# luxS/AI-2 quorum sensingは抗菌剤に対するStreptococcus agalactiaeの感受性低下に関与する


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Research article

# *luxS*/AI-2 Quorum Sensing Is Involved in Antimicrobial Susceptibility in *Streptococcus agalactiae*

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**ABSTRACT**—*luxS*-mediated autoinducer-2 (AI-2) directly or indirectly regulates important physiologic function in a variety of bacteria, including bioluminescent effect, antimicrobial resistance, virulence factor control, biofilm structure stability and so on. In this study, the regulation of susceptibility to antimicrobials by *luxS*/AI-2 was investigated in *Streptococcus agalactiae*. A *luxS*- knockout mutant strain named SX1 was constructed by homologous recombination from *S. agalactiae* CNEP110823 (wild type strain), and a *luxS*-complementation strain named SX2 was constructed from SX1 through transducing a plasmid with the *luxS* gene and its promoter. Then the susceptibility to cefradine and norfloxacin of these two strains, SX1 exposed to AI-2 precursor molecule 4,5-dihydroxy-2,3-pentanedione (DPD) and the wild type strain was determined by viable bacterial counting. SX1 decreased susceptibility to cefradine and norfloxacin, SX2 returned the susceptibility to the same level as the wild type strain and DPD restored the susceptibility of SX1. The results indicate that *luxS*/AI-2 quorum sensing is involved in antimicrobial susceptibility in *S. agalactiae,* which may provide novel clues for antimicrobial therapy in the infection.

**Key words:** *Streptococcus agalactiae*, *luxS* mutant, AI-2, antimicrobial susceptibility, molecular mechanism

*Streptococcus agalactiae* is an important pathogen in humans, cows, fish and so on, which causes pneumonia, sepsis, meningitis and sometimes death in human and mastitis in cows. In fish, *S. agalactiae* can result in massive kills of fish and is responsible for heavy economic losses in many species of freshwater, marine, and estuarine fish worldwide. Many reports showed that the infections characterized by septicemia and meningoencephalitis (Evans *et al.*, 2002, 2006; Mian *et al.*, 2009; Geng *et al.*, 2012; Delannoy *et al.*, 2013). Now, many antimicrobials have routinely been the drugs for prophylactic strategies and treatment of the infections of *S. agalactiae*. But, more and more multidrug resistant strains represent a critical challenge for antimicrobial therapy.

Quorum sensing (QS) is a cell-cell communication mechanism to coordinate social activities. It is well known that one QS signal called autoinducer-2 (AI-2) is shared by both Gram-positive and Gram-negative bacteria (Bassler, 1999; Miller and Bassler, 2001; Waters and Bassler, 2005; Federle, 2009; Zhao *et al.*, 2010; Wang *et al.*, 2011). AI-2 molecule is formed as a result of spontaneous rearrangement of dihydroxy-2,3-pentanedione (DPD), which is catalyzed by the conserved and widespread *luxS* enzyme (Chen *et al.*, 2002; Xavier and Bassler, 2003). Many studies have highlighted the significance of *luxS*/AI-2 in the biological processes of various bacterial species including antimicrobial production, biofilm formation, carbohydrate metabolism and virulence expression and so on (Bassler *et al.*, 1993; Vendeville *et al.*, 2005; Doherty *et al.*, 2006; Ahmed *et al.*, 2007, 2009; Choi *et al.*, 2007; Palaniyandi *et al.*, 2013).

Interestingly, *S. agalactiae* also possesses a functional *luxS* gene and has the ability to produce AI-2 molecule. Analysis of 3D structure showed that deduced LuxS protein contained conserved active center (His-57, His-61, Cys-127) and $Zn^{2+}$ binding site (His-Thr-Ile-Glu-His) (Ma *et al.*, 2013). A recent report showed that inactivation of *S. agalactiae luxS* resulted in reduction of bioluminescence using the response of AI-2 reporter *Vibrio* strain (Qu *et al.*, 2005). Now, we want to investigate whether AI-2 signaling influences other functions in *S. agalactiae*. The aim of the current study is to test the hypothesis that AI-2 QS system may

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affect the antimicrobial susceptibility of *S. agalactiae*. Here, we show that *luxS* inactivation results in a mutant with increased resistance to cefradine and norfloxacin.

## Materials and Methods

*Construction of a* S. agalactiae luxS *mutant strain and* luxS *mutant complementation strain*

