Trail Following by Gliding Bacteria

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Slime trails, which are deposited on surfaces by gliding bacteria and which serve as preferential pathways for gliding motility, were tested for the species specificity of their support of movement. Among the pairs of bacteria tested, a variety of gliding bacteria and a flagellated bacterium moved along trails of unrelated species. Thus, the trails did not serve as pheromones. Rather, they may have guided gliding elasticotactically. Some biological implications of this finding are considered.

Gliding motility is a form of surface-associated translocation characteristic of a variety of procaryotes comprising the orders *Myxobacterales* and *Cytophagales* and several genera of cyanobacteria. The nature and mechanism of gliding and related movements and the taxonomy of these bacteria have been recently reviewed (4, 14, 35).

It has commonly been observed that gliding bacteria leave behind them trails of slime or sheath material (9, 23, 31, 33) which appear phase bright on gels of an appropriate refractive index (e.g., 2.0 to 2.5% agar). In some species, this material has a fibrillar substructure (21, 42; R. Burchard and P. Loch, unpublished data).

This characteristic release of slime comprised of polysaccharides (44) which may have associated polymers (13, 18) must be energetically expensive for the glider. Thus, it is likely that this extracellular material serves one or more vital functions in the biology of these bacteria. Directed expulsion of slime might provide the driving force for gliding (reviewed in references 4, 14, and 46); however, there is no experimental evidence to support this hypothesis. The extracellular material possibly serves as a temporary adhesive, holding the glider to the substratum on which it is moving (18, 39). The unstressed adhesive slime may be sheared into plasticity by waves of compression moving anteriorly in the cell envelope, thereby permitting translocation (B. Hughes and R. Burchard, submitted for publication).

Slime trails deposited by pioneer cells serve as preferential gliding pathways for other cells of the same species. A follower cell, on encountering a preexisting trail at a relatively shallow angle, will move onto and glide on that trail more frequently than it will cross over it (7, 31, 32). Cells glide more rapidly on preexisting trails than on trail-free areas (7, 34; D. Pelto and R. Burchard, unpublished data).

These behaviors would be advantageous to the myxobacteria, which demonstrate communal behavior during their life cycle. Myxobacteria require a critical cell density to undergo morphogenesis culminating in fruiting body formation (19, 29, 47, 48). Formation of swarms of cells which demonstrate "wolf-pack" feeding behavior (36) would also be facilitated. Trails might also serve to bring cells together for putative conjugation. There have been reports of transfer of drug resistance traits between *Myxococcus* species (27, 45).

Implicit in these communal phenomena is species specificity. The development of such intercellular associations would be facilitated if slime trails were species specific, thereby functioning like pheromones. Pheromones are substances released by one individual which elicit a specific reaction in a receiving individual of the same species (20). The activators of luciferase released by *Photobacterium* strains (11) may be classified as pheromones. Similarly, promoters of aggregation of *Streptococcus* sp. (10) and the myxobacterium *Stigmatella aurantiaca* (41) are pheromones. This study was designed to determine whether trails function as pheromones.

(A preliminary report of this work appeared previously [3].)

MATERIALS AND METHODS

The gliding and nongliding bacteria and the growth conditions used are presented in Table 1.

The substrata for gliding were gels of agar (2.5%) or agarose (1.5%), generally made up with stream water. Trails on these gels appeared as bright lines when examined by phase-contrast microscopy.

The strain which was to provide the trails was inoculated onto the gel, which was incubated at a temperature appropriate for that organism. After the organism had commenced gliding and trails with sections free of cells were evident, one or more trails were localized under a 10× objective and inoculated with

TABLE 1. Bacterial strains

Strains	Medium	Incubation temp	Source and reference
<i>B. alba</i> B15LD	Thioglycolate (BBL Microbiology Systems) (1/10 normal concn)	Ambient	W. Strohl (43)
<i>Cytophaga</i> sp. strain U67	<i>Cytophaga</i> agar no. 62 (15)	Ambient	J. Henrichsen (15)
<i>Flexibacter</i> sp. strain FS-1	Yeast extract medium (38) (1/2 yeast extract)	30°C	D. White (38)
<i>F. columnaris</i> 1R43	Modified <i>Cytophaga</i> medium (28) (made up wih stream water)	Ambient or 30°C	J. Pate (25)
<i>M. xanthus</i> DK101 DK306 A2 5A ME RB5 NM SM	P-T medium (1)	30°C	D. Kaiser (17) D. Kaiser (17) I. Sutherland (44) I. Sutherland (44) I. Sutherland (44) My laboratory (2) My laboratory (1) My laboratory (1)
<i>O. animalis</i>	Medium D (6)	Ambient	University of Texas culture collection
<i>P. mirabilis</i>	Nutrient broth	30°C	My laboratory
<i>S. aurantiaca</i> DW4	Casein hydrolysate medium (29)	30°C	D. White (29)

the test strain via a fine glass needle or with a suspension of bacteria sprayed onto the surface with a perfume atomizer. The preparations were incubated at a temperature conducive to gliding (ambient or 30°C) and observed at intervals.

