LsrB-based and temperature-dependent identification of bacterial AI-2 receptor

Yuxi Zhang1,2, Kezong Qi2, Yawei Jing1, Jiakun Zuo1, Jiangang Hu1, Xiaolong Lv1, Zhaoguo Chen1, Rongsheng Mi1, Yan Huang1, Shengqing Yu1* and Xiangan Han1*

Abstract

The luxS gene is required for autoinducer-2 (AI-2) synthesis in many bacterial species. AI-2 is taken up by a specific receptor to regulate multiple bacterial activities. However, the lack of methods to identify AI-2 receptors has impeded investigations into the roles of AI-2. Here, a luxS mutant of Escherichia coli strain BL21 (DE3) was constructed (named BL21∆luxS), and the recombinant LsrB protein of Salmonella enterica was expressed in BL21∆luxS and BL21 cells, which were named LsrB (BL21∆luxS) and LsrB (BL21), respectively. The results of the activity of recombinant LsrB binding showed that LsrB (BL21) bound to endogenous AI-2 (produced from BL21 strain), while LsrB (BL21∆luxS) did not (as BL21∆luxS cannot produce AI-2). However, the results of recombinant LsrB binding showed that LsrB (BL21∆luxS) can bind exogenous AI-2, which was released from LsrB (BL21∆luxS) at 55 °C for 10 min, while LsrB (BL21) could not bind exogenous AI-2 (due to binding of endogenous AI-2 before). Furthermore, analysis of the thermal stability of AI-2 showed that that AI-2 activity was relatively high at incubation temperatures below 65 °C. These findings will be beneficial for screening of new AI-2 receptors in different bacterial species.

Keywords: Escherichia coli BL21, LuxS mutant, LsrB, AI-2 receptor

Introduction

Cell–cell communication in bacteria is accomplished through the exchange of extracellular signalling molecules, called autoinducers, in a process termed quorum sensing (QS) (Pereira et al. 2013). Although most autoinducers are species specific, autoinducer-2 (AI-2) is considered to be a universal signalling molecule for interspecies communication that is synthesised by LuxS, an enzyme that is highly conserved and widespread in diverse bacteria (Han and Lu 2009; Pereira et al. 2013; Even-Tov et al. 2016).

The LuxS/AI-2 type QS system plays important roles in the regulation of bacterial bioluminescence, sporulation, competence, antibiotic resistance, biofilm formation, and virulence factor secretion (Xue et al. 2013; Han et al. 2013, 2015a, b). AI-2 is taken up by a specific membrane receptor transport system. Previous studies have identified several AI-2 receptors in various bacterial species, including LuxP in Vibrio harveyi (Chen et al. 2002), LsrB in Salmonella typhimurium (Miller et al. 2004) and RbsB in Haemophilus influenzae (Armbruster et al. 2011). Some bacterial species lack the AI-2 synthase gene luxS and are, therefore, not capable of producing AI-2 (De Keersmaecker et al. 2006; Rezzonico and Duffy 2008). However, such bacteria, including Sinorhizobium meliloti (Pereira et al. 2013) and Riemerella anatipestifer (Han et al. 2015a, b), might use endogenous AI-2 to regulate physiological functions. Furthermore, the lack of knowledge of the underlying molecular mechanisms of AI-2 recognition, signal transduction and/or processing continues to impede understanding of the function of AI-2 in any given species. Undoubtedly, the lack of appropriate screening procedures is a major drawback in the identification of AI-2 receptors in other bacterial species and impedes further investigations into the roles of AI-2. In this study, a set of criteria was established to identify functional AI-2 receptors using LsrB in S. typhimurium. These findings will be beneficial for future screening of AI-2 receptors in different bacterial species.
Materials and methods

Bacterial strains, plasmids, and culture conditions

*Escherichia coli* strains DH5α and BL21 (DE3) (Invitrogen Corporation, Carlsbad, CA, USA) were grown at 37 °C in Lennox broth (LB) or on solid medium containing 1.5% agar at 37 °C and used for the cloning and expression of recombinant genes. When necessary, LB medium was supplemented with an appropriate dosage of ampicillin (100 μg/ml) or kanamycin (100 μg/ml).

