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Phenol-soluble modulins in staphylococci

What are they originally for?

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Phenol-soluble modulins (PSMs) are amphipathic peptides produced by staphylococci that have multiple functions in pathogenesis. For example, they may function as cytotoxins and pro-inflammatory agents. Additionally, in a recent study we demonstrated that *Staphylococcus aureus* PSMs structure biofilms and cause dissemination during biofilm infection. Based on those results suggesting a surfactant-like mechanism by which PSMs work, we here propose that all PSM functions in pathogenesis arose from an original role in non-pathogenic surface colonization. This original role may have included overcoming surface tension in environments of strongly varying hydrophobicity and emulsification of hydrophobic molecules for use as food sources.

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Phenol-soluble modulins (PSMs) are a family of amphipathic, α-helical peptides that comprise the long-known staphylococcal δ–toxin.[1] PSMs are present in virtually all staphylococci, particularly those that are pathogenic.[2] The name was coined in 1999, when the group of Seymour Klebanoff isolated a pro-inflammatory complex, containing at least three peptides, from the phenol-soluble fraction of *Staphylococcus epidermidis* culture filtrate.[3] Since then, our group has used molecular methodology to identify all PSMs present in *S. aureus* and *S. epidermidis* on the gene and protein level.[4-7]

Within the past 5 y, we demonstrated that PSMs have a variety of biological functions that are crucial to staphylococcal pathogenesis. Some PSMs, such as the PSMα group of *S. aureus* or the PSMα peptide of *S. epidermidis*, efficiently lyse white and red blood cells.[6,8] Consequently, deletion mutants of the *psm*α operon in *S. aureus* are severely attenuated in animal infection models, indicating a central role of PSM peptides in staphylococcal virulence.[6] Furthermore, all PSMs trigger inflammatory responses, such as chemotaxis and priming of human neutrophils, and induction of cytokine expression.[4,6,8] We recently showed that these responses are mediated via activation of the formyl peptide receptor 2.[9] Finally, PSMs may act as weapons in inter-bacterial warfare.[10,11]

Additionally, we showed that the PSMs peptides of *S. epidermidis* contribute to the structuring of biofilms and the dissemination of biofilm-associated infections.[12] Biofilms, sticky agglomerations of microorganisms, represent a key virulence factor in staphylococcal infection, owing to the significantly increased resistance they provide to antibiotics and host defenses.[13] They commonly form on contaminated indwelling medical devices such as catheters. Now, in a recent publication in the *Proceedings of the National Academy of Sciences*, we analyzed the contribution of all PSMs of *S. aureus* to biofilm structuring and detachment/dissemination processes in vitro and in vivo in molecular detail.[14] Notably, these studies were performed using isolates of methicillin-resistant *S. aureus* of high clinical importance. Our studies revealed that all *S. aureus* PSMs are involved in biofilm structuring processes. Apparently, the biofilm structuring mechanism is due to the common amphipathic characteristics of PSMs, which gives them surfactant-like features able to disrupt cellular interactions

within biofilms, thereby loosening up the sticky biofilm agglomerations and introducing channels in the biofilm structure. Such channels are vital components of biofilms, as they enable nutrients to be delivered to deeper biofilm layers, keeping all cells in the biofilm alive. Importantly, non-uniform secretion of PSMs among biofilm cells is an obvious prerequisite for that mechanism; and we showed that it is a consequence of varying activity of the quorum-sensing system Agr that strictly controls PSM expression.[15] Upon strong production of PSMs at a given location in the biofilm, channels form; when this happens at a high rate, entire biofilm clusters may detach. In vivo, this process leads to the dissemination of infection to other sites in the body.

Staphylococci are first and foremost commensal organisms, colonizing different sites of the human body with species-specific predominance.[16] For example, *S. aureus* is a colonizer of the nares in about one-fourth of the population, while *S. epidermidis* is found in all individuals on many body sites, in particular the axillae (armpits), head and nares.[17] Infection with *S. epidermidis* is considered an "accident" rather than a programmed pathogenesis event involving dedicated virulence factors.[17] It is believed that molecules that form an important part of the organism's commensal life on epithelia rise to additional benefit during infection. The same is likely the case for many other opportunistic staphylococcal pathogens, and in principle comparable to subacute and chronic infections with *S. aureus*.

At first glance, PSMs appear to form an exception to the fact that *S. epidermidis* in particular does not produce dedicated virulence factors. However, the widespread occurrence of PSMs among staphylococci and the fact that *psm* genes form part of staphylococcal core genomes indicate that there is an "original" function of PSMs not directly related to virulence. Now, our discovery of the roles of PSMs in biofilm formation may give a hint to that function, inasmuch as the surfactant features enabling biofilm structuring may also be crucial for colonization. Colonization of epithelial surfaces requires dealing with strongly varying physico-chemical environments, ranging from the hydrophobic compounds secreted by sebaceous glands to the aqueous environment in nasal secretions. The production of surfactant molecules in high amounts appears to be perfectly suited to overcome the extreme surface tensions present in such environments, enabling the bacteria to colonize surfaces and emulsify otherwise immiscible molecules to use them as food sources. Notably, PSMs are secreted in extremely high amounts; making them by far the most abundant proteins in staphylococcal culture filtrates. Moreover, the facts that (1) all staphylococcal species are excellent colonizers of mammalian epithelial surfaces, setting them apart from other bacterial genera and (2) PSM production is limited to the genus *Staphylococcus*, further indicate a potential causal link between PSM production and the capacity to colonize epithelia. Thus, we here propose that the multiple functions of PSMs in acute and chronic virulence are derived from an original role of PSMs in the nonpathogenic colonization of mammalian epithelia. We believe that during the evolution of pathogenicity, the amphipathic α-helical structure that is the structural basis of PSM surfactant features also gave rise to the diverse functions of PSMs in virulence. For that purpose, adaptations of the common PSM structural features were likely necessary, for example to efficiently integrate into and lyse mammalian cytoplasmic membranes. While, owing to the lack of appropriate skin colonization models, this general hypothesis is difficult to prove experimentally, the structural adaptations that are linked to specific PSM functions are currently being investigated in our laboratory. Finally, the question remains why other bacteria have not developed such a structurally simple solution as the PSMs to the challenging tasks of surface colonization and survival in the human body. The answer lies probably in the problem of PSM export, which is also under current investigation and may be dependent on the co-evolution of a dedicated PSM secretion system.

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