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## Molecular basis of *Staphylococcus epidermidis* infections<sup>1</sup>

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### Abstract

*Staphylococcus epidermidis* is the most important member of the coagulase-negative staphylococci and one of the most abundant colonizers of human skin. While for a long time regarded as innocuous, it has been identified as the most frequent cause of device-related infections occurring in the hospital setting and is therefore now recognized as an important opportunistic pathogen. *S. epidermidis* produces a series of molecules that provide protection from host defenses. Specifically, many proteins and exopolymers, such as the exopolysaccharide PIA, contribute to biofilm formation and inhibit phagocytosis and the activity of human antimicrobial peptides. Furthermore, recent research has identified a family of pro-inflammatory peptides in *S. epidermidis*, the phenol-soluble modulins (PSMs), which have multiple functions in immune evasion and biofilm development, and may be cytolytic. However, in accordance with the relatively benign relationship that *S. epidermidis* has with its host, production of aggressive members of the PSM family is kept at a low level. Interestingly, in contrast to *Staphylococcus aureus* with its large arsenal of toxins developed for causing infection in the human host, most if not all “virulence factors” of *S. epidermidis* appear to have original functions in the commensal lifestyle of this bacterium.

### Keywords

*Staphylococcus epidermidis*; biofilm; phenol-soluble modulins; polysaccharide intercellular adhesin; device-related infections; hospital-associated infections

## 1. Introduction

Coagulase-negative staphylococci (CNS) are a prominent part of the normal flora of human skin. Certain CNS species prefer specific niches, while others occur almost everywhere on our body [1,2]. In contrast to *Staphylococcus aureus*, CNS colonize every human being. This includes *Staphylococcus epidermidis*, which is the most frequently encountered CNS species on the human body and a common colonizer of the axillae, head, and nares [1,3]. Colonization with *S. epidermidis* may play an important role for the maintenance of a healthy skin flora, by competition with potentially harmful microorganisms such as in particular *S. aureus*. On the other hand, *S. epidermidis* is now also being recognized as an important opportunistic pathogen that can cause significant problems when breaching the epithelial barrier, especially during biofilm-associated infection of indwelling medical devices. Here, I will present molecular factors that are involved in the success of *S. epidermidis* as a colonizer and pathogen, with a specific focus on the interaction with *S.*

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*aureus* and the different “approaches” of these two related bacteria to evade human immune defenses during colonization and infection.

## 2. Diseases caused by *S. epidermidis* and other coagulase-negative staphylococci

Most diseases caused by *S. epidermidis* and other CNS are of a chronic character and occur as device-related infections (such as intravascular catheter or prosthetic joint infections) and their complications [4]. *S. lugdunensis* represents a certain exception among the CNS, as it is more often involved in severe infections that resemble those caused by *S. aureus* [5–7]. In CNS device-related infections, complete removal of the infected device and prolonged antibiotic therapy are often necessary. CNS bloodstream infections originating from intravascular catheter infections are estimated to reach 250,000 cases per year in the U.S., with a mortality rate of 1–25%. These infections represent a significant burden for the public health system, with a cost of \$25,000 per episode and an excess hospital stay of > 7 days [8,9]. More severe manifestations, such as prosthetic valve endocarditis (PVE), are rare compared to device-related infections. However, CNS are among the most frequent causes of PVE (15–40%) [10]. Of note, neonates represent a particularly high-risk group for CNS infections [11], with 31% of all neonatal infections and 73% of neonatal bacteremias attributable to CNS [12].

## 3. Antibiotic resistance

Resistance to methicillin is widespread among hospital isolates of CNS and in particular, *S. epidermidis* (methicillin-resistant *S. epidermidis*, MRSE), ranging globally from 75–90% [13]. It is due to the presence of the *mecA* gene, which codes for a penicillin binding protein, PBP2a, with decreased affinity to methicillin [14]. Heteroresistance, which means that only one in  $10^4$  to  $10^8$  cells shows a high level of methicillin resistance, occurs in CNS similar to *S. aureus* [15]. Resistance to aminoglycosides and macrolides, and to a lesser extent, tetracycline, chloramphenicol, and clindamycin is also frequently observed among hospital-associated *S. epidermidis* strains [4]. Intermediate resistance to vancomycin (VISE, vancomycin intermediate resistant *S. epidermidis*) is on the rise [16]; however, spread of vancomycin high-level resistant strains has not been reported. Among the newer antibiotics, resistance is still very rare to linezolid and streptogramins, and not reported for daptomycin or tigecycline [4]. Of note, non-specific resistance (tolerance) by biofilm formation is of great concern in CNS infections and will be discussed below.

## 4. Virulence factors of *S. epidermidis*

*S. epidermidis* is by far the best studied member of the CNS in terms of the knowledge that we have on molecular mechanisms of virulence (Tab. 1). For the purpose of the present review, the definition of “virulence factors” will be broad, comprising genes and proteins that facilitate the establishment of infection and persistence of the organism in the human body. It will become clear that most of these factors also have important roles in the commensal life of *S. epidermidis* as an innocuous inhabitant of the human skin and may thus not be regarded as “virulence factors” *sensu stricto*.

