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Correlation between biofilm formation and production of polysaccharide intercellular adhesin in clinical isolates of coagulase-negative staphylococci

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Abstract

The ability to form a biofilm seems to play an essential role in the virulence of coagulase-negative staphylococci (CoNS) by permitting them to cause persistent prosthetic device-related infections. The most clearly characterized component of staphylococcal biofilms is the polysaccharide intercellular adhesin (PIA) encoded by the *icaADBC* operon. In the present paper, we assess the link between the ability to form a biofilm (Bf+/−), to synthesize PIA (PIA+/−) and the presence of the *ica* locus (*ica*+/−). For this purpose, 66 CoNS strains were tested in vitro. Seventy three percent of all strains revealed presence of the *ica* locus (*ica*+), and therefore were potentially able to produce PIA and to form a biofilm. However, the characteristics observed indicated that 15% of all strains were biofilm forming without PIA production (Bf+, PIA−, *ica*+/−) while 8% were PIA producers without biofilm formation (Bf−, PIA+, *ica*+). On the basis of the obtained data we conclude that (i) PIA synthesis alone is not sufficient to produce a biofilm and (ii) staphylococci can also form a biofilm without producing PIA.

Keywords: *Staphylococcus*; Biofilm; *ica* operon; PIA

Introduction

Coagulase-negative staphylococci (CoNS) are among the most commonly reported pathogens in nosocomial infections. Nearly 80% of the human isolates are *S. epidermidis* (Von Eiff et al., 1999), which is described as a leading species involved in chronic polymer-associated infections and isolated from immuno-compromised patients (Huebner and Goldmann, 1999; Pessoa-Silva et al., 2001). The pathogenesis of foreign body-associated infections with *S. epidermidis* is thought to be linked to its ability to form multilayered cell clusters on the polymer surfaces (Mack, 1999).

The cells in these layers are embedded and protected by a slimy material known as biofilm (Peters et al., 1982) which represents the key virulence factor of CoNS

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(Raad et al., 1998). The later phases of biofilm formation, in which organisms adhere to one another and elaborate the biofilm, are mediated by polysaccharide intercellular adhesin (PIA) (Rupp et al., 1999).

A clearly characterized component of staphylococcal biofilms is PIA, a specific polysaccharide antigen, which was isolated from *S. epidermidis* strains 1457 and RP62A (Mack et al., 1996a). This polysaccharide is a linear β-(1,6)-linked glucosaminoglycan with different substituents (Mack et al., 1996a). By transposon insertional mutagenesis, an operon of genes responsible for PIA biosynthesis was identified in *S. epidermidis* O-47 and named *ica* for intercellular adhesion (Heilmann et al., 1996). This operon is composed of the *icaADBC* genes (Gerke et al., 1998).

The direct involvement of the biofilm in conferring virulence to CoNS is still a subject of discussion. Ishak et al. (1985) reported that biofilm formation by CoNS blood-stream isolates was similar to that by commensal strains isolated from skin. Vogel et al. (2000) found no clear association between biofilm production and the virulence of *S. epidermidis* isolated from catheter-related infections and nasal colonizers. Götz and Peters (2000) suggested that biofilm production might be an adaptive response of some strains to the presence of a foreign body rather than a necessary virulence factor.

The role of the *ica* operon in infectious staphylococci has been intensively investigated by a number of authors. Eighty-five percent of *S. epidermidis* blood culture isolates contained the *ica* locus compared to 6% of mucosal isolates (Ziebuhr et al., 1997). A high prevalence of *ica*+ strains was also demonstrated in CoNS isolated from prosthetic implants or catheter-related urinary tract infections (Galdbart et al., 2000; Allignet et al., 1999; Cho et al., 2002).

The expression of the *ica* operon and, as a result, the formation of biofilms seems to be highly variable among clinical isolates of staphylococci (Mack et al., 1996b; Ziebuhr et al., 1997; Rohde et al., 2001). The recent finding by Fitzpatrick et al. (2002) suggests that the presence of the *ica* locus alone is not sufficient for biofilm formation. The regulation of biofilm formation under altered growth conditions, which may exist in the in vivo environment, may also play a role in the pathogenesis of biomaterial-related *S. epidermidis* infections (Fitzpatrick et al., 2002).

Since biofilm production seems to play a pivotal role as a virulence factor of CoNS, and while taking into account a large number of publications relating the formation of a biofilm and the presence of the *ica* operon in *S. epidermidis*, the aim of this study was to determine the correlation between the occurrence of the *ica* locus, PIA synthesis, and biofilm formation not only in *S. epidermidis*, but also in other significant CoNS species isolated from patients with infected implants.

Materials and methods

Bacterial strains

As controls, two staphylococci reference strains, kindly provided by Dr. W. Ziebuhr (Würzburg, Germany), were used, the biofilm producing strain *S. epidermidis* RP62A (ATCC 32984) and the non-biofilm-producing strain *S. carnosus* TM300. The 66 CoNS used in this study were collected from patients with infected implanted devices, hospitalized in the Mignot Hospital of Versailles, France (see Table 2). Bacteria were grown in trypticase soy broth TSB (Difco Laboratories, Detroit, USA) at 37 °C overnight and stored at −80 °C in 15% glycerol. Only strains growing on Baird–Parker plates (Difco) and identified as Gram-positive, catalase-positive cocci and negative for the free coagulase test (AES Laboratoire, Combourg, France) were retained for this study.

The identification of strains was confirmed by sequencing of a 430-bp fragment of the 16S rRNA gene. Total cellular DNA was extracted and purified from staphylococcal strains as previously described (Killgore et al., 2000). The extracted genomic DNA was subjected to restriction endonuclease cleavage by HindIII (Boehringer Mannheim, Germany) as specified by the manufacturer. Native and digested DNA were analyzed by electrophoresis in a 1% agarose gel (Eurobio, Les Ulis, France) in Tris–Borate–EDTA (TBE). Less than 1-kb amplification products were separated in 1% agarose gels. PCR experiments were performed in a DNA thermal cycler (Eppendorf, Hamburg, Germany) and the primers were synthesized by Proligo (France SAS). The following high-stringency conditions with primers 5′-CGGCCCAGACTCC-TACGGGAGGCAGCA-3′ and 5′-GCGTGGACTAC-CAGGGTATCTAATCC-3′ were used: preheating for 5 min at 94 °C, 25 cycles (94 °C 60 s, 58.5 °C 45 s and 72 °C 60 s) and a final extension for 2 min at 72 °C. The 16S rDNA of CoNS strains was partially sequenced using an ABI PRISM™ 310 Genetic Analyzer apparatus (Applied Biosystems, Foster City, CA, USA) and the sequences were analyzed using Sequencing Analysis 3.4.1. software.

Biofilm formation assay

Assay of biofilm formation was performed essentially according to (Christensen et al., 1985). Strains were grown in TSB medium supplemented with 0.25% glucose from 6 h to overnight with shaking at 37 °C. Preliminary cultures were diluted 1:200 into TSB medium with 0.25% glucose, volumes of 200 µl were inoculated into each well of 96-well tissue culture polystyrene microtiter plates (Greiner bio-one, Germany) and incubated for 24 h at 37 °C. Tests were performed in