

**Polyphasic Characterization of Phototrophs from a
Microbial Mat in Great Sippewissett Salt Marsh,
Massachusetts, USA**

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Introduction

Microbial mat communities are common in many habitats including salt marshes, coral reefs, hypersaline ponds and hot springs. Frequently these mats are dominated by phototrophs, especially cyanobacteria and purple sulfur bacteria. In laminated mats, there exists an identifiable zonation of phototrophic organisms in the upper few centimeters. This zonation is known to follow a basic pattern in salt marsh mats (Nicholson et al. 1987) determined by the decreasing availability of light to organisms, in addition to oxygen and sulfide levels to which the organisms are exposed. Oxygenic phototrophs, primarily cyanobacteria and eukaryotic algae such as diatoms, typically exist in the largest numbers on the surface of intertidal mats where they excrete extracellular polymeric substances that bind sediments and stabilize the mat structurally (Stal 1993). These organisms contain chlorophyll a and carotenoid pigments, while cyanobacteria also contain phycobilin pigments. Together, these pigments absorb most of the light in the visible (VIS) spectrum. Below this aerobic layer, one or two layers of purple sulfur bacteria are commonly found. These bacteria require sulfide and anaerobic conditions for their metabolism and contain different bacteriochlorophylls and carotenoid pigments which give them their identifiable pink-to-peach color. These pigments absorb at wavelengths not absorbed by cyanobacteria, including infrared radiation (IR) wavelengths, and are used in anoxygenic photosynthesis. Finally, at a depth of a centimeter or more, green sulfur bacteria can be found. These bacteria are the most sulfide tolerant of all three groups of photosynthetic organisms in the mat and are capable of growing under very poor light conditions.

The microbial mats of the Great Sippewissett Marsh, Cape Cod, MA, USA, are distinguished from other intertidal mats by their exceptional number of layers of phototrophs. This mat community has been characterized previously by several authors (Nicholson et al. 1987; Pierson et al. 1987, 1990). These investigations included the use of light microscopy, electron microscopy, pigment analysis and the fiber optic analysis of spectral irradiance within the mats. These techniques have provided valuable information about this ecosystem. However communities are difficult to fully describe by microscopy and pigment analysis alone. We propose in this study to further characterize the community by various enrichment techniques on liquid and solid medium. However, one should be aware that many bacteria fall into the category of "not yet cultivated" elements of a community. In fact, it is probable that only a small percentage (<5%) of the organisms from mats have been cultivated (Castenholz 1993). Therefore, the utilization of molecular techniques may yield new forms of information about the mats. The use of PCR amplification of ribosomal DNA and in situ hybridizations with ribosomal RNA to characterize mats has been suggested in the past (Turner et al., 1989). These approaches

has proven effective in characterizing microbial mats in hot spring microbial mats (Ward et al. 1993). The present study is the first attempt to integrate molecular methods with microscopy, pigment analysis and culture techniques to improve the understanding of the Sippewissett salt marsh mats.

Materials and Methods

I. Sampling

Microbial mat samples were taken on the 10th of July about 2 p.m. from the Great Sippewissett Marsh, a salt marsh located on the western shore of Cape Cod, Massachusetts, USA (41° 40' N; 41° 35'W) (Figure 1). Samples were gathered by scraping sediment of the green and pink layers with a razor blade. The separated layers were stored in petri dishes at 4° C after returning to the laboratory.

II. Enrichments

Enrichments from the separate colored layers were made in each of several types of media. Marine cyanobacterial media with and without combined nitrogen (SNAX and SOX) (Waterbury 1996) were prepared for liquid and solid media enrichments. Each type of media were inoculated with ~0.5 cm³ material and incubated at 22° C with a 14:10 L/D cycle of 12W General Electric™ cool white fluorescent bulbs. Enrichments for purple non-sulfur bacteria were made using agar plates of Basic Salt Medium (Hanselmann 1996). Purple and Green Sulfur bacterial enrichments were also carried out using Basic Salt Medium with adjusted sulfide concentrations and pH. Enrichments included serial dilution (6 X's 10-fold) in liquid cultures in Hungate tubes and shake agar tubes.

