The LuxS/AI-2 Quorum-Sensing System Regulates the Algicidal Activity of *Shewanella xiamenensis* Lzh-2

Jian Liu1, Kaiquan Liu2, Zhe Zhao1, Zheng Wang1, Fengchao Wang1, Yuxiu Xin1, Jie Qu1, Feng Song1* and Zhenghua Li1**

1 Shandong Key Laboratory of Biophysics, Institute of Biophysics, Dezhou University, Dezhou, China, 2 School of Bioengineering, Qilu University of Technology, Shandong Academy of Sciences, Jinan, China

Cyanobacterial blooming is an increasing environmental issue all over the world. Algicidal bacteria are potential tools for the control of algal blooms. The algicidal activity in many bacteria exhibits quorum-sensing (QS) dynamics and the regulatory mechanism of this activity in these bacteria is unclear. In this study, combining genomic sequencing and genome editing, we have identified that the primary quorum-sensing system in the isolated algicidal strain *Shewanella xiamenensis* Lzh-2 is the LuxS/AI-2 signaling pathway. Disruption of the QS system through recombination deletion of the LuxS gene led to a loss of algicides production and algicidal activity. Restoration of the LuxS gene in the deletion mutant compensated the QS system and recovered the algicidal activity. Consequently, we proved that Lzh-2 regulates the algicidal activity through LuxS/AI-2 quorum-sensing system.

**Keywords:** quorum-sensing, algicidal bacteria, LuxS/AI-2, *Shewanella xiamenensis*, bloom control

**INTRODUCTION**

Cyanobacterial blooms, resulting from eutrophication, have substantial harmful effects such as animal deaths and human illness (Huisman et al., 2018). There is a huge need for eco-friendly algal bloom control methods. Algicidal bacteria that decrease the growth of cyanobacteria by producing algicides is a potential option (Yang et al., 2020). To date, many species with algicidal activity have been reported, such as *Rhizobium* strain AQ_MP (Pal et al., 2021), *Shigella* sp. H3, *Alcaligenes* sp. H5 (Xue et al., 2021), *Paracoccus homiensis* (Ding et al., 2021), *Enterobacter* sp. EA-1 (Lu et al., 2021), etc.

Lzh-2 was an efficient algicidal bacterium isolated from Taihu Lake (Li et al., 2014), where *Microcystis aeruginosa* is the dominant bloom-forming cyanobacterium (Ye et al., 2011). Our previous study showed that the main algicidal substances of Lzh-2 were hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (S-2A) and 2, 3-indolinedione (isatin, S-2B) (Li et al., 2014). Interestingly, Lzh-2 exhibited a cell density-dependent behavior that algicidal compounds were produced only when the density exceeded a threshold of about $10^8$ cells·mL$^{-1}$. This behavior was called quorum-sensing (QS). Some algicidal bacteria exhibited the QS behavior (Dow, 2021).

Quorum-sensing relies on the communication between cells through signaling molecules. There are two well-characterized QS signaling systems: N-acylhomoserine lactones (AHLs) and...
Autoinducer 2 (AI-2) signaling (Dow, 2021). LuxI and LuxR are the crucial genes in the AHLs system. LuxI participates in the synthesis of AHLs, while LuxR mediates the transmission of signals from external AHLs to related biological activities (Cui and Harling, 2005). The AHLs and LuxI/LuxR QS system has been identified in several algicidal bacteria (Guo et al., 2016; Zhang et al., 2021). In contrast, LuxS, the core gene of the QS system, participates in the synthesis of AI-2, and the signals are transmitted through different regulators (Zhao et al., 2018). Few LuxS/AI-2 QS system cases in algicidal bacteria have been reported (Zhang S. J. et al., 2020).

In the present study, we used genome sequencing and genome editing strategies to identify the primary QS system in the Lzh-2 strain and reveal how it regulates the algicidal activity through quorum sensing.

**MATERIALS AND METHODS**

### Strains, Plasmids, and Culture Conditions

All strains and plasmids used in this study are listed in Supplementary Table 1. All primers were synthesized by Sangon Biotech (Shanghai, China) and listed in Supplementary Table 2. All bacteria strains were cultivated aerobically in Luria-Bertani (LB) medium (Difco, Detroit, MI) at 30°C (Shewanella xiamenensis) and 37°C (Escherichia Coli), respectively. Where needed, the growth medium was supplemented with chemicals at the following concentrations: 2.6-diaminopimelic acid (DAP), 0.3 mM; ampicillin (Amp), 100 µg/mL; kanamycin (Kan), 50 µg/mL; and gentamicin (Gm), 15 µg/mL. Microcystis aeruginosa was grown in BG11 medium at 25°C under 40 µmol photons/(m²·s) and a 12 h:12 h (light:dark) cycle (Tian et al., 2012). Cell densities were quantified using a hemocytometer under a light microscope (magnification × 400). All chemicals were purchased from Sigma-Aldrich (Shanghai, China) unless otherwise noted. All experiments were repeated at least 3 times.

