An attempt to enrich for phototrophic consortia from the Sippewissett Salt Marsh

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Abstract

An attempt to enrich for phototrophic consortia from Sippewissett salt marsh was made. Samples were grown on complex medium, and various molecular and microscopic techniques were used to determine presence/absence of these epibionts. Unfortunately, epibionts were not enriched for on this medium. In situ hybridization analysis (FISH) was done using a universal oligonucleotide probe and probes specific for sulfate reducing bacteria (SRBs), green sulfur bacteria, and low GC gram positive bacteria. The universal and low GC probes were the only probes that were able to hybridize with rRNA in cells present in the enrichment cultures. RFLP analysis of these cultures showed 12 different eubacterial species, and one archaeal species. When these cultures were examined with differential interference contrast, epifluorescent, and light and phase contrast microscopy, spirochetes, cyanobacteria, rod shaped bacteria, and several encapsulated and flagellated green algae species were observed.

Introduction

Syntrophic associations between phototrophic and nonphototrophic bacteria have been observed primarily in regions of freshwater lakes with low sulfide concentrations. Thus far, there have not been many attempts made to enrich for these aggregates in saltwater environments, however, in 1995 they were observed in samples taken from Oyster Pond (Woods Hole, Massachusetts). Previous examinations of these consortia have shown that they seem to thrive on the surface of anoxic mud or in axoxic hypolimnia. They seem to consist of a central sulfate reducing bacteria which is usually slightly motile surrounded by sulfide oxidizing phototrophs. The physiological basis for these complex associations has not yet been determined. However, it has been speculated that a syntrophic sulfur cycle is occurring within these microbial consortia. According to this model, the central bacterium is capable of sulfur or sulfate reduction, and the sulfide generated from this reduction is phototrophically reoxidized by the epibionts to sulfur or sulfate.

Different environmental conditions seem to stimulate the formation of these aggregates. Overmann et al (1998) were able to show that the epibiont, *Chlorochromatium aggregatum* exhibits chemotaxis towards sulfide, and thiosulfate. In addition, they found that *C. ageratum* exhibits a wavelength-dependent accumulation in

the spectral range between 700 and 770 NM, and a scotophobic response of this aggregate was apparent when it was exposed to white light. Also, they were able to show that the medium used for successful enrichment of this consortia contains significantly less sulfide (300 uM) than the conventional medium used for the enrichment of Green Sulfur Bacteria (2.5 mM). Experiments have indicated that external sulfide and light levels dramatically affect the formation of intact consortia. Therefore, we used optimal sulfide and light levels to enrich for these aggregates.

Materials and Methods

Source of inoculum

Samples were collected from the Sippewissett salt marsh in Falmouth, Massachusetts. They were taken from anoxic layers of cyanobacterial mats ranging from 5 to 11 cm below the surface. These mats are mostly in open nonshaded areas where radiation exposure is high. Sulfide levels within the layers of these mats can vary greatly. Oxygen levels fluctuate dramatically over a 24 hour time period in the top layers of these mats. Diatoms and cyanobacteria produce high levels of oxygen during the day, while aerobic respiration occurs in plants and cyanobacteria at night. These large oxygen fluctuations create completely anoxic conditions and alternative oxidants become important for many bacteria such as sulfate and sulfur. These conditions seemed ideal for the isolation of phototrophic epibionts. Fluctuating sulfide, sulfate, sulfur and light levels in anoxic layers should allow these aggregates to form and dissociate depending on environmental conditions.

Enrichment

Anaerobic cultures for the enrichment of phototrophic consortia were set up in Pfennig bottles (50 ml). A defined medium was used consisting of the following: $NaHCO_3$ (4 g/l); KH_2PO_4 (1 g/l); NH_4Cl (.5 g/l); $MgCl_2$-6 H_2O (0.4 g/l); $CaCl_2$-$2H_2O$ (0.1 g/l); NaCl (300 mM); KCl (5mM); vitamin solution (1 ml); and trace element solution SL12 (1 ml). Na_2S (300 µM) was added to the medium as a reducing agent and electron donor for anoxygenic photosynthesis, and the pH of the medium was adjusted to 7.3. Six

enrichment cultures were made, and allowed to incubate at room temperature for two weeks in front of the light set.

