Implication of quorum sensing in *Salmonella* virulence: the *luxS* gene is necessary for expression of genes in pathogenicity island 1

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Abstract

Despite the regulatory system sensing density of cell population and its signaling molecule were identified in *Salmonella enterica*, biological significance of this phenomenon termed as quorum sensing remains unknown. In this report, we provide evidence that the *luxS* gene is necessary for *Salmonella* virulence phenotypes. Transcription assays showed that the cell-density dependent induction of the *invF* gene was abolished in *Salmonella* strain deleted for the *luxS* gene. The effect of *luxS* deletion was also ensued in other InvF-regulated genes expressed from *Salmonella* pathogenicity island 1 (SPI-1). The decreased expression of SPI-1 genes in the *luxS*-deleted strain could be restored by either addition of a synthetic signal molecule or introduction of plasmid-copy of the *luxS* gene. Thus, the reduced expression of *invF* and its regulated genes in *Salmonella* cells lacking quorum sensing resulted in attenuation of virulence phenotypes both *in vitro* and *in vivo*.
Introduction

Bacterial cells are able to modify gene expression patterns in response to a variety of extracellular signals. In contrast, some bacterial species release and detect signaling molecules, and consequently control gene expression as their populations are growing. This type of bacterial signal transduction termed as quorum sensing was initially reported in marine bacterium *Vibrio fischeri*, where the signaling molecule and its sensing mechanism were identified (29). The quorum sensing system in *V. fischeri* consists of two proteins, LuxI, which is responsible for the synthesis of the acylhomoserine lactone autoinducer, and LuxR, which is activated by this autoinducer, and thus regulates expression of the luciferase operon (15).

Although both Gram-negative and Gram-positive bacteria use species-specific autoinducer 1 (AI-1) for activation of the quorum sensing system, signaling molecules differ: the former use acetylated homoserine lactones (AHLs) (35), whereas peptide pheromones are used in the latter (12). In addition, certain bacterial species including *Vibrio harveyi* also harbor quorum sensing systems activated by the autoinducer 2 (AI-2) in which the signaling molecules may be used as a universal language for communication with other bacteria rather than exclusively used for intra-bacterial communication (41). In both *V. harveyi* and *Salmonella enterica* serovar Typhimurium
(S. Typhimurium), the \textit{luxS} gene encoding the AI-2 synthase (i.e. LuxS protein) is directly involved in production of the AI-2 molecules from S-adenyl-methionine (SAM) (35). The LuxS protein catalyzes the final step in biosynthesis of AI-2 generating the signaling molecule 4,5-dihydroxy-2,3-pentanedione (DPD) (26). \textit{V. harveyi} and \textit{Salmonella} have two distinct AI-2 molecules derived from DPD. It has been identified that the \textit{V. harveyi} AI-2 is \((2S, 4S)-2\text{-methyl-2,3,3,4- tetrahydroxytetrahydrofuran-borate (S-THMF borate)}\) and the \textit{Salmonella}'s AI-2 is \((2R, 4S)-2\text{-methyl-2,3,3,4- tetrahydroxytetrahydrofuran (R-THMF)}\) (26).

It has been reported that a variety of bacterial phenotypes, such as bioluminescence, sporulation, biofilm formation, conjugation, motility, competence, and antibiotic production are regulated in response to signaling molecules of quorum sensing systems (11, 24, 28, 30, 33). The potential role of quorum sensing in virulence has been studied in several different pathogens. In \textit{Escherichia coli} O157:H7, the expression of the type III secretion system and other virulence factors is mediated by another autoinducer, AI-3 (6, 7, 33). In \textit{Streptococcus pyogenes}, inactivation of the \textit{luxS} gene resulted in the increased hemolytic activity but reduced secretion of cystein protease, a putative virulence factor (24). In addition \textit{S. pyogenes luxS} mutant was internalized with greater efficiency by HEp-2 epithelial cells than wild-type parent (25).
In *Porphyromonas gingivalis*, mutation of the *luxS*$_{Pg}$ gene decreased the expression of two cysteine proteases even though the mutant strain displayed the normal virulence phenotype in mice (3). In *Neisseria meningitides*, inactivation of the *luxS*$_{Nm}$ gene reduced the ability to cause bacteremia in the infant rat (40).

