

Quorum sensing in *Escherichia coli* and *Salmonella typhimurium*

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ABSTRACT *Escherichia coli* and *Salmonella typhimurium* strains grown in Luria–Bertani medium containing glucose secrete a small soluble heat labile organic molecule that is involved in intercellular communication. The factor is not produced when the strains are grown in Luria–Bertani medium in the absence of glucose. Maximal secretion of the substance occurs in midexponential phase, and the extracellular activity is degraded as the glucose is depleted from the medium or by the onset of stationary phase. Destruction of the signaling molecule in stationary phase indicates that, in contrast to other quorum-sensing systems, quorum sensing in *E. coli* and *S. typhimurium* is critical for regulating behavior in the prestationary phase of growth. Our results further suggest that the signaling factor produced by *E. coli* and *S. typhimurium* is used to communicate both the cell density and the metabolic potential of the environment. Several laboratory and clinical strains of *E. coli* and *S. typhimurium* were screened for production of the signaling molecule, and most strains make it under conditions similar to those shown here for *E. coli* AB1157 and *S. typhimurium* LT2. However, we also show that *E. coli* strain DH5 α does not make the soluble factor, indicating that this highly domesticated strain has lost the gene(s) or biosynthetic machinery necessary to produce the signaling substance. Implications for the involvement of quorum sensing in pathogenesis are discussed.

Intercellular communication is used to regulate a wide variety of processes in bacteria, including quorum sensing in luminous *Vibrio* (1–3), competence and sporulation in *Bacillus* (4), and sporulation and motility in *Myxococcus* (5, 6). In each case, cell–cell communication is mediated by the synthesis, secretion, and detection of small extracellular signaling molecules. The signaling substances are distinct for the specific processes; acyl-homoserine lactone autoinducers regulate density sensing in *Vibrio*, peptides are used to induce competence and sporulation in *Bacillus*, and a complex mixture of amino acids and fragments of peptidoglycan control sporulation, social, and asocial motility in *Myxococcus*. In the case of the quorum-sensing bacterium *Vibrio harveyi*, two independent cell–cell communication systems are used to control luminescence (*lux*) expression in response to cell density (7). One of the *V. harveyi* systems (signaling system 1) is a high specificity, species-specific system, and the sensor responds to an acyl-homoserine lactone signal. The second system (signaling system 2) is a species-nonspecific system, and the signaling molecule(s) for this system has not yet been identified (8).

There have been preliminary indications that *E. coli* senses cell density (9–11). We took advantage of the species nonselectivity of the signaling system 2 sensor in *V. harveyi* to develop a sensitive assay for detection of extracellular signal molecules produced by *E. coli* and *S. typhimurium*. By using this assay we could determine the conditions under which many strains of *E.*

coli and *S. typhimurium* synthesize, secrete, and degrade a signaling substance that will interact with the *V. harveyi* system 2 detector.

MATERIALS AND METHODS

Preparation of Cell-Free Culture Fluids. *E. coli* strains AB1157 and DH5 α and *S. typhimurium* strain LT2 were grown at 30°C overnight with aeration in Luria–Bertani (LB) broth containing glucose at the concentrations specified in the text. The next morning fresh LB medium containing the same concentration of glucose used for the overnight growth was inoculated at a 1:100 dilution with the overnight-grown cultures. The fresh cultures were grown for various times at 30°C with aeration. Cell-free culture fluids were prepared by removing the cells from the growth medium by centrifugation at 15,000 rpm for 5 min in a microcentrifuge. The cleared culture fluids were passed through 0.2- μ m HT Tuffryn filters (Gelman) and stored at –20°C. Cell-free culture fluids containing *V. harveyi* autoinducer-2 were prepared from *V. harveyi* strain BB152 (autoinducer 1[–], autoinducer 2⁺). *V. harveyi* BB120 (autoinducer 1⁺, autoinducer 2⁺) was used to prepare culture fluids containing autoinducer-1. In both cases, the *V. harveyi* strains were grown overnight at 30°C with aeration in AB (autoinducer bioassay) (7) medium. Cell-free culture fluids from *V. harveyi* were prepared from the overnight culture exactly as described above for *E. coli* and *S. typhimurium*.

