INFECTION AND IMMUNITY, Jan. 2006, p. 488–496 Vol. 74, No. 1
0019-9567/06/$08.00+0 doi:10.1128/IAI.74.1.488–496.2006
Copyright © 2006, American Society for Microbiology. All Rights Reserved.

Role of the *luxS* Quorum-Sensing System in Biofilm Formation and Virulence of *Staphylococcus epidermidis*

Lin Xu,[1,2] Hualin Li,[1,2] Cuong Vuong,[3]† Viveka Vadyvaloo,[3] Jianping Wang,[1,2] Yufeng Yao,[3] Michael Otto,[3] and Qian Gao[1,2]*

Key Laboratory of Medical Molecular Virology, Shanghai Medical College, Fudan University, Shanghai 200032, People's Republic of China[1]; Institute of Medical Microbiology, Fudan University, Shanghai 200032, People's Republic of China[2]; and Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, the National Institutes of Health, Hamilton, Montana 59840[3]

Received 12 June 2005/Returned for modification 18 July 2005/Accepted 5 October 2005

Nosocomial infections caused by *Staphylococcus epidermidis* are characterized by biofilm formation on implanted medical devices. Quorum-sensing regulation plays a major role in the biofilm development of many bacterial pathogens. Here, we describe *luxS*, a quorum-sensing system in staphylococci that has a significant impact on biofilm development and virulence. We constructed an isogenic Δ*luxS* mutant strain of a biofilm-forming clinical isolate of *S. epidermidis* and demonstrated that *luxS* signaling is functional in *S. epidermidis*. The mutant strain showed increased biofilm formation in vitro and enhanced virulence in a rat model of biofilm-associated infection. Genetic complementation and addition of autoinducer 2-containing culture filtrate restored the wild-type phenotype, demonstrating that *luxS* repressed biofilm formation through a cell-cell signaling mechanism based on autoinducer 2 secretion. Enhanced production of the biofilm exopolysaccharide polysaccharide intercellular adhesin in the mutant strain is presumably the major cause of the observed phenotype. The *agr* quorum-sensing system has previously been shown to impact biofilm development and biofilm-associated infection in a way similar to that of *luxS*, although by regulation of different factors. Our study indicates a general scheme of quorum-sensing regulation of biofilm development in staphylococci, which contrasts with that observed in many other bacterial pathogens.

For a long time, *Staphylococcus epidermidis* has been regarded as an innocuous commensal bacterium on the skin and mucous membranes of the human body. However, it is now known to be a major nosocomial pathogen, causing infections on implanted medical devices such as central venous catheters (CVCs), urinary catheters, prosthetic heart valves, orthopedic devices, and contact lenses. The investigation of *S. epidermidis* pathogenesis has become of interest in recent years, as *S. epidermidis* infections have arisen as a major burden to the public health system. They are often responsible for prolonged hospitalization and may even be fatal (51).

In contrast to *Staphylococcus aureus*, *S. epidermidis* produces only a limited amount of toxins and degradative exoenzymes (58). The formation of a biofilm, which is a differentiated structure of surface-attached cells that are embedded in a self-secreted heterogeneous matrix, is thought to be the leading cause for the persistence of *S. epidermidis* infection. A biofilm constitutes a depository that may continuously release bacteria into the bloodstream. Furthermore, the treatment of biofilm-associated infections is especially difficult due to biofilm resistance to antibiotics and attacks from the human immune system. To date, several genes have been identified that are associated with the different stages of biofilm formation, including those carrying autolysin E (*atlE*) (20), fibrinogen binding protein (*fbe*) (41), and the intercellular adhesion operon (*ica*) (17, 21). However, the exact roles of these genes during biofilm formation are not yet entirely clear (11, 26, 32). Possibly, differential expression of these factors in vitro and in vivo and under different environmental conditions has so far been underestimated.

Quorum sensing (QS) is a bacterial intercommunication system that controls the expression of multiple genes in response to population density (16). QS systems use small signal molecules called autoinducers (AIs). When the AIs accumulate to a threshold concentration, the system is activated and directly or indirectly controls the transcription of target genes. Gram-negative bacteria normally use acylated homoserine lactones (AHLs) as AIs, while gram-positive bacteria use oligopeptide AIs, which act through two-component phosphorelay cascades (36, 44, 53).

In the last decade, a novel quorum-sensing system was discovered in the bioluminescent marine bacterium *Vibrio harveyi* (3). *V. harveyi* has three parallel QS systems to induce bioluminescence. One uses AI-1, a typical AHL, as a signal; a second uses CAI-1 (*V. cholerae* autoinducer 1), a signal-mediating intercommunication in closely related marine bacteria; and finally there is a novel signaling molecule called AI-2 (22). There is ample data to suggest that AI-2s have been designed for interspecies cell-cell communication (53). They are found in both gram-negative and gram-positive bacteria (36, 48, 56). AI-2s are not species specific, as AI-2s produced by other bacteria species can also trigger the bioluminescence of *V. harveyi* (47). A gene called *luxS* is required for the synthesis of AI-2 (48). AI-2-like activity and *luxS* homologues are found in

* Corresponding author. Mailing address: Key Laboratory of Medical Molecular Virology, Shanghai Medical College, Fudan University, Shanghai 200032, People's Republic of China. Phone: 86-21-54237195. Fax: 86-21-54237603. E-mail: qiangao@shmu.edu.cn.

† Present address: Department of Anesthesiology, University of Massachusetts, Worster, MA 01655.

TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Description	Source or reference(s)
Strains		
S. epidermidis 1457	*luxS* wild-type strain, biofilm positive	33
S. epidermidis 1457 Δ*luxS* mutant	*luxS* mutant (LuxS$^-$ Erm^r)	This study
E. coli DH5α	*supE44* Δ*lacU169* (ϕ*80lacZ*ΔM15) *hsdR17 recA1 endA1 gyrA96 thi-1 relA1* Δ*luxS*	19, 48
V. harveyi BB170	*luxN*::Tn*5* (sensor 1$^-$ sensor 2$^+$), AI-2 reporter strain	47
Plasmids		
pcDNA2.1	Cloning vector, ampicillin resistant	Invitrogen
pEC4	A vector containing a 1.45-kb ClaI *ermB* fragment of Tn*551* cloned into the ClaI restriction site of pBluescript KS$^+$	6
pBT1	Shuttle vector, temperature sensitive, ampicillin and chloramphenicol resistant	6
pQG23	pcDNA2.1 harboring a 2.5-kb DNA fragment containing the *luxS* gene	This study
pQG24	pcDNA2.1 harboring an Erm^r gene with regions up and down from *luxS*	This study
pQG21	pBT1 harboring Erm^r gene with up- and downstream sequence of *luxS* gene	This study
pQG27	pBT1 harboring 1.0-kb DNA fragment containing the *luxS* gene	This study

a wide range of bacterial species. Database analysis shows that *luxS* exists in 35 of the 89 available complete bacterial genomes (National Center for Biotechnology Information) (56). Besides light production in *V. harveyi* (4) and *Vibrio fischeri* (28), *luxS*/AI-2-dependent QS regulates the growth of *Escherichia coli* (45), *Bacillus anthracis* (23), and *Actinobacillus actinomycetemcomitans* (15). It also plays a role in *E. coli* flagellum motility (45). Furthermore, it modulates the virulence of *Streptococcus pyogenes* (29), *Clostridium perfringens* (39), *Vibrio vulnificus* (25), and *Streptococcus pneumoniae* (24, 46). Interestingly, AI-2 from the resident microflora in the lungs of cystic fibrosis patients can up-regulate pathogenicity factors in non-AI-2-producing *Pseudomonas aeruginosa* (12). In some bacteria, it has been reported that *luxS*/AI-2-dependent QS impacts biofilm formation in various ways. For example, a *luxS* mutant of *Streptococcus gordonii*, a major component of dental plaque biofilm, was unable to form a mixed-species biofilm with a *luxS*-null strain of the periodontal pathogen *Porphyromonas gingivalis* (34) despite the wild-type strains readily doing so. Furthermore, a *luxS* mutant of *Helicobacter pylori* forms biofilm more efficiently than the wild type (9). Finally, a *luxS* mutant of *Streptococcus mutans* shows an altered biofilm structure (35).