Bacterial strains, plasmids and primers used in this study are described in Table 1. The allelic replacement method was performed as described previously (Takamatsu *et al.*, 2001b). The N-terminus and flanking region and the C-terminus and flanking region of *luxS* gene of *S. agalactiae* CNEP110823 were amplified by PCR using primers *up-luxS-f-SphI* plus *up-luxS-r-SalI* and *down-luxS-f-BamHI* plus *down-luxS-r-SmaI* according to the *S. agalactiae* genome sequence on Genbank database (No. CP000114.1), respectively, and digested with *Sph*I plus *Sal*I and *Bam*HI plus *Sma*I, respectively. The *cat* gene of pSET3 (Takamatsu *et al.*, 2001a) was amplified by PCR using primers *CAT-f-SalI* plus *CAT-r-BamHI* and digested with *Sal*I plus *Bam*HI. These PCR derived fragments were ligated to the same enzyme-digested pSET4s. *E. coli* DH5$\alpha$ was transformed with the ligation mixture, several $Cm^rSpc^r$ transformants that harbored recombinant plasmids containing the *cat* gene sandwiched between the *luxS* flanking sequences were obtained. One was designated pS*luxS*. *S. agalactiae* CNEP110823 was cultured in Todd-Hewitt Broth (THB) and harvested in mid-logarithmic phase ($OD_{600}$ = 0.3–0.5), and the cells were then washed with 10% glycerol and resuspended in 10% glycerol. After incubation on ice for 30 min, cells were washed twice with 10% glycerol and resuspended in 10% glycerol. An aliquot of 0.1 mL of the cell suspension was mixed with plasmid DNA. The mixture was then placed in a prechilled sterile electroporation cuvette (Bio-Rad) and pulsed immediately with a Bio-Rad Gene Pulser (2.5 kV, 200 W and 25 mF). The mixture was then diluted with THB broth containing 10% fetal calf serum and chloramphenicol and incubated at 28°C to early logarithmic phase and the cultures were then shifted to 37°C and incubated for 10 h. Subsequently, the cells were spread on THB agar plates without antimicrobial and incubated at 37°C. Cultures were screened for mutants that had lost the vectors and had exchanged their wild-type allele for a genetic segment containing the *luxS* gene as a consequence of homologous recombination via a double cross-over. A resulting mutant strain named SX1 was verified by PCR amplification using Ex-Taq polymerase (Takara) with primers *up-luxS-f-SphI/down-luxS-r-SmaI* and confirmed by DNA sequence, and then RNA was extracted and reverse transcripted, and cDNA sequence was con-

**Table 1.** Strains, plasmids and primers used in the study

| Strain or plasmid | Relenant genotype | Reference or source |
|---|---|---|
| Strain | | |
| *Streptococcus agalactiae* | | |
| CNEP 110823 | Wild-type | Laboratory stock |
| SX1 | 110823△ luxS::*cat* | This study |
| SX2 | 110823△ *luxS*::*cat* pAT*luxS* | This study |
| *Escherichia coli* | | |
| DH5$\alpha$ | Clone host strain | Laboratory stock |
| Plasmid | | |
| pSET4S | Suicide vetor, temperature-sensitive; $Spc^r$ | Takamatsu *et al.* (2001b) |
| pSET3 | Shuttle vetor, $Cm^r$, $Spc^r$ | Takamatsu *et al.* (2001a) |
| pS*luxS* | pSET4S containing 693 bp upstream and 509 bp downstream fragment of *luxS* and *cat* gene, for *luxS* mutagensis; $Spc^r$, $Cm^r$ | This study |
| pAT18 | Shuttle vector; $Em^r$ | Li *et al.* (2010) |
| pAT*luxS* | pAT18-*luxS* and its promoter, $Em^r$ | This study |
| Primer | Oligonucleotide sequence | |
| *up-luxS-f-SphI* | CAT*GCATGC*ATCGTTTCAACTGGTCGTAATC | This study |
| *up-luxS-r-SalI* | GC*GTCGAC*GCGCCACATTGTCTGATCTAAT | This study |
| *down-luxS-f-BamHI* | CGG*GATCC*CTCCTTTTGTATTACATTCTAG | This study |
| *down-luxS-r-SmaI* | CC*CCCGGG*GGATCTGTTAAACGTGCCTCCATC | This study |
| *CAT-f-SalI* | GC*GTCGAC*TAATTCGATGGGTTCCGAGG | This study |
| *CAT-r-BamHI* | CGG*GATCC*CACCGAACTAGAGCTTGATG | This study |
| *pAT18-luxS-f-BamHI* | CGG*GATCC*AAAGTCCACTTTTAAGTTTATCA | This study |
| *pAT18-luxS-r-HindIII* | GCAAGCTTTTTATTAGATCAGACAATGTGGC | This study |
| *parC-f* | TTGTGACTGTTGCTCCTATCCA | This study |
| *parC-r* | TTACACCTGCGGCTTTACTACC | This study |

firmed with primers *up-luxS-f-SphI/down-luxS-r-SmaI*.

The *S. agalactiae* CNEP110823-DNA region containing the intact *luxS* gene was amplified by PCR using primers *pAT18-luxS-f-BamHI* and *pAT18-luxS-r-HindIII*, digested with *Bam*HI plus *Hind*III and ligated to *Bam*HI- and *Hind*III-digested pAT18 (Trieu-Cuot *et al.*, 1991). *S. agalactiae* was transformed with the ligation mixture and one $Em^r$ transformant that harbored the recombinant plasmid containing the entire *luxS* gene was obtained. This recombinant plasmid was designated pAT*luxS*. Then the plasmid pAT*luxS* was electrotransformed into SX1 strain, PCR was performed to confirm that *luxS* gene was complemented. The *luxS*-complemented strain of SX1 was named SX2. The primers used in this study are described in Table 1.

