Biofilm formation by *Staphylococcus capitis* strains isolated from contaminated platelet concentrates

Valerie S. Greco-Stewart,¹ Hamza Ali,¹ Dilini Kumaran,¹ M. Kalab,² Ineke G. H. Rood,³ Dirk de Korte³ and Sandra Ramírez-Arcos¹

¹Canadian Blood Services, Ottawa, Ontario, Canada
²Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada
³Sanquin Blood Supply Foundation, Amsterdam, The Netherlands

Bacterial contamination of platelet concentrates (PCs) poses the greatest infectious risk in modern transfusion medicine despite the implementation of measures such as improved skin disinfection and first aliquot diversion. The majority of PC contaminants are commensal skin flora introduced by venipuncture at the time of blood collection. The predominant organisms are Gram-positive coagulase-negative staphylococci such as *Staphylococcus capitis*. This bacterium has been implicated in numerous instances of infection and sepsis, likely for its ability to form surface-associated communities of micro-organisms encased in extracellular materials, known as biofilms. In the present study, five strains of *S. capitis* isolated from contaminated PCs were assessed for their ability to produce extracellular polysaccharide (slime), a canonical indicator of biofilm-formation ability, on Congo red agar plates. Biofilm formation was evaluated in both glucose-enriched trypticase soy broth (TSBg) and in PCs by using a crystal violet staining assay. The chemical nature of the biofilms was evaluated by disruption assays using sodium metaperiodate and proteinase K. In addition, biofilm architecture was observed by scanning electron microscopy. The presence of the biofilm-associated icaR and icaADBC genes was also examined by PCR.

While only two out of the five *S. capitis* strains formed biofilms in TSBg, all strains formed biofilms in PCs. The ability of strains to produce extracellular polysaccharide and their possession of wild-type ica genes were not exclusive predictors of biofilm formation in TSBg or PCs; different profiles of biofilm markers were observed among isolates. This is likely due to the proteinaceous composition of the *S. capitis* biofilm matrix. Interestingly, an ica-negative, non-slime-producing isolate was capable of biofilm formation in PCs. Together, these data indicate that the platelet storage environment stimulates biofilm formation in *S. capitis* in the absence of extracellular polysaccharide production and that multiple bacterial factors and regulatory elements are likely involved in biofilm formation in this milieu.

INTRODUCTION

Transfusion-associated bacterial infections have been significantly reduced in recent years by the introduction of several interventions, including the implementation of improved skin disinfection methods, first aliquot diversion and bacterial testing (Corash, 2011). However, despite these strategies, bacterial contamination of blood products currently poses the most significant infectious risk in transfusion medicine. Platelet concentrates (PCs) are exquisitely susceptible to contamination since their storage conditions of 20–24 °C with agitation are particularly amenable to bacterial growth (Braine et al., 1986). While Gram-negative organisms are typically implicated in the most severe adverse transfusion reactions, Gram-positive bacteria constitute the majority of organisms responsible for PC contamination (Brecher & Hay, 2005; Corash, 2011). Specifically, coagulase-negative staphylococci (CoNS), the most abundant group of species composing the commensal microflora of human skin, are most frequently implicated in bacterial contamination of PCs. These bacteria are likely introduced at the time of venipuncture during blood collection despite the implementation of the aforementioned prophylactic measures to prevent contamination (Corash, 2011). *S. capitis* is a common CoNS that is often recovered from contaminated PCs (Eder et al., 2009; Pearce et al., 2011; Rood et al., 2011). Since 2009, Canadian Blood Services has detected four strains of *S. capitis* by routine PC

Abbreviations: CoNS, coagulase-negative staphylococci; CRA, Congo red assay/agar; TSBg, glucose-enriched trypticase soy broth; PC, platelet concentrate; PIA, polysaccharide intercellular adhesin.

A supplementary table is available with the online version of this paper.
screening, preventing transfusion of the contaminated platelet units.