### 4.1 Biofilms

*S. epidermidis*, like many other CNS, is an excellent biofilm former, and most *S. epidermidis* infections involve biofilms. Biofilms are sticky, surface-attached agglomerations of bacteria that are embedded in an extracellular matrix and provide protection from antibiotics and mechanisms of host defense [17,18]. For many antibiotics, MIC values against bacteria in

biofilms can be higher by several logs compared to those against planktonic (free-floating) bacteria.

The mechanisms underlying the protective features of biofilms are multiple and may be different for a specific antibiotic or host defense mechanism. First, the matrix represents a mechanical barrier that is hardly penetrable for immune cells. However, there are also reports suggesting that, for example, neutrophils penetrate deeply into bacterial biofilms [19], likely through the matrix-free channels that are characteristic components of the three-dimensional biofilm structure. Nevertheless, phagocyte activity is commonly regarded as severely hampered by biofilm formation. For *S. aureus*, this has recently been shown clearly by Thurlow et al. [20]. Furthermore, limited diffusion through the extracellular matrix contributes to biofilm tolerance towards some antibiotics [21]. This is the case for example for ciprofloxacin and biofilms of *Pseudomonas aeruginosa* [22]. In contrast, other antibiotics have been shown to diffuse easily into the biofilm matrix, such as rifampin, vancomycin, and daptomycin in *S. epidermidis* biofilms [23,24]. Second, biofilms are to some extent physiologically “dormant” - showing reduced activity of many active cell processes such as cell division, protein synthesis, or DNA replication [21]. This specific physiological state of biofilms significantly decreases the efficacies of many antibiotics that target those processes, including for example the large number of protein synthesis inhibitors and the cell wall-active beta-lactam antibiotics. Third, biofilms may comprise an increased number of so-called “persister” cells, which show increased tolerance to antibiotics [25]. Recently, it was shown that *S. epidermidis* biofilms contain a high number of persister cells upon antibiotic exposure [26]. Finally, biofilms may show over-production of proteins or other biopolymers that contribute to resistance or tolerance [27].

#### **4.1.1 *S. epidermidis* biofilms protect from mechanisms of innate host defense**

—Many of the protective mechanisms of biofilms toward host defenses, in particular those of innate host defense, have been analyzed in *S. epidermidis*. Mechanisms of innate host defense allow the human body to respond swiftly to an infection. They are triggered by the recognition of invariant structures on the bacterial surface, such as peptidoglycan, lipopeptides, lipoteichoic acids, or even some species-specific molecules such as the staphylococcal phenol-soluble modulins (PSMs) discussed below. Innate host defense comprises mainly the activity of phagocytes, antimicrobial peptides (AMPs), and the complement system.

It has been suggested early that the main biological purpose of the *S. epidermidis* “slime” substance, now called extracellular biofilm matrix, is to prevent ingestion of bacteria by phagocytes. Beginning in the 1980s, several reports were published providing evidence for that function [28–30]. Recent research has given more mechanistic insight, showing that *S. epidermidis* biofilms protect from neutrophil-dependent killing by complement inactivation via prevention of the deposition of C3b and immunoglobulin G (IgG) [31]. Furthermore, the extracellular matrix decreases the activity of antimicrobial peptides (AMPs), likely mostly by hindering them from reaching their predominant target, the cytoplasmic membrane [32].

#### **4.1.2 Molecular mechanisms contributing to biofilm development**—Biofilm development proceeds in 3 steps: initial adhesion, intercellular aggregation and accumulation, and final detachment [33]. Initial adhesion may occur to abiotic surfaces, such as the uncovered plastic surface of indwelling medical devices, or to biotic surfaces, such as tissues or human matrix protein-covered devices. Intercellular aggregation is accomplished by a series of matrix proteins and non-proteinaceous polymeric substances. In addition, more recent research has indicated a key role of surfactants in biofilm detachment [34–36]. Specific molecules involved in *S. epidermidis* adhesion, aggregation, biofilm structuring, and detachment will be presented in the following. First, proteins and polymers involved in

adhesion and/or accumulation will be discussed. Many of these have been implicated with both adhesion and accumulation. Finally, recent work on biofilm structuring and detachment will be presented.

**MSCRAMMs:** Several MSCRAMMs (microbial surface components recognizing adhesive matrix molecules) [37] play a role in tethering *S. epidermidis* cells to tissue or matrix protein-covered device surfaces. This may or may not ultimately lead to biofilm formation, but in any case is a crucial step for *S. epidermidis* persistence in or on the human body during colonization and infection. Similar to *S. aureus*, *S. epidermidis* has several MSCRAMMs for that purpose, with specificities for a series of different human matrix proteins [38]. This functional redundancy underscores the importance that the establishment of tissue adhesion has for *S. epidermidis* physiology. For example, MSCRAMMs of *S. epidermidis* accomplish binding to fibrinogen (SdrG/Fbe) [39,40], fibronectin (Embp) [41], vitronectin (AtlE, Aae) [42,43], and collagen (GehD) [44]. The fibrinogen and fibronectin binding proteins represent MSCRAMMs *sensu stricto*, with sortase-catalyzed covalent anchoring to peptidoglycan [45] and primary function as adhesins, while AtlE and Aae are non-covalently surface bound autolysins with adhesive in addition to their primary cell wall turnover functions [46]. Finally, GehD is a lipase that also appears to have an unrelated function as binding protein [44].

