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Myxococcus xanthus Does Not Respond Chemotactically to Moderate Concentration Gradients

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Using a number of approaches we were unable to demonstrate a chemotactic response of *Myxococcus xanthus* to a variety of defined and complex materials. These data in addition to a number of prima facie arguments considerably reduce the likelihood that *M. xanthus* possesses a mechanism for chemotactic behavior.

It has been assumed for some time that the myxobacteria are capable of directed movement and that the basis of the directionality is a chemotactic one. This opinion has been expressed in numerous reviews (7, 14, 23), and in fact, one recent review on the myxobacteria (5) is included in the proceedings of a symposium on chemotaxis. The basis of this assumption rests on three lines of evidence: (i) analogy with the developmental behavior of the slime mold *Dictyostelium discoideum*, (ii) time-lapse photomicrographs showing directed movement of myxobacteria during aggregation and feeding (15), and (iii) various claims to have experimentally demonstrated chemotactic behavior (9, 12, 13, 16, 20, 25). First, the analogy between the eucaryotic slime molds and the procaryotic myxobacteria is superficial and, in this case, inappropriate. Second, while Kühlwein and Reichenbach's films (15) do demonstrate directed movement, those authors nowhere claimed that this indicated chemotactic behavior. Finally, all of the claims to have experimentally demonstrated chemotaxis are subject to alternative explanations. In most cases, they have instead demonstrated directed induction of fruiting bodies rather than chemotaxis per se; in some cases, elasticotactic behavior (27) has confused the results. In any case, there has been no direct demonstration that myxobacterial cells (or for that matter, any gliding bacteria) are able to direct their movement by sensing a chemical gradient.

In this paper we shall present experimental evidence which shows that, by a variety of approaches, it has not been possible to demonstrate a chemotactic response by *Myxococcus xanthus* to gradients that are effective in other systems, both procaryotic and eucaryotic. In addition, we shall present several arguments that chemotaxis is neither mechanistically appropriate nor developmentally or ecologically useful for *M. xanthus*. In an accompanying paper Dworkin demonstrates that directed movement by *M. xanthus* does indeed occur; however, it is not necessarily a response to chemical gradients (8).

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MATERIALS AND METHODS

Organisms. *Myxococcus xanthus* MD207, which is a fully motile (A^+ S^+) strain, was used as the wild-type strain. This strain was obtained from D. Kaiser in whose laboratory it is designated as DK1622. Strain MD2 (YS) was also used as a wild-type strain in some experiments. Strain MD14 is a nonmotile mutant. *Escherichia coli* was obtained from the culture collection of the Microbiology Department of the University of Minnesota.

Cultivation. All cultures were grown on CT medium (21) at 32°C on a rotary shaker. Culture turbidity was measured with a Klett-Summerson colorimeter with a no. 54 filter. One Klett unit is equal to 4.5×10^6 cells per ml.

Chemotaxis experiments. In view of the swarming nature of myxobacterial motility it was not possible or appropriate to use the movement of individual cells as a parameter of directed movement; instead, the orientation of flares or tongues of cells gliding out from a drop placed on an agar surface astride a concentration gradient was observed. Nondirected movement consisted of the even and symmetrical emergence of flares around the entire 360° of the circular drop (Fig. 1). Had we observed an asymmetric response we were prepared to quantitate this by determining the length and frequency distribution of flares as a function of their azimuth (18). Three approaches of increasing sophistication were used for establishing the cells in a concentration gradient. In the first, a crystal of the compound to be tested was simply placed on the agar; small drops of cells were placed in a spiral around the crystal. In the second, two 1-cm filter paper disks were placed atop each other on the surface of PM agar (10^{-2} M K_2HPO_4-KH_2PO_4 [pH 7.6], 8 mM $MgSO_4$, 1.5% Difco Noble agar). Microdrops of cells were placed in a spiral arrangement around the disks. The drops were 0.4 μl and contained 10^9 washed cells per ml. The drops also contained a suspension of dialyzed India

