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Cell–Cell Communication and Biofilm Formation in Gram-Positive Bacteria

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1.1
Introduction

It is now widely accepted that naturally, bacteria prefer to live in surface-associated communities called biofilms. In the biofilms, the bacteria are embedded in an extracellular polymeric matrix, and are protected against environmental stresses, antimicrobial treatment, and the host immune system. Biofilms have been implicated in a variety of human infections, such as endocarditis, osteomyelitis, chronic otitis media, foreign-body-associated infections, gastrointestinal ulcers, urinary tract infections, chronic lung infections in cystic fibrosis patients, caries, and periodontitis [1]. The causative agents of biofilm-associated infections are different Gram-positive species of *Staphylococcus*, *Streptococcus*, and *Enterococcus* as well as Gram-negative bacteria, such as *Pseudomonas aeruginosa*, *Escherichia coli*, and *Actinobacillus actinomycetemcomitans*.

Within the biofilm community, bacteria communicate with each other by using chemical signal molecules in response to population density in a process that is called quorum sensing (QS; reviewed in [2]). The cell–cell communication via QS involves the production, release, detection, and response to small hormone-like molecules termed pheromones or autoinducers (AIs). During growth, bacteria produce the AIs, which activate the QS system upon reaching a threshold concentration. Three different types of AIs are currently known: *N*-acyl-homoserine lactones that are mainly used by Gram-negative bacteria and secreted cyclic oligopeptides with a thiolactone structure that are preferred by Gram-positive bacteria. LuxS/AI-2 are produced by both Gram-negative and Gram-positive bacteria, and are believed to function in interspecies communication [2].

Various of physiological activities are regulated via QS in Gram-positive bacteria, including biofilm formation in staphylococci, streptococci, and enterococci, expression of virulence factors in staphylococci, development of competence in streptococci, sporulation in *Bacillus*, and antibiotic biosynthesis in *Lactococcus lactis* [2].

Among the Gram-positive bacteria, biofilm formation and QS has been most intensely studied with staphylococci. In contrast to many biofilms found in natural environments, where a biofilm usually consists of a multispecies microbial community, infections due to staphylococci mostly, but not always, are monospe-

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cific [3]. The most important staphylococcal species involved in biofilm-associated infections are *Staphylococcus epidermidis* (primarily causing foreign-body-associated infections) and *Staphylococcus aureus* (typically causing infections associated with colonization of the host tissue).

1.2 Staphylococcal Infections and Biofilms

Staphylococci are ubiquitous commensals of the skin and mucous membranes of humans and animals. In humans, *S. aureus* and the coagulase-negative *S. epidermidis* are among the most leading causes of nosocomial infections [4]. Infections due to *S. epidermidis* typically are more subacute or even chronic and require a predisposed or immunocompromised host, such as patients with indwelling medical devices (e.g., prosthetic heart valves and joints, artificial pacemakers, and intravascular catheters) [5]. In contrast, *S. aureus* causes more acute infections associated with the colonization of the host tissue, such as endocarditis and osteomyelitis, which may lead to sepsis. However, *S. aureus* is also a common cause of foreign-body-associated infections and, occasionally, *S. epidermidis* may cause native valve endocarditis.

The most critical pathogenicity factor in these infections is the colonization of abiotic or biotic surfaces by the formation of a three-dimensional structure called a biofilm. The presence of large adherent biofilms on explanted intravascular catheters has been demonstrated by scanning electron microscopy [6]. Microorganisms within a biofilm are protected against antimicrobial chemotherapy as well as against the immune system of the host.

To form a biofilm, staphylococci first attach either to host tissue or to the surface of a medical device, and then proliferate and accumulate into multilayered cell clusters, which are embedded in an amorphous extracellular material that mainly is composed of *N*-acetyl-glucosamine, cell wall teichoic acids, DNA, and host products [7–9]. A mature biofilm contains fluid-filled channels that ensure the delivery of nutrients and oxygen to bacterial cells located deeper in the biofilm [1]. From a mature biofilm, individual cells or cell aggregates can detach. Upon detachment from the biofilm, the bacteria may disseminate via the blood stream, which is thought to lead to metastatic infection and/or development of sepsis. In the following, the molecular mechanisms involved in staphylococcal biofilm formation and detachment are summarized (Figure 1.1).