Assay for Production of Signaling Molecules. Cell-free culture fluids from *E. coli*, *S. typhimurium*, and *V. harveyi* strains were tested for the presence of signaling substances that could induce luminescence in the *V. harveyi* reporter strain BB170 or BB886. In the assays, 10 μ l of cell-free culture fluids from *E. coli* AB1157, *E. coli* DH5 α , and *S. typhimurium* LT2 strains grown and harvested as described above were added to 96-well microtiter dishes. The *V. harveyi* reporter strain BB170 or BB886 was grown for 16 hr at 30°C with aeration in AB medium and diluted 1:5,000 into fresh AB medium, and 90 μ l of the diluted cells was added to the wells containing the *E. coli* and *S. typhimurium* cell-free culture fluids. Positive control wells contained 10 μ l of cell-free culture fluid from strain *V. harveyi* BB152 (autoinducer-1[–], autoinducer-2⁺) or *V. harveyi* BB120 (autoinducer-1⁺, autoinducer-2⁺). Negative control wells contained 10 μ l of sterile growth medium. The microtiter dishes were shaken in a rotary shaker at 175 rpm at 30°C. Every hour, light production was measured by using a Wallac (Gaithersburg, MD) Model 1450 Microbeta Plus liquid scintillation counter in the chemiluminescence mode. The *V. harveyi* cell density was measured by diluting the same aliquots of cells used for measuring luminescence, spreading the dilutions onto solid Luria–marine medium (7), incubating the plates overnight at 30°C, and counting the resulting colonies the next day.

Preparation of *E. coli* and *S. typhimurium* Viable and UV-Killed Cells for the Activity Assay. *E. coli* AB1157, *E. coli*

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Abbreviations: LB, Luria–Bertani; AB, autoinducer bioassay.
A commentary on this article begins on page 6571.

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DH5 α , and *S. typhimurium* LT2 cultures were grown for 8 hr in LB medium containing 0.5% glucose at 30°C with aeration. The cultures were subjected to centrifugation for 5 min at 15,000 rpm in a microcentrifuge, and the growth medium was removed from the cell pellets by aspiration. The cell pellets were resuspended in AB medium and washed by vigorous mixing. The cells were again subjected to centrifugation for 5 min at 15,000 rpm. The AB wash medium was removed and discarded, and the cells were resuspended in fresh AB medium. Each cell suspension was diluted to give 1×10^6 cells/10 μ l, and multiple 10- μ l aliquots were added to wells of microtiter dishes. Half of the cell aliquots were treated with short-wavelength UV light for 15 min at a distance of 10 cm. This treatment was sufficient to kill all of the cells as judged by plating and incubating the UV-treated cells, and ensuring that no growth occurred by the next day. Ninety microliters of the diluted *V. harveyi* reporter strain BB170 was next added to the wells containing either the viable or dead *E. coli* and *S. typhimurium* cells, and the activity assay was carried out exactly as described in the previous section.

Analysis of Glucose in *S. typhimurium* LT2 Culture Fluids.

Glucose concentrations were determined in cell-free culture fluids prepared from *S. typhimurium* by using a Trinder assay (Diagnostic Chemicals, Oxford, CT) according to the recommendations of the manufacturer, except that the glucose standards were prepared in LB medium. The assay was sensitive to less than 0.002% glucose. No interfering substances were present in LB medium or spent LB culture fluids.