*Antimicrobial susceptibility assay*

The antimicrobial susceptibility assay was performed as described previously (Ahmed *et al.*, 2007; Xue *et al.*, 2013). Antimicrobial susceptibility of the *S. agalactiae* strains (WT, SX1 and SX2) was determined for antimicrobial agents including cefradine and norfloxacin (Boxiang). Overnight cultures were inoculated into 5 mL THB medium by touching a sterile wire loop and incubated at 30°C. Then the bacteria were pelleted and diluted to a final concentration of $1 \times 10^6$ CFU/mL using fresh THB, dispensed into multi-well culture plates of 96 wells containing two-fold serial dilutions of drugs (0.06–30 μg/mL cefradine and norfloxacin, respectively), then the plates were incubated at 30°C for 6 h and 24 h. Appropriate dilutions from mid-exponential growth (6 h) and overnight bacterial cultures (24 h) were plated on Todd-Hewitt agar plates to determine viable bacterial counts. The minimal inhibitory concentration (MIC) was defined as the lowest concentration that produced inhibition of bacterial growth. The minimal bactericidal concentration (MBC) was defined as the lowest concentration of cefradine and norfloxacin that killed 100% of the bacteria. Experiments were repeated three times with two parallels.

*AI-2 complementation*

The AI-2 complementation assay was performed as described previously (Ahmed *et al.*, 2007; Xue *et al.*, 2013). To verify the association between AI-2 communication and susceptibility to antimicrobials, the pre-AI-2 molecule 4,5-dihydroxy-2,3-pentanedione (DPD) (Omm Scientific Inc.) was used to complement function of the SX1. To determine the appropriate concentration of DPD, SX1 inoculated to a final concentration of $1 \times 10^6$ CFU/mL into THB containing 10-fold serial dilutions of DPD (0.074 nM-7.4 μM) and 7.5 μg/mL cefradine or 3.75 μg/mL norfloxacin was dispensed into sterile borosilicate glasses and incubated at 30°C for 24 h. $OD_{600}$ values were determined by appropriate dilutions using spectrophotometer. Experiments were repeated three times with two parallels.

*RNA isolation and quantification by real-time reverse transcription PCR*

WT, SX1 with and without 7.4 nM DPD and SX2 were grown to late-exponential phase in THB at 30°C. Cells were collected and resuspended in DEPC-treated deionized water containing 20 mg/mL lysozyme. Following incubation at 37°C for 30 min, total RNA was extracted using the TRIzol method, and residual DNA was removed by treating with DNase I (Takara). Reverse transcription was conducted using DNase-treated RNA (250 ng) with the PrimeScript™ 1st Strand cDNA Synthesis Kit (Takara) according to the manufacturer's instructions. Quantification of cDNA levels was performed with the primers *parC-f* and *parC-r* following the instructions of the SYBR® Premix Ex Taq™ (Takara) on an ABI One-step system (Applied Biosystems). The quantity of *parC* cDNA was normalized to the 16S cDNA abundance by the relative quantification method.

*Statistical analysis*

The paired *t*-test (SPSS 17.0 China) was used for statistical comparisons. The level of statistical significance was set at $P \leq 0.05$.

## Results

*Construction of a* S. agalactiae luxS *mutant strain and complementation of the* luxS *mutant*

PCR in DNA/RNA level and sequencing were performed to confirm that the desired *luxS* gene deletion had occurred by double-crossover recombination. As shown in Fig. 1A, a 2,258-bp fragment was amplified from genomic DNA of SX1 by PCR, containing 693 bp upstream of *luxS* gene, 1,056 bp *cat* gene and 509 bp downstream of *luxS* gene.

PCR and RNA transcription PCR were performed on genomic DNA of SX2 to confirm that *luxS* gene was complemented. As shown in Fig. 1B, the *luxS* gene including the promoter was amplified in SX2 and the sequence had 100% homology with *S. agalactiae* A909 (GenBank No. CP000114.1).

*Inactivation of* luxS *in* S. agalactiae *is involved in antimicrobial susceptibility*

As shown in Fig. 2, *S. agalactiae* CNEP110823 (WT strain), *luxS* mutant of CNEP110823 (SX1), SX1 with pAT*luxS* (SX2) and SX1 with exogenous AI-2 reached similar growth values in absence of antimicrobial agents, indicating that inactivation of *luxS* or addition of AI-2 had no effect on growth.

To assess the viability of bacteria, WT, SX1 and SX2 strain were exposed to various concentrations of antimicrobial agents including cefradine and norfloxacin, and viable counts were determined at mid-exponential

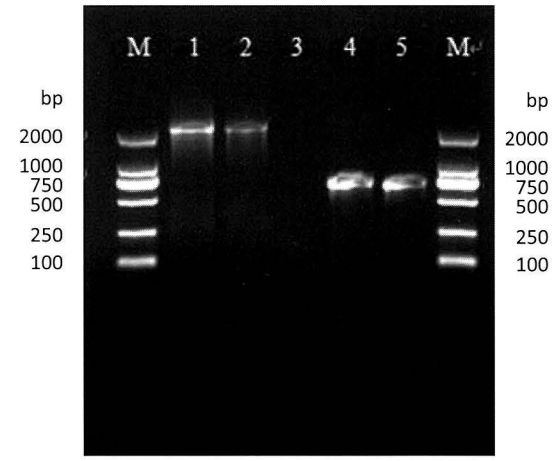


**Fig. 1.** PCR confirmation of the knockout mutant strain SX1 and complementation strain SX2. Lane 1, primers *up-luxS-f-SphI/down-luxS-r-SmaI*, SX1 at DNA level; Lane 2, primers *up-luxS-f-SphI/down-luxS-r-SmaI*, SX1 at RNA level; Lane 4, primers *pAT18-luxS-f-BamHI/pAT18-luxS-r-HindIII*, SX2 at DNA level; Lane 5, primers *pAT18-luxS-f-BamHI/pAT18-luxS-r-HindIII*, SX1 at RNA level.

