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MINIREVIEW

Surface Motility of *Serratia liquefaciens* MG1

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POPULATION MIGRATION OF *SERRATIA LIQUEFACIENS* MG1 AS AN EXAMPLE OF MULTICELLULAR PROKARYOTIC BEHAVIOR

The view of bacteria as unicellular organisms has strong roots in the traditional way of culturing bacteria in liquid medium. Although studies of bacterial activities during conditions of balanced growth have lead to insight into basic life processes and have unraveled complex regulatory networks, it must be emphasized that in nature microbial activity is often associated with surfaces (16). In fact, it appears that the ability to form surface-associated structured and cooperative consortia (referred to as biofilms) is one of the most remarkable characteristics of bacteria. Moreover, the observation that bacteria undergo cell differentiation when they grow in colonies and the discovery of sophisticated intercellular communication systems have shown that bacteria are much more interactive than previously realized (81). Communication capabilities are considered to be essential prerequisites for coordinated bacterial activities. The communication language is in most cases chemical in nature. Signal molecules that are released by specialized cells are thought to modulate the activity of other cells in the vicinity, thus regulating collective activities (involving many different genes connected by joint control factors). This minireview focuses on one example of coordinated bacterial activity, namely, the migration of populations by means of swarming motility in the strain *S. liquefaciens* MG1.

SWARMING PHENOMENON

Bacterial surface translocation. Swarming is one of six described forms of bacterial surface translocation (including swimming, gliding, twitching, darting, and sliding) (45). The ability of members of the genus *Proteus* to swarm on solid medium has interested many microbiologists since the phenomenon was described by Hauser more than a century ago (42). However, swarming is not limited to the genus *Proteus* but has been demonstrated for a wide range of diverse bacteria, and it is thought that this form of motility is ubiquitous among eubacteria (40). Swarming motility is driven by the operation of peritrichously arranged flagella, which are thought to function as helical propellers driven by a biological rotary motor (44). The development of a swarming colony on an agar plate follows three major steps (Fig. 1). First, a regular colony is formed at the inoculation point. Thereafter, the cells at the rim of the colony initiate a differentiation process resulting in long (up to 50-μm) multinucleated, aseptate, hyperflagellated cells, which have the unique ability to move on top of the agar surface (Fig. 1B). Microscopic inspection reveals that the differentiated cells organize in highly motile rafts that form an outer, motile layer that moves in a swirling fashion (Fig. 1C). By analogy to swarming bees, this type of multicellular bacterial behavior is referred to as swarming motility. The rapid outward movement of the swarm cells at the rim of the swarm colony is accompanied by bacterial growth inside the colony, resulting in extremely fast colonization of all available surface space. We have recorded velocities of colony expansion of up to 10 mm/h for *S. liquefaciens* MG1.

Swarming motility is an intrinsically surface-linked and cell density-dependent phenomenon involving cell differentiation, extensive flagellation, contact between neighboring bacteria, and in particular, highly coordinated migration of swarm cells. Various extracellular compounds, such as biosurfactants and polysaccharides (2, 54, 58), facilitate surface translocation. The high degree of coordination between the cells within a swarm colony and the observation that separated swarm cells are unable to swarm suggest that this form of surface translocation has to be considered a social phenomenon. This is in sharp contrast to swimming, where cells move separately in periods of smooth runs interrupted by short tumbles in an apparently unorganized manner.

Medium effects. *S. liquefaciens* MG1 is capable of swimming and swarming motility. The critical factor that determines whether cells swim, differentiate into swarm cells, or form a regular colony is the agar concentration and thus the viscosity of the medium. On media containing low agar concentrations (<0.4%), the strain exhibits swimming motility, while on media solidified with 0.4 to 1.2% agar (with an optimum colony expansion rate at 0.7%), the strain swarms atop the agar surface. On media with higher agar concentrations, migration of the strain is inhibited and consequently a normal-sized colony is formed. This is in sharp contrast to *Proteus mirabilis* and *Vibrio parahaemolyticus*, which are able to form swarming colonies on the surface of 2.0% agar. At the other extreme, *Escherichia coli* will form swarming colonies only on Eiken agar (41).

In *P. mirabilis*, swarming is cyclical in nature. The repetition of alternating phases of swarming and consolidation (dedifferentiation into vegetative cells) leads to the formation swarm colonies with regularly spaced concentric terraces (26). Swarming colonies of *S. liquefaciens* MG1 do not produce concentric zones of consolidation, but on some media like minimal medium containing gelatin, dentritic fractal consolidation patterns have been observed (23). As with *Proteus*, swarming of *S. liquefaciens* MG1 is strongly promoted by rich media (25, 48). Swarming on minimal medium is observed only when the medium is supplemented with a mixture of amino acids (such as Casamino Acids). *V. parahaemolyticus* will form swarm colonies on minimal medium (66). For *P. mirabilis* it has been shown that a single amino acid, glutamine, is sufficient to in-

