JOURNAL OF BACTERIOLOGY, Jan. 2006, p. 431–440 Vol. 188, No. 2
0021-9193/06/$08.00+0 doi:10.1128/JB.188.2.431–440.2006

Coordinated Regulation of Two Independent Cell-Cell Signaling Systems and Swarmer Differentiation in *Salmonella enterica* Serovar Typhimurium

Wook Kim[1] and Michael G. Surette[1,2]*

Department of Microbiology and Infectious Diseases[1] and Department of Biochemistry and Molecular Biology,[2] University of Calgary, Calgary, Alberta, Canada

Received 5 October 2005/Accepted 24 October 2005

Almost all members of the genus *Salmonella* differentiate and migrate on semisolid surfaces in a coordinated population behavior known as swarming. Important virulence determinants are coupled to swarmer differentiation in several other pathogenic organisms, collectively suggesting that conditions that trigger swarming in the laboratory may fortuitously promote the cells to enter a robust physiological state relevant to the host environment. Here, we present evidence that expression of two independent cell-cell signaling systems are also coupled to swarmer differentiation in *S. enterica* serovar Typhimurium. Expression of both *pfs* and *sdiA* genes was up-regulated in the actively migrating swarmers compared to their vegetative counterparts propagated in broth or spread plated on the surface of swim, swarm, and solid media. Accordingly, swarmers produced elevated levels of a universally recognized signaling molecule, autoinducer-2, and exhibited increased sensitivity to *N*-acyl homoserine lactones (AHLs), signaling molecules that *Salmonella* does not produce. Expression of the *rck* operon was concomitantly up-regulated in the swarmers in an SdiA-dependent manner only in the presence of exogenous AHLs. In addition to the previously reported adaptive antibiotic resistance phenotype and global shift in metabolism, this work presents another component of the physiological changes that are specifically associated with swarmer differentiation in serovar Typhimurium and not simply due to growth on a surface.

Bacteria are unicellular organisms that can behave independently within a given population but often exhibit coordinated group behavior. This can be achieved by cell-cell signaling mechanisms generally referred to as quorum sensing (5, 37, 42, 54). Various secreted chemical compounds have been shown to act as signaling molecules, and their accumulation within a given population alters gene expression, ultimately leading to changes in the population's behavior and physiological state. In nature, bacteria often exist in complex communities made up of multiple species, frequently exhibiting temporal and spatial organization (32). Different species of bacteria are capable of producing and/or sensing the same or different signaling molecules, facilitating intra- and interspecies communication (5).

Cell-cell signaling systems are typically integrated into other regulatory circuits and are thus capable of exerting and responding to global influences on individual cells within a given population. Such complexity permits coordinated response and adaptation at the community level. A diverse array of signaling molecules has been reported in recent years, including *N*-acyl homoserine lactones (AHLs) and LuxS-dependent autoinducer-2 (AI-2). AHL communication systems are widely conserved among gram-negative bacteria (19). The LuxIR system in *Vibrio fischeri* serves as the paradigm for AHL-based signaling systems, where LuxI synthesizes the AHL and LuxR regulates the transcription of genes for generating luminescence (via luciferase operon) in response to AHL. AHL-regulated bioluminescence and other AHL-regulated genes play important physiological roles in the symbiotic relationship between *V. fischeri* and its squid host *Euprymna scolopes* (48). LuxIR homologues play important roles in the pathogenesis of numerous bacteria and may serve as novel antivirulence drug targets (17).

AI-2 is often referred to as a universal signal, since many gram-negative and gram-positive bacteria produce the same molecule(s) (55). AI-2 is a product of *S*-adenosylhomocysteine metabolism, which is derived from *S*-adenosylmethionine (SAM) (55). Pfs, an adenosine nucleosidase, converts *S*-adenosylhomocysteine into *S*-ribosylhomocysteine, and LuxS cleaves *S*-ribosylhomocysteine into homocysteine and 1,2-dihydroxypentanedione, which undergoes spontaneous chemical isomerization to form AI-2. Although several researchers have questioned whether AI-2 is a true communication signal, suggesting that it is simply a common, even toxic metabolite (18, 54), there is no evidence that 1,2-dihydroxypentanedione is toxic to the cell. Furthermore, *luxS* mutants exhibit no observable growth defects (44, 50). Given that all signaling molecules are derived from one form of metabolism or another, it is difficult to clearly distinguish whether a molecule is a metabolite or a true signal, especially if an organism has evolved to sense a common metabolite as a function of sensing the presence of others in a complex community. It is likely that LuxS/AI-2 serves various functions among different organisms from its well established roles as a cell-cell signal in *Vibrio* spp. and as a metabolite in other organisms (55).

Salmonella enterica serovar Typhimurium may possess the

* Corresponding author. Mailing address: Department of Microbiology and Infectious Diseases, University of Calgary, 3330 Hospital Dr. NW, Calgary, Alberta, Canada T2N 4N1. Phone: (403) 220-2744. Fax: (403) 270-2772. E-mail: surette@ucalgary.ca.

ability to sense both AI-2 and AHLs (1). Serovar Typhimurium is one of the organisms in which LuxS was first identified (44), and later it was demonstrated that AI-2 production is regulated at the level of *pfs* transcription rather than that of *luxS* (7). It was recently reported that serovar Typhimurium recognizes a stereoisomer of the AI-2 signal chemically distinct from that of *V. harveyi* (35). AI-2 production is stimulated by glucose metabolism, and its extracellular accumulation peaks during mid- to late exponential growth (43). In the postexponential phase of growth, AI-2 is rapidly imported back into the cell by an ABC transporter system encoded by the *lsrACDBFGE* operon, where AI-2, in a phosphorylated state, is believed to control the expression of the operon (45, 46). It is speculated that the removal of extracellular AI-2 may represent a sophisticated mechanism for modulating cell-cell signaling events at the population level within a mixed-species community.

Serovar Typhimurium also possesses a LuxR homologue, SdiA, but lacks the LuxI-like component (2), suggesting that serovar Typhimurium does not itself produce AHLs but may respond to signaling molecules produced by other organisms. Indeed, it was recently shown that *srgE* and the *rck* operon (*pefI-srgDAB-rck-srgC*) are regulated by SdiA in an AHL-dependent manner (41). The *rck* operon encodes products associated with regulating the production of the plasmid-encoded fimbriae and resistance to complement killing, but the function of *srgE* remains unknown (41).

Swarming is a coordinated population behavior associated with surface motility (14). Population density plays an important role in triggering swarmer differentiation, and migration occurs in groups of cells aligned along their long axis, mimicking multicellular rafts (8, 22). Accordingly, disruption of cell-cell signaling systems in several organisms has been shown to abolish their ability to swarm (4, 24, 30, 38). However, quorum sensing does not seem to control swarmer differentiation per se but rather influences the migration of already differentiated swarmer populations. In *Serratia liquefaciens*, quorum sensing regulates the production of an extracellular lipopeptide biosurfactant known as serrawettin (30). The primary role of serrawettin is to decrease surface tension, where its accumulation provides a slime-like milieu, enabling rotation of flagella for surface movement (21). Hence, the population density-dependent aspect of the swarming behavior may be limited to the proportional accumulation of extracellular surfactants. Mutations in the *sdiA* or *luxS* locus do not affect swarming in serovar Typhimurium (47), suggesting that neither SdiA nor AI-2 modulates swarmer differentiation.