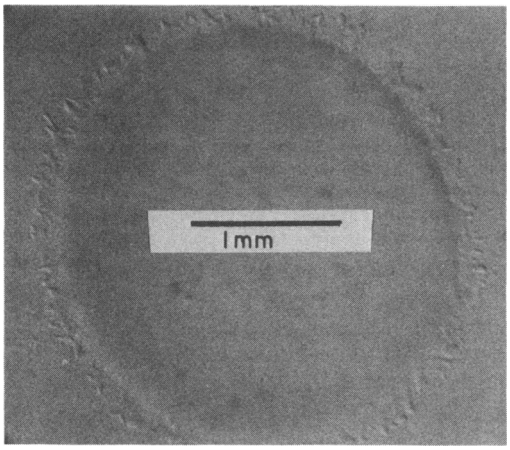

FIG. 1. *M. xanthus* MD207 on PM agar. The suspension contained 10^{10} cells per ml of distilled water.

ink so as to mark the original boundary of the drop. After 2 h, during which time the flares of cells had just begun to emerge from the drop, the top disk was replaced with a disk containing the test compound. The drops of cells were purposely made small so as to minimize the concentration difference between one side of the drop and the other. The third approach used a slide culture technique. While this technique did not generate a stable concentration gradient of the test material, it did permit the calculation of gradient properties. Furthermore, it allowed us to vary the slope of the concentration gradient and the absolute concentrations easily and conveniently. The details are described in the appendix. A glass slide with two glass shelves, one at either end of the slide, was constructed. The two shelves enclosed a volume of PM agar between them. Then one-half of the agar block was removed and replaced with molten PM agar containing the putative attractant. The attractant then diffused across the interface in essentially a one-dimensional fashion, establishing a predictable gradient. Since the slope of the concentration curve varies with time (i.e., as the attractant diffuses toward the end of the slide, the concentration gradient becomes less steep) and the absolute concentration is a function of the distance from the interface, it was possible to vary both of these factors when testing the response of the cells to any particular compound. Thus, 5 drops of cells were evenly spaced along a diagonal line from one end of the gradient interface to the opposite corner of the chamber. The slope was varied by applying these drops to replicate slides at 30 and 180 min after the attractant was supplied.

Preparation of cells. Generally the suspensions for the chemotactic experiments were made by centrifuging and washing cells taken from the exponential phase of growth in liquid CT medium. They were then resuspended in distilled water at 1×10^9 to 4×10^9 cells per ml. In some cases, attempts were made to precondition or sensitize cells so that they presumably would be maximally chemotactic. Cells were placed on CF agar (10) and incubated for 8 to 10 h. Under these conditions the cells will eventually form fruiting bodies; by 8 to 10 h, they had not yet begun to aggregate. These cells were then washed off the plate, diluted to 10^9/ml, and used as the test organism in the slide culture technique. These cells will be referred to as "pregrown on CF." Alternatively, cells preincubated on CF agar were placed on the PM agar in the slide culture and allowed to incubate an additional 12 h. By this time they were just beginning to aggregate, and the chemoattractant was then added to the agar. These cells will be referred to as "aggregating cells."

RESULTS

Using a variety of approaches, we were unable to demonstrate a chemotactic response to any of the defined compounds or complex mixtures that we tested. The materials tested and the conditions under which they were tested are listed in Table 1. The rationales for choosing those materials are as follows.

(i) Cyclic AMP. Cyclic AMP is the chemoattractant for *D. discoideum* (1). It has also been shown to be one of a number of adenine nucleotides that induce fruiting body formation in *M. xanthus* (4), and its intracellular concentration increases during development (30).

(ii) Cyclic GMP. Ho and McCurdy (12) reported that *M. xanthus* responds chemotactically to cyclic GMP. Using the identical conditions as Ho and McCurdy but with a different strain, we have been unable to confirm their observation (unpublished data).

(iii) 5′-AMP. It has been reported (12, 25) that *M. xanthus* was chemotactic toward 5′-AMP. Using the stable gradient device (25) as well as the gradient maker of Ho and McCurdy (12), we have been unable to confirm these results.

(iv) Adenosine. It has been reported that adenosine induced fruiting body formation in low-density populations of *M. xanthus* (24).

(v) Folic acid. Folic acid has been reported to be a chemoattractant for *D. discoideum* (22).

(vi) Methionine, asparagine, leucine, and isoleucine. These amino acids have been shown to be required for the growth of *M. xanthus* (3).

(vii) D-Alanine–*N*-acetylglucosamine–diaminopimelic acid. Shimkets and Kaiser (26) have shown that a mixture of these compounds induces rippling behavior in *M. xanthus*.

