CHEMOTAXIS AND ALGAL ATTACHMENT OF CAULOBACTER

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INTRODUCTION

As a habitat, the ocean is under practically constant nutrient limitation, and the microorganisms living there have had to develop specific features to be able to survive and proliferate with a meager supply of growth substrates.

The prosthecate bacteria, which are distributed generally as oligotrophs in nature, appear to have at least two adaptations that confer on this group of bacteria advantages in scavenging nutrients:
- the stalk, an appendage which during starvation periods elongates and in this manner extends the surface of the cell, and
- a motile swarmer cell, which provides this typically sessile organism with a means of dispersal.

This type of cell is a product of every reproductive event, which implies that its role in the welfare of the total population is significant. Its motility alone would disperse the population because its sibbling is non-motile; however, it is reasonable to expect that its motion would be directed toward a more favorable environment.

In many motile bacteria, it is known that motility can be directed by chemotaxis (a gradient of chemicals). In positive chemotaxis, the net motion of the organism is along the increasing concentration of a chemical. In negative chemotaxis, the net motion of the organism is along the decreasing concentration of a chemical. Obviously, positive chemotaxis would be beneficial in a nutrient limited habitat.

MATERIALS AND METHODS

Bacteria

A marine strain of Caulobacter, VC5, isolated from cover slips left submerged near a hydrothermal vent in E. Pacific, was obtained from Jeanne Poindexter. A strain of *Pseudomonas*, Ps. 2000, was obtained from Carrie Greenberg.

Algae

The following algae were obtained from Robert Guillard, Bigelow Laboratory for Ocean Sciences: *Chlorella capsulata* (Fla.E), *Halochlorococcum saccatum* (Fla.9), *Chlorossarcinopsis halophilia* (T.9), *Occystis minuta* (0.15), *Chlorella autotrophica* (580), and Unidentified (OP.T 10).

Media

For the cultivation of the Caulobacter, the following strain CPS-medium was used: 0.5 g peptone and 0.5g cas amino acids in one liter of 80% seawater (SW). For plates, agar (10 g/l) was added. For the cultivation of the *Pseuduomonas* strain L-broth was used which contained per liter: 5g yeast extract, 10g tryptone, 5g NaCl, 1g glucose, and, for plates, 15g agar. For the cultivation of the algae the methods of Guillard (1975) were used.

Solutions-
The solutions used in the chemotaxis assay for Caulobacter were:

*wash and dilution medium: 80% SW, pH7
*attractants: cas amino acids dissolved in 80% SW, pH 7
1-0.002 g/l
*control: 80% SW, pH 7

The solutions used in the chemotaxis assay for Pseudomonas were:

*wash and dilution medium: potassium phosphate 50 mM pH 7
$(NH_4)_2SO_4$ 1%
tap water
*attractants: cas amino acids 1%
potassium phosphate 50 mM pH 7
EDTA 10µM
deionized H_2O
*control potassium phosphate 50 mM pH 7
EDTA 10µM
deionized H_2O

Incubation Conditions

Caulobacter was precultured in 5 ml CPS-broth from an agar slant and incubated overnight on a shaker at 22°C.

For the experimental population, the preculture was transfered as a 10% inoculum into a 250-ml E-flask containing 30 ml CPS-broth and incubated for 7-12 hrs on a shaker at 30°C.

Pseudomonas was precultured in 10 ml L-broth from an agar plate and incubate overnight on a shaker at 22°C. For the experimental population, the preculture was transfered as a 5% inoculum into a 250-ml E-flask containing 30 ml L-broth and incubated for 10 hrs on a shaker at 30°C.

The algae were cultured according to the methods of Guillard (1975).

Chemotaxis assay

Chemotaxis chamber was constructed as described by Adler (1973). The experimental bacterial culture was transfered to a sterile centrifuge tube. The motility of the bacteria was checked microscopically. The suspension was centrifuged aseptically at a speed of 4000 x g at 10°C. (In the case of Caulobacter this procedure made the swarmers accumulate in the pellet and the stalked cells remain in the supernatant).

The supernatant was decanted and discarded. The pellet was resuspended in 5-10 ml of sterile wash medium. The pellet was manipulated gently in order to prevent breakage of the flagella. The density of the washed cells was adjusted to approximately 10^8 cells/ml (barely turbid suspension), and the suspension was introduced into the chambers as described by Adler (1973) and at the onset and after 60 min at 25°C of the chemotaxis experiment, the motility of the bacteria was checked microscopically. The agar plates were incubated as follows: Caulobacter: 3-4 days, 30°C; Ps. 2000: 1-2 days, 30°C.

Attachment of bacteria to algal surfaces .

Algae were grown for six days in a photoperiod of 14L/10D. The last photoperiod was either light or dark. Meanwhile, a culture of VC5 was grown so that the proportion of swarmers would be maximal at the time the algae were harvested. One ml of bacterial suspension was transfered along with 2 ml of algal suspension to 5 ml of fresh algal medium. The mixed population was incubated at 22°C in light and the status of both organisms were taken immediately and thereafter at 4,8, and 24 hours.

RESULTS

There had not been any previous experiments establishing chemotatic response in Caulobacter. To be sure that the Adler assay was performed correctly, a pilot study was performed with Ps. 2000, known to respond chemotactically to cas-amino acids . As shown in Table 1, the technique was, in our hands, suitable for obtaining a positive response to the attractant.

% cas-amino acids	0	1
bacteria/capillary	$1.6 \cdot 10^5$	$.2 \cdot 10^6$

Table 1: Pseudomonas response to cas-amino acids.

