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Glucose effect on biofilm formations of *S. aureus* strains

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Abstract: Biofilm formations of 25 *S.aureus* strains were investigated on microtitration plates using TSB and TSB media supplemented with glucose (0.25%, 1.0%, or 2.0%, w/v) for 48 hours. The biofilm formations of *S.aureus* isolates incubated in TSB were generally found to be moderate (84%). Majority of strong biofilm formations (84-96%) were determined by *S.aureus* strains incubated in the presence of glucose. It was shown that the presence of glucose had positive effect on *S.aureus* biofilm formations.

Keywords: *S.aureus*, biofilm formation, D-glucose, polystyrene, microtitration plates.

1. Introduction

Staphylococci are the most important pathogens causing major problems in food industry (Tauxe, 2002). The predominant *Staphylococcus* species are *S. epidermidis, S. haemolyticus* and *S. aureus* (Otto, 2013; Becker et al., 2014; Méric et al., 2015). Especially *S.aureus* is known as the third common cause of foodborne illness worldwide (Aydin et al., 2011). *S.aureus* can be easily isolated from surfaces in the food and dairy industries (Ateba et al., 2010; Santos et al., 2014). The products can also be contaminated during food processing by *S.aureus* enterotoxins that cause foodborne illnesses (Cha et al., 2006; Sudagidan and Aydin, 2009). Moreover, *S.aureus* strains create biofilms on different types of surfaces such as stainless steel, plastic, glass, and rubber (Akbas and Kokumer, 2015; Lee et al., 2014, 2016, 2017; Unlu et al., 2018).

In this study, the effects of glucose on biofilm formations by *S. aureus* strains isolated from raw milk samples were investigated.

2. Materials and Method

2.1. Bacterial strains

In this work, 25 *S.aureus* isolates (S1-S25) were evaluated for their biofilm formation abilities in TSB (Tryptic soy broth) or TSB_{Glc} (TSB enriched with D-glucose) media. *S. aureus* strains ATCC 25923 and ATCC 29213 were used as biofilm positive and biofilm negative strains, respectively.

2.2. Biofilm Formation

For determining the biofilm formations, TSB and TSB_{Glc} (TSB enriched with D-glucose at different concentrations, 0.25% $TSB_{Glc0.25}$; 1.0% TSB_{Glc1} and 2.0% TSB_{Glc2}; w/v) media were used. The isolates and reference strains were incubated overnight at 37°C in TSB. Following the incubation, the cultures were diluted at a rate of 1:100 in fresh TSB or TSB_{Glc} media. The cultures were transferred into flat-bottomed 96-well polystyrene microtitration plates (150µL; about 10^5cfu/mL, final concentration) and incubated at 37°C for 48 h. At the end of the incubation, bacterial cells were removed and then the wells were stained with crystal violet solution (0.5% w/v, Sigma, St. Louis, MO, USA) for 20 min. Then, the wells were decanted and treated with glacial acetic acid (33% v/v, Merck) for 10 min. All samples were transferred into a new microtitration plate wells and measured at 570 nm using an ELISA plate reader (Christensen et al., 1985). The absorbance values of TSB or TSB supplemented with D-glucose (OD_c) were used as a blank. Each experiment was performed in triplicate for all isolates.

Biofilm formations were determined according to Stepanović et al. (2000).

- Nonbiofilm producer (-, $OD \leq OD_c$),
- Weak biofilm producer (+, $OD_c < OD \leq 2xOD_c$),
- Moderate biofilm producer (++, $2xOD_c < OD \leq 4xODc$), and
- Strong biofilm producer (+++, $4xOD_c < OD$)

3. Results

In this study, 25 *S.aureus* isolates (S1-S25) and 2 reference strains (ATCC 25923 and ATCC 29213) were used for determination of biofilm formations. The negative reference *S. aureus* ATCC 29213 and the positive reference strain *S. aureus* ATCC 25923 were shown as non and strong biofilm producers in the all media, respectively (Table 1). In addition, S3 and S4 were considered as strong biofilm producers (Table 1).

In general, 21 of 25 *S. aureus* isolates (84%) were considered as moderate biofilm producers when they were incubated in TSB medium (Table 2). The biofilm formation capacities of strains gradually increased by increasing concentrations of glucose in TSB media supplemented with glucose. 24 of 25 *S. aureus* isolates (96%) cultured in TSB_{Glc2} media were determined as strong biofilm producers (Table 2).

According to obtained results, it was shown that the content of media was important parameter for biofilm productions. For this reason, the effects of media components should be investigated in more detail for biofilm formation studies.

Table 1. Biofilm formations (OD_{570nm}) of *S.aureus* strains on TSB and TSB_{Glc} ($TSB_{Glc0.25}$, TSB_{Glc1}, and TSB_{Glc2}) media on microtitration plates after 48 hours. OD, optical density; standard deviations are in parentheses.

