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Abstract

This paper describes work in the areas of chemotaxis and culture work seeking microorganisms capable of growing on particular nitrogen sources. A capillary racetrack was constructed and successfully used to isolate magnetotactic bacteria from marsh sediments. The magnetotactic bacteria were used in chemotaxis assays, but no positive chemotaxis was observed. Chemotaxis of *Chlorochromatium* consortia towards various substances was also tested but not positively observed. Enrichments were established using media designed to investigate the use of man-made nitrogen compounds as sole nitrogen sources for saltwater microorganisms. Growth was observed but could not be attributed solely to the man-made compounds.

Magnetic Bacteria

Certain microaerophilic bacteria contain intracellular iron grains, termed magnetosomes, which render them sensitive to the Earth's geomagnetic field. Because magnetotactic bacteria are gradient organisms and live at the oxic-anoxic interface in sediments, their ability to swim along lines of magnetic force. The local component of the Earth's geomagnetic field points downward (towards the center of the earth) in both hemispheres (Blakemore 1982). No matter which hemisphere they live in, magnetotactic bacteria are magnetically polarized so that their predominant swimming direction is downwards. This work sought to observe chemotaxis behavior in magnetotactic bacteria.

Collection of Magnetotactic Bacteria

Magnetotactic bacteria were collected from the School Street Marsh. Bottom water and the top 3-4 cm sediment was collected with a 1 L scoop and poured into a 50 mL screw cap jar. The jar was stored on a desktop at room temperature with ambient lighting. The lid of the jar was not tightly sealed, to allow for exchange with the atmosphere. 24 hours after collection, the sediment in the jar had settled, and the oxic-anoxic interface had reestablished itself (2-3 mm from the water-sediment interface). Sediment samples from the interface were taken with a Pasteur pipette.

Isolation of Magnetotactic Bacteria

Magnetotactic bacteria were isolated by the use of a capillary racetrack as described by Wolfe et al. (1987). The large end of a drawn Pasteur pipette was broken off the narrow end sealed in a flame. The capillary tube was filled with 0.2 micrometer-filtered bottom water from the sample from which the magnetic bacteria were to be

isolated. A small plug of absorbent cotton was tucked into the neck of the capillary to slow the migration of non-magnetic bacteria down the capillary tube. A small of innoculum of sediment (~ 100 mg) was gently placed in the widest part of the capillary, resting on the cotton. Refer to Wolfe et al. (1987) for a more thorough description of this apparatus.

Construction of a Slide Holder to Monitor Movement of Magnetotactic Bacteria

A holder for the capillary was constructed to allow real-time monitoring of the migration of magnetotactic bacteria. Two microscope slides were sandwiched together outside a piece of 3 mm plexiglass which had a U-shaped channel cut into it. The holder was sealed using silicone (aquarium sealant). By filling the holder with water it was possible to observe movement of the magnetotactic bacteria. By positioning a stirring bar magnet in line with the capillary with the south end of the magnet closest to the tip of the capillary, magnetotactic bacteria were drawn into the narrow end of the capillary. In this manner magnetic bacteria could be separated from nonmagnetic organisms. Magnetotactic bacteria generally began to accumulate in the narrow end of the capillary within 3-5 minutes, though their movement could be severely slowed by cotton which had been packed too tight. By breaking the capillary below the cotton plug, magnetotactic bacteria could be removed with a drawn Pasteur pipette.

Chemotaxis Assays

Chemotaxis was assayed by two different protocols (see accompanying illustrations). In the first method, a U shaped tube was placed between a microscope slide and a cover slip, with the cover slip resting over the curved part of the U tube. Magnetic bacteria and then 0.2 micrometer filtered bottom water were used to fill the chamber created by the U tube and the cover slip. Microcapillary tubes containing the attractant being assayed were filled by first sealing one end of the tube in an open flame, then filling the tube by gently warming the tube (quickly passing it through an open flame) immediately before placing the open end of the capillary in the attractant solution (1 mM concentration). As the air inside the capillary tube cooled and created a vacuum, the attractant solution moved into the capillary. The open end of the microcapillary tube was then placed in the center of the bacterial suspension. Chemotaxis was scored as positive if cells accumulated in a cloud around the tip of the capillary. Verification that chemotaxing cells were indeed magnetotactic could be simply accomplished by placing a stirring bar magnet next to the slide and looking for a response.

