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ORIGINAL PAPER

Staphylococcus epidermidis glucose uptake in biofilm versus planktonic cells

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Abstract The aim of this work was to compare the glucose uptake of biofilms formed by four different *Staphylococcus epidermidis* strains as well as to compare between sessile and planktonic cells of the same strain. Biofilm cells showed a lower level of glucose uptake compared to planktonic cells. Moreover, glucose uptake by cells in the sessile form was strongly influenced by biofilm composition. Therefore, this work helps to confirm the phenotypic variability of *S. epidermidis* strains and the different behaviour patterns between sessile and free cells.

Keywords Biofilms · Glucose uptake · Planktonic cells · *Staphylococcus epidermidis*

Introduction

Staphylococcus epidermidis is a coagulase-negative staphylococcus (CNS) that is one of the main nosocomial pathogens associated with infections of implanted medical devices (Von Eiff et al. 2002). CNS adheres to such devices and has the ability to form biofilms which constitutes one important virulence factor and is considered the main responsible for *S. epidermidis* pathogenesis (Henning et al. 2007). Bacteria in biofilms are more resistant (1,000 up fold) to antibiotics and to the host immune defence system than their planktonic counterparts (Cerca et al. 2005), suggesting that cells in a biofilm may have altered metabolic activity (Resch et al. 2005). This strong resistance regularly requires the removal of the infected biomaterial and leads to substantial morbidity and mortality (Mack et al. 2004). Thus, it is extremely important to know the essential differences, especially concerning physiology and metabolic activity, between a planktonic cell and its sessile counterpart adhered to a surface living in a biofilm.

The aim of this work was to compare the glucose uptake of biofilms formed by four different *S. epidermidis* strains, and between sessile and planktonic cells of the same strain. Thus, the influence of biofilm characteristics such as total biomass formed, cell concentration and polysaccharides content on the glucose uptake was also evaluated.

Materials and methods

All strains used in this work (LE7, 9142, IE186 and IE214) are clinical isolates and were kindly provided by DR. G. B. Pier, Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston.

For all the biofilm assays, cells were first grown for 24 h in 15 ml of TSB (Merck, Darmstadt, Germany) at 37 °C using an orbital shaker (130 rev/min). After this period, 50 µl of each suspension were transferred to 30 ml of fresh TSB broth and incubated for 18 h (late exponential phase), at 37 °C and 130 rev/min. Then, the cells were centrifuged (Sigma 4K10, B. Braun, Germany) for 5 min, at 10,500*g* and 4 °C, washed twice with a saline solution (0.9% NaCl (Merck) in distilled water), and sonicated (Ultrasonic Processor, Cole-Parmer, Illinois, USA) during 10 s, with an amplitude of 22% (previously optimized to avoid cell disruption). The cellular suspension was adjusted to a final concentration of approximately 1×10^9 cells/ml, determined