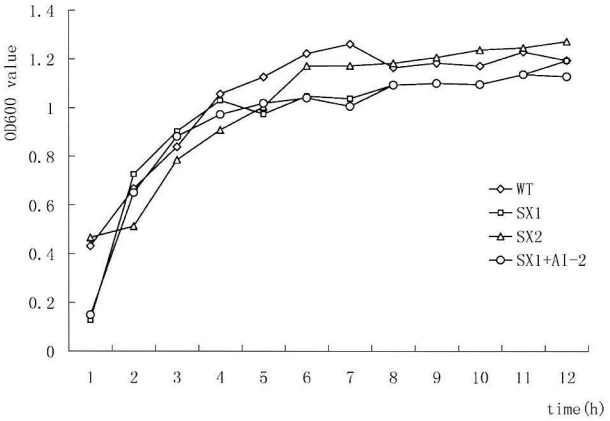


**Fig. 2.** Growth curves of *S. agalactiae* CNEP 110823 (WT), its isogenic *luxS* mutant (SX1) and *luxS* mutant with plasmid pAT*luxS* (SX2) and SX1 with exogenous AI-2 (SX1 + AI-2) at 30°C.

(6 h) and at stationary phase (24 h) (Fig. 3). MICs of cefradine and norfloxacin were tested, which showed that SX1 strain displayed two times higher than WT, and that SX2 recovered to the WT level. MBC also showed that SX1 displayed two times higher than WT, the complementation of *luxS* recovered the antimicrobial susceptibility. And viable counts were almost 2,000 times higher in SX1 in the presence of 1.875 $\mu$g/mL norfloxacin compared with WT, and 500 times higher in SX1 in the presence of 3.75 $\mu$g/mL cefradine compared with WT (Table 2). No significant viable counts were detected in WT and SX2 in 3.75 $\mu$g/mL and 1.875 $\mu$g/mL cefradine, 1.875 $\mu$g/mL and 0.94

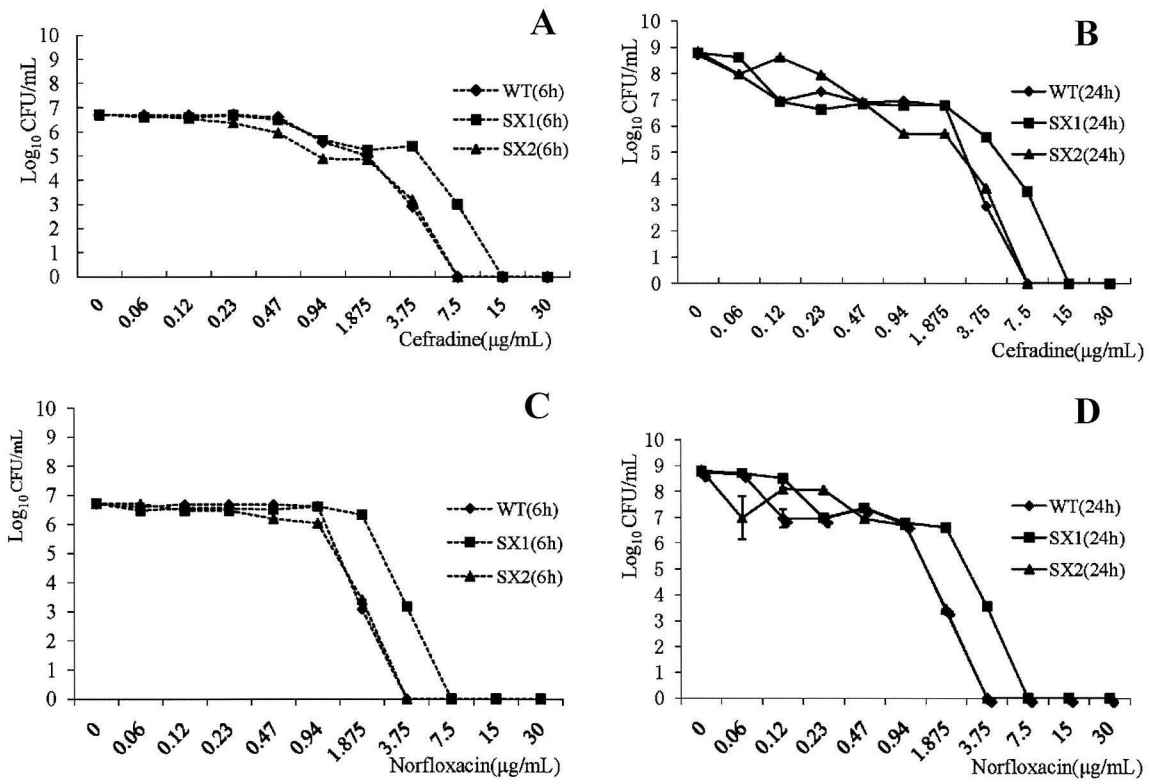


**Fig. 3.** Effect of cefradine (A, B) and norfloxacin (C, D) on growth of *S. agalactiae* CNEP 110823 (WT), its isogenic *luxS* mutant (SX1) and *luxS* mutant with plasmid pAT*luxS* (SX2). Bacterial counts were taken at mid-exponential phase (6 h) (A, C) and after overnight growth (24 h) (B, D).