(viii) *N*-Formyl-L-methionyl-L-leucyl-L-phenylalanine. This tripeptide has been shown to be a potent chemoattractant for human polymorphonuclear leukocytes (29).

(ix) Casitone. Casitone is a mixture of peptides and amino acids that serves as an excellent growth medium for *M. xanthus* (6).

(x) *E. coli* cells. *M. xanthus* will lyse and feed on cells of *E. coli*.

(xi) Various intact cells of *M. xanthus* and extracts of these cells. This is to test the possibility that *M. xanthus* produces and excretes an attractant during developmental aggregation. MD14 is a nonmotile mutant. Its inability to

TABLE 1. Compounds and conditions tested for chemotaxis

Material tested	Concn or amt[a]	Testing method[b]	Pretreatment of cells[c]
Cyclic AMP	10^{-7}–10^{-3} M	GS	E, CF, Ag
	0.01, 0.1 μmol	D	E
		C	E
Cyclic GMP	10^{-6}–10^{-3} M	GS	E
	0.01, 0.1 μmol	D	E
		C	E
5′-AMP	10^{-6}–10^{-2} M	GS	E, CF, Ag
	0.01, 0.1 μmol	D	E
		C	E
Adenosine	10^{-7}–10^{-3} M	GS	E, CF, Ag
Folic acid	3.6×10^{-6} M	GS	E
L-Methionine	0.01, 0.1 μmol	D	E
L-Asparagine	0.01, 0.1 μmol	D	E
L-Leucine	0.01, 0.1 μmol	D	E
L-Isoleucine	0.01, 0.1 μmol	D	E
Mixture of D-alanine, *N*-acetylglucosamine, and *meso*-diaminopimelic acid	20, 20, and 14 μmol, respectively	D	E
N-Formyl-L-methionyl-L-leucyl-L-phenylalanine	10^{-12}–10^{-4} M	GS	E
Casitone (Difco)	1, 5%	GS	E
	3.2, 16 mg	D	E
Lysate of vegetative cells of *M. xanthus* MD207[d]		D	E
Lysate of developing cells of *M. xanthus* MD207[e]		D	E
Intact cells of *E. coli*		—[f]	E
Aggregating cells of *M. xanthus* MD207		—[g]	E
Vegetative cells of *M. xanthus* MD14		—[f]	E

[a] The sequence of concentration increases was 5×, 2×, etc. When the disk method (D) was used, the numbers refer to the amounts applied to the disk.

[b] C, Crystal deposition; D, disk method; GS, gradient slide.

[c] E, Cells from exponential growth phase in liquid CT medium; CF, cells pregrown on CF; Ag, aggregating cells.

[d] *M. xanthus* MD2 was grown in liquid CT medium. Exponential phase cells were washed, resuspended at 7×10^9 cells per ml, and broken by sonic oscillation. The suspension was centrifuged at 10,000 × *g* for 10 min and then filtered through a 0.45-μm pore size Millipore filter.

[e] *M. xanthus* MD2 was plated on CF agar at 5×10^7 cells per cm^2. After 3 to 4 days at 32°C, cells were harvested in PM buffer, washed, and broken by sonic oscillation. The suspension was centrifuged at 10,000 × *g* for 10 min and then filtered through a 0.45-μm pore size Millipore filter.

[f] A 40-μl drop of a suspension of *E. coli* or of the nonmotile mutant of *M. xanthus* (MD14) containing 4×10^8 cells per ml was placed directly on PM agar. Drops (0.4 μl) of *M. xanthus* MD207 were distributed as with the disk method.

[g] *M. xanthus* MD207 (5×10^7 cells per cm^2) was placed on a cellophane disk (1 cm in diameter) and incubated at 32°C on a CF agar plate for 21 h, at which time aggregates have just begun to form. The disks were then removed and placed on PM agar plates. Spots of suspensions of *M. xanthus* MD207 were then distributed as with the disk method.

move prevents it from interfering with the test organism.

DISCUSSION

We have been unable to demonstrate that *M. xanthus* can respond chemotactically to concentration gradients. We have tested amino acids that are required for growth, undefined mixtures of peptides that support rapid growth, various nucleotides and other compounds that have been implicated as regulatory molecules in the metabolism or development of *M. xanthus*, molecules that have been shown to act as chemoattractants in other systems, and various intact cells or extracts of cells. We have tested these materials under a variety of conditions, and the results have been uniformly negative. It is, of course, possible that we have missed the right compound or have not found the proper conditions; however, it is unlikely that we would have seen no evidence of a response at all.