1.3 Molecular Basis of Biofilm Formation in Staphylococci

1.3.1 Attachment to Abiotic Surfaces

Microbial adherence to biomaterials largely depends on the nature of the polymer material and on the cell surface characteristics of the bacteria. The initial interactions

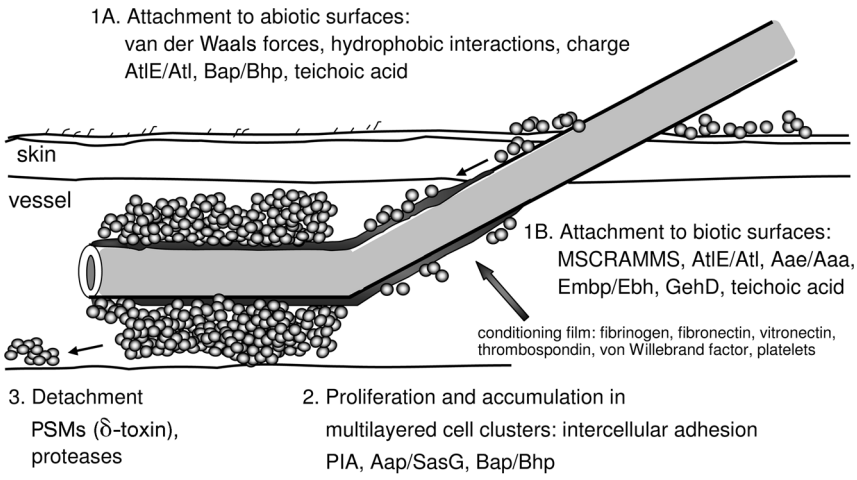

Figure 1.1 Model of different phases of staphylococcal biofilm formation and factors involved. Biofilms develop by initial attachment to surfaces, which may be abiotic (polymer surface) or biotic (polymer surface coated with extracellular matrix and plasma proteins or host tissue), and subsequent proliferation and accumulation into multilayered cell clusters, which requires intercellular adhesion. From a mature biofilm, cells or cell aggregates can detach and disseminate. The different phases and factors involved are indicated.

are believed to occur via nonspecific physicochemical forces such as charge, van der Waals forces, and hydrophobic interactions. The *S. aureus* colonization of abiotic surfaces depends on the charge of its teichoic acid. *S. aureus* teichoic acids are highly charged cell wall polymers, composed of alternating phosphate and ribitol (wall teichoic acids) or glycerol (lipoteichoic acids) groups, which are substituted with D-alanine and *N*-acetyl-glucosamine. A *dltA* mutant lacks D-alanine in its teichoic acid rendering it higher negatively charged. The *dltA* mutant has a biofilm-negative phenotype due to a decreased initial attachment to polystyrene or glass, which is hydrophobic or negatively charged, respectively [10].

Initial adherence has also been attributed to bacterial surface proteins. Using transposon mutagenesis, the autolysin AtlE of *S. epidermidis* O-47 was identified as a surface-associated component that mediates primary attachment of bacterial cells to a polystyrene surface [11]. The 148-kDa AtlE and the homologous autolysin Atl from *S. aureus* are proteolytically cleaved into two bacteriolytically active domains – an N-terminal amidase and a C-terminal glucosaminidase [11, 12]. In the central part of the proteins, there are three repetitive sequences, possibly involved in the adhesive function.

Another protein from *S. aureus*, the 239-kDa biofilm-associated protein Bap, is involved in attachment to a polystyrene surface and intercellular adhesion leading to biofilm formation [13]. The structural features of Bap correspond to those of other typical Gram-positive surface proteins, called MSCRAMMs (microbial surface components recognizing adhesive matrix molecules; see below). The clinical significance of Bap is not clear, because it is apparently present in only 5% of 350 bovine

mastitis and absent in all human clinical *S. aureus* isolates tested so far. However, a gene encoding a Bap-homologous protein, the 258-kDa Bhp, is present in the human clinical strain *S. epidermidis* RP62A [14].

