Coincidence and Association of Caulobacters and Diatoms

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heterotrophic, stalked bacteria are often found in both freshwater and marine environments in which diatoms are abundant. and are often attached to ~~the~~ diatom surfaces. The study of coincidence of these organisms can be approached by studying natural algal samples and two-membered cultures in the laboratory.

Both groups of organisms are found as prominant components of microbial communities ~~found~~ in freshwater systems. (Poindexter, 1981; Tilman and Kesling, 1984). Tilman and Kiesling supported this observation by studying a microbial population succession in the Great Lakes. These researchers noted that during the Spring, an overturn of silicate and an increase in light intensity led to a diatom bloom. An increase in water temperature and a decrease in silicate concentration supported an increase in the green algal population, while a further increase in temperature and depletion of nutrients, ~~particularity~~ nitrogen in particular, shifted the population to a predominance of cyanobacteria. Finally, in the Fall, with another isothermal mixing of the water and release of silicate, a second diatom bloom occurred.

These researchers showed that the composition of this community could not be explained in terms of one factor or one process. A linear regression analysis determined that both nutrient concentration and temperature affected the selection of predominant organisms within the ecosystem.

We studied the Great Sippewissett Salt Marsh as a marine counterpart to determine if the same trends of succession would occur. Nutrient concentration was the only parameter tested. Observations of natural algal samples would also support the notion that both diatoms and Caulobacters occur in marine environments.

Since diatoms and caulobacters

the association of one or more organisms.

METHODS.

Nutrient-limited enrichments.

Inocula. Algal samples were collected from two sources. A golden mass (designated 7-9) developing in a marine aquarium which contained a large section of microbial mat from the Great Sippewisset Salt Marsh was sampled and observed microscopically. A variety of diatoms were observed.

Samples were also taken directly from the marsh. One of these, designated 7-13, was collected in early July from an inlet at Black Beach in the salt marsh. ~~Golden~~ Delicate golden masses of filaments were seen growing on the sandy bottom along the southern bank of the inlet in an area away from the main water tidal flow. The area was fairly clear of other attached growth. These samples were collected in the natural sea water, returned to the lab, and refrigerated. ~~Microscopy~~ Phase contrast microscopy was used to analyze this sample.

A second sample was collected from this same area in early August (designated 8-7). The sand bank was no longer clear of other organisms. There was organic material including sea grass and other types of algae, and there ~~were~~ was no longer an abundance of the ~~stalk~~ diatom mass. However, diatoms as well as a green ~~alga~~ filamentous alga were collected in seawater and returned to the lab.

Medium. A marine medium, Guillard's F/2 (Guillard, 1975), was used for the nutrient-limited enrichment series. This medium was modified by substituting ammonium chloride for nitrate (h/2) and a carbon source, sodium acetate, was also added to the medium (sodium acetate · $3H_2O$, 106.25 mg/L). The medium

Cultures. The nitrogen, phosphorous, and carbon dilution series were normally made of the basic F/2 with sea water and minerals had been sterilized. Medium was added to either 125ml erlenmeyer flasks for light incubation and 250ml screw cap culture bottles or #18 test tubes for dark incubation. Inocula were prepared by either suspending clumps in sterile artificial sea water or placed in F/2 (without the added nitrogen, phosphorus, and carbon sources) and teased or pulled apart using forceps for a greater suspension of cells. Inoculum volume was somewhat arbitrary, but a constant volume was added to each series. Samples to be incubated in the dark (including the carbon, nitrogen, and phosphorus series) were wrapped in foil and placed in a foil-wrapped cardboard box. Replicate nitrogen and phosphorus samples were incubated in the light. All the cultures remained stationary, with the exception of samples from 8-7. The dark tubes were placed on a shaker for aeration. All samples were incubated in the light table (21°C) under fluorescent lighting and observed over time.

Caulobacter - Alga Attachment.