The expression vector pCold-TF was purchased from Takara Bio, Inc. (Shiga, Japan). Restriction enzymes were purchased from MBI Fermentas, Inc. (Waltham, MA, USA). *V. harveyi* strains BB170 (sensor1<sup>−</sup>sensor2<sup>+</sup>) (ATCC BAA-1117) and BB152 (ATCC BAA-1119) were purchased from the American type culture collection (Manassas, VA, USA) and cultivated in modified auto-inducer bioassay (AB) medium (Bassler et al. 1993). BB170 was used as the AI-2 biosensor strain and BB152 as a positive control for AI-2 production. All chemicals used were of analytical grade and purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA).

Construction and identification of luxS mutant strain BL21ΔluxS

The upstream and downstream fragments (845 and 884 bp, respectively) of the BL21 (DE3) luxS gene were amplified by PCR using the primer pairs LuxS-UF/LuxS-Overlap-UR and LuxS-Overlap-DF/LuxS-DR (Table 1). The mutant strain BL21ΔluxS was generated by creating a 394-bp in-frame deletion in the 516-bp luxS open reading frame using the lambda red recombination system (Fig. 1a), as described previously with slight modifications (Datsenko and Wanner 2000). Briefly, the upstream and downstream fragments of the luxS gene were ligated by overlap PCR using the primer pair LuxS-UF/LuxS-DR (Table 1) to produce a 1729-bp PCR product, and then the PCR product was sub-cloned into the pMD19-T vector to construct the recombinant plasmid pMD19-Up-Down. A kanamycin resistance cassette (Kan) was amplified from plasmid pKD4 by PCR using the primer pair pKD4-Kan-F/pKD4-Kan-R (Table 1). Then, the Kan was digested with the nuclease Sall and subsequently inserted into the recombinant plasmid pMD19-Up-Down to form the plasmid pMD19-Up-Kan-Down. The mutagenic construct (containing the insertion sequence of Kan within the luxS gene coding region) was amplified by PCR using the primer pair LuxS-UF/LuxS-DR. Subsequently, the PCR product (3155 bp) was purified and used for electroporation. One microgram of PCR product was added to 100 μl of BL21 (DE3) competent cells containing the lambda red recombinase expression plasmid pKD46, and electroporation was performed using a Gene Pulser II transfection apparatus (Bio-Rad Laboratories, Hercules, CA, USA) at 25 μF, 2.4 kV and 250 Ω. After the electric pulse, the cells were diluted immediately in 1 ml of SOC (super optimal broth with catabolite repression) medium, incubated at 37 °C for 2 h and then plated on LB plates containing 100 μg/ml of Kan. After a 24-h incubation, the resulting Kan-resistant colonies were selected for PCR amplification with the primer pairs LuxS-inF/LuxS-inR and

| Table 1 Primers used in this study |
|-----------------------------------|
| **Primers** | **Oligonucleotide sequence (5′–3′)** | **Description** | **Product size (bp)** |
| LuxS-UF | TTCTGATGCGCGTGTACGT | The upstream sequence of luxS | 845 |
| LuxS-Overlap-UR | TCGCAGGCGCGGTTGTTGATTTTGGG<sup>a</sup> | | |
| LuxS-Overlap-DF | TGAACACCGCGGCGCAGCGCGGAAGAGAG | The downstream sequence of luxS | 884 |
| LuxS-DR | TGAACCAGGATAACCGGA | | |
| pKD4-Kan-F | CGCGTCGACGTAGGCTGGAGCTGCTT | Kanamycin resistance cassette | 1494 |
| pKD4-Kan-R | CGCGTCGACCATATGAAATATCTTTCCTTAGTTC | | |
| LsrB-F | CGCGGATCCATGCCAAAGACACAGCATATACAAAT<sup>b</sup> | lsrB | 1023 |
| LsrB-R | CGCGGATCCATGCCAAAGACACAGCATATACAAAT<sup>b</sup> | | |
| LuxS-F | CGCGGATCCATGCCAAAGACACAGCATATACAAAT<sup>b</sup> | luxS | 516 |
| LuxS-R | CGCGGATCCATGCCAAAGACACAGCATATACAAAT<sup>b</sup> | | |
| LuxS-OutF | GCCGATTGTGTTTCTTCTTCTC<sup>c</sup> | Primers for identification of luxS deletion | 2519 |
| LuxS-OutR | GATCAAGAATCGTACAGG | | |
| LuxS-InF | GAAATGATGCGAGCAAGAGAGAG<sup>a</sup> | Primers for identification of luxS deletion | 307 |
| LuxS-InR | CGCAAATGCTACGGCAACT | | |