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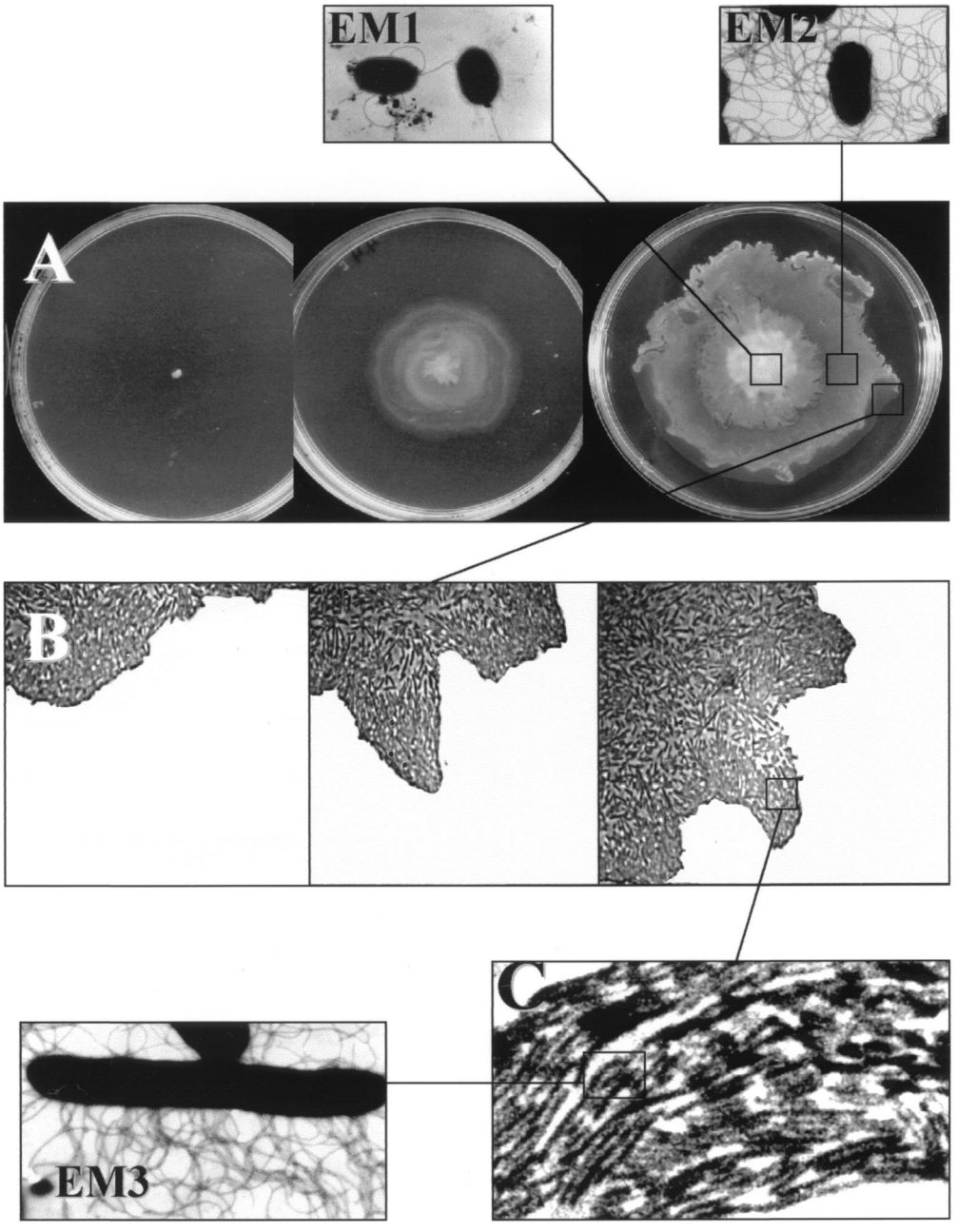

FIG. 1. Growth of an expanding swarm culture on 0.6% agar. (A) Top view. (B) Microscopic inspection of the swirling cells at the outer region of the expanding colony. Cells have been sampled from the part of the colony in the squares and examined by electron microscopy (EM1 to -3). The central part of the colony contains cells that entered stationary phase, as judged from their appearance as round, less-flagellated cells (EM1). In the middle of the colony, the vegetative (biomass-producing) cells exhibit the swim cell morphology (EM2), and finally at the border, the highly motile, flagellated, and elongated swarm cells (EM3) (24) are organized in rafts (C).

duce swarming motility (1). Swarming migration of *S. liquefaciens* MG1, however, cannot be promoted by the addition of any of the naturally occurring amino acids to minimal medium (24, 25). The doubling time of *S. liquefaciens* MG1 grown in liquid minimal medium was found to be significantly increased in the presence of even very low concentrations of Casamino Acids (0.01%), suggesting that the requirement of amino acids for swarming is likely to be attributed to the stimulation of growth (23). At present, we hypothesize that the indispensable requirement of amino acids may reflect a high demand for both building blocks and energy to synthesize and operate the hundreds of flagella produced during swarming differentiation.

GENETICS OF SWARMING

Two regulatory systems. We have identified two key regulators in *S. liquefaciens* MG1 that are involved in the regulation of swarming behavior, namely, the flagellar master FlhD-FlhC and a *N*-acyl-L-homoserine lactone (AHL)-based quorum-sensing system (24, 25). In enteric bacteria, FlhD-FlhC con-

