Negative Chemotaxis of Marine Bacteria to Toxic Chemicals

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The positive chemotactic responses of motile marine bacteria were reversed by the addition of sublethal concentrations of a toxic chemical. The negative chemotactic effect was observed with a wide range of toxicants including heavy metals and hydrocarbons. This phenomenon may be utilized to develop new approaches for control of marine fouling.

A tactic response is the movement of an organism toward or away from a source of stimulation. When the stimulating source is a chemical, it is referred to as chemotaxis. Chemotactic behavior was first observed in bacteria almost 90 years ago (9). More recently, aspects of the mechanism of positive chemoreception have been elucidated by Adler (1). He found that Escherichia coli cannot metabolize all compounds to which it is attracted. Subsequently, it has been shown that the galactose-binding protein required for galactose transport across the membrane also functions as the galactose chemoreceptor (6). A mutation in the binding protein rendered the organism inactive toward galactose. Ecological aspects of positive bacterial chemoreception were studied by Chet et al. (2). They concluded that isolated predatory bacteria which degrade Pythium or Skeletonema are specifically chemotactic toward compounds excreted by the respective organism.

Although most of these investigations have dealt with the positive taxis of bacteria toward a variety of compounds, Pfeffer (9) described the observation of negative taxis. Smith and Doetsch (10) Doetsch and Seymour (5) have demonstrated that Pseudomonas fluorescens displays a distinct negative chemotaxis toward gradients of increasing hydrogen ion concentration.

This report describes the pattern and extent of negative chemotactic behavior in four motile marine bacteria. Sublethal concentrations of toxic organic and inorganic compounds were used as the stimuli. The application of this phenomenon to control of bacterial populations on surfaces in natural waters is discussed.

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MATERIALS AND METHODS

Organisms. Four marine organisms, R3, P2, G4, and MAV, were used in this investigation. All four organisms are identified as pseudomonads and are heterotrophic, facultatively anaerobic, motile, and obligately marine. Their observed differences include cellular and colony morphology, pigmentation, luminescence, and positive and negative chemotaxis patterns.

Culture conditions. All cultures were maintained on either seawater-nutrient agar or nutrient broth prepared with 20% seawater and 80% NaCl solution (2.5%).

Negative chemotaxis assay. Bacteria were grown for 10 to 12 h in liquid medium and harvested by centrifugation. They were washed in artificial seawater, and a working suspension of $10^8$ cells per ml was prepared. The design of the experiment was basically modeled after the method of Chet et al. (2), except that we used a 5-µliter microcapillary to hold the test substances. Test materials were suspended in artificial seawater (7)-nutrient broth. The concentration of toxicants ranged from 0.001 to 5% (wt/vol) for the organic hydrocarbons and 0.0001 to 0.5% (wt/vol) for the inorganic compounds. A random movement control consisted of artificial seawater in the microcapillary, while a positive activity control consisted of artificial seawater-nutrient broth in the microcapillary. The viability of the organisms over the range of concentrations used was determined by incubating a known concentration of cells in each concentration of chemical for 10 min. Numbers were determined by serial dilution in artificial seawater and plating on seawater-nutrient agar. Viability curves were substantiated in duplicate, and the chemotaxis data at least four times. The relative degree of negative chemotaxis was averaged from the four determinations.

RESULTS

Microscopy of bacterial behavior in response to chemical stimuli indicated that, under the influence of a positive stimulus, such as nutrient broth in the capillary, bacteria in the bulk
solution, i.e., artificial seawater, concentrate in and around the capillary mouth. With time, a large population of bacterial cells concentrates inside the capillary. However, when a toxic chemical is combined with the nutrient broth, bacteria do not enter the capillary and avoid the area immediately adjacent to the capillary mouth. A distinct region free of bacterial cells was clearly observed. The size of the bacteria-free region varies with the organisms and is proportional to the concentration of toxic compound used. Under relatively low concentrations of negative stimuli, there is no region, or a very slight one, free of bacteria at the capillary mouth, but the volume inside is clearly free of the cells. The blank controls, with artificial seawater only, were observed to have a similar random bacterial distribution and uniform activity, within the mouth of the capillary, in the outside area adjacent to it, and in the bulk solution. Therefore, upon microscopy, the reaction of the bacterial population to negative stimuli was observed to be markedly different from that to no stimulus.

These observations indicate that bacteria can actively avoid unfavorable areas. However, it was necessary to establish that the low number of bacteria entering the capillary is a direct result of bacterial activity and is not due to a toxicity effect. The negative tactic response can be of ecological significance only if it comes from a physiologically viable cell. Therefore, it is particularly interesting that, in most of the cases tested, negative chemotaxis occurred at nonlethal concentrations of toxic chemicals.

The effect of chloroform on G4 isolates is illustrated graphically in Fig. 1. The lower curve indicates the number of bacteria entering the capillary as a function of toxicant concentration. When there was no toxicant present and only artificial seawater-nutrient broth was placed in the capillary, 10⁴ bacterial cells ingressed. As the concentration of the toxicant increased, the bacterial numbers sharply decreased by nearly 2 orders of magnitude. The top curve illustrates the viability of the organisms over the concentration range tested. The highest concentrations used were lethal as indicated by the drop in the curve. However, over the concentration range in which negative chemotaxis was found to occur, viability was not affected. Under these nonlethal conditions, approximately 97% of the bacterial cells which otherwise would enter the capillary were prevented from doing so.