Besides the motility phenotype, numerous important physiological changes are coupled to swarmer differentiation in several organisms, including expression of important virulence factors (3, 20, 31, 40, 49). Swarmer differentiation in serovar Typhimurium is coupled to adaptive antibiotic resistance to a wide variety of structurally and functionally distinct classes of antibiotics, including cationic peptides (25, 28), and a global shift in metabolism from catabolism to anabolism (26). Expression of type III secretion system genes may also be coordinately regulated with swarming through the regulatory pathways that modulate flagellation (52). Furthermore, virtually all members of the genus *Salmonella* are capable of swarming, suggesting that this is an evolutionarily conserved behavior (27). As part of our ongoing efforts to understand the swarm state of *Salmonella* beyond the motility phenotype, we explored this coordinated population behavior with respect to the two independent cell-cell signaling systems in serovar Typhimurium. Here, we show that expression of both *pfs* and *sdiA* genes are up-regulated in the swarmer population. Accordingly, both AI-2 production and AHL-dependent expression of the *rck* operon were also stimulated. Importantly, the concomitant regulation of these two cell-cell signaling systems was not observed under surface growth conditions that do not support swarmer differentiation.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. All bacterial strains and plasmids used in this study are listed in Table 1. For routine culturing, cells were grown in Luria broth (LB) at 37°C with shaking at 200 rpm. Swarm (NBG [nutrient broth with 0.5% glucose], 0.5% agar) and swim (NBG, 0.25% agar) assays were performed as previously described at 37°C (26, 28). Kanamycin (Sigma) or ampicillin (Sigma) was supplemented at 50 μg/ml (wt/vol) or 100 μg/ml (wt/vol), respectively, when necessary, and all media components were obtained from Difco unless otherwise noted.

Construction of *sdiA*, *rck*, and *lsr* luciferase transcriptional fusions and σ^{70} reporter. The intergenic region between *yecC* and *sdiA* genes was PCR amplified from serovar Typhimurium 14028 by primers PsdiA1 and PsdiA2 and cloned into the plasmid pCR2.1-TOPO as instructed by the manufacturer (Invitrogen). *rck* is a member of the *pefI-srgDAB-rck-srgC* operon (16, 34). The upstream region of *pefI*, containing the promoter of the operon (41), was PCR amplified from strain 14028 by primers Prck1, containing an XhoI restriction site, and Prck2, containing a BamHI restriction site. The upstream region of the *lsrACDBFGE* operon was PCR amplified from strain 14028 by primers Plsr1, containing an XhoI restriction site, and Plsr2, containing a BamHI restriction site. The recombinant TOPO vector containing the *sdiA* promoter and the PCR products containing the *rck* promoter and the *lsr* promoter were digested with XhoI and BamHI and subsequently cloned into plasmid pCS26 as previously described (28) to generate the *sdiA*::*lux* transcriptional reporter vector pSDIA, the *rck*::*lux* reporter vector pRCK, and the *lsr*::*lux* reporter vector pLSR. pSDIA, pRCK, and pLSR were individually transformed into strain 14028 or the Δ*sdiA* mutant WK2. All PCR primer sequences used in this study are listed in Table 1.

The control σ^{70} promoter (sig70-16) has the sequence 5′-CTCGAGAATAA TTCTTTACATTTATGCTTCCGGCTCGTATTCTACGTGCAATTGGATCC-3′ and was cloned into the XhoI-BamHI sites of pCS26 (28). This was one of several control σ^{70}-dependent promoters selected from a library constructed with the above sequence with four degenerate positions in the promoter (underlined) (K. Pabbaraju and M. G. Surette, unpublished data). These synthetic promoters reflect RNA polymerase activity and can be used to normalize expression under different conditions, particularly with respect to any possible variation in luciferase-specific activity.

Construction of an *sdiA* deletion mutant of serovar Typhimurium 14028. An *sdiA* deletion mutant was constructed using the methods and plasmid systems described by White et al. (53). A 739-bp fragment, containing 630 nucleotides upstream of the *sdiA* gene and the first 109 nucleotides of the open reading frame, was PCR amplified from serovar Typhimurium 14028 by primers PsdiA5′A containing an EcoRI restriction site and PsdiA5′B containing a BamHI restriction site. The PCR product was digested with EcoRI and BamHI and then cloned into plasmid pTZ18R, which is a high-copy cloning vector harboring an ampicillin resistance cassette. The recombinant plasmid was named pTZSDIA5′. A 635-bp fragment containing the last 51 nucleotides of *sdiA* and 584 nucleotides immediately downstream was amplified by PsdiA3′A and PsdiA3′B. PsdiA3′A contains a BamHI restriction site, and PsdiA3′B contains a HindIII restriction site. The PCR product was digested with BamHI and HindIII and then cloned into the plasmid pTZSDIA5′, generating a fusion with the previously cloned *sdiA* fragment. The recombinant plasmid was named pTZΔSDIA, which was digested with EcoRI and HindIII, and the fragment containing the truncated *sdiA* gene (i.e., Δ*sdiA*) was subsequently cloned into the low-copy suicide vector pHSG415. The recombinant suicide plasmid (pHSGΔSDIA) was introduced into strain 14028, and those harboring the appropriate deletion in the *sdiA* gene were screened and confirmed by PCR.

Luciferase assays. One microliter of an overnight culture of serovar Typhimurium 14028 harboring individual luciferase transcriptional reporter systems was spot inoculated on the center of swarm and swim plates and incubated at 37°C for 8 to 9 h (26, 28). Cells were harvested from six discrete regions on the

TABLE 1. Strains, plasmids, and PCR primers used in this study

Strain, plasmid, or primer	Relevant characteristic(s)	Source or reference
Strains		
14028	Wild-type *Salmonella enterica* serovar Typhimurium	American Type Culture Collection
WK2	14028 Δ*sdiA*	This study
MM32	*Vibrio harveyi luxN*::cm *luxS*::Tn*5* (Cm^r, Kan^r)	15
Plasmids		
pCS26	Low-copy *luxCDABE* reporter vector (Kan^r)	9
pSDIA	pCS26 containing 14028 *sdiA* promoter	This study
pRCK	pCS26 containing 14028 *rck* operon promoter	This study
pAB13	pCS26 containing 14028 *pfs* promoter	7
pLSR	pCS26 containing 14028 *lsr* operon promoter	This study
pSIG70-16	pCS26 containing σ^{70}-responsive synthetic promoter	K. Pabbaraju
pCR2.1-TOPO	Multicopy cloning vector (Amp^r, Kan^r)	Invitrogen
pTZ18R	Multicopy cloning vector (Amp^r)	53
pTZΔSDIA	pTZ18R containing the Δ*sdiA* construct	This study
pHSG415	Low-copy suicide vector (Cm^r, Amp^r, Kan^r)	53
pHSGΔSDIA	pHSG415 containing the Δ*sdiA* construct	This study
PCR primers		
PsdiA1	GTAAAGGGAGAGCCGAAAGC	This study
PsdiA2	CCGGAGGATAAGTGGTACGA	This study
Prck1	AGTCTCGAGCGACAATGCGTGCTGGAGATG	This study
Prck2	AGTCGGATCCAGCATCGGCAATATCGCGGG	This study
Plsr1	GCCCTCGAGTATCGCTCATTGTCATAACC	This study
Plsr2	GCCGGATCCGTATTGTGACTGATTTGC	This study
PsdiA5′A	AGTCGAATTCGCGGGAACTGTTAGCCAAAG	This study
PsdiA5′B	AGTCGGATCCGCCGCTGTGTCTGATATTGC	This study
PsdiA3′A	AGTCGGATCCTGCAGAAGAAATTCAATGCG	This study
PsdiA3′B	AGTCAAGCTTAAATTTCACGTTCCGCTGTC	This study

plates (i.e., from the center to the edge of the migrating front) with sterile toothpicks and transferred into microcentrifuge tubes containing 400 μl of fresh liquid. Each sample was gently vortexed, and luminescence in three 100-μl aliquots was measured in clear-bottom black 96-well plates (Nunc) as previously described (26) using the Trilux scintillation counter (Wallac). The remaining cell suspension in each microcentrifuge tube was serially diluted and plated out on kanamycin-supplemented Luria agar plates to estimate the number of CFU in each sample. Each luminescence value (counts per minute) was normalized to 10^8 CFU and is represented as relative light units.

Overnight cultures were also diluted 1:1,000 in fresh liquid NBG, and 100-μl aliquots (ca. 10^5 CFU) were gently spread plated on swim, swarm, and solid (1.5% agar) NBG plates. Each plate was left at room temperature with the lid slightly ajar until the inoculum was completely absorbed by the medium. All plates were incubated at 37°C, and cells were harvested over time. At a given time point, 2 ml of fresh liquid NBG was added to the surface of each plate and cells were harvested by repeated motion on a tilting platform. Prior to this procedure, swim plates were gently scarred with a pipette tip to facilitate the harvest. Each sample was processed for luminescence and CFU counting and normalized as described above. For broth assays, 10 ml of the same 1:1,000 overnight cultures was transferred into petri plates and incubated at 37°C and processed as described above over time.

