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Evidence for the involvement of *Staphylococcus epidermidis* LPXTG surface protein SesC in biofilm formation and catheter-related infections

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Introduction

Staphylococcus epidermidis is a frequent cause of infections on indwelling medical devices due to its ability to form biofilm. The surface of these bacteria is decorated with proteins. However, Surface proteins have been shown to play key roles in *S. epidermidis* biofilm formation. (1,2) We have recently shown that active and passive immunization against *S. epidermidis* LPXTG surface protein SesC can decrease S. epidermidis biofilm formation in vitro and in vivo. In this study we will present new evidence on the role of SesC in *S. epidermidis* biofilm formation and its potential as a suitable vaccine target.(2)

Materials and Methods

Bacterial strains, plasmids and media: All (sub)cloning experiments were performed in *E. coli* DH5α-competent cells, obtained from Invitrogen. *E. coli* DH5α transformed with plasmids was grown in Luria-Bertani (LB) medium or on agar plates at 37°C supplemented with 100 µg/ml ampicillin. All *Staphylococcus* spp. strains were grown in brain heart infusion (BHI). Whenever required, media of transformants or mutants were supplemented with suitable antibiotics.The 8325-4 and BH1CC isogenic *ica* and *srtA* mutants were constructed as previously explained. The isogenic *srtA* mutants were complemented by pSR*srtA*5 carrying *S. aureus srtA* gene as previously explained (3).

Cloning and expression of *S. epidermidis sesC* and *sesK* genes in *S. aureus* strains: The entire coding regions of *S. epidermidis sesC* (NP_765787.1) and *sesK* (NP_765056.1) were amplified using genomic DNA (gDNA) of biofilm-forming *S. epidermidis* strain 10b as template and ligated into pCN50 and pCN68 *E.coli-Staphylococcus* shuttle vectors yielding pCN50*sesC*, pCN68*sesC* and pCN50*sesK*. All recombinant plasmids were first electroporated into the restriction-deficient *S. aureus* strain RN4220 and then into other *S. aureus* strains.

Biofilm formation and treatment assay: Briefly, overnight cultures of bacteria, were diluted to an OD_{600} of 0.005 in BHI or in BHI supplemented with 4% NaCl or 1% glucose. 200µl of the diluted overnight cultures of bacteria were pipetted into sterile 96-well polystyrene microtiter plates and incubated overnight at 37°C without shaking. After the incubation, plates were washed with PBS, stained with crystal violet, washed again with water and dried. For quantification, acetic acid was added to each well to dissolve the stain and the OD_{595} of the dissolved stain was measured.
The biofilm stability against sodium metaperiodate (SM) or proteinase K (PK) treatment was tested as described previously [4].

PIA quantification by PIA non-specific immunoblot assay: The relative amount of PIA produced was determined with some modifications as described (5).

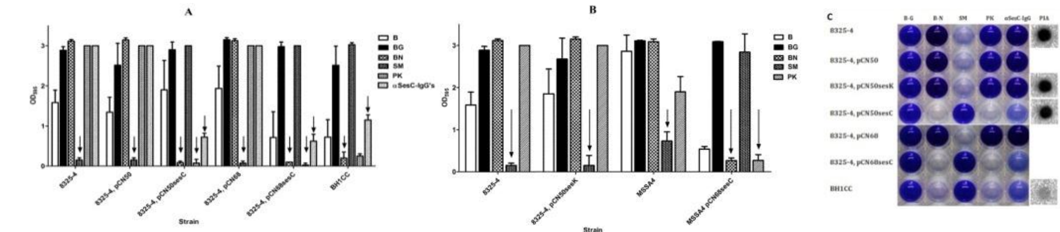
Scanning Electron Microscopy: *In vitro* biofilm formation on glass disks was visualized by scanning electron microscopy (SEM) as explained previously (6).

References

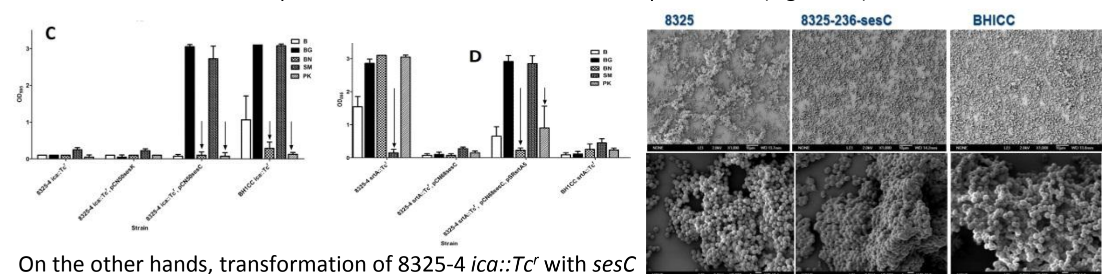
1.Shahrooei et al. IAI, 2012, 2. *Foster* et al.,2014, 3. O'Neill et al. J Bacteriol, 2008, 4. Fitzpatrick et al. J Clin Microbiol, 2005, 5. Singh et al. J Med Microbiol 2010, 6. Pintens et al. Microbiology-Sgm, 2008.

Results

Heterologous expression of *sesC* in S. aureus switches the biofilm phenotype from PIA-dependent to proteinaceous:



Heterologous expression of *sesC* had no effect on the biofilm-type of the protein-type forming strain BH1CC. It inhibited biofilm formation by 8325-4 which makes a PIA-type biofilm in BHI supplemented with 4% NaCl (Fig. A & C). Furthermore, biofilms of 8325-4 expressing SesC grown in BHI glucose, were dispersed with Proteinase K and not Sodium Metaperiodate which dispersed wild type 8325-4 biofilms (Fig. A & C). This is consistent with a biofilm phenotype switch from PIA- to protein-mediated. The biofilm phenotype of MSSA4 also switched from PIA- to protein – mediated after induction of pCN68*sesC* (Fig. B & C).



On the other hands, transformation of 8325-4 *ica*::Tc^r with *sesC* but not *sesK* restored the biofilm formation to approximately wild type levels in BHI supplemented with 1% glucose (Fig. C). Transformation of 8325-4 *srtA*::Tc^r with pCN68*sesC* completely impaired biofilm production, further indicating the dominant role of SesC over PIA-type biofilm production (Fig. D). Immunoblots revealed that PIA synthesis was significantly decreased in 8325-4 *srtA*::Tc^r, pCN68*sesC* (results not shown). However, complementation of 8325-4 srtA::Tcr, pCN68sesC with a plasmid carrying the *S. aureus* srtA gene (pSRsrtA5) restored biofilm formation and increased PIA expression.

Conclusions

Our new data are consistent with our previous findings in suggesting an important role for SesC in *S. epidermidis* biofilm formation. Although we have to be cautious in extrapolating conclusions based on data obtained in *S. aureus* to *S. epidermidis*, we conclude that SesC is a virulence factor associated with the early stages in *S. epidermidis* biofilm formation such as adhesion and colonization. The biofilm formation versatility and flexibility of *S. epidermidis* may be due in part to the presence of *sesC* and similar factors that help *S. epidermidis* to adapt to changing environmental conditions.