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Mini Review

Phenol-soluble modulins

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ABSTRACT

PSMs are a recently discovered family of short, amphipathic, α-helical peptides in staphylococci. Several PSMs are key virulence determinants, particularly in highly virulent *Staphylococcus aureus* strains. PSMα peptides of *S. aureus* facilitate neutrophil lysis after phagocytosis, and are key contributors to several infection types, including skin infection and bacteremia. Furthermore, all PSMs contribute to biofilm structuring and the dissemination of biofilm-associated infection. Cytolytic PSMs as produced by *S. aureus* appear to have evolved from original functions in the non-infectious lifestyle of staphylococci. The surfactant properties of PSMs, which they all share, are believed to facilitate growth on epithelial surfaces. The basic role of PSMs in staphylococcal physiology is underscored, for example, by their exceptionally strict and direct control by quorum-sensing and the presence of a dedicated secretion system. Targeting PSMs for anti-staphylococcal drug development may be a promising approach to overcome the problems associated with widespread antibiotic resistance in staphylococci.

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Introduction

Phenol-soluble modulins (PSMs), a family of amphipathic, α-helical peptides found in staphylococci, have recently drawn much attention owing to the key contribution of some PSM peptides to staphylococcal virulence, in particular in highly virulent *Staphylococcus aureus* (Wang et al., 2007). However, it is often overlooked that only some PSMs have aggressive properties that make them virulence determinants. Many PSMs, especially those found in less pathogenic species, appear to have different roles in staphylococcal physiology (Periasamy et al., 2012a), which is why in order to understand the evolution of PSMs, it is crucial to consider both commensal and pathogenic species, or lifestyles, of staphylococci.

Staphylococci are commensals on the epithelia of humans and mammals (Kloos and Musselwhite, 1975). While there is a certain specification among staphylococci regarding which niches on the human body they prefer, all have to deal with the challenges that colonization of surfaces with frequently changing environmental conditions entails. In addition to having to be able to endure high osmotic stress and changing temperatures, staphylococci have to live and acquire nutrients in an environment that contains a high number of hydrophobic molecules, such as lipids and waxes. This is especially true for life in and around sebaceous glands or hair follicles, which are preferred niches for many staphylococci (Grice et al., 2009). There are reports on staphylococci expressing enzymes that detoxify harmful fatty acids; and many staphylococci secrete lipases, which may have a function in degrading lipids for nutrient acquisition (Otto, 2004). However, how staphylococci manage to live in such an environment with the specific challenges regarding aqueous/oily interfaces is poorly understood.

For many opportunistic pathogens among the staphylococci, such as *S. epidermidis* and others, infection may be regarded as an "accident" rather than a program (Otto, 2009). Many molecules that these species produce may rise to additional benefit during infection, but judging from the fact that they usually have additional, or rather original, roles in their commensal lifestyles, they appear not to have evolved for a role in pathogenesis. Examples are the polyglutamate capsule of *S. epidermidis* (Kocianova et al., 2005) or surface proteins needed to attach to epithelial surfaces (Bowden et al., 2005). In contrast, *S. aureus* produces a large series of molecules whose production is directly related to infection (Foster, 2005; Kim et al., 2012; Lowy, 1998). Most of these subvert mechanisms of host defense. It is poorly understood why *S. aureus* is largely immune to elimination by antibody-mediated mechanism of the acquired immune system. In contrast, many mechanisms are known by which *S. aureus* escapes the innate immune system, including complement and phagocytosis by leukocytes (Rooijakkers et al, 2005).

Here, the genetics, biochemistry, and roles of PSMs in the commensal and infectious lifestyles of staphylococci will be reviewed. It will also be discussed whether and how PSMs could be targeted for anti-staphylococcal drug development.

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PSMs are widespread in staphylococci

The term "phenol-soluble modulin" was coined by the group of Seymour Klebanoff. This group isolated what they described as a complex of three peptides from *S. epidermidis* culture filtrate by hot phenol extraction (Mehlin et al., 1999). The peptides were named PSMα, PSMβ, and PSMγ, with PSMγ being identical to the long-known δ-toxin (McKevitt et al., 1990). Afterwards, the PSM composition of *S. epidermidis* and then *S. aureus* was analyzed more systematically, using the exceptionally late elution behavior of PSMs on reversed-phase columns as an analytical and preparative tool (Vuong et al., 2004a; Wang et al., 2007; Yao et al., 2005). PSMs can be grouped into the smaller (~20–25 amino acids) α-type PSMs and the longer (~44 amino acids) β-type PSMs (Fig. 1).

As many *psm* genes are smaller than the open reading frame cut-off length used in most genome annotations, similarity searches such as by BlastP or BlastN do not reliably find *psm* genes. This approach has worked only to identify the somewhat larger β genes; and many genes encoding PSMβ-like peptides were found in the genomes of recently sequenced coagulase-negative staphylococci. These PSMβ-like peptides from different staphylococcal species show relatively strong similarity. Unfortunately, they were not always annotated as PSMβ peptides. Recently, they received a pfam group identifier: pfam05480 (*Staphylococcus* haemolytic protein). In contrast, for α-type PSMs, isolation of the PSM in question, N-terminal sequencing to obtain the amino acid sequence, and search of available whole-genome databases to identify the *psm* gene(s) was required in most cases. Using a combination of these approaches, it was found that *S. aureus* produces 7 PSMs named PSMα1-α4, PSMβ1 and PSMβ2, and the *S. aureus* δ-toxin (Wang et al., 2007) (Fig. 1). *S. epidermidis* also produces 7 PSMs, named PSMα, PSMβ1 and PSMβ2, PSMδ, PSMε, and the *S. epidermidis* δ-toxin (McKevitt et al., 1990; Mehlin et al., 1999; Vuong et al., 2004a; Yao et al., 2005). While all these PSMs are core genome-encoded, specific staphylococcal cassette chromosome (SCC)*mec* elements, which carry methicillin resistance genes, encode a PSM that was termed PSM-mec (Queck et al., 2009). It is found in SCC*mec* types II, III, and VIII, which are present in many MRSA and MRSE (methicillin-resistant *S. aureus* and *S. epidermidis*, respectively) strains (Chatterjee et al., 2011). The *psm-mec* gene also encodes a regulatory RNA that works via inhibition of translation of AgrA (Kaito et al., 2013). Notably, PSMs are different in different species, even when they carry the same name (such as for example *S. epidermidis* versus *S. aureus* PSMβ1). It is thus best to always identify a PSM peptide by the producing species in addition to its name.