Figure 2 illustrates a corresponding set of data for a different bacterial isolate, MAV, and a different toxicant, ethanol. Again the top curve indicates that there was no lethal effect over the concentration range in which negative chemotaxis was observed. In this case, the threshold concentration which triggered the negative tactic response is clearly indicated on the bottom curve. The inclusion of 10⁻¹ M ethanol along with the nutrient broth in the
capillary again inhibited a significant fraction of bacteria, which normally would do so, from entering the capillary.

A total of four hydrocarbon compounds, chloroform, ethanol, benzene, and toluene, and two heavy metal compounds, lead and copper, were used as toxicants. The results of their effect on bacterial chemotaxis are summarized in Table 1. The last column of the table shows that, under sublethal conditions, more than 90% of the cells were inhibited from entering the capillary. In all but one case, more than 70% of the bacteria which otherwise would enter the favorable zone in the capillary were prevented from doing so. In terms of the overall effectiveness of the toxic chemicals, no consistent pattern appears to emerge.

Of the bacteria tested, G4, MAV, and P2 in general displayed similar responses to the toxic chemicals (Table 1). However, R3 was repelled to a lesser degree than the other bacteria. Only 70 to 80% were inhibited from entering the capillary by any of the chemicals tested, although the concentrations were similar. These data suggest that R3 was more sensitive than the other isolates to sublethal effects of the toxicants on bacterial motility.

### DISCUSSION

The chemotactic behavior of bacteria has been categorized as phobotaxis (7), whereby a stimulus triggers the bacterium to change its direction of movement in a random manner. The new direction bears no special relationship to the direction of stimulation. This “Schreckbewingung,” or shock-reaction response, results in a net movement toward a positive stimulus whereby an organism displays many changes in direction when an unfavorable, but not when a favorable, area is entered (7). Therefore, a favorable region retains and accumulates those bacteria which have entered. The same mode of behavior can describe a negative taxis whereby many changes of direction will occur until the organism reaches a nontoxic area, resulting in a net movement away from the negative stimulus. Theoretical interpretations of the means by which a stimulus is translated into locomotion have been discussed by Clayton (3) and Doetsch (4).

Previous investigations have indicated that positive chemotactic responses are inhibited by hydrocarbons without immobilization of the bacteria (2, 8). The results presented here demonstrate that isolated marine bacteria also have the capability of detecting and removing themselves from areas containing toxic compounds. The two statements may appear to be contradictory without clarification of the differences in procedure. Inhibition of positive chemotactic responses (2, 8) was demonstrated when the bacterial population was suspended in a nonlethal concentration of inhibitor. No net movement was directed toward the positive stimulant. Negative chemotaxis toward toxic compounds was observed with the bacteria suspended in a blank solution. Net movement was directed away from a zone (the region around the mouth of the capillary) containing the toxic material. Therefore, if both a favorable and an unfavorable area are available, the negative chemotactic response allows the population to demonstrate a net shift away from the unfavorable and consequently toward the favorable area. This ability of bacteria to remove themselves from injurious regions provides, then, a survival mechanism operating not only at lethal concentrations but at sublethal concentrations as well. Consequently, the organism can selectively avoid potentially toxic areas. As a survival mechanism, a margin of safety or a degree of warning is provided for the organism.

### Table 1. Negative chemotactic response of four motile marine bacteria to sublethal concentrations of toxic chemicals

| Bacteria | Compound     | Effective nonlethal conc (%) | Degree of negative chemotaxis (%) |
|----------|--------------|------------------------------|----------------------------------|
| R3       | Chloroform   | 0.1                          | 72                               |
|          | Toluene      | 0.1                          | 80                               |
|          | Ethanol      | 0.2                          | 80                               |
|          | CuSO₄        | 0.05                         | 70                               |
| G4       | Chloroform   | 0.3                          | 97.5                             |
|          | Toluene      | 0.1                          | 95.6                             |
|          | Ethanol      | 3.0                          | 75                               |
|          | Benzene      | 0.2                          | 87                               |
|          | Pb(NO₃)₂      | 0.001                        | 56                               |
| P2       | Chloroform   | 0.5                          | 96                               |
|          | Toluene      | 0.1                          | 95                               |
|          | Ethanol      | 2.0                          | 96                               |
|          | Benzene      | 0.2                          | 92                               |
|          | Pb(NO₃)₂      | 0.001                        | 87                               |
| MAV      | Chloroform   | 0.3                          | 89                               |
|          | Toluene      | 0.5                          | 93.7                             |
|          | Ethanol      | 3.5                          | 95                               |
|          | Benzene      | 0.05                         | 91                               |
|          | Pb(NO₃)₂      | 0.001                        | 96                               |

* Fraction of bacterial population which was prevented from entering the capillary as compared to positive control conditions.
The toxicants alone were found to elicit the negative tactic response. Furthermore, the incorporation of a food source, such as nutrient broth, along with the toxicant, did not alter the negative taxis. The same low background numbers of bacteria entered the capillary regardless of the presence of food. It appears, therefore, that the negative taxis overrides the positive response. The negative chemotactic response of bacterial populations thus provides a selective advantage for motile bacteria since (1) they possess a mechanism for detecting and removing themselves from lethal as well as sublethal regions, and (2) the negative response takes precedence over a positive response for food.

The evidence presented here suggests that toxicity is not the only means by which microbial fouling of surfaces is prevented. Heavy metal paints currently used for the control of microbial as well as macrobiological fouling may also be functioning by inhibiting the approach of both motile bacteria and fouling larvae to the surface. This negative tactic phenomenon, therefore, could be utilized to explore alternative methods of control for microbial fouling. It should be possible to coat surfaces with less universally toxic chemicals at nonlethal concentrations which prevent fouling by repelling a potential primary microbial film and fouling larvae.

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