<sup>a</sup> Fl, BamHI and HindIII restriction sites are underlined, respectively
<sup>b</sup> A 307 bp PCR product was amplified from wild-type strain BL21(DE3), and no product was amplified from mutant strain BL21ΔluxS, using primers LuxS-inF/LuxS-inR
<sup>c</sup> A 2519 or 2209 bp PCR product was amplified from wild-type strain BL21(DE3), or mutant strain BL21ΔluxS without kan, respectively, using primers LuxS-OutF/LuxS-OutR
LuxS-OutF/LuxS-outR (Table 1) to identify the deletion of the luxS gene from BL21 cells. The mutant strain was transformed further with the pCP20 plasmid to cure the Kan and produce a Kan-sensitive mutant strain that was named BL21∆luxS.

**AI-2 assay**

AI-2 activity is expressed in relative light units (RLU). The AI-2 bioassay was performed according to a previously described method with some modifications (Bassler et al. 1993; Han and Lu 2009; Han et al. 2013). Cell-free culture fluid (CF) was prepared as follows: E. coli strains BL21 (DE3), BL21∆luxS and DH5a were grown in LB at 37 °C, and then pelleted by centrifugation at 12,000g at 4 °C for 10 min. Then, the resulting supernatants were filtered through a 0.22-μm filter (EMD Millipore, Bedford, MA, USA) to obtain CF samples. The reporter strain V. harveyi BB170 was diluted to 1:5000 in fresh AB medium and then 180 μl of bacterial culture was mixed with 20 μl of the CF sample and incubated at 30 °C for 4 h. After incubation, 100-μl aliquots were added to white, flat-bottomed, 96-well plates (Thermo Labsystems, Franklin, MA, USA) for detection of AI-2 activity. A positive control was obtained from overnight cultures of BB152, while

---

**Fig. 1** Identification of the luxS mutant BL21∆luxS. **a** Schematic chart of strategy for producing the BL21 luxS deletion mutant. The luxS was deleted by replacing the partial gene sequence of luxS with kanamycin resistance cassette at SalI cleavage sites. The primers used for the confirmation of the luxS deletion are also indicated. **b** Identification of the luxS mutant BL21∆luxS. Lane M: DL 2000 DNA marker (D501A; Takara); lane 1: the wild-type strain BL21 showed a 2519-bp PCR product using primers LuxS-OutF/LuxS-OutR; lane 2: the mutant BL21∆luxS with cure of the kanamycin resistance cassette showed a 2209-bp PCR product using primers LuxS-OutF/LuxS-OutR; lane 3: negative control; lane 4: the wild-type strain DE17 showed a 307-bp PCR product using primers LuxS-inF/LuxS-inR; lane 5: the mutant DE17∆pfs showed no PCR products using primers LuxS-inF/LuxS-inR; lane 6: negative control.
the CF from *E. coli* DH5a was used as a negative control. Luminescence was measured with a Tecan GENios Plus microplate reader in luminescence mode (TECAN Austria GmbH, Grödig, Austria). The AI-2 activity in CF of *V. harveyi* BB170 is reported as RLU. All samples were assayed in triplicate.

**Influence of temperature on AI-2 activity**

To investigate the influence of temperature on AI-2 activity, AI-2 (Omm Scientific, Inc., Dallas, TX, USA) was dissolved in phosphate-buffered saline to form different concentrations of AI-2 solution (2.5, 10.0 and 40.0 μM). Then, the AI-2 solution was incubated for 10 min at 37, 45, 50, 55, 60, 65 or 70 °C, respectively. After a 10-min incubation, the AI-2 solution was filtered through a 0.22-μm filter (EMD Millipore) to obtain cell-free CF for detection of AI-2 activity. The AI-2 bioassay was performed as previously described (Han et al. 2013; Han et al. 2015a, b). All samples were assayed in triplicate.