A number of bacterial virulence proteins are translocated into host cells during *Salmonella* infection via type III secretion systems (16, 20). Especially, a type III secretion system encoded by genes clustered in *Salmonella* pathogenicity island 1 (SPI-1) is required for invasion of *Salmonella* into epithelial cells (17), and is activated by various environmental cues reflecting complex conditions present in the intestinal lumen before host cell invasion (18). A series of studies in *Salmonella enterica* indicated that a quorum sensing signal molecule, AI-2, is imported during transition from the mid-exponential phase to the early-stationary phase, and the *lsr* operon encoding the Lsr transport apparatus and proteins for processing of phospho-AI-2 is also maximally expressed in the mid-exponential phase (36). The coincidence between the fact that the expression of SPI-1 is upregulated at the moment from mid-exponential to stationary growth of *Salmonella* cells (23) and the induction kinetics of the *Salmonella* quorum sensing system prompted us to investigate whether quorum sensing system is implicated in the regulation of SPI-1. In this study, we provide evidence that
the luxS gene is required for induction of InvF regulator, and consequently, its target
genes in SPI-1. Our findings in this report may provide a link between quorum sensing
and regulatory cascades of virulence genes in SPI-1.

Materials and methods

Bacterial strains, plasmids and growth conditions. Bacterial strains and plasmids
used in this study are listed in Table 1. Salmonella Typhimurium strains used in this
study are derived from strain SL1344. Phage P22-mediated transductions were
performed as described (4). Vibrio harveyi strains were kindly gifted by Dr. Rhee, J. H.
(21). All Salmonella strains were grown aerobically or anaerobically at 37°C in Luria-
Bertani broth (LB). V. harveyi strains were grown aerobically in Autoinducer Bioassay
(AB) medium (13) at 30°C. Antibiotics were used in the following concentrations;
ampicillin 50 µg/ml, chloramphenicol 25 µg/ml, kanamycin 50 µg/ml and streptomycin
50 µg/ml.

Construction of strains. We constructed a Salmonella strain deleted for the luxS gene
using the method by Datsenko and Wanner (10). The CmR cassette from plasmid pKD3
was amplified using primers luxS-1 (5’
AGTTCAAGTTATTTTTAAAAATTATCGGAGGTGACTAATGTAGGCTGGAGC
TGCTTCG 3’) and luxS-2 (5’
CTAAAATATGCAGTTCTGCAGTTTTTCTTTCGGCAGCGCCATATGAATATCC
TCC 3’) and the resulting PCR product was used to transform the wild-type strain selecting for luxS::Cm transformants. Finally, the Cm\textsuperscript{R} cassette was removed using plasmid pCP20 (10).

**Construction of plasmids.** Plasmid pJJ2 was constructed by cloning of the luxS gene from *S. typhimurium* strain SL1344 into the pACYC184 plasmid vector (5). The luxS gene was amplified by PCR using primers luxS-F4 (5’
GATAATCCTGAACTAAGCTTCT CCGC 3’) and luxS-R4(5’GGTTATGAGAAAAGCATGCACCGATCA 3’) and the resulting product was cloned between HindIII and SphI sites of pACYC184.

**β-galactosidase assay.** β-galactosidase assays were carried out in duplicate and the activity was determined as described (27).

