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Dimorphic transition in *Escherichia coli* and *Salmonella typhimurium*: Surface-induced differentiation into hyperflagellate swarmer cells

(swarming motility/surface translocation/chemotaxis/flagella/multicellular behavior)

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ABSTRACT **We describe a new behavioral response in *Escherichia coli* and *Salmonella typhimurium* in which the bacteria differentiate into filamentous, multinucleate, hyperflagellate cells that navigate the surface of solid media by means of coordinated swarming motility. The cue for differentiation into swarmer cells is provided by the concentration and composition of the agar. Examination of the behavior of various mutants shows that the flagellar apparatus used for swimming motility and the chemotaxis system are indispensable for swarming motility.**

Escherichia coli and *Salmonella typhimurium* have remarkable metabolic and physiological versatility that facilitates their survival and growth under a wide range of environmental conditions. These bacteria are usually short rods (2–4 μm long) with peritrichous flagella that rotate to allow movement in liquid environments (see refs. 1 and 2). Information about attractants and repellents is conveyed to the flagellar motors through a signal transduction network, allowing chemotactic movement in response to external chemical stimuli. We have found that these two well-studied organisms are also capable of a hitherto unknown physiological response triggered by growth on certain agar surfaces. This response allows the bacteria to differentiate into motile swarmer cells that move as a coordinated group to effectively colonize the entire growth surface.

Swarming is a specialized form of surface translocation well known in *Proteus, Bacillus, Clostridium*, and *Vibrio* species (see ref. 3) and more recently in *Serratia marcescens* (4). Typically, cells inoculated at the center of an appropriate agar medium differentiate at the periphery of the colony into filamentous swarmer cells that are multinucleate and can have 50-fold more flagella per unit of cell surface. With the exception of *Vibrio* species (see ref. 5), swarming bacteria are generally peritrichously flagellated both as swarmer and swimmer cells.

Swarming is a coordinated multicellular activity during which swarmer cells maintain lengthwise contact with each other. Although individual swarmer cells move rapidly back and forth within the swarming bacterial mass, the colony as a whole migrates outwards. This manner of surface translocation is generally not observed in isolated cells. Different species vary in the macroscopic appearance of the swarm colony. *Proteus* species produce concentric zones because of repeated cycles of swarming and consolidation. Periods of consolidation define a phase of "dedifferentiation" in which the cells stop moving and divide into short cells (see ref. 6).

In *Vibrio parahaemolyticus* and *Se. marcescens*, differentiation into swarmer cells is also triggered by growth in viscous media (4, 7). In a number of swarming bacteria, mutants defective in chemotaxis have defects in swarming (8–10). It is not yet understood how information about the surface environment is sensed and processed to elicit differentiation into swarmer cells. Demonstration of swarming in *E. coli* and *S. typhimurium* should hasten research into understanding this signal transduction pathway.

MATERIALS AND METHODS

Bacterial Strains and Growth Conditions. Common strains were as follows: Wild-type *E. coli*, ATCC 25922; wild-type *S. typhimurium*, LT2; and *E. coli* C600 (*thr, leu, tonA, lacY, gal, supE*). The *fliC* mutant of *E. coli* was from M. Simon (California Institute of Technology, Pasadena). The *fljB* and *fliC* mutants of *S. typhimurium* LT2 were from K. Hughes [University of Washington, Seattle: TH714 (*fljB5001*::Mu*dJ*), TH1134 (*fliC5050*::Mu*dJ*, *hin-101, zfg-3516*::Tn*10*dTc), and TH2173 (*fliC1116 fljB5001*::Mu*dJ*)]. The *che* and *mot* mutants of *E. coli* (see Tables 2 and 3), derivatives of RP437, were from J. Parkinson [University of Utah, Salt Lake City; RP437 = *thr*(Am)*-1, leuB6, his-4, metF*(Am)*-159, eda-50, rpsL136, thi-1, lacY1, ara*-14, *xyl-5, mtl-1, tonA-31, tsx-78*]. Membrane transducer mutants (RP437 derivatives) were from M. Manson (Texas A&M University, College Station).