Staphylococci have one known QS system called *agr*, for accessory gene regulator, which secretes modified peptides as signals (38, 40). In *S. epidermidis*, *agr* represses biofilm formation and influences the structure of a biofilm (49, 50) by regulation of biofilm factors, such as AtlE and δ-toxin (49). *luxS* homologues are present in the *S. aureus* and *S. epidermidis* genomes (54, 58). However, although Winzer et al. have purified the LuxS protein from *S. aureus* that can synthesize the AI-2 (54), whether *S. epidermidis* or other staphylococci use an AI-2-type QS system to control gene expression has not been investigated previously.

In this study, we explored the *luxS*/AI-2-dependent QS system of *S. epidermidis* by construction and analysis of an allelic replacement mutant of *S. epidermidis luxS*. We found that the *luxS*/AI-2-dependent QS system of *S. epidermidis* is functional. Of note, similarly to the *agr* system (50), *luxS* of *S. epidermidis* limits biofilm formation and virulence in an animal model of device-associated infection. Our data point towards a common scheme of QS control of biofilm formation and biofilm-associated infection in staphylococci.

MATERIALS AND METHODS

Bacterial strains and growth media. The bacteria and plasmids used are listed in Table 1. *E. coli* DH5α was grown in Luria-Bertani medium. Plasmid-containing *E. coli* strains were grown in the same medium but with ampicillin (100 μg/ml) included. *S. epidermidis* and its derivative strains were grown in TSB (tryptic soy broth, soybean-casein digest medium USP; Oxoid) medium, and when necessary, erythromycin (10 μg/ml), erythromycin (2.5 μg/ml), and chloramphenicol (20 μg/ml) were supplemented. *V. harveyi* BB170 was kindly provided by B. Bassler (Princeton University) and was grown in autoinducer bioassay (AB) medium at 30°C (48). Media were solidified with 1.5% (wt/vol) agar as needed.

DNA manipulation. Genomic DNA of *S. epidermidis* 1457 was prepared by a standard protocol for gram-positive bacteria (13). Plasmid DNA from *E. coli* was extracted using a plasmid purification kit (TianWei Co.) according to the manufacturer's instructions. Plasmid DNA from *S. epidermidis* and *S. aureus* was extracted using the same kit except that the cells were incubated for at least 15 min at 37°C in solution P1 with lysostaphin (25 μg/ml; Sigma) before solution P2 was added. *Taq* DNA polymerase and restriction enzymes were obtained from Takara; and incubation conditions were as recommended by the suppliers. *S. epidermidis* was transformed by electroporation as described previously (2).

Construction of an *S. epidermidis* Δ*luxS* mutant strain. A 2.5-kb DNA fragment from *S. epidermidis* 1457 genomic DNA with *luxS* and 1-kb upstream and 1-kb downstream flanking sequences was PCR amplified using the primers luxS(a)5 and luxS(a)3, introducing BamHI and EcoRI sites, respectively. The amplified PCR products were cloned into pcDNA2.1, yielding pQG23. An erythromycin resistance (Erm^r) gene was PCR-amplified by primers erm5 and erm3 from pEC4 (6). The product was ligated to the NdeI and SpeI sites inside the *luxS* gene on pQG23, yielding pQG24. A BamHI/EcoRI fragment was excised

TABLE 2. Oligonucleotides used in this study

Oligonucleotide	DNA sequence (5′→3′)[a]
luxS(a)5	CG**GGATCC**ATCAAGTTCTTTCCGTGAAG
luxS(a)3	CG**GAATTC**ATAGTGGTTATGATGTAATCG
luxS(b)5	CGGGATCCGCAATATCCGTTTCGATTTC
luxS(b)3	CGGAATTCAGAGAAACATCGAAGGGAAG
erm5	GGAATTG**CATATG**GATACAAATTCCCCGTAGGC
erm3	GG**ACTAGT**GAAATAGATTTAAAAATTTCGCTG
icaC5	TGCTTACACCAACATATTTGAAGATAATAC
icaC3	GACGCCTATACAAATTCCTAGAATCATT
icaCP	TTTCTTGGCGATTTCACT

[a] Incorporated restriction sites are underlined and boldfaced.

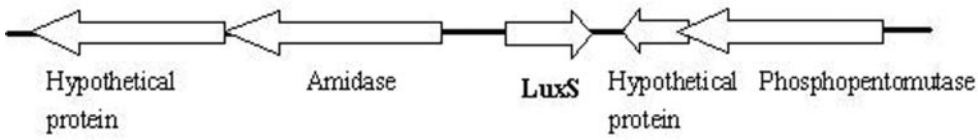

FIG. 1. Identification of the *luxS* gene of *S. epidermidis*. Shown are the *luxS* gene and its surrounding region according to genetic information (58).

from pQG24, purified, and ligated to the vector pBT1 (6) to create pQG21. Allelic replacement of the native *luxS* gene with the resulting plasmid in genomic DNA of *S. epidermidis* 1457 with Erm^r was performed as described previously (6). Erythromycin-resistant and chloramphenicol-sensitive colonies were selected. PCR and sequencing were performed to confirm that the desired gene inactivation had occurred by double-crossover recombination.

Construction of pQG27 for complementation. To complement *E. coli* DH5α or the Δ*luxS* mutant strain of *S. epidermidis* 1457 with a functional copy of *luxS*, a 1.0-kb fragment containing an intact *luxS* open reading frame (ORF), including its promoter, was amplified from *S. epidermidis* 1457 genomic DNA by using primers luxS(b)5 and luxS(b)3. The amplified product was ligated into pBT1, creating pQG27. pQG27 was then introduced into *E. coli* DH5α or the *S. epidermidis* 1457 Δ*luxS* mutant strain by electroporation. Clones exhibiting resistance to ampicillin (100 μg/ml) or chloramphenicol (20 μg/ml) were chosen for further study. The presence of *luxS* on pQG27 was confirmed by sequencing. To exclude the possible influence from the vector, the empty vector was electroporated into *E. coli* DH5α or *S. epidermidis* 1457 as a control strain.

AI-2 assay. The AI-2 reporter assay was performed according to the method of Surette and Bassler (47) and Surette, Miller, and Bassler (48), which is based on the reporter strain *V. harveyi* BB170 responding to the presence of exogenous AI-2 by induction of bioluminescence. Cell-free medium (CM) was prepared as follows: tester strains were grown overnight at 37°C and then diluted 1:100 into fresh medium, and 1.5-ml samples were taken every 2 h. Cells were pelleted by centrifugation, and the resulting supernatant was further clarified by filtering through a 0.22-mm-pore-size filter (Millipore). Samples (20 μl) of CM were added into white 96-well microtiter plates. To determine bioluminescence, the *V. harveyi* BB170 reporter strain, grown for 16 h with aeration (180 rpm) at 30°C in AB medium, was diluted 1:5,000 in fresh AB medium and then 180 μl was added to each sample. The bioassay medium was incubated at 30°C. Luminescence was monitored hourly by Fluoroskan Ascent FL (Labsystems). In every case, conditioned medium from *V. harveyi* BB170 served as an additional positive control. For all the complementation experiments, strains were grown at 30°C.

Semiquantitative biofilm formation. The method for quantification of biofilm was performed as described previously (8) and modified as follows. *S. epidermidis* strains were grown in TSB for 16 h and diluted 1:100 into fresh TSB. The diluted cultures were pipetted into sterile 96-well flat-bottomed tissue culture plates and incubated at 37°C for 24 h. Culture supernatants were gently removed with a pipette, and the wells were washed four times with PBS (phosphate-buffered saline; pH 7.2). The adherent organisms remaining at the bottom of the well were fixed by Bouin fixative. After 1 h, the fixative was removed and wells were washed with PBS. Following that, the adherent bacteria were stained with crystal violet and the excessive stain was washed off gently by slowly running water. After drying, the stained biofilm was read with a MicroELISA autoreader (Bio-Rad Co.) using a wavelength of 570 nm under single-wavelength mode. For all complementation experiments, strains were incubated at 30°C.

