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Evidence for a Signaling System in *Helicobacter pylori*: Detection of a *luxS*-Encoded Autoinducer

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Helicobacter pylori **possesses a homolog of the *luxS* gene, initially identified by its role in autoinducer production for the quorum-sensing system 2 in *Vibrio harveyi*. The genomes of several other species of bacteria, notably *Escherichia coli*, *Salmonella enterica* serovar Typhimurium, and *Vibrio cholerae*, also include *luxS* homologs. All of these bacteria have been shown to produce active autoinducers capable of stimulating the expression of the luciferase operon in *V. harveyi*. In this report, we demonstrate that *H. pylori* also synthesizes a functional autoinducer (AI-2) that can specifically activate signaling system 2 in *V. harveyi*. Maximal activity is produced during early log phase, and the activity is diminished when cells enter stationary phase. We show that AI-2 is not involved in modulating any of the known or putative virulence factors in *H. pylori* and that a *luxS* null mutant has a two-dimensional protein profile identical to that of its isogenic parent strain. We discuss the implications of having an AI-2-like quorum-sensing system in *H. pylori* and suggest possible roles that it may play in *H. pylori* infection.**

Since its discovery in 1982, *Helicobacter pylori* has attracted a great deal of attention, both because it occupies the unusual and inhospitable niche of the gastric lumen and because of the diversity of clinical outcomes associated with this infection. Research efforts have focused on identifying genes and the functions of their protein products that allow this organism to colonize and cause disease in humans. In addition, much effort has been dedicated to investigating how expression of these genes is regulated in the host.

Only a handful of the genes characterized in *H. pylori* have been definitively shown to be transcriptionally regulated. Studies of the *H. pylori* flagellar loci indicate that these genes are transcriptionally controlled by a regulatory hierarchy including sigma 54 and sigma 28 homologs and FlgR, an NtrC homolog (33). The pattern of flagellar transcription shows similarities to those of both the family *Enterobacteriaceae* and *Caulobacter* (30, 33). There have also been reports suggesting that environmental conditions, such as pH, temperature, metal concentrations, and iron availability (7, 8, 26, 37), can influence *H. pylori* transcription. One surprising feature of the genetic makeup of *H. pylori* revealed by the full genome sequence is the absence of most of the regulatory genes present in other gastrointestinal bacteria (38). Since the genome of *H. pylori* differs significantly from those of other organisms occupying the human gastrointestinal tract, we were interested in identifying and studying mechanisms utilized by *H. pylori* that contribute to its unique ability to survive and persist in the gastric lumen.

Despite the relative lack of regulatory-gene homologs, the *H. pylori* genome does contain putative homologs of some sensor kinase and response regulator proteins belonging to the family of two-component systems. These proteins are widespread in other organisms and are central to the transcriptional regulation of a variety of processes that respond to changing environmental conditions (22).

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The *H. pylori* genome contains a homolog of *luxS*, a gene involved in autoinducer (AI) production in one of the two quorum-sensing systems of *Vibrio harveyi* (36). Quorum sensing involves the production and detection of membrane-permeating signaling molecules, or AIs, that function to communicate information to a population of cells about their external environment and cell density (19). The interaction of an AI with its cognate sensor protein results in activation of a signal transduction cascade that ultimately leads to changes in gene expression (19). In *V. harveyi*, quorum sensing controls the expression of bioluminescence.

Quorum sensing was first characterized in the regulation of bioluminescence in the marine bacterium *Vibrio fischeri* (reviewed by Dunlap and Greenberg [13]). In *V. fischeri*, quorum sensing depends on the action of two regulatory proteins, LuxI and LuxR. The product of the *luxI* gene directs the synthesis of the AI, an acylated homoserine lactone derivative. As cell density increases, the amount of AI also increases. At a critical concentration, the accumulated AI interacts with LuxR, the AI-binding protein. When bound to AI, LuxR acts as a transcriptional activator of the luciferase structural operon, and the bacteria emit light.