Luria–Bertani (LB) broth contained 10 g of tryptone, 5 g of yeast extract, and 10 g of NaCl per liter. Eighteen grams of Difco nutrient broth was used per liter. Glucose or other sugar supplements were added at 4 g/liter. Minimal medium contained 6 g of Na_2HPO_4, 3 g of KH_2PO_4, 0.5 g of NaCl, 1 g of NH_4Cl, 2 g of glucose, 0.014 g of $CaCl_2$, 0.24 g of $MgSO_4 \cdot 7H_2O$, and 0.01 g of thiamine per liter (pH 7.2). Minimal medium was supplemented with 2 g of Casamino acids per liter where indicated. Eiken agar was from Eiken Chemical (Tokyo). The final agar concentration was 0.5%. Different batches of media sometimes supported different levels of filamentation, flagellation, and rates of swarming.

Saizawa and Sugawara's Flagella Staining Procedure. A 5- to 10-μl drop of water was placed on a clean glass slide and tilted to wet a larger surface area. The drop was touched in several places with small amounts of bacteria on a toothpick and allowed to air-dry. The slide was flooded with solution I (100 ml of distilled water containing 5 g of tannic acid, 1.5 ml of 9% $FeCl_3$, 2 ml of formalin, and 1 ml of 1% NaOH; tannic acid was dissolved in water first, and the other ingredients were added in the order shown), allowed to stand for 20–30 min, and washed gently with distilled water. Solution II (2 g of silver nitrate in 100 ml of distilled water to which 5–6 drops of 10% aqueous ammonia solution were added until the

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Abbreviations: Dps, defective in progressive swarming; Dis, defective in swarming.

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precipitate formed just dissolved) was added for 10 min after which the slide was washed with distilled water. Lastly, the slide was flooded with a solution of Ziel's Carbol-fuchsin (Muto Pure Chemicals, Tokyo) for 10 min, washed with distilled water, and air dried.

Flagellar Filament Isolation. Cells were propagated in nutrient broth supplemented with glucose, either as liquid cultures or on plates (0.5% Eiken agar). Flagellar filaments were prepared by the method of DePamphilis and Adler (11) as described (4). Flagellin protein was solubilized and incubated with trypsin [1:1 (wt/wt) ratio of trypsin/flagellin] for 1 h for *E. coli* and for 2 h for *S. typhimurium* at 37°C as described (4).

RESULTS

Swarming in *E. coli* and *S. typhimurium*. In liquid media, *E. coli* and *S. typhimurium* move in alternating episodes of smooth swimming produced by counterclockwise flagellar rotation and of tumbling produced by clockwise (CW) rotation. Swimming motility can also be observed in semisolid agar (0.2–0.4% agar), where cells swim through water-filled channels in the agar. Higher agar concentrations inhibit swimming motility.

We discovered that agar obtained from a Japanese manufacturing company (Eiken) enabled both *E. coli* and *S. typhimurium* to carry out classical swarming motility atop the agar surface. As with other swarming bacteria, a few hours after inoculation of the center of an agar plate, streams of cells moved outward, colonizing the entire surface within the next few hours. Consolidation phases were generally not evident, although a layered macroscopic appearance was sometimes observed (Fig. 1 *A* and *B*). The swarming colony often had a glistening, mucoid appearance. Filamentous (5–20 μm long) cells were observed near the periphery of the colony (Fig. 1 *C* and *D*), while in the internal regions of the colony the cells were shorter. The long cells were multinucleate and generally devoid of the external constrictions that mark the presence of cell septa (Fig. 2). 4,6-Diamidino-2-phenylindole (DAPI)-stained nucleoids appeared relatively evenly distributed along the length of the cell filaments. The extent of filamentation depended on the composition of the medium, with the longest filaments (200–300 μm long) observed on solidified medium prepared with nutrient broth. Compared with broth-grown cells, swarmer cells showed increased flagellation (Fig. 3 *A* and *C*; 2–3 times as many filaments were counted per unit area of cell surface). Cells examined from central regions of the swarm colony had fewer flagella (Fig. 3*B*), more typical of swimmer cells.

