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Quorum sensing in *Escherichia coli*, *Salmonella typhimurium*, and *Vibrio harveyi*: A new family of genes responsible for autoinducer production

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ABSTRACT **In bacteria, the regulation of gene expression in response to changes in cell density is called quorum sensing. Quorum-sensing bacteria produce, release, and respond to hormone-like molecules (autoinducers) that accumulate in the external environment as the cell population grows. In the marine bacterium *Vibrio harveyi* two parallel quorum-sensing systems exist, and each is composed of a sensor–autoinducer pair. *V. harveyi* reporter strains capable of detecting only autoinducer 1 (AI-1) or autoinducer 2 (AI-2) have been constructed and used to show that many species of bacteria, including *Escherichia coli* MG1655, *E. coli* O157:H7, *Salmonella typhimurium* 14028, and *S. typhimurium* LT2 produce autoinducers similar or identical to the *V. harveyi* system 2 autoinducer AI-2. However, the domesticated laboratory strain *E. coli* DH5α does not produce this signal molecule. Here we report the identification and analysis of the gene responsible for AI-2 production in *V. harveyi*, *S. typhimurium*, and *E. coli*. The genes, which we have named $luxS_{V.h.}$, $luxS_{S.t.}$, and $luxS_{E.c.}$ respectively, are highly homologous to one another but not to any other identified gene. *E. coli* DH5α can be complemented to AI-2 production by the introduction of the *luxS* gene from *V. harveyi* or *E. coli* O157:H7. Analysis of the *E. coli* DH5α $luxS_{E.c.}$ gene shows that it contains a frameshift mutation resulting in premature truncation of the $LuxS_{E.c.}$ protein. Our results indicate that the *luxS* genes define a new family of autoinducer-production genes.**

Many species of bacteria regulate gene expression in response to increasing cell population density, and, collectively, this phenomenon is termed quorum sensing (1). Quorum-sensing bacteria produce and release acyl-homoserine lactone-signaling molecules (called autoinducers) that accumulate in the environment as the cell density increases. When a threshold stimulatory concentration of autoinducer is achieved, a signal transduction cascade is initiated that ultimately is translated into a change in behavior of the organism (2–5). *Vibrio harveyi*, a free-living marine bacterium, possesses two quorum-sensing systems that function in parallel to control the density-dependent expression of bioluminescence (*lux*). Each of the *V. harveyi* density-sensing systems (signaling system 1 and signaling system 2) is composed of a sensor and a cognate autoinducer. Therefore, system 1 is composed of sensor 1 and it responds to autoinducer 1 (AI-1), and system 2 is composed of sensor 2 and this system detects autoinducer 2 (AI-2) (6). Sensory information from both systems is integrated via a shared regulatory protein to control the output, light emission (7–9). Genetic analysis of *V. harveyi* has shown that either system 1 or system 2 is sufficient for the density-dependent expression of luminescence.

The *V. harveyi* system 1 autoinducer (AI-1) has been purified and identified as hydroxybutanoyl-L-homoserine lactone (10), and its synthesis dependents on the genes *luxL* and *luxM* (11). Neither the structure nor the biosynthetic gene(s) for the system 2 autoinducer (AI-2) has been reported. The sensor proteins that detect AI-1 and AI-2 are members of the bacterial family of two-component adaptive regulatory proteins, and the mechanism of signal transduction is a phosphorylation/dephosphorylation cascade (11, 12). Reporter strains of *V. harveyi* have been constructed that respond specifically to only AI-1 or AI-2. These strains were used in the development of a bioassay capable of detecting autoinducers produced by other species of bacteria. It was observed that many species of bacteria produce an AI-2-like activity; however, only very rarely were species identified that produced an AI-1-like substance (13).

Using the *V. harveyi* bioassay, several strains of *Escherichia coli* and *Salmonella typhimurium* were shown to produce an AI-2-like activity. Analysis of autoinducer production in these enteric bacteria showed that the AI-2 activity is produced maximally in midexponential phase in the presence of certain preferred carbon sources. However, unlike other described quorum-sensing systems, in *E. coli* and *S. typhimurium* the AI-2 signal is degraded when the bacteria enter stationary phase (14). Several environmental cues were shown to influence the levels of autoinducer production and degradation in *E. coli* and *S. typhimurium*. Rapid logarithmic growth, preferred carbon sources, low pH, and/or high osmolarity all resulted in increased autoinducer production, whereas conditions of stationary phase, the lack of a preferred carbon source, neutral pH, and low osmolarity induced degradation of the AI-2 signal. Protein synthesis was required for both induction of signal production and signal degradation in *E. coli* and *S. typhimurium* (15). Interestingly, we showed that the domesticated laboratory strain *E. coli* DH5α is not capable of AI-2 production or degradation (14).

In the present manuscript we report the analysis of a gene responsible for AI-2 production in *V. harveyi*, *E. coli*, and *S. typhimurium*. The gene identified in all three species of bacteria is highly homologous, and we propose that these genes define a new family of proteins involved in autoinducer production. The genes, which we named $luxS_{V.h.}$, $luxS_{E.c.}$, and $luxS_{S.t.}$ have been identified in many species of bacteria by genome sequencing projects, but until now no function has been ascribed to this gene in any organism. The *luxS* genes do

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Abbreviations: AI-1 and AI-2, autoinducer 1 and 2, respectively.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. AF120098).