1.3.2 Attachment to Biotic Surfaces

Implanted material rapidly becomes coated with plasma and extracellular matrix proteins, such as fibronectin, fibrinogen, vitronectin, thrombospondin, bone sialoprotein, collagen, and von Willebrand factor, or platelets. Thus, all these host factors could serve as specific receptors for colonizing bacteria [15, 16]. Moreover, *S. aureus* is especially able to directly adhere to host tissue, such as the host epithelium or endothelium. Staphylococcal host-factor-binding proteins typically belong to the MSCRAMM family [17]. MSCRAMMs have a common overall organization including an N-terminal signal peptide, an exposed ligand-binding domain, a characteristic cell-wall-spanning region that often contains repetitive sequences, and a C-terminal LPXTG motif responsible for covalent cell wall anchorage. While the *S. aureus* genomes contain a larger number of genes encoding MSCRAMMs (at least 20), there are only 12 genes in the *S. epidermidis* RP62A genome [18]. MSCRAMMs can bind to one or more host extracellular matrix and plasma protein, and include in *S. aureus* fibronectin-binding proteins (FnBpA, FnBpB), fibrinogen-binding proteins (clumping factors ClfA and ClfB), a collagen-binding protein (Cna), a bone-sialoprotein-binding protein (Bbp), and a von Willebrand factor-binding protein A (Spa) [17, 19–22]. However, not all the ligands of all MSCRAMMs have yet been identified. Less data on host factor-binding MSCRAMMs of *S. epidermidis* are available. The fibrinogen-binding 119-kDa Fbe and the almost identical 97-kDa SdrG show significant similarity to the ClfA of *S. aureus* [23].

Staphylococcal surface-associated proteins that are anchored to the cell surface by different means (noncovalently) include the giant 1.1-mDa fibronectin-binding protein Ebh of *S. aureus* and the homologous Embp of *S. epidermidis* [24, 25], whose fibronectin-binding sites seem to be unrelated to those of the *S. aureus* FnBPs, autolysins, the collagen-binding GehD lipase in *S. epidermidis* [14], and the elastin-binding protein EbpS [26]. Further examples of noncovalently associated surface proteins of *S. aureus* are two proteins with a broad binding spectrum, the extracellular matrix and plasma-binding protein Emp and the extracellular adherence protein Eap [27, 28]. Aside from proteins, the cell wall teichoic acid is involved in the adherence of *S. epidermidis* to fibronectin [29].

The autolysin AtlE from *S. epidermidis* not only mediates primary attachment to a polystyrene surface (see Section 1.3.1), but also binds vitronectin [11]. By using a catheter-associated infection model, an *in vivo* role for AtlE was suggested [30]. Further multifunctional autolysin/adhesins include the Aae from *S. epidermidis* and the homologous Aaa from *S. aureus*. Aae and Aaa have bacteriolytic activity, and bind to fibrinogen, fibronectin, and vitronectin in a dose-dependent and saturable fashion and with high affinity [31, 32].

1.3.3 Accumulation Process

After successful attachment to a surface, bacteria proliferate and accumulate in multilayered cell clusters, which requires intercellular adhesion. Probably the same mechanisms are involved in biofilm accumulation on biotic and abiotic surfaces. Staphylococcal biofilm accumulation can be mediated by polysaccharide as well as protein factors.

1.3.3.1 Polysaccharide-Associated Biofilm Accumulation

Transposon mutants not able to accumulate in multilayered cell clusters lack a specific polysaccharide antigen referred to as polysaccharide intercellular adhesin (PIA) [33, 34], which was later also designated as poly-*N*-acetyl-glucosamine (PNAG) [33, 35]. Purification and structural analysis of PIA revealed that it is a linear β-1–6-linked *N*-acetyl-glucosaminoglycan with 15–20% of the *N*-acetyl-glucosaminyl residues being non-*N*-acetylated [8]. Thus, the designation as PNAG is certainly not correct.