A ubiquitous constituent of the human skin microbiome (Kloos & Schleifer, 1975), \textit{S. capitis} accounts for \textasciitilde5\% of all clinical CoNS isolates (Oren & Merzbach, 1990). While \textit{S. capitis} is typically apathogenic, it has occasionally been shown to cause illness such as skin infection, pneumonia and urinary tract infection (Oren & Merzbach, 1990; Pal & Ayyagari, 1989). Serious instances of \textit{S. capitis} infection, including prosthetic valve-endocarditis (Nalmas \textit{et al}, 2008) and shunt-related and community-acquired meningitis (Oud, 2011; Oren & Merzbach, 1990), have also been reported. Furthermore, it has been estimated that \textit{S. capitis} accounts for up to 20\% of cases of neonatal sepsis (Van Der Zwet \textit{et al}, 2002), a life-threatening illness in this highly vulnerable population. \textit{S. capitis} has been shown to survive and proliferate in blood components (Walther-Wenke \textit{et al}, 2010) and has been known to escape automated bacterial detection methods (Murphy \textit{et al}, 2008), posing a potential threat to blood product recipients.

While bacteria associated with the skin microflora are traditionally considered non-pathogenic, the ability of certain strains to form biofilms, surface-associated populations of cells encased in an extracellular polymeric matrix, increases their virulence by enhancing their capacity to adhere to and colonize biotic and abiotic surfaces, evade the immune response, and resist antimicrobial chemotherapy [reviewed by Flemming & Wingender (2010)]. We have previously demonstrated that several strains of Gram-positive (\textit{Staphylococcus epidermidis}) and Gram-negative (\textit{Serratia marcescens}) bacteria recovered from contaminated platelet units form biofilms in the platelet storage environment; remarkably, some strains that were unable to form biofilms in traditional laboratory medium adopt a biofilm-forming phenotype when grown under platelet storage conditions (Greco \textit{et al}, 2007; Greco-Stewart \textit{et al}, 2012). Additionally, strains of \textit{S. marcescens} which form biofilms in PCs demonstrate an increased propensity to evade detection by automated culture screening (Greco-Stewart \textit{et al}, 2012).

Many genes have been identified in staphylococcal species, including CoNS such as \textit{S. epidermidis}, which contribute to biofilm formation [reviewed by Götz (2002) and by Fey & Olson (2010)]. Proteins, carbohydrates, teichoic acids and DNA have all been shown to compose staphylococcal biofilms; thus a variety of biofilm-associated genes and operons involved in the four stages of biofilm growth, namely adherence (primary attachment), accumulation, maturation and dispersal, have been identified. While little is known about biofilm formation in \textit{S. capitis}, homologues of some biofilm-associated genes, such as the ica\textit{ADCB} operon, have been identified (de Silva \textit{et al}, 2002). The ica genes compose a genetic locus whose products are responsible for the synthesis and export of polysaccharide intercellular adhesin (PIA) in \textit{S. epidermidis} (Heilmann \textit{et al}, 1996). PIA is a \textbeta\textdash1,6-linked \textit{N}-acetylglycosamine homoglycan (Mack \textit{et al}, 1996) involved in the adhesion and accumulation phases of biofilm growth and accounts for the ‘slime’ produced in biofilm-positive isolates of \textit{S. epidermidis} (Heilmann \textit{et al}, 1996; McKenney \textit{et al}, 1998).

The ambition of the present study was to characterize strains of \textit{S. capitis} (Rood \textit{et al}, 2011) recovered from contaminated platelet units with respect to their capacity to form biofilms under platelet storage conditions and to determine which bacterial factors contribute to biofilm formation in this environment.

**METHODS**

**Bacterial strains and growth conditions.** Five strains of \textit{S. capitis} captured during routine platelet screening by automated culture using the BacT/ALERT System (bioMérieux) were recovered from contaminated PCs at Sanquin Blood Supply Foundation (strains 512, 517, 521 and 525; Rood \textit{et al}, 2011) and Canadian Blood Services (strain 07/2010). Prior to experimentation, strains were examined using API Staph identification strips (bioMérieux) according to the manufacturer’s protocols to verify culture purity and to assess urease production. Two reference strains of \textit{S. epidermidis}, ATCC 35984 and ATCC 12228, obtained from the American Type Culture Collection, were included as biofilm-positive and biofilm-negative controls, respectively. All strains were cultivated in trypticase soy broth (TSB; Difco) and grown on trypticase soy agar plates incubated overnight at 37\°C unless otherwise stated. Strains were preserved at \textless80\°C in brain–heart infusion (BHI) broth (BD Biosciences) supplemented with 15\% glycerol (w/v).

**Platelet concentrates (PCs).** Blood was obtained from healthy, deferred volunteer donors after receipt of signed, informed consent. Whole blood units were collected, screened for transmissible diseases, and processed at the Canadian Blood Services Network Centre for Applied Development (netCAD). PCs were prepared by either the buffy-coat or apheresis methods in accordance with Canadian Blood Services standard operating procedures. The use of PCs and experimental approaches were approved by the Canadian Blood Services Research Ethics Board.