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by optical density at 640 nm. Acrylic was used as substratum and cut into 2 cm × 2 cm squares. Prior to use, the coupons were washed with sterile distilled water and with a solution of 70% ethanol diluted in sterile distilled water. Each clean acrylic coupon was placed into an individual well of a 6-well tissue culture plate (Sarstedt, Newton, NC, USA) containing 5 ml of TSB enriched with 0.25% of glucose (Merck). For every strain, an inoculum of 50 µl with 1×10^9 cells/ml was added to each well. Assays were performed in triplicate and repeated 3 times. The plates were incubated for 192 h (eight days) at 37 °C in an orbital shaker (120 rev/min). The medium was withdrawn and replaced by fresh TSB + 0.25% glucose every 12 h (fed-batch mode). The total attached biomass to the acrylic coupons was measured by methanol fixation, crystal violet staining and acid acetic elution as previously described (Stepanović et al. 2000). The eluted dye of each well was removed and placed in a 96-well microtitre plate (Sarstedt, Newton, NC, USA) and its absorbance was read in an ELISA reader (Bio-Tek Instruments Inc., Vermont, USA) at 570 nm. In order to determine the biofilm cell concentration, the acrylic coupons with biofilm were washed twice with NaCl 0.9%. Then, the biofilm was removed from the coupons by scrapping with a spatula and resuspended into 25 ml of TSB + 0.05% Tween 20 (Merck). Next, the suspension was sonicated (20 s with 22% of amplitude), centrifuged (5 min, 10,500*g*, 4 °C), resuspended in 30 ml of NaCl 0.9% and sonicated again (10 s, 22% amplitude) to promote biofilm disruption. The absorbance was read at 640 nm. The number of c.f.u in biofilm for each *S. epidermidis* strain was determined by plating onto TSA in triplicate using decimal dilutions and incubating for 24–48 h. The extraction of the exopolymeric matrix, for the quantification of the polysaccharides, was assessed using the Dowex resin extraction method as described by Azeredo et al. (2003). The biofilm matrix polysaccharides were quantified by the method of Dubois et al. (1956). In order to determine the glucose uptake in the biofilm and to remove the cells loosely attached and residual medium, each coupon was consecutively transferred to three 100 ml glass beakers containing distilled water, and was allowed to rest there for approximately 10 s. Then the coupons were transferred to a new 6-well tissue plate containing 5 ml of 0.10% glucose solution (w/v). The glucose concentration in the medium was measured immediately after transferring the coupons and also 60 min later, maintaining the plates under slow agitation. Glucose was quantified with the enzymatic kit Glucose–TR (Spinreact, SA, Spain) using the ELISA reader at 505 nm.

For the assays to determine the glucose uptake by the planktonic cells, all strains were incubated in 15 ml of TSB and grown for 24 (±2) h at 37 °C in an orbital shaker (130 rev/min). Then, 100 µl of each cell suspension were transferred to 60 ml of fresh TSB and incubated for 18 h (to reach late exponential phase) at 37 °C and 130 rev/min. Cells were harvested by centrifugation (for 5 min at 10,500*g* and 4 °C) and resuspended in TSB to the same concentration as previously determined by c.f.u. plating of biofilm cells. For all strains, 1 ml of the suspension obtained was centrifuged at 9,500*g* and the pellet resuspended in 1 ml of 0.10% glucose solution. These samples were then transferred to the wells of a 6-well tissue culture plate containing 4 ml of 0.10% glucose solution. Glucose present in the medium was measured at time zero and after 60 min with the enzymatic kit Glucose–TR, as described for biofilm assays.

The data obtained was analyzed using a statistical program, SPSS (Statistical Package for the Social Sciences). One-way ANOVA with Tukey test was used to compare the results of the different assays. All tests were performed with a confidence level of 95%.

Results and discussion

In this study, strain variability in terms of biofilm formation ability was assessed through the crystal violet assay (Fig. 1) and the data obtained revealed a significantly ($p < 0.05$) higher ability of biofilm formation by strains 9142, IE186, IE214 in contrast to strain LE7. This shows that crystal violet staining is a reliable method, having the advantage of being easy and expedite, i.e., providing the results almost immediately, with a great objectivity and accuracy. The assays to determine the glucose uptake by cells in suspension were performed with the same cellular concentration of the biofilm assays. This cellular concentration was determined by c.f.u. plating of the biofilm cells, in terms of c.f.u./ml, for the four *S. epidermidis* strains. Strain 9142 presented the highest cellular concentration (1.5×10^{18} c.f.u./ml) in comparison with strains IE214

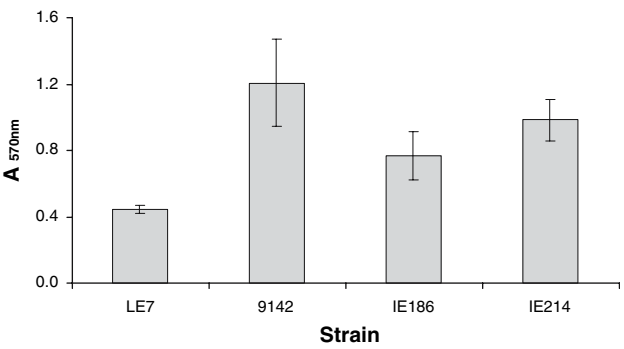

Fig. 1 Biofilm biomass of four S*taphylococcus epidermidis* strains, accumulated over 192 h, expressed as crystal violet absorbance (A_{570nm}). Results represent means plus standard deviations (errors bars) from three independent experiments