**PIA:** The exopolysaccharide polysaccharide intercellular adhesin (PIA), also named poly-N-acetylglucosamine (PNAG) was identified as the main constituent of the *S. epidermidis* extracellular matrix substance (“slime”) in 1996 by the group of Dietrich Mack in Hamburg, Germany [47]. While minor uncertainties persist about partial N-succinylation and yet not further identified variants of PIA, the main PIA exopolysaccharide is a linear N-acetylglucosamine polymer with partially N-deacetylated amine groups. It has a chain length of more than 130 residues in average. Notably, the sugar moieties are linked in  $\beta$ 1–6 linkage, which contrasts one of the most abundant poly-glucosamines in nature, chitin, which has  $\beta$ 1–4 linkages [47] (Fig. 1).

PIA is synthesized by the gene products of the *ica* operon, *icaA*, *icaD*, *icaB*, and *icaC* [48]. Adjacent to the *ica* operon is a regulatory gene, *icaR*, that is involved in the multifaceted regulation of *ica* gene expression [49–54]. IcaA is a glucosaminyl transferase that was shown to transfer glucosamine residues from UDP-glucosamine to a growing chain of pre-PIA [55]. It requires IcaD for full activity, but the detailed role of IcaD is not known. Both proteins are located in the cytoplasmic membrane. Presumably, IcaC, another membrane protein, exports the growing chain of pre-PIA out of the cell. This was speculated based on membrane location and the fact that in an *icaC* mutant, chain elongation stops at about 15 residues, also suggesting that chain elongation and export are coupled and the IcaA, IcaD, and IcaC proteins may form a complex [55]. Pre-PIA is N-deacetylated after export by the IcaB enzyme, which is a surface-attached PIA N-deacetylase [56]. Deacetylation is crucial for the multiple roles of PIA discussed below [56]. It also significantly increases immunogenicity of the PIA polymer [57].

As expected, a correlation between PIA production and biofilm formation among a collection of clinical *S. epidermidis* strains was found early after discovery of the polymer [58], and PIA has long been regarded as indispensable for *S. epidermidis* biofilm formation. However, strains were found more recently, in which in-vitro and in-vivo biofilm formation is independent of PIA and in which proteins functionally substitute for PIA [59,60]. Interestingly, there is evidence indicating that PIA-dependent biofilms form more robust biofilms than those exclusively formed by proteinaceous factors [60]. Furthermore, PIA was found to have multiple, additional roles in immune evasion that exceed a mere contribution to intercellular aggregation in biofilms [56]. For example, PIA was described as the major *S.*

*epidermidis* hemagglutinin [61]. Moreover, PIA reduces the activity of AMPs and killing by human neutrophils [56]. As the latter effects were observed after disruption of cell clusters by sonication, they are likely at least in part independent of intercellular aggregation, due only to the layer of PIA that surrounds a single *S. epidermidis* cell. PIA protects from the activity of both positively and negatively charged AMPs, indicating that different mechanisms may contribute to PIA-mediated resistance to AMPs, such as sequestration in addition to repulsion [56,32]. PIA was also reported to induce IL-8 expression in human astrocytes in a TLR2-dependent fashion [62]. However, this was not substantiated using isogenic *ica* deletion mutants, which is crucial when analyzing pro-inflammatory activities of bacterial compounds, because they almost never can be purified without leaving traces of known pro-inflammatory agents such as lipopeptides. This is especially true in case of the complicated purification of PIA [47,63].

The contribution of PIA to virulence has been monitored in a multitude of animal studies of device-related infection. These studies used mice, rats, rabbits, or *Caenorhabditis elegans* and mutants in the *ica* operon of *S. aureus* or *S. epidermidis*. A majority of studies found that PIA contributed to virulence in those models [64–67]. This includes a study in which specifically the role of IcaB-dependent deacetylation was investigated and found to have a significant impact on virulence [56]. However, some studies failed to detect a role of PIA in the pathogenesis of biofilm-associated infection [68,69]. While those latter studies are often exclusively cited when aiming to emphasize the importance of protein-dependent biofilm formation, it must be stressed that (i) differences in the experimental setup may have led to the negative outcome and (ii) those studies contradict a larger series of studies showing a significant role of PIA in biofilm-associated infection.

**Teichoic acids:** Teichoic acids (TA) are characteristic and virtually omnipresent surface components of Gram-positive bacteria. They consist of alternating phosphate and polyol (ribitol or glycerol) moieties. Teichoic acids occur in two major forms, lipoteichoic acids (LTA), which are linked to the cell membrane via a membrane-spanning lipid anchor, and wall teichoic acids (WTA), which are covalently linked to peptidoglycan [70]. Extracellular TA are loosely attached to the cell surface and may originate from WTA that have lost covalent anchoring. The structure of cell wall and extracellular TA of *S. epidermidis* have been analyzed in detail [71]. They consist of poly(glycerol phosphate) units, which are substituted at the 2-position of glycerol with alpha-glucose, alpha-glucosamine, D-alanine, or alpha-6-D-alanyl-glucose. Reports on “lipid S”, an alleged pro-inflammatory short-chain form of LTA in *S. epidermidis* [72], are most likely erroneous and caused by co-purification and misinterpretation of mass spectra originating from a phenol-soluble modulin peptide (PSM-mec) present in MRSE [73].