**Biofilm assays.** A semiquantitative crystal violet (CV) staining assay was used to assess the amounts of biofilm-related materials and cells deposited on the surface of six-well tissue culture-treated polystyrene plates (Fisher Scientific), similar to methods originally described by Christensen \textit{et al}, (1985) and modified in our laboratory (Greco \textit{et al}, 2007; Greco-Stewart \textit{et al}, 2012). Biofilm assays were performed on cultures grown in either TSB supplemented with 0.5\% glucose (TSBG; Difco) or in in-date (<5 day old) PCs. Each well was inoculated with \textasciitilde10\textsuperscript8 c.f.u. ml\textsuperscript{-1} of each bacterial strain in duplicate. Strains grown in TSBg were incubated at 37\°C without agitation overnight (~16 h) while bacteria grown in PCs were incubated under platelet storage conditions [22 ± 2\°C for 5 days with agitation of ~64 rocks min\textsuperscript{-1} on a thermal rocker ( Fisher)]. Following incubation, planktonic cells and culture medium were removed and wells were washed three times with sterile PBS (pH 7.4) and stained with 3 ml of a 3\% Gram CV solution (BD Biosciences) for 30 min with agitation (~60 r.p.m. on a horizontal shaker). Upon removal of the dye, wells were rinsed three times with PBS and materials were eluted with 3 ml of 80:20 ethanol:acetone (v/v) solution. Measurements of samples from each well were taken in triplicate at 492 nm using a microplate reader (Expert Plus). Independent experiments were performed a minimum of nine times on different days.
**Scanning electron microscopy (SEM).** Biofilm formation of S. capitis strains on polystyrene pegs was assessed by SEM. Biofilms were formed on the 96-peg lid of a 96-well plate known as the Minimum Biofilm Eradication Concentration (MBEC) device (Physiology & Genetics, Innovotech). MBEC devices were inoculated with ~10^10 c.f.u. ml^-1 of each strain in either TSBg or PCs and incubated as described above. Following incubation, pegs were removed with sterile forceps, rinsed five times in PBS, preserved in 0.1 M cacodylate [Na(CH3)2AsO2•3H2O; J. B. EM Services] containing 2.5% glutaraldehyde, and stored at 4°C prior to examination. Pegs were critical-point dried, sputtered with a 20 nm layer of gold, and visualized by using the XL30 ESEM microscope (Phillips) with an accelerating voltage of 7.5 kV, beam spot of 2, and working distance 7.5 mm as previously described (Greco-Stewart et al., 2012).

**Biofilm disruption experiments.** The chemical nature of the S. capitis biofilm matrix was investigated by treatment with sodium metaperiodate (disrupts polysaccharides) or proteinase K (disrupts proteins) following previously published protocols (Stevens et al., 2009; Fredheim et al., 2009). Pre-formed S. capitis biofilms grown in TSBg or in PCs in six-well plates (as described above) were washed with sterile 0.9% saline solution (SS), followed by the addition of 2.9 ml of either 10 mM sodium metaperiodate in 50 mM sodium acetate buffer (pH 4.5) or 100 µg ml^-1 proteinase K in 20 mM Tris (pH 7.5) and 100 mM NaCl. The plates were then incubated at 37°C for 2 h or overnight with proteinase K or sodium metaperiodate, respectively. The treated biofilm cells were washed with SS, air-dried and stained with CV as described above. The assays were repeated in duplicate (non-disrupted control and disrupted sample) four and two independent times in PCs and TSBg, respectively.

**Assessment of slime production.** Congo red assay (CRA) for the assessment of slime (extracellular polysaccharide) production was performed based on the protocol of Freeman et al. (1989) and modified as previously described (Greco et al., 2008). Congo red dye (Sigma-Aldrich) and sucrose (Sigma-Aldrich) were dissolved in sterile water, filter-sterilized, and added to autoclaved BHI agar to achieve final concentrations of 0.8 g l^-1 and 36 g l^-1, respectively. Strains were plated in duplicate and incubated for 24 h at 37°C. Positive slime production was scored when the majority of colonies had a rough, black morphology whereas slime-negative strains appeared as smooth, red-to-pink colonies. Assessment was performed in duplicate with the assistance of a blinded volunteer who was not involved in the research and was unaware of strain identity. The assay was performed four times in duplicate.