Organisms. A caulobacter sp. (VC13) isolated from the deep sea vents was used for attachment studies. Several species of algae, including diatoms and a green alga that were used are listed below:

* Phaeodactylum tricornutum (TFX-1), a photothrophic diatom.
* Thalassiosira weissFlogii (Actin). " " "
* Chaetoceros simplex (BBsm) " " "

Nitzschia sp. - isolated from Fucus sp. in Woods Hole - a heterotrophic

supplemented with 0.5% peptone and 100 µg/ml of each of penicillan G and streptomycin.

Two-culture experiments. Actively motile Cantobacter cultures were produced by adding 30 or 90 µM of phosphate to the growth medium. Incubation time for good swarmer formation was usually over 12 hours at 30°C with good aeration. All algae used in these experiments were taken directly from stock culture tubes (organisms growing in F/2) with the exception of the Heterotophic diatom which was grown within 12-15 hours to a grazing turbidity at 30°C with aeration.

All cultures were checked microscopically for bacterial contamination. Cultures were mixed in small, sterile test tubes using ratios of 3:1 or 4:1 of bacterial volume to algal volume. Drop quantities were added by sterile pasteur pipette. These mixtures were placed on a shaker at 30°C and observed by phase microscopy at various time intervals, beginning with one hour after mixing. Samples were often fixed with 2% acetic acid or glutaraldehyde, and filtered onto 0.2µm Nuclepore filters for scanning electron microscopy.

RESULTS.

Nutrient-limited enrichments.

Observations of the diatom series revealed the following:

After one week, the golden diatom mass became lighter in color in the nitrogen dilution series. The diatom pigmentation in the phosphate dilution series did not show as much a decrease in color. After two to three weeks, the nitrogen series had developed green areas of growth in the more dilute nitrogen tubes. Microscopic analysis of these green areas revealed cyanobacteria. There was no visible change noted in the phosphorus series.

Within three to four weeks, the same trend was noted in

dilute nitrogen tubes and the diatom mass became paler and more feathery in texture.

Dark samples were analyzed for bacterial development, especially that of prosthecate bacteria. (Samples from the mixed enrichment were not analyzed due to lack of time in the course.) Bacteria were seen, a variety of types, but no trends were noted due to the difficulty in observing and quantifying the bacteria in these cultures.

Caulobacter-diatom attachment.

When a motile culture of Caulobacter was mixed with an algal culture, attachment was observed within an hour of mixing. Attachment of the bacteria was equally abundant in all strains of algae, with the exception of P. tricornutum. Attachment was often very good to this organism, but it was noted when a culture containing many triradiate cells was used, attachment was poor relative to other algae tested. Upon further incubation of the cultures, stalk development was seen on the bacteria attached to the diatom. Stalked bacteria were also seen attached to the microscope coverslip, empty diatom frustules, and to themselves in rosette formation. Scanning electron micrographs of the mixture incubated for 9 hours revealed ~~numerous stalked bacteria a further~~ an increase in stalk development of bacteria attached to the ~~diatom~~ algal formation. This increase in stalk formation was also seen with phase microscopy. (see Figures 1-3).

DISCUSSION.

The nutrient dilution enrichment of marine diatoms and other algae revealed similar patterns of succession as noted by Tilman. n a freshwater study. Cyanobacteria developed under nitrogen-limiting conditions and diatoms were seen to better withstand

predominant prosthecate bacterial population attached whereas other types of algae (green and red) had a greater variety of attached bacteria. In early August, the same area revisited revealed a new predominant algal population. It was difficult to locate the sheathed diatom as there was much rotting vegetation along with red algae.

These observations support Tilman's contention that nutrients and seasonal influence, such as temperature, affect the predominance of algae found in freshwater, and in our case, the marine environment.

That caulobacters attached to the diatoms as well as a heterotrophic diatom and a green alga seems to indicate that diatoms are not a specific substrate for caulobacter attachment. Good attachment did depend on having a motile bacterial culture. The only population to which caulobacters did not attach well to was the pleiomorphic. P. tricornutum(. Triradiate cells of this diatom, to which poor attachment was seen, lack their mucilaginous capsule and ~~silified~~ silicified wall (Lewin, et al., 1958). Though the triradiate culture used in some of the attachment studies was not tested for the absence of a capsule, that they are known not to have a capsule in this form implies a reason for the lack of attachment.