The source of the agar and the composition of the medium had profound effects on swarming (Table 1). Eiken agar consistently evoked a strong swarming response on rich media with both organisms. For *E. coli*, the optimal agar concentration to elicit swarming was 0.5%, whereas for *S. typhimurium* (LT2), concentrations of 0.5%–0.8% worked well. The rate of movement of the swarming bacterial front varied widely, depending on the temperature, wetness of the surface (freshly poured plates were better), and batch of the medium, generally ranging between 2 to 10 μm/s at room temperature, although speeds as fast as 30–50 μm/s were sometimes observed with LT2. When compared at 24°C, 30°C, 37°C, and 42°C, swarming was optimum at 30°C for *E. coli*, being very much reduced at 37°C; *S. typhimurium* swarmed optimally at 37°C. Minimal medium did not generally support swarming, although the colonies often showed (see Fig. 4*A*) a "Dps" phenotype—i.e., under the microscope, active swarming movement was observed only within the inoculated area. Thus, the bacteria "swarmed in place." [The Dps phenotype cannot be distinguished by eye from a "Dis" (defective in swarming; ref. 10) phenotype (Fig. 4*B*), in which cells lack all movement, unless observed by microscope (see below).] On LB medium solidified with Difco agar, *E. coli* gave a very poor swarming response. This agar

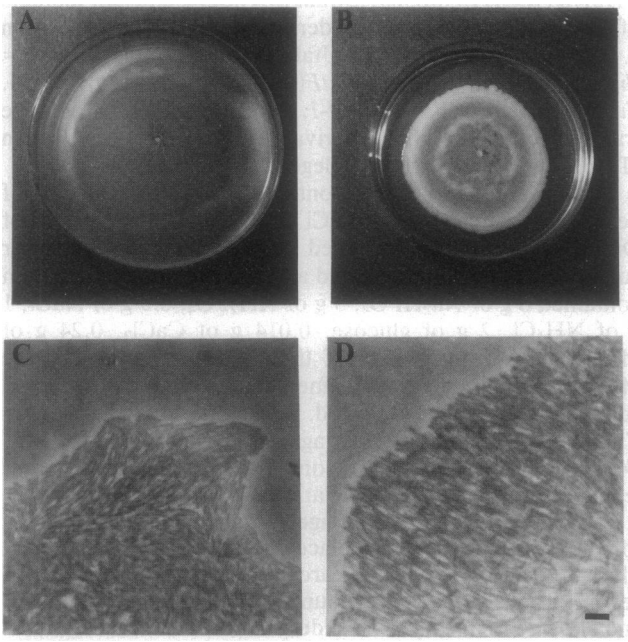

FIG. 1. Morphologies of swarm colonies. (*A*) An *E. coli* swarm colony propagated on nutrient broth medium solidified with 0.5% Eiken agar, photographed after overnight growth at 30°C. (*B*) A *S. typhimurium* swarm colony grown as in *A* except with 0.8% Difco agar, photographed after 8 hr at 37°C. (*C*) Advancing edge of an *E. coli* swarm colony growing on LB medium containing 0.5% Eiken agar, viewed with a ×40 long-working-distance objective (10). (*D*) Similar view of a *S. typhimurium* swarm colony growing on Difco nutrient broth containing glucose and 0.7% Difco agar. Note the parallel arrays of cells at the edges of both colonies in *C* and *D*. (Bar = 5 μm.)

