Involvement of LuxS in the Regulation of Motility and Flagella Biogenesis in Vibrio alginolyticus

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The fish pathogen Vibrio alginolyticus contains two unique flagellar systems. The LuxS quorum sensing system is reported to regulate the expression of virulence factors in a wide variety of pathogenic bacteria. Our previous work demonstrated that inactive luxS led to decreased virulence in V. alginolyticus. In this study, LuxS-dependent regulation of motility and flagella biogenesis, the potential virulence factors in V. alginolyticus, were further investigated. A luxS-deleted mutant showed deficiency in motility and flagella formation, and an intact luxS complemented the defect. Since motility is flagella dependent, V. alginolyticus flagella biogenesis related genes, including the flagella regulator genes fliK and lafK and the sub-hierarchical flagellar genes fliS and lafA, were cloned and identified. Based on quantitative real-time reverse transcription-PCR, differential expression of these genes was confirmed in wild-type and luxS mutants. Our results indicate that LuxS plays an important role in the regulation of motility and flagella biogenesis in V. alginolyticus.

Key words: flagella; LuxS; motility; quorum sensing; Vibrio alginolyticus

Bacterial mobility, a shared mechanism among numerous microorganisms, is essential for pathogenic bacteria during their initial invasion and colonization process of injection. Hence it is considered to be an important virulence factor in many pathogens.1) Bacteria use flagella through liquids (swimming) and through highly viscous environments and along surfaces (swarming) to survive in different niches.2) Because flagella biogenesis and the powering of flagella rotation are costly energy expending processes, bacteria have to assemble their flagella in a hierarchical regulatory pattern to avoid energy waste.3) The most well known flagellar gene hierarchical system belongs to Enterobacteriaceae, in which three classes of flagellar genes are controlled by the master regulator FlhDC.4,5) In Vibrio sp., a σ28-dependent regulator, belonging to the NtrC family, is responsible for the early flagellar gene expression of Vibrio cholerae and Vibrio parahaemolyticus.6) Particularly, it has been demonstrated that a σ28-dependent regulator controls late flagellar gene expression in V. parahaemolyticus.7)

Quorum sensing is a bacterial cell-cell communication system using autoinducers (AIs) to modulate diverse behaviors in response to population density.3) AI-1 is considered to be an intra-species communication signal molecule due to its distinct structure in various species. By contrast, AI-2, which is produced through the action of the LuxS enzyme, is believed to be an inter-species communication autoinducer.9) Indeed, luxS homologs have been found in more than 55 species in gram positive and negative bacteria.10) Mutation of the luxS gene can not only entirely eliminate AI-2 activity, but can also severely impact various cellular events, including biofilm formation,11–13) hemin and iron acquisition,14) leukotoxin production,15) and type III secretion.16,17)

The gram-negative bacterium V. alginolyticus is one of the most invasive and highly fatal fish pathogens in the South China Sea. It can cause vibriosis in the large yellow croaker, sea bream, grouper, and Kuruma prawn, as well as in the larvae of fish and shellfish species.18,19) Moreover, it has been reported to cause gastroenteritis, conjunctivitis, and otitis in humans.20) V. alginolyticus has an unique character in that it has two types of flagella: (i) a single polar flagellum, suited to swim in a liquid environment and synthesized constitutively; (ii) numerous lateral flagella, suited to swarm over the surfaces of animate and inanimate objects, and produced only under conditions in which the polar flagellum is not functional.21,22) Previous detailed research on the V. alginolyticus flagellar system was focused on the polar flagellum rotating mode,23,24) ion-motive...
forces,\textsuperscript{22,25} and chemotaxis,\textsuperscript{22} but until now there have no reports on the motile factor regulated by the LuxS quorum sensing system in \textit{V. alginolyticus}.

In a previous study, we found that \textit{V. alginolyticus} MVP01, a pathogen isolated from a vibriosis outbreak in caged-cultured Pseudociona crocea (Richardon) in the South China Sea in 1999, possessed the luxS gene and produced functional Al-2. luxS-null mutation of \textit{V. alginolyticus} has been found to exhibit decreased virulence in a fish model (Ye \textit{et al.}, unpublished). Since motility is an important virulence factor in many pathogens, especially in invasion and colonization in the initial infection phase, investigation of the role of luxS in the regulation of motility is important in understanding the virulence mechanism of \textit{V. alginolyticus}.