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not bear homology to any other gene known to be involved in autoinducer production.

MATERIALS AND METHODS

Bacterial Strains, Media, and Recombinant DNA Techniques. *V. harveyi* BB120 is the wild-type strain (13). *S. typhimurium* strain LT2 was obtained from K. Hughes (University of Washington), *S. typhimurium* 14028 is ATCC strain 14028 Organism: *Salmonella choleraesuis. E. coli* O157:H7 is a clinical isolate supplied by Paddy Gibb (University of Calgary). Luria–Bertani medium (LB) contained 10 g bacto tryptone (Difco), 5 g yeast extract (Difco), and 10 g NaCl per liter (16). The recipe for autoinducer bioassay (AB) medium has been reported (17). Where specified, glucose was added from a sterile 20% stock to a final concentration of 0.5%. Antibiotics were used at the following concentrations (mg/liter): ampicillin (Amp), 100; chloramphenicol (Cm), 10; gentamycin (Gn), 100; kanamycin (Kn), 100; and tetracycline (Tet), 10. DNA isolation, restriction analysis, and transformation of *E. coli* was performed as described by Sambrook *et al.* (16). Probes for Southern blot analysis were labeled by using the Multiprime DNA labeling system of Amersham. Sequencing was carried out by using an Applied Biosystems sequencing apparatus. The *V. harveyi* BB120 genomic library was constructed in the cosmid pLAFR2 as described (11). The method for Tn*5* mutagenesis of cloned *V. harveyi* genes and the allelic replacement technique for inserting Tn*5* mutated genes into the *V. harveyi* chromosome have been reported (11).

Autoinducer Assay. The AI-2 bioassay using the *V. harveyi* reporter strain BB170 (sensor 1^-, sensor 2^+) has been reported (14, 15). Cell-free culture fluids from *V. harveyi*, *E. coli*, or *S. typhimurium* strains to be tested for AI-2 activity were prepared as described (14, 15) and assayed at 10% (vol/vol). AI-2 activity is reported as the fold-induction of the reporter strain over background or as the percentage of activity obtained from *V. harveyi* BB120 (wild type) cell-free culture fluid.

Mutagenesis and Analysis of the AI-2 Production Gene in *S. typhimurium* LT2. MudJ insertion mutants of *S. typhimurium* LT2 were generated by using a phage P22 delivery system as described (18). After growth to midexponential phase in LB containing 0.5% glucose, the *S. typhimurium* insertion mutants were tested for AI-2 production using the *V. harveyi* BB170 bioassay. The site of the MudJ insertion that inactivated the AI-2 production function in *S. typhimurium* was identified by PCR amplification and sequencing of the chromosomal DNA at the insertion junction. A two-step amplification procedure was used (19). In the first PCR, the arbitrary primer 5′-GGCCACGCGTCGACTAGTCANNNNNNNNN-NACGCCC-3′ and the MudJ specific primer 5′-GCACTA-CAGGCTTGCAAGCCC-3′ were used. Next, 1 μl of this PCR was used as the template in a second PCR amplification employing a second arbitrary primer (5′-GGCCACGCGTC-GACTAGTCA-3′) and another MudJ specific primer (5′-TCTAATCCCATCAGATCCCG-3′). The PCR product from the second reaction was purified and sequenced.

Cloning and Sequencing of the *E. coli* MG1655, *E. coli* O157:H7, and *E. coli* DH5α AI-2 Production Genes. The DNA sequence obtained from the *S. typhimurium* LT2 MudJ screen was used to search the *E. coli* MG1655 genome sequence to identify the corresponding *E. coli* region (20). The gene identified from the *E. coli* genome project had the designation *ygaG*. Primers that flanked the *ygaG* gene and incorporated restriction sites were designed and used to amplify the *E. coli* MG1655, *E. coli* O157:H7, and *E. coli* DH5α *ygaG* genes. The primers used were 5′-GTGAAGCTTGTTTACTGACTA-GATC-3′ and 5′-GTGTCTAGAAAAACACGCCTGACAG-3′. The PCR products were purified, digested, and cloned into pUC19. In each case, the PCR products from three independent reactions were cloned and sequenced.

RESULTS

Identification and Cloning of the Gene Responsible for AI-2 Production in *V. harveyi*. We recently have reported that unlike many other *E. coli* strains, *E. coli* strain DH5α does not produce an AI-2 signal molecule that can be detected by *V. harveyi* (14). We reasoned, therefore, that we could use *E. coli* DH5α as a mutant to clone the *V. harveyi* AI-2 production gene. A library of wild-type *V. harveyi* BB120 genomic DNA was transformed into *E. coli* strain DH5α, and the transformants were screened for AI-2 production in the *V. harveyi* BB170 AI-2 detection bioassay. The library consisted of 2,500 clones, each containing roughly 25 kb of *V. harveyi* genomic DNA. Five DH5α clones were identified that resulted in upwards of 300-fold stimulation of the reporter strain in the bioassay.