The second chemotaxis assay was performed inside the capillary in which the magnetotactic bacteria were isolated and was designed to avoid dilution of the isolated magnetotactic cells. The capillary was broken below the cotton plug to remove the sediment and nonmagnetic bacteria. A microcapillary tube was filled with attractant as described above and inserted into the open end of the broken capillary. The capillaries were gently placed in the slide holder to facilitate observation of cell movement. As before, chemotaxis was considered positive if a cloud of cells accumulated around the tip of the capillary.

Results

Compounds assayed for chemotaxis included acetate, citrate, propionate, fumarate, ammonium, nitrate, cas-amino acids, and yeast extract. No chemotaxis to any of these compounds was observed. Potential reasons include that magnetotactic bacteria

do not chemotax, they do not chemotaxis to any of the compounds tested, or they were suffering from oxygen stress because they were in too oxic of an environment.

References

Blakemore R. 1982. Magnetotactic Bacteria. Annu. Rev. Microbiol. 36: 217-138.
Wolfe RS, RK Thauer, and N Pfennig. 1987. A capillary racetrack method for isolation of magnetotactic bacteria. FEMS Microbiol. Ecol. 45: 31-35.

Chlorochromatium chemotaxis

Chlorochromatium is a symbiosis between one large motile rod, member of the beta class of the Proteobacteria, and several small nonmotile green sulfur bacteria which cluster around the central rod. Tight communication appears to exist between members of the consortia, as the small nonmotile cells seem to direct the movement of the rod. Overmann and colleagues have investigated this consortia previously (eg. Frostl and Overmann 1998). Chemotaxis towards alpha-ketoglutarate has been previously observed, and the purpose of this work was to seek evidence of alpha-ketoglutarate chemotaxis in nascent *Chlorochromatium* enrichments.

Methods

Chlorochromatium enrichments from the Trunk River (Falmouth, MA) were maintained by J. Overmann. The cultures used for these experiments were pale green with ~ 2 mm diameter green flocs of *Chlorochromatium* resting on the bottom of the culture vials. Chemotaxis towards five substances was tested: alpha-ketoglutarate, hydrogen sulfide, acetate, citrate, and glutamate. As a control, 0.2 micrometer-filtered culture media was used. Concentrated stock solutions of attractants were diluted to 1 mM concentration with sterile media. The microcapillary tubes used were rectangular, with flat sides that allowed viewing of the contents of the capillary under a 10x or 40x microscope objective.

Chemotaxis assays were performed in Corning 30 mL tissue culture flasks. A red hot wire was used to punch ~ 1 mm diameter holes in the side of the bottle in a 3x4 grid spaced ~ 1 cm apart (see accompanying illustration and photo). Five attractants plus one control were assayed in duplicate to allow observation of the chemotaxis experiment at two time points.

The same assay was repeated twice, once on a benchtop using a stream of N_2/CO_2 gas to keep the *Chlorochromatium* anoxic and once under anoxic conditions in a glove box with a N_2/H_2 atmosphere. The chemotaxis apparatus was constructed with the tissue culture flask laying on its side with the holes facing upwards. A gassing needle with a

slow stream of N_2/CO_2 was inserted through the neck of the flask to keep the inter of the flask anoxic. The flask was filled approximately halfway with *Chlorochromatium* culture, including all flocs. Capillary tubes were broken in half so that they were ~ 8 cm long. By placing one end in the attractant solution, capillary action filled the microcapillary tube with attractant. The broken end of the capillary was sealed by gently plunging the capillary into a plug of plasticene clay. The unbroken, open end of the capillary was gently lowered into the tissue culture flask through one of the 12 holes until the tip of the capillary was submerged about 5 mm. A small dot of plasticene was used to plug the hole and keep the capillary in place. Great care was taken not to jar the *Chlorochromatium* cells or stir up the flocs. When all 12 capillaries were in place, the apparatus was incubated on a shelf in ambient light at room temperature. Four hours after the experiment commenced, one replicate of each attractant was removed and observed under a microscope at 10x or 40x magnification. The second replicate capillary was left in the tissue culture flask for observation at a later time.