SEM. For SEM (scanning electron microscopy), biofilm was grown in TSB with 0.5% glucose for 24 h at 37°C on the surface of sterile glass slides, which were deposited in advance in each well of 24-well hydroxylapatite disks. The contents of each well then were carefully aspirated with a pipette, rinsed with PBS three times, and then fixed with glutaral fixative solution overnight. Following dehydration through a graded series of ethanol rinses, the slides were coated with gold-palladium. SEM micrographs were taken in the Electron Microscopy Core Facility at Shanghai Medical College, Fudan University.

Quantitative RT-PCR (TaqMan). Oligonucleotide primers and probes were designed using Primer Express version 2.0 (Applied Biosystems) and were synthesized by Applied Biosystems. The primers (icaC5 and icaC3) and probes (icaCP) used are listed in Table 2. Fluorescent probes were used to continuously monitor formation of PCR products during PCR. Isolated RNA was quantified by spectrophotometry. A total of 100 ng of RNA was used per reaction. Reverse transcription of the RNA template and real-time (RT)-PCR were carried out in a single step using TaqMan one-step RT-PCR autoinducer 2 containing master mix (Applied Biosystems). Reactions were performed in a MicroAmp optical 96-well reaction plate with a model 7700 sequence detector (Applied Biosystems). Reverse transcription was performed at a temperature of 48°C for 30 min. PCRs were performed as described previously (57). Standard curves were determined for each gene by use of purified chromosomal template DNA at concentrations of 0.0005 to 500 ng/ml. Assays were performed in triplicate using 16S rRNA as a control.

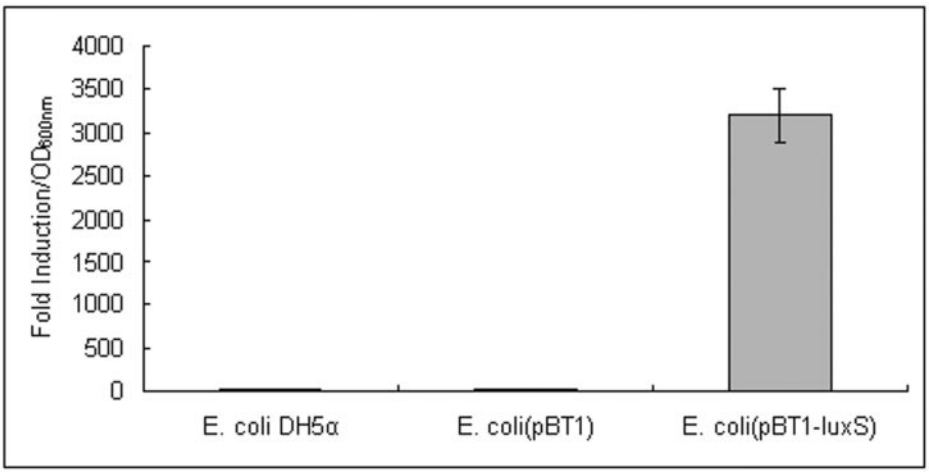

FIG. 2. Complementation of the AI-2 synthesis deficiency of *E. coli* DH5α by *luxS* from *S. epidermidis*. The capacity to produce AI-2 was examined by an AI-2 reporter assay as described previously (47, 48). *V. harveyi* BB170 (AI-1 sensor$^-$, AI-2 sensor$^+$) served as a positive control. Cell-free medium was prepared from the strains at logarithmic growth phase, when their light production reached maximum. Luminescence was expressed as fold induction relative to the background values and normalized to cell OD of 1 at 600 nm. Data were obtained from four independent experiments.

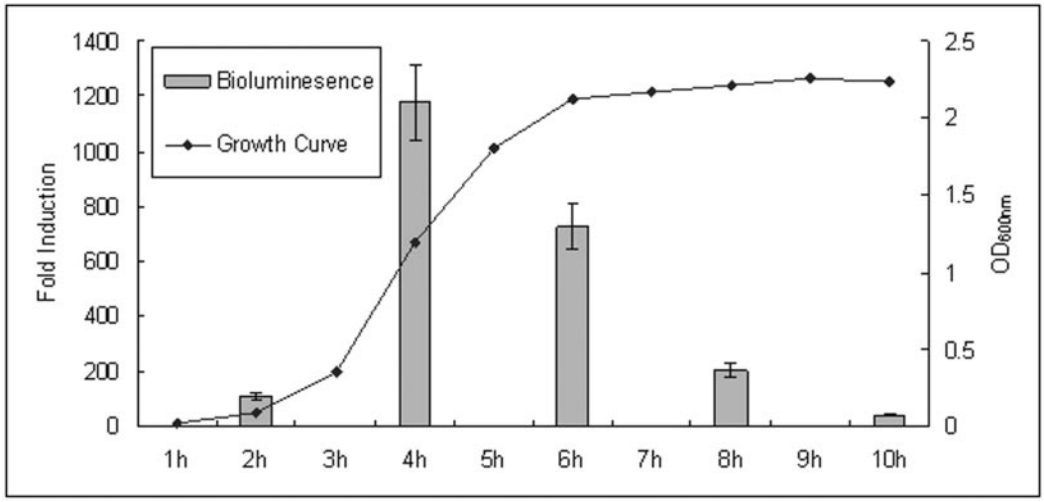

FIG. 3. Detection of AI-2 activity throughout the growth of *S. epidermidis* 1457. Cells were grown overnight and diluted 1:100 into fresh TSB medium. The absorbance (OD) at 600 nm was then examined hourly by spectrophotometry. Every 2 h, conditioned cell-free supernatants were examined for the capacity to induce light production in *V. harveyi* BB170. Data were obtained from four independent experiments.

Immuno-dot blot assay. To quantify *p*olysaccharide *i*ntercellular *a*dhesin (PIA) production, equal amounts of *S. epidermidis* cells were grown to stationary growth phase (24 h) and PIA was extracted by incubation with 0.5 mol/liter EDTA (pH 8.0) for 5 min at 100°C. Two aliquots of the samples were spotted on a nitrocellulose membrane, air-dried, and blocked with 5% skim milk in TBS buffer overnight. PIA was detected using anti-PIA antiserum as described previously (52) by using a scanner and Total Lab version 2003 software (Nonlinear USA, Inc., Durham, NC). Assays were performed in triplicate.

Complementation with conditioned medium. Cell-free conditioned medium (CM) from *S. epidermidis* 1457 was prepared as follows. Cultures were grown overnight and then incubated and diluted 1:100 followed by resumption of shaking at 37°C for 4 h for the *S. epidermidis* wild-type strain and 6 h for the *luxS*-complemented strain (both achieved exponential phase). Cell-free supernatant was harvested by centrifugation and filtered as described above. CM was added to the culture at the start of incubation of biofilm formation for the Δ*luxS* mutant in 96-well flat-bottomed tissue culture plates, at 10% (vol/vol) final concentration.

A rat model of intravascular catheter-associated infection. A rat model of CVC-associated infection was developed according to the method of Mack et al. (43). Thirty healthy male Sprague-Dawley rats (weight, 500 g) (Academia Sinica) were surgically dissected, and Silastic lumen-within-lumen catheters (inside diameter, 0.12 mm) were inserted into their right external jugular veins. The proximal portion of the catheter was tunneled subcutaneously and came out from the midscapular space. A metal column was used to block up the end of the proximal portion of the catheter, preventing contamination from outside. Twenty-four hours after CVC placement, 4×10^5 CFU of *S. epidermidis* 1457 or its isogenic Δ*luxS* mutant were inoculated into the catheters. In our study, 15 rats were infected with *S. epidermidis* 1457 and 15 were infected with its Δ*luxS* mutant. The catheters were flushed daily with heparin. One week after inoculation, the rats were killed and the bacteria recovered from catheters, blood, hearts, kidneys, and livers were counted.