**RNA isolation and analysis of gene expression using real-time PCR.** *Salmonella* strains were grown in LB anaerobically to stationary phase, and total RNA was isolated using RNaesy Mini Kit (Qiagen). After DNase treatment of the RNA solution, cDNA was synthesized using Omnitranscript Reverse Transcription reagents (Qiagen) and
random hexamers. Quantification of cDNA was carried out using 2×iQ SYBR Green Supermix (Bio-Rad Laboratories), and real-time amplification of PCR product was analyzed using iCycler iQ real-time detection system (Bio-Rad Laboratories). The relative amount of cDNA was calculated using a standard curve obtained from PCR on serially diluted genomic DNA as templates. The mRNA expression level of the target gene was normalized to 16S rRNA expression level. The sequences of the primers used are presented in Table 2.

**Vibrio harveyi bioluminescence assay.** The autoinducer activity in *Salmonella* Typhimurium was determined using *V. harveyi* reporter system as described (34). Overnight culture of *Salmonella* strain was diluted 1:100 into 50 ml of fresh LB broth, and the supernatant was collected with the specific time intervals. Cell-free culture fluids were prepared by removing the cells by centrifugation, passed through 0.22 µm nitrocellulose membrane filters (Millipore), and stored at -20°C. The cell-free culture fluid from *V. harveyi* strain was obtained from overnight culture grown at 30°C in autoinducer bioassay (AB) medium. Ten µl aliquots of cell-free culture fluid from *Salmonella* were added into 96-well microtiter dishes (BD bioscience). Overnight culture of the *V. harveyi* BB170 strain was diluted 1:5000 with fresh AB medium and 90 µl of the diluted cells was added into the wells containing 10 µl of the *Salmonella*
culture fluids. We also performed the same experiment using the cell-free culture fluid from either *V. harveyi* BB152 (autoinducer-1\(^{-}\), autoinducer-2\(^{+}\)) or *V. harveyi* BB120 (autoinducer-1\(^{+}\), autoinducer-2\(^{-}\)) as a positive control. For negative control experiment, 10 \(\mu\)l of sterile growth medium was mixed with the *V. harveyi* BB170 strain. The microtiter dishes were incubated at 30\(^{\circ}\)C with agitation and the resulting light production was measured with a chemiluminometer Autolumat LB953 (EG & G Derthold) after 5 hr of incubation. The AI-2 activity was presented as the fold activity obtained by dividing the relative light unit (RLU) value obtained from test supernatants with that of the negative control.

**Invasion assay.** HEp-2 cells were grown in Dulbecco’s modified Eagle medium (DMEM; Invitrogen) supplemented with 10% fatal bovine serum (FBS), penicillin (50 U/ml), and streptomycin (50 U/ml). Confluent monolayers for infection with bacteria were prepared in 24-well tissue culture plates. Each well was seeded with 2x10\(^{5}\) cells suspended in DMEM-10% FBS without antibiotics and incubated for 1hr at 37\(^{\circ}\)C under 5% CO\(_{2}\). The wells were washed three times with phosphate-buffered saline (PBS) before bacterial cells were added. Bacterial cells were washed with PBS, suspended in pre-warmed DMEM medium, then added onto cell monolayer with a multiplicity of infection (MOI) of 10:1. Following 1 hr of incubation, the wells were washed three
times with pre-warmed PBS to remove extracellular bacteria and incubated for 1 hr with
pre-warmed medium supplemented with 100 µg/ml of gentamicin (Gm) to kill
extracellular bacteria. Afterward, the wells were washed three times with PBS, lysed in
1% triton X-100 for 30 min, and then diluted with PBS. Dilution of the suspension was
plated on LB agar medium to enumerate colony forming unit (CFU). To show the
relative invasion abilities of wild type and mutants, each number of colonies was
divided by the mean value of the colonies in wild-type *Salmonella*.