PIA is also produced by *S. aureus*. It has been reported that the *N*-acetyl-glucosamine residues of PIA from *S. aureus* are completely succinylated, which led to its designation as poly-*N*-succinyl β1–6-glucosamine [36]. However, it is now clear that the succinyl groups were an artifact [35].

The partial deacetylation of 15–20% of the *N*-acetyl-glucosaminyl residues renders the polysaccharide positively charged, which determines its biological activity. Possibly, it functions as an intercellular adhesin by electrostatically attracting the negatively charged teichoic acid at the bacterial cell surface. The structure of PIA so far is unique. However, PIA-mediated biofilm formation might represent a common principle, because PIA-related structures have also been identified to play a role in the biofilm formation of other pathogenic bacteria, such as the Gram-negative *E. coli* and *A. actinomycetemcomitans* [37].

PIA is produced by the gene products encoded by the *icaADBC* operon. The *icaADBC* operon was first identified in *S. epidermidis*, and is also present in *S. aureus* and other staphylococcal species [34, 38]. The *N*-acetyl-glucosaminyltransferase activity is carried out by IcaA, which requires IcaD for full activity. With its transmembrane helices, IcaC very likely is an integral membrane protein that putatively transports the *N*-acetyl-glucosamine oligomers across the membrane [39]. IcaB is mainly found in the culture supernatant and deacetylates PIA [39, 40].

The importance of PIA as a pathogenicity factor has been confirmed in various foreign-body animal infection models with different *S. epidermidis icaADBC* mutants [30, 41]. However, in *S. aureus* conflicting results were obtained: PIA production did not increase the capacity to induce persistent infections in a tissue cage model [42]. A study investigating the pathogenic properties of *S. epidermidis* strains obtained from polymer-associated septicemic disease compared with saprophytic skin and mucosal isolates demonstrated a strong correlation of biofilm formation and presence of the *ica* gene cluster essentially associated with disease isolates [43].

1.3.3.2 Extracellular DNA

Another polymeric molecule, extracellular DNA, has been identified as an important component of the biofilm matrix of several bacterial species, such as *Streptococcus pneumoniae*, *P. aeruginosa*, and *Enterococcus faecalis* [44–46]. Although it does not seem to mediate biofilm accumulation by itself, it contributes to *S. aureus* biofilm development [47]. DNA is a negatively charged molecule that upon its release could interact with the positively charged extracellular polymer PIA, thus acting as an additional "glue."

1.3.3.3 Protein-Associated Biofilm Accumulation

Staphylococcal biofilm formation is not always polysaccharide-mediated. There are examples of infection-related biofilm-forming *S. epidermidis* strains that do not carry the *icaADBC* gene cluster [48]. In these strains, biofilm formation may be mediated by surface proteins. Surface proteins conferring biofilm accumulation include the 220-kDa accumulation-associated protein Aap from *S. epidermidis* and the homologous *S. aureus* surface protein G (SasG) [49, 50]. The function of Aap in the accumulation process was speculated to be the anchoring of PIA to the cell surface. However, recently, it was shown that Aap is able to mediate intercellular adhesion and biofilm accumulation in a completely PIA-independent background. Intercellular adhesion is mediated by a repeat domain B, which becomes active only after proteolytic cleavage of the N-terminal A domain [49]. Most recently, the B repeats of Aap (also known as G5 domains) were found to be zinc-dependent adhesion modules and a "zinc zipper" mechanism was suggested for G5 domain-based intercellular adhesion in Aap- or SasG-mediated biofilm accumulation [51]. Recently, transmission electron microscopy revealed that Aap has a fibrillar structure [52].

The biofilm-associated protein Bap mentioned above is involved in *S. aureus* adherence to a polystyrene surface, intercellular adhesion, and biofilm accumulation, [13]. The Bap-homologous protein Bhp may be involved in biofilm accumulation in *S. epidermidis* [14].

