APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Oct. 2006, p. 6615–6622 Vol. 72, No. 10
0099-2240/06/$08.00+0 doi:10.1128/AEM.01291-06


# Quantitative Effect of *luxS* Gene Inactivation on the Fitness of *Helicobacter pylori*[▽]

Woo-Kon Lee,[1]† Keiji Ogura,[1]‡ John T. Loh,[2] Timothy L. Cover,[2,3,4] and Douglas E. Berg[1]*

*Departments of Molecular Microbiology, Genetics and Medicine, Washington University Medical School, St. Louis, Missouri 63110[1]; Department of Medicine[2] and Department of Microbiology and Immunology,[3] Vanderbilt University School of Medicine, Nashville, Tennessee 37232; and Veterans Affairs Tennessee Valley Healthcare System, Nashville, Tennessee 37212[4]*

Received 6 June 2006/Accepted 15 August 2006

**Furanone metabolites called AI-2 (autoinducer 2), used by some bacterial species for signaling and cell density-regulated changes in gene expression, are made while regenerating *S*-adenosyl methionine (SAM) after its use as a methyl donor. The *luxS*-encoded enzyme, in particular, participates in this activated methyl cycle by generating both a pentanedione, which is transformed chemically into these AI-2 compounds, and homocysteine, a precursor of methionine and SAM. *Helicobacter pylori* seems to contain the genes for this activated methyl cycle, including *luxS*, but not genes for AI-2 uptake and transcriptional regulation. Here we report that deletion of *luxS* in *H. pylori* reference strain SS1 diminished its competitive ability in mice and motility in soft agar, whereas no such effect was seen with an equivalent Δ*luxS* derivative of the unrelated strain X47. These different outcomes are consistent with *H. pylori*'s considerable genetic diversity and are reminiscent of phenotypes seen after deletion of another nonessential metabolic gene, that encoding polyphosphate kinase 1. We suggest that synthesis of AI-2 by *H. pylori* may be an inadvertent consequence of metabolite flux in its activated methyl cycle and that impairment of this cycle and/or pathways affected by it, rather than loss of quorum sensing, is deleterious for some *H. pylori* strains. Also tenable is a model in which AI-2 affects other microbes in *H. pylori*'s gastric ecosystem and thereby modulates the gastric environment in ways to which certain *H. pylori* strains are particularly sensitive.**

There has been great interest in the ability of some bacterial taxa to signal their presence and abundance by secreting specific metabolites; to use these metabolites to monitor the density of members of their own species and of other species; and to respond with changes in patterns of gene expression, cellular phenotypes, and interactions with other microbes or host tissues—a set of behaviors termed "quorum sensing" (6, 17, 25, 27). Quorum sensing within individual species is often achieved using acyl-homoserine lactones (collectively called autoinducer 1 or AI-1), with much of the species specificity of AI-1 action stemming from differences in acyl chain length.

Chemically distinct furanone metabolites called AI-2 are also used by some species as signals for sensing cell density, both of unrelated taxa and of the same species. These furanones are by-products of a cyclic pathway that uses *S*-adenosyl methionine (SAM) as a methyl donor and then regenerates it (Fig. 1). In this pathway, methyl group transfer from SAM generates *S*-adenosyl homocysteine (SAH), a potentially toxic methyltransferase inhibitor. SAH is then deadenylated by a nucleosidase (Pfs) to generate *S*-ribosyl homocysteine (SRH). In the final enzymatic step in AI-2 synthesis, SRH is cleaved by the LuxS enzyme to generate 4,5-dihydroxyl-2,3-pentanedione, which undergoes chemical rearrangement and in some cases addition of boron or other substituents to generate a variety of furanones, some of which have AI-2 signaling activity (6, 25). The other cleavage product, homocysteine, serves as a precursor for methionine and then SAM synthesis. Despite use of AI-2 for quorum sensing by some taxa, AI-2s synthesized in other taxa could be simple by-products of the activated methyl cycle and of no regulatory significance (20, 25). It is not known if LuxS-mediated SRH consumption contributes importantly to depletion of the potentially toxic SAH intermediate and thus to fitness, or if Pfs-catalyzed conversion of SAH to SRH is sufficient, in any species in which AI-2 synthesis has been studied.

*Helicobacter pylori*, the genetically diverse pathogen implicated in peptic ulcer disease and gastric cancer (4, 15), contains a *luxS* gene and exhibits *luxS*-dependent AI-2 synthesis but seems to lack close homologs of genes known to be involved in AI-2 uptake or AI-2-responsive transcriptional regulation (2, 20, 25). Furthermore, no effect of *luxS* gene inactivation on overall growth in culture, VacA toxin synthesis, protein profiles in two-dimensional gels, or motility in liquid cultures was detected for the *H. pylori* strains tested (10, 12). Additional studies indicated that a functional *luxS* gene diminishes the capacity of *H. pylori* to form a biofilm at air-liquid-glass interfaces (9) and that a functional *luxS* gene contributes to a cell density-dependent induction of expression of *flaA-lacZ* and *flaA-cat* reporter constructs (14). However, *flaA* expression was less stimulated by conditioned medium than was typical of responses to AI-2 in well-established models. These studies were carried out using pure cultures of *H. pylori*, however, whereas *H. pylori*'s natural habitat consists primarily of gastric epithelial cell surfaces and overlying mucin, a niche that also can contain numerous other microbial species (3). Thus, if AI-2 signaling

* Corresponding author. Mailing address: Department of Molecular Microbiology, Campus Box 8230, 4940 Parkview Place, Washington University Medical School, St. Louis, MO 63110. Phone: (314) 362-2772. Fax: (314) 362-1232. E-mail: berg@borcim.wustl.edu.

† Present address: Department of Microbiology, Gyeongsang National University School of Medicine, Jinju, Republic of Korea.

‡ Present address: Department of Gastroenterology, University of Tokyo, Tokyo, Japan.

▽ Published ahead of print on 25 August 2006.