**Animal experiments.** Six-week-old female BALB/c mice were purchased from
Institute of Laboratory Animal Resources in Seoul National University. Mice in each
group were infected by oral gavage with ~10^7 of *Salmonella* cells in 100 µl of PBS.
Water and food were withdrawn 4 hr before infection, and were provided again at 2 hr
post infection (8). For analysis of bacterial colonization in organs, all mice were
euthanized with mix of ketamine and xylamine at designated time points, and the spleen
and liver were taken aseptically and placed in 1.5 ml tubes. The organs were then
homogenized in 1 ml of ice cold PBS and serially diluted. Bacterial loads were
determined by plating these samples on MacConkey agar plates containing 50 µg
streptomycin per ml.
Results

**Induction of the invF gene expression is dependent on the AI-2 activity.** A quorum sensing system that is activated by anutoinducer-2 (AI-2) has been identified in *Salmonella enterica* (37). It has also been reported that the AI-2 activity of *Salmonella* begins to significantly increase during entry of *Salmonella* cells into exponential growth, peaks at late exponential phase, then decreases to ~25% of the maximum level reaching the new steady-state (Fig. 1A) (36). In *Salmonella* Typhimurium, the luxS gene is required for a burst of AI-2 activity because the luxS gene encodes an enzyme (i.e. AI-2 synthase) catalyzing biosynthesis of the AI-2 molecule (Fig. 1A) (36).

It has been known that transcription of a subset of genes related to the SPI-1-encoded type III secretion system is regulated in a growth dependent manner (23). Thus, we examined whether quorum sensing of *Salmonella* affects expression of genes within SPI-1. We initially chose to study the transcription kinetics of the hilA and the invF genes because these two respectively encode the HilA and the InvF proteins which are primary regulators activating expression of the SPI-1 genes (22). When *Salmonella* cells carrying a chromosomal *invF-lacZ* transcriptional fusion were diluted into fresh medium, transcription of the *invF* gene began to elevate at the similar timing for the
initial increase of AI-2 activity then reached the steady-state levels (Fig. 1B). However, in isogenic luxS-deleted mutant, the growth dependent induction of invF gene transcription was not observed (Fig. 1B). Interestingly, the effect of quorum sensing appears to be biased to the InvF regulator because time-course transcription of the hilA gene showed no correlation with kinetics of the AI-2 activity in both the wild-type and the luxS-deleted strains (Fig. 1B).

To verify further that quorum sensing is necessary for induction of the invF transcription, we carried out complementation experiments. Real-time PCR analysis using total RNA isolated from isogenic strains reproduced the result that the transcription level of invF gene was decreased in the mutant strain deleted for the luxS gene (Fig. 2A). This result was indeed due to the LuxS function because the phenotype of luxS deletion was restored by either introduction of the plasmid-linked luxS gene or addition of 4, 5-dihydroxy-2, 3-pentanedione (DPD), a synthetic AI-2 molecule (26), into luxS-deleted mutant (Fig. 2A). In the case of the hilA gene, the moderately decreased level of mRNA expression (i.e. less than two fold) was also observed in the absence of the luxS gene (Fig. 2B). However, given that the introduction of the LuxS-expressing plasmid rescued the hilA gene transcription but the addition of DPD did not (Fig. 2B), the effect of LuxS on the expression of hilA might result from one of multiple
functions of this protein other than quorum sensing (39).

In sum, the data presented in this section suggest that expression of the InvF regulator is regulated in a cell-density dependent manner by which the quorum sensing system is mediated.

The *luxS* gene is necessary for expression of a subset of virulence genes in SPI-1.

The InvF regulator positively controls expression of the *sicA* gene encoding a chaperone then these two protein together activate transcription of *sigD* and *sopE* that encode secreted effector proteins that promote invasion (22). Thus, we hypothesized that the reduced expression of the InvF regulator in *luxS*-deletion mutant could consequently affect expression of its target genes. To test this idea, we directly measured the mRNA levels of the *sicA*, *sigD* and *sopE* genes, using real-time PCR analysis. The results in Fig. 2A showed that the decreased expression of *invF* gene in a *luxS*-deleted strain caused 40~50% reduction of the *sicA*, the *sigD*, and the *sopE* transcription levels. Consistent with the complementation experiment for the *invF* gene, the decreased transcription of the InvF-regulated genes were restored by either addition of DPD or introduction of plasmid copy of the *luxS* gene to the *luxS*-deletion mutant (Fig. 2A).