RESULTS AND DISCUSSION

***E. coli* AB1157 and *S. typhimurium* LT2 Produce a Signaling Substance That Induces One Specific Quorum-Sensing System of *V. harveyi*.** The *V. harveyi* reporter strain BB170 has the quorum-sensing phenotype sensor 1⁻, sensor 2⁺. It induces *lux* expression in response to extracellular signals that act exclusively through the signaling system 2 detector (7). Addition of 10% cell-free spent culture fluid prepared from *V. harveyi* strain BB152 (which contains the system 2 autoinducer) stimulates the reporter strain roughly 1,000-fold over the endogenous level of luminescence expression. In Fig. 1, the light

production by *V. harveyi* BB170 induced by the addition of 10% cell-free spent culture fluids is normalized to 100% activity.

E. coli strain AB1157 and *S. typhimurium* strain LT2 were grown for 8 hr in LB broth or LB broth containing 0.5% glucose. The *E. coli* and *S. typhimurium* cells were removed from the growth medium, and the cell-free culture fluids were prepared and assayed for an activity that could induce luminescence expression in *V. harveyi*. Addition of 10% cell-free culture fluid from *S. typhimurium* LT2 or *E. coli* AB1157 grown in LB medium containing glucose maximally induced luminescence in the reporter strain BB170, similar to culture fluids from *V. harveyi* BB152 (Fig. 1A). Specifically, *E. coli* AB1157 produced 106% and *S. typhimurium* produced 237% of the *V. harveyi* BB152 activity. When the *E. coli* and *S. typhimurium* were grown in LB medium without added glucose they did not produce the signaling factor. Substitution of 10% (vol/vol) of LB medium containing 0.5% glucose did not stimulate luminescence in the reporter strain, indicating that there is no substance in the LB-glucose growth medium that induces luminescence expression in *V. harveyi*. We tested obvious candidates for the signal, including glucose, amino acids, cAMP, acetate, homoserine lactone, α -ketoglutarate, and other keto acids that are known to be excreted (12). None of these compounds has activity. These results suggest that *V. harveyi* BB170 can respond to some substance secreted by *E. coli* AB1157 and *S. typhimurium* LT2 when they are grown on LB medium containing glucose.

Analogous experiments were performed with the *V. harveyi* reporter strain BB886 (sensor 1⁺, sensor 2⁻). *V. harveyi* BB886 is defective in its response to signaling molecules that act through the signaling system 2 detector, but it is an otherwise wild-type strain (13). Fig. 1B shows the normalized 100% activation of *V. harveyi* BB886 by cell-free spent culture fluids prepared from *V. harveyi* BB120. *V. harveyi* BB120 produces the system 1 autoinducer *N*-(3-hydroxybutanoyl)-L-homoserine lactone (7). Addition of *S. typhimurium* LT2 and *E. coli* AB1157 cell-free culture fluids to *V. harveyi* strain BB886 caused a 5% and a 1% increase, respectively, above the control level (Fig. 1B). Together the results of Fig. 1A and B show that the signaling molecule produced by *E. coli* and *S. typhimurium* must act specifically through *V. harveyi* signaling system 2 and not some other, unidentified pathway.