The recombinant cosmid DNA from the five AI-2-producing *E. coli* DH5α clones was analyzed by restriction analysis and Southern blotting. All five of the cosmids contained an overlapping subset of identical *V. harveyi* genomic restriction fragments, indicating that we had cloned the same locus several times. One cosmid, called pBB2929, was selected for further analysis. Random mutagenesis using transposon Tn*5* was carried out on cosmid pBB2929, and pools of cosmids harboring Tn*5* insertions subsequently were transformed into *E. coli* DH5α. We tested 962 individual *E. coli* DH5α/pBB2929::Tn*5* strains for the loss of the ability to produce AI-2. Four *E. coli* DH5α strains harboring Tn*5* insertions in pBB2929 were identified that failed to produce AI-2. We mapped the locations of these Tn*5* insertions in pBB2929 and found that all four transposon insertions resided in the same 2.6-kb *Hin*dIII *V. harveyi* genomic DNA fragment (Fig. 1*A*).

Cosmid pBB2929 was digested with *Hin*dIII, and the eight resulting fragments were subcloned in both orientations into pALTER (Promega). The pALTER subclones were transformed into *E. coli* DH5α and subsequently tested for AI-2 production. The only strains capable of producing AI-2 contained the 2.6-kb *Hin*dIII fragment identified in the Tn*5* mutagenesis. This fragment was sequenced and only one ORF could be identified. Its location corresponded to the map positions of the four Tn*5* insertions that eliminated AI-2 production. We named the ORF $luxS_{V.h.}$ (Fig. 1*A*).

Mutagenesis of $luxS_{V.h.}$ in *V. harveyi*. We analyzed the effects of $luxS_{V.h.}$ null mutations on AI-2 production in *V. harveyi*. The four Tn*5* insertions that mapped to the $luxS_{V.h.}$ gene and the control Tn*5* insertion adjacent to the $luxS_{V.h.}$ locus were transferred to the corresponding locations in the *V. harveyi* BB120 chromosome to make strains MM37, MM30, MM36, MM38, and MM28, respectively (Fig. 1*A*). Southern blotting was used to confirm the correct placement of all five Tn*5* insertions in the *V. harveyi* chromosome. The four *V. harveyi* $luxS_{V.h.}$::Tn*5* insertion strains were tested for the ability to produce AI-2, and all four strains gave identical results.

In Fig. 2*A*, we show the AI-2 production phenotypes of the wild-type control Tn*5* insertion strain MM28 and one representative $luxS_{V.h.}$::Tn*5* insertion strain, MM30. *V. harveyi* MM28 and MM30 were grown to high cell density, after which cell-free culture fluids were prepared. The culture fluids were assayed for AI-2 activity by the ability to induce luminescence in the AI-2 detector strain BB170. Fig. 2*A* shows that addition of culture fluids from the control Tn*5* insertion strain MM28 induced luminescence in the reporter 780-fold, whereas culture fluid from the $luxS_{V.h.}$::Tn*5* insertion strain MM30 did not induce the expression of luminescence in the reporter. Therefore, a null mutation in $luxS_{V.h.}$ in *V. harveyi* eliminates AI-2 production.

Identification and Analysis of *S. typhimurium* Autoinducer-Production Mutants. To identify the gene responsible for AI-2 production in *S. typhimurium*, we randomly mutagenized *S.*