TA have multiple roles in staphylococcal physiology and pathogenesis, contributing to adhesion, colonization, and inflammation [74–76]. In *S. epidermidis*, TA were shown to enhance adhesion to fibronectin [77]. Furthermore, recent research substantiated the long suspected role of WTA in *S. epidermidis* biofilm formation by analyzing a *tagO* mutant of *S. epidermidis* that exhibited decreased attachment and aggregation phenotypes [78]. A direct role of TA in immune evasion of *S. epidermidis* has not yet been evaluated, but as a component that has a significant impact on biofilm formation, TA can be assumed to have such a function. Furthermore, *S. epidermidis* also contains the genes for D-alanylation of TA, a modification that is known from a series of other bacteria, including *S. aureus*, to protect from the activity of AMPs [79]. These genes, together with others that encode AMP protection systems, namely the VraFG transporter and the phospholipid lysylation enzyme MprF [80,81], are regulated by a system that senses AMP presence and was discovered in *S. epidermidis* [82].



**Accumulation-associated protein (Aap):** In 1997, Hussain et al. described a 140-kilodalton extracellular protein that is essential for the accumulation of *S. epidermidis* on surfaces [83]. This protein was called accumulation-associated protein (Aap). Aap was later shown to form polymeric fibrils on the surface of *S. epidermidis* cells, also mediating intercellular adhesion [84]. One subpopulation of cells expresses fibrils, while another does not. The mechanism underlying this specification is not known. The formation of fibrils is zinc-dependent and proceeds via dimerization of so-called G5 domains and modular association of tandem G5 domains [85]. This mechanism is analogous to that involved in the formation of mammalian cadherin domains. Of note, Aap needs proteolytic processing to obtain its biofilm-active form [86]. In addition to mediating intercellular adhesion and adhesion to surfaces, Aap has also recently been shown to attach *S. epidermidis* to human corneocytes via its terminal A domain [87]. Aap is considered the most important factor contributing to protein-dependent (non-PIA-dependent) biofilm formation in *S. epidermidis*.

**Embp:** The extracellular matrix-binding protein (Embp) is a giant covalently attached surface protein of 1 MDa [41]. A truncated form of 460 kDa is necessary and sufficient to promote protein-dependent biofilm formation of *S. epidermidis* [88]. In addition, Embp mediates adherence to fibronectin and is thus involved in both adhesion and accumulation stages of biofilm formation [41,88].

**Bap/Bhp:** The biofilm-associated protein (Bap) is a 239 kDa surface protein involved in biofilm adhesion and accumulation [89]. While in *S. aureus*, Bap is present only in a minority of bovine mastitis isolates, located on a pathogenicity island, it is found frequently in *S. epidermidis* strains [90]. A protein with high similarity to Bap, the Bap homologue protein Bhp, occurs in some *S. epidermidis* strains, but does not appear to be essential for biofilm formation [91].

Structuring and detachment: PSMs. While molecular mechanisms contributing to aggregation during biofilm development have been intensely studied, we lack knowledge about cell-cell disruptive mechanisms. These are required for the formation of the characteristic biofilm structure with fluid-filled channels and mushroom-shaped cellular aggregations, and for the detachment of cells from a biofilm, which controls biofilm thickness and expansion. In several bacteria, including *S. aureus* and *S. epidermidis*, quorum-sensing systems have been implicated in the control of biofilm thickness and biofilm structuring [92–96]. However, the quorum-sensing controlled factors involved in those processes on a mechanistic level have remained largely undefined. In *P. aeruginosa* and *B. subtilis*, surfactant molecules with different chemical natures (rhamnolipid and surfactin, respectively) are involved in biofilm structuring [36,35]. In a recent study, the PSM $\beta$  peptides of *S. epidermidis* were shown to structure *S. epidermidis* biofilms and thus fulfill a task similar to that accomplished by rhamnolipid in *P. aeruginosa* [34]. Of note, in that study it was demonstrated for the first time, using the PSM $\beta$  peptides of *S. epidermidis* and a murine device-related infection model, that biofilm structuring molecules trigger the dissemination of biofilm-associated infection. Isogenic *S. epidermidis* psm $\beta$  deletion mutants showed significantly less systemic dissemination from the biofilm and anti-PSM $\beta$  antibodies blocked dissemination.

**4.1.3 *S. epidermidis* biofilms have a specific, protective gene expression profile**—Biofilms represent a strongly divergent mode of growth compared to that of free-floating (often called “planktonic”) bacteria, a situation that for many bacteria is encountered almost exclusively under laboratory conditions. Not surprisingly, gene expression profiles of biofilms show considerable differences when compared to planktonic bacteria in whole genome expression experiments using microarrays. In *S. epidermidis*, similar to other bacteria, gene expression in biofilms is characterized by a suppression of

many active cell processes and an adaptation of metabolic processes to fermentative growth [27]. However, very specific adaptations were also observed. These comprised down-regulation of the Agr (accessory gene regulator) system and the Agr-controlled PSM peptides, which as discussed above were recently attributed a key role in biofilm development [34]. In addition, there was increased expression of genes encoding the biosynthetic machinery for production of the protective surface polymer poly- $\gamma$ -glutamic acid PGA (see below), exemplifying that over-expression of protective factors not directly involved in aggregation may also contribute to the increased protective characteristics of biofilms.