Since its discovery in *V. fischeri*, quorum sensing has been recognized as a widespread mechanism utilized by many gram-negative bacteria for the control of gene expression. Many elegant studies of cell-cell signaling in bacteria have recently been published (2, 9, 10, 12, 15, 16, 21, 23, 34, 36). The results of these studies underscore the importance of microbial cell-cell signaling in a variety of processes, including the development of disease in *Pseudomonas aeruginosa*- and *Staphylococcus aureus*-associated infections (1, 18, 29, 39, 40), the formation of microbial biofilms (27), and the production of light in luminous bacteria (3, 14, 24).

The expression of bioluminescence in *V. harveyi* is similarly under the control of a quorum-sensing mechanism. However, in this case, the regulated expression of luciferase is not mediated by homologs of the *V. fischeri* LuxI and LuxR proteins. Rather, *V. harveyi* accomplishes quorum sensing through the integration of two parallel two-component regulatory systems

(system 1 and system 2). Each system is composed of a sensor protein that contains both kinase and response regulatory domains (LuxN and LuxQ) (5, 6) and its cognate AI (AI-1 and AI-2). Interaction of either AI-1 or AI-2 with its cognate sensor activates a phosphorelay cascade that ultimately converges on the response regulator protein, LuxO (5, 6). Expression of bioluminescence is determined by the phosphorylation state of the LuxO protein. At low cell density, neither AI-1 nor AI-2 is present at high concentration and LuxO is phosphorylated and acts to repress luminescence (5, 6, 16, 17). The accumulation of AI-1 or AI-2 at high cell density leads to dephosphorylation of LuxO and derepression of the *lux* operon. A positive regulator, LuxR (the *V. harveyi* LuxR is not a homolog of *V. fischeri* LuxR), is also necessary to activate transcription of the *lux* operon.

The recently described *V. harveyi luxS* gene is required for the production of the AI (AI-2) in signaling system 2 (36), and *luxS* homologs exist in over 25 other bacterial species, including *Vibrio cholerae*, *Salmonella enterica* serovar Typhimurium, *Escherichia coli*, and *H. pylori*. Surette et al. (36) assayed several different genera possessing the *luxS* homolog and found that AI-2 activity was produced by many of these organisms. In this study, we present data indicating that *H. pylori* possesses AI-2 activity and that this activity can specifically activate *lux* in *V. harveyi* through signaling system 2.

MATERIALS AND METHODS

Bacterial strains and recombinant DNA techniques. The bacterial strains used were the clinical isolate *H. pylori* Alston, a generous gift from David Cave (St. Elizabeth's Hospital, Boston, Mass.), *V. harveyi* BB170 (14), and *E. coli* DH5α (Bio-Rad Laboratories). EJ103 is a *luxS* deletion derivative of *H. pylori* Alston constructed by the following procedure. One kilobase of sequence directly flanking both the upstream and downstream regions of *luxS* in *H. pylori* was PCR amplified with *Taq* polymerase (Gibco BRL, Grand Island, N.Y.) using the following primers: upstream region, A (5′ TCTAGAGGTGTTTTCATGTTTTAACTCC) and (5′ GGTACCTCATCGCTGATTTAGAAGG); downstream region, C (5′ GCGGCCGCCCTTTGCCAGATAAG) and D (5′ TCTAGAGTGGTCTGAAGTGGGG). A directed ligation containing the resulting two PCR fragments, a kanamycin (Tn*903*) cassette, and pBluescript II SK vector resulted in the plasmid pEJ32, in which the kanamycin cassette is flanked by the two PCR fragments such that it replaces the complete *luxS* sequence. pEJ32 was electroporated into *H. pylori* Alston, resulting in the *luxS* mutant strain EJ103. PCR analysis with primers B and C gave rise to a 1,300-bp product, confirming that *luxS* had been replaced by the kanamycin cassette. To express LuxS, the *H. pylori luxS* gene was amplified with the primers XP1 (5′ ATGAAAACACCAAAAATGAATGTAG) and XP2 (5′ AACCCCCACTTCAGACCAC), and the 465-bp DNA fragment was cloned into the pBAD TopoIV expression vector (Invitrogen, Carlsbad, Calif.), resulting in pEJ30, which was expressed in the *luxS* mutant *E. coli* strain DH5α (34).