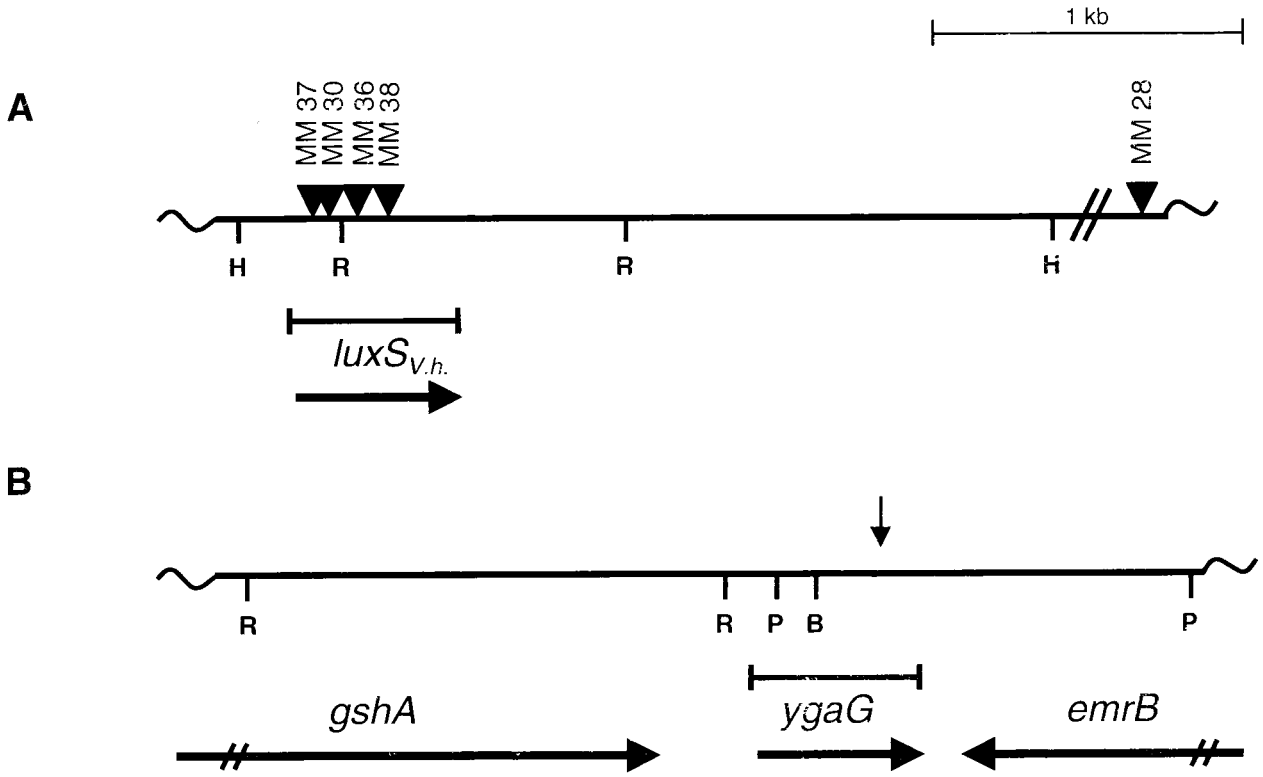

FIG. 1. The *luxS* and *ygaG* genes from *V. harveyi* and *E. coli* MG1655. (*A*) A restriction map of the *V. harveyi* $luxS_{V.h.}$ chromosomal region that was defined by Tn*5* insertion. The sites of Tn*5* insertions that disrupted the AI-2-production function and one control Tn*5* insertion outside of the $luxS_{V.h.}$ locus are shown (triangles). (*B*) The *ygaG* region in the *E. coli* MG1655 chromosome. This ORF is flanked by the *emrB* and *gshA* genes. The direction of transcription of each gene is indicated by horizontal arrows. The corresponding position of the MudJ insertion that eliminated AI-2 production in *S. typhimurium* LT2 is shown by a vertical arrow. H, R, P, and B denote *Hin*dIII, *Eco*RI, *Pst*I, and *Bam*HI restriction sites, respectively.

typhimurium LT2 using the MudJ transposon (18). Ten thousand *S. typhimurium* LT2 insertion mutants were assayed for AI-2 production in the *V. harveyi* BB170 bioassay. One *S. typhimurium* MudJ insertion mutant (strain CS132) was identified that lacked detectable AI-2 in culture fluids at midexponential phase.

Fig. 2*B* shows the AI-2 production phenotypes of *S. typhimurium* strain LT2 and the corresponding MudJ insertion strain CS132. The strains were grown to midexponential phase in LB containing glucose, and cell-free culture fluids were prepared and assayed for AI-2. *S. typhimurium* LT2 culture fluids induced the reporter strain 500-fold, whereas culture fluids from strain CS132 contained no AI-2 activity. Furthermore, strain CS132 did not produce AI-2 under any of the growth conditions that we have reported previously that induce AI-2 production in *S. typhimurium* (not shown).

The site of the MudJ insertion in *S. typhimurium* CS132 was determined by PCR amplification followed by sequencing of the 110 bp of chromosomal DNA adjacent to the transposon. This sequence was used to search the database for DNA homologies. The sequence matched a site (89-/105-bp identity) in the *E. coli* MG1655 genome that corresponded to an ORF of unknown function denoted *ygaG* (20). In the chromosome, the *E. coli ygaG* gene is flanked by the *gshA* and *emrB* genes (Fig. 1*B*). The *ygaG* gene is transcribed from its own promoter, which is located immediately upstream of the gene, indicating that it is not in an operon with *gshA* (A. Beeston, M.G.S., and B.L.B., unpublished results). The *emrB* gene is transcribed in the opposite direction. We PCR-amplified the *ygaG* region from the chromosomes of *E. coli* O157:H7 and *E. coli* MG1655, and the two *E. coli ygaG* genes were cloned into pUC19.

Complementation of *S. typhimurium* and *E. coli* AI-2$^-$ Mutants. We tested whether the *E. coli* O157:H7 *ygaG* gene and the *V. harveyi* $luxS_{V.h.}$ gene could restore AI-2 production in the AI-2$^-$ strains *S. typhimurium* CS132 and *E. coli* DH5α. In Fig. 3*A*, we show the AI-2 activity produced by wild-type *V. harveyi* BB120, *E. coli* O157:H7, and *S. typhimurium* LT2. In this figure, the level of AI-2 activity present in *V. harveyi* BB120 cell-free culture fluids was normalized to 100%, and the activities in cell-free culture fluids from *E. coli* and *S. typhimurium* were compared with that. In this experiment, *E. coli* O157:H7 produced 1.5 times and *S. typhimurium* LT2 produced 1.4 times more AI-2 activity than *V. harveyi* BB120 (i.e., 150% and 141%, respectively).