This study, we constructed a luxS in-frame deletion mutant, investigated the effect of luxS deletion on \textit{V. alginolyticus} motility (both swimming and swarming) and flagellar morphology, and further elucidated the LuxS-dependent regulation of \textit{V. alginolyticus} flagellar system genes by quantitative real-time reverse transcription-PCR (qRT-PCR). Our work strongly confirmed LuxS involvement in the regulation of motility and flagella biogenesis in \textit{V. alginolyticus}.

**Materials and Methods**

**Bacterial strains, plasmids, and growth conditions.** The strains and plasmids utilized in this study are listed in Table 1. \textit{V. alginolyticus} was grown at 30 °C in Luria-Bertani (LB) broth containing 3% NaCl (LBS). For bioluminescence assay, \textit{V. alginolyticus} was cultured in Al bioassay (AB) medium.\textsuperscript{9} \textit{E. coli} strains were grown in LB broth (Oxoid, Basingstoke, UK) or on LB agar (1.5%) at 37 °C. \textit{V. harveyi} reporter strain BB170 (sensor 1\textsuperscript{+}, sensor 2\textsuperscript{+}) was kindly provided by B. Bassler (Princeton University), and was grown in LB broth (Oxoid, Basingstoke, UK) or on LB agar (1.5%) at 37 °C. Antibiotics, when necessary, were used at concentrations: for \textit{V. alginolyticus} and \textit{E. coli}, Ap at 100 μg/ml and chloramphenicol (Cm) at 7 μg/ml; for \textit{E. coli}, Ap at 100 μg/ml and Cm at 20 μg/ml.

DNA manipulation. General recombinant DNA techniques were performed according to standard protocol.\textsuperscript{26} Restriction enzyme digestion, ligation, and plasmid purification were done in accordance with the manuals of the manufacturers (Takara, Dalian, China). DNA sequencing and primer synthesis were carried out by Invitrogen (Shanghai, China).

Construction of \textit{V. alginolyticus} ΔluxS, luxS\textsuperscript{+} and flagellar gene mutant strains. All primers used in mutant construction are given in Table 2. The luxS in-frame deletion mutant of \textit{V. alginolyticus} was constructed by allelic replacement. A DNA fragment containing 90 bp of the 5' end of luxS and 432 bp upstream of the ATG initiation codon was amplified from chromosomal DNA by PCR using primers luxSdeup-F and luxSdeup-R. A DNA fragment containing 39 bp of the 3' end of luxS and 342 bp downstream of the stop codon was amplified using primers luxSdown-F and luxSdown-R. Both fragments were purified and fused in a subsequent PCR reaction using primers luxSdeup-F and luxSdown-R. The fused segment was sequenced and ligated into suicide vector pDM4.\textsuperscript{27} The resulting plasmid, pDM-luxS, was mated from \textit{E. coli} SM10 \textit{Apr}\textsuperscript{28} into \textit{V. alginolyticus} MVP01, and the transconjugants with the plasmid integrated into the chromosome by homol-
Table 2. Primers Used in Cloning and qRT-PCR