Measurement of *rck* operon activity in response to HHL. Swarm, swim, and solid plates were either spot inoculated or spread plated as described above with wild-type 14028 or WK2 (Δ*sdiA*) harboring the *rck* reporter and incubated at 37°C for 4 h. Filter disks impregnated with 200 nmol of *N*-hexanoyl-homoserine lactone (HHL; Fluka) (20 μl of 10 mM stock solution in methanol) or methanol (20 μl) were then placed on the surface near the edges of each plate, and the plates were further incubated for 5 to 6 h at 37°C. Cells were harvested with sterile toothpicks from areas surrounding the impregnated filter disks or areas remote from the disks. Luciferase activity and CFU in each sample were measured as described above.

Digital images of the motility plates were captured using the Alpha Imager FluorChem 8900 CCD camera system (Alpha Innotech Corp.) under reflective light. Luminescence images were captured with the same camera system without any exogenous light. Photons emitted by the plates were captured for 5 min. Snowflake-like pixels present in some luminescence images represent background thermal noise.

AI-2 assays with the biosensor *Vibrio harveyi* MM32. AI-2 production was estimated using the methods described by Surette and Bassler (43). Swarm plates were inoculated with serovar Typhimurium 14028 and incubated at 37°C for 8 to 9 h until the migrating swarm front nearly reached the edges of the plate. The "flower-like" central portion was physically cut out from the swarm plates using a pipette tip and transferred into a fresh petri dish with ethanol-sterilized microscope slides. The cells from the central portions and the remaining swarm-front portions were harvested with 2 ml liquid NBG as described above. Each cell suspension was gently spun down and resuspended in 1.2 ml liquid NBG to an optical density at 600 nm of 1.0, and two 100-μl aliquots were removed for CFU counting as described above. The remaining sample was spun down and resuspended in 0.4 M NaCl (1 ml) for 1 h at room temperature. Each sample was spun down, and the supernatant was filter sterilized. This salt-extracted material is referred to as osmotic shock fluid (OSF). Serovar Typhimurium 14028 was also diluted into broth plates or spread plated onto swim, swarm, and solid NBG plates and incubated as described for the luciferase assays. At each time point, cells were processed for CFU counting and OSF extraction as described above.

Vibrio harveyi MM32 was used as the reporter strain to compare AI-2 levels present in each of the 14028 OSF samples. MM32 does not produce any endogenous AI-2 but possesses the ability to emit light proportional to exogenous levels of AI-2 (15). MM32 was grown overnight at 30°C in AB medium (17.5 g NaCl, 12.3 g $MgSO_4$, 2 g Casamino Acids, pH 7.5 [KOH], 10 ml K_2HPO_4 [1 M], 20 ml glycerol [50%], and 10 ml arginine [0.1 M]) and diluted 1/5,000 in fresh AB medium. Luminescence was measured at 30°C in 96-well black plates (Nunc) with the Victor^2 (Wallac) luminometer at 30-min intervals and recorded as counts per second. Each well contained a 150-μl volume of the diluted MM32 culture supplemented with 1%, 2%, 5%, or 10% (vol/vol) of the various OSFs or 0.4 M NaCl as control. Counts-per-second values were individually adjusted to the respective CFU counts estimated from each sample, and luminescence values were normalized to 10^8 CFU (i.e., relative light units).

RESULTS

Expression of *sdiA* is up-regulated in the swarmers of serovar Typhimurium, and *sdiA* is not autoregulated. SdiA is a LuxR homologue which has been previously demonstrated to

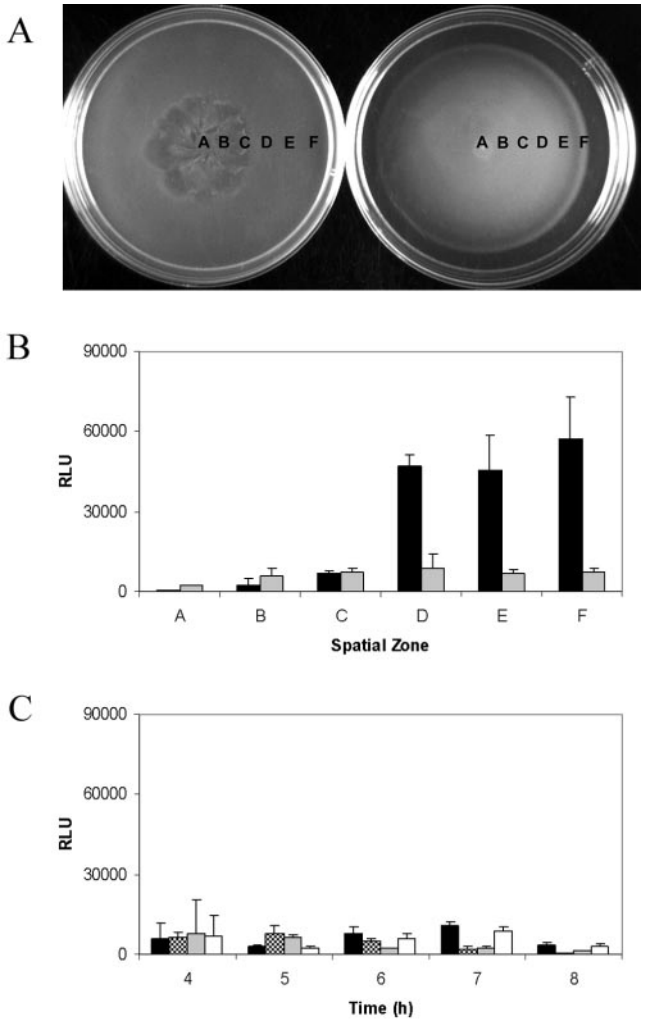


FIG. 1. Comparison of the *sdiA*::*lux* reporter activity in serovar Typhimurium 14028 under conditions that promote or do not support swarmer differentiation. (A) Digital images of spot-inoculated cells on swarm (left) and swim (right) plates after 9 h. The letters A to F indicate the different areas where the cells were harvested. (B) CFU-normalized spatial expression profiles of migrating swarmer (black) or swimmer (gray) cells harvested from the corresponding regions indicated in panel A. (C) CFU-normalized temporal expression profiles of cells propagated in broth (checkered) or spread plated on swarm (black), swim (gray), or solid (white) plates. The error bars indicate the standard deviation among three independent samples. RLU, relative light units.

respond to purified AHLs or to AHL-producing species (41). A luciferase transcriptional reporter system was constructed to investigate whether *sdiA* is differentially regulated in the swarming population of serovar Typhimurium. The *sdiA*::*luxCDABE* fusion system was introduced into the wild-type strain 14028, and its activity was measured throughout actively migrating swarmer and swimmer populations (Fig. 1A). Luminescence varied spatially across the swarm plate (Fig. 1B), suggesting the presence of unique populations. In particular, increased luminescence coincided spatially with areas on the plate occupied by actively migrating swarmer populations (26). Luminescence was much lower in the central portion of the plate, exhibiting the "flower-like" morphology, which represents mostly undifferentiated vegetative cells (22). In contrast, luminescence was generally uniform throughout the swimmer population and was at levels similar to those detected in the flower-like portion on the swarm plates (Fig. 1B). These results suggest that *sdiA* expression is up-regulated in the actively migrating swarmer population.

A recent microarray study reported that most genes in serovar Typhimurium LT2 are similarly regulated when cells are spread plated on swarm or solid plates compared to those propagated in broth (51). To address whether or not the observed differences in *sdiA* expression was simply due to surface-specific growth, *sdiA*::*lux* activity was measured over time in cells propagated in broth and those spread plated on the surface of swarm, swim, and solid plates. Under these conditions, luminescence values were generally similar between cells propagated on swarm and solid plates, and the maximum values measured under all four conditions (Fig. 1C) were comparable to those of the actively migrating swimmer population and the flower-like portion on the swarm plates (Fig. 1B). Thus, elevated expression of *sdiA* in the actively migrating swarmer population is independent from surface-specific conditions.