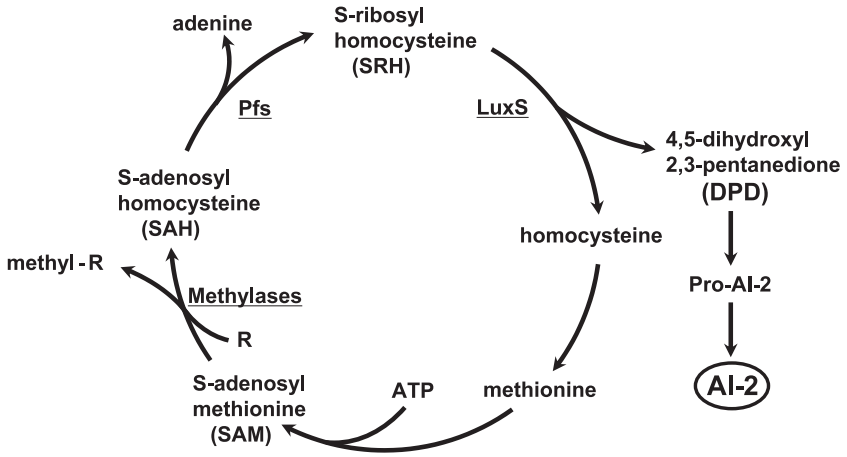


FIG. 1. AI-2 synthesis as a by-product of metabolic flux in the activated methyl cycle. For simplicity, only those metabolites and enzymes most relevant to the present studies are presented. More-detailed descriptions of this cycle, including structures of intermediates, are presented elsewhere (20, 25).

were important for *H. pylori* at all, this might be most evident in vivo, where AI-2 might affect coexisting microbes in ways that, in turn, impact on receptivity of the gastric mucosal environment to *H. pylori*.

Here we studied effects of *luxS* inactivation on colonization using two unrelated mouse-colonizing *H. pylori* strains, SS1 and X47. These two strains were chosen because they differ in their preferred sites of gastric colonization (antrum versus corpus, respectively [1]) and in their need for another gene of central metabolism, that encoding polyphosphate kinase 1 (PPK1) (responsible for inorganic polyphosphate synthesis [23]).

## MATERIALS AND METHODS

*H. pylori* strains SS1 and X47-2AL (for simplicity, here called X47) and derivatives diagrammed in Fig. 2 were used in these experiments. Standard growth conditions were used, including culture on brain heart infusion or brucella agar (supplemented with 7% blood or 7% serum) in microaerobic atmospheres (5% $O_2$, 10% $CO_2$, or simply 5% $CO_2$, at Washington and Vanderbilt universities, respectively) (1, 14, 22, 23). These media were supplemented with 0.4% Isovitalex and the antibiotics amphotericin B (8 μg/ml), trimethoprim (5 μg/ml), and vancomycin (6 μg/ml). Nalidixic acid (10 μg/ml), polymyxin B (10 μg/ml), and bacitracin (200 μg/ml) were added to this medium when culturing *H. pylori* from mouse stomachs (referred to as *H. pylori* selective agar), thereby ensuring that all colonies recovered are *H. pylori* (1). Motility was assayed by inoculating patches

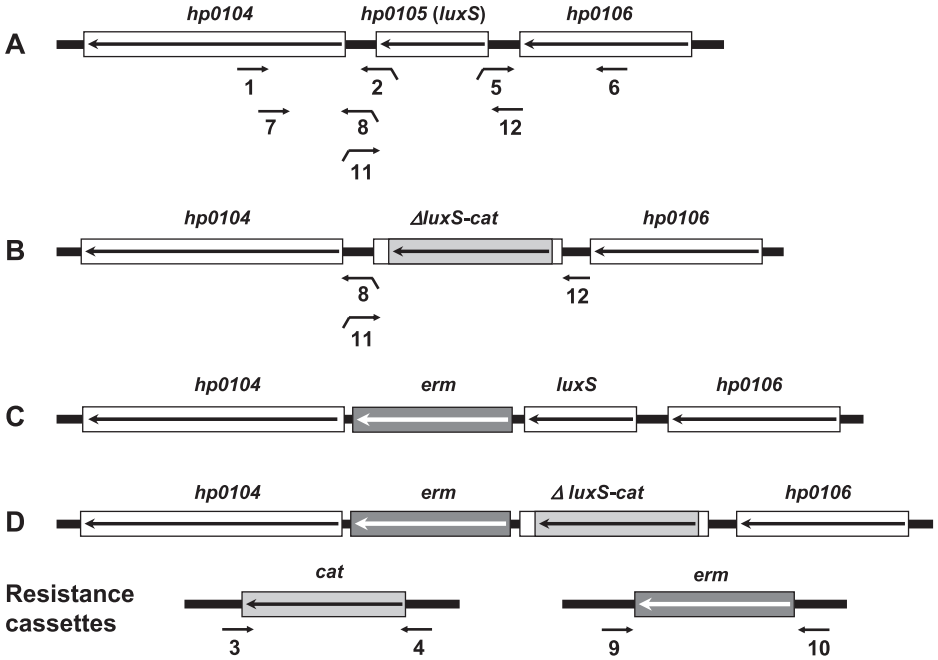


FIG. 2. Structures of *luxS-cat* deletions and *erm*-marked insertions used in these studies. Alleles were constructed by assembly of separate PCR products using primers 1 through 12 diagrammed here, with sequences given in Table 1. Downward tails indicate extensions at 5′ ends of primers that overlap with and are complementary to other specific primers used here and allow assembly of alleles from individual PCR products, as diagrammed in reference 22. The extensions on primers 2 and 5 overlap with primers 3 and 4, respectively, and extensions on primers 8 and 11 overlap with primers 9 and 10, respectively.

TABLE 1. PCR primers[a]