To investigate further whether the effect LuxS-mediated quorum sensing is
limited to InvF-regulatory events, we also measured transcription of prgH, the first gene of the prg operon, which is directly regulated by the HilA regulatory protein (2). We observed that transcription of the prgH gene is reduced in the Salmonella strain lacking the luxS gene, and that this phenotype was not complemented by addition of DPD as observed for the hilA transcription (Fig. 2B). Therefore, these data suggest that quorum sensing is implicated in the expression of the InvF regulator and its regulated gene targets.

The luxS gene is required for Salmonella invasion into eukaryotic cells.

Salmonella’s entry into mucosal tissues of animal hosts is mediated by various gene products expressed from SPI-1 (16). Especially, Salmonella strain deleted for the invF gene significantly impairs in its ability to enter host cells as a result of decreased expression of the InvF-controlled type III secreted proteins (14). Therefore, we tested the possibility that the reduced expression of SPI-1 genes by luxS-deletion might affect the invasiveness of Salmonella into eukaryotic cells. Murine epithelial HEp-2 cells were infected with isogenic strains of Salmonella grown in SPI-1 inducing growth condition (i.e. oxygen-limited condition) (2, 31). We found that invasion of Salmonella mutant deleted for the luxS gene occurred significantly less (i.e. ~33%) than that of the wild-
type strain, and this phenotypic defect was recovered in a mutant strain grown with either extracellular DPD or plasmid copy of the luxS gene (Fig. 3). This result is consistent with the transcription profiles of the invF gene and its targets that were changed by the quorum sensing activity (Fig. 2A) and suggests that the LuxS-mediated quorum sensing is required for efficient invasion of Salmonella into host tissue.

*Salmonella luxS mutant displays attenuation of virulence in mice.* The expression of SPI-1 genes is required for the lethality of orally infected Salmonella in mice (19). Therefore, we assessed the virulence phenotype of wild-type and its isogenic luxS-deleted mutant *in vivo*. We infected a group of five mice with ~10^7 CFU of Salmonella cells via an oral route and investigated bacterial colonization in liver and spleen. The mice were sacrificed after 2 and 5 days after infection then the numbers of bacteria in spleen and liver were determined. We observed that the colonization of luxS-deleted mutant was lower than that of wild-type Salmonella both in liver and spleen (Fig. 4). Notably, on day 5 post infection, the wild-type Salmonella and luxS-deleted mutant showed 2-log differences in the mean bacterial loads in these organs (Fig. 4). Thus, this experiment demonstrates that the luxS gene contributes to Salmonella virulence in mice by controlling the expression levels of SPI-1 genes.
Discussion

Although the LuxS quorum sensing system has been identified in *Salmonella enterica* (36), its biological significance has remained unaddressed. In this paper, we have now demonstrated that the luxS-mediated quorum sensing is necessary for *Salmonella* virulence.

*Salmoneilla* must express a series of proteins from SPI-1 to invade epithelial cells and move into deeper tissue of animal hosts during course of infection (22). We initially observed that transcription of the invF gene began to increase in *Salmonella* cells at similar timing that the initial burst of AI-2 activity occurred, and this cell-density dependent induction of the invF transcription was abrogated in *Salmonella* mutant deleted for the luxS gene (Fig. 1B). This was due to the lack of quorum sensing activity because the reduction of the invF transcription was restored either by addition of DPD or by introduction of the LuxS-expressing plasmid into luxS-deletion mutant (Fig. 2). The following transcription assay also showed that the mRNA levels of other SPI-1 components such as sicA, sigD, and sopE were decreased to 50–60% of the wild-type levels (Fig. 2), which is consistent with the report that the InvF protein is a
transcriptional activator of these genes (14, 22). Thus, the absence of quorum sensing activity resulted in decreased invasiveness of *Salmonella* into epithelial cells (Fig. 3), which in turn attenuated its virulence in orally infected mice (Fig. 4).