Fig. 3 *B* and *C* shows the AI-2 complementation results for *S. typhimurium* CS132 and *E. coli* DH5α. Fig. 3*B* demonstrates that introduction of the *E. coli* O157:H7 *ygaG* gene into *S. typhimurium* CS132 restored AI-2 production beyond the level of production of wild-type *S. typhimurium* (i.e., 209% activity). Comparison of the data in Fig. 3 *A* and *B* shows that the *E. coli ygaG* gene in *S. typhimurium* resulted in AI-2 production, exceeding that produced *in vivo* by *E. coli* O157:H7. Introduction of the *V. harveyi* $luxS_{V.h.}$ gene into *S. typhimurium* resulted in AI-2 production at slightly less than the level produced by wild-type *V. harveyi* BB120 (i.e., 73% of the level of *V. harveyi* BB120). Fig. 3*C* shows that introduction of the cloned *E. coli* O157:H7 and the *V. harveyi* BB120 AI-2 production genes in *E. coli* DH5α resulted in AI-2 production. However, expression of *E. coli* O157:H7 *ygaG* and *V. harveyi* BB120 $luxS_{V.h.}$ in *E. coli* DH5α resulted in only 31% and 43% of the *V. harveyi* BB120 AI-2 activity, respectively. Fig. 3 *B* and *C* shows that the control vectors produced no activity in the complementation experiments.

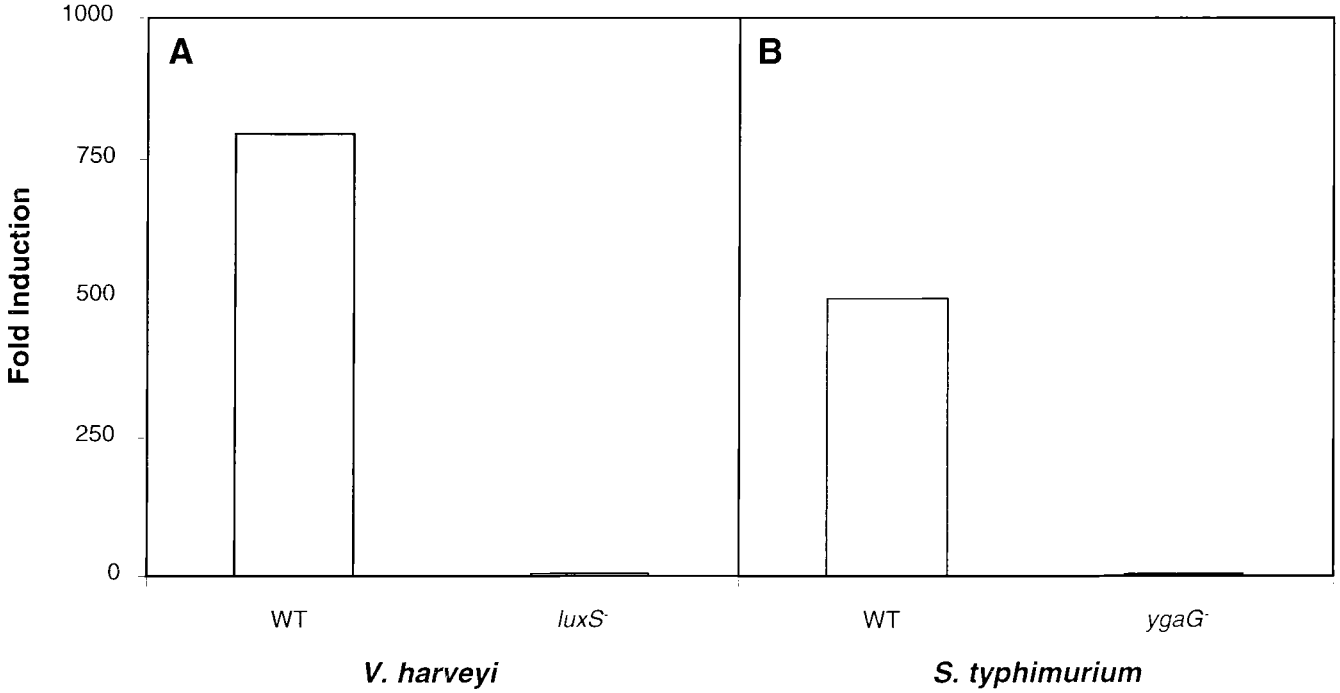

FIG. 2. Autoinducer-production phenotypes of *V. harveyi* and *S. typhimurium* strains. Cell-free culture fluids from *V. harveyi* and *S. typhimurium* strains were prepared and tested for AI-2 activity in the *V. harveyi* BB170 bioassay. (*A*) AI-2 production phenotypes of the wild-type *V. harveyi* strain MM28, which contains a Tn*5* insertion outside of $luxS_{V.h.}$ (denoted WT) and the $luxS_{V.h.}$::Tn5 mutant strain MM30 (denoted $luxS^-$). (*B*) AI-2-production phenotypes of wild-type *S. typhimurium* LT2 (denoted WT) and the *ygaG*::MudJ insertion mutant strain CS132 (denoted $ygaG^-$). Activity is reported as fold-induction of luminescence expression of the *V. harveyi* BB170 reporter strain over that when sterile medium was added.