| Primer function, no., and name | Sequence | Location of 5′ end |
|---|---|---|
| Generation of Δ*luxS* deletion allele marked with *cat* | | |
| 1. HP105F2 | 5′-GCTATTGCCTTGCAACAAATCCCCGC | 242 bp after 5′ end of *hp0104* |
| 2. HP105R3 | 5′-CCCAGTTTGTCGCACTGATAATTAGACAA ACGCGCTGAGTGGTC | Complementary to catR primer and then 38 bp before 3′ end of *hp0105* (*luxS*) |
| 3. catR | 5′-TTATCAGTGCGACAAACTGGG | 24 bp after 3′ end of *cat* |
| 4. catF | 5′-GATATAGATTGAAAAGTGGAT | 94 bp before 5′ end of *cat* |
| 5. HP105F3 | 5′-ATCCACTTTTCAATCTATATCGGTGTTTTC ATGTTTTTAACTCC | Complementary to catF primer and then 10 bp after 5′ end of *hp0105* (*luxS*) |
| 6. HP105R2 | 5′-ATACTTAGGCGGGCATAGCGATG | 558 bp before 3′ end of *hp0106* |
| Insertion of *erm* determinant downstream of *luxS* locus and restoration of *luxSwt* or Δ*luxS* | | |
| 7. HP105CF2 | 5′-ATCCTTGTCAAGCCGTTATTGG | 160 bp after 5′ end of *hp0104* |
| 8. HP105CR3 | 5′-TTCAATAGCTATAAATTATTTAATAAGTA AGTGGTCTGAAGTGGGGGTTTGA | Complementary to eryR primer and then 21 bp before 3′ end of *hp0105* (*luxS*) |
| 9. eryR | 5′-TTACTTATTAAATAATTTATAGCTATTGAA | Coincides with 3′ end of *erm* |
| 10. eryF | 5′-CAATAATCGCATCAGATTGCAGTA | 118 bp before 5′ end of *erm* |
| 11. HP105CF3 | 5′-TACTGCAATCTGATGCGATTATTGTCAAA CCCCCACTTCAGACCAC | Complementary to eryF primer and then coincides with 3′ end of *hp0105* (*luxS*) |
| 12. HP105CR2 | 5′-GGAGTTAAAAACATGAAAACACC | 12 bp before 5′ end of *hp0105* |

[a] Overlaps between primers that allow assembly of separate PCR products are indicated by underlining: primers 2 with 3 and 5 with 6 for assembly of Δ*luxS-cat* allele by PCR with primers 1 and 6; primers 8 with 9 and 11 with 10 for assembly of the *erm*-linked *luxSwt* or Δ*luxS-cat* alleles with primers 7 and 6 or 7 and 12, respectively (see Fig. 2).

of *H. pylori* growth into plates containing brucella serum medium solidified with 0.35% agar, as described previously (22). Deletion of most of the *luxS* gene and its replacement with a nonpolar *cat* (resistance) cassette, as well as insertion of a nonpolar *erm* resistance cassette just downstream of both Δ*luxS-cat* and *luxS* wild-type alleles (Fig. 2), was done by PCR without cloning (7, 22), using primers listed in Table 1. The structures of transformants made with PCR products diagrammed in Fig. 2 were tested by PCR (as in reference 23); all transformants had the expected allele replacements. Inoculation of 8- to 10-week-old BALB/cJ and C57BL/6J mice, their sacrifice 2 weeks later, culturing of *H. pylori* from them, and testing for bacterial genotype were carried out according to Washington Animal Studies Committee-approved protocols, as described previously (1, 23).

Two precautions were taken to guard against inadvertent attenuation of mouse-colonizing ability. First, cultures used for transformation with DNA containing the Δ*luxS* allele were derived from pools of about 10 colonies that had been recovered by culture on *H. pylori* selective agar from infected mice. Second, cells from pools of about 10 Δ*luxS* transformant colonies were then used to infect mice, and another set of pools of 10 colonies recovered by culture 2 weeks later (all carrying the Δ*luxS* allele, as expected) was then tested for ability to compete with isogenic mouse-passaged *luxS* wild-type ($luxS_{wt}$) strains. No effect of mouse passage on the chloramphenicol or erythromycin resistance phenotype of the various transformants used here was detected. This use of pooled colonies and frequent mouse passage avoided the risk of inadvertently using a single colony that might have lost colonization ability due to mutation at loci unrelated to the *luxS* gene under study (see also reference 23).

The ability of conditioned medium from *H. pylori* cultures to induce luminescence was tested with the *Vibrio harveyi* reporter strain BB170 (10, 21). *H. pylori* cells were grown overnight in a 24-well dish (1 ml per well) and then subcultured into fresh brucella broth (20 ml) with shaking for various lengths of time. At each time point, aliquots of culture were collected, most cells were removed by centrifugation, and the supernatant-conditioned medium was sterilized by passage through a 0.22-μm filter. Supernatants were then stored until assayed at −80°C.

To detect AI-2-type compounds produced by *H. pylori*, *V. harveyi* BB170 was grown overnight in autoinducer bioassay (AB) medium (10), washed with fresh AB medium, and inoculated (1:5,000) into fresh AB medium containing 10% (vol/vol) of either *H. pylori* conditioned medium or, as a control, sterile brucella broth or medium conditioned by growth of this same *V. harveyi* strain. The *V. harveyi* reporter cultures were then grown at room temperature with shaking (150 rpm) for 6 h, and luminescence was measured using a Monolight 3010 luminometer. *n*-fold luminescence induction values were calculated as values obtained after adding conditioned medium versus values obtained after adding sterile brucella broth. The analysis was done five times with conditioned medium prepared on four different occasions.

## RESULTS

To test if *luxS* is important in vivo, we constructed a PCR product containing a deletion of the *luxS* gene marked with a nonpolar *cat* resistance marker (Fig. 2). Wild-type strains were then transformed with the DNA containing this Δ*luxS* allele, and chloramphenicol-resistant (Δ*luxS*) transformant colonies were selected. Initial tests indicated that deletion of *luxS* from SS1 or X47 did not severely impair the ability of either strain to colonize mice: *H. pylori* densities were in the range of ~$10^6$ CFU/stomach 2 weeks after inoculation in each of five mice tested with each *H. pylori* strain, which is similar to results obtained with isogenic wild-type parents (1).

Competition tests were used to examine more critically the possibility of an effect of *luxS* inactivation on colonization ability. Mice were each inoculated with 1:1 mixtures of Δ*luxS* mutant and isogenic $luxS_{wt}$ parent strains and then sacrificed 2 weeks later. *H. pylori* was cultured from separated antrum and corpus tissues, and at least 20 separate colonies from each tissue from each mouse were tested for chloramphenicol resistance (Δ*luxS*) versus susceptibility ($luxS_{wt}$). In the case of X47, the Δ*luxS* derivative comprised on average ~63% of *H. pylori* from the antrum and corpus of each mouse line (Fig. 3A; see legend for details). In no case was there any indication that the Δ*luxS* allele decreased X47 fitness. Indeed, the X47 Δ*luxS* strain was slightly *more* abundant than its wild-type parent in the corpus of BALB/c mice (68% [±17%]; significantly greater than 50% [$P = 0.01$], one-sample sign test).