We should also mention that the LuxS protein was necessary for full expression of other SPI-1 genes such as HilA and PrgH (Fig. 2B). However, it does not seem that quorum sensing via production of AI-2 is solely responsible for this phenomenon, because there was complementary effect of the luxS-expression from plasmid but no recovery effect of DPD addition on transcription of these two genes (Fig. 2B). Based on the report that the *E. coli* LuxS protein plays a role in metabolic pathways, which are unrelated with quorum sensing (39), we assume that one of multiple functions of LuxS is involved in transcriptional regulation of *hilA*, where the controlled expression of the LuxS protein (i.e. at a specific timing with appropriate amounts) from the original locus on chromosome might be critical for this regulation.

In the *Salmonella* quorum sensing system, a regulatory protein, LsrR, negatively controls expression of the *lsr* operon encoding the Lsr transport apparatus and this operon is derepressed by the processing of phospho-AI-2 through LsrK (36). However, it is unlikely that expression of the *invF* gene and its regulated targets are regulated by the LsrR protein as a mutant *Salmonella* strain deleted of the *lsrR* gene does not show
any defect in growth dependent SPI-1 gene expression (data not shown). In enterohemorrhagic *E. coli*, quorum sensing via the AI-3 molecules increased levels of the QseA regulatory protein, which in turn activated type III secretion gene transcription and protein secretion in chromosomal pathogenicity islands named LEE (6, 32, 38). We found that *Salmonella* also harbors the *yhcS* gene encoding a putative regulatory protein that displays high amino acid identity to the QseA regulator (our unpublished data). Thus, it is tempting to test whether the YhcS protein of *Salmonella enterica* would connect quorum sensing and the expression of SPI-1 mimicking a regulatory circuit found in *E. coli* O157:H7.

In response to environmental signals such as high osmolarity and low oxygen, transcription of SPI-1 genes is upregulated in a highly complicated manner (2, 14). Among several transcriptional regulators, the HilA protein, a key player for SPI-1 expression, activates transcription of the *prgH* operon encoding type III secretion apparatus plus another transcription factor InvF (1) thereby expression of a number of effector proteins (i.e. SigD and SopE) are promoted (14). Because of this regulatory hierarchy, it would occur that the effect of quorum sensing was limited to a part of genes (i.e. InvF-regulated genes) in SPI-1 (Fig. 2). Although we do not know the reason, this finding implies that *Salmonella* may require AI-2 signaling to produce and deliver
enough effector proteins into host cells even after the HilA-mediated expression of type III secretion system. This might be a mechanism for *Salmonella* to secure the initial infection process by delivering virulence factors for modification of host response only when its population size attains to a certain level.

**Acknowledgments**—We thank Dr. Michael M. Meijler for kindly providing DPD and Dr. Joon-Heang Rhee for *V. harveyi* strains BB170, BB152 and BB120. And we also thank Dr Miller, VL for the given *Salmonella* strains which have invF- and hilA-lacZ fusions in chromosome, respectively. J. Choi was the recipient of a graduate fellowship provided by the Ministry of Education through the Brain Korea 21 Project. This work was supported by the 21C Frontier Microbial Genomics and Applications Center Program, Ministry of Science and Technology, Republic of Korea (MG05-0201-04-0).
References


Infect Immun 70:3085-3093.


Figure legends

Fig. 1. Induction kinetics of the invF gene shows correlation with the AI-2 activity.

(A) Extracellular AI-2 activities were determined over growth of Salmonella strains. Aliquots of wild-type (closed circle) and its isogenic luxS-deletion mutant (open circle) that were grown anaerobically in LB broth were removed with the specific time intervals and the AI-2 activity in the cell culture fluids was measured using the V. harveyi BB170 bioassay (34). Identical growth of two Salmonella strains is indicated as dashed lines. (B) Time-course transcription of the invF and the hilA genes. Aliquots of wild-type (closed symbols) and luxS-deletion mutant (open symbols) strains harboring a lacZ-transcriptional fusion to either the invF (squares) or the hilA (triangles) promoter were obtained as described above and the β-galactosidase activities (Miller units) were determined (27). The AI-2 activities determined above are also shown as dashed lines.