**PCR and DNA sequencing.** PCR was used to amplify the icaAD region and its cognate promoter (~1.6 kb). Primers were designed based on published sequence data (AY146582; Merrett et al., 2003) and comprised a forward and reverse primers (5’-ggcct cta ctc taa aat ctc ccc-3’ and 5’-gcgcc agc acc ttt ctt ttt ggg cct-3’, respectively). Similarly, primers to amplify icaR (icaR-FW: 5’-gggc ggg ggg gat ttg aga gaa gaa-3’ and icaR-REV: 5’-cgccc ctc ccc gaa gaa gga-3’) and icaBC (icaCB-FW: 5’-gcgc tca gtt tta ctt ctc act agg-3’ and icaCB-REV: 5’-gcgc aag aag agg aga gga ctc gtc-3’) were designed based on the sequence of the S. capitis ica locus available at the accession number JF930147.1 (Cui et al., 2011). Reactions were performed in a final reaction volume of 50 µl using OneTaq Polymerase (New England Biolabs) according to the manufacturer’s recommended protocol. Template DNA (5 µl per reaction) was obtained from cell suspensions of S. capitis strains lysed in sterile, nuclease-free water adjusted to a McFarland turbidity standard of 0.5. The reaction was as follows: 94°C for 30 s, 30 cycles of 94°C for 30 s/53°C for 45 s/68°C for 1.45 min, 68°C for 5 min, and hold at 4°C. Products were resolved by electrophoresis in 1% agarose and purified using the QIAquick PCR Purification kit (Qiagen). Sequencing was performed by Stemcore Laboratories (Ottawa, ON) using the forward primer from the PCR as well as internal primer 5’-ggcct gtt gac tgg gat act atg-3’ (annealing at position 830 of icaAD) to ensure complete gene coverage. Sequencing of the icaAT and icaBC genes was accomplished by using the amplification primers and internal primers icaCB1358-FW (5’-ggcct aat ggc ttg tca ctt act-3’) and icaCB1935-FW (5’-ggcct att caa att ctt taa ctc cta cac-3’). Multiple alignment of the Ica proteins was performed using Multalin (Corpet, 1988) and CLUSTAL W (Thompson et al., 1994) web-based software. Sequences of the S. capitis Ica proteins available at JF930147.1 (Cui et al., 2011) were included as references.

**Statistical analysis.** For comparison of biofilm formation in TSBg and PCs, means and standard deviations were calculated using Statistical Analysis System SAS 9.1.3 software (SAS Institute) and plotted using Microsoft Excel. Error bars represent the mean plus one standard deviation. Statistical significance (P-value) was calculated by pairwise comparison of data by non-parametric t-test using SAS and a value of P<0.05 was interpreted as statistically significant. For the biofilm disruption experiments, means and SDs were calculated by treatments and strain types for each environment. Differences and their 95% confidence intervals were calculated to show the magnitude of the difference. For each type of strain, the paired t-test was applied to find if the difference between treatment and control was statistically significant. Overall treatment effects were evaluated through the mixed model analysis. In the model, potential correlation between paired control and treatment units was controlled by fitting random effects. A P-value of <0.05 was considered statistically significant.

**RESULTS**

**Clinical isolates of S. capitis form biofilms in PCs**

Five strains of CoNS recovered from contaminated PCs that were previously identified as S. capitis (Rood et al., 2011) were confirmed to be S. capitis subspecies capitis by their negative urease reaction (Bannerman & Kloos, 1991). Biofilm formation assays showed that S. capitis strains 512 and 525 do not form biofilms in TSBg while strains 517, 521 and 07/2010 were biofilm-positive in this medium (Fig. 1; P<0.0005). In the PC environment, all clinical strains of S. capitis formed biofilms based on the biofilm-negative threshold established using the negative control strain S. epidermidis ATCC 12228 grown in TSBg (Fig. 1, baseline indicated with dotted line). Notably, S. capitis strains 512 and 525 became biofilm-positive when grown in PCs as previously reported with S. epidermidis ATCC 12228 (Greco et al., 2007); biofilm formation was significantly different between growth in TSBg and in PCs for these strains (P<0.05). Biofilm-positive control strain S. epidermidis ATCC 35984 remained biofilm-positive in PCs although it exhibited diminished biofilm formation capacity compared with when grown in TSBg (P<0.001). While lack of biofilm formation in TSBg correlated with lack of slime production on CRA for strains 512 and 525, biofilm formation by strain 521 was not associated with slime production on CRA (Table 1).
S. capitis biofilm matrix is mainly composed of proteins