#### 4.2. Poly- $\gamma$ -glutamic acid (PGA)

Poly- $\gamma$ -glutamic acid (PGA) is a linear homopolymer of glutamic acid residues that are linked via the  $\gamma$ -carboxy group of glutamic acid [97]. Thus, it is strictly speaking a peptoid. PGA is found in many microorganisms, in particular in halophilic bacteria, where it is assumed to play a role in osmoprotection [98]. Until its discovery in *S. epidermidis* in 2005 [99], *Bacillus anthracis* was the only pathogenic organism in which PGA was attributed a role in immune evasion, namely in protecting from phagocytosis [100].

In *S. epidermidis*, PGA is composed of a roughly equal amount of D- and L-glutamic acid, while in *B. anthracis*, PGA is composed entirely of D-glutamic acid residues [99]. However, all forms of PGA (100% D-glutamic acid, 100% L-glutamic acid, and mixed) are found in different organisms in nature. Using isogenic deletion mutants in the *cap* locus of *S. epidermidis*, which encodes the PGA biosynthesis genes and is similar to the *cap* locus of *B. anthracis*, PGA of *S. epidermidis* was shown to protect from AMPs, neutrophil killing, and virulence in a mouse model of device-related infection [99]. Of note, while PIA and TA exhibit at least part of their role in protecting from host defenses via their contribution to biofilm formation, PGA did not have a detectable role in biofilm formation [99].

#### 4.3 Toxins in *S. epidermidis*

*S. epidermidis* is a bacterial species that is commonly described as relatively innocuous, which is in part due to the notion that it lacks secreted toxins – in stark contrast to its aggressive cousin *S. aureus*, whose virulence is based on a large repertoire of secreted molecules that are toxic to humans, such as  $\alpha$ -toxin, enterotoxins, and a series of leukocidins [101]. There are reports that describe the sporadic occurrence of toxic shock syndrome toxin (TSST) and enterotoxins in CNS including *S. epidermidis* [102,103]. Recently, a pathogenicity island, termed SePI, has been found and sequenced in one clinical *S. epidermidis* strain [104]. This island contains staphylococcal enterotoxin C3 (SEC3) and staphylococcal enterotoxin-like toxin L (SEIL). However, the production of such toxins by *S. epidermidis* has to be regarded as exceptional and the acquisition of toxin gene-harboring MGEs, likely from *S. aureus*, as extremely rare. Possibly, this is due to the presence of so-called CRISPR sequences that appear to be common to *S. epidermidis* while they are absent from *S. aureus* [105]. These genetic elements provide a system that allows distinguishing between self and non-self DNA and the destruction of intruding foreign DNA sequences, such as phages. The mechanism of CRISPR interference has been studied intensively using *S. epidermidis* as a model organism [105].

**4.3.1 Phenol-soluble modulins (PSMs)**—Recently, the notion that *S. epidermidis* and other CNS are virtually toxin-free had to be somewhat revised with the identification and characterization of the PSMs, a family of genome-encoded peptides with frequently cytolytic character. The term phenol-soluble modulins was coined by the group of S. Klebanoff in 1999, when they described a peptide “complex” with several pro-inflammatory activities that was purified from *S. epidermidis* culture filtrate using hot

phenol extraction [106]. The PSM “complex”, which was then shown to comprise three components,  $\delta$ -toxin and the newly described PSM $\alpha$  and PSM $\beta$ , activated the HIV1-long terminal repeat (LTR), induced cytokine release, activated nuclear factor B in cells of macrophage lineage [106], primed neutrophils and activated the neutrophil oxidative burst [107]. The same group also reported the pro-inflammatory activities of the PSM “complex” to be mediated via the Toll-like receptor 2 (TLR2) [108]. However, this was never substantiated using synthetic peptides or isogenic deletion mutants of *psm* genes, leaving the possibility that TLR2 induction may have been due, at least in part, to co-purified pro-inflammatory impurities. This is even more likely given the recent identification of the formyl peptide receptor 2 (FPR2) as a receptor that binds PSMs and triggers inflammatory responses to PSMs [109]. It still needs to be investigated whether TLR2 is involved in PSM pro-inflammatory activities using *psm* deletion mutants and pure PSM peptides.