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5'-3')</th>
</tr>
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<tbody>
<tr>
<td>lux5deup-F</td>
<td>ACTAGTCCGCTGACGTTCCGTATGTT</td>
</tr>
<tr>
<td>lux5deup-R</td>
<td>CACGACTAGTACGGCAACCTCCCTTTGGGTCTGCTGATGG</td>
</tr>
<tr>
<td>lux5deown-F</td>
<td>CAGACCCCCCAAAAGGGTTGGCAGTGATGTTAAA</td>
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<tr>
<td>lux5deown-R</td>
<td>AGATCTCGCTCCCTCAGGTAAGCTACG</td>
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<tr>
<td>luxS-F</td>
<td>CGATGATGCACAGATGTAAGT</td>
</tr>
<tr>
<td>luxS-R</td>
<td>ACCACTGAACTGCAGTAATGTGAAG</td>
</tr>
<tr>
<td>luxScomF</td>
<td>GAAATTCCGAGATACATGCTTGGAAGAAGC</td>
</tr>
<tr>
<td>luxScomR</td>
<td>CTGGAGACGCCACACACTGACGTAAGCATG</td>
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<td>lafKinF</td>
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<tr>
<td>lafKinR</td>
<td>ACTAGTGCGAGGTCTTGGGAGAAGCAAAGAG</td>
</tr>
<tr>
<td>flaKinF</td>
<td>ACTAGTGGCGAGTCTTGGGAGAAGCAAAGAG</td>
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<tr>
<td>flaKinR</td>
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</tr>
<tr>
<td>flaK2</td>
<td>GTCACGCACAAACACAGCTTTAGT</td>
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<tr>
<td>lafAinF</td>
<td>CTGGAGGGTAAAGCAACAGACGATCTCTAT</td>
</tr>
<tr>
<td>lafAinR</td>
<td>AGATCTGTTACCAGAAGTGGCAGCATGTCAG</td>
</tr>
<tr>
<td>flaG2</td>
<td>TCTAAACGACAGCTACGCTGAGG</td>
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<tr>
<td>flsInF</td>
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<td>flsInR</td>
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<td>flaS</td>
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<td>pNQdown</td>
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<tr>
<td>flaSR</td>
<td>CTCGCGGTCGAGGAGCCGGCTTATTCA</td>
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*aNucleotides in bold represent restriction enzyme sites added to the 5' region of the primer.

- AI-2 assay. AI-2 activity in the cell-free V. alginolyticus MVP01 culture supernatants was measured by V. harveyi BB170 bioluminescence reporter assay. Briefly, culture supernatants were prepared by concentration and filtration with 0.22-μm filtration membranes. The reporter strain V. harveyi BB170, grown for 16 h with shaking at 30°C in AB medium, was diluted 1:10,000 in fresh AB medium, and then 90 μl of culture was mixed with 10 μl of cell-free sample and incubated at 30°C. Luminescence was measured hourly using a FB12 Single Tube Luminometer (Berthold, Bad Wildbad, Germany). The supernatants of V. harveyi ATCC 33342 and E. coli DH5α, prepared by the same method, were served as positive and negative control respectively.

- Swimming and swarming motility assays. Swimming and swarming motility assays were performed on LBS agar plates that contained 0.3% or 1.5% agar (Genebase, Shanghai, China) respectively. Each strain was inoculated into 5 ml of LBS broth and incubated for 16 h with shaking. Overnight culture of each V. alginolyticus strain (5 μl) was spotted onto the swimming or swarming assay plate for 16 h of incubation.

- Conditioned medium preparation. Cell-free conditioned medium (CM) from V. alginolyticus MVP01 was prepared as follows: cultures were grown overnight and then incubated and diluted 1:100, followed by resumption of shaking at 30°C for 5 h (achieved exponential phase). Cell-free culture was harvested by centrifugation and filtered as described above.

- Transmission electron microscopy. Bacterial cells were cultured on either swimming or swarming plates overnight at 30°C, collected, and suspended in 2.5% glutaraldehyde. A drop was spotted onto a copper grid Formvar coated, and negatively stained with 2% phosphotungstate (pH 6.0) for 30 s. The samples were examined under a HITACHI 600 transmission electron microscope (TEM).

- Quantitative real-time reverse transcription PCR. Overnight cultures were incubated on LBS agar plates containing either 0.3% or 1.5% agar at 30°C. The cells were collected and RNA was isolated with an RNA isolating kit (Tiangen, Beijing, China). The RNA was subjected to DnaseI (Promega, Madison, WI) treatment and subjected to DnaseI (Promega, Madison, WI) treatment and allowed to recombine into the chromosome. The insertion of the plasmid into the corresponding gene of V. alginolyticus MVP01 and generation of the lafK′, flaK′, lafA′, and fls′ mutants were confirmed by PCR and sequencing with primers complementary to the pNQ705-1 vector just outside the linker region (pNQdown), and to regions upstream of the internal fragments (lafKF2, flaKF2, lafAF2, and flsF2 respectively).
to exclude the genomic DNA contaminant. A first-strand cDNA synthesis Kit (Toyobo, Tsuruga, Japan) was used to generate cDNA from RNA (1 μg) templates with the relevant specific primer. qRT-PCR was carried out by performing three independent experiments, each in triplicate, with a FTC-200 detector (Funglyn Biotech, Shanghai, China), and transcript levels were normalized to 16S rRNA in each sample by the ΔΔCt method. The primers for qRT-PCR listed in Table 2 were designed using Primer Express software (Applied Biosystems, Foster city, CA) with predicted product sizes in the 100 to 200 bp range.