Difficulties arose during the attempt to adopt the technique to Caulobacter. To verify when the swarmer stage was most pronounced, a culture was followed through the growth curve in CPS-broth. Microscopic examination of the culture at frequent intervals revealed that the swarmer proportion was suitably high between 7-12 hrs of growth. After 12 hrs, the swarmers seemed to be less active.

During the centrifugation step, not all of the swarmers were found in the pellet, leaving a fair number in the supernatant. An even greater problem was that the stalked cells were also present in the pellet. Nevertheless, the procedure described above provided a pellet that was sufficiently enriched in swarmers to be suitable for the chemotaxis assay.

The results obtained with a swarmer enriched cell suspension (Table 2) clearly showed a positive chemotactic response to cas-amino acids. Since only the swarmers can account for any kind of motility in this organism, this indicated that the swarmer state of Caulobacter can react chemoacticaly to amino acids as attracants.

% cas-amino acids	0	0.002	0.01	0.05	0.1
bacteria/capillary	$1x10^5$	$>3x10^7$	$>3x10^7$	$4x10^6$	$>3x10^6$

Table 2: Caulobacter response to cas-amino acids

Attachment of Caulobacter to algae

To initiate the studies of Caulobacter response to the presence of algae, six species of algae were screened. The microscopical studies are summarized in Table 3.

t,hr / Algae	0		4		8		24	
	L	D	L	D	L	D	L	D
Fla E	-	-	(+)	+	+	++	++	++
Fla 9	-	-	+	+	+	+	++	++
T 9	-	-	+	+	++	++	+++	+++
O 15	-	-	+	++	+++	++	+++	+++
580	-	-	-	(+)	-	(+)	-	-
OP T 10	-	-	-	(+)	(+)	(+)	(+)	(+)

Table 3: Swarmers or stalked cells of Caulobacter clustered around different algae.

L Last photographed was light for algae
D Last photographed was dark for algae
\- no attachment/clustering
(+) a few bacteria in the vicinity of a few algal cells
+,++, +++ relative numbers of bacteria clustering around algal cells or algal clusters

These preliminary observations suggest that Caulobacter attaches preferentially to certain algae.

In those cases where significant numbers of Caulobacter cells attached during the observation period, there was not any significant influence of the illumination history of the algal population on the tendency of the Caulobacter to attach.

DISCUSSION

The initial intent of this study was to determine for the first time whether a marine strain of Caulobacter would show a chemotactic response to compounds likely to be exuded by algae.

The overall design of the project was three-fold: (a) to determine whether the bacteria exhibited any chemotactic behavior, (b) to determine whether the bacteria exhibited any preference for algae as substrate, and (c) to determine whether there was any correlation between the chemotactic behavior and the preference for particular algae.

The principal problems encountered were obtaining a sufficient proportion of swarmers and maintenance of their motility in the chamber, and so to develop

a dependable and reproducible assay. Nevertheless, it was possible to detect a chemotactic response in the marine Caulobacter strain and also to discern a preference on its part for certain algae as substrata.

PRELIMINARY INVESTIGATION OF ORGANISMS CAPABLE OF DEGRADING DIELDRIN AND AROMATIC COMPOUNDS: TECHNIQUES FOR ISOLATION AND CHARACTERIZATION

Brad Harten

INTRODUCTION

The degradation of most organic compounds is usually due to microbial activity since nonenzymatic reactions seldom lead to the breakdown of introduced chemicals. Many microorganisms have been shown to degrade aromatic and other hydrocarbon compounds.

Two different catabolic processes are responsible for the removal of chemicals from the environment. The first, primary metabolism, refers to the mineralization process in which carbon and energy are derived from the breakdown of the organic molecule by the microorganism. The second, cometabolism or cooxidation, refers to the process in which biotransformation of the chemical occurs without the net production of energy or carbon. Organisms use carbon and energy derived from a second substrate for growth and to carry out the cometabolic transformation.

The distinction between these two processes may have significant ramifications. Mineralization results in the removal of organic, and potentially toxic, compounds from the environment. Because these compounds are used as substrates, they may be eliminated relatively quickly. Cometabolic processes, however, generally do not transform the chemical quickly since compounds subject to attack are not used as substrates for energy. In addition, only small changes in molecular structure may result from these transformations. Cometabolic products similar to the original compound may then accumulate in the environment. Byproducts may even be more toxic than the parent compound. Horvath (1972) stated that cometabolism may be important in the degradation of recalcitrant molecules including halogenated pesticides such as DDT and aldrin.

Interestingly, the enzymes involved in some aromatic degradative pathways have been shown to be plasmid-encoded, particularly in Pseudomonas species (Williams and Worsey, 1976). This is usually the primary metabolic enzymes. The role of plasmids in cometabolism is unclear.

The purpose of this study was to test for the presence of primary and cometabolic organisms in the marine environment and to develop techniques for their isolation. Both aromatic compounds and the insecticide dieldrin were used as test compounds. Partial characterization of organisms known to metabolize aromatics was carried out using plasmid screening procedures and thin layer chromatography. One isolate which produced clearing zones on dieldrin was also screen for plasmids.

MATERIALS AND METHODS

All minimal salts broth and plates were prepared using the procedure of Baumann and Baumann, Chapter 104, p. 1308, The Prokaryotes. Neopeptone broth and plates were prepared using the procedure of Kiyohara et al. (1982), modified as follows:

0.5ml glycerol, 0.1g neopeptone, and 0.1g yeast extract were added to 1 liter minimal salts media. 1.5% agar was used to make plates. Spread plates were made by spreading 0.4ml of a stock solution of aromatic or