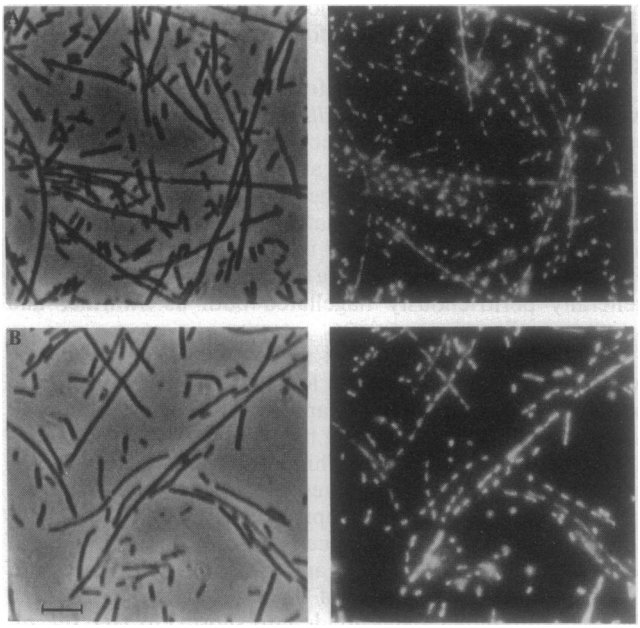

FIG. 2. Arrangement of nucleoids in elongated swarmer cells. (*Left*) Phase-contrast micrographs of *E. coli* (*A*) and *S. typhimurium* (*B*) swarmer cells propagated on Difco nutrient broth containing glucose and 0.5% Difco agar and stained with 4,6-diamidino-2-phenylindole (DAPI) as described earlier (10). (*Right*) DAPI fluorescence of the same cells under UV excitation. (Bar = 5 μm.)

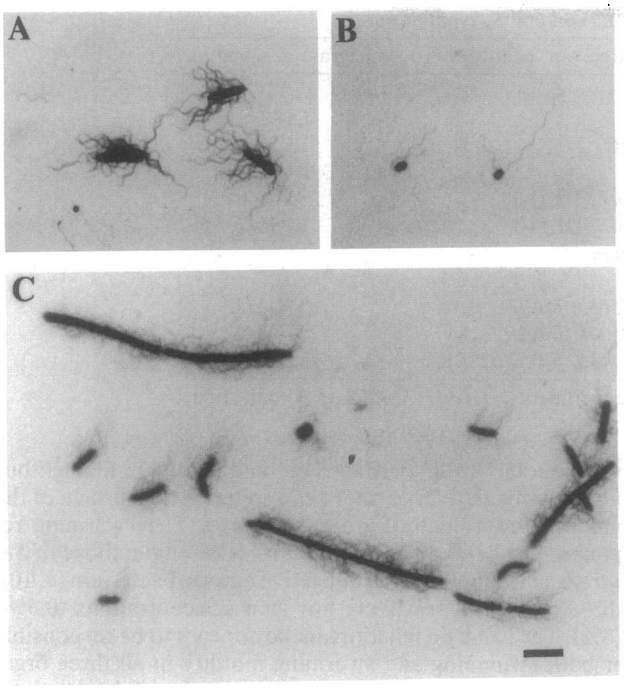

FIG. 3. Hyperflagellation of *E. coli* and *S. typhimurium* swarmer cells. Bacteria were grown on Eiken nutrient broth containing glucose and 0.5% Eiken agar. Flagella were visualized by silver staining (see text) followed by light microscopy. (*A*) Cells from the periphery of an *E. coli* swarm colony. (*B*) Cells from a central region of an *E. coli* swarm colony. (*C*) Cells from the edge of a *S. typhimurium* swarm colony. (Bar = 5 μm.)

supported vigorous swarming of *S. typhimurium*, however, provided 0.5% glucose was added. Other sugars that could substitute for glucose in this medium were mannose, mannitol, *N*-acetylglucosamine, fructose, galactose, and arabinose. Sugars that did not support swarming under these conditions were sucrose, maltose, ribose, and glycerol.

A number of the sugars that promoted swarming are transported by the phosphotransferase system (PTS; see ref. 12). Indeed, *S. typhimurium* mutants defective in the PTS enzymes were also defective in swarming. Enzyme II mutants were unresponsive to their specific sugars (glucose and mannose) but could swarm with other PTS sugars, while mutations in *ptsI* and the *ptsH* gene abolished the response to all PTS sugars (data not shown). In the absence of added sugars, *S. typhimurium* showed a Dps phenotype on LB Difco agar. In the presence of the nonmetabolizable glucose analog 2-deoxyglucose, which is also transported by the PTS,

Table 1. Effect of medium composition and agar sources on swarming

Medium	*E. coli*	*S. typhimurium*
LB		
+ Eiken agar	++++	++++
+ Difco agar	Dps	Dps
+ Difco agar/glucose	Dps	++++
Difco nutrient broth		
+ Difco agar	+	+
+ Difco agar/glucose	++	++++
Minimal salts		
+ Glucose/Eiken agar	−	−/+
+ Glucose/Eiken agar/CAA	−/Dps	Dps/+

Agar concentration was 0.5% throughout. ++++, Complete colonization (8 cm) of the Petri dish after overnight incubation at 30°C; ++, ≈4 cm; +, 1–2 cm; −, no movement. Dps denotes a colony that "swarms in place" (see text). A slash separates two observed phenotypes. CAA, Casamino acids.