All S. capitis pre-formed biofilms were disrupted when treated with proteinase K (Fig. 2, Table 2). A mixed model analysis showed that treatment with proteinase K significantly reduced pre-formed biofilms by all strains in comparison with the non-treated controls in both TSBg (P<0.0038) and PCs (P<0.0001). In addition, no differences in susceptibility to treatment were observed between biofilms formed in TSBg (P=0.0745) or in PCs (P=0.1615). Only biofilms formed by strain 07/2010 in PCs were significantly disrupted after treatment with sodium metaperiodate (P<0.0001), indicating that a mix of polysaccharide and proteins is present in the biofilm matrix of this strain. Interference of the platelet milieu with the sodium metaperiodate treatment is suggested by the observation that in some cases (e.g. strains 512, 521 and 525) an increase in absorbance at 492 nm was observed after treatment (data not shown).

Biofilm architecture varies between cells grown in TSBg and in PCs

To further characterize biofilm formation by S. capitis strains in different environments, SEM was used to observe differences in biofilm architecture and composition. Macroscopically, S. capitis biofilms grown in PCs acquire a pale tan to orange hue when grown under platelet storage conditions whereas S. epidermidis biofilms remain white; biofilms are visible by dissection microscopy for biofilm-positive strains, whereas biofilm-negative pegs appear smooth. SEM of representative fields of biofilm-positive and -negative strains are shown in Fig. 3. It was observed that biofilms grown on pegs in TSBg existed primarily as confluent (ATCC 35984) or patchy (517) monolayers in

Table 1. Biofilm-related characteristics of S. capitis strains isolated from contaminated PCs

<table>
<thead>
<tr>
<th>Strain</th>
<th>BF in TSBg</th>
<th>BF in PCs</th>
<th>Slime production on CRA</th>
<th>Presence of ica genes</th>
<th>Amino acid substitutions in Ica proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>icaR</td>
<td>icaADBC</td>
</tr>
<tr>
<td>35984</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>12228</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>512</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
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<tr>
<td>517</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>521</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>525</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>07/2010</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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</table>
biofilm-forming strains whereas scattered cells or small cell clusters were observed for biofilm-negative strains (ATCC 12228 and 512). When strains were grown in PCs, biofilms appeared as confluent masses composed of cells and extracellular materials which were several layers thick. Discrete platelets were not observed although much of the extracellular matrix was consistent with previous observations of platelet-derived materials (Greco-Stewart et al., 2012).

Profile of biofilm-associated genes present in clinical strains of S. capitis

Many genes have been identified in S. aureus and S. epidermidis that contribute to various stages of biofilm formation (reviewed by Fey & Olson, 2010). Since little is known about biofilm-related genes in S. capitis, we chose to selectively amplify genes bearing homology to biofilm-associated genes of S. epidermidis, a closely related CoNS. Genes of the icaADBC locus and its transcriptional regulator icaR were successfully amplified for all strains with the exception of S. capitis 512 (Table 1). The upstream regulatory sequence of icaADBC was also amplified since regulatory elements are contained therein; specifically, the IcaR binding region is located in a 28 nt region positioned 17 nt upstream from the start codon of icaA (Jeng et al., 2008).

S. capitis icaR, icaADBC and cognate upstream element were sequenced and genes translated for strains 517, 521, 525 and 07/2010 and compared with each other and with a previously reported sequence (JF930147.1; Cui et al., 2011). Of the clinical isolates, only strain 521 deviated from the IcaA (N107D, G162D, A197D and L288I), IcaB (K136E and I150V) and IcaC (L67F and M326V) consensus sequences (Table 1). Potential regulatory elements in the region upstream of the icaA start codon were also examined and the presence of a G→T transversion at the −10 position and a G→A transition at position −29 was noted in strain 521. The IcaR and IcaD sequences were completely identical among clinical and reported strains.