Growth of bacterial cell cultures and HEp-2 tissue cultures. *H. pylori* strains were grown on campylobacter agar (Difco, Detroit, Mich.) supplemented with 10 μg of vancomycin (Sigma, St. Louis, Mo.)/ml, 2.5 U of polymyxin B (Sigma)/ml, 5 μg of trimethoprimsulfamethoxazole (Elkins Sinn, Cherry Hill, N.J.)/ml, and 20 μg of kanamycin/ml (where appropriate) plus 5% defibrinated sheep blood (Binax-NEL, Waterville, Maine). *H. pylori* liquid cultures were grown in brucella broth (BBL; Becton Dickinson Labware, Lincoln, N.J.; and Difco) supplemented with 5% horse serum (Gibco BRL). All *H. pylori* cultures were incubated in GasPak jars (BBL) with Campypaks (BBL) to generate a microaerobic environment. *V. harveyi* was cultured in heart infusion medium (25 g of heart infusion broth [Difco], 20 g of NaCl/liter), and *E. coli* DH5α was grown in Luria broth (LB). The AI bioassay (AB) medium has been previously described (37). The laryngeal carcinoma cell line HEp-2 was maintained in RPMI 1640 medium containing 5% fetal calf serum and glutamine (Gibco BRL). The cells were grown at 37°C in an atmosphere of 5% CO_2.

Microscopic examination, growth, and urease activity of *H. pylori* strains. Bacterial growth was monitored by optical density of broth cultures and viable plate counts. Motility was determined by microscopic examination. The urease status of the strains was determined by osmotically shocking 24-h cultures of *H. pylori* strains in ice water and adding 1/10 of the supernatant to a solution of 300 mM urea, 85 mM NaCl, 10 mM KH_2PO_4, and 0.00045% phenol red (pH 6.4). Hydrolysis of urea results in a qualitative color change of the buffer from orange to pink.

Preparation of cell-free culture fluids for AI-2 assays. Confluent growth from 24-h *H. pylori* plate cultures was collected by flooding each plate with 5 ml of brucella broth supplemented with 5% horse serum (BBH) followed by swabbing the agar surface with a sterile cotton swab. Cell suspensions were used for broth inoculations. BBH was inoculated with the appropriate culture to a starting optical density at 600 nm of 0.1, and the culture was incubated at 37°C under microaerobic conditions with vigorous shaking (220 rpm). Cell-free culture fluids were prepared by centrifugation in an Eppendorf microcentrifuge (1 min at 15,000 rpm) and filtration of the supernatant through Nalgene 0.2-μm-pore-size filters (Nalge Co., Rochester, N.Y.). The supernatants were stored on ice before being assayed for vacuolating cytotoxin and AI-2 activity (34).

AI-2 assay. Cell-free culture fluids were prepared from the *H. pylori* parental and *luxS* mutant strains, from *E. coli* DH5α, and from *E. coli* DH5α expressing the *H. pylori luxS* gene as described above. Ten microliters of each preparation was added to wells of 96-well microtiter dishes and assayed for AI-2 activity with the *V. harveyi* BB170 AI-2 bioassay as described previously (34, 35). For each preparation, 10 μl of the corresponding sterile medium was added to the wells as a negative control, and 10 μl of serovar Typhimurium 14028 cell-free culture fluid prepared from a culture grown to mid-exponential phase in LB containing 0.5% glucose at 37°C with aeration was used as a positive control for AI-2 activity. These conditions are known to promote maximal AI-2 production in serovar Typhimurium 14028 (34). *V. harveyi* BB170 was grown overnight with aeration at 30°C in AB medium and then diluted 1:5,000 in fresh AB medium, and 90 μl of the diluted culture was added to the wells containing the cell-free culture fluids or medium controls. The microtiter dishes were shaken at 200 rpm in a rotary shaker at 30°C. Light production was quantified every 30 min with a Wallac Model 1450 Microbeta Plus liquid scintillation counter. The data are reported as the fold stimulation of light emission by *V. harveyi* BB170 over that obtained for the corresponding growth medium alone.