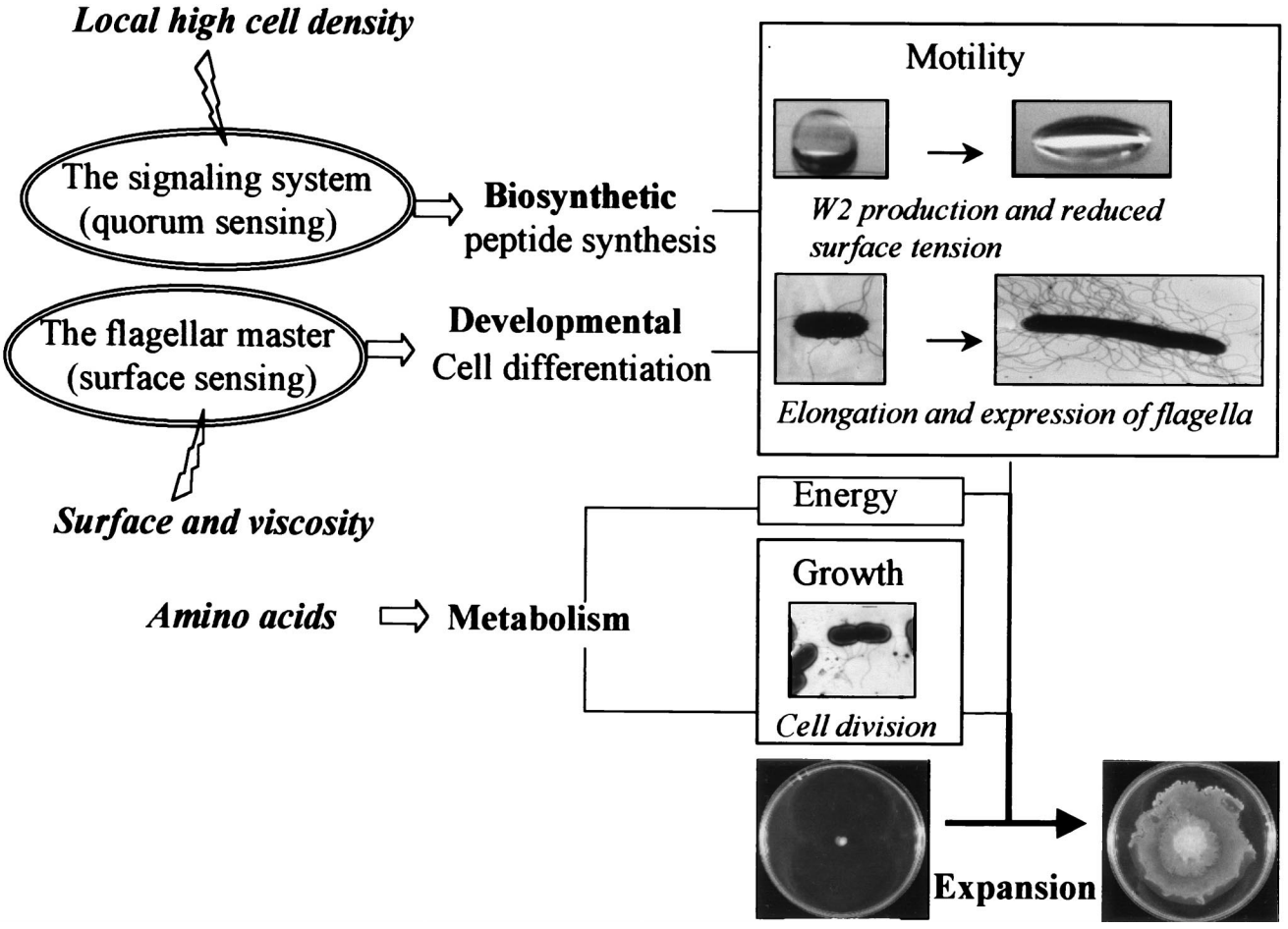

FIG. 2. Summary of the two major sensory, regulatory systems (ovals) involved in swarm cell differentiation and surface motility. Inducing stimuli (lightning) point to their respective sensory system. The fat horizontal arrows indicate the pathways targeted by the regulatory systems. The rectangles summarize the biological processes the combined action of which leads to expansion of the colony.

trols expression of the entire flagellar hierarchy (56). Quorum-sensing systems based on AHL signal molecules have been reported for a variety of gram-negative bacteria and were demonstrated to control diverse physiological processes in concert with cell density (27, 28, 88). In most cases, these cell-cell communication circuits are involved in the production of extracellular products that are essential for the interaction of bacteria with each other and their surroundings. We recently demonstrated that the flagellar master and the quorum-sensing system control two separate regulons (33). In our model for control and development of a swarming colony, the flagellar master and the quorum-sensing system control two equally important pathways, a developmental pathway and a biosynthetic pathway, respectively (Fig. 2). In addition to this, energy-generating metabolism is required for swarming behavior of *S. liquefaciens* MG1.

DEVELOPMENTAL PATHWAY

Regulation of the flagellar master. In *E. coli* and *Salmonella typhimurium*, the *flhDC* operon encodes the transcriptional regulators FlhD and FlhC that controls the expression of approximately 50 genes related to flagellar structure, chemotaxis, and cell division (56). A recent analysis of transposon insertion mutants of *S. liquefaciens* MG1 that were isolated on the basis of their inability to swim indicates a substantial degree of homology with the *E. coli* flagellar hierarchy (13). In *E. coli*, expression of the *flhDC* operon is tightly regulated in response to environmental signals by the complex interplay of various regulatory systems and factors. Among those are catabolite repression (84), the phosphorylation status of the osmoregulator OmpR (77, 87), heat shock proteins (DnaK, DnaJ, and GrpE), acetyl phosphate (83), autogenous control, and cell cycle regulation (78, 79). In *E. coli* as in *S. liquefaciens* MG1, overexpression of *flhDC* causes inhibition of growth, and a connection between synthesis of flagella and cell division has been suggested (24, 78). In *E. coli* this coupling is mediated by FlhD which has been demonstrated to regulate the cell division rate via the acid response gene *cadA* (80).

Flagellar master and cell differentiation. A *flhDC* null mutant of *S. liquefaciens* MG1 is devoid of flagella and is thus unable to swim or swarm (24). Controlled expression of the *flhDC* operon from an isopropyl-β-D-thiogalactopyranoside (IPTG)-inducible *Ptac* promoter, however, leads to flagellar synthesis and restores both swimming and swarming. Moreover, overexpression of *flhDC* in liquid medium has been demonstrated to induce swarm cell differentiation. As a result, filamentous, multinucleated, and hyperflagellated cells are formed; these cells are indistinguishable from swarm cells isolated from the edge of a swarm colony (24). Thus, artificial

stimulation of *flhDC* expression can overcome the otherwise obligatory requirement of surface contact. This indicates that at least the sensing of surface contact, which is the major stimulus for swarm cell differentiation, is channeled through the *flhDC* operon. Recent studies with *P. mirabilis* (29) and *Yersinia enterolitica* (96) were consistent with our results in *S. liquefaciens* MG1 and strengthened the view that the *flhDC* master operon is a major checkpoint for swarming behavior in different bacteria. The formation of a swarming colony would be most readily explained by assuming that the level of *flhDC* mRNA is specifically increased. In fact, Northern analysis of mRNA levels in *P. mirabilis* has demonstrated that the amount of *flhDC* mRNA is more than 30-fold higher in swarm cells than the amount found in vegetative cells (29). With *S. liquefaciens* MG1, we have not been able to detect any significant increase in *flhDC* transcription in differentiated swarm cells (89).