RESULTS

Identification of *luxS* from *S. epidermidis*. In order to determine whether *S. epidermidis* has a *luxS*/AI-2-dependent QS system, we examined the available *S. epidermidis* genome information (58) and identified a candidate ORF whose predicted protein product was 43% identical and 63% similar to the *V. harveyi* LuxS protein. *S. epidermidis* LuxS is very similar

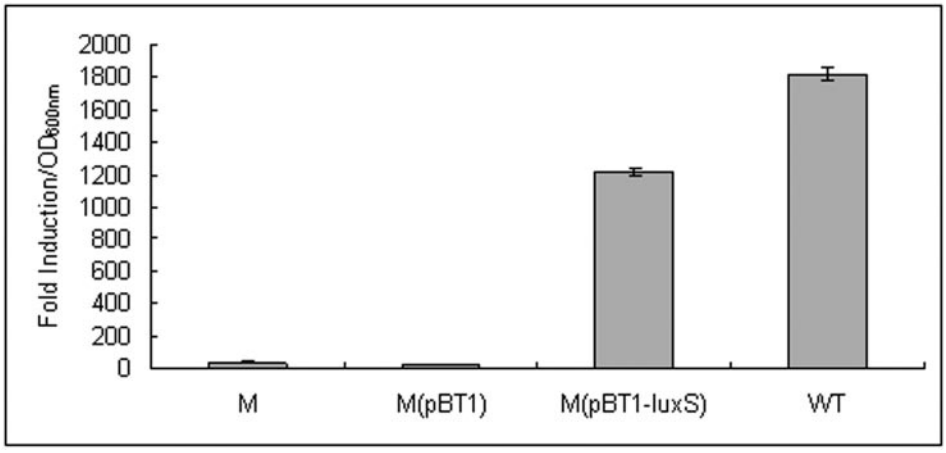

FIG. 4. AI-2 activity of *S. epidermidis* 1457, its isogenic Δ*luxS* mutant strain and/or the mutant strain with empty vector complemented with *luxS*. Cell-free medium was prepared from those strains during the log phase. All experiments were performed in triplicate. WT, *S. epidermidis* 1457 wild-type strain; M, *S. epidermidis* 1457 isogenic Δ*luxS* mutant strain; M(pBT1), *S. epidermidis* Δ*luxS* mutant strain containing vector pBT1; M(pBT1-luxS), *luxS*-complemented *S. epidermidis* mutant strain.

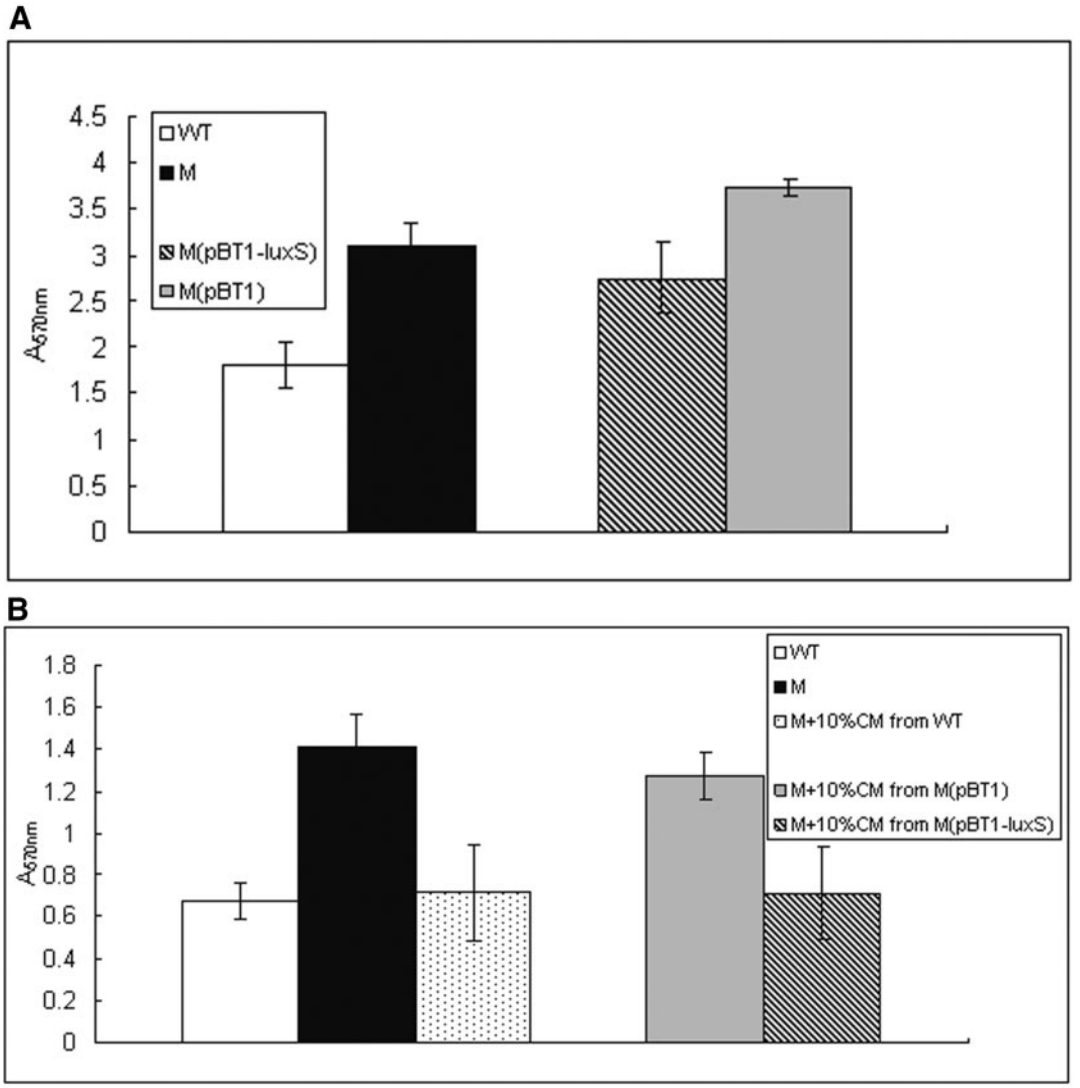

FIG. 5. Semiquantitative biofilm assay. Each experiment was repeated eight times. A, Biofilm formation of *S. epidermidis* 1457, its isogenic Δ*luxS* mutant, the mutant with the empty vector, and the *luxS*-complemented mutant at 24 h. B, Biofilm formation of *S. epidermidis* 1457, its isogenic Δ*luxS* mutant, and the mutants with exogenous AI-2 prepared from wild-type and complemented mutant strains at 24 h using mutant with CM from mutant strain transformed with empty vector as control.

to *S. aureus* LuxS (identity at the amino acid level of 91%; similarity, 98%) and also shows significant similarity (63%) to LuxS of *E. coli*. The *luxS* gene of *S. epidermidis* is an independent ORF (Fig. 1), and its promoter is most likely located upstream of the ORF.

Functional analysis of the AI-2 QS system in *S. epidermidis*. To investigate whether the *luxS* gene of *S. epidermidis* is functional and required for AI-2 production, we complemented an *E. coli* DH5α strain deficient in AI-2 synthesis (47) with a plasmid (pQG27) containing the *luxS* gene of *S. epidermidis* under control of its putative native promoter. The presence of the plasmid containing *luxS* was sufficient to induce light production over 3,000-fold, whereas the control *E. coli* strains did not produce light (Fig. 2). Furthermore, to determine whether *S. epidermidis* produces AI-2 and uses AI-2 QS control, we measured AI-2 activity during the growth of the *S. epidermidis* wild-type strain 1457 using the same reporter assay. AI-2 accumulates during logarithmic phase and is reduced in stationary phase in *S. epidermidis* (Fig. 3). These analyses demonstrated that *S. epidermidis* contains a pathway to synthesize and secrete AI-2.