1.3.4 Biofilm Escape Factors

Biofilm detachment may lead to the dissemination of a staphylococcal infection, and thus to colonization of new sites and metastatic infection. Factors involved in biofilm detachment may include enzymatic activities that lead to the disintegration of the "glue." Depending on the nature of the substance that mediates the "stickiness", enzymatic activities like glycosyl hydrolases that would degrade PIA, proteases that would degrade protein components (such as Aap/SasG or Bap/Bhp), or nucleases that would degrade extracellular DNA, might be involved. Indeed, the Gram-negative periodontal pathogen *A. actinomycetemcomitans* produces dispersin B, which is a soluble glycosyl hydrolase that degrades the self-synthesized extracellular polysaccharide PGA. Like PIA, PGA is a linear polymer of β1–6-linked *N*-acetyl-glucosamine residues [37]. Dispersin B is also able to dissolve biofilms of clinical *S. epidermidis* strains by hydrolyzing the glycosidic linkages of PIA [37, 53]. However,

the *S. aureus* and *S. epidermidis* genomes do not seem to encode analogous enzymatic activities.

Extracellular DNA has been shown to be an important component of the *S. aureus* biofilm matrix (see Section 1.3.3.2) [47]. Accordingly, the addition of DNase I inhibits biofilm formation of *S. aureus* and promotes the detachment of preformed *S. aureus* biofilms [54]. Therefore, it may be speculated that the activity of an extracellular *S. aureus* nuclease would also contribute to biofilm detachment. The expression of the *S. aureus* nuclease gene (*nuc*) is under control of the *agr* QS system (see Section 1.4.1) [55]. In contrast to *S. aureus*, DNase I only slightly inhibits biofilm formation in *S. epidermidis*, but does not promote the detachment of preformed biofilms. Thus, in *S. epidermidis* extracellular DNA seems to effect initial attachment to a surface, rather than biofilm accumulation and detachment [54].

Several studies indicate that the biofilm matrix of a significant proportion of biofilm-forming staphylococcal strains mainly contained teichoic acid and proteins, but not PIA [48, 56]. In this case, protease treatment disintegrated the biofilms, although sometimes only partially [48, 57]. At least in *S. aureus*, protease-mediated biofilm detachment is dependent on a functional *agr* QS system (see Section 1.4.1) [58].

Another strategy leading to biofilm detachment involves the production and release of small peptides called phenol-soluble modulins (PSMs). PSMs were first described as proinflammatory agents in *S. epidermidis* [59]. According to their length, the PSMs can be divided in two classes: α-type peptides have a length of approximately 20 amino acids and β-type peptides are 40–45 amino acids in length. PSMs are supposed to have a surfactant-like effect due to their amphipathic α-helical character, which might be responsible for their role in biofilm detachment [60]. The expression of the genes encoding the PSMs is under the control of the *agr* QS system (see Section 1.4.1) [61].

1.4 QS in Staphylococcal Biofilms

In staphylococci, two QS systems have been described so far: the accessory gene regulator (*agr*) system, which has been studied in great detail in *S. aureus* and is also present in other staphylococcal species [55], and the *luxS*/AI-2 system identified in *S. epidermidis* as well as in *S. aureus* [62, 63].

1.4.1 agr QS Locus

S. aureus uses a biphasic strategy to cause disease. At low cell density, the bacteria produce protein factors, such as the MSCRAMMS and other adhesins that promote attachment and biofilm accumulation. In contrast, at high cell density, the bacteria repress the genes encoding the colonization factors, and initiate secretion of a variety of toxins (such as α-toxin, δ-toxin, and toxic shock syndrome toxin-1) and enzymes

(such as proteases, lipases, hyaluronidase, and nuclease) that are involved in tissue destruction and/or biofilm detachment probably required for dissemination of the infection and colonization of new sites.