There are obvious differences among the enterics with respect to *flhDC* regulation. For example, in sharp contrast to *E. coli*, activation of the *S. liquefaciens* MG1 *flhDC* operon is independent of a functional Crp protein as *crp* mutants remain fully motile (13). Differentiated swarm cells of *Proteus* express a 50-fold increase in surface flagella, while MG1 displays only a modest increase (31, 53, 89). In contrast to *S. liquefaciens* MG1, several loci affecting *flhDC* expression at the transcriptional level such as *ppaA* and *lrp* have been identified in *P. mirabilis* (53). In *P. mirabilis*, expression of *flhDC* is positively regulated by the leucine-responsive regulatory protein Lrp (43). More recently four genes of unknown function have been identified in *P. mirabilis* that up-regulate expression of the *flhDC* operon when provided on a multicopy plasmid in *trans* (22). Inspection of the DNA sequences upstream of the *flhDC* genes from the different bacteria revealed a great degree of variation, while the coding regions were found to be highly conserved (29, 30).

Surface sensing. *V. parahemolyticus* has been demonstrated to sense its presence on a surface with its polar flagellum that functions as a tactile sensor measuring external forces influencing its motion (65). Under conditions that render the polar flagellum nonfunctional, e.g., in highly viscous medium or on surfaces, expression of swarm cell-specific genes, in particular the lateral swarm flagella, is induced. As in *P. mirabilis*, *S. liquefaciens* MG1 produces only one type of flagellum, and so far the sensor of surface contact in these bacteria has not been identified.

BIOSYNTHETIC PATHWAY

Quorum sensing. *S. liquefaciens* MG1 produces two extracellular signal molecules, *N*-butyrylhomoserine lactone (BHL) and *N*-hexanoylhomoserine lactone (HHL), that are used for sensing the density of the population (25). In recent years this type of regulatory system has been identified in various gram-negative bacteria and is referred to as the quorum-sensing system (27, 28, 88). It relies upon the presence of two proteins, a signal generator (AHL synthase) and a receptor for the specific AHL which functions as a transcriptional activator. In *S. liquefaciens* MG1, the *swrI* gene encodes an AHL synthase that directs the synthesis of BHL and HHL in a molar ratio of 10 to 1 (Fig. 3) (25). Downstream of *swrI* and transcribed convergently, an open reading frame codes for a polypeptide (SwrR) with substantial similarity to members of the LuxR family of AHL-dependent regulators (32). A knockout mutation of the *swrI* gene was found to strongly reduce the swarming capability of the strain. However, swarming motility could be restored to the wild-type level by supplementing the medium with 150 nM BHL or 900 nM HHL. Other signal molecules such as *N*-3-oxohexanoylhomoserine lactone (OHHL) or *N*-3-oxo-octanoylhomoserine lactone complement the *swrI* mutation at 9 μM (25). Thus, BHL is by far the most efficient in promoting swarming motility. The *swrI* mutation did not affect growth rate in liquid medium or swimming motility or the ability to differentiate into swarm cells when exposed to a surface (25, 31).

Target genes of the quorum-sensing system. By transposon mutagenesis, a nonswarming mutant was isolated. Insertion has occurred in a quorum sensing-controlled gene denoted *swrA* (54). DNA sequence analysis of the *swrA* gene revealed the presence of a translation product that exhibits homology to a large family of giant, multidomain enzyme complexes responsible for nonribosomal peptide synthesis (86, 92). The Srf complex of *Bacillus subtilis*, which catalyzes the synthesis of the biosurfactant surfactin (a small cyclic peptide consisting of seven amino acids and a 3-hydroxy-13-methyl-tetradecanoic fatty acid side chain), is one of the best-characterized examples of this type of enzyme complex (8, 14). The Srf complex contains seven highly homologous amino acid binding domains, which are encoded by four large open reading frames, that determine the seven specific amino acids and their order in the final surfactin molecule (11). *B. subtilis* mutants that are unable to produce surfactin have been demonstrated to be defective in swarming motility (68). From an evolutionary point of view, the ubiquitous peptide synthetases are highly interesting, being present in both gram-negative and -positive bacteria as well as in certain filamentous fungi (11, 86). The different products of the peptide synthetases display a range of powerful biological properties such as antibiotic, antifungal, hemolytic, antitumor, and surface-conditioning activities (50, 69, 70, 74).

Surface-grown cells of *S. liquefaciens* MG1 create a conditioning film that changes the wettability and surface tension of the medium (Fig. 3) (54). The formation of this film is dependent on functional *swrI* and *swrA* genes. In a *swrI* mutant, surface conditioning is restored when BHL is added exogenously to the medium (Fig. 3B) (54). Since swarming motility is a cell density-dependent phenomenon, the finding that *S. liquefaciens* MG1 employs a quorum-sensing mechanism in the process of surface conditioning was not unexpected (Fig. 3). Secretion of the extracellular lipopeptide serrawettin W2 causes reduction in the surface tension (54). Serrawettin W2 was originally isolated from spent culture supernatants of *Serratia marcescens* NS25, and its structure was proposed by Matsuyama et al. (59, 61). Detailed spectroscopic analyses identified the biosurfactant produced by *S. liquefaciens* MG1 as serrawettin W2 and at the same time confirmed the proposed chemical structure of this compound (54). Serrawettin W2 is a cyclic lipodepsipentapeptide carrying a 3-hydroxy C_{10} fatty acid side chain (Fig. 3). Interestingly, this biosurfactant is almost identical to kailuin A, a compound that was isolated from a gram-negative marine bacterium capable of swarming motility, except for the exchange of D-Leu in kailuin A with D-Phe in serrawettin W2 (39).

Although 18 transposon inserts were isolated in BHL-controlled genes (23) and expression of a minimum of 28 genes is regulated by the BHL signal molecule (33), the only identified gene involved in swarming motility is *swrA*. This suggests that, at least in our experimental setup, only a minor fraction of quorum sensing-responsive genes is involved in swarming motility. In *P. aeruginosa*, synthesis of the biosurfactant rhamnolipid is controlled by BHL-dependent quorum sensing (71).

