Diverse Microbiological Investigations

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Introduction

Three diverse projects during the 1999 summer microbiological diversity course were investigated. The purpose of this report is to provide sufficient information, regarding both what worked and what did not, so that a subsequent investigator would be able to pick up one of these projects following where these investigations left off. (Please feel free to contact me at chyba@seti.org.)

Extracellular DNA

The first project investigated required an attempt to obtain extracellular DNA from a variety of environmental sources (ocean, sewage, etc.). DeFlaun et al. (1986) report an extracellular DNA abundance in coastal waters of about 10 micrograms per liter. Since the typical mass of DNA per prokaryotic cell is about 10^{-15} g, this corresponds to about 10^{7} bacteria/ml equivalent DNA, a remarkable concentration not that different from the bacterial number densities themselves.

We pursued the method reported by DeFlaun et al. (1986), as it had apparently been previously successful. This involved filtering the sample through a sterilized (autoclaved) 0.2 micron filter apparatus (prefiltering was typically done as well), under a vacuum of about 150 mm Hg. We were able to attain such a vacuum via aspiration , using a rubber stopper with two holes, through one of which passed a tube to a vacuum gauge to allow the

vacuum to be monitored. The strength of the vacuum was chose to avoid either lysing cells or pulling small cells intact through the filter; control experiments by DeFlaun et al. (1986) suggest that this procedure was largely successful in meeting these two requirements.

After filtration, the DNA in the filtrate was precipitated by treatment with 1:10 sodium acetate (3 M) and 2:1 ethanol (volume per volume filtrate). The filtrate was then passed to sterile 250 ml plastic bottles and spun in an ultracentrifuge for 30 min at 6,500 g.

The dilemma was that, with the exception of a positive control experiment involving the injection of DNA into autoclaved seawater, no experiment yielded a visible pellet of DNA at the base of the ultracentrifuged samples. This made harvesting and resuspending the DNA problematic, and certainly problematic for quantitative conclusions. Further attempts to acquire extracellular DNA from environmental samples should consider at a minimum adopting a procedure that will allow centrifugation using clear tubes (as opposed to the opaque plastic which we employed); this might allow the identification of any DNA pellet obtained.

As we approached the end of the course, the author made a decision to pursue less ambitious projects that would ensure that he had the opportunity to practice a variety of important molecular techniques.

Enrichments of soil bacteria utilizing methanol or formaldehyde as their sole carbon source

Some prosthecate bacteria that are common in soils (e.g. *hyphomicrobium*) are reported to be able to grow on a number of one-carbon compounds, including formaldehyde, as their sole carbon source (Madigan et al. 1997). This seems especially intriguing, as formaldehyde is likely produced in substantial abundance by radiation chemistry in the uppermost layer of Europa's ice crust (Delitsky and Lane 1997). Along with such simple organics, oxidants are simultaneously produced. If melt-through events occur, these molecules will be mixed into the Europan ocean where they could serve as the redox couples to drive a europan biosphere (Chyba 1999).

To cultivate these organisms, we produced the following culture medium, roughly following that presented in the second edition of *The Prokaryotes*. To 1 liter of distilled water, we added 1.36 g KH_2PO_4, 2.13 g $Na_2H\ PO_4$, 0.2 g $MgSO_4 \cdot 7\ H_2O$, 10 mg $CaCl_2$, 2 mg $FeSO_4 \cdot 7\ H_2O$, 0.8 mg $MnSO_4 \cdot 7\ H_2O$, 2.5 mg $Na_2MoO_4 \cdot 2\ H_2O$. The pH of the solution was adjusted to 6.8. This solution was divided in half; to one half was added 5 g KNO_3; to the other was added 0.5 g $(NH_4)_2SO_4$. Media was dispensed into 50 ml flasks, and 5 ml methanol per liter was added. The flasks were inoculated using 0.1 g soil from a local woods, and stored within a sealed container under air.

After several days of growth the flasks were visibly cloudy; after a week of growth an inoculum was removed (avoiding withdrawing any visible soil particles) and added to 50 ml flasks with fresh media. After an additional four days, the second generation flask containing KNO_3 was used to inoculate two petri dishes containing the medium (with no carbon source added) and 15 g agar per liter. Onto the upper lid of one of these petri dishes was pipetted 10 microliters of formaldehyde; onto the lid of the second was pipetted 100 microliters. The dishes were stored under a hood. After 8 more days, visual inspection revealed no growth on the plate to which 100 microliters formaldehyde had been added. However, visible colonies had just begun to appear on the plate onto the lid of which 10 microliters had been pipetted.

Due to the approach of the end of the course, rather than wait to grow pure cultures, we proceeded to lyse cells (using the PrepMan protocol) from the second-generation liquid suspension and amplified this DNA using PCR (EB8F forward primer and 1492R reverse primer). We ran 1:1, 1:10, and 1:100 serial dilutions on a gel, and cloned (using *E. coli*) the DNA from the dilution corresponding to the cleanest bands (1:100). After incubation for two days, 32 visible colonies were obtained. PCR amplification using EB8F and 1492R was performed on all 32 colonies, and 4 showed product when run on a gel. DNA from these four colonies was digested overnight and RFLP analysis was performed. Two different populations seemed to be present. As this paper is being written, sequencing has been delayed due to problems with the sequencer over the past several days.