With SS1, in contrast, the Δ*luxS* mutant comprised, on average, only 2% (±7%) and 6% (±11%) of colonies from the antrum of 23 BALB/cJ mice and 24 C57BL/6J mice, respec-

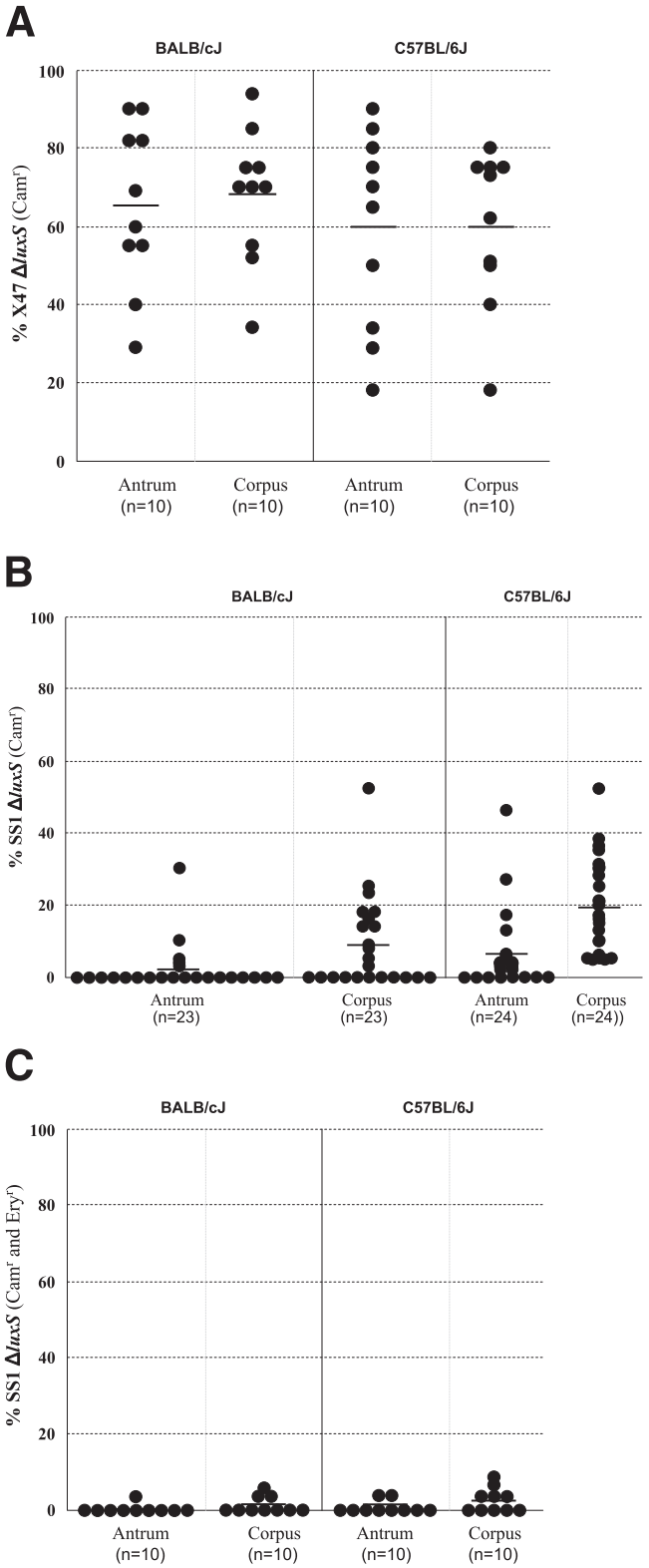


FIG. 3. Competition tests of the importance of a functional *luxS* gene for mouse colonization. Mice of two different inbred lines (C57BL/6J and BALB/cJ) were inoculated with 1:1 mixtures of isogenic $luxS_{wt}$ and Δ*luxS* derivative strains (chloramphenicol sensitive and resistant, respectively) shown in Fig. 2. Mice were sacrificed 2 weeks later, antrum and corpus were separated, and *H. pylori* was recovered from each tissue by colony formation (see Materials and Methods). At least 20 single colonies from each tissue from each mouse were scored as resistant (Δ*luxS*) or susceptible ($luxS_{wt}$) to chloramphenicol. Each point represents the ratio of two types from a different mouse. Horizontal lines depict mean ratios. Panel A. Strain X47 derivatives diagrammed in Fig. 2, lines A and B. Panel B. Strain SS1 derivatives diagrammed in Fig. 2, lines A and B. Panel C. Derivatives of the SS1 Δ*luxS* strain used in panel B that had been transformed with DNA containing a $luxS_{wt}$ allele linked to a downstream *erm* (resistance) insertion or with DNA containing the original Δ*luxS* allele linked to this *erm* cassette at the same site (see Fig. 2, lines C and D).

tively, and only 9% (±13%) and 20% (±13%) of colonies from the corpus of 23 BALB/cJ mice and 24 C57BL/6J mice, respectively (Fig. 3B). These yields were, in each case, far less than the 50% expected if Δ*luxS* had been neutral in the SS1 genetic background ($P < 0.001$).

**Restoration of wild-type *luxS*.** In principle the lower fitness of SS1 Δ*luxS* could have been due to secondary mutations, selected if *luxS* inactivation had decreased bacterial fitness in culture, rather than to an effect of loss of *luxS* function itself. To test such a possibility, we first made PCR products in which a selectable erythromycin resistance marker (*erm*) had been inserted in the intergenic space just downstream of $luxS_{wt}$ and also at the same site downstream of a Δ*luxS* allele (Fig. 2C and D). These DNAs were each used to transform the SS1 Δ*luxS* strain (Fig. 2B) that had competed poorly with its $luxS_{wt}$ parent. This generated a new pair of isogenic $luxS_{wt}$ and Δ*luxS* derivatives of strain SS1, each marked with the *erm* gene just downstream of the *luxS* locus but again distinguishable by chloramphenicol susceptibility versus resistance. Preliminary tests indicated that each type of $Erm^r$ transformant colonized mice efficiently when inoculated alone, as expected. A mixture of these new isogenic $luxS_{wt}$ and Δ*luxS* strains was then used to inoculate 10 C57BL/6J mice and 10 BALB/cJ mice, as described above. Analyses of colonies recovered 2 weeks later showed that the strain that had retained the Δ*luxS* allele was less fit than its sibling, in which $luxS_{wt}$ had been restored (Fig. 3C). Indeed, it seemed that the cost of the Δ*luxS* mutation was more severe in the strain carrying the *erm* insertion than in a strain without the *erm* gene (compare Fig. 3B and C). One possible explanation invokes perturbation by *erm* in expression of downstream genes, which putatively encode a cyclic-nucleotide phosphodiesterase and a methyl-accepting chemotaxis protein (2, 24), despite this cassette having been engineered to remove likely transcription termination sequences (23). This model would invoke synergism between effects of the *erm* insertion on downstream genes and those caused by the Δ*luxS* allele. The Δ*luxS* allele is marked with *cat*, but studies using other chromosomal loci had indicated that this resistance determinant itself does not decrease strain SS1's fitness in mice (23). Hence, we conclude that SS1 Δ*luxS*′s lower fitness in vivo stems primarily from loss of *luxS* activity per se, not the resistance marker used for selection.