Members of the PSM peptide family show distinct physico-chemical characteristics [110–112]. All PSMs contain an amphipathic  $\alpha$ -helix that confers surfactant-like properties. The shorter  $\alpha$ -type PSMs are about 20–25 amino acids in length with the amphipathic  $\alpha$ -helix stretching over the entire peptide.  $\beta$ -type PSMs are about double in size with the C-terminal part containing the  $\alpha$ -helix. These characteristics also cause an unusual behavior of PSMs during reversed-phase chromatography compared to all other proteins found in culture filtrate, namely elution at extremely high percentages of organic solvent. This, together with the fact that concentrations of PSMs in culture filtrates are commonly very high, allowed the relatively easy detection of PSMs in several CNS [111]. In *S. epidermidis*, all genetic loci coding for PSMs detected by reversed-phase HPLC/mass spectrometry have been identified either using the detected masses or further purification and N-terminal amino acid sequencing [27,106,113] (Fig. 2). In addition to the 3 PSMs described by Mehlin et al., *S. epidermidis* was found to contain 3 additional PSMs: 2  $\alpha$ -type PSMs (PSM $\delta$ , PSM $\epsilon$ ) and an additional  $\beta$ -type PSM, which is now called PSM $\beta$ 1, while the original PSM $\beta$  is now called PSM $\beta$ 2. The *S. epidermidis* PSMs are encoded in 4 different genetic loci on the *S. epidermidis* chromosome: The genes coding for PSM $\alpha$  and PSM $\delta$  form an operon as do those coding for PSM $\beta$ 1 and PSM $\beta$ 2. Interestingly, the *psm* $\beta$  locus also contains a gene for an additional  $\beta$ -type PSM, which however was never found to be produced [110]. Furthermore, the gene coding for PSM $\beta$ 1 is found in 2 copies in some *S. epidermidis* strains [34]. The  $\delta$ -toxin (gene name *hld*) is encoded within RNAIII, the regulatory RNA of the Agr quorum-sensing system, similar to *S. aureus* and other staphylococci. The gene coding for PSM $\epsilon$  is not linked to another *psm* gene. Of note, all PSMs are under strict regulation by Agr [113]. In addition to the aforementioned PSMs, a PSM, PSM-mec, was recently discovered on the staphylococcal cassette chromosomes (SCC) mec of types II, III, and VIII, which contain the genes responsible for resistance to methicillin [73]. As many clinical *S. epidermidis* isolates are MRSE and belong to one of those SCCmec types, PSM-mec production is frequent among *S. epidermidis* strains isolated from infection. PSM-mec significantly contributes to virulence in *S. aureus* strains that show relatively high production of that peptide [73]. The role of PSM-mec in virulence of *S. epidermidis* has not yet been investigated directly.

The PSM pattern is characteristic for a given staphylococcal species, with different species producing different PSM peptides and only occasionally occurring significant amino acid sequence similarity between PSMs from different species. For example, the  $\delta$ -toxin of *S. epidermidis* is only different from that of *S. aureus* in 2 amino acids [114], suggesting a common ancestor. Similarity is also found among the PSM $\beta$  peptides of different species. PSM $\beta$  peptides were frequently annotated in staphylococcal genomes, including those of *S. epidermidis* that have been sequenced [115,116], whereas the shorter  $\alpha$ -type PSMs usually missed the threshold for minimal length of open reading frames. Of note, PSMs lack a signal



peptide and are therefore secreted as the primary translation product, harboring an N-terminal N-formyl methionine. The mechanism of PSM secretion is unknown.

The earlier reports on *S. epidermidis* PSMs passed without much appreciation by the staphylococcal research community, possibly because the pro-inflammatory capacities of PSMs were not further substantiated using deletion mutants and there was no evaluation of the impact that PSMs have on staphylococcal infection. This changed when PSM $\alpha$  peptides were discovered in *S. aureus* and recognized as key contributors to virulence in bacteremia and skin infection caused by community-associated methicillin-resistant *S. aureus* (CA-MRSA) [112]. In particular, *S. aureus* PSM $\alpha$  peptides were demonstrated to exhibit a strong capacity to lyse human neutrophils and other cell types. Mechanistically, there is evidence indicating that the cytolytic capacities of PSMs are not receptor-mediated, but likely caused by their surfactant characteristics [109].

The capacities of *S. epidermidis* PSM peptides to lyse neutrophils and erythrocytes have only recently been analyzed [110]. Similar to *S. aureus* PSM $\beta$  peptides, the PSM $\beta$  peptides of *S. epidermidis* are not cytolytic, indicating that the lack of cytolytic capacity may be a common feature of the  $\beta$ -type class of PSMs. Most  $\alpha$ -type PSMs of *S. epidermidis* have low to moderate cytolytic activities with the notable exception of PSM $\delta$ , which reaches a cytolytic capacity comparable to that of the strongly cytolytic *S. aureus* PSM $\alpha$ 3 [110].

The discovery that PSM $\delta$  is strongly cytolytic to human neutrophils and erythrocytes is of special importance, as it represents the first strongly potent toxin to be discovered in *S. epidermidis* that is conserved in all known members of the species. However, so far, PSM $\delta$  was found to be produced only at relatively low levels [110], in accordance with the commonly low overall aggressiveness of *S. epidermidis* as a colonizer and opportunistic pathogen. Possibly, the low production of cytolytic PSMs in *S. epidermidis* is linked to the much lower resistance of *S. epidermidis* compared to *S. aureus* against killing by human neutrophils. A detailed investigation of the contribution of *S. epidermidis* PSMs to infection has not yet been performed, mostly due to the fact that isogenic deletion mutants are difficult to produce in clinical *S. epidermidis* strains and have not yet been constructed except for the *psm $\beta$*  genes.