In situ hybridization (FISH)

rRNA targeted probes were applied to the enrichment cultures. Fluroescently labeled oligonucleotide probes complementary to the ribosomal rRNA of low GC gram positive bacteria, sulfate reducing bacteria, green sulfur bacteria, and a universal probe were applied to slides containing these enrichments. The samples were first fixed with 4% paraformaldehyde for one hour. They were then immobilized on slides, counterstained with a nonspecific dye (DAPI), and hybridized with the appropriate fluorescently labeled probes. Epifluorescent microscopy was used to view the hybridization results.

Molecular Analysis

MoBio UltraClean Soil DNA isolation kit was used to isolate genomic DNA from enrichment cultures. The bead beating step was done for only 10 seconds to avoid shearing of the DNA. Genomic DNA was visualized on a .7% agarose gel, and PCR amplification was done using eubacterial and archaeal forward and reverse primers for 16S rDNA. The PCR amplification step with eubacterial primers was carried out at 55 degrees, while gradient PCR was used with the archaeal primers. PCR products were then run on a 1.25% agarose gel to determine efficacy of amplification. The TOPO TA cloning kit was used to ligate the 16S fragment into the TOPO vector, and *E. coli* cells were transformed. Transformed cells were grown on LB agar plates containing ampicillin and X-gal. Eighteen white colonies were selected from these plates. Six of these colonies were amplified with archaeal primers; and 12 were amplified with eubacterial primers. Prior to amplification, cells were lysed in the thermocycler during brief exposure to 95 degrees. PCR amplification with the appropriate primers was then able to confirm the presence of insert. RFLP analysis with the restriction enzymes MinPI and HinPI was used to determine which vectors contained unique inserts. The restriction digests were run on a 2% MetaPhor gel. Sequences are currently being determined on the ABI sequencer, however, sequences are not yet available.

Microscopy

Routine light microscopy was carried out with a Zeiss microscope. Pictures were taken with a Zeiss axioscope using phase contrast and Nomarski microscopy. A UV filter was used to visualize cells stained with DAPI, a non-specific nucleic acid stain; fluorescent rRNA-targeted oligonucleotide probes were visualized using the rhodamine filter in epifluorescent microscopy.

Results

Enrichment results

The phototrophic epibionts were not grown on the defined medium. Although organic carbon was not added to these cultures, a fermentation reaction occurred within the enrichment cultures.

Microscopy

Figure 1: DAPI staining of cells from enrichment culture detected cyanobacterial species with what appears to be a heterocyst.

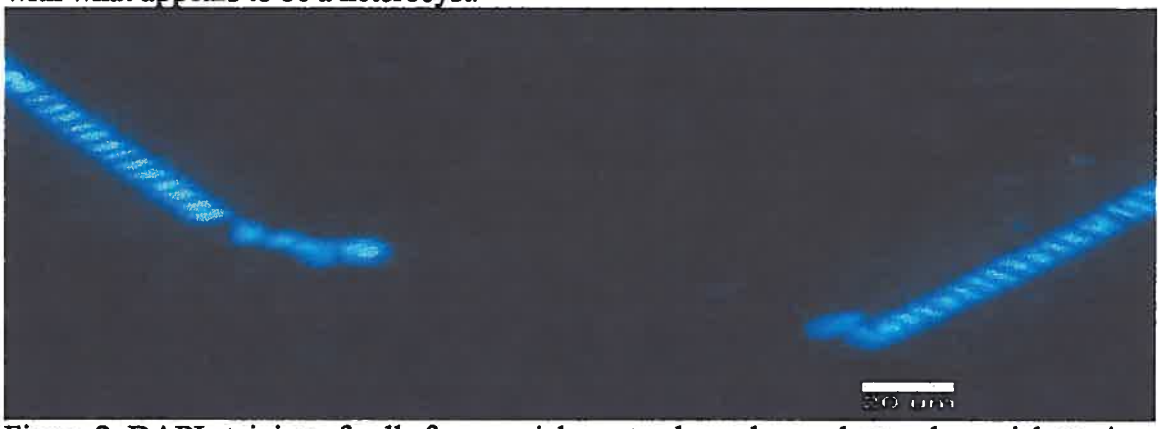


Figure 2: DAPI staining of cells from enrichment culture detected cyanobacterial species.