Enrichments for gliding bacteria were carried out on LTY-Seawater plates at room temperature. Inoculation was made from the blue-green layer only.

III. Microscopy

Field samples and enrichments were observed under light and epifluorescence microscopy on Zeiss™ Axioplan II microscopes.

IV. Pigment Analysis

Extraction of water soluble phycobilin pigments were performed by sonication of cells suspended in 50 mM phosphate buffer solution (pH 7.3). Subsequently, cells were extracted overnight at 4° C with 7:2 Acetone:Methanol solution to obtain the spectrum of

water-insoluble pigments (chlorophyll a, bacteriochlorophylls, and carotenoids) . Acidification was performed to shift the chlorophyll a peak to distinguish it from the bacteriochlorophyll b peak. Relative pigment concentrations were determined by spectral analysis using a Shimadzu™ UV-3101 PC spectrophotometer.

V. In Situ 16S rRNA Hybridization

Cells from the green layer were washed in sterile, filtered sea water (SFSW) and extracted in 100% methanol until the extractant was colorless. This was an attempt to minimize background fluorescence. The hybridization was performed as directed by Nierzwicki-Bauer (1996). The samples were suspended in 3 mls of phosphate-buffered saline (PBS) (pH 7.4) and vortexed repeatedly with glass beads to disrupt the filaments. The supernatant was removed and placed into an microfuge tube. Samples were microfuged for 8 minutes at 14,000 rpm, washed and resuspended in 0.1% gelatin. The gelatin slurry was spotted (10 spots) onto baked slides and dried at 37° C.

Slides were treated with Ethanol:formaldehyde (90:10) for 45 minutes, rinsed twice with dH₂O and air dried. 16S rRNA oligonucleotide probes were suspended at a concentration of 0.34 ng/μl in hybridization buffer (see below). 40 μl were then spotted onto the cells. The probes used were specific to bacterial ribotypes as follows: universal probe mixture, alpha-proteobacteria, beta-proteobacteria/purple bacteria, delta-proteobacteria/sulfate reducing bacteria, Archaea, high G+C bacteria, low G+C bacteria, flavobacteria, and enteric bacteria. A blank of hybridization buffer was also included as a negative control.

After probing, slides were washed (3 X's 20 minutes) in 1X SET (see below) solution at 37° C, then dried vertically in a dark place until epifluorescence microscopic analysis could be performed. Coumarin, an amine staining compound was included in the probe mixture. This allowed for comparison of staining by coumarin and by fluorescence due to labeled probe binding.

Solutions:

Hybridization Buffer

1.2 ml 33% dextran sulfate
2 ml 10 SET
400 μl 1% polyadenylic acid
400 μl BSA

10X SET Solution

1.5 M NaCl
200 mM Tris Cl
(pH 7.8)
10 mM EDTA

VI. 16S rDNA Analysis

Samples from the green layer were vortexed to remove sediment from the matrix of cells. Tufts of filaments were seized by sterile forceps and washed in several drops of SFSW, then suspended in TE buffer. Similarly, purple sulfur bacteria layers were suspended in SFSW and vortexed. All samples were then centrifuged, the supernatant removed and the pellet resuspended in 50 μ l TE buffer. 15 μ l Gene Releaser™ (5-3 Prime Inc.) was added to extract DNA from the cells following the thermocycler method described in the manufacturer's instructions. PCR amplification was performed with a 'hot start' procedure on an Ericomp Powerblock™ thermocycler using universal 16S forward and reverse primers¹ and cyanobacterial-specific 16S forward and 23s reverse primers². The primers were used at a concentration of 0.6 μ M. 2.5 or 3 mM MgCl₂ were added to the buffer recommended by the manufacturer of the Taq enzyme (AmpliTaq from Perkin-Elmer or Taq from Fisher Products). An incubation of 10 min. at 94° C followed by 5 min. at 35° C to solidify the wax bead was carried out. Then, the enzyme was added (2.5 units/100 μ l) and 35 PCR-cycles were performed (1 min. at 94 ° C, 1 min. at 50° C, 3 min. at 72° C) followed by a last incubation of 7 min. at 72 ° C). The PCR products were purified using the Promega Wizard™ PCR Preps kit and then cloned into the pCNTR shuttle vector using the blunt-end ligation protocol of the General Contractor™ DNA Cloning System (5-3 Prime, Inc.). The plasmids were checked for proper insertions using restriction fragment analysis with BamHI . Plasmid preparations using the alkaline lysis method, chloroform purification protocol (used in the laboratory of M. Sogin) or the Perfect Prep™ kit (5-3 Prime, Inc.) were performed and sequences determined using cycle sequencing with the LI-COR™ automated sequencing system. Partial sequences obtained were compared to existing 16S sequences in the Genbank database using BLAST. The neighbor-joining method was used to construct a distance tree from the sequences. In the case of the cyanobacterial sequences, they were analyzed using the software package TREECON for DOS (Van De Peer and De Wachter, 1993). The sequences were aligned manually with their closest relative. Pairwise evolutionary distances were calculated using the Jukes and Cantor correction for multiple mutations. This distance matrix was used to construct a tree topology by the Neighbor joining method. *Escherichia coli* was used as an