Fig. 2. The quorum sensing activity is required for expression of a subset of SPI-1 genes. Total RNA was isolated from wild-type (WT), luxS mutant (luxS), luxS mutant supplemented with 24 µM DPD (luxS + DPD), and luxS mutant harboring pluxS plasmid (luxS + pluxS) that were grown anaerobically to the stationary phase. (A) The mRNA levels of the invF gene and its regulated targets, sicA, sigD, and sopE were determined.
with three different samples using real-time PCR analysis. (B) Transcription of the hilA gene and the HilA-regulated prgH was also examined. Expression levels of the target genes were normalized to that of 16S rRNA gene. Shown is the average of three independent experiments and error bars indicate standard deviation from the mean.

**Fig. 3.** *Salmonella luxS* mutant displays invasion defect for epithelial cells. Wild-type (WT), luxS mutant (*luxS*), luxS mutant supplemented with 24 µM DPD (*luxS* + DPD), and luxS mutant harboring *pluxS* plasmid (*luxS* + *pluxS*) were grown anaerobically for 3 hr and ~10⁶ CFU of bacterial cells were used for infection of HEp-2 cells in 24-well plates. Note that DPD was added before infection into DMEM without antibiotics. Invasion of *Salmonella* was allowed for 1 hr and extracellular bacterial cells were eliminated by washing with PBS and treatment of 100 µg/ml of gentamicin. The invasion ability of strain was determined by plating the intracellular bacteria on LB agar after cell lysis with 1% TritonX-100. The relative *Salmonella* invasion was calculated from dividing the numbers of intracellular bacteria with those of bacteria used for initial infection and the resulting values were normalized further to the wild-type level.

*P<0.01 (Student’s *t*-test)
Fig. 4. The luxS deletion attenuates *Salmonella* virulence in mice. Isogenic

*Salmonella* strains grown aerobically were used for infection of BALB/c mice. A group of five mice was orally inoculated with $1.3 \times 10^7$ CFU of *Salmonella*. To analyze bacterial colonization in organs, mice were sacrificed at 48 hr and 120 hr after infection. The spleen and liver were removed, homogenized, and then plated on MacConkey agar plates containing 50 µg streptomycin per ml.
### Table 1. Bacterial strains and plasmids

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**Plasmids**

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<td>pACYC184</td>
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<td>pJJ2</td>
<td>pACYC184::luxS (containing its own promoter region) (cm^[R]) This study</td>
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**Table 2. Primers used in this study.**

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RT-rrsH-F1  CGGGAGGAAGGTGTTGTG
RT-rrsH-R1  GAGCCCGGGGATTTCACATC
Fig. 1

![Graph showing OD600 and RLU over time for different genotypes.](image)

- **OD600** (■, □)
- **RLU** (▲, △)

*Graph legend: invF::lacZ (■, □) and hilA::lacZ (▲, △).*
Fig. 2

A

mRNA expression

invF  sicA  sigD  sopE

B

mRNA expression

hilA  prgH

WT  luxS  luxS + DPD  luxS + pluxS
Fig. 3

**Relative invasion of Salmonella**

![Graph showing relative invasion of Salmonella WT, luxS, luxS + DPD, and luxS + pluxS. The graph indicates a significant difference (marked with an asterisk) between WT and luxS strains.]
Fig. 4

The figure shows a comparison of colony-forming units (CFUs) per gram of spleen and liver between WT and luxS strains at 48hr and 120hr. The data is presented as log-transformed CFUs, with error bars indicating variability. There is a noticeable difference in CFU levels between the WT and luxS strains, especially at 120hr.