**Motility.** No effect of inactivation of *luxS* on *H. pylori* motility in broth was found by light microscopy (as in reference 12), although other studies using different strains had indicated that *luxS* inactivation decreased expression of *flaA* (14), which

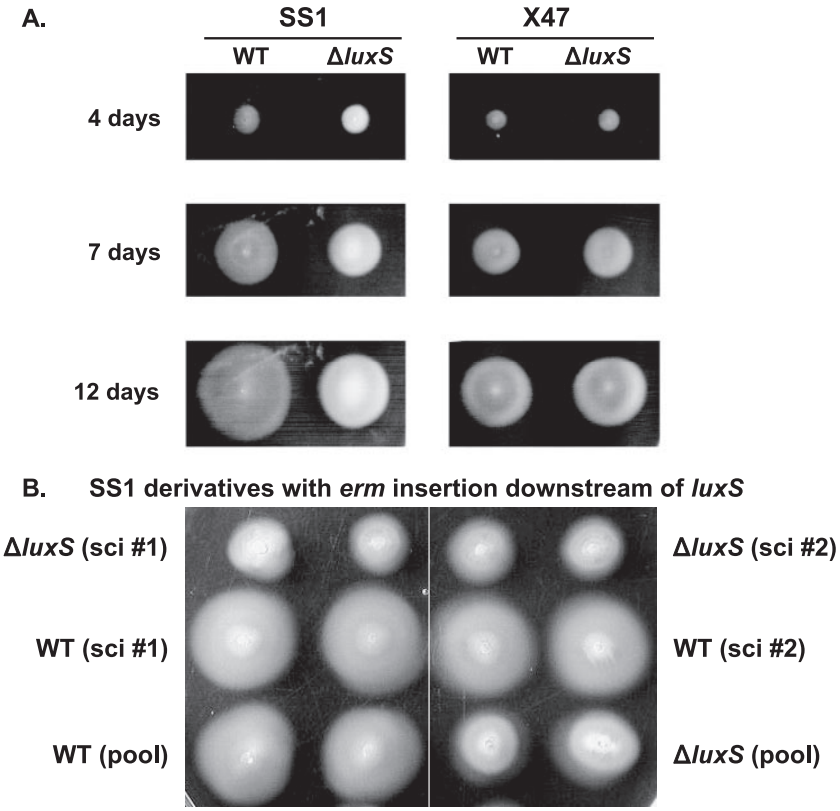


FIG. 4. Motility assays. Panel A. Colonies of SS1 $luxS_{wt}$, X47 $luxS_{wt}$, or Δ*luxS* derivatives of these two strains were stabbed once into soft agar and incubated for 4, 7, or 12 days, as indicated on the left. Panel B. Derivatives of strain SS1 Δ*luxS* used in panel A that had been transformed with DNA containing an *erm* insertion downstream of either the Δ*luxS* or $luxS_{wt}$ allele (see Fig. 2, lines C and D) were tested. Two single colonies (sci #1 and sci #2) and also a pool from each transformation (separated by white vertical line) were tested by stabbing in duplicate into soft agar and incubation for 7 days.

encodes one of the two *H. pylori* flagellins. We tested for effects of Δ*luxS* on motility with strains SS1 and X47, using a soft agar assay that detects changes in the strength of flagellum-driven swimming in a viscous environment, and also in chemotaxis along gradients that arise as nutrients are depleted by growth of bacterial colonies (18, 19). Figure 4A shows that Δ*luxS* mutant strains did indeed produce ever-expanding halos of growth, indicative of motility, although the SS1 Δ*luxS* halos were smaller than those of the isogenic SS1 wild type. In contrast, the halo sizes of X47 Δ*luxS* and isogenic X47 wild-type strains were not distinguishable either early or late during this growth period (Fig. 4A). Further tests showed that normal motility was restored if the Δ*luxS* allele was replaced with $luxS_{wt}$ linked to the *erm* resistance marker, whereas it was not restored in cells that had received the Δ*luxS* allele linked to this same *erm* insertion (Fig. 2C and D and 4B).

To test for possible intercellular "complementation" of the Δ*luxS* strain's motility defect by AI-2 or any other metabolite produced by SS1 $luxS_{wt}$, the Δ*luxS-cat* and $luxS_{wt}$ SS1 strains were mixed in a ratio of 1:10, 1:1, or 10:1, and these strain mixtures were inoculated in soft agar as for Fig. 4 and incubated for 7 days. Cell populations from the edges and centers of halos of growth were streaked to form single colonies, and these colonies were tested on chloramphenicol agar to estimate ratios of Δ*luxS* versus $luxS_{wt}$ strain types at each site. Both strain types were found at the centers of halos after 7 days in ratios equivalent to those used in the original inoculations (data not shown). In contrast, the Δ*luxS* strain was found at the halo edge only when Δ*luxS* had been in a 10-fold excess in the original inoculum, and then it comprised 27% of isolates on average. In contrast, the Δ*luxS* strain comprised <0.01% of bacteria found at the halo edge when the starting inoculum consisted of a 1:1 or 1:10 (Δ*luxS*:$luxS_{wt}$) mixture (each of two separate trials). This indicated that the defect caused by *luxS* inactivation was not restored by metabolites from the wild-type strain. Based on transmission electron microscopy (as described in reference 23), SS1 $luxS_{wt}$ and SS1 Δ*luxS* cells did not seem to be different morphologically: most cells of each type were slightly curved and rod shaped and typically bore several flagella of similar lengths at one pole (data not shown).