The transcription of the virulence genes is regulated by a 514-nucleotide RNA molecule, termed RNAIII. RNAIII is a component of the global *agr* QS system that activates the transcription of genes encoding secreted toxins and enzymes and represses the transcription of genes encoding cell surface proteins (Figure 1.2) (reviewed in [55]). The *S. aureus agr* locus, approximately 3.5 kbp in size, consists of the genes *agrA*, *agrC*, *agrD*, and *agrB*, which are cotranscribed (RNAII), and the divergently transcribed gene for the regulatory RNAIII molecule, which also encodes the gene for the 26-amino-acid δ-toxin (*hld*). Transcription of RNAII is controlled by the P2 promoter and transcription of RNAIII is controlled by the P3 promoter. The autoinducing peptide (AIP) is a post-translationally modified cyclic peptide that

Autoinducing peptides

YSTCDFIM	*S. aureus* I	YNPCASYL	*S. epidermidis* I
GVNACSSLF	*S. aureus* II	DSVCASYF	*S. epidermidis* II
YINCDFLL	*S. aureus* III	YNPCSNYL	*S. epidermidis* III
YSTCYFIM	*S. aureus* IV	YNPCANYL	*S. epidermidis* IV

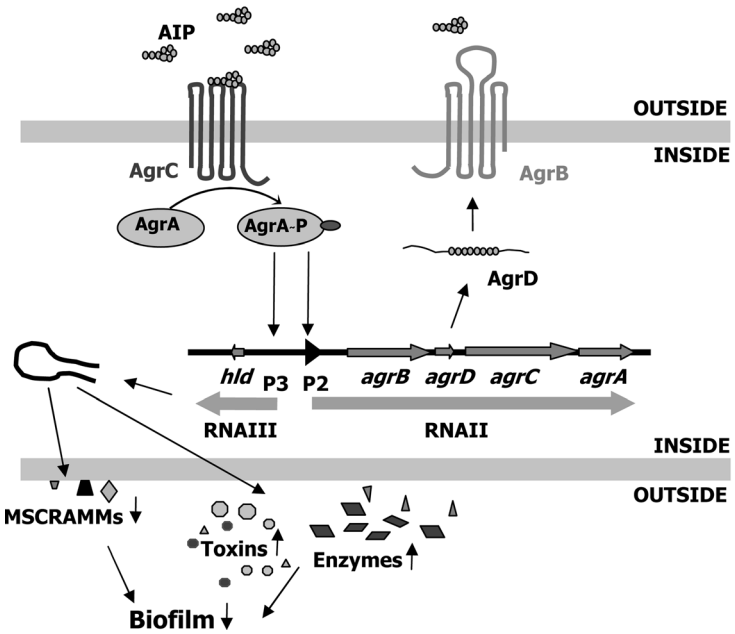

Figure 1.2 Model of the *Staphylococcus agr* QS system [55]. AIP is encoded by *agrD* and processed by *agrB*. The response regulator AgrA∼P activates promotors P2 and P3 to transcribe RNAII encoding *agrBDCA* and the effector molecule RNAIII, respectively. RNAIII also contains the δ-toxin gene (*hld*). RNAIII inhibits the expression of the genes encoding MSCRAMMs, and stimulates the expression of genes encoding extracellular enzymes and toxins, and as a consequence downregulates biofilm formation. The amino acid sequences of autoinducing peptides of *S. aureus* and *S. epidermidis* specificity groups I–IV are listed.

contains a thiolactone ring structure and is encoded by *agrD*. The AgrB protein processes, modifies, and exports the AIP. The modification is a cyclic thiolactone bond between the central cysteine and the C-terminal carboxyl group. The proteins encoded by *agr*A and *agr*C constitute a classical two-component regulatory system. Binding of the AIP to AgrC leads to phosphorylation of AgrA. Phospho-AgrA then activates the promotor P3 and thus induces the expression of the regulatory RNAIII. Moreover, phospho-AgrA activates the promotor P2 leading to the autoinduction of the *agr* system. The *agr* system is induced when the AIP reaches a certain threshold concentration in the culture medium, which usually occurs in the late exponential growth phase. In *S. aureus*, four different classes of AIPs have been identified, each belonging to another specificity group. The AIP of one specificity group activates the respective homologous *agr* system, while inhibiting the heterologous *agr* systems [64]. The *agr* specificity groups can be correlated with different pathotypes (e.g., most menstrual toxic shock syndrome strains belong to *agr* group III) [55].