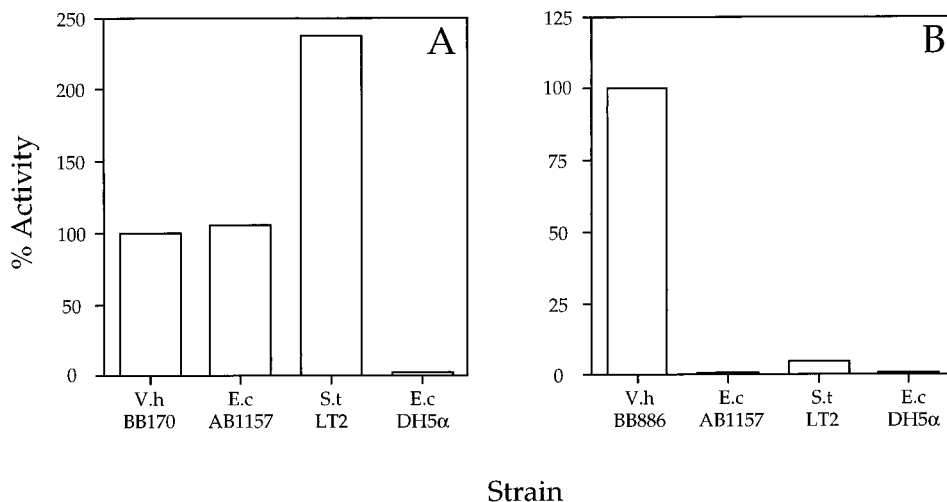


FIG. 1. *E. coli* AB1157 and *S. typhimurium* LT2 cell-free culture fluids contain a signaling substance that induces luminescence in *V. harveyi*. The responses of *V. harveyi* reporter strains BB170 (sensor 1⁻, sensor 2⁺) (A), and BB886 (sensor 1⁺, sensor 2⁻) (B) to signaling substances present in cell-free culture fluids from *E. coli*, *S. typhimurium*, and *V. harveyi* strains are shown. A bright culture of each reporter strain was diluted 1:5,000 into fresh medium, and the light production per cell then was measured during the growth of the diluted culture. Cell-free culture fluids or sterile growth medium were added at a final concentration of 10% (vol/vol) at the start of the experiment. The data for the 5-hr time point are shown and are presented as the percent of the activity obtained when *V. harveyi* cell-free spent culture fluids are added. V.h, *V. harveyi*; S.t, *S. typhimurium*, and E.c, *E. coli*.

Viable *E. coli* AB1157 and *S. typhimurium* LT2 Are Required for Secretion of the Signaling Molecule. We considered the possibility that growth of *E. coli* AB1157 and *S. typhimurium* LT2 in LB medium containing glucose simply allowed them to use and therefore remove some pre-existing inhibitor of induction of luminescence. To show that the cells themselves produce the soluble signaling factor, we added washed *E. coli* and *S. typhimurium* cells directly to the luminescence assay. These results are presented in Fig. 2. In this experiment, *E. coli* AB1157 and *S. typhimurium* LT2 were grown for 8 hr in LB medium containing 0.5% glucose, the conditions for maximal production of the signaling factor. The cells were removed from the LB-glucose growth medium by centrifugation, and sterile *V. harveyi* luminescence assay medium was used to wash and resuspend the cell pellets. *E. coli* AB1157 or *S. typhimurium* LT2 cells (1×10^6) were added to the diluted *V. harveyi* BB170 culture at the start of the experiment. In Fig. 2, the empty bar in each series shows that the presence of washed *E. coli* AB1157 or *S. typhimurium* LT2 cells is sufficient to fully induce luminescence in *V. harveyi* BB170. *E. coli* AB1157 and *S. typhimurium* LT2 stimulated *lux* expression in *V. harveyi* BB170 821-fold and 766-fold, respectively. Identical aliquots of the washed *E. coli* or *S. typhimurium* cells were killed with short-wave UV light before addition to the assay. When dead cells were included in the assay, no stimulation of luminescence occurred. In Fig. 2, these results are shown in the filled bars for each strain. Taken together, the results show that the stimulatory factor is produced by the *E. coli* AB1157 and *S. typhimurium* LT2 cells themselves during the time course of the experiment; the factor could not have come from the medium in which the cells had been grown. This factor is actively released into the medium by *E. coli* and *S. typhimurium* because dead cells have no activity.