The role of the AI-2 QS system in biofilm formation of *S. epidermidis*. To investigate the role of *luxS* in the virulence of *S. epidermidis*, a Δ*luxS* mutant strain was constructed by an allelic exchange strategy (6). The successful exchange of the *luxS* gene with the antibiotic resistance cassette was confirmed by PCR and DNA sequencing (data not shown). The mutant and wild-type strains were unaffected in growth rate and extent in a variety of media (data not shown). The Δ*luxS* mutant of *S. epidermidis* 1457 produced no detectable AI-2 activity (Fig. 4), and the difference between the wild type and the Δ*luxS* mutant was most significant after 4 h of growth, at which point both

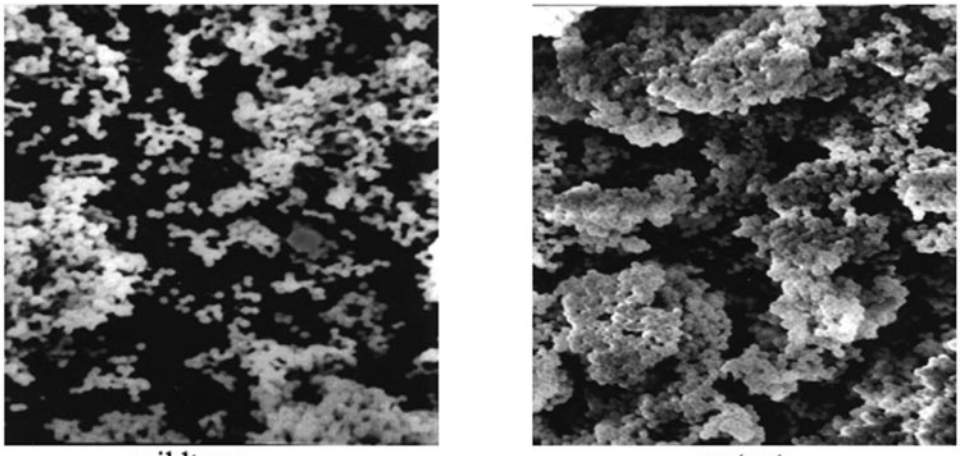

FIG. 6. Scanning electron micrographs of *S. epidermidis* 1457 and its Δ*luxS* mutant. Twenty-four-hour biofilms were grown on hydroxyapatite disks that were deposited in 24-well cell culture clusters in TSB with 0.5% (wt/vol) glucose. Images shown are at a magnification of ×3,000.

strains entered logarithmic growth phase (optical density, ~1.2). After complementation of the mutant with plasmid pQG27, AI-2 activity was restored to almost the wild-type level (Fig. 4). These analyses demonstrated that *luxS* is responsible for AI-2 production in *S. epidermidis*.

Biofilm formation is considered the main virulence factor of *S. epidermidis*. To investigate whether *luxS* influences biofilm formation in *S. epidermidis*, we first performed semiquantitative biofilm assays using microtiter plates. The Δ*luxS* mutant of *S. epidermidis* showed significantly ($P < 0.0001$, two-tailed *t* test) increased biomass (1.7 times) compared to that of the wild-type strain (Fig. 5A). Biofilm formation of the complemented strain was reduced compared to the Δ*luxS* mutant strain (Fig. 5A).

In addition, we used SEM to detect any quantitative or qualitative alteration in the biofilm formation of wild-type and Δ*luxS* mutant strains. SEM analysis showed that the Δ*luxS* mutant strain generated a more compact and thicker biofilm than that generated by the wild-type strain (Fig. 6).

To examine whether the increased biofilm formation of the *S. epidermidis* Δ*luxS* mutant strain was due to a genuine AI-2 signaling event or a mere result of metabolic changes accompanied by the inactivation of the *luxS* gene, we incubated the Δ*luxS* mutant strain with exogenous AI-2. To that end, we used CM prepared from exponential-growth-phase cultures of *S. epidermidis* 1457, the *luxS*-complemented mutant strain, and the mutant strain with the empty vector as control. CM was mixed with Δ*luxS* mutant cells grown overnight, which were then incubated for 24 h to test for biofilm formation. The Δ*luxS* mutant strain showed reduced biofilm formation when 10% CM was added (Fig. 5B) while no growth inhibition was detected (data not shown). These data indicate that the *luxS* gene of *S. epidermidis* is involved in repressing biofilm formation through a cell-cell signaling mechanism based on AI-2 secretion.

Secretion of the exopolysaccharide PIA is a major factor that determines biofilm formation in *S. epidermidis* and other bacteria (18, 30, 31). To test whether the altered biofilm formation that we observed in the Δ*luxS* mutant strain was due to a change in the transcription of the *ica* operon responsible for PIA production (21), we analyzed transcription of the *icaC* gene by quantitative real-time PCR. Expression of *icaC* during mid-exponential growth phase was 4.4 times higher ($P = 0.01$, two-tailed *t* test) in the Δ*luxS* mutant strain than in the wild-type strain (Fig. 7A). The *luxS*-complemented mutant strain showed an *icaC* expression level significantly lower than that of the control strain with the empty cloning vector ($P = 0.01$, two-tailed *t* test) and comparable to that of the wild-type strain. This indicates that *luxS* negatively regulates the expression of the *ica* gene at the transcriptional level. To determine whether *luxS* regulates the synthesis of PIA, we extracted PIA from stationary-phase cultures of *S. epidermidis* 1457, the Δ*luxS* mutant strain, and the complemented and control strains and determined the amount of PIA by use of immuno-dot blot analysis. PIA production was about three times higher in the Δ*luxS* mutant strain than in the wild-type strain ($P = 0.03$, two-tailed *t* test). Thus, *luxS*/AI-2-dependent QS regulates the expression of PIA. By visual comparison, PIA production was also decreased in the *luxS*-complemented strain compared to that produced by the mutant strain with the empty vector. Photodigital analysis of this experiment was, however, not possible due to uneven distribution of the reaction on the spotted dots (data not shown).

The role of the AI-2 QS system in the virulence of *S. epidermidis*. To determine whether *luxS* has an effect on the pathogenicity of *S. epidermidis* during biofilm-associated infection, we used a rat intravascular CVC-associated infection model. Twenty-four hours after insertion of Silastic lumen-within-lumen catheters into the necks of the rats, 4×10^5 CFU bacteria were injected into the catheters. Bacteria in blood, the tissue surrounding the catheters, and other organs were counted after sacrificing the rats on day 8 after infection. As shown in Table 3, the Δ*luxS* mutant had a higher capacity to cause CVC infection ($P = 0.006$, two-tailed *t* test) and also showed more significant bacteremia and a greater burden of metastatic disease in liver ($P < 0.05$, Wilcoxin test). The bacterial counts in other tissues (kidney and heart) did not differ significantly between the two strains, but the Δ*luxS* mutant strain was found more frequently in tissue