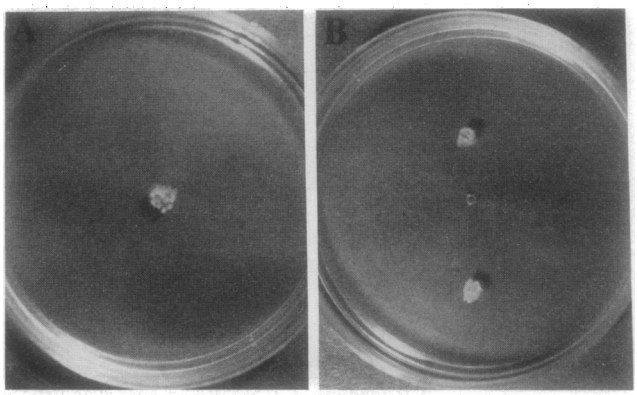

FIG. 4. Dps (defective in progressive swarming; ref. 10) and Dis (defective in swarming; see below) phenotypes of *E. coli* swarm colonies. (*A*) Wild type *E. coli* inoculated on minimal medium supplemented with Casamino acids (see text). Although the colony remains confined to the inoculated area, cells are seen to actively move when examined under the microscope. This phenotype is referred to as Dps. (*B*) Two *che* mutants (*cheA* and *cheY*) were inoculated on rich medium. Although indistinguishable from the Dps phenotype to the unaided eye, under the microscope these mutants truly do not move. This is the Dis phenotype.

only a Dps response was observed, suggesting that sugar metabolism is important for swarming.

The Motive Organelle for Swarming. To determine whether swarming requires the flagella that are used for swimming, we examined mutants lacking various components of the flagellar system. Mutations in the *E. coli* flagellar filament gene *fliC* or in the *motA* or *motB* gene (the *mot* genes are required for flagellar motor rotation) abolished swarming motility (Table 2). In *S. typhimurium*, either of the two flagellin genes [*fljB* (serotype *H2*) or *fliC* (serotype *H1*)] was sufficient for swarming, whereas the double mutant could not swarm. These results indicate that a rotating flagellum is essential for swarming but do not directly address whether the flagella induced on the swarmer cells are the same as those required for swimming. Therefore, we compared flagellin protein profiles of swimmer and swarmer cell flagella from both *E. coli* and *S. typhimurium* (Fig. 5). The molecular weight of the flagellin protein subunits isolated from both types of filaments is similar, although the protein amounts are different (compare lane 2 with lane 3 and lane 6 with lane 7), with more protein isolated from swarmer cells (increase in swarmer-cell flagellin varied between 2- to 6-fold in different experiments), consistent with their hyperflagellated phenotype. Further evidence of the similarity of these two filament protein subunits is provided by their similar trypsin digestion patterns (compare lanes 4 and 5 and lanes 8 and 9). Any significant differences in primary, secondary, or tertiary structure of the two proteins would have been manifest in such an analysis. These results strongly suggest that the swimmer and swarmer flagellar filaments are built from the same protein and that very likely the basal bodies to which these filaments are attached are also the same.

Importance of the Chemotaxis System in Swarming. To test whether the chemotactic signaling system plays a role in swarming motility, various *E. coli* chemotaxis mutants were examined (Table 3). Mutants lacking any one of the inner membrane chemoreceptors (Trg, Tsr, Tar, or Tap) swarmed normally. However, strains missing three or all four of these transducers showed no significant swarming movement under the microscope—i.e., showed a Dis phenotype.