Results

During the assay conducted on the benchtop, some chemotaxis was observed. All cells seen in the capillary were motile, and the density of cells decreased away from the end of the capillary which had been submerged in the media, but very few consortia were seen. In some cases there was a band of motile cells beyond which eukaryotes did not pass, indicating that the assay was contaminated with oxygen and aerotaxis was occurring. The second assay was conducted in the glove box in an attempt to circumvent this problem. The results of the second assay were likewise negative, in that even fewer consortia were seen in the capillaries than in the first assay. J. Overmann suggested that the enrichments were too old for this experiment to have worked properly, and that next time this experiment should be attempted with a younger culture.

Reference

Frostl JM and J Overmann. 1998. Physiology and tactic response of the phototrophic consortium "*Chlorochromatium aggregatum*." Arch. Microbiol. 169: 129-135.

Novel Compounds as Nitrogen Sources

In this project, novel (ie. entirely manmade) chemicals were used as the sole nitrogen and carbon sources in saltwater enrichment media. Presumably, the nitrogen was present in forms that were new to marine bacteria. This project examined the ability of microorganisms to utilize new nutrient sources and the relative bioavailibility of nitrogen bound by various N-C bonds.

Methods

Seawater salts were autoclaved for the purpose of making aerobic and anaerobic media. Heat-labile media components were filter sterilized and added after the seawater

salts had cooled. For the anaerobic media, autoclaving was performed in a Widdel flask. The Widdel flask was cooled under a N_2 atmosphere in an ice bath. Media was dispensed into serum vials under an N_2/CO_2 atmosphere, and the serum vials were sealed with stoppers. Aerobic media was dispensed by glass pipette into 18x150 mm test tubes, and the test tubes were capped. After the basal media had been dispensed, individual tubes were supplemented as necessary. The media composition is described in the table below. When more than one substance is listed as a media component (ie. succinate and novel compound are both carbon sources), this indicates that two types of media were made, one for each case.

Both aerobic and anaerobic media were inoculated with the same two samples: Sippewissett microbial mat and Sippewissett black mud. Approximately 200 mg of mud was inoculated into 10 mL of media for each enrichment.

Enrichments were monitored twice weekly by microscopic examination under 10x pseudo-darkfield and 40x phase contrast magnification. Transfers were made of some enrichments approximately 10 days after inoculation.