**Expression and purification of recombinant LsrB and LuxS**

The *lsrB* gene of *S. enterica* (NC_003198.1) and *luxS* gene of *E. coli* strain BL21 (DE3) (WP_001130211.1) were amplified using the oligonucleotides LsrB-F/LsrB-R and LuxS-F/LuxS-R, respectively (Table 1). The primers generated *BamH*I and *HindIII* restriction site (underlined) that were used to clone the *BamH*I/*HindIII*-digested PCR fragments into a *BamH*I/*HindIII*-digested pCold TF vector to construct the two expression plasmids pColdTF-lsrB and pColdTF-luxS. *E. coli* strains BL21 and BL21ΔluxS were transformed with the expression vectors pColdTF-lsrB and pColdTF-luxS, respectively, and grown on LB agar plates containing ampicillin (100 μg/ml). The *E. coli* strain BL21 containing the plasmid pColdTF-lsrB or pColdTF-luxS was named BL21 (pColdTF-lsrB) or BL21 (pColdTF-luxS), respectively. The *E. coli* strain BL21ΔluxS containing the plasmid pColdTF-lsrB was named BL21ΔluxS (pColdTF-lsrB). The addition of 1 mM isopropyl β-D-1-thiogalactopyranoside induced the expression of recombinant LsrB or recombinant LuxS. The recombinant LsrB of *S. enterica* was expressed in BL21ΔluxS and BL21 *E. coli* cells, which were named LsrB (BL21ΔluxS) and LsrB (BL21), respectively. Recombinant LsrB and LuxS proteins were isolated and purified using a HisTrap™ column according to the manufacturer’s protocol (Amersham Biosciences Corporation, Amersham, UK). The final protein concentration was determined by the Bradford method using the SmartSpec™ 3000 Spectrophotometer (Bio-Rad Laboratories). Bovine serum albumin was used as a standard.

**Binding of recombinant LsrB to AI-2**

To determine whether recombinant LsrB can bind to endogenous AI-2, LsrB (BL21) and LsrB (BL21ΔluxS) proteins were isolated from *E. coli* strain BL21 (pColdTF-lsrB) or BL21ΔluxS (pColdTF-lsrB), respectively. Purified LsrB (BL21) and LsrB (BL21ΔluxS) proteins (5 mg/ml) were incubated for 10 min at 37, 50 and 60 °C to release endogenous AI-2, respectively. After incubation, the LsrB proteins were removed by ultrafiltration (10,000-Da cut-off; EMD Millipore), and the filtered reaction products were tested for AI-2 activity using a *V. harveyi* BB170 bioassay, performed essentially as described above. The LuxS protein was isolated from BL21 (pColdTF-lsrB) cells as a negative control and AI-2 (10 μM) was used as a positive control. All samples were assayed in triplicate.

Different concentrations of exogenous AI-2 (2.5, 10.0 and 40.0 μM) were incubated with 5 mg/ml of LsrB (BL21ΔluxS) protein for 10 min at 37 °C. After
incubation, the LsrB (BL21ΔluxS) protein was removed by ultrafiltration (10,000-Da cut-off, EMD Millipore) and the filtered reaction products were tested for AI-2 activity by investigating the binding activity of LsrB (BL21ΔluxS) to exogenous AI-2. Further, the isolated LsrB (BL21ΔluxS) proteins (5 mg/ml) were incubated for 10 min at 37, 50 and 60 °C to release exogenous AI-2, respectively. The LsrB (BL21ΔluxS) protein was denatured for 10 min at 100 °C and then also incubated with exogenous AI-2 for 10 min at 37 °C. Afterward, the filtered reaction products were tested for AI-2 activity, as described above, with a negative control. Increasing concentrations of AI-2 (2.5, 10.0, and 40.0 μM) were used as positive controls, respectively. All samples were assayed in triplicate.

Statistical analysis
All statistical analyses were conducted using SPSS ver. 19.0 software (SPSS Inc., Chicago, IL, USA). One-way analysis of variance was used to identify differences in biofilm formation and gene transcription levels. A probability (p) value of < 0.05 was considered statistically significant.

Results
Identification of the BL21ΔluxS mutant strain
Two primer pairs, LuxS-OutF/LuxS-OutR and LuxS-inF/LuxS-inR, were used for PCR analysis (Fig. 1a) to confirm the deletion of the luxS gene from BL21 (DE3) cells. A 2519-bp PCR product was amplified from wild-type strain BL21 (DE3) (Fig. 1b, lane 1), while the mutant BL21ΔluxS with cure of the kanamycin resistance

![Fig. 3](https://via.placeholder.com/150)