The spatial expression patterns of *sdiA* may also have risen simply due to the intrinsic nature of the luciferase reporter system. Oxygen is essential for bioluminescence (33), thus, reduced activity in the flower-like portion of the swarm plates may be due to relative differences in oxygen tension resulting indirectly from variations in population density. To address this issue, the activity of a σ^{70}-responsive promoter (sig70-16) was analyzed under the same set of conditions applied to the *sdiA*::*lux* reporter. This synthetic promoter serves as an internal control for comparing expression profiles of a given gene under various growth conditions (7 and K. Pabbaraju and M. G. Surette, unpublished results). Unlike *sdiA*, sig70-16 was active spatially throughout the swarm plate, including the flower-like central portion, and its activity was similar throughout the actively migrating swimmer population (Fig. 2A). Luminescence in the spread-plated populations remained uniform over time (Fig. 2B) and was comparable to the spatial measurements obtained from the migrating swarmer and swimmer populations. In broth, sig70-16 activity was similar to that of other growth conditions at 4 h and 5 h; however, it was significantly reduced at later time points (Fig. 2B). This temporal pattern was conserved among all reporters tested in this study, suggesting that the broth environment negatively influences luciferase activity compared to the other growth conditions. Nevertheless, these results indicate that the observed differences in *sdiA*::*lux* activity among surface-propagated populations were not simply due to growth condition-specific influences on luciferase activity.

The transcriptional fusion data collectively suggest that *sdiA* expression is coregulated with swarmer differentiation, but the regulatory elements that modulate its expression are unknown. To determine whether SdiA regulates its own expression, an *sdiA* deletion mutant (WK2) was constructed in strain 14028 and the same fusion system was introduced into the mutant strain. There were no detectable differences in its activity between the two strains on either swarm or swim plates (with or without AHLs) (data not shown), indicating that *sdiA* is not

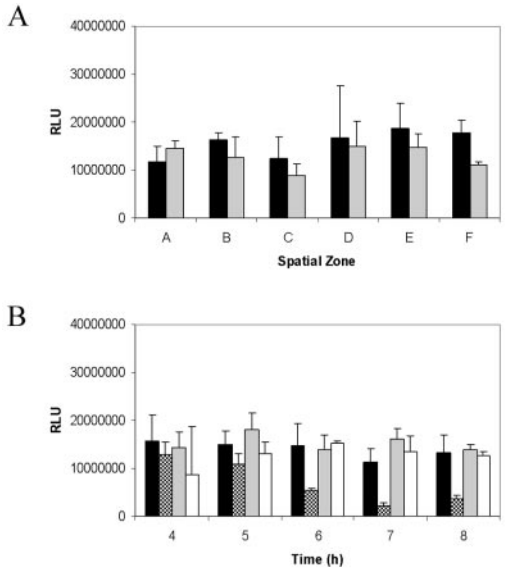


FIG. 2. Activity of the synthetic sig70-16::*lux* reporter in serovar Typhimurium 14028 under conditions that promote or do not support swarmer differentiation. (A) CFU-normalized spatial expression profiles of migrating swarmer (black) or swimmer (gray) cells harvested from the corresponding regions indicated in Fig. 1A. (B) CFU-normalized temporal expression profiles of cells propagated in broth (checkered) or spread plated on swarm (black), swim (gray), or solid (white) plates. The error bars indicate the standard deviation among three independent samples. RLU, relative light units.

autoregulated and other regulatory elements modulate its expression at the level of swarmer differentiation.

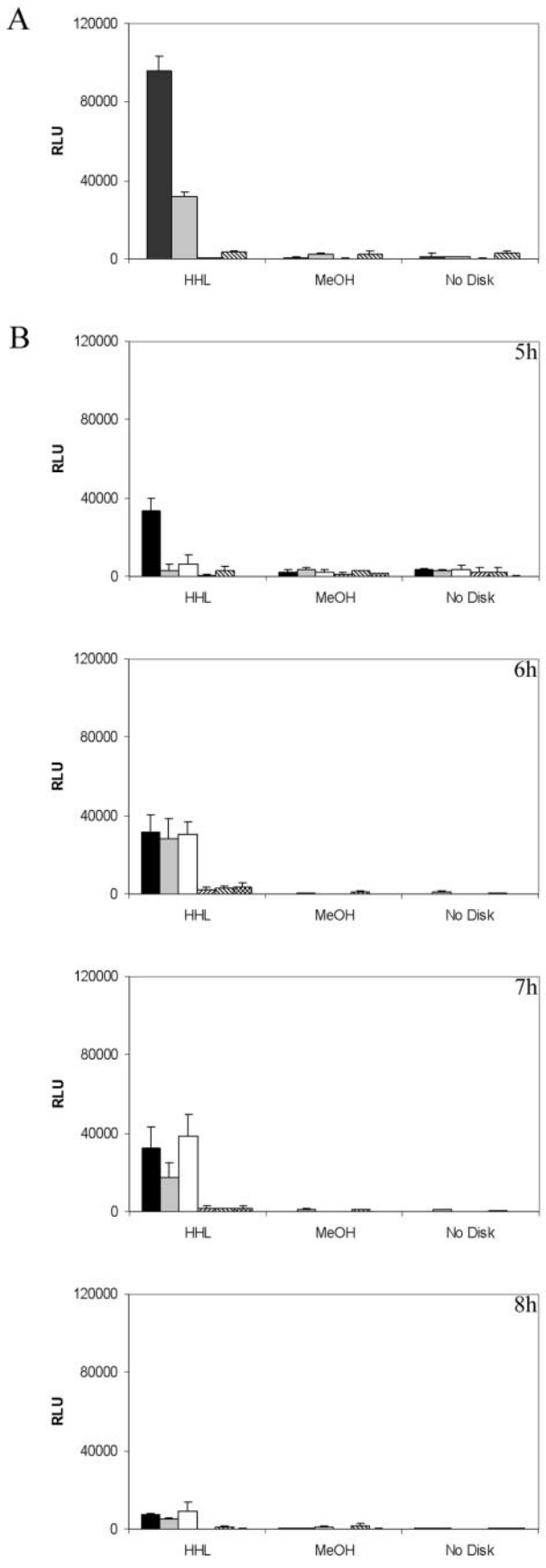
Expression of the *rck* operon is regulated by SdiA in an AHL-dependent manner and coincides with elevated expression of *sdiA* in the swarming population. The *rck* operon is regulated by SdiA strictly in an AHL-dependent manner (41). Since *sdiA* expression is up-regulated in the swarmer population, expression of the *rck* operon should also be coordinately regulated. To test this hypothesis, an *rck*::*luxCDABE* transcriptional fusion was constructed and introduced into 14028 and WK2 (Δ*sdiA*). When spot inoculated in either swarm or swim plates, *rck* expression was activated by *N*-hexanoyl-homoserine lactone (HHL) but not by methanol alone (Fig. 3A). Similar results were obtained when cells were exposed to *N*-octanoyl-homoserine lactone (OHL) (data not shown). No luminescence was detected in the mutant background, regardless of HHL exposure in either migrating swimmer or swarmer populations (Fig. 3A). In the wild-type background, *rck*::*lux* activity was higher in the swarmer population (Fig. 3A), in accordance with up-regulation of *sdiA* expression (Fig. 1B).

To address whether the observed differences can be entirely attributed to growth on the surface, *rck* reporter activity was monitored over time in cells spread plated on swarm, swim, and solid plates. Similar to the migrating populations, luminescence was detected only in the cell populations that were exposed to HHL (Fig. 3B). The temporal luminescence patterns were conserved under all conditions tested, with the exception of those spread plated on swarm plates at 5 h. Regardless of the growth conditions, the maximum activity observed in these spread-plated populations did not exceed that detected in the migrating swimmer population. These results clearly indicate that the *rck* operon is SdiA regulated strictly in an AHL-dependent manner and further support the notion that *sdiA* is up-regulated specifically in the actively migrating swarmer population.