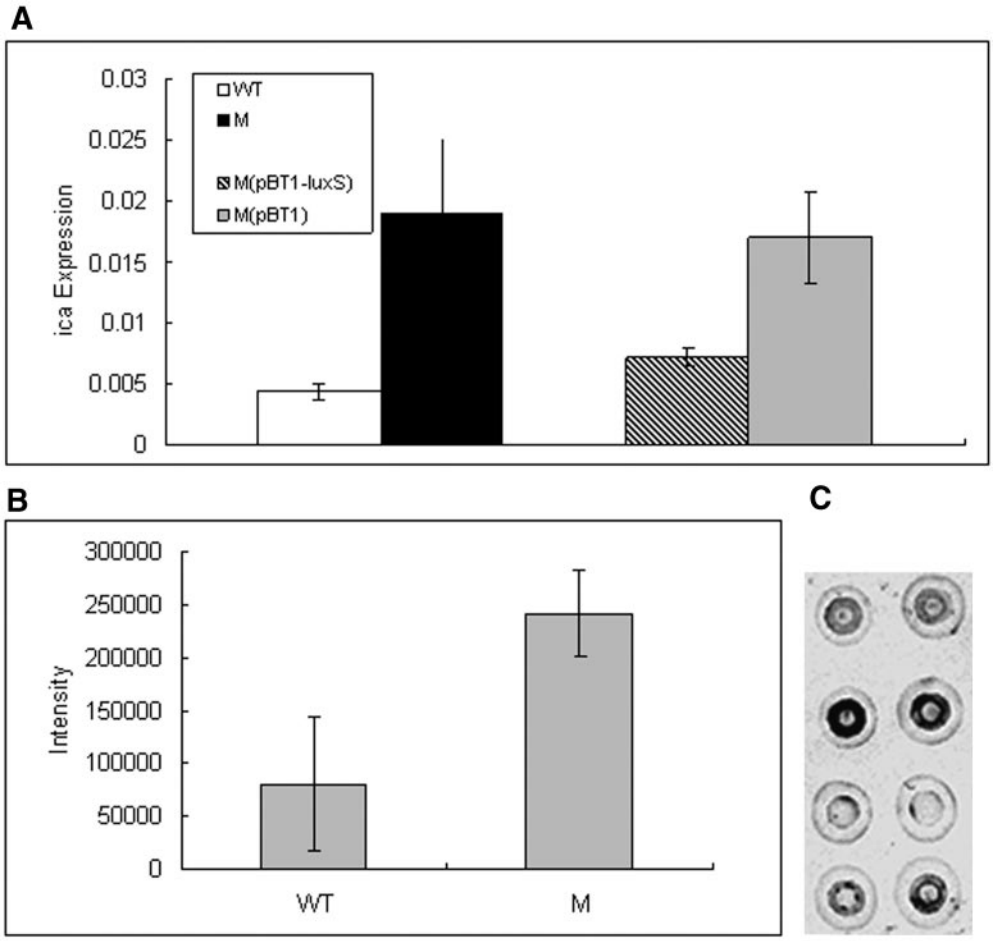

FIG. 7. A, TaqMan analysis of *ica* gene expression. TaqMan analysis was performed using an *icaC* probe, and cells were grown to mid-exponential phase. The levels of *ica* mRNA had been normalized to the level of 16S rRNA mRNA. Assays were performed in triplicate. Values given are the means plus or minus standard errors of the means. B, PIA production. PIA samples were isolated from the surface of cells grown to stationary growth phase by boiling with 0.5 mol/liter EDTA. PIA production was determined by immuno-dot blot analysis using anti-PIA antisera and quantified by photodigital analysis. Values are the means plus or minus standard errors of the means from three independent experiments with three measurements (nine dots per strain). C, Representative immuno-dot blot. First row, wild-type strain; second row, isogenic Δ*luxS* mutant strain; third row, empty vector control strain; fourth row, *luxS*-complemented strain.

samples. Thus, the deletion of *luxS* in *S. epidermidis* increased pathogen success during biofilm-associated infection.

DISCUSSION

Many bacteria use signaling systems to adapt gene expression to environmental changes. For example, QS systems control gene expression in response to cell density. The *luxS* QS system, which uses AI-2 signal, is a more recently described QS system (7). Importantly, it appears to be the most widespread QS system known so far. However, we lack information about the role of *luxS* QS control in important bacterial pathogens. Here we describe the *luxS*/AI-2 QS system in the genus *Staphylococcus*, which contains several important pathogens such as *S. aureus* and *S. epidermidis*.

The *luxS* system is not always accompanied by endogenous AI-2 activity, suggesting that the AI-2 QS system may not be operative in some bacteria in spite of the existence of *luxS*. For example, the *luxS* gene of *Borrelia burgdorferi* functionally complements a *luxS* deficiency in *E. coli* DH5α but AI-2 activity is not detectable in high-density culture supernatants of *B. burgdorferi* (5). Similar observations have been made in *Streptococcus pneumoniae* (24). Here we show that the *luxS* gene of *S. epidermidis* complements AI-2-deficient *E. coli*, and AI-2 is endogenously produced by *S. epidermidis* in a growth-phase-dependent fashion, with a peak observed during exponential growth. In contrast, AI-2 activity was minimal during stationary growth phase. This is consistent with previous reports for *E. coli* (47), *Salmonella typhimurium* serovar Typhimurium (47), *H. pylori* (27), *A. actinomycetemcomitans* (14), *S. mutans* (35), and *C. perfringens* (39).

To date, the *V. harveyi* AI-2 structure has been determined, but it is possible that the AI-2s synthesized by different bacteria are similar, resulting in a species-nonspecific language. Accordingly, *S. epidermidis*-conditioned medium containing AI-2 stimulated the *luxS* gene system in *V. harveyi*. However, the magnitude of light stimulation in *S. epidermidis* was lower than that of AI-2 in *V. harveyi* BB170 (data not shown). This might be due to unique modifications to the basic AI-2 structure or to

TABLE 3. Metastatic disease caused by *S. epidermidis* 1457 and its isogenic Δ*luxS* mutant in the rat CVC-associated infection model[b]

Organ	*S. epidermidis* 1457 (n = 15)		*S. epidermidis* 1457 Δ*luxS* mutant (n = 15)		P value
	No. rats infected/total	Median (range) CFU/g tissue	No. rats infected/total	Median (range) CFU/g tissue	
Liver[a]	4/15	0 (0–66,000)	11/15	333 (0–2,216,667)	0.0164
Kidney[a]	5/15	0 (0–308,000)	9/15	2,000 (0–3,673,333)	0.1002
Heart[a]	3/15	0 (0–66,667)	6/15	0 (0–1,140,000)	0.2195
Blood[a]	3/10	0 (0–130)	8/11	460 (0–10,980)	0.0154
Tube[a]		2,050 (1,750–3,050)		4,525 (2,050–7,600)	0.1084
Infection rate[b]		27.5 ± 2.8%		61.5 ± 7.8%	0.0064

[a] The difference in results among liver, kidney, heart, blood, and tube between the two strains was assessed by a Wilcoxon test.
[b] The overall infection rate is defined as recovery of *S. epidermidis* from the blood, catheter, or organs at the time of sacrifice. The P value for the infection rates of the mutant versus the wild type was 0.0064. The difference of infection rate between the two strains was assessed by a two-tailed t test.

a lower production level of AI-2 in *S. epidermidis* under the growth conditions that were used. In fact, interspecies signals secreted by *Salmonella* serovar Typhimurium have recently been demonstrated to be a distinct form of the AI-2 signal, although they are both derived from the same precursor (37).

Inactivation of the *luxS* gene of *S. epidermidis* did not result in noticeable changes in growth patterns compared with those of the wild-type strain in different media. This finding indicates that *luxS* has no significant effect on basic cellular metabolic processes required for growth of *S. epidermidis* in vitro. This is in contrast to some other bacteria in which *luxS* had an effect on growth (23, 29).

Our data showed that biofilm formation, the main virulence mechanism of *S. epidermidis*, was considerably enhanced in the Δ*luxS* mutant strain. This repressive effect on biofilm formation by *luxS* in vitro was also observed in *S. mutans* (35) and *H. pylori* (9), while the Δ*luxS* mutant of *Salmonella enterica* serovar Typhimurium was unable to develop a complete biofilm (42). Thus, *luxS* has contrasting effects on biofilm formation in different strains. A repressive effect on biofilm formation in *Staphylococcus* has also been described for the only other known QS system of *Staphylococcus*, *agr*, suggesting a common scheme of QS-dependent repression of the biofilm mode of growth in this genus. In contrast to *agr*, whose impact on biofilm formation is most likely via regulation of the production of the autolysin AtlE and the detergent-like phenol-soluble modulin peptides and which does not impact PIA production, *luxS* of *S. epidermidis* regulates transcription of the *ica* genes and production of PIA. This impact on the major biofilm exopolysaccharide PIA is most likely the cause for the different degree of biofilm formation of the Δ*luxS* mutant.

Decreased virulence has been seen in Δ*luxS* mutants of several pathogenic bacteria. A *Serratia marcescens* 274 Δ*luxS* mutant exhibits modestly attenuated virulence in a *Caenorhabditis elegans* model (10), a *Vibrio vulnificus* Δ*luxS* mutant has a significantly delayed time required to kill mice (25), and a *Neisseria meningitidis* Δ*luxS* mutant is attenuated for bacteremic infection in an infant rat model (55). In contrast, the *S. epidermidis* Δ*luxS* mutant, similar to the *agr* mutant of *S. epidermidis*, shows increased virulence in a model of catheter-associated infection. Most likely, the increased virulence may be partly attributed to the increased synthesis of PIA and more-intense biofilm formation.