**Fig. 3** Thermal stability of AI-2. The different concentrations of AI-2 solution 40.0 μM (a), 10.0 μM (b) and 2.5 μM (c), respectively. Then, the AI-2 solution was incubated for 10 min at 37, 45, 50, 60, 65 or 70 °C, respectively. After a 10-min incubation, the AI-2 solution was filtered through a 0.22-μm filter (EMD Millipore) to obtain cell-free CF for detection of AI-2 activity. AI-2 activity was expressed as relative light units (RLUs). The results of the thermal stability assay showed that AI-2 activity was relatively high at incubation temperatures of less than 65 °C. Furthermore, an incubation temperature of 45–65 °C had no significant effect on AI-2 activity. Experiments were repeated three times.
cassette showed a 2209-bp PCR product using the primer pair LuxS-OutF/LuxS-OutR (Fig. 1b, lane 2). No product was amplified from the mutant strain BL21ΔluxS (Fig. 1b, lane 5), while a 307-bp (Fig. 1b, lane 4) PCR product was amplified from wild-type strain BL21 (DE3) using the primer pair LuxS-InF/LuxS-InR.

**The BL21ΔluxS mutant strain lacked AI-2 activity**

No AI-2 activity was detected from bacterial CF prepared from mutant strain BL21ΔluxS, in which the luxS gene was deleted (Fig. 2). However, *E. coli* wild-type strain BL21 and *V. harveyi* strain BB152 (positive control) both secrete AI-2-like molecules that can induce bioluminescence of *V. harveyi* strain BB170. The results showed that the luxS mutant BL21 (DE3) strain did not produce AI-2-like signalling molecules.

**Thermal stability of AI-2**

The results of the thermal stability assay showed that AI-2 activity was relatively high at incubation temperatures of less than 65 °C. However, AI-2 activity was significantly reduced at 70 °C and decreased by 20% (Fig. 3a), 55% (Fig. 3b) and 90% (Fig. 3c), respectively, at concentrations of 2.5, 10.0 and 40.0 μM. Furthermore, an incubation temperature of 45–65 °C had no significant effect on AI-2 activity.

**Expression and purification of LsrB and LuxS**

The recombinant vectors pColdTF-lsrB and pColdTF-luxS were expressed successfully in *E. coli* BL21 and BL21ΔluxS cells. The transformant supernatants were analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and the fusion proteins were clearly observed as bands with the expected molecular weights of 84.8 kDa (including a 48.0-kDa fusion protein tag) for LsrB and 57.4 kDa (including a 48.0-kDa fusion protein tag) for LuxS (Fig. 4).

The purity of the proteins was checked by 12% SDS-PAGE, which demonstrated single bands with the expected molecular weights (Fig. 4). The final concentrations of the LsrB and LuxS proteins were both 5 mg/ml.

**Binding of LsrB to endogenous AI-2**

The extent of LsrB binding to endogenous AI-2 was evaluated using an AI-2 assay, which showed that recombinant LsrB (BL21) bound to endogenous AI-2 (produced by wild-type strain BL21) and was released from

![Fig. 4 SDS-PAGE analysis of total cellular proteins and purified fusion proteins from BL21ΔluxS cells. Lane 1 and lane 5: SDS-PAGE analysis of total cellular proteins from BL21ΔluxS containing pCold TF (serve as negative control). Lane 2 and lane 6: SDS-PAGE analysis of total cellular proteins containing expression plasmids pCold-TF-lsrB (a) and pCold-TF-luxS (b), respectively. Lane 3 and lane 7: SDS-PAGE analysis of total precipitation proteins containing expression plasmids pCold-TF-lsrB and pCold-TF-luxS, respectively. Lane 4 and lane 8: elution of the LsrB and LuxS purified fusion protein from the affinity column, respectively](image-url)
LsrB (BL21) at 50 or 60 °C, respectively (Fig. 5a). However, since the luxS mutant BL21ΔluxS did not produce endogenous AI-2, no AI-2 could be released from the recombinant LsrB (BL21ΔluxS) (Fig. 5b). Moreover, the recombinant LuxS protein, which was expressed in strain BL21 (pColdTF-lsrB) as a negative control, also showed no AI-2 binding activity (Fig. 5c). AI-2 (10 μM) was used as a positive control.
Binding of LsrB to exogenous AI-2
Different concentrations of AI-2 (2.5, 10.0 and 40.0 μM) were incubated with 5 mg/ml of LsrB (BL21∆luxS). The results of AI-2 binding activity indicated that 5 mg/ml of LsrB (BL21∆luxS) could bind exogenous AI-2 from 2.5 to 40 μM. Furthermore, the assay of AI-2 releasing activity showed that bound AI-2 was released from LsrB (BL21∆luxS) at 55 °C for 10 min. However, denatured LsrB (BL21∆luxS) was not able to bind to AI-2.