Analysis of the AI-2 Production Genes from *V. harveyi*, *E. coli*, and *S. typhimurium*. We sequenced the AI-2 production gene $luxS_{V.h.}$ from *V. harveyi* BB120 and the *ygaG* loci from *E. coli* O157:H7, *E. coli* MG1655, and *E. coli* DH5α. The translated protein sequences encoded by the *ygaG* ORFs are shown in Fig. 4, and they are aligned with the translated LuxS protein sequence from *V. harveyi*. The underlined amino acids indicate the residues in the *E. coli* proteins that differ from the *V. harveyi* LuxS protein. The *ygaG* loci from *E. coli* encode proteins that are highly homologous to one another and also to LuxS from *V. harveyi*. The *E. coli* MG1655 and the *E. coli* O157:H7 YgaG proteins are 77% and 76% identical to LuxS from *V. harveyi* BB120. The DNA sequence we determined for *ygaG* from *E. coli* O157:H7 differs at five sites from the reported (and our) sequence for the *E. coli* MG1655 *ygaG* gene. Four of the changes are silent; the fifth results in a conservative Ala-to-Val alteration at amino acid residue 103 in the *E. coli* O157:H7 protein.

Identification of the *ygaG* locus in *E. coli* MG1655 and *E. coli* O157:H7 allowed us to investigate the AI-2 production defect

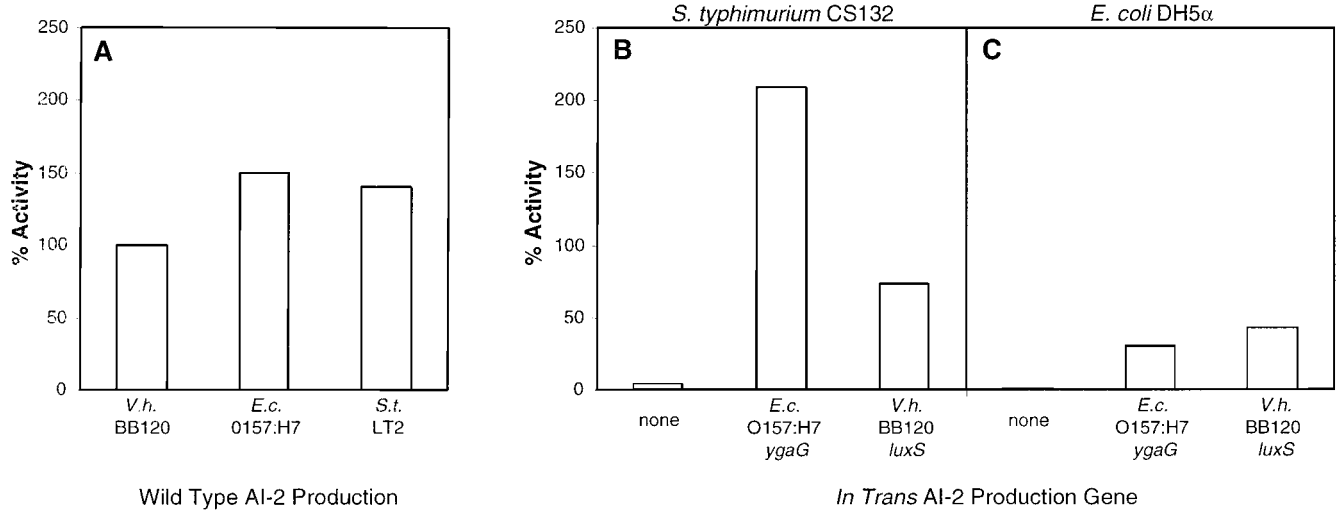

FIG. 3. Complementation of AI-2 production in *S. typhimurium* CS132 and *E. coli* DH5α. Cell-free culture fluids from *E. coli* and *S. typhimurium* strains were tested for AI-2 activity in the bioassay. The activity present in these fluids was compared with that produced by wild-type *V. harveyi* BB120. The level of BB120 activity was normalized to 100%. (*A*) AI-2 activity in cell-free fluids from wild-type *V. harveyi* BB120, *E. coli* O157:H7, and *S. typhimurium* LT2. (*B*) Complementation of *S. typhimurium* CS132 (*ygaG*::MudJ). (*C*) Complementation of *E. coli* DH5α. In *B* and *C*, the *in trans* AI-2 production genes are the following: vector control ("none"), *E. coli* O157:H7 *ygaG*, and *V. harveyi* BB120 $luxS_{V.h.}$. E.c., *E. coli*; *V.h.*, *V. harveyi*.