The results from this study indicate that the attachment and therefore the association of caulobacters and diatoms is nonspecific. Even though these organisms are coincident in nature, their association may be purely one which has developed because they are both well-suited to grow in low nutrient environments.

Whether their association is beneficial to one or both partners warrants

xperience in microbial diversity. I especially want to thank Dr. Jeanne Poindexter for such an inspiration in science and insights into the human condition. And finally, I thank Nancy Benson and Louie Kerr for assistance in scanning electron microscopy and Dr. Joel Goldman and Dr. Keith Cooksey for algal cultures.

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Figure 1. [illegible] after a 9 hour incubation.

Figure 2. Carlobacters attacked to *Oocystis minuta* after a 9 hour incubation.

Figure 3. Carlobacters attacked to a *Nitzschia* sp. after a 9 hour incubation.

Aerobic, heterotrophic, stalked bacteria of the genus Caulobacter are commonly found in freshwater and marine samples in which diatoms are abundant, often attached to the diatoms' frustules. In freshwater, both groups of organisms are found to persist as prominent components of the microbiota particularly during periods and in regions of phosphorus limitation (Poindexter 1981, Microbiol. Rev. 45: 123-179; Tilman 1984, Curr. Persp. Microbial Ecol. pp. 314-319). Our preliminary experiments revealed that marine diatoms, also, were especially favored in enrichment cultures prepared with extremely low levels of phosphate, but not in those prepared with low levels of nitrogen. The present studies were undertaken to determine whether caulobacters and diatoms occurred together in Great Sippewissett Marsh, and whether caulobacters exhibited a preference for diatoms as attachment substrata. Phase-contrast and scanning electron microscopical studies of samples of algal masses attached to rocks or sand in the marsh creeks revealed caulobacters among the variety of bacteria attached to red and green algae and the sheaths of Schizonema, a tubular diatom. Unicellular diatoms, in contrast, were generally either free of attached bacteria or supported a small population of almost exclusively stalked bacteria. In two-membered cultures of a marine caulobacter (VC13) and each of several test algae, motile caulobacters attached rapidly to each type of alga tested, resulting in 15-20 caulobacters per alga within 60 min. of inoculation. The test algae included phototrophic and apochlorotic diatoms and unicellular green algae. The only population to which caulobacters attached only poorly was a pleiomorphic population of Phaeodactylum tricornutum in which the presence of triradiate cells implied that the cells lacked their mucilaginous capsule and

are less capable of attachment to diatoms. The apparent lack of preference of caulobacters for diatoms as substrata further implies that the coincidence and association of these organisms in natural samples is determined principally by environmental (probably nutritional) factors; the question of whether their association is of benefit to either organism remains to be explored.

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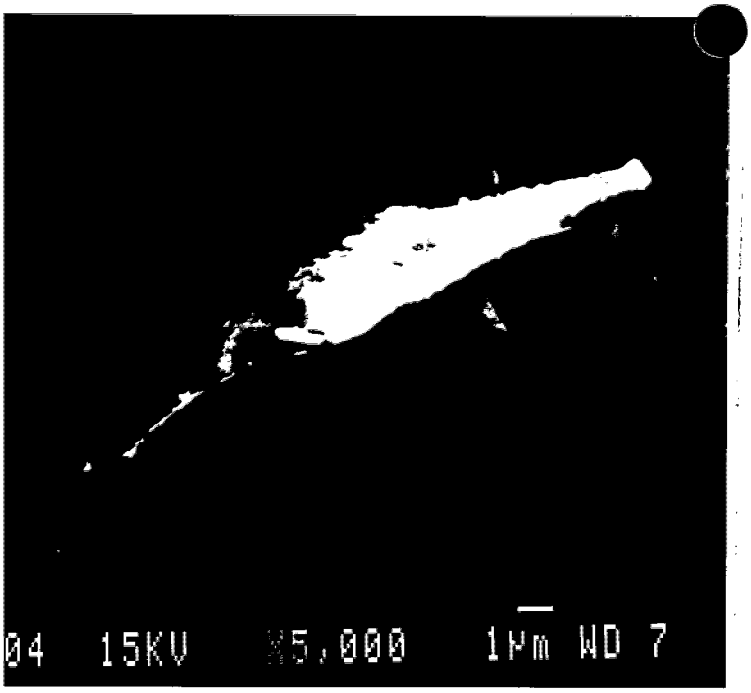