Discussion
AI-2, which was first identified in the marine bacterium *V. harveyi*, is produced by many Gram-negative and -positive bacteria (Surette et al. 1999). AI-2 is produced by the activated methyl cycle by the AI-2 synthase Pfs and LuxS. The LuxS-mediated QS system play roles in biofilm formation, virulence gene expression, the type III secretion system, and drug sensitivity changes in different bacterial species by AI-2 (Xue et al. 2013; Novotny et al. 2015; Mou and Plummer 2016). AI-2 is taken up by specific membrane receptors in bacterial transport systems to regulate physiological functions, such as LuxP in *V. harveyi* (Chen et al. 2002), LsrB in *S. typhimurium* (Miller et al. 2004) and RbsB in *H. influenza* (Armbuster et al. 2011). Furthermore, the AI-2 receptors also play roles in some host-microbial relationships. For example, an AI-2 mimic detected by the bacterial AI-2 receptor, LuxP/LsrB, can activate QS-controlled gene expression in the enteric pathogen *S. typhimurium* (Ismail et al. 2016). Therefore, further research of the AI-2 receptors will help to elucidate the mechanisms underlying the LuxS/AI-2 type QS system.

In this study, the combination of endogenous AI-2 produced by BL21 (DE3), which is known to interfere with the binding of AI-2 to LsrB (BL21), resulted in the loss in the ability of LsrB (BL21) to bind to exogenous AI-2. Hence, the BL21 (DE3) luxS mutant, which was incapable of producing endogenous AI-2, was constructed to express the recombinant protein LsrB and eliminate the interference from endogenous AI-2. Since the BL21∆luxS mutant eliminated endogenous AI-2, the LsrB (BL21∆luxS) mutant, which was overproduced in strain BL21∆luxS, had no AI-2 activity upon denaturation, but could bind to exogenous AI-2. However, the LsrB (BL21) overproduced in strain BL21 had AI-2 activity upon denaturation, but could not bind exogenous AI-2 (Fig. 7).

Research is needed on the discovery of molecules, like LsrB, that are AI-2 receptors and can release endogenous AI-2 upon denaturation or bind exogenous AI-2 when expressed in BL21 or BL21∆luxS, respectively. In the present study, the luxS gene of *E. coli* strain BL21 was expressed in BL21. However, the LuxS could not bind to endogenous or exogenous AI-2 (Fig. 5). The results confirmed that the proposed assay based on LsrB could be used for identification of new AI-2 receptors in future studies.

Furthermore, the present study also optimized the conditions of LsrB binding or releasing AI-2. Although AI-2 activity was high, an incubation temperature of 45–65 °C had no significant effect on AI-2 activity. Interesting, the results of AI-2 releasing activity showed that the optimal temperature for release of AI-2 bound to LsrB was 55 °C, within the range of 45–65 °C, and not affect the detection of AI-2 activity in *V. harveyi* BB170 bioluminescence.
These findings will be beneficial for future screening of new AI-2 receptors and to further study the roles of AI-2 in different bacterial species.

**Abbreviations**
AI-2: autoinducer-2; QS: quorum sensing; LB: lennox broth; AB: autoinducer bioassay; Kan: kanamycin resistance cassette; CF: cell-free culture fluid.

**Authors’ contributions**
HXG designed the work; ZYX, JYW, ZJK, HJG and LXL performed the research study; YSQ, QKZ, CZG, MRS and HY analysis the data; HXG drafted the manuscript. All authors read and approved the final manuscript.

**Author details**
1 Shanghai Veterinary Research Institute, The Chinese Academy of Agricultural Sciences (CAAS), 518 Ziyue Road, Shanghai 200241, People’s Republic of China. 2 College of Animal Science and Technology, Anhui Agricultural University, Hefei 230036, People’s Republic of China.

**Acknowledgements**
Not applicable.

**Competing interests**
The authors declare that they have no competing interests.

**Availability of data and materials**
The data on which the conclusions are made are all presented in this paper.

**Consent for publication**
Not applicable.

**Ethics approval and consent to participate**
This article does not contain any studies with human participants or animals performed by any of the authors.