***E. coli* DH5 α Does Not Produce the Signaling Activity.** Clinical isolates of *E. coli* and *Salmonella* also produce the

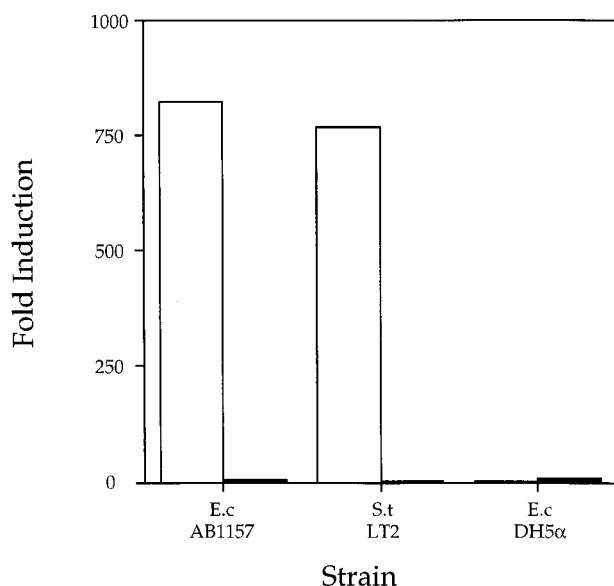


FIG. 2. Viable *E. coli* and *S. typhimurium* actively secrete the signaling molecule. The response of the *V. harveyi* reporter strain BB170 (sensor 1⁻, sensor 2⁺) to a signaling substance produced and secreted by *E. coli* AB1157 and *S. typhimurium* LT2 but not *E. coli* DH5 α is shown. *V. harveyi* reporter strain BB170 was diluted 1:5,000 in AB medium and light output per cell was monitored during growth. At the start of the experiment, either 1×10^6 *E. coli* AB1157, *S. typhimurium* LT2, or *E. coli* DH5 α washed and resuspended viable cells (empty bars) or UV-killed cells (filled bars) was added. The data are presented as the fold-activation above the endogenous level of luminescence expressed by *V. harveyi* BB170 at the 5-hr time point. S.t, *S. typhimurium*; E.c, *E. coli*. Results for replicates were within 10%.

signaling compound. Ten clinical isolates of *Salmonella* and five pathogenic isolates of *E. coli* O157 were assayed, and all produced the activity (not shown). It was conceivable that the signal was some normal byproduct of glucose metabolism that simply diffuses out of the cells. This postulation is not the case, however, because we show that *E. coli* DH5 α , which is equally capable of using glucose as *E. coli* AB1157 and *S. typhimurium* LT2 (14), does not produce the signaling activity. Fig. 1A demonstrates that unlike *E. coli* AB1157 and *S. typhimurium* LT2, the addition of 10% cell-free culture fluid prepared from *E. coli* DH5 α grown 8 hr in LB medium containing 0.5% glucose does not stimulate light production in *V. harveyi* BB170. Similarly, inclusion of washed viable or killed *E. coli* DH5 α cells in the luminescence assay does not stimulate *V. harveyi* BB170 to produce light (Fig. 2). The inability of *E. coli* DH5 α to produce the activity indicates that this highly domesticated strain lacks the gene or genes necessary for either the production or the export of the signaling activity. We assayed other laboratory strains of *E. coli* for the signaling activity (Table 1). Only *E. coli* DH5 α was completely defective in producing the extracellular signal.

Glucose Regulates the Production and Degradation of the Signaling Factor by *S. typhimurium* LT2. Cell-free culture fluids from *S. typhimurium* LT2 and *E. coli* AB1157 cells grown in LB medium without added glucose did not stimulate the expression of luminescence in the reporter strain, indicating that metabolism of glucose is necessary for the production of the signal. We tested other carbohydrates, including mannose, mannitol, fructose, glucosamine, sucrose, and maltose. In general, growth in the presence of phosphotransferase system (PTS) sugars (15) enabled *E. coli* AB1157 and *S. typhimurium* LT2 to produce the signal. Of the sugars tested, growth on glucose induced the synthesis of the highest level of activity. Growth on other carbon sources, for example tricarboxylic acid cycle intermediates and glycerol, did not induce significant production of the signaling activity.