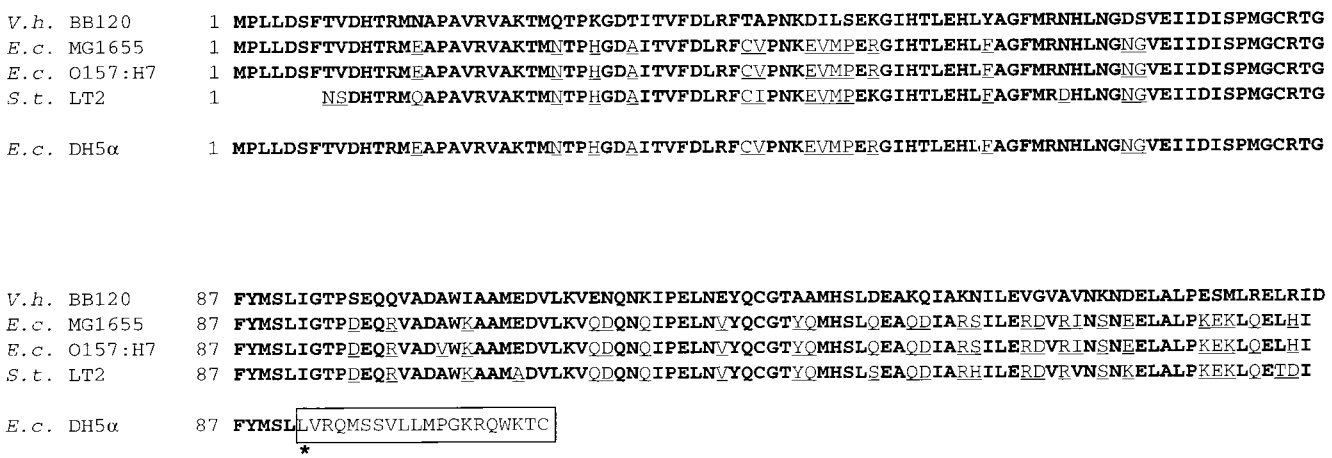

FIG. 4. Alignment of LuxS and YgaG protein sequences. The translated protein sequences for the AI-2 production family of proteins are shown. We determined the sequences for the $luxS_{V.h.}$ gene from *V. harveyi* BB120 and the *ygaG* genes from *E. coli* MG1655, *E. coli* O157:H7, and *E. coli* DH5α. The *S. typhimurium* LT2 *ygaG* partial sequence came from the *S. typhimurium* database. Amino acid residues that are not identical to the $LuxS_{V.h.}$ protein are underlined. The site of the frameshift mutation in the *E. coli* DH5α DNA sequence is denoted by ∗. The 20 altered amino acid residues that are translated after the frameshift are enclosed by the box.

in *E. coli* DH5α. *E. coli* DH5α possesses the *ygaG* gene because we could PCR-amplify this region from the chromosome using the same primers we employed to amplify it from *E. coli* MG1655 and *E. coli* O157:H7. Examination of the *E. coli* DH5α *ygaG* promoter showed that it is identical to that of *E. coli* MG1655, indicating that the AI-2 defect in *E. coli* DH5α is not simply a result of decreased transcription of *ygaG* (not shown). However, sequence analysis of the *E. coli* DH5α *ygaG* coding region showed that a 1-bp G-C deletion and a T-to-A transversion exist at base pairs 222 and 224, respectively. The frameshift mutation resulting from the G/C deletion causes premature truncation of the *E. coli* DH5α protein. Fig. 4 shows that the truncated *E. coli* DH5α protein is 111 aa, whereas the *E. coli* MG1655 and *E. coli* O157:H7 proteins are 171 residues. Twenty altered amino acids are translated after the frameshift and before termination of the protein. Our complementation results (Fig. 3) demonstrate that the AI-2 production defect in *E. coli* DH5α is recessive to *in trans* expression of *ygaG*, which is consistent with the defect being a result of a null mutation caused by the frameshift in the *E. coli* DH5α *ygaG* gene.

We searched the *S. typhimurium* database by using the sequence we obtained adjacent to the MudJ that inactivated the AI-2-production function in *S. typhimurium* CS132. A perfect match (110/110 bp) was identified to fragment B_TR7095.85-T7 in the *S. typhimurium* LT2 genome sequencing database (Genome Sequencing Center, Washington University, St. Louis). However, the *S. typhimurium* LT2 database *ygaG* sequence is incomplete (Fig. 4). The translated sequence matches the *E. coli* and *V. harveyi* sequences beginning at amino acid residue 8. The translated sequence shows that the *S. typhimurium* protein is 75% identical to LuxS of *V. harveyi*. To align the *S. typhimurium* sequence with the *V. harveyi* LuxS protein, we corrected three apparent frameshift errors in the database sequence. Considering that only crude, unannotated sequence data are currently available for *S. typhimurium*, we predict that the *S. typhimurium* protein contains seven more amino acids and that the frameshift mutations are sequencing errors. We were unsuccessful at PCR-amplifying either the *S. typhimurium* 14028 or the *S. typhimurium* LT2 *ygaG* gene using the primers designed for *E. coli*, so we do not yet have the complete sequence of the *S. typhimurium* gene.

DISCUSSION

Our results indicate that the genes identified and analyzed in this report encode a novel family of proteins responsible for autoinducer production. We have designated the members of this family of autoinducer-production genes as $luxS_{E.c.}$, $luxS_{S.t.}$, and $luxS_{V.h.}$ for *E. coli*, *S. typhimurium*, and *V. harveyi*, respectively.