Expression of *pfs* is up-regulated in the swarmer population, which coincides with increased AI-2 production under conditions in which the *lsr* operon is not expressed. AI-2 production in serovar Typhimurium is regulated at the level of *pfs* transcription rather than that of *luxS* (7). Hence, monitoring the transcriptional activity of *pfs* can indicate changes in AI-2 production. The activity of the *pfs*::*luxCDABE* reporter system was compared between migrating swarmer and swimmer populations to examine whether AI-2 production is also differentially regulated. *pfs*::*lux* activity was stimulated in the swarmer population, but the spatial pattern differed from that generated by the *sdiA* reporter. Unlike the *sdiA*::*lux* spatial pattern, where elevation in luminescence coincided specifically between the regions divided by the flower-like portion and the actively migrating swarmer population (Fig. 1B), an increasing gradient of *pfs*::*lux* activity was observed within the flower-like portion (Fig. 4A). Nonetheless, maximum activity was observed within the actively migrating swarmer population and was maintained uniformly throughout, analogous to *sdiA*::*lux* activity.

The observed stimulation of *pfs* expression in the swarmer population was not solely due conditions associated with surface growth, since maximum activity detected in spread-plated populations was comparable to that in migrating swimmer population (Fig. 4B). To further validate the transcriptional data, AI-2 production was compared spatially within point-inoculated swarm plates and temporally in broth-propagated cells and spread-plated cells on swarm and solid plates. Swim plates were not utilized in this assay, as there is no reliable method for harvesting sufficient amounts of cells for AI-2 extraction. The flower-like portion was physically removed from the swarm plates, and cells were harvested independently from the actively migrating portion (see Materials and Methods). Cells were also harvested over time from broth and spread-plated swarm and solid plates. All samples were shocked in 0.4 M NaCl, as this method was shown to optimize AI-2 extraction while minimizing degradation (43), thus facilitating the measurement of the samples' maximum capacity to produce AI-2. Osmotic shock fluid (OSF) obtained from each sample was assayed for AI-2 activity using the *Vibrio harveyi* bioluminescence assay. *V. harveyi* MM32 is an ideal reporter strain for comparing AI-2 production, since it possesses an intact sensory machinery to bind exogenous AI-2 and transduce the signal to generate light, while being incapable of producing its own AI-2 (15). AI-2 production was significantly higher in the actively migrating swarmer population (Fig. 4C, Front) compared to those in the flower-like region (Fig. 4C, Center). AI-2 production was also consistently higher in the swarm front compared to cells propagated in broth or those spread plated on swarm and solid plates (Fig. 4D), the relative differences being similar to those in *pfs* transcriptional activity (Fig. 4A and B). These results collectively indicate that the actively migrating swarmer population of serovar Typhimurium possesses a greater capacity to produce AI-2 in a *pfs* transcription-dependent manner.

Since the *lsrACDBFGE* operon has been demonstrated to be regulated by AI-2 (46), we examined whether the activity of the



lsr::*luxCDABE* reporter system is also differentially regulated between swim and swarm populations. No detectable activity was observed in either population (Fig. 5A), indicating that the *lsr* operon was not expressed under these conditions. Recent studies have reported that transcription of the *lsr* operon in *Escherichia coli* is stimulated by the cyclic AMP (cAMP)-cAMP receptor protein complex (50, 56), and glucose strongly represses its activity accordingly (50). Consistent with these observations, *lsr* expression was observed in the same swim medium (NB) in the absence of glucose (Fig. 5B). In contrast to the standard swim medium composed of NBG (nutrient broth with 0.5% glucose), *lsr*::*lux* activity was detectable in LBG (LB with 0.5% glucose), albeit at a markedly reduced level compared to that in LB (Fig. 5B). These results suggest that expression of the *lsr* operon in 14028 is also strongly repressed by glucose, potentially due to low levels of cAMP. In addition, *lsr* expression was spatially localized (at the detectable limit) to the central region of the swim colonies (Fig. 5B), where the occupying cells are likely exposed to relatively more stressful conditions than those near the outermost regions. Consistent with this view is that expression of the *lsr* operon is stimulated during the early phase of stationary growth in broth (45, 46), especially in the absence of glucose (50). More importantly, the observed increase in AI-2 production in the swarmer population in NBG was not affected by relative differences in the uptake rate of extracellular AI-2.

DISCUSSION

The data presented here on the regulation of two independent signaling systems implies that swarmer differentiation in serovar Typhimurium may be coregulated with a heightened sensitivity for intra- and interspecies communication. This potential was highlighted by the observations that the swarmers possess a higher capacity to generate a universally recognized signaling molecule (AI-2) in addition to increasing their ability to sense the presence of other bacterial species by signaling molecules that they themselves do not produce (AHL). However, as reviewed by Ahmer (1), the physiological roles of these signaling systems in serovar Typhimurium remain largely unknown.

Schneider et al. (39) previously reported that LuxS-dependent AI-2 production in *Proteus mirabilis* is correlated to the initial stages of swarming and is maintained throughout the migration process. The data presented here are in general agreement with this previous report: *pfs* activity is elevated in

FIG. 3. Effect of HHL on *rck*::*lux* reporter activity in serovar Typhimurium 14028 and Δ*sdiA* mutant WK2. (A) CFU-normalized expression profiles of migrating 14028 (black) or WK2 (upward diagonal) swarmer cells and 14028 (gray) or WK2 (downward diagonal) swimmer cells. (B) CFU-normalized temporal expression profiles of 14028 cells spread plated on swarm (black), swim (gray), or solid (white) plates and WK2 cells spread plated on swarm (upward diagonal), swim (downward diagonal), or solid (checkered) plates. Cells were harvested from areas surrounding filter disks impregnated with *N*-hexanoyl-homoserine lactone (HHL) or methanol (MeOH) and areas remote from the disks (No Disk). The error bars indicate the standard deviation among three independent samples. RLU, relative light units.

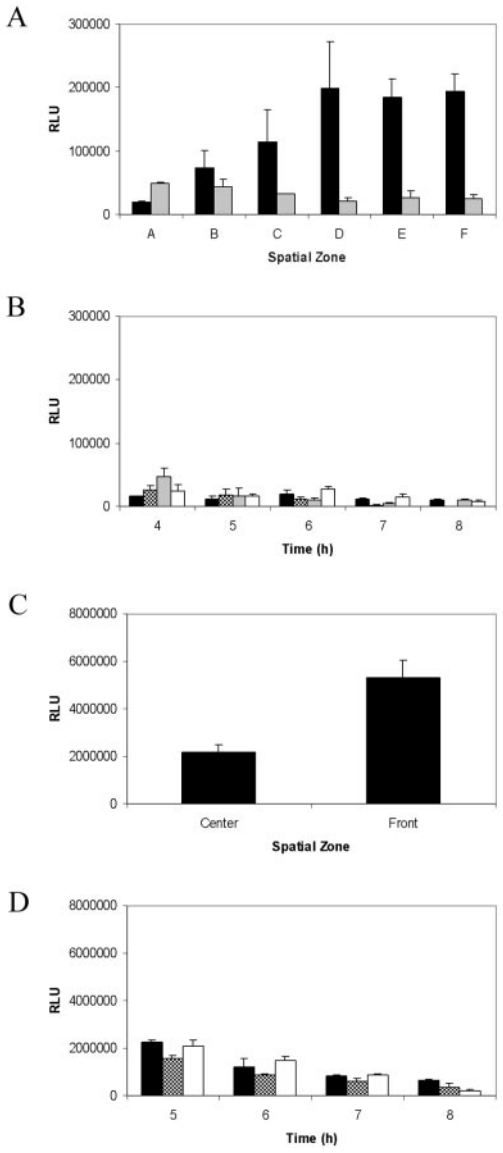


FIG. 4. Comparison of the *pfs*::*lux* reporter activity and AI-2 production in serovar Typhimurium 14028 under conditions that promote or do not support swarmer differentiation. (A) CFU-normalized spatial expression profiles of migrating swarmer (black) or swimmer (gray) cells harvested from the corresponding regions indicated in Fig. 1A. (B) CFU-normalized temporal expression profiles of cells propagated in broth (checkered) or spread plated on swarm (black), swim (gray), or solid (white) plates. (C) AI-2 production in the flower-like region (Center) or actively migrating swarmer population (Front) measured by the *Vibrio harveyi* MM32 bioassay. (D) Temporal AI-2 production in cells propagated in broth (checkered) or spread plated on swarm (black) or solid (white) plates. Osmotic shock fluid (OSF) obtained from each sample was added at 5% (vol/vol) in the MM32 bioassay. The maximum CFU-normalized luminescence values are shown. The error bars indicate the standard deviation among three independent samples. RLU, relative light units.