Absent in humans, the LuxS enzyme is an attractive target for the development of novel therapeutic agents for bacterial infection. Inhibitors of LuxS have been synthesized (1). However, our present data, showing the increased capacity of biofilm formation of the Δ*luxS* mutant of *S. epidermidis* in vitro and increased virulence of the mutant in a rat model, suggest that such inhibitors are not suited for the treatment of biofilm-associated *S. epidermidis* infection.

In conclusion, we show that *luxS* in *S. epidermidis* is functional and *luxS*-dependent gene regulation represses biofilm formation in vitro and pathogen success during biofilm-associated infection. Although by regulating different biofilm factors, the two QS systems of *Staphylococcus*, *agr* and *luxS*, thus have very similar effects on the biofilm mode of growth.

ACKNOWLEDGMENTS

We thank Bonnie Bassler for the gift of *V. harveyi* strain BB170, Friedrich Gotz for providing *S. aureus* strain RN4220, and Reinhold Bruckner for kindly providing plasmid pBT1. We thank Steven D. Goodman for modification of the manuscript.

This work was supported by the Chinese National Natural Science Foundation Grant (30570087) and 211 Project Grant—Functional Genomics of Important Pathogenic Microorganisms. This work was also supported by the China National 973 Program (2002CB512804).

REFERENCES

1. **Alfaro, J. F., T. Zhang, D. P. Wynn, E. L. Karschner, and Z. S. Zhou.** 2004. Synthesis of LuxS inhibitors targeting bacterial cell-cell communication. Org. Lett. **6:**3043–3046.
2. **Augustin, J., and F. Gotz.** 1990. Transformation of Staphylococcus epidermidis and other staphylococcal species with plasmid DNA by electroporation. FEMS Microbiol. Lett. **54:**203–207.
3. **Bassler, B. L., E. P. Greenberg, and A. M. Stevens.** 1997. Cross-species induction of luminescence in the quorum-sensing bacterium *Vibrio harveyi*. J. Bacteriol. **179:**4043–4045.
4. **Bassler, B. L., M. Wright, and M. R. Silverman.** 1994. Multiple signalling systems controlling expression of luminescence in Vibrio harveyi: sequence and function of genes encoding a second sensory pathway. Mol. Microbiol. **13:**273–286.
5. **Blevins, J. S., A. T. Revel, M. J. Caimano, X. F. Yang, J. A. Richardson, K. E. Hagman, and M. V. Norgard.** 2004. The *luxS* gene is not required for *Borrelia burgdorferi* tick colonization, transmission to a mammalian host, or induction of disease. Infect. Immun. **72:**4864–4867.
6. **Bruckner, R.** 1997. Gene replacement in Staphylococcus carnosus and Staphylococcus xylosus. FEMS Microbiol. Lett. **151:**1–8.
7. **Chen, X., S. Schauder, N. Potier, A. Van Dorsselaer, I. Pelczer, B. L. Bassler, and F. M. Hughson.** 2002. Structural identification of a bacterial quorum-sensing signal containing boron. Nature **415:**545–549.
8. **Christensen, G. D., W. A. Simpson, J. J. Younger, L. M. Baddour, F. F. Barrett, D. M. Melton, and E. H. Beachey.** 1985. Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. J. Clin. Microbiol. **22:**996–1006.