(2.7×10^{17} c.f.u./ml) and IE186 (3.9×10^{17} c.f.u./ml). The number of cells on LE7 biofilms was very low (6.5×10^{16} c.f.u./ml) compared to the other three strains which are strong biomass producers (Fig. 1). As far as the amount of polysaccharides present in the polymeric matrix is concerned, strain IE214 produced the largest amount of polysaccharides (0.442 $mg/g_{(dry\ weight/wet\ weight)} \pm 0.173$) and this is the most likely explanation for the large value of its total biomass production. In the same way, LE7 was the strain that produced the smallest amount of polysaccharides (0.189 $mg/g_{(dw/ww)} \pm 0.045$) and is the one that produced the least quantity of total biomass. Strain 9142 and strain IE186 presented intermediary values, 0.378 $mg/g_{(dw/ww)} \pm 0.172$ and 0.228 $mg/g_{(dw/ww)} \pm 0.092$, respectively. The present study shows that biofilm glucose uptake can differ between strains of the same species (Fig. 2). In absolute terms, strain 9142 biofilms removed from the liquid phase the highest amount of glucose (23.98%), followed by the biofilms of strains IE186 (12.7%) and IE214 (8.29%), while strain LE7 displayed a significantly less glucose uptake (1.11%). These results correlate with the number of c.f.u./ml in the biofilms. This is also in agreement with previous studies that showed that the amount of biofilm produced by individual *S. epidermidis* strains is highly variable phenotypically and regulated by several factors (Cramton et al. 2001; Mack et al. 2004). The percentage of glucose uptake by planktonic cells (Fig. 2) shows the same tendency but is significantly higher than their biofilm counterparts. This would be expected because it has already been shown that cells in sessile form display a lower metabolic activity (Walters et al. 2003). The reduction of the percentage of glucose uptake from planktonic cells to biofilm cells is on average twofold for the three highest biofilm producing strains. Low levels of glucose uptake might be an indicative of a lower metabolic activity and consequently slow growth has been considered one of the main reasons for biofilm resistance and success against antimicrobial agents (Fux et al. 2005). Biofilms cells are immersed in a matrix which provides the bacterial population protection from the host defence mechanisms and antimicrobial agents. In addition, the surface attached populations are exposed to a different nutrient environment than the planktonic cells (Møller et al. 1995). Thus, many factors may affect the response of biofilm cells in terms of metabolic activity and antibiotic susceptibility.

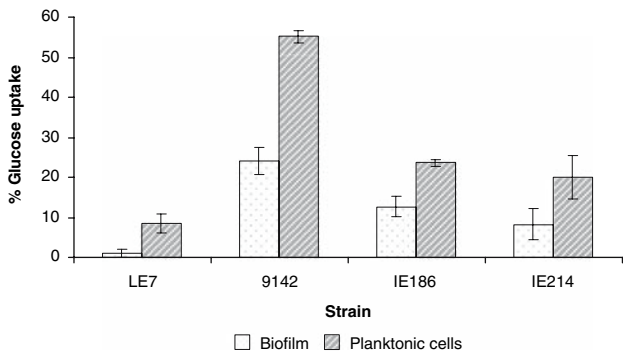

Fig. 2 Glucose uptake expressed in % for the four *Staphylococcus epidermidis* strains in biofilm and in the planktonic form. Results represent means plus standard deviations (errors bars) from three independent experiments

Conclusions

This study shows that the determination of glucose uptake by cells in suspension and in biofilm is an expedite method to evaluate the different behaviour of *S. epidermidis* strains in both forms. According to the results obtained, the four *S. epidermidis* strains revealed different abilities of biofilm formation, with sessile cells displaying lower glucose uptake in comparison to planktonic cells for all the strains studied. In the sessile form, glucose uptake seems to be dependent on the strain specific properties of the biofilm such as the cell concentration and the production of extracellular matrix.

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