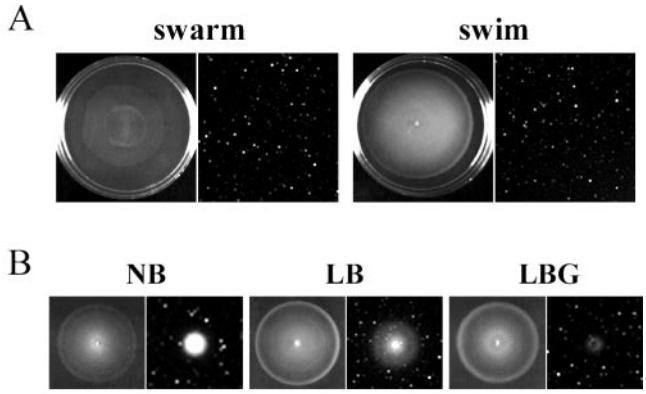


FIG. 5. Activity of the *lsr*::*lux* reporter in swarmer and swimmer populations of strain 14028. Digital images captured by reflected light (left) or luminescence (right) on (A) the standard NBG (nutrient broth, 0.5% glucose) swarm or swim plates and (B) alternative swim plates as indicated. NB, nutrient broth; LB, Luria broth; LBG, LB with 0.5% glucose.

the actively migrating swarmer population of serovar Typhimurium which coincides with elevated AI-2 production. However, the absence of AI-2 production does not have any bearing on swarmer differentiation or migration in either organism (39, 47). The link between AI-2 production and swarmer differentiation may lie at the level of metabolic differentiation described in actively swarming serovar Typhimurium (26). Although there is no direct evidence at present, given the metabolic potential of the differentiated swarmers, SAM utilization may also be elevated (since SAM is the primary methyl donor), consequently generating higher levels of AI-2. Elevated de novo biosynthetic pathways and the prerequisite for glucose utilization (26) may indirectly stimulate AI-2 production via elevated *pfs* expression and repression of the *lsr* system. Although the physiological role of AI-2 in *Salmonella* remains to be elucidated, a recent report suggested that LuxS/AI-2 is essential for biofilm formation on the surface of gall stones (36). Although disrupting the *luxS* gene does not adversely affect the motility aspect of swarming (47), AI-2 may be intimately associated with the differentiation and/or maintenance of the metabolic state. Analogous to the SdiA signaling system in *Salmonella*, *Pseudomonas aeruginosa* does not produce AI-2 but is able to respond to AI-2 secreted by other bacteria. Expression of important virulence genes in *P. aeruginosa* was shown to be modulated by exogenous AI-2, which overlapped with the expression profiles generated in the presence of the resident oropharyngeal microflora (11). Thus, increased production of AI-2 in the swarmer population may be geared towards modulating the dynamics of the resident microflora.

Under our standard swim and swarm assay conditions (glucose-supplemented nutrient broth), expression of the *lsr* operon was not detectable. This is consistent with the recent observations that the *lsr* operon in *E. coli* is strongly influenced by catabolite repression, where the cAMP-cAMP receptor protein complex directly binds to the *lsr* operon promoter and stimulates its transcription in the absence of preferred carbon sources (50). We have observed similar results on *lsr* expression in *Salmonella* (A. L. Beeston and M. G. Surette, unpublished results). Accordingly, we showed that the *lsr* operon is expressed in the absence of glucose in NB or LB swim media. However, it is puzzling that low levels of *lsr* operon expression were detected in glucose-supplemented LB (LBG). Nonetheless, the observed differences in AI-2 production between the swimmer and swarmer populations were not due to relative

differences in their ability to internalize extracellular AI-2 but were at the level of AI-2 synthesis.

AHL-based cell-cell signaling systems in several organisms contribute to the motility aspect of swarming but do not influence swarmer differentiation. Biosurfactant production is controlled by AHLs in *Serratia* (12), *Pseudomonas* (29), and *Burkholderia* (24) species, which is crucial for surface motility. Similar to LuxS and AI-2, SdiA is not essential at least for the mechanistic aspect of surface motility (47). Here, we demonstrated that expression of *sdiA* and the *rck* operon (AHL dependent) were both significantly up-regulated in the actively migrating swarmer population. Although it is not overly forceful to envision that a complex polymicrobial environment such as the gastrointestinal tract may serve as a rich source of AHL signaling molecules, there is currently no evidence that AHLs are produced by the gastrointestinal microflora. In fact, there is only one known report that bears a relatively close mark, wherein several forms of AHLs were detected within bovine ruminal contents (13). As reviewed by Ahmer (1), the potential physiological role(s) of SdiA may be deduced from the established functions of genes known to be regulated by SdiA, in particular the *rck* operon. *rck* encodes an outer membrane protein which confers significant resistance to killing by human complement by interfering with the formation of polymerized C9 tubular membrane attack complexes (23). When *Salmonella* Rck was expressed in a nonadherent, noninvasive laboratory strain of *E. coli*, the recombinant strain was able to adhere to both epithelial monolayers and extracellular matrix proteins, including laminin (10). Another member of the *rck* operon is *pefI*, which encodes a transcriptional regulator of the *pef* operon (16). Expression of both *pefI* and *rck* genes is controlled by the *rck* promoter used in this study (34). The *pef* operon encodes the components of the plasmid-encoded fimbriae that mediate adhesion to the murine intestinal epithelium (6). Thus, expression of the SdiA-regulated genes would be beneficial to *Salmonella* within the gastrointestinal environment, and the observed up-regulation of *sdiA* and the concomitant regulation of the *rck* operon in the swarmer population brings the relevance of the differentiated state closer to the milieu of the host environment. To substantiate this potential link, however, the aforementioned physiological attributes associated with the *rck* operon must be individually assessed in the swarmer population.

The spatial gene expression patterns presented here indicate that the cells occupying the central flower-like region are quite distinct from those actively swarming. This was not entirely due to differences in the relative age of the central population, since expression levels were consistent from the inner edge (i.e., directly proximal to the flower-like region) to the outer edge of the actively swarming population. Moreover, expression of both *sdiA* and *pfs* genes in the flower-like portion of the swarm colonies are comparable to the levels expressed throughout the vegetative swimmer population. This is consistent with the view that the cells in the flower-like region on the swarm plates (i.e., undifferentiated and/or dedifferentiated) are phenotypically similar to the vegetative populations propagated in either liquid or swim media (26). Furthermore, an actively migrating swarmer population does not generally exhibit transcriptional heterogeneity and is metabolically distinct (26), which was also evident by the absence of spatial pH fluctuations in contrast to the central portion of the swarm colony (47). Thus, the actively migrating swarmer population, as a whole, is physiologically distinct from the flower-like population.

Wang and colleagues (51) reported that nearly one-third of the functional genome of serovar Typhimurium was differentially regulated when cells were spread plated on either swarm or solid plates compared to those propagated in broth. They clearly demonstrated that growth on the surface represents a physiologically distinct environment compared to that in broth. In this study, we have shown that the two cell-cell signaling systems are stimulated specifically in the actively migrating swarmer population and are not entirely due to adaptation to conditions innately associated with the surface. Spread plating on swarm plates undoubtedly produces differentiated swarmer cells (51); however, this approach also inherently leads to a heterogeneous mixture of differentiated and undifferentiated cells (26). The gradient-like spatial patterns of the *pfs* reporter within the flower-like portion may be indicative of relative differences in the heterogeneity of the population. As evidenced by the relative homogeneity of transcriptional activity in the actively migrating swarmer population and the clear spatial separation from the flower-like region, the spot-inoculation approach inherently separates undifferentiated or dedifferentiated populations from those that are fully differentiated. We encourage future studies that utilize both experimental approaches to discern physiological changes associated swarmer differentiation and those associated with growth on the surface.