9. **Cole, S. P., J. Harwood, R. Lee, R. She, and D. G. Guiney.** 2004. Characterization of monospecies biofilm formation by *Helicobacter pylori*. J. Bacteriol. **186:**3124–3132.
10. **Coulthurst, S. J., C. L. Kurz, and G. P. Salmond.** 2004. *luxS* mutants of *Serratia* defective in autoinducer-2-dependent 'quorum sensing' show strain-dependent impacts on virulence and production of carbapenem and prodigiosin. Microbiology **150:**1901–1910.
11. **Dobinsky, S., K. Kiel, H. Rohde, K. Bartscht, J. K. Knobloch, M. A. Horstkotte, and D. Mack.** 2003. Glucose-related dissociation between *icaADBC* transcription and biofilm expression by *Staphylococcus epidermidis*: evidence for an additional factor required for polysaccharide intercellular adhesin synthesis. J. Bacteriol. **185:**2879–2886.
12. **Duan, K., C. Dammel, J. Stein, H. Rabin, and M. G. Surette.** 2003. Modulation of Pseudomonas aeruginosa gene expression by host microflora through interspecies communication. Mol. Microbiol. **50:**1477–1491.
13. **Flamm, R. K., D. J. Hinrichs, and M. F. Thomashow.** 1984. Introduction of pAMβ1 into *Listeria monocytogenes* by conjugation and homology between native *L. monocytogenes* plasmids. Infect. Immun. **44:**157–161.
14. **Fong, K. P., W. O. Chung, R. J. Lamont, and D. R. Demuth.** 2001. Intra- and interspecies regulation of gene expression by *Actinobacillus actinomycetemcomitans* LuxS. Infect. Immun. **69:**7625–7634.
15. **Fong, K. P., L. Gao, and D. R. Demuth.** 2003. *luxS* and *arcB* control aerobic growth of *Actinobacillus actinomycetemcomitans* under iron limitation. Infect. Immun. **71:**298–308.
16. **Fuqua, W. C., S. C. Winans, and E. P. Greenberg.** 1994. Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators. J. Bacteriol. **176:**269–275.
17. **Gerke, C., A. Kraft, R. Sussmuth, O. Schweitzer, and F. Gotz.** 1998. Characterization of the *N*-acetylglucosaminyltransferase activity involved in the biosynthesis of the *Staphylococcus epidermidis* polysaccharide intercellular adhesin. J. Biol. Chem. **273:**18586–18593.
18. **Gotz, F.** 2002. Staphylococcus and biofilms. Mol. Microbiol. **43:**1367–1378.
19. **Hanahan, D.** 1983. Studies on transformation of Escherichia coli with plasmids. J. Mol. Biol. **166:**557–580.
20. **Heilmann, C., M. Hussain, G. Peters, and F. Gotz.** 1997. Evidence for autolysin-mediated primary attachment of Staphylococcus epidermidis to a polystyrene surface. Mol. Microbiol. **24:**1013–1024.
21. **Heilmann, C., O. Schweitzer, C. Gerke, N. Vanittanakom, D. Mack, and F. Gotz.** 1996. Molecular basis of intercellular adhesion in the biofilm-forming Staphylococcus epidermidis. Mol. Microbiol. **20:**1083–1091.
22. **Henke, J. M., and B. L. Bassler.** 2004. Three parallel quorum-sensing systems regulate gene expression in *Vibrio harveyi*. J. Bacteriol. **186:**6902–6914.
23. **Jones, M. B., and M. J. Blaser.** 2003. Detection of a *luxS*-signaling molecule in *Bacillus anthracis*. Infect. Immun. **71:**3914–3919.
24. **Joyce, E. A., A. Kawale, S. Censini, C. C. Kim, A. Covacci, and S. Falkow.** 2004. LuxS is required for persistent pneumococcal carriage and expression of virulence and biosynthesis genes. Infect. Immun. **72:**2964–2975.
25. **Kim, S. Y., S. E. Lee, Y. R. Kim, C. M. Kim, P. Y. Ryu, H. E. Choy, S. S. Chung, and J. H. Rhee.** 2003. Regulation of Vibrio vulnificus virulence by the LuxS quorum-sensing system. Mol. Microbiol. **48:**1647–1664.
26. **Kozitskaya, S., S. H. Cho, K. Dietrich, R. Marre, K. Naber, and W. Ziebuhr.** 2004. The bacterial insertion sequence element IS*256* occurs preferentially in nosocomial *Staphylococcus epidermidis* isolates: association with biofilm formation and resistance to aminoglycosides. Infect. Immun. **72:**1210–1215.
27. **Loh, J. T., M. H. Forsyth, and T. L. Cover.** 2004. Growth phase regulation of *flaA* expression in *Helicobacter pylori* is *luxS* dependent. Infect. Immun. **72:**5506–5510.
28. **Lupp, C., and E. G. Ruby.** 2004. *Vibrio fischeri* LuxS and AinS: comparative study of two signal synthases. J. Bacteriol. **186:**3873–3881.
29. **Lyon, W. R., J. C. Madden, J. C. Levin, J. L. Stein, and M. G. Caparon.** 2001. Mutation of luxS affects growth and virulence factor expression in Streptococcus pyogenes. Mol. Microbiol. **42:**145–157.
30. **Mack, D., W. Fischer, A. Krokotsch, K. Leopold, R. Hartmann, H. Egge, and R. Laufs.** 1996. The intercellular adhesin involved in biofilm accumulation of *Staphylococcus epidermidis* is a linear β-1,6-linked glucosaminoglycan: purification and structural analysis. J. Bacteriol. **178:**175–183.
31. **Mack, D., M. Haeder, N. Siemssen, and R. Laufs.** 1996. Association of biofilm production of coagulase-negative staphylococci with expression of a specific polysaccharide intercellular adhesin. J. Infect. Dis. **174:**881–884.
32. **Mack, D., H. Rohde, S. Dobinsky, J. Riedewald, M. Nedelmann, J. K. Knobloch, H. A. Elsner, and H. H. Feucht.** 2000. Identification of three essential regulatory gene loci governing expression of *Staphylococcus epidermidis* polysaccharide intercellular adhesin and biofilm formation. Infect. Immun. **68:**3799–3807.
33. **Mack, D., N. Siemssen, and R. Laufs.** 1992. Parallel induction by glucose of adherence and a polysaccharide antigen specific for plastic-adherent *Staphylococcus epidermidis*: evidence for functional relation to intercellular adhesion. Infect. Immun. **60:**2048–2057.
34. **McNab, R., S. K. Ford, A. El-Sabaeny, B. Barbieri, G. S. Cook, and R. J. Lamont.** 2003. LuxS-based signaling in *Streptococcus gordonii*: autoinducer 2 controls carbohydrate metabolism and biofilm formation with *Porphyromonas gingivalis*. J. Bacteriol. **185:**274–284.
35. **Merritt, J., F. Qi, S. D. Goodman, M. H. Anderson, and W. Shi.** 2003. Mutation of *luxS* affects biofilm formation in *Streptococcus mutans*. Infect. Immun. **71:**1972–1979.
36. **Miller, M. B., and B. L. Bassler.** 2001. Quorum sensing in bacteria. Annu. Rev. Microbiol. **55:**165–199.
37. **Miller, S. T., K. B. Xavier, S. R. Campagna, M. E. Taga, M. F. Semmelhack, B. L. Bassler, and F. M. Hughson.** 2004. Salmonella typhimurium recognizes a chemically distinct form of the bacterial quorum-sensing signal AI-2. Mol. Cell **15:**677–687.
38. **Novick, R. P.** 2003. Autoinduction and signal transduction in the regulation of staphylococcal virulence. Mol. Microbiol. **48:**1429–1449.
39. **Ohtani, K., H. Hayashi, and T. Shimizu.** 2002. The luxS gene is involved in cell-cell signalling for toxin production in Clostridium perfringens. Mol. Microbiol. **44:**171–179.
40. **Otto, M., R. Sussmuth, G. Jung, and F. Gotz.** 1998. Structure of the pheromone peptide of the Staphylococcus epidermidis agr system. FEBS Lett. **424:**89–94.
41. **Pei, L., and J. I. Flock.** 2001. Functional study of antibodies against a fibrogenin-binding protein in Staphylococcus epidermidis adherence to polyethylene catheters. J. Infect. Dis. **184:**52–55.
42. **Prouty, A. M., W. H. Schwesinger, and J. S. Gunn.** 2002. Biofilm formation and interaction with the surfaces of gallstones by *Salmonella* spp. Infect. Immun. **70:**2640–2649.
43. **Rupp, M. E., J. S. Ulphani, P. D. Fey, and D. Mack.** 1999. Characterization of *Staphylococcus epidermidis* polysaccharide intercellular adhesin/hemagglutinin in the pathogenesis of intravascular catheter-associated infection in a rat model. Infect. Immun. **67:**2656–2659.
44. **Schneider, K. B., T. M. Palmer, and A. D. Grossman.** 2002. Characterization of *comQ* and *comX*, two genes required for production of ComX pheromone in *Bacillus subtilis*. J. Bacteriol. **184:**410–419.
45. **Sperandio, V., A. G. Torres, J. A. Giron, and J. B. Kaper.** 2001. Quorum sensing is a global regulatory mechanism in enterohemorrhagic *Escherichia coli* O157:H7. J. Bacteriol. **183:**5187–5197.
46. **Stroeher, U. H., A. W. Paton, A. D. Ogunniyi, and J. C. Paton.** 2003. Mutation of *luxS* of *Streptococcus pneumoniae* affects virulence in a mouse model. Infect. Immun. **71:**3206–3212.
47. **Surette, M. G., and B. L. Bassler.** 1998. Quorum sensing in Escherichia coli and Salmonella typhimurium. Proc. Natl. Acad. Sci. USA. **95:**7046–7050.
48. **Surette, M. G., M. B. Miller, and B. L. Bassler.** 1999. Quorum sensing in Escherichia coli, Salmonella typhimurium, and Vibrio harveyi: a new family of genes responsible for autoinducer production. Proc. Natl. Acad. Sci. USA **96:**1639–1644.
49. **Vuong, C., C. Gerke, G. A. Somerville, E. R. Fischer, and M. Otto.** 2003. Quorum-sensing control of biofilm factors in Staphylococcus epidermidis. J. Infect. Dis. **188:**706–718.
50. **Vuong, C., S. Kocianova, Y. Yao, A. B. Carmody, and M. Otto.** 2004. Increased colonization of indwelling medical devices by quorum-sensing mutants of Staphylococcus epidermidis in vivo. J. Infect. Dis. **190:**1498–1505.
51. **Vuong, C., and M. Otto.** 2002. Staphylococcus epidermidis infections. Microbes Infect. **4:**481–489.
52. **Vuong, C., H. L. Saenz, F. Gotz, and M. Otto.** 2000. Impact of the *agr* quorum-sensing system on adherence to polystyrene in Staphylococcus aureus. J. Infect. Dis. **182:**1688–1693.
53. **Winans, S. C., and B. L. Bassler.** 2002. Mob psychology. J. Bacteriol. **184:**873–883.
54. **Winzer, K., K. R. Hardie, N. Burgess, N. Doherty, D. Kirke, M. T. Holden, R. Linforth, K. A. Cornell, A. J. Taylor, P. J. Hill, and P. Williams.** 2002. LuxS: its role in central metabolism and the in vitro synthesis of 4-hydroxy-5-methyl-3(2H)-furanone. Microbiology **148:**909–922.
55. **Winzer, K., Y. H. Sun, A. Green, M. Delory, D. Blackley, K. R. Hardie, T. J. Baldwin, and C. M. Tang.** 2002. Role of *Neisseria meningitidis luxS* in cell-to-cell signaling and bacteremic infection. Infect. Immun. **70:**2245–2248.
56. **Xavier, K. B., and B. L. Bassler.** 2003. LuxS quorum sensing: more than just a numbers game. Curr. Opin. Microbiol. **6:**191–197.
57. **Yao, Y., D. E. Sturdevant, and M. Otto.** 2005. Genomewide analysis of gene expression in Staphylococcus epidermidis biofilms: insights into the pathophysiology of S. epidermidis biofilms and the role of phenol-soluble modulins in formation of biofilms. J. Infect. Dis. **191:**289–298.
58. **Zhang, Y. Q., S. X. Ren, H. L. Li, Y. X. Wang, G. Fu, J. Yang, Z. Q. Qin, Y. G. Miao, W. Y. Wang, R. S. Chen, Y. Shen, Z. Chen, Z. H. Yuan, G. P. Zhao, D. Qu, A. Danchin, and Y. M. Wen.** 2003. Genome-based analysis of virulence genes in a non-biofilm-forming Staphylococcus epidermidis strain (ATCC 12228). Mol. Microbiol. **49:**1577–1593.

Editor: F. C. Fang