**Funding**
This work was supported by the fund of the National Natural Science Foundation of China (31370045, 31572546, 31372402 and 31772707), Shanghai Key Project on Agricultural Development through Science and Technology.
References
Armbruster CE, Pang B, Murrah K, Juneau RA, Perez AC, Weimer KE, Swords WE (2011) RbsB (NTHI_0632) mediates quorum signal uptake in nontypeable Haemophilus influenzae strain 86-028NP. Mol Microbiol 82(4):836–850
Bassler BL, Wright M, Showalter RE, Silverman MR (1993) Intercellular signalling in Vibrio harveyi: sequence and function of genes regulating expression of luminescence. Mol Microbiol 9(4):773–786
Chen X, Schauder S, Potier N, Van Dorsselaer A, Pelczer I, Bassler BL, Hughson FM (2002) Structural identification of a bacterial quorum-sensing signal containing boron. Nature 415(6871):545–549
Datsenko KA, Wanner BL (2000) One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. Proc Natl Acad Sci USA 97(12):6640–6645
De Keersmaecker SC, Sonck K, Vanderleyden J (2006) Let LuxS speak up in AI-2 signaling. Trends Microbiol 14(3):114–119
Even-Tov E, Bendori SO, Valastyan J, Ke X, Pollak S, Bareia T, Ben-Zion I, Bassler BL, Eldar A (2016) Social evolution selects for redundancy in bacterial quorum sensing. PLoS Biol 14(2):e1002386
Han X, Lu C (2009) Biological activity and identification of a peptide inhibitor of LuxS from Streptococcus suis serotype 2. FEMS Microbiol Lett 294(1):16–23
Han X, Bai H, Liu D, Dong H, Liu R, Song J, Ding C, Qi K, Liu H, Yu S (2013) The luxS gene functions in the pathogenesis of avian pathogenic Escherichia coli. Microb Pathog 55:21–27
Han X, Bai H, Tu J, Yang L, Xu D, Wang S, Qi K, Fan G, Zhang Y, Zuo J, Tian M, Ding C, Yu S (2015a) Deletion of luxS further attenuates the virulence of the avian pathogenic Escherichia coli aroA mutant. Microb Pathog 88:39–47
Han X, Lu L, Fan G, Zhang Y, Xu D, Zuo J, Wang S, Wang X, Tian M, Ding C, Yu S (2015b) Riemerella anatipestifer lacks luxS, but can uptake exogenous autoinducer-2 to regulate biofilm formation. Res Microbiol 166(6):486–493
Ismail AS, Valastyan JS, Bassler BL (2016) A host-produced autoinducer-2 mimic activates bacterial quorum sensing. Cell Host Microbe 19(4):470–480
Miller ST, Xavier KB, Campagna SR, Taga ME, Semmelhack MF, Bassler BL, Hughson FM (2004) Salmonella typhimurium recognizes a chemically distinct form of the bacterial quorum-sensing signal AI-2. Mol Cell 15(5):677–687
Mou KT, Plummer PJ (2016) The impact of the LuxS mutation on phenotypic expression of factors critical for Campylobacter jejuni colonization. Vet Microbiol 192:43–51
Novotny LA, Jurcisek JA, Ward MO Jr, Jordan ZB, Goodman SD, Bakaletz LO (2015) Antibodies against the majority subunit of type IV Pili disperse nontypeable Haemophilus influenzae biofilms in a LuxS-dependent manner and confer therapeutic resolution of experimental otitis media. Mol Microbiol 96(2):276–292
Pereira CS, Thompson JA, Xavier KB (2013) AI-2-mediated signalling in bacteria. FEMS Microbiol Rev 37(2):156–181
Rezzonico F, Duffy B (2008) Lack of genomic evidence of AI-2 receptors suggests a non-quorum sensing role for luxS in most bacteria. BMC Microbiol 8:154
Surette MG, Miller MB, Bassler BL (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(4):1639–1644
Xue T, Zhao L, Sun B (2013) LuxS/AI-2 system is involved in antibiotic susceptibility and autolysis in Staphylococcus aureus NCTC 8325. Int J Antimicrob Agents 41(1):85–89

Submit your manuscript to a SpringerOpen journal and benefit from:
► Convenient online submission
► Rigorous peer review
► Open access: articles freely available online
► High visibility within the field
► Retaining the copyright to your article

Submit your next manuscript at ► springeropen.com