Although we have demonstrated that regulation of the two cell-cell signaling systems is coordinated with swarmer differentiation in serovar Typhimurium, specific regulatory elements and the corresponding mechanisms remain to be determined. We have previously presented evidence that the swarmer population represents a physiologically differentiated state at the level of metabolism (26). Thus, even in the absence of specific stimuli, systemic changes associated with swarmer differentiation may be integrated to regulatory pathways resulting in the coregulation of genes such as *sdiA* and *pfs* as components of the differentiation program. Concurrent to the examples of synchronicity observed between host-related gene expression and swarmer differentiation in other bacteria (3, 20, 31, 40, 49), global changes in metabolism independent of nutritional inputs (26), adaptive antibiotic resistance in the absence of overt pressure (25, 28), and differential regulation of cell-cell signaling systems described here may collectively represent an evolved strategy of serovar Typhimurium and other *Salmonella* isolates (27) in adaptation to the host environment. Although there is no direct evidence that *Salmonella* exists in the swarm state in the gastrointestinal environment, ruling out this possibility is not a trivial task. Unlike in *Proteus* spp., morphological changes associated with swarmer differentiation in *Salmonella* spp. are not nearly as readily apparent (22, 47). Further characterization of other components of the swarmer differentiation program should continue to provide fresh insight into a bacterial behavior often viewed as a simple laboratory motility phenotype.

ACKNOWLEDGMENTS

We thank A. P. White and K. Pabbaraju for constructing the *lsr*::*luxCDABE* and the σ^{70} reporters, respectively, and the late R. J. Kadner for providing insightful comments on the manuscript.

This work was supported by a grant from the Canadian Institutes of Health Research (CIHR). M.G.S. is supported as an Alberta Heritage Foundation for Medical Research (AHFMR) Senior Scholar and Canada Research Chair in Microbial Gene Expression.