It is possible to rationalize the unexpected failure to demonstrate a chemotactic response ex post facto from mechanistic, developmental, and ecological points of view. When Macnab and Koshland (17) addressed the question of the mechanism of gradient perception by bacteria, they rejected prima facie the notion of an instantaneous spatial sensing mechanism. They were able to demonstrate that the cells used instead a temporal sensing mechanism. Conversely, in *M. xanthus*, we can reject, prima facie, the temporal sensing mechanism as highly unlikely. *M. xanthus* moves, by gliding, at about 1/3,000 the speed of *E. coli*. This has two consequences. (i) The cell is moving extremely slowly in comparison to the net flow of the molecules by diffusion. Thus, depending on the shape and size of the source, in a time-varying gradient, even though the cell is moving away from the source of the molecules, the concentration of the molecular attractant is likely to be increasing at each point sufficiently rapidly that the cell always senses an increasing concentration. While it is true that the rate of increase in concentration will not be as great as if the cell were moving toward the source, that would be asking the cell to make an extremely subtle computation. (ii) The cell would have to have an extremely long memory to make the kind of temporally based comparison characteristic of swimming bacteria.

In the title of this paper we use the term "moderate concentration gradients." This is, of course, a relative judgment, and it is based on comparisons with gradients to which other microbial systems can respond. Macnab and Koshland (17) have estimated that the gradient that exists between the two ends of *E. coli* that can be perceived chemotactically is 1 part in 10^4 or a 0.01% gradient. Using the gradient slide described in this paper, cells put down at 30 min and close to the interface see a gradient that is 25 times steeper, about 1 in 400 or 0.25%. Leukocytes respond quite well to a gradient of about 1 part in 50 or 2% (N. J. Scott, M.S. thesis, University of Minnesota, Minneapolis, 1982), and *D. discoideum* measures a gradient of about 1 part in 100 (19). Both of these organisms use a spatial rather than a temporal sensing mechanism (19, 31) and thus require a steeper gradient. The possibility must therefore be left open that *M. xanthus* also uses a spatial sensing mechanism; to respond chemotactically, it must see a concentration gradient far steeper than is the case for other procaryotes which use a temporal sensing mechanism, and about 5 to 10 times steeper than we have been able to generate experimentally.

In terms of absolute concentrations, when a range of concentrations of 10^{-6} to 10^{-2} M is used, as in the case of 5'-AMP, this can be compared with a range of 10^{-6} to 10^{-4} M maltose that can be chemotactically perceived by *E. coli* (11) and 10^{-9} to 10^{-4} M for the leukotactic peptides perceived by neutrophils (28).

The two possible functions of chemotaxis in *M. xanthus* are for developmental aggregation and feeding. Yet chemotaxis does not seem to be a useful mechanism for *M. xanthus* from either of these points of view. In the case of the slime mold *D. discoideum*, the myxamoebae before aggregation are well separated from each other; i.e., under conditions that are optimal for development, the amoebae occupy only about 1% of the surface area (2). Aggregation thus requires a mechanism with which to call in cells that are widely dispersed and relatively distant from each other. In the case of *M. xanthus*, optimal development requires that the cells literally be atop one another (24). Thus there is no developmental need for the cells to communicate with each other over long distances via diffusible, chemotactic messages. It is rather more likely that the directed and cooperative behavior of the cells is mediated by cell-cell contact interactions. From an ecological point of view, the myxobacteria feed on insoluble macromolecular debris and on living or dead cells. Thus, their need is to perceive objects, not concentration gradients of soluble nutrients. Nevertheless, there may be some value in perceiving the presence of soluble nutrient. For example, if the low-molecular-weight products of the extracellular hydrolysis of a food source diffuse back to the cell, it would be useful if the cell did not then move off in the opposite direction. There is already evidence (unpublished data) that the rate of motility in *M. xanthus* is responsive to nutrient concentration. Thus, a higher concentration of nutrient tends to inhibit motility and to trap