Nucleotide sequence accession number. The NCBI accession numbers for the sequences described in this study were assigned as EF623983 (lafK), EF634326 (lafA), EF634327 (flaK), and EF623982 (fltS).

Results

Construction of luxS in-frame deletion mutant and complemented strain

A luxS in-frame deletion mutant, referred as ΔluxS, was constructed by allelic replacement as described above. To ensure that the observed phenotypes of the mutant strain were due to disruption of luxS, a complemented strain, referred to as luxS+, was constructed by introducing the wild-type (WT) copy luxS gene with its putative promoter region. Culture supernatants of wild-type strain MVP01, the ΔluxS mutant, and complemented strain luxS+ at the logarithmic growth phase were isolated, and their abilities to induce bioluminescence of V. harveyi reporter strain BB170 were detected. Compared with MVP01, ΔluxS obviously lost the ability to produce AI-2, while luxS+ restored AI-2 activity to a level equal to that of the wild-type strain (Fig. 1). This indicates that ΔluxS and luxS+ were constructed successfully and the complement strain restored AI-2 production.

Effects of the luxS mutation on the motility of V. alginolyticus

V. alginolyticus contains two unique flagellar systems, polar and lateral flagella, which are responsible for swimming and swarming respectively. In order to investigate the effect of luxS mutation on motility, wild-type strain MVP01, deletion mutant ΔluxS, and complemented strain luxS+ were inoculated on soft agar plates for swimming analysis and on hard agar plates for swarming analysis. On soft agar plates, the wild-type strain moved so fast that it spread across the whole surface of the plates, but ΔluxS did not move at all unless it was complemented with the wild-type luxS gene (Fig. 2A). When grown on hard agar plates, ΔluxS produced a much smaller swarm halo than the wild-type strain did, and luxS+ produced almost the same size halo as that of MVP01 (Fig. 2B). Moreover, since LuxS is responsible for the biosynthesis of AI-2, we further investigated whether the decreased motility of V. alginolyticus ΔluxS mutant strain was due to an AI-2 signaling event. CM, containing exogenous AI-2, was prepared from logarithmic growth phase culture of wild-type MVP01 and added to the swimming and swarming plates. As shown in Fig. 2, when growing on 50% CM supplemented plates, ΔluxS showed the same swimming and swarming motility as that of the wild type, indicating the effect of V. alginolyticus LuxS on motility is through a signaling mechanism based on AI-2 secretion.

Effects of luxS mutation on the flagellar morphology of V. alginolyticus

To determine whether the swimming and swarming motility deficiency of ΔluxS was caused by non-functional or impaired flagella, we examined the swimming and swarming cells of wild-type strain MVP01, ΔluxS, and luxS+ by TEM. For swimming cells, a polar flagellum 3–5 times the length of the cell body endowed the wild-type strain with strong swimming ability, while ΔluxS did not possess a polar flagellum any more, and the complemented strain luxS+ restored the ability to produce polar flagellum (Fig. 3A). For swarming cells, MVP01 produced numerous lateral flagella around the cell body, while the ΔluxS mutant had few lateral flagella, and intact luxS completely restored the flagellar phenotype in the luxS+ strain (Fig. 3B). These results indicate that LuxS plays an important role in motility and flagella formation, and that the loss of swimming and swarming motility shown by ΔluxS was due to deficient synthesis of flagella.