Media Composition

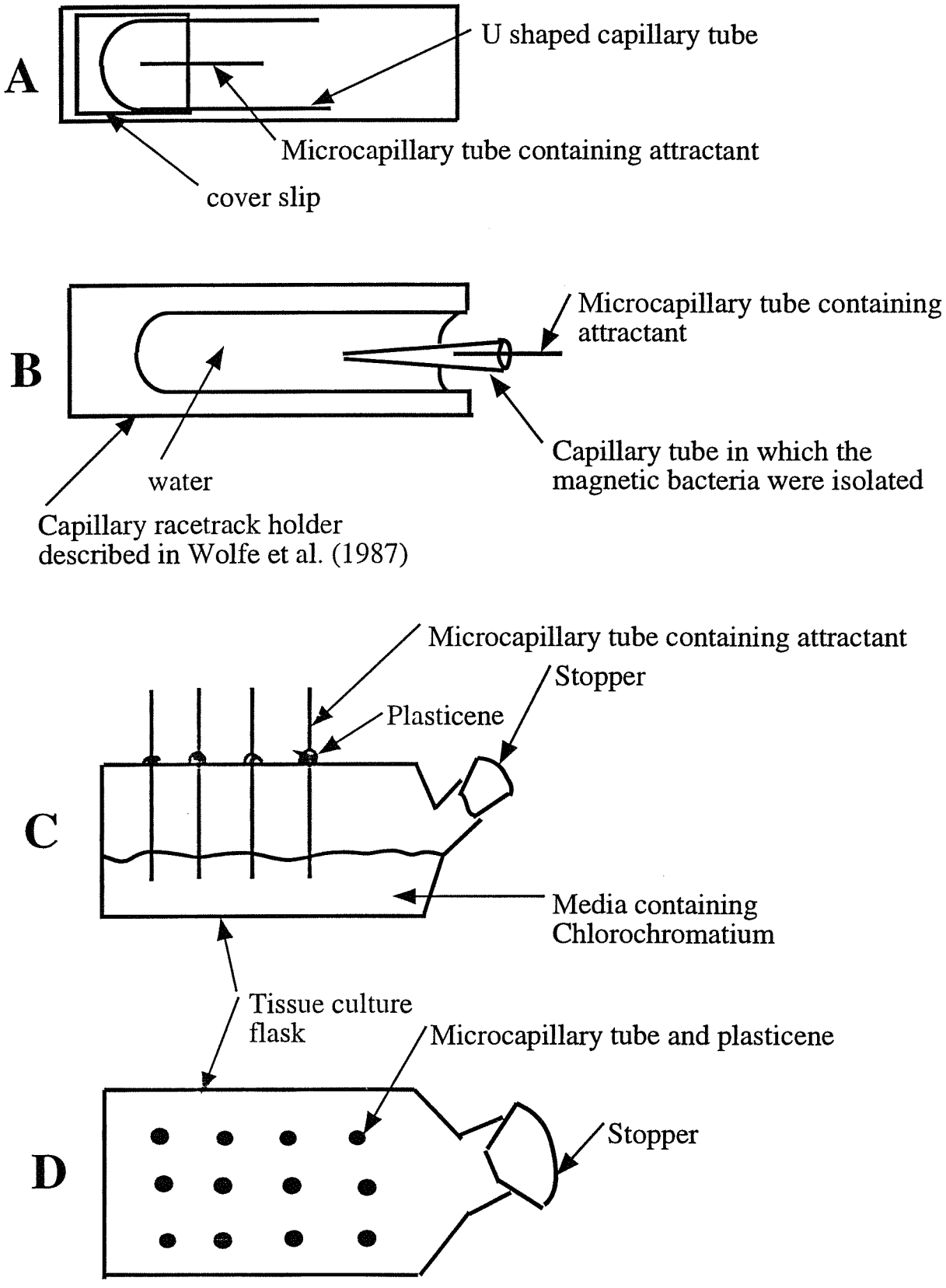
	Aerobic Media	*Anaerobic Media*
N source	Novel compound (2 mM)	Novel compound (2 mM)
C source	1. Novel compound (2 mM) 2. Succinate (1 mM)	1. Novel compound (2 mM) 2. Succinate (1 mM)
S source	Sulfate (500 μM)	1. Sulfate (30 mM) 2. None
Reductant	None	Sulfide (1 mM)
Buffer	MOPS 10 mM, pH 7.2	MOPS 10 mM, pH 7.2 Bicarbonate (30 mM)
Atmosphere	Air	N_2/CO_2
Incubation conditions	Room temp, shaken 250 rpm	Darkness, 28C
Media base	Seawater salts, trace metals, vitamins	Seawater salts, trace metals, vitamins