E. coli carrying defects in the *cheA*, *-B*, *-R*, *-W*, *-Y*, or *-Z* genes also exhibited no swarming on rich media (Table 3). All of these mutants have aberrant flagellar rotation patterns that might be causally related to their swarming defect. However,

Table 2. Effect of flagellar mutations on swarming of *E. coli* and *S. typhimurium*

	Swarm phenotype	Relevant genotype	Strain designation
		E. coli	
C600 parent	++++		C600
C600Δ(*fliC*)$^-$	−	Δ*fliC*	LC2a
RP437 parent	+++		RP437
motA	−	(*motA*) DE12-14	RP6666
motB	−	(*motB*) DE14-1	RP6647
		S. typhimurium	
LT2 parent	++++		LT2
fliC(H1)$^-$	++++	*fliC5050*::Mu*dJ*	TH1134
fljB(H2)$^-$	+++	*fljB5001*::Mu*dJ*	TH714
fliC/*fljB*(H1, H2)$^-$	−	*fliC1116*, *fljB5001*::Mu*dJ*	TH2173

LB medium + 0.5% Eiken agar was used in these experiments. ++++, Complete colonization; +++, ≈6 cm; −, no movement.

a *cheR cheB* double mutant can rotate its flagellum in both directions, yet failed to swarm. The products of cheA and *cheW* genes detect the state of ligand occupancy of the membrane transducers and convey this information to the flagellar motor through a phospho-relay pathway that generates a tumble signal in the form of phosphorylated CheY (CheY-*P*; see ref. 2). The *cheZ* gene encodes a protein that regulates the levels of CheY-*P*. The products of *cheB* and *cheR* genes control the state of methylation of the cytoplasmic signaling domain of the membrane transducers, which mediates the sensory adaptation phase of the chemotactic responses. The inability of all of these mutants to swarm shows that the chemotactic signaling system is indispensable for swarming.

Most of the swarming mutants shown in Table 3 were capable of cell elongation, but none were hyperflagellate when examined under the optimal swarming conditions cited in Table 1. Thus, the chemotaxis pathway appears to be necessary for inducing hyperflagellation.

DISCUSSION

We have shown that *E. coli* and *S. typhimurium* have the capacity to differentiate into swarmer cells when propagated on agar of appropriate consistency. The differentiated swarmer cells are hyperflagellate, and many are filamentous and multinucleate. Swarmer cells colonize the surface of the agar as a coordinated, group response. The swarming responses of *E. coli* and *S. typhimurium* resemble those of *Se. marcescens*, another gram-negative enteric bacterium (4, 10). All take place at relatively low agar concentrations (0.5%–0.8%). The same flagellar organelle appears to be responsible for both swimming and swarming motility in all three organisms as judged from the similarity in the size and proteolytic fragmentation pattern of the flagellar filament proteins isolated from swimmer and swarmer cell flagella, as well as from the common effects of disrupting flagellar function on both types of motility.

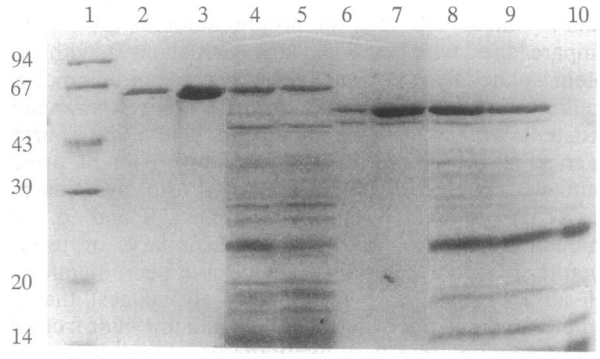

FIG. 5. Flagellin protein profiles of swimmer and swarmer cell flagella. Flagellar filaments were isolated and electrophoresed on a 12% polyacrylamide gel with or without trypsin digestion as described (see text). Molecular mass (kDa) of size markers (lane 1) is indicated on the left. Aliquots of flagellin protein isolated from an equivalent starting cell mass (as measured by optical density) of swimmer and swarmer cells of *E. coli* or *S. typhimurium* were electrophoresed on lanes 2 and 3, respectively, for *E. coli* and lanes 6 and 7, respectively, for *S. typhimurium*. For comparison of their trypsin digestion profiles, equivalent amounts of flagellin protein from swimmer and swarmer cells were digested with trypsin and electrophoresed on lanes 4 and 5, respectively, for *E. coli* and lanes 8 and 9, respectively, for *S. typhimurium*. Lane 10 is a control containing trypsin similar in amount to that used for flagellin digestion.