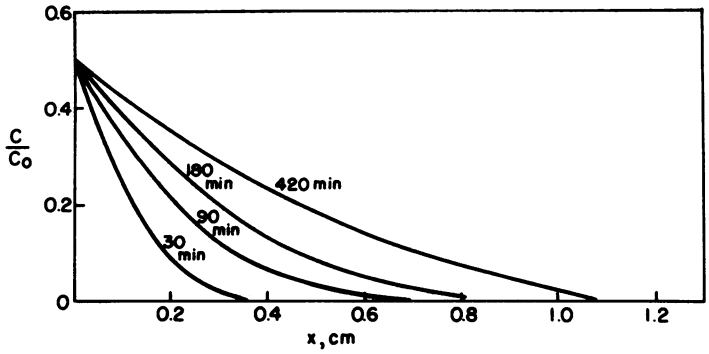

FIG. 3. Calculated diffusion profiles at various times. The concentration of a molecule with a molecular weight of 300 and a diffusion coefficient of 37°C of 6×10^{-6} cm²/s is plotted as a function of distance, x, from the interface. The various curves illustrate the concentration profiles at various times after the gradient has been initially established.

the cells in areas of high nutrient concentration. In other words, chemokinesis may play a role in the directed movement of *M. xanthus*.

To summarize, there is a theoretical basis for suspecting that known mechanisms of chemotaxis may be inappropriate for the myxobacteria due to their slow rate of movement. Furthermore, we have made prima facie arguments that chemotaxis does not seem to be necessary for developmental aggregation or to be useful for feeding. And lastly, we have been unable to demonstrate chemotaxis to moderate gradients of a variety of defined and complex materials, using a number of experimental approaches.

APPENDIX

This appendix describes the slide culture used to generate unstable but predictable concentration gradients (Fig. 2). It also presents calculations and a graph describing the resultant concentration profiles as a function of time (Fig. 3).

The slide culture technique and the associated calculation of the diffusion profiles were generated by N. J. Scott (M.S. thesis, University of Minnesota, Minneapolis, 1982).

For the system shown, if the agar on the right side is initially free of attractant and the agar on the left side initially contains the attractant at a concentration C_0, the equation expressing the concentration in the right half as a function of time and position is the following:

$$\frac{C}{C_0} = \frac{1}{2}\left(1 - \text{erf}\,\frac{x}{2\sqrt{Dt}}\right)$$

where erf $x/2\sqrt{Dt}$ is the error function, defined as

$$\text{erf}\,\frac{x}{2\sqrt{Dt}} = \frac{2}{\sqrt{\pi}}\int_0^{\frac{x}{2\sqrt{Dt}}} e^{-y^2}\,dy$$

The variables in these equations are defined as follows: x = distance from interface; t = time from start of experiment; C_0 = initial concentration of attractant; C = concentration of attractant at time t and distance x, i.e., C = f(x, t); D = diffusion coefficient of attractant. For a small molecule, e.g., one with a molecular weight of approximately 300, at 37°C, $D \simeq 6 \times 10^{-6}$ cm²/s. For such a molecule, the concentration profiles predicted by the above equation are plotted in Fig. 3. Note that $C/C_0 = 1/2$ for all times at the interface.

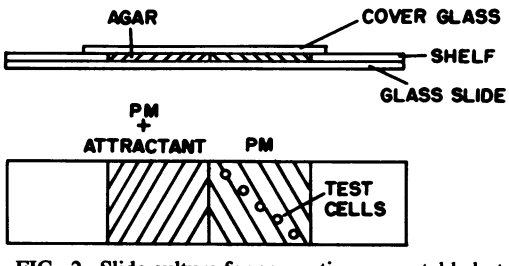

FIG. 2. Slide culture for generating an unstable but predictable gradient. The construction of the slide culture is described in the text.

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We thank Kenneth H. Keller for advice on the analysis of concentration gradients. Randy Conley performed some of the chemotaxis experiments.

ADDENDUM IN PROOF

Berg and Purcell (Biophys. J. 20:193–219, 1977) have calculated that a relatively small cell could still use a true spatial sensing mechanism to perceive a gradient if it time-averaged the results. In such a case, the cell would average the concentrations at either end over a period of time and then compare the average values from the two ends. Berg has calculated that this would be theoretically feasible for a cell with the properties of *M. xanthus* (personal communication). Thus, according to this reasoning, the concentration gradients we have been able to generate should have been steep enough for the cells to perceive them.

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