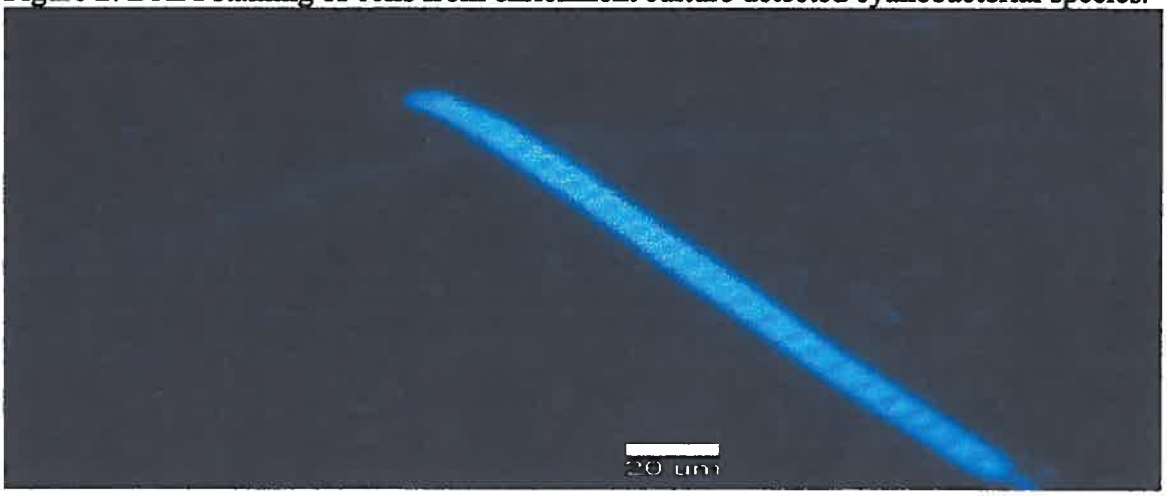


Figure 3: DAPI staining of another cyanobacteria.