Properties of serrawettins. The function of serrawettin W2 can be visualized by its surface tension-reducing properties that cause water droplets to collapse (Fig. 3B). Media supple-

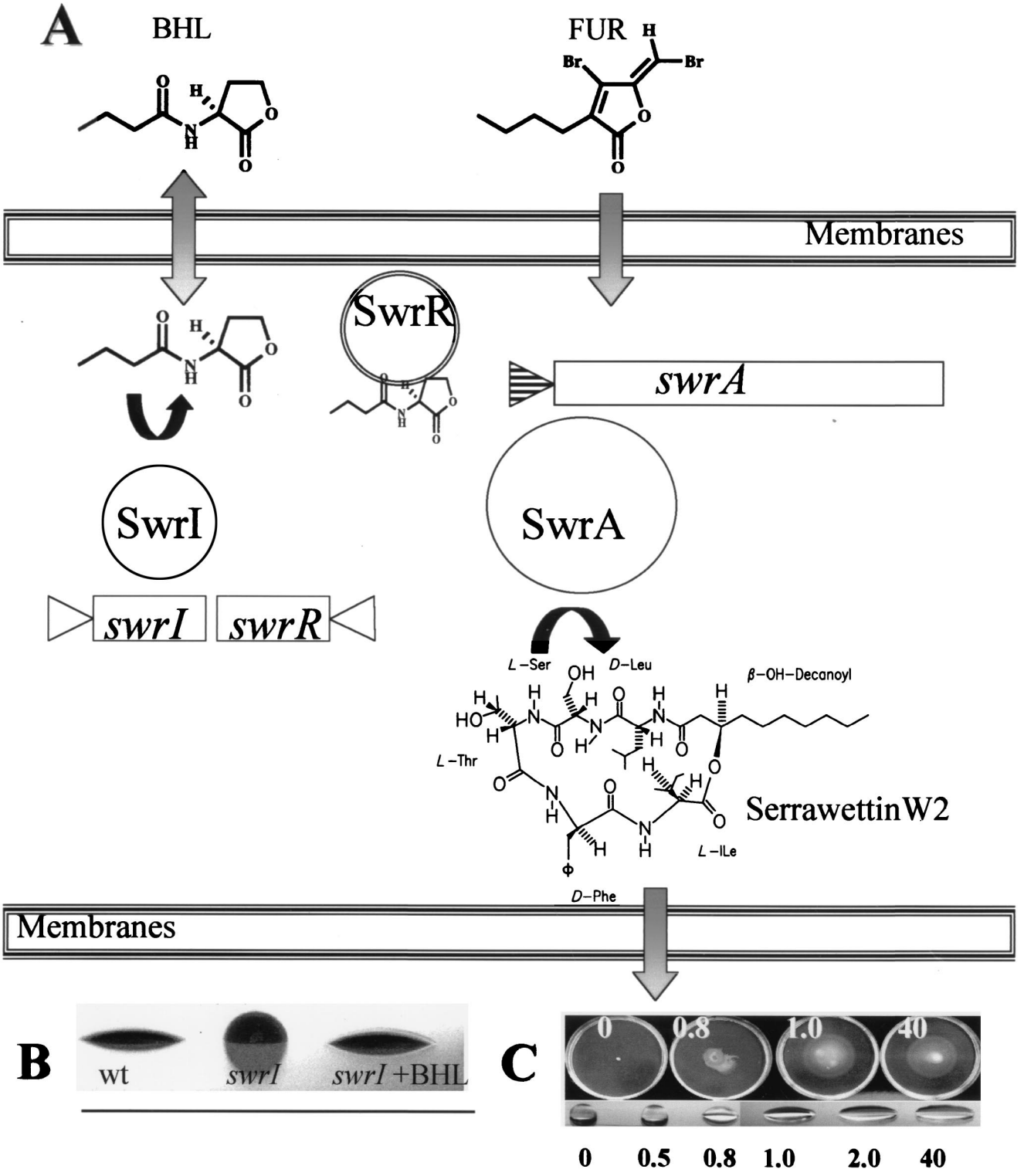

FIG. 3. Quorum sensing and control of biosurfactant synthesis in *S. liquefaciens* MG1. (A) The divergently arranged genes *swrI* and *swrR* encode an AHL synthase (LuxI homologue) and a putative regulatory LuxR homologue, SwrR, respectively. The arrowheads indicate the direction of transcription. BHL is freely diffusible over the bacterial membranes as indicated by the shaded arrow pointing up and down. FUR, one of the furanone compounds produced by *D. pulchra*. The signal molecule, BHL, is thought to bind to SwrR which in turn up-regulates transcription of the *swrA* gene (the position of the *swrA* promoter is indicated by the arrowhead with horizontal lines). The signal inhibitor, FUR, is thought to pass through the bacterial membranes and compete with BHL for the binding site present on SwrR. SwrA encodes a peptide synthetase, SwrA, which catalyzes production of the surfactant serrawettin W2. It is not known whether passage of W2 through the bacterial membranes is passive or mediated by a transport system. (B) Side views of cultures by the drop-collapsing test (54). Small volumes of bacterial cultures were placed on the lid of a petri dish. wt, wild type; *swrI*, the *swrI* mutant; +BHL, the strain was grown in the presence of 200 nM BHL. (C) Swarming motility of the *S. liquefaciens* MG1 *swrI swrA* double mutant on medium supplemented with serrawettin W2 and drop-collapsing test (54) of water supplemented with serrawettin W2 at the concentrations (in micrograms per milliliter) indicated at the bottom of the panel.

mented with pure W2 allows surfactant-defective *S. liquefaciens* MG1 (*swrI* mutant and the *swrI swrA* double mutant) cultures to travel atop the agar surface (Fig. 3C). The full effect of W2 is achieved within a narrow range around 1 μg/ml, probably reflecting the critical concentration of micelle formation. The biological importance of the biosurfactant is illustrated by fact that a nonflagellated mutant of *S. liquefaciens* MG1 is capable of colonizing the surface of plates with a low percentage of agar by means of spreading motility (which is solely driven by the biosurfactant), as has been observed previously with *S. marcescens* (58–63, 72).