**Table 2.** Viable counts of *S. agalactiae* with 3.75 μg/mL cefradine or 1.875 μg/mL norfloxacin

| Growth condition | Viable count ± SD (CFU/mL) | |
|---|---|---|
| | *S. agalactiae* WT | SX1 |
| 6 h | | |
| Control (without antibiotics) | $5.0 \pm 0.4 \times 10^6$ | $5.2 \pm 0.9 \times 10^6$ |
| Cefradine (3.75 μg/mL) | $8.1 \pm 0.2 \times 10^2$ | $2.6 \pm 0.5 \times 10^5$ [a] |
| Nofloxacin (1.875 μg/mL) | $1.2 \pm 0.3 \times 10^3$ | $2.5 \pm 0.8 \times 10^6$ [a] |
| 24 h | | |
| Control (without antibiotics) | $5.1 \pm 0.9 \times 10^8$ | $6.1 \pm 0.8 \times 10^8$ |
| Cefradine (3.75 μg/mL) | $9.0 \pm 0.2 \times 10^2$ | $3.9 \pm 0.5 \times 10^5$ [a] |
| Norfloxacin (1.875 μg/mL) | $2.5 \pm 0.2 \times 10^3$ | $4.1 \pm 0.6 \times 10^6$ [a] |

[a]: Significantly different from *S. agalactiae* WT ($p < 0.05$).

μg/mL norfloxacin, respectively. These results show that the antimicrobial susceptibility decrease in SX1 may result from the *luxS* mutation.

*AI-2 signal complementation reversed antimicrobial susceptibility of SX1*

To provide evidence for the involvement of AI-2 in complementation of antimicrobial susceptibility, various concentrations in a range of 0.074 nM–7.4 μM of the pre-AI-2 molecule DPD were used to complement the *luxS* mutant in the presence of 7.5 μg/mL cefradine and 3.75 μg/mL norfloxacin. $OD_{600}$ values were significantly decreased in SX1 complemented with exogenous DPD (0.074 nM–7.4 μM) compared with that with no DPD complementation, optimum SX1 complementation was reached at DPD threshold concentration at 7.4 nM (Fig. 4).

*Quantification by real-time reverse transcription PCR*

Previous report showed that *parC* was involved in quinolone resistance. To further determine whether the decreased susceptibility to norfloxacin is associated

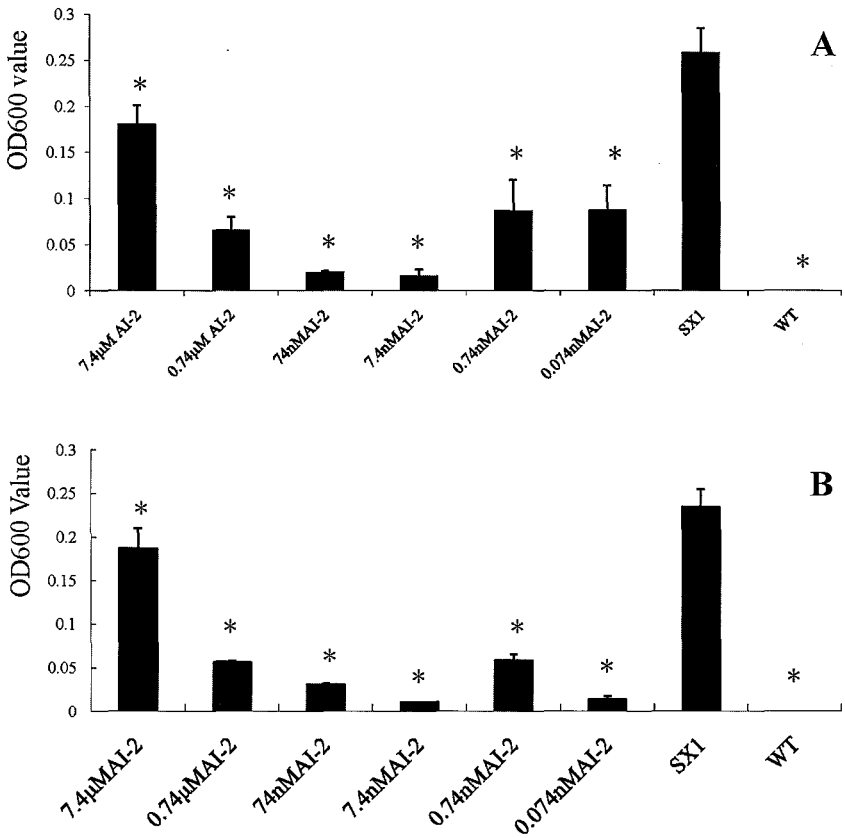


**Fig. 4.** $OD_{600}$ values of *S. agalactiae* CNEP 110823 (WT), its isogenic *luxS* mutant (SX1) and the *luxS* mutant with 0.074 nM to 7.4 μM AI-2 complementation following 24 h of incubation at 30°C in the presence of 7.5 μg/mL cefradine (A) and 3.75 μg/mL norfloxacin (B). *: Significantly different from SX1 ($p < 0.05$).