For some studies, the trail-forming bacteria were

killed with toluene vapors or UV light. This procedure ensured that the trail-formers did not backtrack and carry test bacteria with them.

To form microscopic grooves in the substratum, I scored gels with 0.5- and 3- μ m abrasive papers (3M Imperial PSA lapping films) in a manner analogous to

FIG. 1. *S. aurantiaca* gliding on trails of *M. xanthus* 1 h (A) and 8 h (B) after inoculation. Before the plate was inoculated with *S. aurantiaca*, it was irradiated with UV light to kill the *Myxococcus* trail formers. Arrow marks cell-free trails. Bar, 50 μ m.

FIG. 2. *M. xanthus* on *B. alba* trails at time of inoculation (A) and 4 h later (B). The field was shifted; arrows mark the same point. Bar, 50 μ m.

that described by Burton and Lee (5). Parallel score lines were observed by phase-contrast microscopy.

RESULTS

A number of heterologous pairs of gliding bacteria were examined for the ability of one to glide on trails of the other (Table 2). *Beggiatoa alba* and *Oscillatoria animalis* were frequently employed, since they deposited lengthy stretch-

es of cell-free trails, facilitating placement and examination of heterologous bacteria. It was evident that a variety of gliding bacteria could move on trails laid down by gliding bacteria of a different species. Preferential gliding on such heterologous trails was most readily demonstrated on agarose gels.

Myxococcus xanthus ME glided on trails produced by *M. xanthus* A2; the latter produces an extracellular polysaccharide which is chemically distinct from that of the former (44; it is presumed that the polysaccharide is a major if not the sole component of the slime trail). Similarly, *S. aurantiaca*, another myxobacterium, glided on *M. xanthus* trails (Fig. 1), and *M. xanthus* glided on *B. alba* trails (Fig. 2). Two nonmotile mutant strains of *M. xanthus*, NM and DK306, demonstrated no movement on or off trails, as anticipated.

Flexibacter sp. strain FS-1, which cannot glide on agarose gels unless they have been

TABLE 2. Gliding of test strains on trails of pioneer strains^a

Test strain	Gliding on trails of:			
	<i>B. alba</i>	<i>O. animalis</i>	<i>M. xanthus</i>	
			A2	DK101 RB5
<i>M. xanthus</i>				
DK101	+	+		+
5A			+	
ME			+	
SM	+			
NM	—			
DK306	—			
<i>S. aurantica</i> DW4	+			+
<i>Cytophaga</i> sp. strain U67	+	+		
<i>Flexibacter</i> sp. strain FS-1	+	+		+
<i>F. columnaris</i> 1R43	+	+		
<i>B. alba</i> B15LD	+	+		
<i>O. animalis</i>	+	+		

^a Test strain cells were inoculated onto trails formed by pioneer strain cells. +, Preferential gliding; —, no gliding; no symbols, not determined.

FIG. 3. *Flexibacter* sp. strain FS-1 (arrow) on *B. alba* trail on agarose. $\times 170$.

FIG. 4. *F. columnaris* 1R43 on *B. alba* trails on agarose. Bar, 50 μ m.

supplemented with the cold water-soluble fraction of agar or another plant or bacterial polysaccharide (J. Arlauskas and R. Burchard, in press), glided on trails deposited on agarose by *B. alba* (Fig. 3).

Flexibacter columnaris 1R43 was able to glide on agarose in the absence of trails but preferred to follow a trail once on one (Fig. 4). On agar gels, this organism appeared unable to discriminate between trailed and trail-free areas (Fig. 5).

Trichomes of the cyanobacterium *O. animalis* are typically straight or slightly curved; their gliding paths are of similar form. *B. alba* trichomes may glide in straight paths, curves, circles, or sine wave patterns. When an *Oscillatoria* trichome encountered and glided onto a *Beggiatoa* trail, its shape conformed to the trail (Fig. 6).

Swarming *Proteus mirabilis* cells tend to follow the slime tracks deposited by other swimmers (12). These flagellated bacteria were also guided by a trail deposited by a gliding bacterium (Fig. 7).