Mutagenesis of *luxS* in *V. harveyi*, *S. typhimurium*, and *E. coli* eliminates AI-2 production in all three species of bacteria (Fig. 2 and DH5α results). *S. typhimurium* could be complemented to full AI-2 production by the introduction of either the *E. coli* O157:H7 $luxS_{E.c.}$ gene or the *V. harveyi* BB120 $luxS_{V.h.}$ gene (Fig. 3). These results indicate that both the *E. coli* and *V. harveyi* LuxS proteins can function with *S. typhimurium* cellular components to produce AI-2. *E. coli* DH5α was only partially complemented to AI-2 production by the introduction of either the *E. coli* O157:H7 $luxS_{E.c.}$ or the *V. harveyi* BB120 $luxS_{V.h.}$ gene (Fig. 3). Because in *trans* expression of *luxS* genes in *E. coli* DH5α did not completely restore autoinducer production, we hypothesize that other biochemical or physiological factors may contribute to signal production. We already know that the regulation of AI-2 production differs between pathogenic and nonpathogenic strains. For example, *E. coli* O157:H7 strains produce AI-2 at 30° and 37°C with or without glucose whereas *E. coli* K-12 strains do not produce AI-2 in the absence of a preferred carbon source. Also, all of the *E. coli* O157 strains that we have tested produce greater AI-2 activity than nonpathogenic *E. coli* strains. Likewise, pathogenic *S. typhimurium* 14028 produces significantly more AI-2 than does *S. typhimurium* LT2 (K. Knapp, M.G.S., and B.L.B., unpublished results). We have begun investigating these differences in regulation of autoinducer production and whether they impact virulence in these bacteria.

Our sequence analysis shows that the LuxS proteins are highly similar (Fig. 4), and our complementation data suggest that the proteins can function across species (Fig. 3). These results indicate that the enzymatic activity carried out by the LuxS proteins and any other cellular machinery that contributes to AI-2 synthesis must be conserved. We could not identify any amino acid sequence motif in the LuxS proteins that is indicative of a particular function. Therefore, we hypothesize that the LuxS proteins catalyze one specific enzymatic step in biosynthesis of the autoinducer. The remainder of the steps involved in AI-2 biosynthesis could be a consequence of normal intermediary metabolic processes.

As we have reported, we do not yet know the structure of AI-2 from *V. harveyi*, *E. coli*, or *S. typhimurium*. Furthermore, the AI-2s cannot be purified by conventional techniques used for the isolation of acyl-homoserine lactone autoinducers (14, 15). These results have led us to suspect that the AI-2s are not acyl-

homoserine lactones. The *luxS* genes identified here bear no homology to other genes known to be involved in production of HSL autoinducers [*luxI*-like (1), *luxLM-ainS*-like (11, 21)], further indicating that the AI-2 class of autoinducers is novel.

Database analysis of finished and unfinished bacterial genomes revealed that many other species of bacteria possess a gene homologous to *luxS* from *V. harveyi*, *S. typhimurium*, and *E. coli*. The species of bacteria identified and the percent homology/identity (H/I) to the LuxS protein of *V. harveyi* are as follows: *Haemophilus influenzae* (88/72), *Helicobacter pylori* (62/40), *Bacillus subtilis* (58/38), *Borrelia burgdorferi* (52/32), *Neisseria meningitidis* (89/80), *Neisseria gonorrhoeae* (89/80), *Yersinia pestis* (85/77), *Campylobacter jejuni* (85/74), *Vibrio cholerae* (95/90), *Deinococcus radiodurans* (65/45), *Mycobacterium tuberculosis* (59/41), *Enterococcus faecalis* (60/44), *Streptococcus pneumoniae* (57/36), and *Streptococcus pyrogenes* (57/36). In an earlier report (13), a few of these species were tested for AI-2 production. We showed that *V. cholerae* and *Y. enterocolitica* but not *B. subtilis* produced AI-2 activity. We predict that *B. subtilis* does produce AI-2 but we have not uncovered the environmental conditions that induce its synthesis. We further predict that all of the species identified in the database analysis produce an AI-2-like molecule.

We have suggested that the cues influencing autoinducer production and degradation in *E. coli* and *S. typhimurium* indicate that AI-2 is important for regulating the transition from a nonpathogenic existence outside a host to a pathogenic existence inside a host (14, 15). In support of this hypothesis, conditioned medium from *E. coli* O157:H7 that had been enriched for AI-2 has been shown to induce the expression of the Type III secretion system, a known virulence target. This result indicates further that AI-2 plays a role in pathogenicity (J. Kaper, personal communication). We have constructed *luxS* null mutants in *S. typhimurium* 14028 and *E. coli* O157:H7, and, using animal models, we currently are testing whether these mutant strains have virulence defects.

Note Added in Proof. Recently a full-length *Salmonella luxS* gene sequence became available from the *Salmonella typhi* sequencing group at The Sanger Centre. The sequence can be obtained from the following web site: http://www.sanger.ac.uk/Projects/S_typhi/blast_server.shtml. The *S. typhi* protein is the same length as the *V. harveyi* and *E. coli* LuxS proteins and it differs from the *E. coli* MG1655 LuxS protein at 11 positions.

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