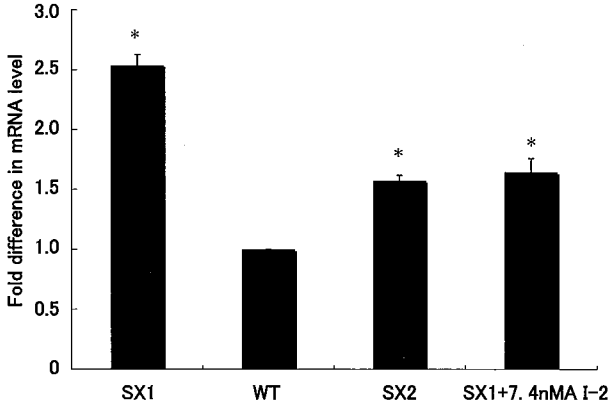


**Fig. 5.** *ParC* gene expression of different strains in vitro. Total RNA was extracted from WT, SX1, SX2 and SX1 + exogenous DPD grown in THB medium at the late-exponential phase and used for qRT-PCR. The mRNA level of each gene was normalized to that of 16S rRNA. Results are shown as relative expression ratios compared to expression in WT. *: Significantly different from WT ($p < 0.05$).

with the *parC* gene in the *luxS* mutant strain. Real-time PCR was used to quantify *parC* expression at the transcriptional level. Expression of *parC* was high-regulated approximately 2.5-fold in SX1 compared with the WT. In contrast, the *parC* mRNA level of SX2 and addition of AI-2 reversed to WT to some extent (Fig. 5). This suggests that change in antimicrobial resistance caused by the *luxS*/AI-2 signaling might be associated with the regulation of *parC* expression in *S. agalactiae*.

## Discussion

Two groups of antimicrobials with different antibacterial mechanisms were studied in this work. Cefradine is a $\beta$-lactam antimicrobial inhibiting peptidoglycan cross-linking and subsequently bacterial cell wall synthesis (Chen and Guo, 2012). Norfloxacin in contrast, inhibits ATP-dependent DNA supercoiling reaction catalyzed by DNA gyration and relaxation of supercoiled DNA, and promotes breakage of double-stranded DNA (Souto *et al.*, 2013). Some reports have shown that *luxS*/AI-2 is involved in antimicrobial susceptibility. Xue *et al.* (2013) reported that AI-2 in *Staphylococcus aureus* decreased susceptibility to antibiotics that inhibit cell wall synthesis including penicillin, oxacillin, vancomycin and teicoplanin. Ahmed *et al.* (2007) reported that AI-2 increased susceptibility to erythromycin and ampicillin in *Streptococcus anginosus*. The study on the role of *luxS*/AI-2 in *Streptococcus intermedius* biofilm formation and cell viability at sub-MICs of ampicillin, ciprofloxacin, or tetracycline showed that bacterial viability of biofilm and planktonic cell cultures of the *luxS* mutant strain was more susceptible to antimicrobials than the WT, and DPD complemented the *luxS* mutant phenotype (Ahmed *et al.*, 2009). But the relation between AI-2 QS and antimicrobial susceptibility in *S. agalactiae* has not been reported. We have compared antimicrobial susceptibilities among WT (CNEP 110823), *luxS* mutant strain (SX1) and *luxS* mutant complementation with either plasmid (SX2) or adding AI-2 to SX1 using disk-diffusion method containing quinolones, $\beta$-lactam, aminoglycosides, macrolide, lincosamides. As a result, there are only significant differences in quinolones and $\beta$-lactam (data not shown). So, we selected cefradine and norfloxacin and compared antimicrobial susceptibility of WT, SX1 and SX2 through antimicrobial susceptibility assay. The result showed that SX1 displayed decreased susceptibility to the two drugs and SX2 recovered to WT level. Different concentrations of DPD in a range of 0.074 nM to 7.4 $\mu$M to complement antimicrobial susceptibility in 7.5 $\mu$g/mL cefradine and 3.75 $\mu$g/mL norfloxacin. Optimum of complementation reached at DPD threshold concentration at 7.4 nM was determined. However, *S. agalactiae* WT, SX1, SX2 and SX1 + DPD showed similar growth profiles in the absence of drugs, the decreasing antimicrobial susceptibility of SX1 was not associated with growth alterations caused by inactivation of *luxS*. Thus, *luxS* was involved in antimicrobial susceptibility in *S. agalactiae* and this involvement was probably mainly due to AI-2 signaling.

Susceptibility to cefradine associates with change of the bacterial cell wall synthesis gene and the cell wall biosynthesis regulating gene. And susceptibility to norfloxacin associates with change of DNA gyrase gene, DNA topoisomerase gene, efflux pump genes and so on (Surcouf *et al.*, 2011; Selvam *et al.*, 2012). DNA topoisomerase IV subunit A gene (*parC*) is a target gene of quinolone drug. Previous study suggested that susceptibility of norfloxacin was associated with RNA transcription level of the *parC* gene (Kim *et al.*, 2013). In this study, *luxS* mutation resulted in high-regulation of the *parC* gene transcription. The higher ParC level in SX1 suggested that norfloxacin can be slower damage of DNA topoisomerase than WT. The in-depth molecular mechanism between *luxS*/AI-2 and susceptibility to cefradine and norfloxacin remains to be elucidated.