#### 4.4. Exoenzymes

Several enzymes that *S. epidermidis* secretes have been implicated in virulence. *S. epidermidis* produces a series of secreted proteases, all of which may contribute to virulence via the destruction of host tissue and host proteins. Specific mechanisms could be attributed to SepA, which degrades human AMPs [117], and Esp, which degrades fibrinogen and complement factor C5 [118]. Esp may also contribute to interspecies interference during colonization (see below) [119]. The roles that secreted lipase plays are poorly understood [120,121]. Finally, *S. epidermidis* secretes a fatty acid-modifying enzyme activity (FAME), which detoxifies fatty acids that are harmful to bacteria [122,120,121]. The proteins or genes responsible for FAME activity are not known.

#### 4.5 Competition with *S. aureus*

It has been speculated for a long time that colonization with *S. epidermidis* prevents overgrowth of the more aggressive *S. aureus* and thus, *S. aureus* infection, which is linked to *S. aureus* colonization [123]. However, evidence for such interference was not available. When cross-interfering quorum-sensing pheromones were discovered in staphylococci [124], notably including *S. epidermidis* pheromones that inhibited quorum-sensing activity of *S. aureus* [125], it was believed that quorum-sensing cross-inhibition could be the source

for a potential *S. epidermidis*/*S. aureus* interference. However, this could not be verified in vivo [126].

Recently, Iwase et al. reported that *S. epidermidis* strains that express a certain secreted protease prevent nasal colonization with *S. aureus* [119]. In contrast, *S. epidermidis* lacking expression of that previously well characterized protease [118], termed Esp, do not interfere with *S. aureus* in vivo. The underlying mechanism was described as protease-dependent destruction of biofilms. While Esp showed such activity in vitro, it is debatable whether the observed in vivo phenomenon of interspecies competition is due to that mechanism [127]. Nevertheless, this study clearly demonstrated that interspecies competition between *S. aureus* and *S. epidermidis* exists in the nares and is, at least in part, responsible for the observed individual differences in nasal colonization with *S. aureus*.

#### 4.6 Are there virulence factors in *S. epidermidis* that distinguish invasive from commensal strains?

It has been noted that the aggressiveness of *S. aureus* and the relatively benign relationship that *S. epidermidis* has with its host allows the two organisms to survive and spread, each by using a different approach [128]. The *S. epidermidis* approach would consist of a series of “passive” mechanisms to evade host defenses, such as the production of protective matrix polymers, without the production of aggressive toxins. This notion has been largely confirmed with the recent more thorough investigation of *S. epidermidis* virulence mechanisms. Interestingly, despite the discovery of PSMs as a series of novel potentially aggressive toxins in *S. epidermidis*, a detailed look at PSM expression in *S. epidermidis* confirmed rather than negated that notion: the expression pattern of PSM peptides is clearly shifted, compared to *S. aureus*, to those that lack cytolytic activity, such as the PSM $\beta$  peptides [110].

It has become clear that no virulence mechanism exists in *S. epidermidis* that clearly distinguishes invasive from colonizing strains. In a study using comparative genomic hybridization, a genome-wide comparison of strains isolated from infections of prostheses versus strains from healthy individuals, no factor could be identified that was solely present in infectious strains, but absent from control strains [129]. Nevertheless, several studies confirmed the in average more frequent presence of the *ica* genes encoding PIA synthesis and the insertion element IS256 in invasive strains [130–133]. These studies underscored the importance of PIA in infection and suggested that IS256 may contribute to genetic adaptations allowing optimization of gene content and expression to an infectious lifestyle. Insertion of IS256 was shown for example to occur in the *ica* operon, abolishing PIA synthesis [134]. Furthermore, a study with human volunteers indicated that *ica*-positive strains are less adapted to colonization [135]. Moreover, strains belonging to ST2, the by far most frequently found clonal type of hospital-associated invasive *S. epidermidis*, are all *ica*- and IS256-positive [132,136]. Then again, it has also been pointed out that when using higher stringency controls, *ica* and other virulence genes are not correlated with invasiveness [137]. Taken together, these studies indicate that there are no genetic markers that can be used to clearly predict the aggressiveness of *S. epidermidis* strains. In further support of that notion, the detailed investigation of virulence mechanisms of *S. epidermidis* in recent years has indicated that most if not all virulence factors in that bacterium have original roles in its commensal lifestyle. Likely, PSMs, PIA and other biofilm factors all have such original roles in establishing growth and allowing survival in microbial agglomerations on the skin [138].

As a consequence, any therapeutic directed against *S. epidermidis* virulence factors would also target commensal strains. Any such therapeutic would have to be evaluated for its impact on *S. epidermidis* colonization and its role in controlling the composition of the

human microflora, especially in light of the recent findings regarding *S. epidermidis*-*S. aureus* interference.