A search for magnetotactic bacteria in Salt Pond

Magnetotactic bacteria were collected by Frankel et al. (1997) from an estuary in Rhode Island with an oxic-anoxic transition zone (OATZ) similar to that found in Salt Pond. (Data obtained from our own water sampling in Salt Pond is shown in the figure (Fig. 1) labeled "Salt Pond data 6/25/99"; the Salt Pond OATZ is readily seen in these data.) Frankel et al. (1997) designated their strain MC-1 and were able to grow it in the laboratory. We attempted to collect similar bacteria from Salt Pond and, following their procedures, to grow these organisms.

MC-1 is thought to use magnetotaxis to migrate to correct oxygen level in the OATZ. MC-1 senses [O_2], and uses this magnitude to determine its swimming direction relative to the local magnetic field. This enables it to move towards the necessary microaerophilic level.

We attempted to grow these or similar strains from the samples of the water column in Salt Pond taken from 3.0, 3.5 (pink layer), and 4.0 meters depth. We followed Frankel et al. (1997) as closely as possible in preparation of a growth medium. Closed test tubes with O_2 in headspace and 0.2% agar in the medium were used to establish an oxygen gradient as organisms consume the O_2. Magnetotactic organisms should swim to and grow in microaerophilic band at boundary of oxidized (pink from resazurin) and reduced (colorless) layers in the tubes. The medium used consisted of autoclaved 2/3 seawater, with 10^{-3} M ferric citrate (to be reduced by organisms to magnetite); 10^{-2} M sodium thiosulfate (the presumed energy source); 10^{-3} M $NaHCO_3$ (the sole carbon source); vitamins, minerals, and potassium phospate. We applied the standard magnetotactic assay, *viz.* microscopic examination of the response of swimming bacteria in suspended water drops to changes in the direction of magnetic field imposed in the lab. Depsite repeated examination of the samples over a period of several weeks, no magnetotactic organisms were found.

Overnight, all tubes developed what appeared to be a dark black precipitate in their lower volumes. After 1-2 weeks, all tubes had also developed a white turbid band directly below the meniscus. In tubes from all three depths, organisms of the same visually striking appearance (4-pointed stars at low magnification) were recovered from this region. Organisms of

identical appearance could be obtained from greater depths in the tubes as well, but always in much lower abundance. Figure 2 shows the appearance of these organisms in bright field photographs. They appear to be greenish in color and seem to be composed of a succession of disk-like cells stacked in each vertex of the star. The author regrets not having been able to attempt to isolate and perform molecular analysis on these organisms, which may represent a species of branched filamentous cyanobacteria. He would very much like to know what they are!

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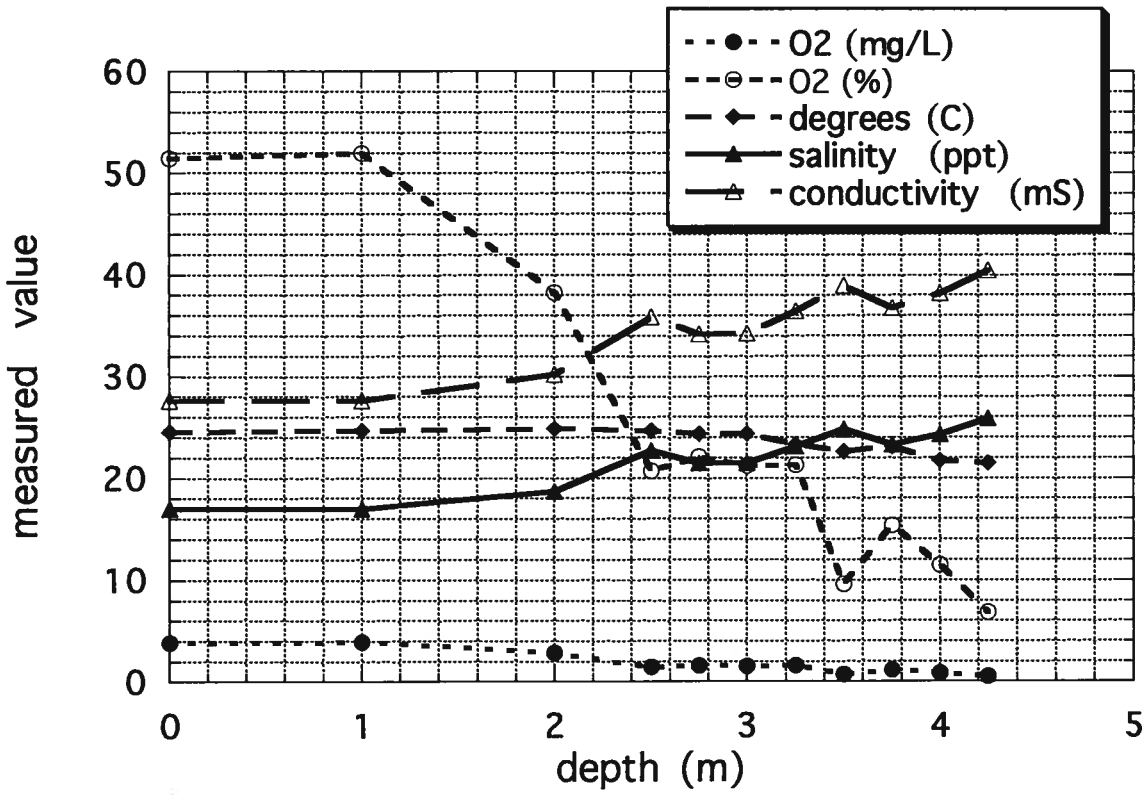
Salt Pond data 6/25/99
O2 (mg/L)
O2 (%)
degrees (C)
salinity (ppt)
conductivity (mS)
measured value
60
50
40
30
20
10
0
0
1
2
3
4
5
depth (m)