Vacuolating cytotoxin assay. Cell-free supernatants were assayed for the ability to produce vacuoles in a HEp-2 epithelial monolayer using a method adapted from Cover et al. (11). Cell supernatant volumes from *H. pylori* grown under conditions that result in AI-2 activity in BBH were concentrated 30- to 40-fold using Centriprep30 and Centricon30 concentrators (Amicon, Beverly, Mass.). HEp-2 cells were seeded in 24-well tissue culture plates to a density of 10^3 cells/ml and incubated overnight in a 5% CO_2 atmosphere at 37°C. After incubation, the media were aspirated, and the cells were washed with phosphate-buffered saline (PBS). Fresh medium was added, and concentrated *H. pylori* supernatants were applied to the cells in 5-, 10-, and 100-μl aliquots and allowed to incubate in a 5% CO_2 atmosphere at 37°C overnight. The presence of vacuoles was determined by microscopic examination.

Assay for IL-8 induction by HEp-2 cells. The assay for interleukin 8 (IL-8) was carried out as described by Sharma et al. (31). Briefly, HEp-2 cells were seeded at a density of 10^5/well. After a 24-h incubation period, the cells were washed twice with PBS and infected with 10^8 CFU of either the parent *H. pylori* strain or EJ103 harvested from a fresh overnight plate/ml in RPMI. The bacteria were pelleted onto the epithelial monolayer by centrifugation in an Eppendorf microcentrifuge for 5 min at 1,000 rpm and incubated for 8 h in a 5% CO_2 atmosphere at 37°C. Following incubation, the supernatants were removed, centrifuged at 15,000 rpm for 5 min, and filtered as described above. The resulting cell-free culture fluid was assayed for IL-8 by enzyme-linked immunosorbent assay (catalog no. EH2-IL8-10; Endogen, Woburn, Mass.) following the manufacturer's instructions.

Protein preparation and 2-D gel analysis. Cell pellets from both the parent *H. pylori* strain and EJ103 grown under conditions resulting in AI-2 activity were resuspended in an osmotic lysis buffer containing 10 mM Tris (pH 7.4) and 0.3% sodium dodecyl sulfate, plus 1× (final concentration) of the following 100× protease inhibitor stock: pepstatin (0.34 mg/ml), leupeptin (1 mg/ml), E64 (0.36 mg/ml), benzamidine (5.6 mg/ml), and phenylmethylsulfonyl fluoride (0.2 mg/ml). RNase (500 mg/ml) (Worthington Biochemical, Lakewood, N.J.) and 350 U of DNase (Worthington Biochemical) were added, and the suspension was incubated on ice for 2 min. Aliquots were removed for protein concentration determination with the Bio-Rad (Hercules, Calif.) protein assay (catalog no. 500-0006), and the remaining sample was solubilized in an equal volume of 5% sodium dodecyl sulfate–5% β-mercaptoethanol boiling buffer. Samples were boiled for 5 min, frozen in dry ice, and stored at −70°C. Two-dimensional (2-D) gel electrophoresis services were performed by Kendrick Laboratories (Madison, Wis.).

RESULTS

Identification of AI-2 activity in *H. pylori*. Analysis of the *H. pylori* genome database revealed a hypothetical protein with 40% identity and 60% homology to LuxS of *V. harveyi* (Fig. 1). To determine if *H. pylori* possessed an AI-2-like activity, we tested *H. pylori* Alston cell-free supernatants derived from late-log-phase growth for the ability to induce luminescence in the *V. harveyi* reporter strain BB170 (sensor 1^- sensor 2^+) (4). The results shown in Fig. 2A demonstrate that the addition of 10% *H. pylori* cell-free supernatant stimulates luminescence in the *V. harveyi* reporter strain BB170 equally to addition of the positive control AI-2 from serovar Typhimurium. Specifically,

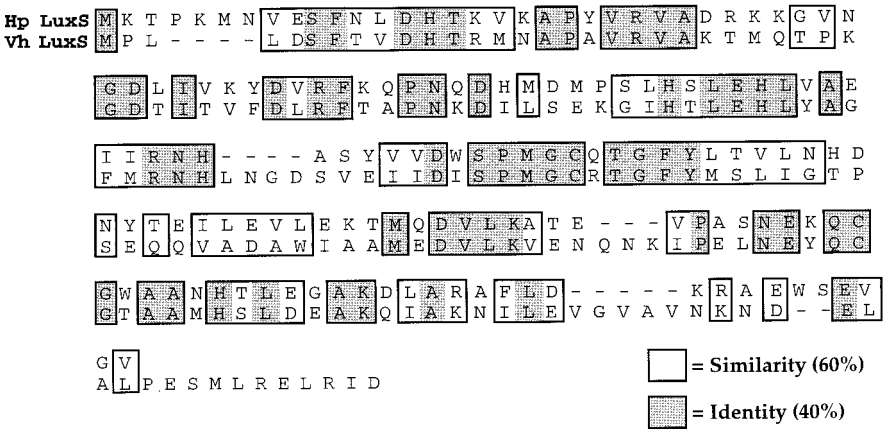

FIG. 1. Alignment of the *V. harveyi* and putative *H. pylori* LuxS proteins.