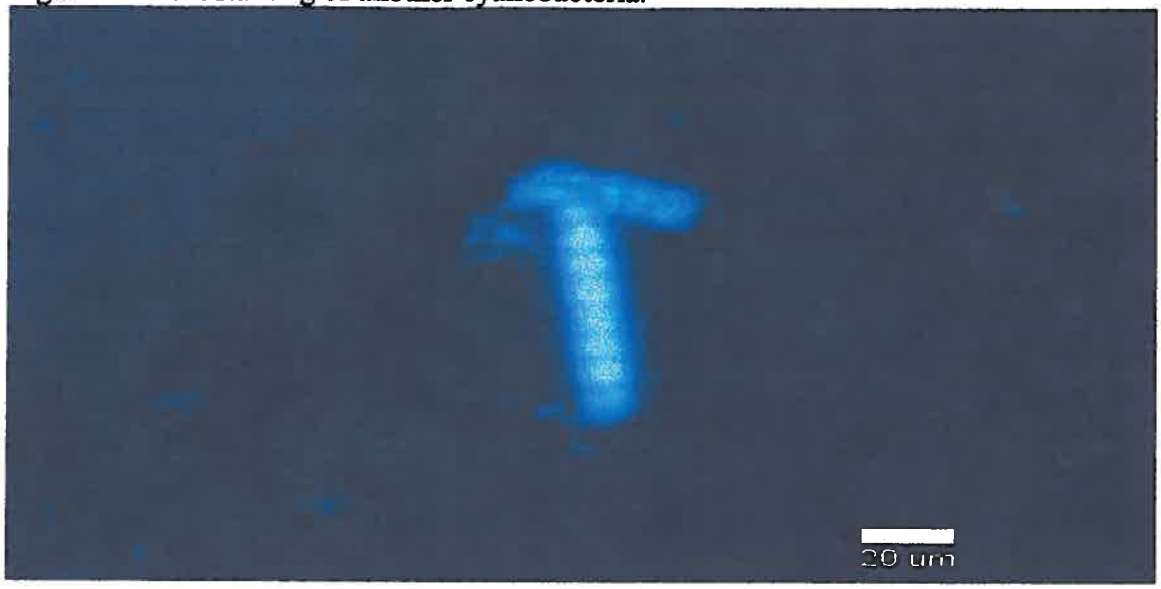


Figure 4: Rods detected with phase contrast microscopy.

Figure 5: Spirochete detected with phase contrast microscopy.

Figure 6: Flagellated green algae detected with differential interference contrast microscopy.

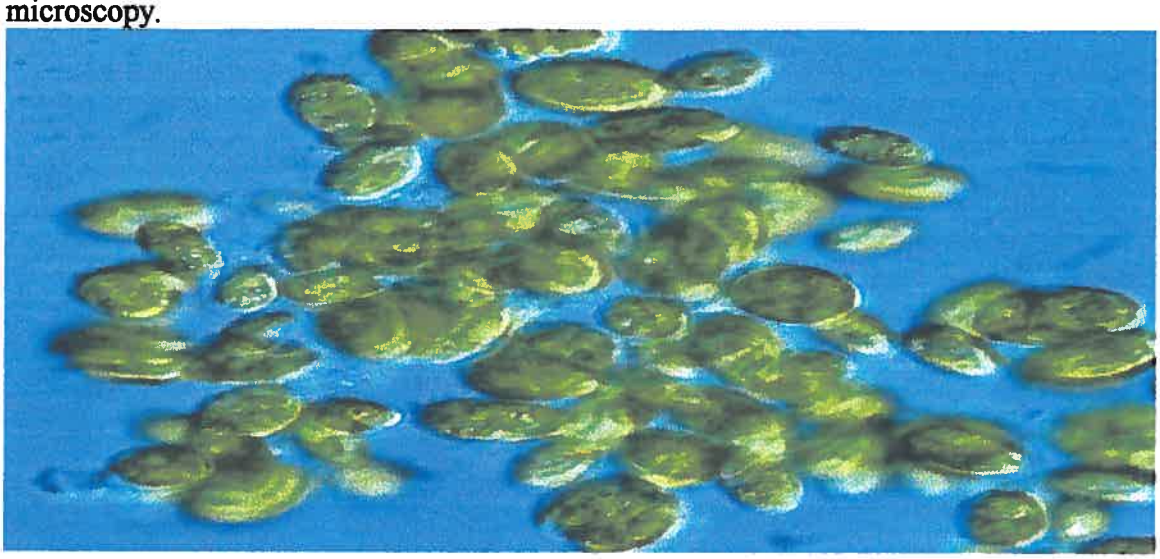
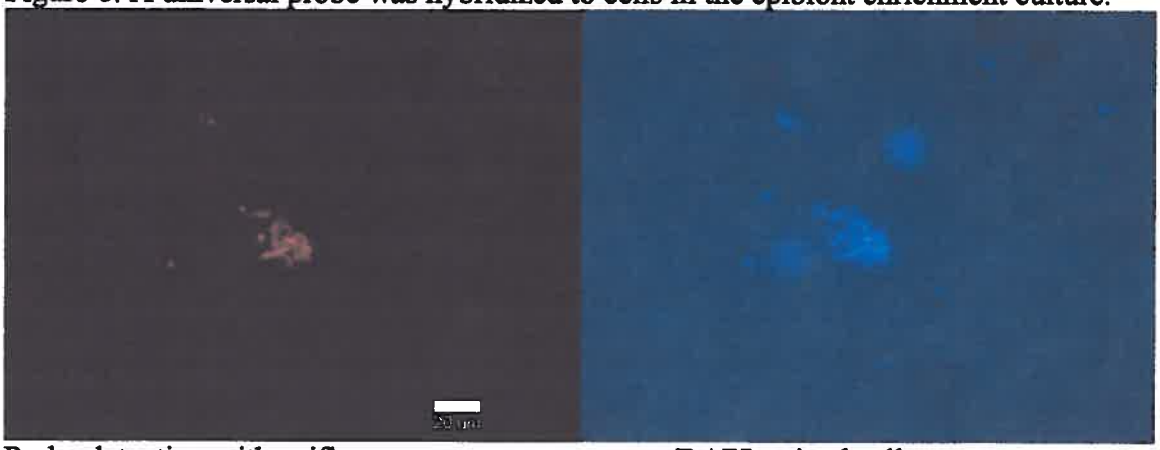