Strain	Media			
	TSB	$TSB_{Glc0.25}$	TSB_{Glc1}	TSB_{Glc2}
S1	0.42 (0.00)	0.88 (0.01)	1.25 (0.02)	1.09 (0.04)
S2	0.46 (0.02)	0.52 (0.15)	0.77 (0.21)	0.69 (0.00)
S3	0.99 (0.20)	1.51 (0.04)	1.49 (0.05)	1.52 (0.04)
S4	0.57 (0.07)	0.76 (0.09)	1.32 (0.01)	1.16 (0.17)
S5	0.43 (0.06)	0.98 (0.07)	1.36 (0.00)	1.31 (0.02)
S6	0.39 (0.11)	0.87 (0.12)	0.87 (0.12)	0.97 (0.01)
S7	0.64 (0.17)	0.70 (0.00)	0.77 (0.23)	0.91 (0.10)
S8	0.31 (0.04)	0.80 (0.04)	0.75 (0.03)	0.82 (0.23)
S9	0.57 (0.03)	0.94 (0.02)	1.26 (0.01)	1.23 (0.15)
S10	0.56 (0.11)	1.03 (0.09)	1.08 (0.04)	1.31 (0.01)
S11	0.55 (0.04)	1.03 (0.10)	0.99 (0.07)	1.16 (0.10)
S12	0.69 (0.02)	0.94 (0.10)	1.60 (0.15)	1.72 (0.23)
S13	0.63 (0.09)	0.89 (0.08)	0.84 (0.00)	0.93 (0.04)
S14	1.13 (0.11)	1.54 (0.05)	1.16 (0.06)	1.64 (0.18)
S15	0.63 (0.01)	0.89 (0.13)	0.94 (0.12)	0.93 (0.05)
S16	0.78 (0.13)	0.97 (0.10)	1.15 (0.39)	1.08 (0.00)
S17	0.50 (0.08)	1.00 (0.00)	1.13 (0.18)	0.97 (0.10)
S18	0.49 (0.05)	0.86 (0.08)	1.06 (0.05)	1.13 (0.08)
S19	0.50 (0.05)	0.99 (0.05)	0.92 (0.12)	1.16 (0.11)
S20	0.62 (0.09)	1.03 (0.07)	0.96 (0.10)	0.90 (0.36)
S21	0.79 (0.10)	1.02 (0.00)	0.94 (0.12)	1.11 (0.01)
S22	0.60 (0.10)	1.05 (0.01)	1.22 (0.04)	1.09 (0.02)
S23	0.51 (0.06)	1.05 (0.06)	1.01 (0.19)	0.98 (0.18)
S24	0.69 (0.19)	0.91 (0.05)	1.10 (0.02)	0.95 (0.00)
S25	0.47 (0.00)	0.98 (0.01)	1.14 (0.14)	1.02 (0.12)
ATCC 29213	0.22 (0.04)	0.25 (0.03)	0.26 (0.04)	0.28 (0.07)
ATCC 25923	1.18 (0.19)	1.04 (0.06)	1.46 (0.05)	1.45 (0.00)

Table 2. The biofilm formations of *S.aureus* isolates (n) with different types of TSB media (TSB, $TSB_{Glc0.25}$, TSB_{Glc1} and TSB_{Glc2}) on microtitration plates after 48 hours.

	***S.aureus* (n)**			
	TSB	**$TSB_{Glc0.25}$**	**TSB_{Glc1}**	**TSB_{Glc2}**
Nonbiofilm (-)	-	-	-	-
Weak (+)	2	-	-	-
Moderate (++)	21	4	3	1
Strong (+++)	2	21	22	24

4. Discussion

In this study it was shown that *S. aureus* strains could produce moderate biofilm formations in the TSB, or strong biofilms in TSB supplemented with glucose, The obtained results were consistent with previous studies (Stepanovic et al., 2000; Rode et al., 2007; Manandhar et al., 2018). The environmental factors including the presence of glucose, or salt, temperature, and pH could be effective on the biofilm formations (Michu et al., 2011; Khangholi and Jamalli, 2016; Kyoui et al., 2016).

Biofilm formations on a solid surface also depends on the type of the surface materials (Jo et al., 2010). Generally, glass and stainless steel are described as hydrophilic materials while rubber and plastic are assumed as hydrophobic materials (Sinde and Carballo, 2000; Donlan and Costerton, 2002). The biofilm formations were found to be higher on hydrophobic surfaces than on hydrophilic surfaces (Simões et al., 2008). In this study, *S.aureus* isolates produced biofilms on hydrophobic polystyrene microtitration plates. Therefore, the biofilm formation of *S.aureus* isolates should also be assessed on various surfaces such as stainless steel, glass, or polypropylene in the presence of different media supplements.

5. Conclusions

In this work, the biofilm formations of 25 *S.aureus* isolates and 2 biofilm reference *S.aureus* strains were investigated by using TSB and TSB_{Glc} media on microtitraiton plates. When *S.aures* isolates were cultured in the TSB media, 21 of 25 isolates (84%) produced moderate biofilm formations. On the other hand, nearly all of isolates (96%) cultivated in TSB supplemented with glucose produced strong biofilm formations. According to these results, it can be concluded that presence of glucose is an important factor for *S.aureus* biofilm formations.

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Authors' contributions:

Taner Sar: Performed the experiment, analyzed data, and wrote the manuscript.

Meltem Yesilcimen Akbas: Analyzed data, supervised the experiment's progress, and wrote the manuscript.

Conflict of interest disclosure:

There are no ethical issues after the publication of this manuscript.

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