We tested whether the presence of glucose was required for the cells to continue to produce the signal. Fig. 3 shows results with *S. typhimurium* LT2 grown in LB medium containing limiting (0.1%) and nonlimiting (0.5%) glucose concentrations. Fig. 3A shows that when glucose is limiting, *S. typhimurium* LT2 produces the signal in midexponential phase (after 4 hr of growth), but stops producing the signaling activity once glucose is depleted from the medium. Fig. 3B shows that when glucose does not become limiting, *S. typhimurium* LT2 produces greater total activity and continues to produce the signaling activity throughout exponential phase, with maximal

Table 1. The induction of luminescence in *V. harveyi* reporter strain BB170 by cell-free culture fluids from *V. harveyi*, *S. typhimurium*, and *E. coli*

Species and strain	Induction of luminescence, %
<i>V. harveyi</i>	
<i>V. harveyi</i> BB152	100
<i>Salmonella</i>	
<i>S. typhimurium</i> LT2	237
<i>E. coli</i>	
<i>E. coli</i> AB1157	106
<i>E. coli</i> DH5 α	5
<i>E. coli</i> JM109	76
<i>E. coli</i> MG1655	100
<i>E. coli</i> MC4100	93

Cell-free culture fluids were prepared from various strains of *V. harveyi*, *S. typhimurium*, and *E. coli* as described and tested for production of a signaling substance that could stimulate light production in the reporter strain *V. harveyi* BB170. The level of *V. harveyi* stimulation was normalized to 100%. The data for the 5-hr time point are shown.

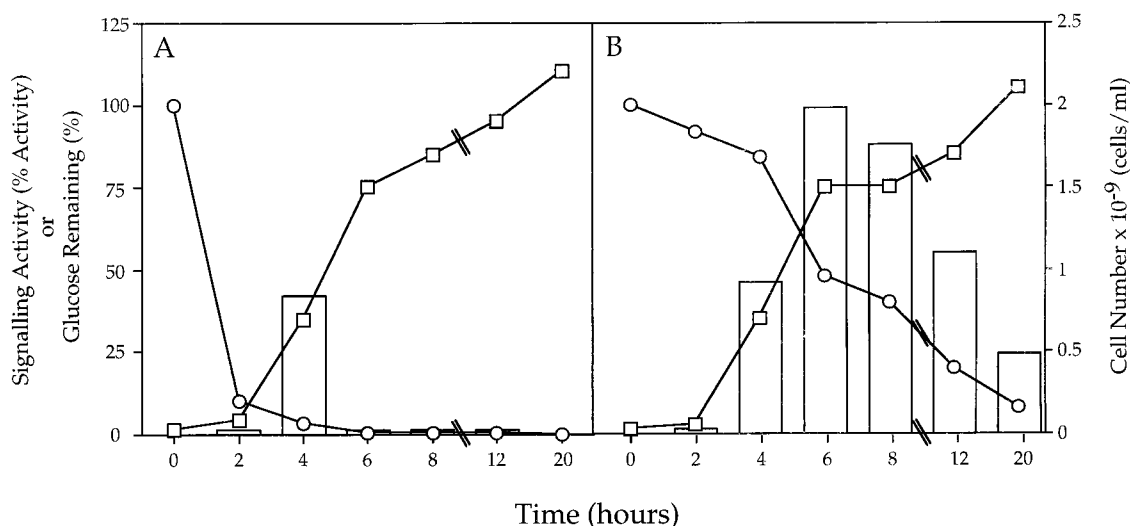


FIG. 3. Effect of glucose depletion on the production and degradation of the signaling activity by *S. typhimurium* LT2. *S. typhimurium* LT2 was grown in LB medium containing either 0.1% glucose (A) or 0.5% glucose (B). At the specified times cell-free culture fluids were prepared and assayed for signaling activity in the luminescence stimulation assay (bars), and the concentration of glucose remaining (\circ). The cell number was determined at each time by diluting and plating the *S. typhimurium* LT2 on LB medium and counting colonies the next day (\square). The signaling activity is presented as the percent of the activity obtained when *V. harveyi* cell-free spent culture fluids are added. These data correspond to the 5-hr time point in the luminescence stimulation assay. The glucose concentration is shown as % glucose remaining. Cell number is cells/ml $\times 10^{-9}$. \backslash indicates that the time axis is not drawn to scale after 8 hr. Replicate samples agreed within 10%.