cell-free supernatants from both *H. pylori* and serovar Typhimurium induced signaling system 2 in the *V. harveyi* reporter strain approximately 500-fold. To determine when *H. pylori* produced this activity, cell-free supernatants obtained from early-, middle-, and late-log-phase cultures were assayed with BB170. These results, shown in Fig. 3, suggest that AI-2 activity per cell reaches a maximum during early log phase and is greatly diminished in late-log-phase cultures. Activation of luminescence was not observed when *H. pylori* supernatants were tested in the *V. harveyi* reporter strain BB886 (sensor 1^+ sensor 2^-), which does not have the AI-2 sensor LuxQ (data not shown). Taken together, these data indicate that *H. pylori* produces an AI-2-like activity at early log phase that activates luminescence specifically through LuxQ and system 2 in *V. harveyi*.

***luxS* is responsible for AI-2 activity in *H. pylori*.** To determine whether *H. pylori luxS* is involved in AI-2 production, we constructed a *luxS* null mutant (EJ103). Taking advantage of *H. pylori*'s natural tendency for homologous recombination, plasmid pEJ32 was electroporated into *H. pylori* Alston, followed by selection for the double-crossover event, resulting in the replacement of *luxS* with the kanamycin marker. The chromosomal *luxS* deletion was confirmed by PCR analysis. Cell-free supernatants prepared from EJ103 were tested for the ability to activate expression of luminescence in *V. harveyi* BB170. Unlike the parent *H. pylori* strain, results from the supernatants prepared from the *luxS* deletion strain, EJ103, did not activate luminescence of BB170 above background levels, as shown in Fig. 2A.

H. pylori luxS can complement a LuxS null *E. coli* mutant in *trans*. *H. pylori luxS* was expressed under the control of the inducible *ara* promoter in pEJ30. After transforming this expression vector into the $LuxS^-$ *E. coli* DH5α strain, the cells were grown to mid-log phase and induction from the arabinose promoter was accomplished by pelleting the cells and resuspending them in LB plus 2% arabinose. After a 2-h incubation period, cell-free supernatants were tested for the ability to activate expression of luminescence in BB170. The supernatants prepared from the DH5α/pEJ30 culture grown under inducing conditions showed a 400-fold activation of light production in *V. harveyi* BB170 (Fig. 2B). In contrast, supernatants prepared from DH5α/pEJ30 culture grown under repressing conditions showed only minimal activation (less than 10-fold) of the reporter strain (Fig. 2B). These results suggest that the inability of EJ103 supernatants to activate luminescence in BB170 is due to the absence of *luxS*. Neither growth medium nor supernatants prepared from *E. coli* DH5α alone caused stimulation of bioluminescence expression in *V. harveyi* BB170.

AI-2 activity in *H. pylori* does not affect expression of known virulence factors. Only a few genes in *H. pylori* have been demonstrated to play a role in virulence. Mutation of the *luxS* homolog in *E. coli* O157 results in loss of virulence, suggesting that cell-cell communication plays a role in pathogenesis (32, 36). By analogy, we wanted to test if the expression of any of the known or putative virulence factors described for *H. pylori* was under the regulatory control of AI-2. The parental strain, Alston, and the *luxS* deletion strain, EJ103, were grown under conditions that result in AI-2 activity and compared for any differences in the growth kinetics in BBH medium, motility, urease activity, Cag-mediated induction of IL-8 production in HEp-2 cells, and *H. pylori*-induced vacuolization of HEp-2 cells. The growth kinetics of the two strains in BBH (liquid) medium were similar, and microscopic examination of these cultures showed that both strains were motile. There were no discernible differences between Alston and EJ103 in urease activity or in the ability to induce IL-8 production. In addition, when equivalent amounts of concentrated supernatants isolated from Alston and EJ103 were used in a vacuolating cytotoxin assay (11, 28), the *luxS* mutant was as effective as the parent strain in causing accumulation of vacuoles in HEp-2 cells (data not shown). Taken together, these data indicate that *luxS* is not involved in the in vitro expression of these factors previously shown to play a role in virulence in *H. pylori*.