Swarmer cells displayed at least twice as many flagella per unit area of the cell surface as did swimmer cells. This number is smaller than that observed on swarmer cells of *Proteus mirabilis* or *V. parahaemolyticus*, which have 10–50 times more flagella than their swimmer cells. These species can swarm even on 2% agar, so the degree of hyperflagellation may set the limit for the highest concentration of the agar on which these bacteria can swarm.

The importance of the chemotaxis system in swarming has been shown in *V. parahaemolyticus*, *Se. marcescens*, and *P. mirabilis*, where the mutants defective in chemotaxis exhibit various swarming defects (8–10). The *che* mutants of *E. coli*, like those described for *Se. marcescens*, are completely blocked in surface translocation. These mutants do not hyperflagellate on swarm agar, suggesting that the primary block to swarming in these mutants is their inability to induce hyperflagellation.

Swarming is not dependent on the presence of any specific chemoreceptor, since the four distinct membrane transducers could be mutated individually (Tar, Tap, Trg, or Tsr) or together (Tar and Tap). However, deleting three (Tar, Tap, and Tsr) or all four of the transducers abolished swarming. The swarming defects could be due to an altered pattern of flagellar rotation (predominantly counterclockwise) found in these mutants. Alternatively, since Tar and Tsr are the abundant transducers in the cell, the swarming defects in these mutants might reflect a need for a certain threshold number of transducer molecules for swarming, rather than a requirement for any specific combination of transducers. Strains VB12 (*tsr*, *tar*, *tap*) and MM580 (*tsr*, *tar*, *tap*, *trg*) could be complemented for swarming by providing Tsr alone from a plasmid.

In *V. parahaemolyticus*, which has two different motility organelles, a constrained state of the polar flagellum found on swimmer cells has been implicated in generating the signal for induction of lateral flagella on swarmer cells (13). One model for achieving this invokes "chemotaxis in reverse," where additional viscous drag on the polar filament is proposed to slow the rotary motor. Conceivably, a slowed motor could promote altered interactions with critical components of the

Table 3. Effect of mutations in the chemotaxis pathway on swarming in *E. coli*

	Swarm phenotype	Relevant genotype	Strain designation
Parent	+++		RP437
trg	++	*trg*::Tn*10*	MM502
tsr	++	*tsr14 thr*$^+$	MM508
tar	+++	(*tar*) DE386-2	MM548
tap	+++	(*tap*) DE365-4	MM552
tar tap	+++	(*tar-tap*) DE5201, *ton*A$^+$, Tn*10*	MM509
tsr tar tap	− (Dis)	(*tsr*) DE7021, (*tar-tap*) DE5201, *thr*$^+$, *eda*$^+$	VB12
tsr tar tap trg	− (Dis)	(*tsr*) DE7021, (*tar-tap*) DE5201, *trg*::Tn*10*, *thr*$^+$, *eda*$^+$	MM580
cheA	− (*Dis*)	(*cheA*) DE1643	RP9535
cheB	− (Dis)	(*cheB*) DE63-216	RP4971
cheR	− (Dis)	(*cheR*) DE58-13	RP4968
cheW	− (Dis)	*cheW* (Am) 113	RP4606
cheY	− (Dis)	(*cheY*) DE60-21	RP5232
cheZ	− (Dis)	(*cheZ*) DE67-25	RP1616
cheB cheR	− (Dis)	(*tap cheR cheB*) DE2241	RP2867

Growth conditions were as in Table 2. Dis denotes a colony truly defective in swarming (see text). +++, ≈6 cm; ++, ≈4 cm.

phospho-relay pathway of chemotaxis and eventually to divert the signal to produce altered gene expression specific to swarm-cell differentiation.

The swarming response of *E. coli* and *S. typhimurium* also depends critically on the source of the agar (Eiken or Difco), although no significant differences between the two agars were apparent when analytical data on their physical and chemical characteristics (pH, water content, jelly strength, viscosity, gelation temperature, gel melting temperature, lucidity, ashes, SO_4^{2-}, Ca, and Fe) were examined. [We did note, however, a viscosity measurement of 8.6 centipoises (cP; 1 P = 0.1 Pa·sec) vs. 5.7 cP, SO_4^{2-} content of 2.2% vs. 0.92%, and Fe content of 21 ppm vs. 71 ppm for Difco vs. Eiken agar, respectively.] It is not immediately obvious to us why one source of agar (Eiken) supports swarming more extensively than the other (Difco). Interestingly, mutants of *Se. marcescens* that show a Dps phenotype on Difco agar (10) swarm normally on Eiken agar (unpublished data).