***luxS* integrity and AI-2 synthesis.** Given the reproducible quantitative effects of *luxS* inactivation on fitness in vivo and motility in strain SS1, one class of explanations for the lack of effect of the Δ*luxS* allele in strain X47 invokes a naturally occurring mutation in *luxS* or elsewhere that prevents AI-2 synthesis or speeds its removal. PCR amplification and DNA sequence analysis of the X47's *luxS* gene revealed a complete 155-codon open reading frame (GenBank accession no. DQ777750), with 96% amino acid sequence identity to *luxS* genes in reference strains 26695 and J99 (2, 24). This suggested that *luxS* should be functional.

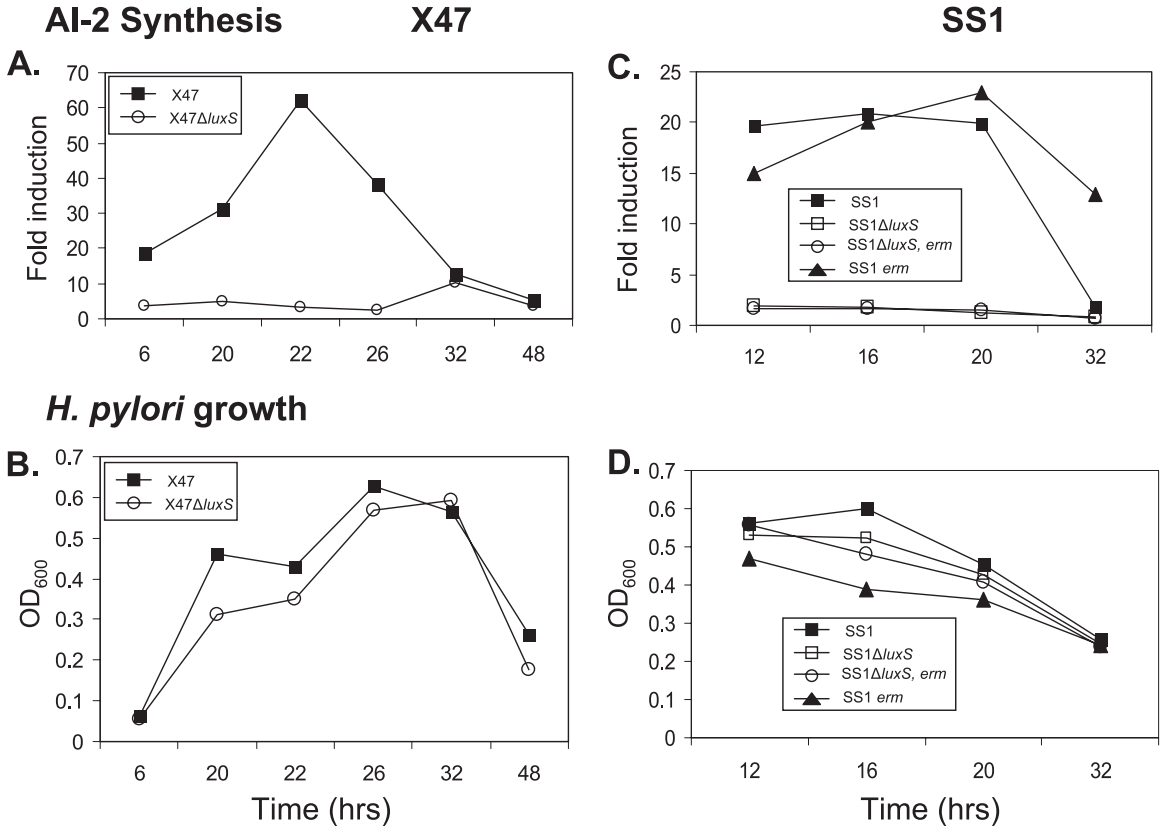


FIG. 5. Representative analyses of AI-2 production. AI-2 activity in *H. pylori* broth supernatants (i.e., conditioned medium) was measured in a bioluminescence assay using *V. harveyi* BB170 as an AI-2 reporter strain (Materials and Methods). Panels A and C show induction by conditioned brucella broth medium relative to results with sterile brucella broth. Panels B and D show optical densities at 600 nm ($OD_{600}$) of the corresponding *H. pylori* cultures at the times when aliquots were withdrawn. The Δ*luxS* allele used was marked with a *cat* (resistance) gene (see Fig. 2). *luxS* status, when not specified in graph caption, implies a wild-type allele.

More critically, we bioassayed AI-2 levels in filter-sterilized culture supernatants by testing the abilities of these supernatants to induce luminescence in the *Vibrio harveyi* reporter strain BB170. Figure 5A and B show that the inducing activity from X47 wild type increased severalfold as cells grew from mid- to late log phase, ultimately attaining levels that were about 12-fold higher than the nonspecific background seen with media from cultures of the isogenic Δ*luxS* mutant. This level was about 30% of that seen using conditioned media from the canonical AI-2-producing *Vibrio harveyi* grown in parallel (J. T. Loh and T. L. Cover, unpublished data). The level of AI-2 activity in *H. pylori*-conditioned media decreased sharply after prolonged (>~22 h) incubation, much as had been seen previously with an unrelated *H. pylori* strain (10). This might reflect intrinsic instability or degradation of AI-2 in culture. Data similar to those shown here were obtained in three other experiments, involving aliquots taken from fewer points in the growth curve (data not shown). It was also noteworthy that the maximum levels of AI-2-type activity produced by strain SS1 (in which the vigor of colonization is affected by *luxS* status) were substantially lower than those produced by strain X47 (Fig. 5A and C). Thus, the lack of effect of *luxS* on motility or colonization by X47 is not likely to be due to a defect in AI-2 accumulation.