Figure 1

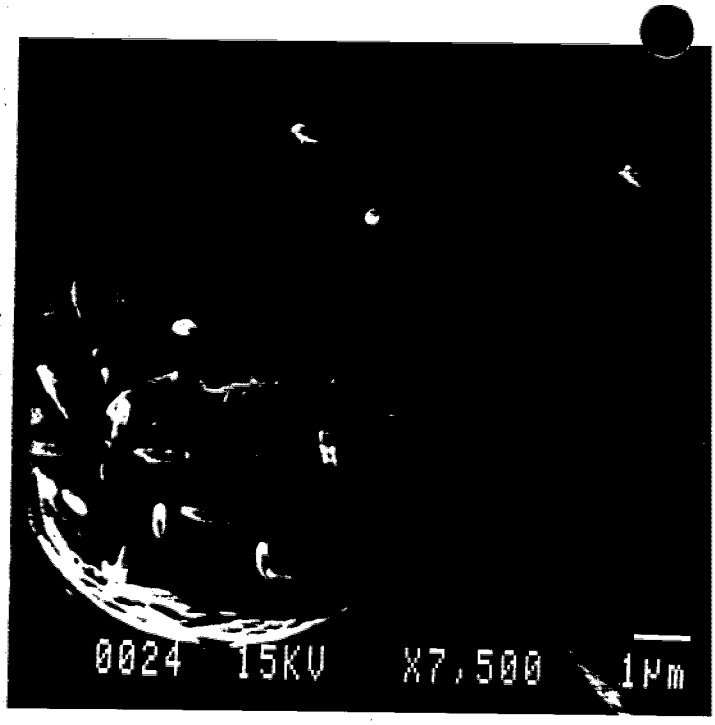

Figure 2.

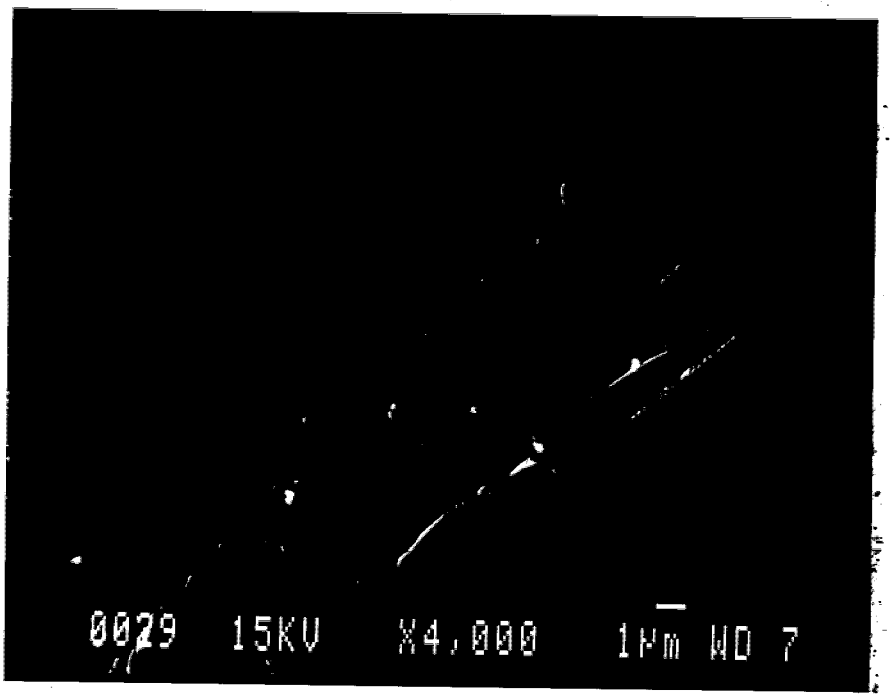

Figure 3