Addition of sugars to rich media stimulates swarming of *E. coli* and *S. typhimurium*. Swarming and gliding bacteria are known to produce an extracellular polysaccharide matrix or "slime," which is thought to aid their coordinated motility (see refs. 6 and 14). In addition, lipolysaccharides found on the outer membrane have been shown to be important in cell–cell interactions in the gliding motility of some bacteria. It is likely that the sugars are used to produce components such as these, which might be essential for bacterial swarming motility.

Although bacteria are generally viewed as unicellular, the bacterial colony shows a high degree of structural and biochemical differentiation (see refs. 15 and 16). The multicellular behavioral response of swarmer cells is only one example. Others include the multicellular response of myxobacteria to nutritional stress, the alternation between the dimorphic states of vegetative and dormant spore cells in *Bacillus* and *Streptomyces* species, and the production of stalked and swarmer cells in *Caulobacter* species (see ref. 17). All differentiated cell types perform specialized functions by coordinately expressing special genetic programs.

The multicellular swarming response, which allows the bacteria to forage for nutrients on solid surfaces, may also promote pathogenic associations. Swarmer cells of *P. mirabilis*, for example, coordinately express high levels of virulence proteins such as intracellular urease, extracellular hemolysin, and metalloprotease (see ref. 6). These cells proficiently invade human urothelial cells *in vitro*. This invasion is impaired in mutants defective in swarming. It would be of interest to determine if the swarming response affects pathogenicity of *E. coli* and *Salmonella* strains. Studies on characterization of the swarming response will increase our understanding of the pathogenesis of bacterial infectious diseases and perhaps shed light on a primordial form of intercellular communication. Discovery of the swarming phenomenon in such genetically well-characterized organisms as *E. coli* and *S. typhimurium* will facilitate these studies.

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1. Macnab, R. M. (1992) *Annu. Rev. Genet.* **26**, 131–158.
2. Parkinson, J. S. (1993) *Cell* **73**, 857–871.
3. Henrichsen, J. (1972) *Bacteriol. Rev.* **36**, 478–503.
4. Alberti, L. & Harshey, R. M. (1990) *J. Bacteriol.* **172**, 4322–4328.
5. McCarter, L. & Silverman, M. (1990) *Mol. Microbiol.* **4**, 1057–1062.
6. Allison, C. & Hughes, C. (1991) *Sci. Prog.* **75**, 403–422.
7. Belas, R., Simon, M. & Silverman, M. (1986) *J. Bacteriol.* **167**, 210–218.
8. Sar, N. L., McCarter, L., Simon, M. & Silverman, M. (1990) *J. Bacteriol.* **172**, 334–341.
9. Belas, R., Erskine, D. & Flaherty, D. (1991) *J. Bacteriol.* **173**, 6279–6288.
10. O'Rear, J., Alberti, L. & Harshey, R. M. (1992) *J. Bacteriol.* **174**, 6125–6137.
11. DePamphilis, M. L. & Adler, J. (1971) *J. Bacteriol.* **105**, 376–383.
12. Potsma, P. (1987) in *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, eds. Neidhardt, F. C., Ingraham, J. L., Low, K. B., Magasanik, B., Schaechter, M. & Umbarger, H. E. (Am. Soc. Microbiol., Washington, DC), Vol. 1, pp. 127–141.
13. McCarter, L., Hilmen, M. & Silverman, M. (1988) *Cell* **54**, 345–351.
14. Shimkets, L. J. (1990) *Microbiol. Rev.* **54**, 473–501.
15. Shapiro, J. A. (1988) *Sci. Am.* **256**, 82–89.
16. Matsuyama, T. & Matsushita, M. (1993) *Crit. Rev. Microbiol.* **19**, 117–135.
17. Kaiser, D. & Losick, R. (1993) *Cell* **73**, 873–885.