Previous reports showed that *luxS*/AI-2 QS was involved in up- or down-regulation of several genes. For example, AI-2 QS regulated up to 10% of the *Escherichia coli* genes and more than 70 genes in *Vibrio cholerae* (Sperandio *et al.*, 2001; Zhu *et al.*, 2002). Köhler *et al.* (2001) showed that expression of multidrug-resistant pumps may be regulated by the AI-1 QS used in intraspecies communication. Our study can well be that evidence that regulation of antibiotic efflux systems is also associated with AI-2 QS in *S. agalactiae*. This finding provides valuable clues for understanding the role of AI-2 QS in antibiotic susceptibility and antimicrobial therapy in infection of *S. agalactiae*.

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## References

Ahmed, N. A., F. C. Petersen and A. A. Scheie (2007): AI-2 quorum sensing affects antimicrobial susceptibility in *Streptococcus anginosus*. *J. Antimicrob. Chemother.*, **60**, 49–53.

Ahmed, N. A., F. C. Petersen and A. A. Scheie (2009): AI-2/*luxS* is involved in increased biofilm formation by *Streptococcus intermedius* in the presence of antimicrobials. *Antimicrob. Agents Chemother.*, **53**, 4258–4263.

Bassler, B. L. (1999): How bacteria talk to each other: regulation of gene expression by quorum sensing. *Curr. Opin. Microbiol.*, **2**, 582–587.

Bassler, B. L., M. Wright, R. E. Showalter and M. R. Silverman (1993): Intercellular signaling in *Vibrio harveyi*–sequence and function of genes regulating expression of luminescence. *Mol. Microbiol.*, **9**, 773–786.

Chen, J. Q. and R. X. Guo (2012): Access the toxic effect of the antimicrobial cefradine and its UV light degradation products on two freshwater algae. *J. Hazard. Mater.*, **30**, 209–210.

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.

Choi, J., D. Shin and S. Ryu (2007): Implication of quorum sensing in *Salmonella* virulence: the *luxS* gene is necessary for expression of genes in pathogenicity island 1. *Infect. Immun.*, **75**, 4885–4890.

Delannoy, C. M., M. Crumlish, M. C. Fontaine, J. Pollock, G. Foster, M. P. Dagleish, J. F. Turnbull and R. N. Zadoks (2013): Human *Streptococcus agalactiae* strains in aquatic mammals and fish. *BMC Microbiol.*, **13**, 41.

Doherty, N., M. T. Holden, S. N. Qazi, P. Williams and K. Winzer (2006): Functional analysis of *luxS* in *Staphylococcus aureus* reveals a role in metabolism but not quorum sensing. *J. Bacteriol.*, **188**, 2885–2897.

Evans, J. J., P. H. Klesius and P. M. Gilbert (2002): Characterization of beta-haemolytic group B *Streptococcus agalactiae* in cultured seabream, *Sparus auratus L.*, and wild mullet, *Liza klunzingeri* (Day), in Kuwait. *J. Fish Dis.*, **25**, 505–513.

Evans, J. J., D. J. Pasnik, P. H. Klesius and S. Al-Ablani (2006): First report of *Streptococcus agalactiae* and *Lactococcus garvieae* from a wild bottlenose dolphin (*Tursiops truncatus*). *J. Wildl. Dis.*, **42**, 561–569.

Federle, M. J. (2009): Autoinducer-2-based chemical communication in bacteria: complexities of interspecies signaling. *Contrib. Microbiol.*, **16**, 18–32.

Geng, Y., K. Y. Wang, X. L. Huang, D. F. Chen, C. W. Li, S. Y. Ren, Y. T. Liao, Z. Y. Zhou, Q. F. Liu, Z. J. Du and W. M. Lai (2012): *Streptococcus agalactiae*, an emerging pathogen for cultured ya-fish, *Schizothorax prenanti*, in China. *Transbound Emerg. Dis.*, **59**, 369–375.

Kim, J., J. Noh and W. Park (2013): Insight into norfloxacin resistance of *Acinetobacter oleivorans* DR1: target gene mutation, persister, and RNA-Seq analyses. *J. Microbiol. Biotechnol.*, **23**, 1293–1303.

Köhler, T., C. van Delden, L. K. Curty, M. M. Hamzehpour and J. C. Pechere (2001): Overexpression of the MexEF-OprN multidrug efflux system affects cell-to-cell signaling in *Pseudomonas aeruginosa*. *J. Bacteriol.*, **183**, 5213–5222.

Ma, Y. P., J. B. Li, L. Hao, Z. X. Liu, G. Q. Feng, J. S. Zhou and H. Ke (2013): Cloning of *Streptococcus agalactiae luxS* gene from tilapia and 3D structure prediction of deduced protein. *South China Fisheries Science*, **9**, 43–47.

Mian, G. F., D. T. Godoy, C. A. Leal, T. Y. Yuhara, G. M. Costa and H. C. Figueiredo (2009): Aspects of the natural history and virulence of *S.agalactiae* infection in Nile tilapia. *Vet. Microbiol.*, **136**, 180–183.

Miller, M. B. and B. L. Bassler (2001): Quorum sensing in bacteria. *Annu. Rev. Microbiol.*, **55**, 165–199.