In *S. epidermidis* and also in other staphylococci, *agr* homologs and different *agr* specificity groups have been identified [55, 64]. DNA sequence analysis revealed a pronounced similarity between the *S. epidermidis* and *S. aureus agr* system. However, there is no striking sequence similarity between the AIPs of *S. epidermidis*, *S. aureus* or *S. lugdunensis* (hepta-, octa-, or nonapeptides) except for the central cysteine and its distance to the C-terminus, suggesting that these conserved structural features are necessary for the thiolactone formation.

The influence of the *agr* QS system on staphylococcal biofilm formation is multifaceted, as expected for a global regulator. Since in *S. aureus*, the *agr* system downregulates the expression of genes encoding colonization factors and upregulates the expression of genes encoding detachment factors, the *agr* system might influence several stages of biofilm formation. Generally, the *agr* system downregulates biofilm formation in both *S. aureus* and *S. epidermidis*: *agr* mutants of *S. aureus* and *S. epidermidis* form a more pronounced biofilm than their parental counterparts [65–68].

The *S. epidermidis agr* mutant showed an increased attachment to polystyrene and expression of the autolysin AtlE, which is involved the attachment phase [11, 68]. Moreover, the *agr* mutant revealed significantly enhanced binding to epithelial cells, suggesting that decreased *agr* activity promotes the colonization of *S. epidermidis*. These results could be confirmed by *in vivo* data – the *agr* mutant revealed a higher infectivity in a rabbit model of device-associated infection. Furthermore, it has been observed that nonfunctional *agr* variants occur at a higher rate among clinical infection strains associated with joint prostheses (36%) in comparison to strains isolated from healthy individuals (4.7%), suggesting an inactive *agr* enhances the success of *S. epidermidis* to cause polymer-associated infections [67].

Further comparison of the *S. epidermidis agr* mutant with its wild type revealed that it showed a significantly altered protein expression: the expression of surface-associated proteins was increased, whereas the expression of extracellular proteins, such as lipases and proteases, was decreased [66]. Accordingly, microarray transcriptional analysis of the *agr* mutant showed that the expression of lipases and proteases as well as that of PSMs is upregulated by the *agr* system [69]. Proteome analysis confirmed that

these proteins were produced in a significantly lower amount in the *agr* mutant [70]. However, the same proteome analysis also indicated that the production of the autolysin AtlE was reduced in the *agr* mutant, which contradicts earlier findings (see above) [68]. The higher level of biofilm formation in the *S. epidermidis agr* mutant could not be explained by an enhanced expression of genes associated with biofilm accumulation, such as the *icaADBC* gene cluster or *aap* [69, 70].

Generally, *agr* transcription was significantly downregulated in *S. epidermidis* cells grown in a biofilm in comparison with planktonically grown bacteria as shown by genome microarray transcription analyzes [60]. More specifically, *agr* expression was restricted to the externally located regions of the biofilm, whereas no *agr* expression was detected in deeper, internally located biofilm layers. This suggested that *agr* might be involved in the biofilm detachment process [67].

Similar results were obtained with *S. aureus*. In a large collection of 105 clinical *S. aureus* isolates, a strong correlation between *agr* and biofilm formation has been found: 78% of *agr*-negative, but only 6% of *agr*-positive strains formed a biofilm [65]. In contrast to *S. epidermidis*, this effect did not correlate with an altered production of the autolysin Atl, because in the *agr* mutant the expression of *atl* was even slightly reduced. Furthermore, PIA production was unchanged und therefore is not under the control of *agr*. Rather, this effect might at least in part be due to an increased production of PSMs, because the surfactant-like structure of PSMs led to a decreased attachment of the bacterial cells to polystyrene [65]. Another study confirmed that *agr* repressed biofilm formation, but only under static growth conditions. In a flow cell, no significant differences in biofilm formation were observed with the wild-type and an *agr* mutant strain [71]. The same study also indicated that cells detaching from a biofilm revealed a highly activated *agr* system, while bacteria within the biofilm repressed the *agr* system, which is consistent with the observations made in *S. epidermidis*. Recently, it was reported that the repression of the *agr* system is required to form a biofilm and that the induction of the *agr* system in established biofilms promotes detachment, which at least in part depends on extracellular protease activity (see Section 1.3.4) [58].

