.

Sequence type	Biofilm assembly category [a]	Isolates (n/%)	
		RPMI-glucose	RPMI-glucose+15%IHP
ST17 (n=18)	Non-producer	0	0
	Week	7 (38.9%)	1 (5.6%)
	Moderate	8 (44.4%)	1 (5.6%)
	Strong	3 (16.7%)	16 (88.9%)
ST 19 (n=20)	Non-producer	0	0
	Week	2 (10%)	1 (5%)
	Moderate	16 (80%)	0
	Strong	2 (10%)	19 (95%)

[a] Biofilm assembly was classified according to Stepanovic and colleagues [3]

For *S. agalactiae* reference strain NEM316, biofilm assembly was monitored by SEM after 48 h incubation in THB supplemented with 1% glucose pH 7.6 and RPMI-glucose e supplemented with 15% IHP. Comparison of the biofilm assembled by NEM316 clearly showed an increase of EPS in the last condition, giving rise to denser biofilms (Fig. 1).

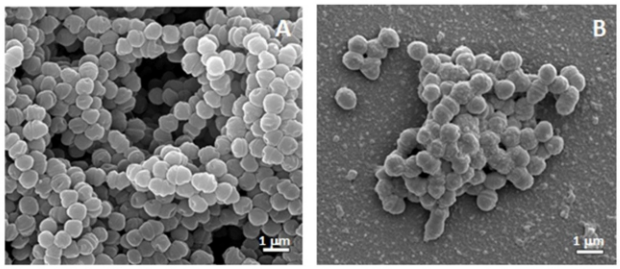

Fig. 1. Effect of inactivated human plasma on *S. agalactiae* reference strain NEM316 biofilms. Biofilm assembly by NEM316 on polystyrene incubated at 37 C/5% CO2 for 48 h in THB supplemented with 1% glucose at pH 7.6 (A) and in RPMI-glucose supplemented with 15% IHP (B) were monitored by scanning electron microscopy (SEM). The presence of EPS forming "bridges" between bacteria could be observed in Fig. 3B. Scale bar 1 mm.

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 (Fig.2).

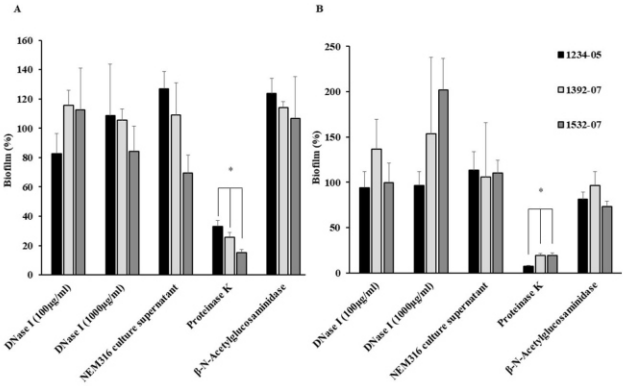

Fig. 2. The extracellular polymeric substance matrix of *S. agalactiae*. Analysis of the effect of DNase I (100 mg/ml and 1000 mg/ml), culture supernatant from stationary phase of reference strain NEM316, Proteinase K (100 mg/ml) and b-N-acetylglucosaminidase (1.2 units) on 48 h-old biofilms in THB supplemented with 1% glucose (A) and in RPMI-glucose supplemented with 15% IHP (B). A significant statistical difference was observed for biofilm dispersion by proteinase K (*$P < 0.05$).

CONCLUSION

Proteins seem to be a major component of the *S. agalactiae* EPS matrix. In addition, slightly alkaline pH, glucose and plasma supplementation favour biofilm formation for ST17 and ST19 strains. No statistical significant correlation between biofilm formation and the strain source (colonization or invasive infection), nor with DNase production, could be established. Further research on *S. agalactiae* biofilms is crucial to develop new curative approaches, namely new enzymatic therapeutics targeting biofilms (avoiding antibiotic overtreatment and resistance), or preventive approaches by the identification of potential targets for new vaccines.

REFERENCES

[1] Florindo C, Barroco CA, Silvestre I, Damião V, Gomes JP, Spellerberg B, et al. Capsular type, sequence type and microbial resistance factors impact on DNase activity of *Streptococcus agalactiae* strains from human and bovine origin. Eur J Microbiol Immunol 2018;8(4):149-54.
[2] Bandeira M, Carvalho PA, Duarte A, Jordão L. Exploring dangerous connections between *Klebsiella pneumoniae* biofilms and healthcare-associated infections. Pathogens 2014;3:720-31.
[3] Stepanovic S, Vukovic D, Hola V, Di Bonaventura G, Djukic S, Cirkovic I, et al. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by *Staphylococci*. Apmis 2007;115(8):891-9.

Manuscript published with the following reference: Silvestre I, Borrego MJ, Jordão L. Biofilm formation by ST17 and ST19 strains of *Streptococcus agalactiae*. Research in Microbiology 2020. 171(8):311-318. DOI: 10.1016/j.resmic.2020.08.001.