### Genome Sequencing and Analysis

Total DNA was extracted using the E.Z.N.A.® Bacterial DNA Kit (Omega Bio-Tek) according to the manufacturer’s instructions. The quality of the extracted DNA was verified through agarose electrophoresis and fluorometrically qualified with the Qubit dsDNA HS kit (Thermo Fisher Scientific) through Qubit Flex Fluorometer (Thermo Fisher Scientific). Two libraries were constructed separately. Paired-end (PE) library preparation with an insert size of 300 bp was constructed with TruSeq™ DNA Sample Prep Kit (Illumina). The library was sequenced using Illumina NovaSeq 6,000 (Illumina) with 150 PE reads at Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China). The Pacbio library with an insert size of 20 kb was constructed using the Template Prep Kit 1.0 (Pacbio) and sequenced with the PacBio Sequel (Pacbio) apparatus at the above place. The obtained sequence was uploaded to the NCBI Sequence Read Archive repository (accession number PRJNA779223).

All reads were quality-filtered. The results from Pacbio Sequel were assembled by HGAP (Chin et al., 2016). Then the complete genome was obtained by error correction with reads from PE library using Pilon (Walker et al., 2014). The complete genome sequences have been submitted to the genebank database of NCBI with the accession number NZ_CP069350.1. GeneMarkS (Besemer et al., 2001), Barrnap, and tRNAscan-SE (Lowe and Eddy, 1997) were used to predict open reading frames and non-coding RNAs. The function of the predicted genes was obtained by aligning the ORFs with the NR database. The circular genome visualization was generated with CGView (v2.0). The taxonomy classification of Lzh-2 was determined by GTDB-tk (Chaumeil et al., 2020). LuxS DNA sequences of other organisms were obtained from GenBank (Supplementary Table 3). The phylogenetic tree was constructed by MEGA11 (Tamura et al., 2021) using the Neighbor-joining algorithm with default parameters.

### AI-2 Bioassay

The AI-2 bioassay was modified from Bodor et al. (2008), Vibrio harveyi BB152 ATCC® BAA1119 and V. harveyi BB170 ATCC® BAA-1117 (Bassler et al., 1993) were used to detect AI-2. V. harveyi BB170 was a biosensor for AI-2 only, while V. harveyi BB152 was used as a positive control. 1 mL culture supernatant of Shewanella xiamenensis strains was sampled and concentrated by centrifugation (13,000 rpm, 2 min) and filtration (0.22 µm) into 100 µL stock. The sensor strain V. harveyi BB170 was freshly inoculated and incubated (160 rpm, 30°C) to an OD<sub>600</sub> nm of 1.0. 180 µL of 5,000 times diluted V. harveyi BB170 culture was mixed with 20 µL samples in a microplate. The plates were incubated at 30°C with shaking at 600 rpm for 4 h. The luminescence of the samples was recorded by a multi-channel microplate reader (HBS-1096, DeTie). The percentage of AI-2 activity was calculated as the ratio of the test sample divided by the positive control (V. harveyi BB152).

### In-Frame Deletion and Complementation

We applied the Fusion PCR method (Gao et al., 2006) to construct the in-frame deletion strains of LuxS. In brief, two fragments flanking LuxS were amplified independently first and joined together by overlap PCR. The resulting fusion fragment was introduced into the plasmid pD53.0 through the In-Fusion HD cloning kit from Takara Bio (Kusatsu, Japan). The resulting plasmid was transformed into E. coli WM3064 (Saltikov and Newman, 2003), and then transferred into S. xiamenensis by conjugation. Integration of the plasmids on the chromosome was selected by gentamycin resistance and confirmed by PCR. The transconjugants were grown in LB broth without NaCl and plated on LB plates containing 10% sucrose. Sucrose led to the deletion of the integrated plasmid. Colonies without the plasmid sequence were selected by gentamycin-sensitive and sucrose-resistant phenotype. Half of these colonies contained the complete LuxS gene, while the other half contained the deletion mutants. They were verified through PCR and partial sequencing. The generation of the deletion mutant was illustrated in Supplementary Figure 1.

For the complementation of LuxS in the deletion mutant, LuxS and its native promoter were amplified by PCR and integrated with pBR1MCS (Kovach et al., 1995) through the
In-Fusion HD cloning kit (Takara Bio). The achieved plasmid was transformed to the deletion mutant by mating with *E. coli* WM3064 containing the vector. The presence of the plasmid with LuxS was further confirmed by plasmid extraction and partial sequencing. The generation of the complementation stain was illustrated in Supplementary Figure 2.

**Quantification of S-2A and S-2B by LC-MS**

Culture supernatants of *S. xiamenensis* were sampled and mixed with an equal volume of ethyl acetate. After keeping the mixture in a separation funnel for 24 h, the ethyl acetate layer was collected and evaporated. The resulting materials were dissolved in 1 mL of water and filtered through a 0.22 µm membrane filter. The filtrates and standard solutions of S-2A and S-2B were subjected to LC-MS analysis in positive mode. MS data were acquired and processed using the LC-MS Qualitative Analysis B.04.00 software supplied with the instrument. Using this program, mass chromatograms corresponding to S-2A and S-2B were extracted and integrated from the total ion chromatogram. The concentrations of S-2A and S-2B in the filtrate were determined by comparing the peak areas of S-2A (or S-2B) with those of the standards (Armando et al., 2012).