The expression of extracellular enzymes and toxins seems to be regulated by *agr* in the same way in *S. epidermidis* and *S. aureus*. In contrast, different regulatory mechanisms seem to be involved in the regulation of the genes encoding colonization factors between *S. epidermidis* and *S. aureus*: while the *agr* system in *S. aureus* downregulates the MSCRAMMs, several cell surface proteins of *S. epidermidis* are expressed mainly in the stationary growth phase rather than in the exponential phase [72].

However, as shown in numerous reports, in *S. aureus* as well as in *S. epidermidis*, biofilm formation is significantly reduced by the *agr* QS system. At least in part, this may be explained by an increased biofilm detachment via the upregulation by *agr* of different genes that might be involved in biofilm detachment, such as nucleases, proteases, and PSMs [55, 58, 61]. Partially conflicting results sometimes obtained for the role of the *agr* QS system in staphylococcal biofilm formation may be explained by different growth conditions, such as static or under flow, different growth phases observed, different supply of nutrients, or strain differences [71].

1.4.2
luxS/AI-2 System

The *luxS* QS system has been identified in several Gram-positive and Gram-negative bacterial species, and affects biofilm formation not only in staphylococci, but also in *Streptococcus mutans*, *Actinomyces naeslundii*, and *Helicobacter pylori* [73, 74]. The *luxS* gene encodes the production of the autoinducer AI-2, which is a furanone derivative, in *S. epidermidis* as well as in *S. aureus*. [62, 63]. The production of AI-2 is growth-phase dependent with a peak production observed during exponential growth. The inactivation of the *luxS* gene in *S. epidermidis* had the same effect as the inactivation of the *agr* system: an *S. epidermidis lux*S mutant was able to form a thicker and stronger biofilm than its parental strain. Transcriptional analysis indicated that the *luxS* system repressed biofilm formation by downregulating the *icaADBC* expression. Accordingly, the production of PIA was elevated in the *luxS* mutant compared with the wild type [62]. This contrasts the effects of the *agr* system, which does not influence *icaADBC* transcription and PIA production. In a rat central venous catheter infection model, the *luxS* mutant turned out to be a more successful colonizer and had a higher capacity to cause infection [62]. However, a recent genome-wide gene expression study indicated that in *S. epidermidis*, mostly genes involved in metabolism, such as sugar, nucleotide, amino acid, and nitrogen, are under the control of the AI-2 [75]. Additionally, *luxS* controls virulence-associated genes encoding lipase and PSMs, suggesting that the stronger biofilm formation in the *luxS* mutant may at least partially be due to a decreased production of PSMs and thus a reduced detachment rate. Surprisingly, the *icaADBC* genes were not found to be differentially expressed in the *luxS* mutant, contradicting earlier findings [75].

In contrast to *S. epidermidis*, a role of the *luxS* system in *S. aureus* biofilm formation and expression of virulence-associated genes could not be detected. Instead, a role for *luxS* in metabolism was suggested [63]. Thus, there seem to exist important species-specific differences in *luxS*-dependent gene regulation among staphylococci. A contrasting effect of *luxS* on biofilm formation has also been observed with other bacterial species. While *luxS* represses biofilm formation in *S. mutans* and *H. pylori*, a *luxS* mutant of *Salmonella* was not able to develop a complete biofilm.

Taken together, the *luxS* QS system has a profound effect on biofilm formation and pathogenicity in *S. epidermidis*, but not in *S. aureus*. Thus, at least in *S. epidermidis* both known QS systems, *agr* and *luxS*, repress biofilm formation.

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