Media supplemented with pure serrawettin W2, W1 (another biosurfactant produced by *S. marcescens*), or surfactin from *B. subtilis* restores the swarming phenotype of the *S. liquefaciens* MG1 *swrI swrA* double mutant. In fact, trace amounts of sodium dodecyl sulfate or Nonidet P-40 were found to be sufficient to promote swarming migration of the double mutant (34). This result demonstrates that the production of molecules lowering the surface tension of the medium is crucial for swarming motility of *S. liquefaciens* MG1. It also explains our previous observation that the *swrI* gene is dispensable for swarming motility on LB or brain heart infusion medium, since they were found to contain small amounts of surfactant (34). In *P. mirabilis*, a capsular polypeptide that facilitates swarming motility by reducing surface friction has been identified (38). Synthesis of this polypeptide is mediated by a 40.6-kDa enzyme that has strong homology to putative sugar transferases required for lipopolysaccharide core modification in *Shigella* and *Salmonella*.

EXOENZYMES

In *S. liquefaciens* MG1, expression of an extracellular phospholipase, which represents a potential virulence determinant, is coupled to the synthesis of flagella via the *flhDC* master regulator (30). This is reminiscent to the situation found with *P. mirabilis* for which it has been demonstrated that expression of virulence factors, such as intracellular urease, extracellular hemolysin, and metalloprotease, are differentially up-regulated in swarm cells (3, 4). However, in *S. liquefaciens* MG1, none of the other extracellular enzymes, which include two proteases, several chitinases, a lipase, and a nuclease, are coregulated with flagella. The quorum-sensing system in *S. liquefaciens* MG1 was found to be involved only in modulating expression of proteolytic and chitinolytic activity (23, 25). The regulation of the synthesis of the various exoenzymes in *S. liquefaciens* MG1 has been the subject of a recent review (32).

ORGANIZATION AND BEHAVIOR OF SPECIALIZED SUBCULTURES IN A SWARM COLONY

A swarming colony consists of specialized cells organized in subpopulations (Fig. 1). The immobilized, stationary-phase cells in the center do not contribute to the overall dynamics of the colony. However, the ability of the cells comprising the swimming and swarming subpopulations to go through cycles of differentiation and dedifferentiation is a major factor determining expansion of the moving culture. A mathematical approach to describe macroscopic pattern formation in *P. mirabilis* was recently presented by Esipov and Shapiro and demonstrated that the expansion rate and periodicity can be explained based on internal population dynamics of age structure (26). During the differentiation process, swarming cells arise from swimming cells that have ceased septation but continue to grow and form long, multinucleated, hyperflagellated cells. The model of Esipov and Shapiro assumes that once formed, the swarm cells do not give rise to new swarm cells. Instead, they age and reach a septation stage at which they divide into swimmers which then in turn gives rise to the formation of new swarm cells. In *P. mirabilis*, these interconversion cycles occur in a synchronized fashion, and as a result, the colony either grows or expands. *Serratia* differs from *Proteus* with respect to the secretion of serrawettins that enables continuous spreading of the growing culture (54, 60, 62, 63, 82).

Population analysis. The following experiments provide novel information regarding community organization and population behavior in a swarming *S. liquefaciens* MG1 colony. Since the two major regulatory systems (Fig. 2) are organized separately, they can be disconnected in individual cells by means of mutations in key regulatory genes (*flhD* and *swrI*). A swarming culture composed of a mixture of green fluorescent protein (GFP)-tagged "green" *flhD* and "dark" *swrI* mutants (and vice versa) can be monitored in situ by combined phase-contrast and epifluorescence microscopy. This technique has shed some light on the function and significance of the swirling rafts in swarm colonies. The central part of the colony is densely populated with vegetative, nondifferentiated *flhD* cells (Fig. 4A and B). In addition, they are clearly present in the outer swirling layer that is dominated by differentiated *swrI* cells (Fig. 4A to C). Furthermore, the nonmotile *flhD* cells travel rapidly in the outer layer. We assume that the production of serrawettin creates a liquid interface layer in which the flow caused by the vigorous movement of the raft *swrI* cells distributes the *flhD* cells to the periphery of the expanding colony. Dividing cells are easily spotted among the transported *flhD* cells, demonstrating that growth and division are not restricted to the more-central parts of the expanding colony. We suggest that the behavior of the differentiated swarm cells serves two major purposes: it leads to the formation and spreading of a surface-conditioning film, and it circulates cells between the two specialized subcultures of swarmers and vegetative cells present at the border and the more-central parts of the colony, respectively. This in turn continuously creates new zones of growth and abolishes the formation of distinct consolidation and motility phases as seen with *P. mirabilis*.

Cell-cell signaling in the swarm. Similar experiments highlight the assignment of the AHL molecules as messengers between specialized subcultures. "Dark" *flhD* cells are mixed with *swrI* cells harboring a plasmid-borne AHL monitor system in which expression of the GFP is controlled by LuxR (7). Many bright green, differentiated *swrI* cells are seen in the swarm, indicating that AHL signals originating from the transported *flhD* cells are received by the *swrI* cells and transformed into gene expression (Fig. 4D). *E. coli* or *Pseudomonas putida* strains (non-AHL producers), harboring a *swrI*$^+$-containing plasmid can form swarming colonies in conjunction with the *S. liquefaciens* MG1 *swrI* mutant. Similarly, swarming colonies can form among *Pseudomonas aeruginosa* PAO1 cells (AHL producers) and the *swrI* mutant (Fig. 4E). The appearance of bright green *swrI* cells harboring the AHL monitor system is indicative of interspecies communication (Fig. 4F). Neither the *Pseudomonas* nor the *E. coli* strains produce serrawettin, and they are unable to differentiate into swarm cells in this particular setup. This indicates that AHL signals originating from the AHL producers trigger surfactant synthesis in the population of *swrI* cells. Thus, the organisms interact by means of chemical signals originating from the *Pseudomonas* cells. An additional level of community complexity arises from the interaction of surface, cells, and cellular exoproducts, which in turn drives the community members to self-organize into a functional community which expresses its complex phenotypic traits.