**Statistical Analysis**

Data are presented as mean ± standard deviation of triplicate cultures. Statistical analyses and figures were performed with Origin 8.5 (Origin Lab Corporation, United States) software. A two-way t-test was used to analyze the significance level, and a *p*-value < 0.05 was considered statistically significant.

**RESULTS**

**Genome Sequencing of the Strain Lzh-2**

The isolated Lzh-2 strain was sequenced by a combined strategy of next-generation sequencing and PacBio long reads sequencing.
The complete genome was obtained with a total length of 4.6 Mbp (Figure 1) and an average GC content of 46.31%. A total of 4,023 protein-coding genes and 310 RNA-coding genes were predicted in the genome. By comparing the genome of Lzh-2 with known genomes, it was classified as *Shewanella xiamenensis*.

### Identification of the LuxS/AI-2 System in Lzh-2

Our previous study showed that Lzh-2 exhibited quorum-sensing behavior (Li et al., 2014). To explore the mechanism, we firstly screened the genome of Lzh-2 for known quorum-sensing genes. We found the existence of the LuxS gene in Lzh-2, which is the crucial gene for the AI-2 quorum-sensing system. LuxS from Lzh-2 and *S. xiamenensis* showed 99% identity with only three different nucleotides, which were synonymous mutations (Supplementary Figure 3).

To further confirm the activity of this quorum-sensing system, we tested the density dynamics of Lzh-2 and the related activity...
of AI-2 in Lzh-2 (Figure 2). The results showed that AI-2 activity was positively correlated with the density of Lzh-2 and then deceased when Lzh-2 reached the stationary phase.

**Disruption of LuxS Suppresses the Quorum-Sensing Behavior and Algicidal Activity**

Lzh-2 had significant algicidal activity, as shown in Figure 3. The density of *Microcystis aeruginosa* 9110 decreased when co-cultured with Lzh-2. To study the relationship between quorum-sensing and algicidal activity, we completely deleted the LuxS gene from the Lzh-2 genome by two-step recombination, resulting in the strain Lzh-2dS. LuxS deleted strain didn’t show the algicidal activity, and *Microcystis aeruginosa* 9110 grew normally with Lzh-2dS (Figure 3). To further confirm the function of LuxS, the whole LuxS cassette was reinserted into Lzh-2dS, which was named Lzh-2dC. Lzh-2dC restored the algicidal activity that *Microcystis aeruginosa* 9110 could not grow with Lzh-2dC (Figure 3).

The algicidal products of Lzh-2 were hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (S-2A) and 2, 3-indolinedione (isatin, S-2B). The production of S-2A and S-2B was positively correlated with the density of Lzh-2 (Figure 4A), while the deletion of LuxS led to deficient production of S-2A and S-2B even at a high cell density of Lzh-2dS (Figure 4B). However, the complementary expression of LuxS in Lzh-2dC restored the production of S-2A and S-2B at high cell density (Figure 4C).

**DISCUSSION**

*Shewanella xiamenensis* is a widely spread species in many environments such as water, soil, and animal guts (Nguyen et al., 2020). The genome size and the average GC content of Lzh-2 are similar to other reported strains of *S. xiamenensis* (Leangapichart et al., 2021). *S. xiamenensis* is an important antibiotic resistance gene (ARG) harboring species that contains genomic *bla*OXA genes, which encode a lactamase conferring resistance to carbapenem (Nguyen et al., 2020). The isolates from the hospital and human gut contain plasmids carrying transposon and ARGs, which may transmit ARGs to other bacteria (Yousfi et al., 2017; Leangapichart et al., 2021). In contrast, Lzh-2 has the genomic *bla*OXA but not the plasmids. Thus, the risk of Lzh-2 transmitting ARGs is low.

Quorum-sensing has been observed in the genus *Shewanella* and plays a crucial role during biofilm formation and interaction with other species (Zhu et al., 2015; Mukherjee et al., 2020). Two quorum-sensing systems were identified in *Shewanella* sp. The quorum-sensing molecules AHLs have been identified in certain *S. xiamenensis* strains (Li et al., 2014). The LuxS/AI-2 quorum-sensing system by inducing the production of algicides S2-A and S2-B.

**DATA AVAILABILITY STATEMENT**

The data presented in the study are deposited in the NCBI repository, accession number PRJNA779223 (sequencing) and NZ_CP069350.1 (genome).

**AUTHOR CONTRIBUTIONS**

JL and ZL designed the research. KL, ZZ, ZW, FW, YX, and JQ performed the experiments. JL, KL, FS, and ZL analyzed the data. JL, FS, and ZL wrote and revised the manuscript. All authors contributed to the article and approved the submitted version.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2021.814929/full#supplementary-material

**Supplementary Figure 1** | In-frame deletion of LuxS.

**Supplementary Figure 2** | The complementation of LuxS in the deletion mutant.

**Supplementary Figure 3** | Phylogenetic tree of the LuxS genes.
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