2-D gel analysis of proteins from the wild-type *H. pylori* strain and the Δ*luxS* mutant EJ103. We next examined the 2-D protein profiles of both strains grown under conditions that result in AI-2 activity. This experiment was designed to identify candidate proteins that could be under AI-2 regulatory control in *H. pylori*. The resulting profiles showed a complex pattern of approximately 400 to 500 expressed proteins with high resolution. Under the conditions used, no apparent differences between the parent strain and EJ103 were detectable (data not shown).

DISCUSSION

The identification of *luxS* and examination of its critical role in signaling system 2 quorum sensing in *V. harveyi* led to the discovery of *luxS* homologs in a variety of other bacteria, including many in which quorum-sensing systems had not been

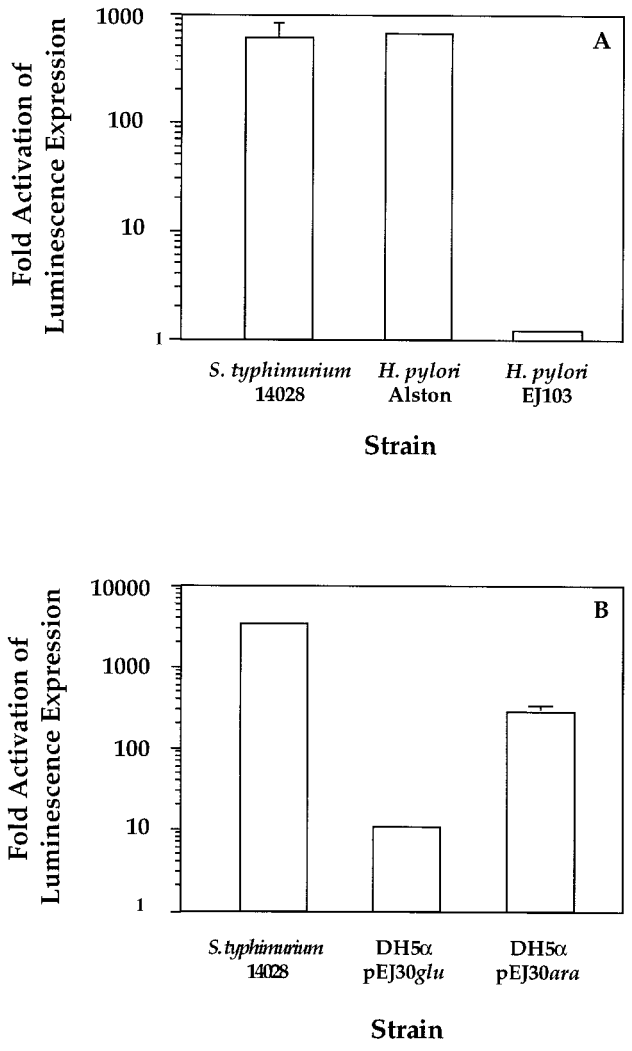

FIG. 2. *H. pylori* produces a *luxS*-dependent AI-2 activity. Cell-free supernatants were tested for the ability to induce luminescence expression in *V. harveyi* BB170. Ten percent cell-free supernatants or sterile media were mixed with the reporter strain in microtiter plates and incubated at 30°C on a rotary shaker. Aliquots were taken, and both cell density and light production were determined. Activity is reported as fold activation of luminescence of BB170 over the level of luminescence when sterile media were added. Assays were repeated at least two times. (A) Wild-type *H. pylori* and Typhimurium 14028 supernatants contain signaling substances that induce expression of luminescence in *V. harveyi* BB170, while the *luxS* deletion strain EJ103 does not. (B) *H. pylori luxS* expression in *E. coli* DH5α restores AI-2 activity. The error bars indicate standard deviations.