Li, P., J. Liu, L. W. Zhu, C. Qi, W. C. Bei, X. H. Cai, Y. Sun and S. Z. Feng (2010): *VirA*: A virulence-related gene of *Streptococcus suis* serotype 2. *Microb. Pathog.*, **49**, 305–310.

Palaniyandi, S., A. Mitra, C. D. Herren, X. Zhu and S. Mukhopahyay (2013): *luxS* contributes to virulence in avian pathogenic *Escherichia coli* O78:K80:H9. *Vet. Microbiol.*, **166**, 567–575.

Qu, Y. Q., W. L. Ma, C. H. Liu, R. Shi and W. L. Zheng (2005): Detection of quorum-sensing pathway and construction of *luxS* gene deletion mutants of group B *Streptococcus*. *Di Yi Jun YiDa Xue Xue Bao.*, **25**, 1135–1139.

Selvam, A., D. Xu, Z. Zhao and J. W. Wong (2012): Fate of tetracycline, sulfonamide and fluoroquinolone resistance genes and the changes in bacterial diversity during composting of swine manure. *Bioresour. Technol.*, **126**, 383–390.

Souto, A., M. A. Montaos, M. Balado, C. R. Osorio, J. Rodríguez, M. L. Lemos and C. Jiménez (2013): Synthesis and antibacterial activity of conjugates between norfloxacin and analogues of the siderophore vanchrobactin. *Bioorg. Med. Chem.*, **21**, 295–302.

Sperandio, V., A. G. Torres, J. A. Girón and J. B. Kaper (2001): Quorum sensing is a global regulatory mechanism in enterohemorrhagic *Escherichia coli* O157:H7. *J. Bacteriol.*, **183**, 5187–5197.

Surcouf, C., S. Heng, C. Pierre-Audigier, V. Cadet-Daniel, A. Namouchi, A. Murray, B. Gicquel and B. Guillard (2011): Molecular detection of fluoroquinolone-resistance in multidrug resistant tuberculosis in Cambodia suggests low association with XDR phenotypes. *BMC Infect. Dis.*, **11**, 255.

Takamatsu, D., M. Osaki and T. Sekizaki (2001a): Construction and characterization of *Streptococcus suis-Escherichia coli* shuttle cloning vectors. *Plasmid*, **45**, 101–113.

Takamatsu, D., M. Osaki and T. Sekizaki (2001b): Thermosensitive suicide vectors for gene replacement in *Streptococcus suis*. *Plasmid*, **46**, 140–148.

Trieu-Cuot, P., C. Carlier, C. Poyart-Salmeron and P. Courvalin (1991): Shuttle vectors containing a multiple cloning site and a *lacZ* $\alpha$ gene for conjugal transfer of DNA from *Escherichia coli* to Gram-positive bacteria. *Gene*, **102**, 99–104.

Vendeville, A., K. Winzer, K. Heurlier, C. M. Tang and K. R.

Hardie (2005): Making 'sense' of metabolism: autoinducer-2, *luxS* and pathogenic bacteria. *Nat. Rev. Microbiol.*, **3**, 383–396.

Wang, Y., W. Zhang, Z. Wu, X. Zhu and C. Lu (2011): Functional analysis of *luxS* in *Streptococcus suis* reveals a key role in biofilm formation and virulence. *Vet. Microbiol.*, **152**, 151–160.

Waters, C. M. and B. L. Bassler (2005): Quorum sensing: cell-to-cell communication in bacteria. *Annu. Rev. Cell Dev. Biol.*, **21**, 319–346.

Xavier, K. B. and B. L. Bassler (2003): LuxS quorum sensing: more than just a numbers game. *Curr. Opin. Microbiol.*, **6**, 191–197.

Xue, T., L. Zhao and B. Sun (2013): *luxS*/AI-2 system is involved in antibiotic susceptibility and autolysis in *Staphylococcus aureus* NCTC 8325. *Int. J. Antimicrob. Agents.*, **41**, 85–89.

Zhao, L., T. Xue, F. Shang and B. Sun (2010): *Staphylococcus aureus* AI-2 quorum sensing associates with the KdpDE two-component system to regulate capsular polysaccharide synthesis and virulence. *Infect. Immun.*, **78**, 3506–3515.

Zhu, J., M. B. Miller, R. E. Vance, M. Dziejman, B. L. Bassler and J. J. Mekalanos (2002): Quorum-sensing regulators control virulence gene expression in *Vibrio cholerae*. *Proc. Natl. Acad. Sci. USA*, **99**, 3129–3134.

## *luxS*/AI-2 quorum sensing は抗菌剤に対する *Streptococcus agalactiae* の感受性低下に関与する

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*S. agalactiae* の薬剤感受性への quorum sensing の関与を検討するため，*S. agalactiae* CNEP110823株（親株）から autoinducer-2（AI-2）生合成酵素 LuxS の遺伝子破壊変異株（SX1）を作製し，抗菌剤に対する感受性を調べた。その結果，cefradine と norfloxacin に対する感受性が低下した。*luxS* 遺伝子を組み込んだプラスミドを SX1 株に導入した菌株（SX2）は親株と同等の感受性を示した。また，AI-2 の前駆物質である 4,5-dihydroxy-2,3-pentanedione を SX1 株に作用させたとき，感受性の回復が観察された。以上のことから，*luxS*/AI-2 quorum sensing は *S. agalactiae* の cefradine と norfloxacin に対する感受性に関与することが分かった。

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