activity at 6 hr of growth. Furthermore, Fig. 3 also shows that the signaling activity synthesized by midexponential phase cells is degraded by the time the cells reach stationary phase. In conditions of limiting glucose, no activity remained at stationary phase, and when glucose was plentiful, only 24% of the activity remained. Increasing the concentration of glucose in the growth medium did not change these results, i.e., the activity was secreted during midexponential growth and severely reduced activity remained in the spent culture fluids by stationary phase.

A Possible Role for Quorum Sensing in *E. coli* and *S. typhimurium*. Our results show that *E. coli* and *S. typhimurium* produce a signaling substance that stimulates one specific quorum-sensing system in *V. harveyi*. Many other bacteria previously have been assayed for such an activity, and only rarely were species identified that are positive for production of this factor (8). Furthermore, as shown here, the *E. coli* and *S. typhimurium* signal is potent; these bacteria make activity equal to that of *V. harveyi*. The degradation of the *E. coli* and *S. typhimurium* signal before stationary phase indicates that quorum sensing in these bacteria is tuned to a lower cell density community than described in other quorum-sensing bacteria. This result suggests that quorum sensing in *E. coli* and *S. typhimurium* is modulated so that the response to the signal does not persist into stationary phase. Additionally, quorum sensing in *E. coli* and *S. typhimurium* is influenced by several environmental factors. The production and the degradation of the signal are sensitive not only to growth phase but also to the metabolic activity of the cells. These results indicate that the quorum-sensing signal in *E. coli* and *S. typhimurium* has two functions; it allows the cells to communicate their growth phase and also the metabolic potential of the environment to one another.

Understanding the regulation of quorum sensing in *E. coli* and *S. typhimurium* could be important for understanding community structure and cell-cell interactions in pathogenesis. In the wild, pathogenic *E. coli* and *S. typhimurium* may never reach stationary phase because dispersion is critical. It is therefore appropriate that quorum sensing in *E. coli* and *S. typhimurium* should be functioning before stationary phase. This situation is in contrast to that of *V. fischeri*, the luminescent marine symbiont, where the quorum-sensing system is

only operational at high cell densities, cell densities indicative of existence inside the specialized light organ of the host. The specific quorum-sensing systems of *V. fischeri* as well as *E. coli* and *S. typhimurium* appear appropriately regulated for the niche in which each organism exists. In both cases, quorum sensing could be useful for communicating that the bacteria reside in the host and are not free-living in the environment. Additional complexity exists in the *E. coli* and *S. typhimurium* systems because these bacteria channel both cell density information and metabolic cues into the quorum-sensing circuit. Again, signals relaying information regarding the abundance of glucose or other metabolites could communicate to the bacteria that they should undergo the transition from a free-living mode to the mode of existence inside the host.

Purification of the *E. coli*, *S. typhimurium*, and *V. harveyi* signal currently is underway. Under all the conditions we have tested, the activity does not extract quantitatively into organic solvents and it does not bind to either a cation or anion exchange column. Our preliminary characterization indicates that the signal is a small (less than 1,000 M_r) polar, but apparently uncharged, organic compound. The activity is acid stable and base labile; it is heat resistant to 80°C but not 100°C. Identification of the signaling molecule, the sensory transduction pathway, and downstream targets will enable us to address the different roles that quorum sensing plays in community interactions and intercellular signaling in *V. harveyi*, *E. coli*, and *Salmonella*.

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