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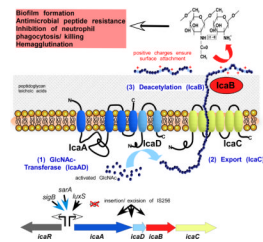
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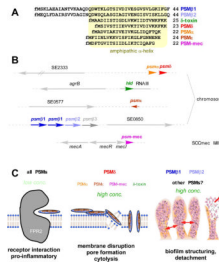
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**Fig. 1. The exopolysaccharide PIA/PNAG**

PIA/PNAG is a partially de-acetylated homopolymer of N-acetylglucosamine residues in  $\beta$ 1–6 linkage (top right). It is synthesized by the gene products of the *ica* operon (bottom). Transcription of *ica* is under control of multiple global regulators and other regulatory influences (but not Agr). It also may be inactivated by insertion of IS256. IcaA and IcaD form a glucosaminyltransferase. IcaC likely functions as exporter of the growing PIA chain. IcaB de-acetylates pre-PIA at the cell surface after PIA export. Positive charges introduced by deacetylation are crucial for surface location and the multiple functions of PIA shown at the top left.



**Fig. 2. Phenol-soluble modulins of *S. epidermidis***

A, Amino acid sequences of all *S. epidermidis* PSMs. PSMs are exported as the primary translation products with N-terminal N-formyl-methionine (fM). B, Genetic location of *psm* genes in the chromosome or in the case of *psm-mec*, on SCCmec elements. Gene numbers are according to the genome of ATCC12228. C, Roles of PSMs in inflammation, cytolysis, and biofilm development. All PSMs trigger inflammatory responses by interaction with the FPR2 receptor. PSM $\delta$  and to some extent, some other PSMs, function as cytolysins. PSM $\beta$  peptides and possibly other PSMs contribute to biofilm structuring and detachment.

Tab. 1

Virulence factors of *S. epidermidis*

Virulence factor	Gene	Function
<b>Biofilm formation</b>		
<u>Primary attachment</u>		
AtlE	<i>atlE</i>	Bifunctional autolysin/adhesin
Aae	<i>aae</i>	Bifunctional autolysin/adhesin
Teichoic acids	(multiple biosynthetic genes)	Attachment (shown in <i>S. aureus</i> )
SdrF	<i>sdrF</i>	Binds to collagen
SdrG (Fbe)	<i>sdrG</i>	Binds to fibrinogen
SdrH	<i>sdrH</i>	Putative binding function only
Ebp	<i>ebp</i>	Binds to elastin
Embp	<i>embp</i>	Binds to fibronectin
AtlE/Aae	<i>atlE/aae</i>	Bind to multiple matrix proteins
Aap	<i>aap</i>	Binds to comeocytes
<u>Intercellular aggregation</u>		
PNAG/PIA	<i>icaADBC</i>	Intercellular polysaccharide adhesin
Bap	<i>bap</i>	Intercellular protein adhesin
Aap	<i>aap</i>	Intercellular protein adhesin precursor (requires proteolytic processing for activity)
Embp	<i>embp</i>	Intercellular protein adhesin
Teichoic acids		Component of biofilm matrix
<u>Protective exopolymers</u>		
PNAG/PIA	<i>icaADBC</i>	Protects from IgG, AMPs, phagocytosis, complement
PGA	<i>capABCD</i>	Protects from AMPs, phagocytosis
<u>Resistance to AMPs</u>		
SepA protease	<i>sepA</i>	AMP degradation
Dlt, MprF, VraFG	<i>dltABCD</i> <i>mprF</i> <i>vraFG</i>	In analogy to <i>S. aureus</i> : D-alanylation of teichoic acids (Dlt), lysislation of phospholipids (MprF), putative AMP export (VraFG)
Aps system	<i>apsR (graR), apsS (graS), apsX</i>	AMP sensor, regulator of AMP resistance mechanisms
<b>Toxins</b>		
<u>Enterotoxins</u>		
SEC3, SE1L	<i>sec3, sell</i>	Pathogenicity island-located enterotoxins
<u>Phenol-soluble modulins (PSMs)</u>		
PSM $\alpha$	<i>psm <math>\alpha</math></i>	Cytolysin (moderate activity), pro-inflammatory
PSM $\beta$ 1, PSM $\beta$ 2	<i>psm<math>\beta</math>1, psm<math>\beta</math>2</i>	Biofilm-structuring surfactants, pro-inflammatory
PSM $\delta$	<i>psm <math>\delta</math></i>	Cytolysin (strong activity), pro-inflammatory
PSM $\epsilon$	<i>psm <math>\epsilon</math></i>	Cytolysin (moderate activity), pro-inflammatory
$\delta$ -toxin	<i>hld</i>	Cytolysin (moderate activity), pro-inflammatory
PSM-mec	<i>psm-mec</i>	Cytolysin (moderate activity), pro-inflammatory
<b>Exoenzymes</b>		
<u>Proteases</u>		
Cysteine protease (SspB, Ecp)	<i>sspB</i>	Unknown, tissue damage?

<b>Virulence factor</b>	<b>Gene</b>	<b>Function</b>
Metalloprotease/elastase (SepA)	<i>sepA</i>	Tissue damage?, AMP resistance
Glutamylendopeptidase (GluSE, SspA, Esp)	<i>sspA</i>	Degradation of fibrinogen and complement factor C5, competition with <i>S. aureus</i> (by proteolysis of biofilm matrix proteins?)
Lipases GehC, GehD	<i>gehC, gehD</i>	unknown
<b>Others</b>		
Fatty acid modifying enzyme unidentified (FAME)		Detoxification of bactericidal fatty acids