FIG. 1

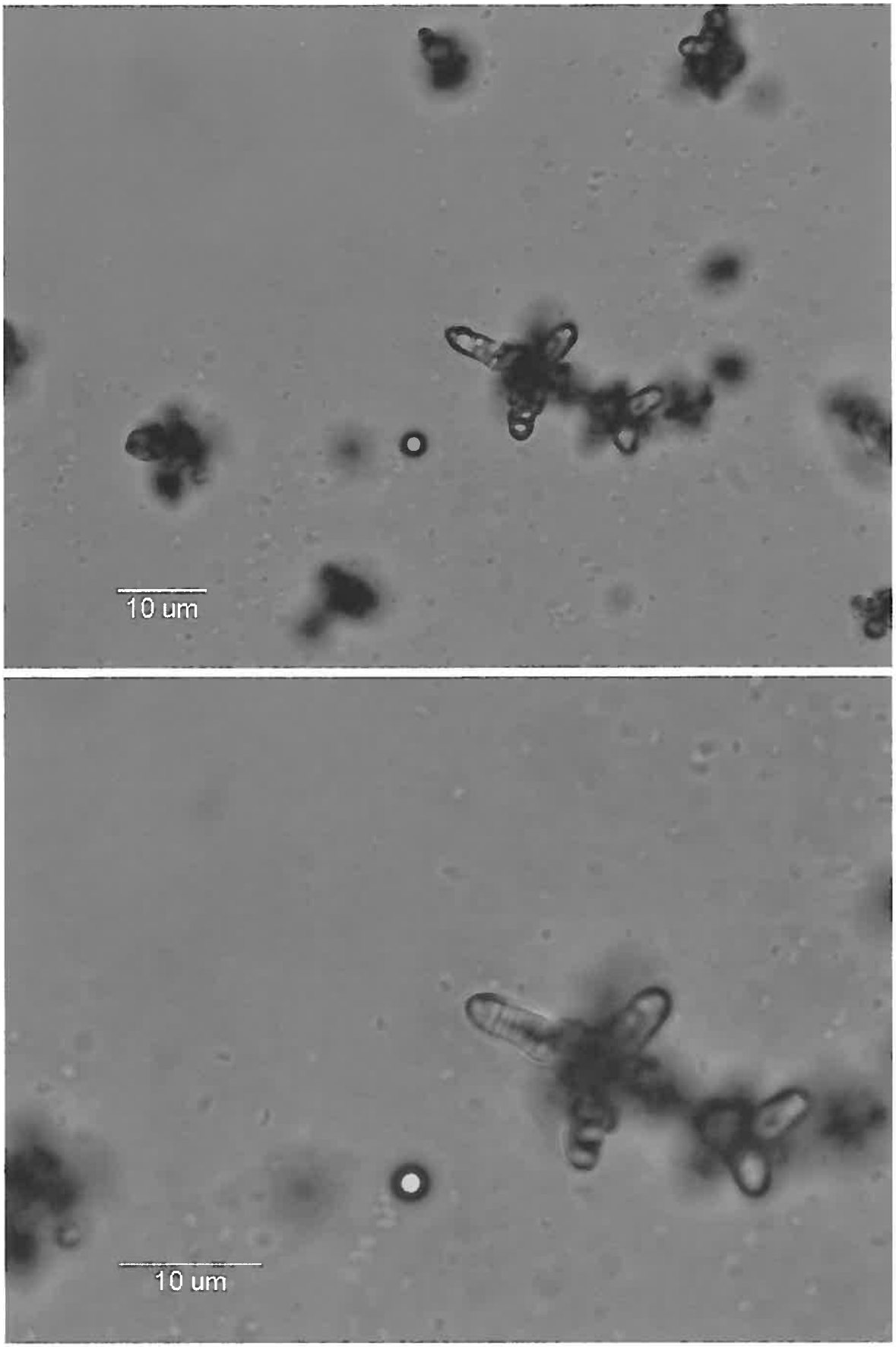

FIG. 2