previously described. One of these organisms, *H. pylori*, has a LuxS homolog that shares 40% identity and 60% similarity with the *V. harveyi* LuxS protein (Fig. 1). Homology exists over the entire length of the protein. Despite its clear role in quorum sensing in *V. harveyi*, an exact function in AI-2 production has not been assigned. Furthermore, there are no motifs in the protein that suggest a particular enzymatic or regulatory activity. Since *H. pylori* possesses an open reading frame whose predicted protein product has such high homology to the *V. harveyi* LuxS, we wanted to determine if *H. pylori* could synthesize AI-2 activity and investigate whether this activity might be associated with a potential quorum-sensing system. Our results indicate that *H. pylori* (*luxS*$^+$) does produce an active signaling molecule (AI-2) that activates expression of bioluminescence in *V. harveyi* in a system-2-specific manner (Fig. 2). Activation of luminescence by supernatants harvested at various times during the growth of *H. pylori* revealed that AI-2-specific activity reaches a maximum early in log phase and diminishes as cells enter late log phase. This suggests that the activity of the molecule is density dependent and, specifically, that conditions of low cell density favor activity (Fig. 3). The loss of activity in stationary phase indicates that early-log-phase activity is degraded when *H. pylori* reaches stationary phase, an observation that mirrors the findings in serovar Typhimurium (34).

Despite the association between infection with *H. pylori* and gastric disease, very few gene products have been conclusively shown to play a role in *H. pylori*-mediated pathogenesis. Since LuxS has been implicated in pathogenesis (32), we wanted to test whether our *luxS* null mutation would have an effect on the expression or activities of any of the confirmed or putative virulence factors described for *H. pylori*. Our results indicate that none of the factors or processes that we examined, including growth, motility, urease activity, vacuolating cytotoxin activity, and induction of IL-8 response in epithelial cells, were affected in EJ103. It is possible that any impact that the *H. pylori* system 2 may have on regulating gene expression involves processes other than those directly involved in pathogenesis. In addition to several reports implicating a role for quorum sensing in pathogenesis (20, 25, 32, 34–36, 39), it is known to control a variety of other phenotypes not directly associated with pathogenesis, such as biofilm formation, luminescence, sporulation, and natural competence. Nevertheless, as the molecular mechanisms and factors underlying *H. pylori*-mediated pathogenesis become more fully understood and characterized, a necessity for cell density-dependent regulation via *luxS* may be revealed.

In an effort to undertake a global examination of *luxS*-mediated gene expression in *H. pylori*, we performed 2-D gel analysis of the parental strain and an isogenic *luxS* deletion strain, EJ103. This analysis suggested that a *luxS* deletion has little effect on protein complexity and abundance during steady-state analysis, since no obvious and reproducible differences in protein profiles between the two strains were detected. There are several potential explanations for this result. Several studies have reported that levels of target gene activation in response to quorum-sensing signals are as little as two- or threefold (20, 25, 27). In these cases, 2-D gel analysis may not be sensitive enough to detect modest changes in protein synthesis.

One limitation of all studies of expression of pathogenic factors in vitro is the difficulty in approximating an in vivo environment. Controlling for multiple environmental factors that may simultaneously influence gene expression is difficult, if not impossible, to accomplish. In this regard, it is also possible that sensory information from several environmental cues may be channeled through other, AI-2-independent, pathways that converge at some critical junction. Thus, a combination of transmitted signals may be required to influence downstream gene expression. If so, elimination of any single pathway may not necessarily result in an obvious phenotype. Our burgeoning understanding of the variety and combinations of molecular mechanisms employed by bacteria to establish quorum sensing as a critical mode of communicating information suggests that this is very likely to be the case. For instance, in *V. harveyi*, the expression of bioluminescence is only partially due to density-dependent signaling. Other environmental cues, such as the availability of iron, oxygen, and carbohydrates, also contribute to the regulation of bioluminescence in an AI-2-independent fashion.