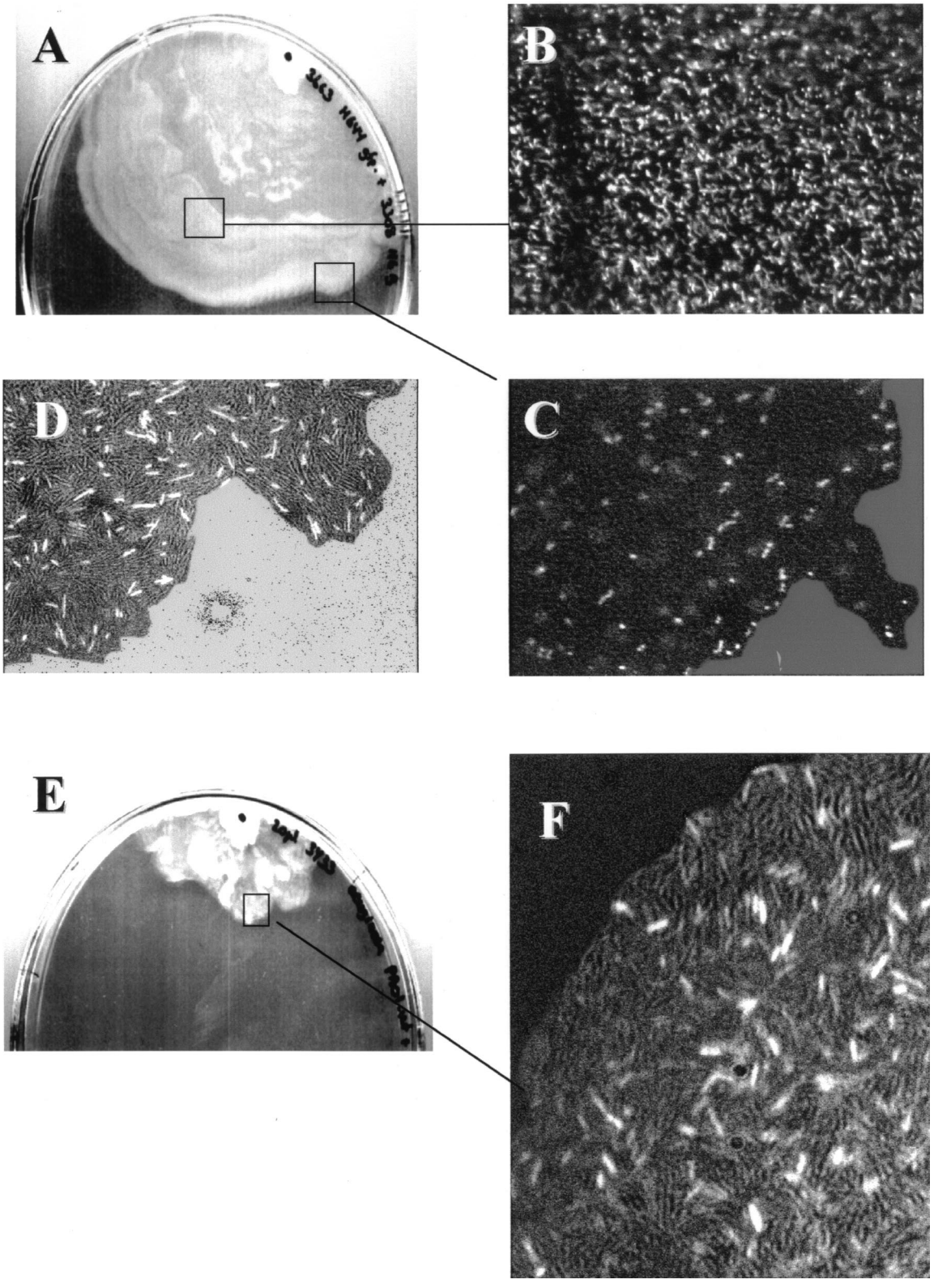

FIG. 4. Swarm colonies consisting of two strains. (A) Top view of a mixed culture of the *S. liquefaciens* MG1 *flhD* strain and the *swrI* mutant. The strains were applied at the dark spot in a 50:50 ratio. (B and C) Detection of GFP-tagged *flhD* cells by means of epifluorescence microscopy in the more-central part (B) and in the outer swirling layer of the colony (C). (D) Detection of *swrI* cells harboring a LuxR-based AHL monitor system (*PluxI-gfp* fusion) expressing GFP in response to the presence of extracellular AHL signal molecules by epifluorescence-light microscopy. (E) Top view of a mixed culture of *P. aeruginosa* PAO1 and *S. liquefaciens* MG1 *swrI* harboring the AHL monitor. (F) Microscopic inspection of the square in the outer part of the colony by epifluorescence-light microscopy.

Although the above-mentioned organisms might not meet under natural conditions, such random communities may display activities and functionalities indicative of coordinated performance. On the other hand, *Erwinia carotovora* regulates carbanapem synthesis by means of an OHHL-based quorum-sensing system (67) and are able to eliminate bacterial competitors such as *Serratia*. It does not form a swarming colony with the *swrI* mutant, indicating that in nature quorum-sensing systems can be employed to favor either collaboration or competition.

ECOLOGICAL RELEVANCE

Swarm cells and virulence. Many pathogenic members of the genera *Serratia*, *Proteus*, *Vibrio*, *Bacillus*, *Clostridium*, *Escherichia*, and *Salmonella* are able to swarm (2, 41). *Serratia* is a common cause of infections in insects and cold-blooded vertebrates (37). *S. marcescens* and *S. liquefaciens* are opportunistic human pathogens that cause respiratory and urinary tract infections (17). Does the ability of swarm cell differentiation in mucus or in urinary or respiratory tracts contribute to the pathogenicity of *Serratia* strains? For the uropathogen *Proteus*, swarming behavior is closely associated with modulation of virulence characteristics and the ability to invade human urothelial cells (2–5). For *V. parahaemolyticus*, differentiation into swarm cells plays an important role in adsorption and colonization of chitinaceous shells of crustaceans (9, 10). Taken together with our recent observation that differentiated *S. liquefaciens* MG1 cells are resistant to predation (6), it suggests that the ability of bacteria to differentiate into the swarm cell state is a general and ecologically important phenomenon not necessarily related to motility. Based on the experiments described above, it might be considered an important social phenomenon, since cultures of different species in certain conditions might be able to collaborate in the process of surface colonization.