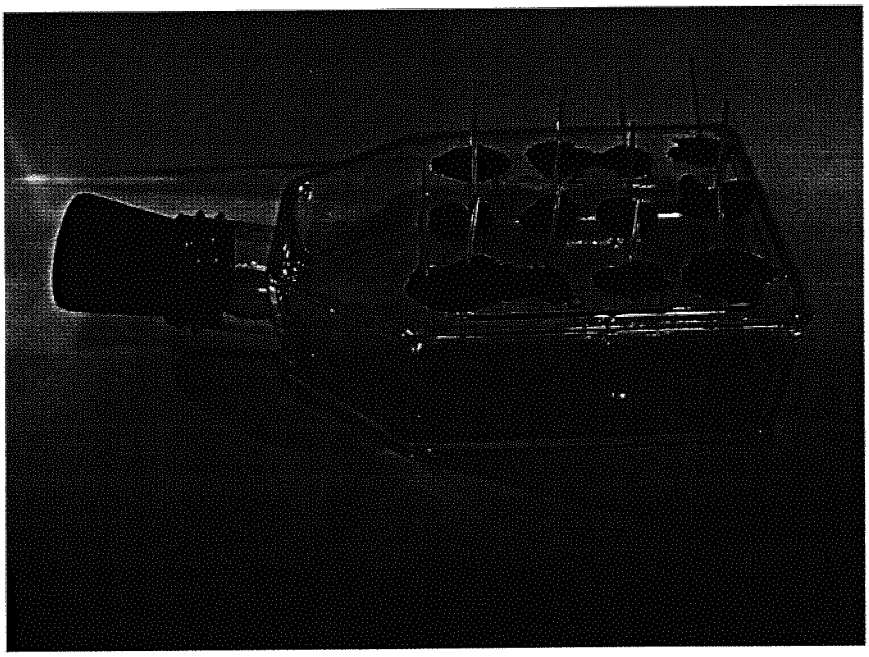
Results

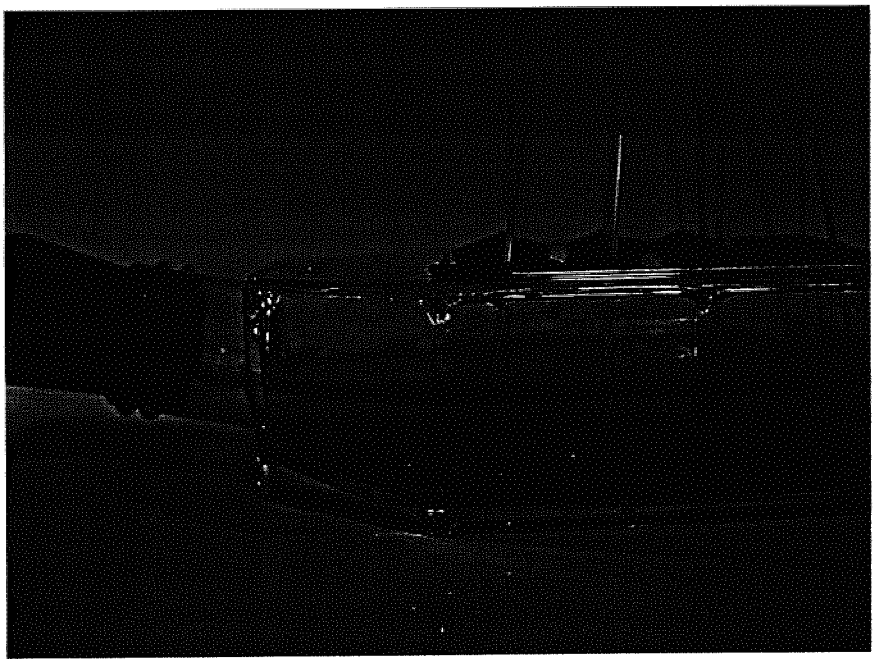
Bacterial cells of diverse morphologies were observed in all enrichments. Growth (turbidity) was observed in some enrichments. However, the growth in controls (media plus mud or mat, but without C or N source) was equivalent to or greater than growth in enrichments containing C and N sources. Flagellate grazers were observed n some enrichments.

Others in this class (Lester and Hansel) suggest that some bacteria can grow on MOPS, which was used as the buffer in these media. Perhaps this explains the cell growth in controls as well as sample enrichments.

Chemotaxis assay equiment. A and B for magnetotactic bacteria. C and D side and top views of Chlorochromatium setup.







Chlorochromatium chemotaxis assay