The initial identification of *luxS* in *V. harveyi* took advantage

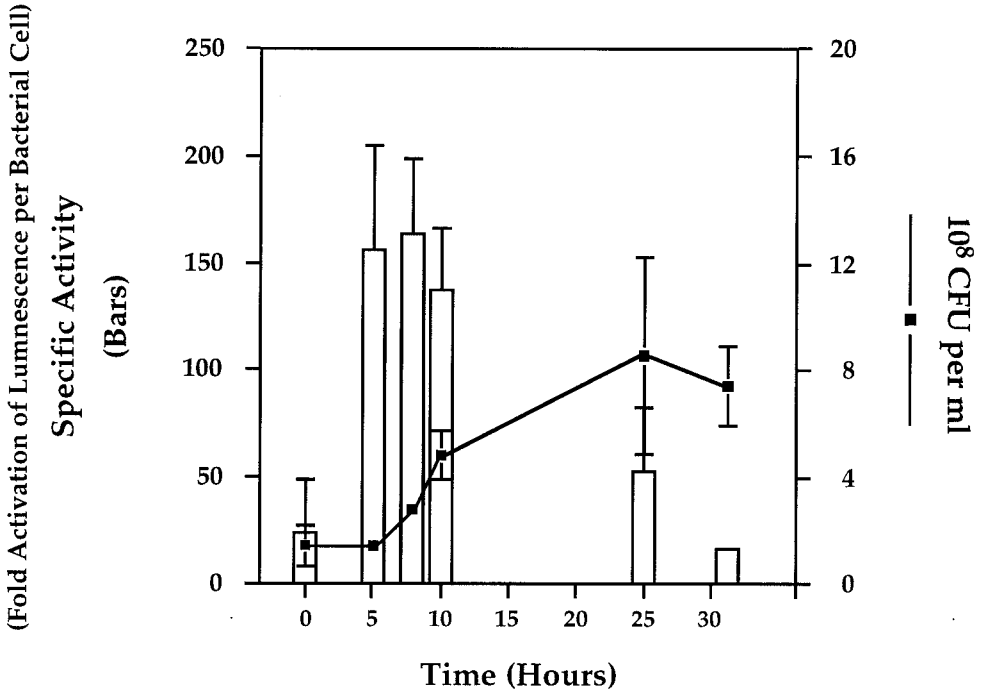

FIG. 3. Specific activity of *H. pylori* AI-2 is maximal in early log phase and decreases in late log phase. *H. pylori* Alston was grown for 31 h under microaerobic conditions in BBH. At 0, 5, 8, 10, 25, and 31 h, cell-free culture fluids were prepared. Cell density was measured by diluting and plating cell aliquots at each time point (line). AI-2 activity was assayed for the ability to stimulate expression of bioluminescence in the *V. harveyi* reporter strain BB170 (bars). Specific activity was determined by dividing the fold activation of luminescence values by the total number of bacterial cells present at each time point.

of the observation that *E. coli* DH5α was phenotypically LuxS$^-$ by complementing the LuxS defect with a *V. harveyi* library (34). Subsequently, the *E. coli* DH5α *luxS* gene was found to have a frameshift mutation. The observation that *luxS* genes from many other organisms complement the defect in *E. coli* DH5α suggests that LuxS is a highly conserved protein. The availability of several bacterial genome sequences reveals that LuxS homologs exist in at least 25 different species. Our data from the trispecies complementation experiment shown in Fig. 2B demonstrating that expression of the *H. pylori luxS* gene in *E. coli* DH5α results in active AI-2 molecules that complement the AI-2 defect in *V. harveyi* underscores the high level of LuxS conservation among species. There is mounting evidence from a few organisms that have a *luxS* homolog for the existence of LuxS-homologous signaling systems (32). Taken together, while it is currently unknown what functions are controlled by AI-2 in *H. pylori*, these observations suggest that a LuxS signaling system is present in *H. pylori*. Understanding how this complex microbial communication process contributes to the biology of *H. pylori* will necessitate developing a facility for "bacterial lexicology."

Since *H. pylori* is unique in its ability to colonize the gastric mucosa, it likely has developed specialized strategies allowing it to exploit, grow, and persist in the restrictive niche of the stomach. The lack of any other characterized reservoir may imply that the organism spends most of its life cycle in this unique environment, the gastric lumen of its host. This raises the possibility that *H. pylori* has little need to regulate gene expression to survive the low pH of the stomach. However, because *H. pylori* depends on a host organism for its survival, it must strike a dynamic balance between growing in the gastric niche and avoiding the host immune response in order to successfully persist. Density-dependent cell signaling could provide a mechanism for *H. pylori* to keep its population size in check in response to changes in its host's environment in order to avoid alerting the host immune system to the infection.

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