Biofilm formation. Pratt and Kolter (76) suggested recently a dual role for flagellum-mediated motility in *E. coli* and *P. aeruginosa* biofilm formation in which flagella promote initial cell-to-surface contact and also contribute to the spreading of a growing biofilm along an abiotic surface. In the formation of *P. aeruginosa* biofilms, the buildup of microcolonies on the tightly packed monolayer is highly dependent on type IV pili-mediated twitching motility (73). For *P. aeruginosa*, it has been recently demonstrated that both the formation of biofilms and twitching motility are dependent on the quorum-sensing system operating in this organism (18, 36). These results argue in favor of functional overlaps between factors necessary for biofilm formation, bacterial pathogenesis, and attachment in vivo as suggested by Kolter and coworkers.

Eukaryotic defense systems. The ability of pathogenic bacteria to form biofilms within the human body is a major medical problem, since this growth mode substantially increases the resistance level of bacteria to antibiotics (15, 47, 51). Biofilm formation is also a major challenge for marine eukaryotes (52, 55). Bacteria can be highly detrimental to marine algae and other eukaryotes (55). Moreover, because bacteria are generally the first colonizers of submerged surfaces, the abundance and composition of the bacterial community on the surface will significantly affect the subsequent development of a macrofouling community (9, 46). To cope with this, eukaryotes have developed chemical defense mechanisms (19, 20, 93, 94), which in several cases are based on nontoxic secondary metabolites that specifically inhibit bacterial colonization-relevant phenotypes (50, 64, 85, 95). Such secondary metabolites (furanones) are produced by the marine alga *Delisea pulchra* (20, 31, 49). The effects of furanones on bacterial colonization phenotypes are due to interference with specific cell processes rather than to toxicity or general surface modification (49, 64). Several of the furanone compounds that exhibit structural similarity to the short-chain AHL molecules (Fig. 3) inhibit swarming motility of *S. liquefaciens* MG1 (31, 57). The *D. pulchra* compounds reduce the motility of the swarm cells by means other than influencing flagellar synthesis or growth rate (31). Our data strongly suggest that furanone compounds inhibit the communication system and reduce serrawettin W2 production (31, 35). The direct interaction with the AHL signaling systems has gained further strength by the displacement of labeled OHHL from LuxR by specific furanones (57). The furanones are likely competitive inhibitors of the AHL signal molecules competing for the same site of the receptor protein. In other words, furanone compounds enable growth and cell differentiation but disable the expansion. Defense systems based on nontoxic metabolites may have the advantage of allowing formation of discrete surface-bound bacterial colonies whose presence is beneficial to the eukaryotic host.

Newly discovered signals. Recently, a group of cyclic dipeptides were found to cross talk with quorum-sensing systems (12). Cyclo(ΔAla–L-Val) and cyclo(L-Pro–L-Tyr) were found to be produced by unrelated gram-negative bacteria including *P. aeruginosa*, *Enterobacter agglomerans*, *P. mirabilis*, and *Citrobacter freundii*. A third peptide, cyclo(L-Phe–L-Pro) was identified in *P. fluorescens* and *P. alcaligenes*. Cyclo(L-Pro–L-Met), cyclo(L-Pro–L-Val), and cyclo(L-Pro–L-Leu) were identified in *E. coli* (21). Some of the peptides were found to react with different AHL monitor strains such as *E. coli* harboring the bioluminescent LuxR-based system and *Chromobacterium violaceum*. Cyclo(L-Pro–L-Tyr) showed competitive (to BHL) inhibition of swarming motility in the *swrI* mutant, whereas cyclo(L-Pro–L-Met) stimulated swarming motility of the *swrI* mutant as efficiently as OHHL. Importantly, the observed responses were found to be specific to individual molecules, which indicates that cyclic peptides do cross talk with AHL-based quorum-sensing systems. Cyclo(L-Phe–L-Pro) and cyclo(L-Pro–L-Tyr) have been shown to act on the central nervous system (75). Based on the structural similarity between some cyclic dipeptides and for example the thyrotropin-releasing hormone, Chhabra et al. suggest the possibility that these cyclic dipeptides may influence interactions between bacterial pathogens and their hosts (12).

FUTURE PERSPECTIVES

Many interesting questions remain to be answered. For example, what is the underlying molecular mechanism by which the *Serratia* quorum-sensing system operates? A direct involvement of SwrR in quorum sensing, such as binding of BHL and other signal molecules, and its function as a transcriptional regulator remain to be elucidated. Serrawettin W2 is produced in vast amounts when *S. liquefaciens* MG1 is grown on plates but is hardly detectable in liquid-grown cultures (54). Is the attachment to a surface the primary signal for the expression of the biosurfactant? Is this signal then just boosted by the quorum-sensing system that would function as a signal amplifier? Alternatively, population densities that can be attained in liquid medium may be insufficient to trigger the quorum-sensing system, in contrast to the high cell density within a plate-grown colony. Is surfactant production differentially up-regulated in swarm cells? Investigation to address these questions will to a large degree depend on the possibility to analyze gene expression in single cells. A recently developed technique that allows detection of mRNA levels in single cells (90, 91) and GFP-

based reporter gene technology (Fig. 4) appear to be suitable tools for this purpose.

So far, only a few of the *S. liquefaciens* MG1 quorum-sensing target genes have been identified (23, 54), and work in progress aims at the identification of these genes. Some of these genes may encode potential virulence determinants whose expression is sensitive to furanone compounds and cyclic dipeptides. This may form a valuable model system in the process of gaining knowledge of the structure and function of bacterial signaling systems. The study of the interaction with cognate signals as well as other modulatory signals may help develop new strategies in the battle against infectious diseases.

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