Cloning and identification of flagellar system genes in V. alginolyticus

Since changes in mobility and flagellar morphology exist in the ΔluxS mutant, it is of interest to elucidate the LuxS-dependent regulation of flagellar system genes in
According to their counterparts in the closely related *V. cholerae* and *V. parahaemolyticus*, the flagellar system genes of *V. alginolyticus* were selected and cloned by PCR, as listed in Table 3. These flagella related genes shared high homology with that of *V. parahaemolyticus* (Table 3). Their corresponding disrupted mutants, *lafK<sup>−</sup>*, *lafA<sup>−</sup>*, *flaK<sup>−</sup>* and *fliS<sup>−</sup>* were constructed as described in “Materials and Methods.” In the swimming motility assay (Fig. 4A), the polar flagellar system mutant *fliS<sup>−</sup>* completely lost swimming motility, but *flaK<sup>−</sup>* showed residual swimming motility, implying that some regulators, such as *rpoN* and lateral flagellar genes, cooperating with *flaK* to modulate the polar flagellar system, might be responsible for compensating the motility activation in *V. alginolyticus*. The lateral flagellar system mutants *lafK<sup>−</sup>* and *lafA<sup>−</sup>*, owing to a normal polar flagellum, showed swimming ability equivalent to MVP01. In the swarming motility assay (Fig. 4B), the lateral flagellar system mutants *lafK<sup>−</sup>* and *lafA<sup>−</sup>* did not move on the hard agar plate, indicating destroyed lateral flagella, and the polar flagellar system mutants *flaK<sup>−</sup>* and *fliS<sup>−</sup>* moved on the swarming plate as the wild-type did, indicating intact lateral flagella.

**Transcriptional regulation of flagellar system genes by LuxS**

The differential expression of these identified flagellar system genes in the ΔluxS mutant was investigated by...
qRT-PCR. On semi-solid swimming plates, the polar flagella related genes flaK and fliS were notably down-regulated in the ΔluxS mutant, but the lateral flagella related genes lafK and lafA were up-regulated about 2-fold (Fig. 5A), suggesting that impaired polar flagellum activates expression of the lateral flagella.\(^{21,33}\) On the solid swarming plates, expression of the lateral flagella related genes lafK and lafA in ΔluxS apparently decreased (Fig. 5B), consistently with the deficiency in swarming motility and the abnormal lateral flagella (Figs. 2B and 3B), while the transcript of the polar flagella related genes flaK and fliS was down-regulated and kept the same level as that in the wild-type strain (Fig. 5B). A further experiment confirmed that the intact luxS gene recovered the transcription of these genes in the luxS\(^+\) strain to the level of the wild-type strain in both swimming and swarming cells, though the expression level of lafA in the swimming cells was about 2-fold higher than the wild-type level (Fig. 5). The qRT-PCR results, combined with the phenotypes observed on the swimming and swarming plates, clearly indicate that LuxS was indeed involved in flagella biosynthesis by controlling the flagellar gene transcription hierarchy, and consequently influenced \(V.\) alginolyticus motility of both swimming and swarming.

**Discussion**

Most bacteria use single/multiple polar flagella or peritrichous flagella to move in a wide variety of environments to adapt to favorable and unfavorable conditions to survive in the nature. However, a few bacteria, including \(V.\) parahaemolyticus, Aeromonas sp., Azospirillum brasilense, and Rhodospirillum centenum, are found to possess dual flagellar systems to express polar and lateral flagellar systems.\(^{33}\) \(V.\) alginolyticus was reported to be one of the most invasive and highly fatal fish pathogens in the South China Sea, possessing two types of unique flagellar systems. Though some factors in this organism have been reported to be related to virulence,\(^{34–36}\) to date no key virulent mechanism has been established in detail in \(V.\) alginolyticus.\(^{19}\)

The LuxS quorum sensing system has been reported to regulate the expression of virulence factors in a wide
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Motility compensation.21) In our work, the deficiency in LuxS-regulated motility and flagella was observed under LuxS mutation and complementation, 41 suggesting that the LuxS-dependent regulation of motility is not common in Vibrio sp.