Figure 7: Nonflagellated green algae with sheath detected with differential contrast microscopy.

In situ hybridization results (FISH)

Figure 8: A universal probe was hybridized to cells in the epibiont enrichment culture:



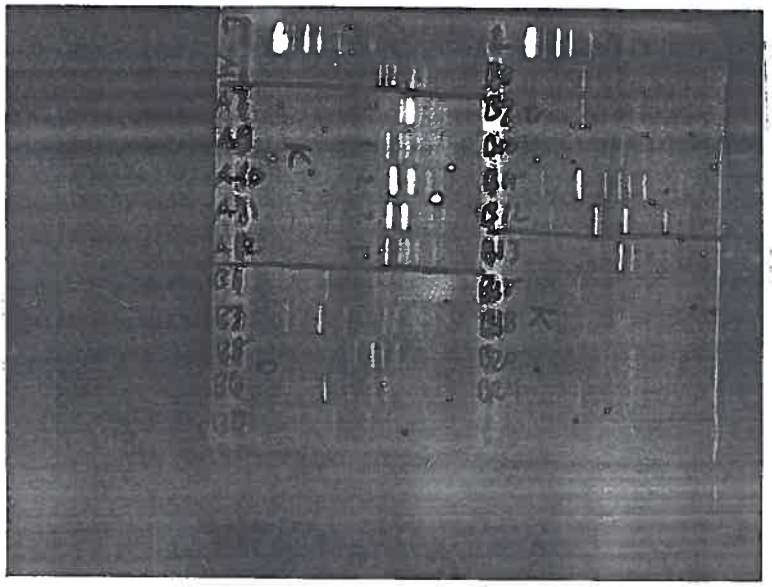
Probe detection with epifluorence DAPI stained cells

Figure 9: A low GC gram positive probe was hybridized to cells in the epibiont enrichment culture. Filamentous bacteria and rods were detected with these probes.

Molecular analysis

Eighteen colonies were selected from the LB ampicillin plates. PCR amplification with archaeal and eubacterial primers revealed one archaeal and eleven eubacterial inserts. RFLP analysis was then carried out on these inserts.

Figure 11: 2% MetaPhor gel showing MinPI and HinPI restriction digest results.



Discussion

The medium used in this experiment was designed for the enrichment of freshwater epibionts. It is possible that a different medium more specific to enrichment of a

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The medium used in this experiment was designed for the enrichment of freshwater epibionts. It is possible that a different medium more specific to enrichment of a phototrophic epibiont that might be found in a saltwater environment would have yielded better results. In addition, the only explanation for the fermentation reaction that occurred in the cultures is that organic carbon was introduced into the medium with the inoculum. This is a definite possibility, considering the fact that the samples were collected from a region of the mat near dense vegetation. The introduction of an organic carbon source to the medium explains the relatively high numbers of low GC gram positive bacteria that were detected by in situ hybridization analysis. In the future, it might also be a good idea to add cycloheximide to the cultures to kill off any eukaryotic growth.

References

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