Table 2. Disruption of the S. capitis biofilm matrix

<table>
<thead>
<tr>
<th>Strain</th>
<th>Biofilm formation</th>
<th>Biofilm disruption</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>TSBg</td>
<td>PCs</td>
</tr>
<tr>
<td>512</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>517</td>
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<tr>
<td>521</td>
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<td>525</td>
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<td>07/2010</td>
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**DISCUSSION**

In the present study, we have characterized the biofilm formation properties of strains of *S. capitis*, an important neonate pathogen (Van Der Zwet *et al.*, 2002), recovered from contaminated platelet units, using several criteria and have observed various unique profiles with respect to biofilm-associated phenotypes among these strains. We have also noticed that strains which display a biofilm-negative phenotype in conventional laboratory media have also noticed that strains which display a biofilm-negative phenotype in PCs, as observed for other Gram-negative (Greco-Stewart *et al.*, 2012) and Gram-positive species (Greco *et al.*, 2007). Differences were unrelated to subspecies, since all strains were determined to be *S. capitis* subspecies *capitis*, and unrelated to plasma sensitivity since all strains were able to survive and grow in PCs.

Biofilm disruption studies indicate that, with the exception of *S. capitis* 07/2010, all strains have a biofilm matrix composed mainly of proteins. Since incomplete biofilm disruption was accomplished by proteinase K treatment and disruption with sodium metaperiodate had little effect, the matrix of *S. capitis* biofilms must be of a heterogeneous nature including other components such as DNA. Similar observations have been reported for the coagulase-negative *Staphylococcus haemolyticus*, another recognized neonate pathogen (Fredheim *et al.*, 2009).

In the present study, the *S. capitis* biofilm phenotype does not always bear the hallmark properties of classic PIA-mediated biofilm formation. The presence of wild-type or mutant Ica proteins, responsible for PIA biosynthesis, does not have a relevant impact on biofilm formation by this species. The phenotypes of strains 521 and 525, for example, are suggestive of PIA-independent biofilm formation in PCs. While *S. capitis* 521 has multiple mutations in the IcaA, IcaB and IcaC proteins, its ability to form biofilms in TSBg and in PCs is not impaired. Strain 525 possesses wild-type Ica proteins and yet is still unable to form biofilms in regular media. Notably, *ica*-negative *S. capitis* strain 512 displayed a typical biofilm-negative phenotype in TSBg but adopted a biofilm-positive phenotype when grown in PCs, similar to what has been observed with the biofilm-negative strain *S. epidermidis* ATCC 12228 (Greco *et al.*, 2007). It thus appears that *ica*-negative strains of *S. capitis* are indeed capable of biofilm formation under certain circumstances and that environmental cues might contribute to *ica*-independent biofilm formation in these strains.

Biofilms confer protective benefits to bacteria, including reduced susceptibility to antimicrobials, protection from the immune system and resistance to sheer stresses. However, it has been demonstrated that *S. epidermidis* *ica*-positive strains are at a disadvantage with respect to skin colonization when compared with *ica*-negative commensal strains (Rogers *et al.*, 2008). It can be speculated that strains lacking the *ica* operon, but that are able to form biofilms *in vivo*, would have a heightened potential for opportunistic pathogenicity. This could explain why *ica*-negative biofilm-forming strains are often isolated from clinical samples (Petrelli *et al.*, 2006; Rohde *et al.*, 2007; Qin *et al.*, 2007; Fredheim *et al.* 2009). It has also been shown that biofilm-associated proteins such as Embp are sufficient to stimulate staphylococcal biofilm formation *in vitro* in the absence of the *icaADBC* operon and other biofilm-related genes such as *aap* (Christner *et al.*, 2010; Los *et al.*, 2010). These observations suggest that a delicate balance exists with respect to the possession and expression of biofilm-associated genes in order to optimize bacterial survival under various physiological conditions.

It has been shown that the *ica* genes are required for attachment of *S. epidermidis* to polystyrene (and potentially other hydrophilic surfaces; Heilmann *et al.*, 1996); however, pre-conditioning these surfaces with biomaterials (such as by the deposition of serum proteins resulting from incubation with PCs) could stimulate biofilm initiation in *ica*-negative strains. Serum conditioning has been shown to promote binding of bacteria to surfaces via microbial adhesins recognising extracellular matrix macromolecules.
and have increased virulence if tainted PC units are transfused. During transfusion, dislodged biofilms can be transferred to the recipient and then become foci of infection, particularly if the patient has implanted biomedical devices. *S. capitis* is particularly dangerous to premature neonates, a vulnerable demographic who often receive multiple transfusions to treat complications associated with preterm birth and who are particularly susceptible to development of sepsis and death as a result of staphylococcal infection (de Silva et al., 2002; Van Der Zwaet et al., 2002). Characterization of biofilm formation of common blood contaminants is thus recommended in order to develop improved methods of detection and eradication of these organisms, contributing to the ultimate goal of improving the safety of our blood supply.

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