There have been reports that trails are also grooves etched into gels by gliding bacteria (22, 31, 33). To test the possibility that trail following may be an elasticotactic response (40) to such grooves, I looked for gliding on agarose gels etched with 0.5- or 3- μ m abrasive paper. *Flexibacter* sp. strain FS-1 and *Cytophaga* sp. strain U67, which did not glide on unsupplemented agarose gels (Arlauskas and Burchard, in press) but which did glide on trails (Table 2), did not glide on scored agarose. On agar, both organisms glided freely. They demonstrated elasticotaxis on agar scored with 3- μ m abrasive paper.

DISCUSSION

The observations reported here indicate that trails of gliding bacteria do not carry species-specific properties which restrict gliding by heterologous bacteria. Thus, they do not have the

properties of pheromones. Rather, the extracellular slime or sheath material which most likely forms the trails may alter the substratum in some fashion to guide and permit or facilitate gliding motility. The guidance may be attributed to the fibrillar substructure of the extracellular material, which might determine alignment of the gliding bacteria in a manner analogous to the elasticotaxis described initially by Stanier (40). It is striking that this postulated elasticotactic behavior results in the typically straight or slightly curvilinear trichomes of *O. animalis* assuming a very different form on a heterologous bacterial trail (Fig. 6).

In the case of *Flexibacter* sp. strain FS-1, the slime trail provides the actual substratum for gliding over agarose, which itself does not support gliding of this bacterium. Slime may have electrostatic or structural properties that are required by this *Flexibacter* strain and several cytophagae for the expression of gliding motility on a substratum (Arlauskas and Burchard, in press).

Indiscriminate trail following would be advantageous to gliding bacteria, such as the myxobacteria and species of *Saprospira* and *Herpetosiphon*, which prey on other gliding bacteria (8, 30, 37), since it would allow these predators to track prey cells. However, such behavior may

FIG. 5. *F. columnaris* 1R43, 30 min (A) and 130 min (B) after being inoculated onto *B. alba* trails on agar. Arrows mark the same point. $\times 130$.

FIG. 6. *O. animalis* gliding on a *B. alba* trail (wave pattern). The frame was shifted to the right (C; 13 min); stars mark the same point on an *Oscillatoria* trail. (A, B) *Oscillatoria* trichome (arrow) assumed wave shape of the trail (0 and 2 min, respectively), (C) straightened on the straight portion of the trail (13 min), (D, E) and moved back, reassuming the wave form (25 and 34 min, respectively). Other straight *Oscillatoria* trichomes moved onto the *Beggiatoa* trail from lower left (D and E). Bar, 5.0 μ m.

pose a problem to the myxobacteria during fruiting: heterologous trail following may result in the formation of fruiting bodies comprised of cells of more than one species. It has been assumed that fruiting bodies are species specific. Species specificity may, nonetheless, be ensured by aggregating pheromones, such as that described for *S. aurantiaca* (41), or by species-specific chemoattractants. Furthermore, the formation of *Myxococcus* fruiting bodies is stimulated by adenine compounds, whereas the formation of *Stigmatella* fruiting bodies responds to guanine derivatives (47). Production of inhibitors directed against other myxobacteria (24) may also result in single-species fruiting bodies.

Since bacterial gliding trails on agar may also be grooves etched by the gliding bacteria (22, 31, 34), such grooves might serve to elastictactically guide the gliding of heterologous bacteria (40). In the case of gliding bacteria (e.g., some cytophagae) which produce agarase, the grooves may be explained by localized hydrolysis of the substratum. Otherwise, such grooves might be

optical artifacts. When observed by phase-contrast microscopy, a trail appearing to be flush with the gel surface may be made to appear as a groove in the agar by lowering the substage

FIG. 7. *P. mirabilis* (arrow) on *B. alba* trail. $\times 170$.

condenser of the microscope. Oblique illumination, which has been reported to highlight trails as though they are grooves (31), might cause a ribbon of slime on an agar surface to appear either as a ridge or as a groove (16).

Simonsiella trails appear as depressions in the agar when examined by scanning electron microscopy; stereomicrographs support this observation (26). It is, however, possible to account for such grooves as artifacts of the preparative procedure for scanning electron microscopy. Slime may be highly hygroscopic, withdrawing water from the underlying agar. During critical point drying, differential dehydration of the hydrated slime and the partially dehydrated underlying agar may give rise to grooves.

That physically etched agarose did not support translocation of bacteria which were unable to glide on this substratum in the absence of preexistent trails suggests that grooves alone cannot explain trail following behavior. Furthermore, if trails were grooves, it would be difficult to explain why *F. columnaris* appeared to ignore such grooves on agar but to be guided by them on agarose (Fig. 4 and 5).

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