## DISCUSSION

We found that deletion of *luxS* from *H. pylori* reference strain SS1 caused quantitative decreases in halo size (motility) in soft agar and in the ability to compete with isogenic SS1 wild type during mouse infection. Restoration of $luxS_{wt}$ by cotransformation with an *erm* resistance determinant inserted just downstream of *luxS* restored full motility and vigor in vivo relative to its isogenic Δ*luxS* sibling, also carrying *erm* at the same site. Thus, the Δ*luxS*-associated decrease in SS1 fitness likely stems from loss of *luxS* itself, not altered expression of downstream genes (predicted to encode a cyclic phosphodiesterase and a methyl-accepting chemotaxis protein [2, 24]) or mutation elsewhere in the genome. With the unrelated strain X47, in contrast, deletion of *luxS* did not significantly affect motility or vigor in vivo. DNA sequencing revealed a complete *luxS* open reading frame in this strain, and bioassays revealed normal *luxS*-dependent AI-2 production. Thus, the lack of effect of the Δ*luxS* allele on X47 phenotypes stems from this strain's tolerance of *luxS* inactivation, rather than a preexisting *luxS* deficiency in X47 wild type.

The decrease in SS1 halo size caused by the Δ*luxS* allele in soft agar is in accord with the previously reported ~2- to ~10-fold decrease in expression of *flaA-lacZ* or *flaA-cat* tran-

scription fusions after *luxS* inactivation in other *H. pylori* strains (14). However, transmission electron microscopy (as described in reference 23) indicated that most cells in SS1 Δ*luxS* cultures carried several flagella of apparently normal length, as did their SS1 wild-type parents (data not shown). Alternatively, this decrease in halo size might reflect altered chemotaxis, a bacterial behavior linked to the activated methyl cycle via methylation of key chemotactic regulators (5, 16). In either case, it will be interesting to test if the thicker biofilms at a glass-broth-air interface found after *luxS* inactivation truly stem from direct suppression of biofilm formation by AI-2 (9) or less-efficient swimming or chemotaxis away from the biofilms into planktonic phase. That others had not noticed an effect of *luxS* inactivation on motility (12) could be ascribed to methodologic differences in assays (liquid culture versus soft agar) or a feature of background genotype in the "Aston" strain they used that could have resulted in tolerance of a *luxS* deficiency, much as invoked here with strain X47.

In terms of possible fitness mechanisms, the in vivo cost of deleting *luxS* in SS1 could be ascribed to a lack of AI-2-directed signaling in this strain when at high density. We do not favor this explanation, however, because (i) the sequenced *H. pylori* genomes each seemed to lack homologs of genes that in other systems participate in responses to AI-2 signals (2, 20, 24) (although, given diversity in gene content in *H. pylori*, the possibility of unrecognized AI-2 response genes in certain strains, SS1 included, is not completely excluded); and (ii) density-dependent *flaA* gene transcription, one of the few events affected by *luxS* inactivation, was only weakly stimulated by adding conditioned medium to a culture of a *luxS*-null strain (14). Alternatively, the decreased fitness of SS1 Δ*luxS* might stem from metabolic disturbances caused by the loss of *luxS*, specifically disruption of the cycle of SRH consumption and homocysteine synthesis (Fig. 1): e.g., if rates of homocysteine synthesis were limiting or SRH consumption was needed to deplete SAH, its potentially toxic precursor (Fig. 1) (8). The possibility of less-direct metabolic network explanations is well illustrated by the finding with *Escherichia coli* of a third quorum sensing signal, AI-3, that is chemically distinct from AI-2 but whose synthesis is also *luxS* dependent (26). AI-3's synthesis was traced to oxaloacetate, a metabolite also used in a second *luxS*-independent path for homocysteine biosynthesis. AI-3's *luxS* dependence was ascribed to siphoning of substrates from its synthesis into the alternative homocysteine biosynthesis pathway in Δ*luxS* strains (23). Although the types of metabolic connections and their relative importances vary among species, this study emphasizes the significance of network architecture and the potential of seemingly indirect effects to shape quantitative strain-variable phenotypes.

In summary, although previous reports of *luxS*-mutant-associated *H. pylori* phenotypes had tended to favor AI-2 signaling-based explanations, alternative physiologic explanations, such as those just outlined, seem more parsimonious to us at present. Further studies will be needed to define mechanisms underlying the differences in effects of Δ*luxS* alleles in strains X47 and SS1. Possibilities include differences between them in resistance to SAH and/or SRH intermediates, in LuxS-independent pathways for disposing of these intermediates, or in *luxS*-influenced pathways for generating other important metabolites. Also not excluded are models in which differences in dependence on AI-2 (if such metabolites are ever used by *H. pylori*) reflect interactions with coexisting bacterial species, some of which are AI-2 responsive and whose own activities affect host permissiveness for particular *H. pylori* strains. Formally, these *luxS* results are reminiscent of our finding that consequences of a polyphosphate kinase 1 deficiency varied with *H. pylori* genetic background. However, a functional polyphosphate kinase 1 gene was more important in vivo for X47 than for SS1 (23), the reverse of the *luxS* dependence seen here. Such contrasting outcomes illustrate that the spectra of potentially limiting metabolic factors vary among *H. pylori* strains and that no one strain is fully representative of this genetically diverse species. This consideration will become increasingly important as more *H. pylori* genomes are sequenced and as metabolic reconstructions and the discipline of systems biology (11, 13) become more refined.

## ACKNOWLEDGMENTS

We thank Mark Forsyth for stimulating discussions.

This work was supported by a fellowship from the Sankyo Foundation of Science (K. Ogura); by grants RO1 DK063041, P30 DK52574, and RO1 DK53623 from the National Institutes of Health; and by a grant from the Department of Veterans Affairs (T. L. Cover).