REFERENCES

1. **Ahmer, B. M.** 2004. Cell-to-cell signalling in *Escherichia coli* and *Salmonella enterica*. Mol. Microbiol. **52:**933–945.
2. **Ahmer, B. M., J. van Reeuwijk, C. D. Timmers, P. J. Valentine, and F. Heffron.** 1998. *Salmonella typhimurium* encodes an SdiA homolog, a putative quorum sensor of the LuxR family, that regulates genes on the virulence plasmid. J. Bacteriol. **180:**1185–1193.
3. **Allison, C., H. C. Lai, and C. Hughes.** 1992. Co-ordinate expression of virulence genes during swarm-cell differentiation and population migration of *Proteus mirabilis*. Mol. Microbiol. **6:**1583–1591.
4. **Ang, S., Y. T. Horng, J. C. Shu, P. C. Soo, J. H. Liu, W. C. Yi, H. C. Lai, K. T. Luh, S. W. Ho, and S. Swift.** 2001. The role of RsmA in the regulation of swarming motility in *Serratia marcescens*. J. Biomed. Sci. **8:**160–169.
5. **Bassler, B. L.** 2002. Small talk. Cell-to-cell communication in bacteria. Cell **109:**421–424.
6. **Baumler, A. J., R. M. Tsolis, F. A. Bowe, J. G. Kusters, S. Hoffmann, and F. Heffron.** 1996. The *pef* fimbrial operon of *Salmonella typhimurium* mediates adhesion to murine small intestine and is necessary for fluid accumulation in the infant mouse. Infect. Immun. **64:**61–68.
7. **Beeston, A. L., and M. G. Surette.** 2002. *pfs*-dependent regulation of autoinducer 2 production in *Salmonella enterica* serovar Typhimurium. J. Bacteriol. **184:**3450–3456.
8. **Belas, R., R. Schneider, and M. Melch.** 1998. Characterization of *Proteus mirabilis* precocious swarming mutants: identification of *rsbA*, encoding a regulator of swarming behavior. J. Bacteriol. **180:**6126–6139.
9. **Bjarnason, J., C. M. Southward, and M. G. Surette.** 2003. Genomic profiling of iron-responsive genes in *Salmonella enterica* serovar Typhimurium by high-throughput screening of a random promoter library. J. Bacteriol. **185:**4973–4982.
10. **Crago, A. M., and V. Koronakis.** 1999. Binding of extracellular matrix laminin to *Escherichia coli* expressing the *Salmonella* outer membrane proteins Rck and PagC. FEMS Microbiol. Lett. **176:**495–501.
11. **Duan, K., C. Dammel, J. Stein, H. Rabin, and M. G. Surette.** 2003. Modulation of *Pseudomonas aeruginosa* gene expression by host microflora through interspecies communication. Mol. Microbiol. **50:**1477–1491.
12. **Eberl, L., M. K. Winson, C. Sternberg, G. S. Stewart, G. Christiansen, S. R. Chhabra, B. Bycroft, P. Williams, S. Molin, and M. Givskov.** 1996. Involvement of N-acyl-L-hormoserine lactone autoinducers in controlling the multicellular behaviour of *Serratia liquefaciens*. Mol. Microbiol. **20:**127–136.
13. **Erickson, D. L., V. L. Nsereko, D. P. Morgavi, L. B. Selinger, L. M. Rode, and K. A. Beauchemin.** 2002. Evidence of quorum sensing in the rumen ecosystem: detection of N-acyl homoserine lactone autoinducers in ruminal contents. Can. J. Microbiol. **48:**374–378.
14. **Fraser, G. M., and C. Hughes.** 1999. Swarming motility. Curr. Opin. Microbiol. **2:**630–635.
15. **Freeman, J. A., and B. L. Bassler.** 1999. A genetic analysis of the function of LuxO, a two-component response regulator involved in quorum sensing in *Vibrio harveyi*. Mol. Microbiol. **31:**665–677.
16. **Friedrich, M. J., N. E. Kinsey, J. Vila, and R. J. Kadner.** 1993. Nucleotide sequence of a 13.9 kb segment of the 90 kb virulence plasmid of *Salmonella typhimurium*: the presence of fimbrial biosynthetic genes. Mol. Microbiol. **8:**543–558.
17. **Fuqua, C., and E. P. Greenberg.** 2002. Listening in on bacteria: acyl-homoserine lactone signalling. Nat. Rev. Mol. Cell Biol. **3:**685–695.
18. **Fuqua, C., M. R. Parsek, and E. P. Greenberg.** 2001. Regulation of gene expression by cell-to-cell communication: acyl-homoserine lactone quorum sensing. Annu. Rev. Genet. **35:**439–468.
19. **Fuqua, C., S. C. Winans, and E. P. Greenberg.** 1996. Census and consensus in bacterial ecosystems: the LuxR-LuxI family of quorum-sensing transcriptional regulators. Annu. Rev. Microbiol. **50:**727–751.
20. **Givskov, M., L. Eberl, G. Christiansen, M. J. Benedik, and S. Molin.** 1995. Induction of phospholipase and flagellar synthesis in *Serratia liquefaciens* is controlled by expression of the flagellar master operon *flhD*. Mol. Microbiol. **15:**445–454.
21. **Givskov, M., J. Ostling, L. Eberl, P. W. Lindum, A. B. Christensen, G. Christiansen, S. Molin, and S. Kjelleberg.** 1998. Two separate regulatory systems participate in control of swarming motility of *Serratia liquefaciens* MG1. J. Bacteriol. **180:**742–745.
22. **Harshey, R. M., and T. Matsuyama.** 1994. Dimorphic transition in *Escherichia coli* and *Salmonella typhimurium*: surface-induced differentiation into hyperflagellate swarmer cells. Proc. Natl. Acad. Sci. USA **91:**8631–8635.
23. **Heffernan, E. J., S. Reed, J. Hackett, J. Fierer, C. Roudier, and D. Guiney.** 1992. Mechanism of resistance to complement-mediated killing of bacteria encoded by the *Salmonella typhimurium* virulence plasmid gene *rck*. J. Clin. Investig. **90:**953–964.
24. **Huber, B., K. Riedel, M. Hentzer, A. Heydorn, A. Gotschlich, M. Givskov, S. Molin, and L. Eberl.** 2001. The cep quorum-sensing system of *Burkholderia cepacia* H111 controls biofilm formation and swarming motility. Microbiology **147:**2517–2528.
25. **Kim, W., T. Killam, V. Sood, and M. G. Surette.** 2003. Swarm-cell differentiation in *Salmonella enterica* serovar Typhimurium results in elevated resistance to multiple antibiotics. J. Bacteriol. **185:**3111–3117.
26. **Kim, W., and M. G. Surette.** 2004. Metabolic differentiation in actively swarming *Salmonella*. Mol. Microbiol. **54:**702–714.
27. **Kim, W., and M. G. Surette.** 2005. Prevalence of surface swarming behavior in *Salmonella*. J. Bacteriol. **187:**6580–6583.
28. **Kim, W., and M. G. Surette.** 2003. Swarming populations of *Salmonella* represent a unique physiological state coupled to multiple mechanisms of antibiotic resistance. Biol. Proc. **5:**189–196. [Online.] http://www.biologicalprocedures.com.
29. **Kohler, T., L. K. Curty, F. Barja, C. van Delden, and J. C. Pechere.** 2000. Swarming of *Pseudomonas aeruginosa* is dependent on cell-to-cell signaling and requires flagella and pili. J. Bacteriol. **182:**5990–5996.
30. **Lindum, P. W., U. Anthoni, C. Christophersen, L. Eberl, S. Molin, and M. Givskov.** 1998. *N*-Acyl-L-homoserine lactone autoinducers control production of an extracellular lipopeptide biosurfactant required for swarming motility of *Serratia liquefaciens* MG1. J. Bacteriol. **180:**6384–6388.
31. **Macfarlane, S., M. J. Hopkins, and G. T. Macfarlane.** 2001. Toxin synthesis and mucin breakdown are related to swarming phenomenon in *Clostridium septicum*. Infect. Immun. **69:**1120–1126.
32. **McLean, R. J., M. Whiteley, D. J. Stickler, and W. C. Fuqua.** 1997. Evidence of autoinducer activity in naturally occurring biofilms. FEMS Microbiol. Lett. **154:**259–263.
33. **Meighen, E. A.** 1993. Bacterial bioluminescence: organization, regulation, and application of the lux genes. FASEB J. **7:**1016–1022.
34. **Michael, B., J. N. Smith, S. Swift, F. Heffron, and B. M. Ahmer.** 2001. SdiA of *Salmonella enterica* is a LuxR homolog that detects mixed microbial communities. J. Bacteriol. **183:**5733–5742.
35. **Miller, S. T., K. B. Xavier, S. R. Campagna, M. E. Taga, M. F. Semmelhack, B. L. Bassler, and F. M. Hughson.** 2004. *Salmonella typhimurium* recognizes a chemically distinct form of the bacterial quorum-sensing signal AI-2. Mol. Cell **15:**677–687.
36. **Prouty, A. M., W. H. Schwesinger, and J. S. Gunn.** 2002. Biofilm formation and interaction with the surfaces of gallstones by *Salmonella* spp. Infect. Immun. **70:**2640–2649.
37. **Redfield, R. J.** 2002. Is quorum sensing a side effect of diffusion sensing? Trends Microbiol. **10:**365–370.
38. **Reimmann, C., N. Ginet, L. Michel, C. Keel, P. Michaux, V. Krishnapillai, M. Zala, K. Heurlier, K. Triandafillu, H. Harms, G. Defago, and D. Haas.** 2002. Genetically programmed autoinducer destruction reduces virulence gene expression and swarming motility in *Pseudomonas aeruginosa* PAO1. Microbiology **148:**923–932.
39. **Schneider, R., C. V. Lockatell, D. Johnson, and R. Belas.** 2002. Detection and mutation of a *luxS*-encoded autoinducer in *Proteus mirabilis*. Microbiology **148:**773–782.
40. **Senesi, S., F. Celandroni, S. Salvetti, D. J. Beecher, A. C. Wong, and E. Ghelardi.** 2002. Swarming motility in *Bacillus cereus* and characterization of a *fliY* mutant impaired in swarm cell differentiation. Microbiology **148:**1785–1794.
41. **Smith, J. N., and B. M. Ahmer.** 2003. Detection of other microbial species by *Salmonella*: expression of the SdiA regulon. J. Bacteriol. **185:**1357–1366.
42. **Sturme, M. H., M. Kleerebezem, J. Nakayama, A. D. Akkermans, E. E. Vaugha, and W. M. de Vos.** 2002. Cell to cell communication by autoinducing peptides in gram-positive bacteria. Antonie Leeuwenhoek **81:**233–243.
43. **Surette, M. G., and B. L. Bassler.** 1999. Regulation of autoinducer production in *Salmonella typhimurium*. Mol. Microbiol. **31:**585–595.
44. **Surette, M. G., M. B. Miller, and B. L. Bassler.** 1999. Quorum sensing in *Escherichia coli*, *Salmonella typhimurium*, and *Vibrio harveyi*: a new family of genes responsible for autoinducer production. Proc. Natl. Acad. Sci. USA **96:**1639–1644.
45. **Taga, M. E., S. T. Miller, and B. L. Bassler.** 2003. Lsr-mediated transport and processing of AI-2 in *Salmonella typhimurium*. Mol. Microbiol. **50:**1411–1427.
46. **Taga, M. E., J. L. Semmelhack, and B. L. Bassler.** 2001. The LuxS-dependent autoinducer AI-2 controls the expression of an ABC transporter that functions in AI-2 uptake in *Salmonella typhimurium*. Mol. Microbiol. **42:**777–793.
47. **Toguchi, A., M. Siano, M. Burkart, and R. M. Harshey.** 2000. Genetics of swarming motility in *Salmonella enterica* serovar typhimurium: critical role for lipopolysaccharide. J. Bacteriol. **182:**6308–6321.
48. **Visick, K. L., J. Foster, J. Doino, M. McFall-Ngai, and E. G. Ruby.** 2000. *Vibrio fischeri* lux genes play an important role in colonization and development of the host light organ. J. Bacteriol. **182:**4578–4586.
49. **Walker, K. E., S. Moghaddame-Jafari, C. V. Lockatell, D. Johnson, and R. Belas.** 1999. ZapA, the IgA-degrading metalloprotease of *Proteus mirabilis*, is a virulence factor expressed specifically in swarmer cells. Mol. Microbiol. **32:**825–836.

50. **Wang, L., Y. Hashimoto, C. Y. Tsao, J. J. Valdes, and W. E. Bentley.** 2005. Cyclic AMP (cAMP) and cAMP receptor protein influence both synthesis and uptake of extracellular autoinducer 2 in *Escherichia coli*. J. Bacteriol. **187:**2066–2076.
51. **Wang, Q., J. G. Frye, M. McClelland, and R. M. Harshey.** 2004. Gene expression patterns during swarming in *Salmonella typhimurium*: genes specific to surface growth and putative new motility and pathogenicity genes. Mol. Microbiol. **52:**169–187.
52. **Wang, Q., A. Suzuki, S. Mariconda, S. Porwollik, and R. M. Harshey.** 2005. Sensing wetness: a new role for the bacterial flagellum. EMBO J. **24:**2034–2042.
53. **White, A. P., S. K. Collinson, J. Burian, S. C. Clouthier, P. A. Banser, and W. W. Kay.** 1999. High efficiency gene replacement in *Salmonella enteritidis*: chimeric fimbrins containing a T-cell epitope from *Leishmania major*. Vaccine **17:**2150–2161.
54. **Winzer, K., K. R. Hardie, and P. Williams.** 2002. Bacterial cell-to-cell communication: sorry, can't talk now—gone to lunch! Curr. Opin. Microbiol. **5:**216–222.
55. **Xavier, K. B., and B. L. Bassler.** 2003. LuxS quorum sensing: more than just a numbers game. Curr. Opin. Microbiol. **6:**191–197.
56. **Xavier, K. B., and B. L. Bassler.** 2005. Regulation of uptake and processing of the quorum-sensing autoinducer AI-2 in *Escherichia coli*. J. Bacteriol. **187:**238–248.