Regarding bacteria motility, two types of mutation can cause non-motile cells: impaired flagella biosynthesis and deficient flagella motor.42) For example, the mutation of luxS led to decreased motility in H. pylori and C. jejuni, 37,43 and at the same time normal morphological flagella were observed, indicating that the luxS mutation probably affected the flagella motor and chemotaxis, but not flagella biosynthesis.43 In our work, obvious morphological changes in the polar and lateral flagella in the V. alginolyticus luxS mutant were found (Fig. 3), so it was assumed that LuxS-regulated motility in V. alginolyticus might occur through controlling flagella biogenesis system genes. Based on this assumption, two key early flagellar regulator genes flaK and lafK, the sub-hierarchical polar flagellin chaperone gene fliS, and the major lateral flagellin gene lafA were chosen to elucidate the regulatory function of LuxS on flagellar system. Transcription analysis by qRT-PCR confirmed that LuxS regulated both the polar and the lateral flagellar systems (Fig. 5). However, with the notable down-regulation of flagellar genes flaK and fliS, the lateral flagellar genes lafK and lafA were up-regulated about 2-fold in semi-solid swimming plates (Fig. 5A). Since the polar flagellum is considered to be a mechanosensor, 21 it was assumed that the polar flagellum could sense decreased swimming motility and then active the expression of the lateral flagella for motility compensation.21)

LuxS, as an AI-2 synthase, is believed to regulate virulence factors of pathogens through the AI-2 signaling mechanism.14,45,46 In our work, the deficiency in V. alginolyticus motility due to the luxS mutation was also found to be complemented by exogenous AI-2. Since the LuxS quorum sensing system includes AI-2 synthesis and the AI-2 signaling system, we further investigated the effects of PfS, an important nucleosidase involved in AI-2 synthesis, and LuxP, AI-2 receptor, on V. alginolyticus motility. The pfs mutant lacked AI-2 production disrupting AI-2 synthesis pathway, and the luxP mutant accumulated extracellular AI-2 blocking the AI-2 signaling pathway. Both of these mutants showed the same decreased motility that luxS mutant did (Tian et al., unpublished). These findings strongly confirm that LuxS involvement in the regulation of motility is dependent on AI-2 signaling.

It is well known that flagella biogenesis is an expensive, energy-consuming process, that possesses a precise regulation mechanism directed not only by regulators belonging to the flagellar system, but also by some higher central regulators outside that system.2 In this study, based on homologous analysis with V. parahaemolyticus, a hierarchical flagellar system was found in the V. alginolyticus chromosome, and the luxS/AI-2 quorum sensing system was also confirmed to regulate not only the sub-hierarchical flagella related genes fliS and lafA, but also the important top flagella regulator genes flaK and lafK, which strongly suggests that the luxS/AI-2 system is in a higher regulatory hierarchy than the flagellar system regulators. Hence we suggest that the luxS/AI-2 quorum sensing system regulates the flagella biogenesis system in V. alginolyticus (Fig. 6).

In E. coli strain K-12, FlhDC, the master regulator of the flagellar system, was found strictly to control a series of sub-hierarchical flagella-related genes, and simultaneously to be regulated by the luxS/AI-2 quorum sensing system through a key intermediate regulator, QseBC, 47 but no homolog of QseBC was found in V. alginolyticus in a genome wide research in our study. Hence we speculate that there might exist some unknown factors that bridge the luxS/AI-2 quorum signaling system and the flagella biogenesis system.
sensing system and the flagellar hierarchy, conferring more precise control over flagella biosynthesis or assembly in *V. alginolyticus* (Fig. 6). Nevertheless, our previous work indicates that rpoN mutant loses motility in *V. alginolyticus* MVP01,\(^5\) and it has been demonstrated that σ\(^54\) plays a role in motility by activating transcription of at least one flagellar gene in *V. flacheri*.\(^6\) Hence we assumed that σ\(^54\) might be among the unknown factors mediating the regulation of flagella biogenesis by the luxS/AI-2 system in *V. alginolyticus*. Further characterization of the intermediates between the luxS/AI-2 system and flagella biogenesis is definitely of interest.

**Acknowledgments**

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**References**

25. Atsumi, T., McCarter, L., and Imae, Y., Polar and lateral