## REFERENCES

1. **Akada, J. K., K. Ogura, D. Dailidiene, G. Dailide, J. M. Cheverud, and D. E. Berg.** 2003. *Helicobacter pylori* tissue tropism: mouse-colonizing strains can target different gastric niches. Microbiology **149:**1901–1909.
2. **Alm, R. A., L. S. Ling, D. T. Moir, B. L. King, E. D. Brown, P. C. Doig, D. R. Smith, B. Noonan, B. C. Guild, B. L. deJonge, G. Carmel, P. J. Tummino, A. Caruso, M. Uria-Nickelsen, D. M. Mills, C. Ives, R. Gibson, D. Merberg, S. D. Mills, Q. Jiang, D. E. Taylor, G. F. Vovis, and T. J. Trust.** 1999. Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen *Helicobacter pylori*. Nature **14:**176–180.
3. **Bik, E. M., P. B. Eckburg, S. R. Gill, K. E. Nelson, E. A. Purdom, F. Francois, G. Perez-Perez, M. J. Blaser, and D. A. Relman.** 2006. Molecular analysis of the bacterial microbiota in the human stomach. Proc. Natl. Acad. Sci. USA **103:**732–737.
4. **Blaser, M. J., and J. C. Atherton.** 2004. *Helicobacter pylori* persistence: biology and disease. J. Clin. Investig. **113:**321–333.
5. **Bourret, R. B., and A. M. Stock.** 2002. Molecular information processing: lessons from bacterial chemotaxis. J. Biol. Chem. **277:**9625–9628.
6. **Camilli, A., and B. L. Bassler.** 2006. Bacterial small-molecule signaling pathways. Science **311:**1113–1116.
7. **Chalker, A. F., H. W. Minehart, N. J. Hughes, K. K. Koretke, M. A. Lonetto, K. K. Brinkman, P. V. Warren, A. Lupas, M. J. Stanhope, J. R. Brown, and P. S. Hoffman.** 2001. Systematic identification of selective essential genes in *Helicobacter pylori* by genome prioritization and allelic replacement mutagenesis. J. Bacteriol. **183:**1259–1268.
8. **Challan Belval, S., L. Gal, S. Margiewes, D. Garmyn, P. Piveteau, and J. Guzzo.** 2006. Assessment of the roles of LuxS, *S*-ribosyl homocysteine, and autoinducer 2 in cell attachment during biofilm formation by *Listeria monocytogenes* EGD-e. Appl. Environ. Microbiol. **72:**2644–2650.
9. **Cole, S. P., J. Harwood, R. Lee, R. She, and D. G. Guiney.** 2004. Characterization of monospecies biofilm formation by *Helicobacter pylori*. J. Bacteriol. **186:**3124–3132.
10. **Forsyth, M. H., and T. L. Cover.** 2000. Intercellular communication in *Helicobacter pylori*: *luxS* is essential for the production of an extracellular signaling molecule. Infect. Immun. **68:**3193–3199.
11. **Francke, C., R. J. Siezen, and B. Teusink.** 2005. Reconstructing the metabolic network of a bacterium from its genome. Trends Microbiol. **13:**550–558.
12. **Joyce, E. A., B. L. Bassler, and A. Wright.** 2000. Evidence for a signaling system in *Helicobacter pylori*: detection of a *luxS*-encoded autoinducer. J. Bacteriol. **182:**3638–3643.
13. **Kell, D. B., M. Brown, H. M. Davey, W. B. Dunn, I. Spasic, and S. G. Oliver.** 2005. Metabolic footprinting and systems biology: the medium is the message. Nat. Rev. Microbiol. **3:**557–565.
14. **Loh, J. T., M. H. Forsyth, and T. L. Cover.** 2004. Growth phase regulation of *flaA* expression in *Helicobacter pylori* is *luxS* dependent. Infect. Immun. **72:**5506–5510.
15. **Mobley, H. L. T., G. L. Mendz, and S. L. Hazell (ed.).** 2001. *Helicobacter pylori.* ASM Press, Washington, D.C.
16. **Parkinson, J. S.** 2003. Bacterial chemotaxis: a new player in response regulator dephosphorylation. J. Bacteriol. **185:**1492–1494.

17. **Parsek, M. R., and E. P. Greenberg.** 2005. Sociomicrobiology: the connections between quorum sensing and biofilms. Trends Microbiol. **13:**27–33.
18. **Pittman, M. S., M. Goodwin, and D. J. Kelly.** 2001. Chemotaxis in the human gastric pathogen *Helicobacter pylori*: different roles for CheW and the three CheV paralogues, and evidence for CheV2 phosphorylation. Microbiology **147:**2493–2504.
19. **Suerbaum, S., C. Josenhans, and A. Labigne.** 1993. Cloning and genetic characterization of the *Helicobacter pylori* and *Helicobacter mustelae flaB* flagellin genes and construction of *H. pylori flaA*- and *flaB*-negative mutants by electroporation-mediated allelic exchange. J. Bacteriol. **175:**3278–3288.
20. **Sun, J., R. Daniel, I. Wagner-Döbler, and A. P. Zeng.** 2004. Is autoinducer-2 a universal signal for interspecies communication: a comparative genomic and phylogenetic analysis of the synthesis and signal transduction pathways. BMC Evol. Biol. **4:**36.
21. **Surette, M. G., and B. L. Bassler.** 1998. Quorum sensing in *Escherichia coli* and *Salmonella typhimurium*. Proc. Natl. Acad. Sci. USA **95:**7046–7050.
22. **Tan, S., and D. E. Berg.** 2004. Motility of urease-deficient derivatives of *Helicobacter pylori*. J. Bacteriol. **186:**885–888.
23. **Tan, S., C. D. Fraley, M. Zhang, D. Dailidiene, A. Kornberg, and D. E. Berg.** 2005. Diverse phenotypes resulting from polyphosphate kinase gene (*ppk1*) inactivation in different strains of *Helicobacter pylori*. J. Bacteriol. **187:**7687–7695.
24. **Tomb, J. F., O. White, A. R. Kerlavage, R. A. Clayton, G. G. Sutton, R. D. Fleischmann, K. A. Ketchum, H. P. Klenk, S. Gill, B. A. Dougherty, K. Nelson, J. Quackenbush, L. Zhou, E. F. Kirkness, S. Peterson, B. Loftus, D. Richardson, R. Dodson, H. G. Khalak, A. Glodek, K. McKenney, L. M. Fitzegerald, N. Lee, M. D. Adams, E. K. Hickey, D. E. Berg, J. D. Gocayne, T. R. Utterback, J. D. Peterson, J. M. Kelley, M. D. Cotton, J. M. Weidman, C. Fujii, C. Bowman, L. Watthey, E. Wallin, W. S. Hayes, M. Borodovsky, P. D. Karp, H. O. Smith, C. M. Fraser, and J. C. Venter.** 1997. The complete genome sequence of the gastric pathogen *Helicobacter pylori*. Nature **388:** 539–547.
25. **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.
26. **Walters, M., M. P. Sircili, and V. Sperandio.** 2006. AI-3 synthesis is not dependent on *luxS* in *Escherichia coli*. J. Bacteriol. **188:**5668–5681.
27. **Walters, M., and V. Sperandio.** 2006. Quorum sensing in *Escherichia coli* and *Salmonella*. Int. J. Med. Microbiol. **296:**125–131.