In other staphylococcal species, *psm* gene sequences have not been systematically searched for. Some peptides that were described before the term PSM was coined are now known to belong to the PSM family, such as the PSMβ-like SLUSH peptides from *Staphylococcus lugdunensis* or the gonococcal growth inhibitor (GGI) peptides from *Staphylococcus haemolyticus* (Donvito et al., 1997; Frenette et al., 1984). Analysis of the culture filtrates of a series of staphylococcal species by reversed-phase high-pressure chromatography/mass spectrometry (RP-HPLC/MS), which is the optimal method to measure PSM production, showed that many staphylococci produce PSMs (Rautenberg et al., 2011). However, only the masses of these peptides were identified; further analyses to obtain amino acid sequences and *psm* genes, as performed in *S. aureus* and *S. epidermidis*, have not yet been undertaken. Based on these findings, the sequence similarity-based discovery of PSMβ-like peptides in virtually every sequenced staphylococcal genome, and the recent discovery of a PSM-specific secretion system that is present in all staphylococcal genomes sequenced so far, it is evident that most staphylococcal species produce a species-specific repertoire of PSMs (Chatterjee et al., 2013).

The "original" role of PSMs in surface colonization

With the focus of research on staphylococci being pathogenesis, it is understandable that in general as well as specifically for the roles of PSMs, our knowledge on the commensal lifestyle of staphylococci is relatively poor. However, several observations support an evolutionarily old and conserved role of PSMs in staphylococcal surface colonization (Fig. 2). First, all PSMs have a pronounced amphipathic α-helical structure (Cheung et al., 2010; Wang et al., 2007), optimally suited to produce surfactant-like characteristics that facilitate growth in environments with oil/water interfaces, such as on the skin. As is necessary for this basic physiological role, PSMs are produced in extraordinarily high amounts. In *S. aureus*, it was demonstrated that they represent more than half of the protein mass secreted into the media (Chatterjee et al., 2013). Second, PSMs and their dedicated secretion system are encoded on the core genome, their regulation by Agr is exceptionally direct (Queck et al., 2008) (see below), and they appear in most species (Rautenberg et al., 2011). This clearly contrasts many other toxins, which often are encoded on mobile genetic elements (MGEs) and are highly specific for certain strains and species (Novick et al., 2001). Third, there is experimental evidence indicating that PSMs facilitate spreading on surfaces (Tsompanidou et al., 2013), owing to their surfactant properties, which is one example underlining their assumed role in epithelial colonization (Fig. 2). Fourth, PSMs contribute to biofilm development, a phenotype believed to be crucial for staphylococcal colonization (Periasamy et al., 2012b; Schwartz et al., 2012; Wang et al., 2011) (Fig. 2). Thus, the toxic functions of PSMs that most research has focused on so far appear to have evolved from an original role of PSMs in the non-infectious lifestyle of staphylococci.

PSMs in pathogenesis

Cytolysis. Cytolysis by PSMs most likely occurs in a non-specific, receptor-independent manner (Fig. 2). This is supported by the fact that the PSM receptor FPR2 that mediates PSM pro-inflammatory PSM activities (see below) is not necessary for cytolysis (Kretschmer et al., 2010). In a way similar to what has been described for δ-toxin (Talbot et al., 2001), other cytolytic PSMs are also assumed to destroy membrane integrity by initial membrane attachment and membrane perturbation at high peptide density. Cytolytic properties of PSMs were analyzed mainly for human neutrophils and sheep or human erythrocytes (Cheung et al., 2012; Wang et al., 2007). *S. aureus* PSMα3 and *S. epidermidis* PSMδ have exceptionally high capacities to lyse human neutrophils (Cheung et al., 2010; Wang et al., 2007). Interestingly, the genetic location encoding *S. epidermidis* PSMδ (and PSMα) corresponds to that of the *S. aureus psm*α operon, indicating that they have a common ancestor. This operon thus appears primarily responsible for the evolution of cytolytic PSMs. Furthermore, in case of the *S. aureus psm*α operon, the evolution of PSMs toward cytolytic functions can be followed, as among the four PSMα peptides of *S. aureus*, which are quite similar to each other and apparently arose from gene duplication events, only one, PSMα3, has high cytolytic activity, whereas the others are only moderately (PSMα1, PSMα2) or not cytolytic (PSMα4). The unrelated *S. epidermidis* PSMε, and the two δ-toxins of *S. aureus* and *S. epidermidis* have moderate cytolytic capacities toward human neutrophils. Other PSMs in *S. aureus* and *S. epidermidis*, most notably all PSMβ peptides, are barely or not cytolytic to that cell type (Cheung et al., 2010; Wang et al., 2007).

Highly virulent *S. aureus*, such as community-associated methicillin-resistant *S. aureus* (CA-MRSA) strains, have an extraordinary capacity to escape elimination by the innate immune system by leading to neutrophil killing after phagocytosis (Voyich et al., 2005). Notably, this phenotype is believed to be mainly