¹ Universal Forward Primer: AGAGTTTGATYMTGGC (From B. Paster)
Universal Reverse Primer: GYTACCTTGTTACGACTT (From B. Paster)

² Cyanobacterial-specific Forward Primer : GAGAGTTTGATYCTGGCTCAG (From B. Paster)
Cyanobacterial-specific Reverse Primer : TCTGTGTGCCTAGGTATCC (Wilmotte et al. 1993)

outgroup. A bootstrap analysis, involving 100 resamplings, was performed. In the case of clones 9 and 7, the program 'rna1' (B. Paster, pers. com.) was used.

Results:

I. Sampling

Sampling of the mats revealed distinct zonation as had been previously described (Nicholson et al. 1987). Distinct zonation by color was found with a green layer tightly bound by polysaccharides above a pink-purple layer which extended down about 1 mm. Below that occasionally was found a lighter, peach-colored layer and below that were dark, reduced iron bands. Only the upper two layers were further characterized in this study.

II. Microscopy

Microscopic analysis of field samples revealed a very wide diversity of microorganisms. The upper, green layer contained mostly pennate diatoms and cyanobacteria. The most conspicuous cyanobacteria were *Microcoleus* sp that formed bundles (Figure 2a). In addition, the sheaths surrounding these bundles were colonized by numerous colorless bacteria and small filamentous cyanobacteria (*Phormidium*-type). In the pink layer, we observed coccoid, purple sulfur bacteria found singly as in *Chromatium* spp. and in tetrads like *Thiopedia* spp (Figure 2b). Additionally, several cyanobacterial species were found in this layer that were rarely observed in the upper layer. These included *Lyngbya* sp., *Spirulina* sp., *Oscillatoria* sp., and other small *Phormidium*-type filamentous cyanobacteria (Figure 2c). No green sulfur bacteria were distinguished by bulk microscopic examination although these have been previously described (Nicholson et al. 1987).

II. Enrichments

Microscopic examination of enrichments for cyanobacteria revealed morphotypes similar to the ones observed in the field samples (Figure 2d). For example, bundles of *Microcoleus* sp., highly motile *Oscillatoria* sp, small *Phormidium*-types and a large *Lyngbya* sp. were present. However, there was not enough time to isolate pure cultures from any of the enrichments due to the slow growth of phototrophs. The cyanobacterial liquid and agar plate enrichments all showed growth after ~2 weeks. They contained mixed cultures of cyanobacteria and diatoms. The liquid and shake tube cultures for sulfide-oxidizing phototrophic bacteria yielded a considerable array of purple and green sulfur bacteria as well as heterotrophs and some cyanobacterial growth was observed as well.

The purple non-sulfur bacterial enrichment plates showed growth of what appeared to be a uniform colony type of phototrophic bacteria as well as several heterotrophic bacterial types. No microscopic examination of non-sulfur enrichments was attempted, as we ran out of time and the non-sulfur bacteria were not the central focus of our study. The LTY-SW enrichments were begun only after sequence data (see below) revealed the presence of gliding bacteria, so there was not enough time for them to grow up by the end of this study.

IV. Pigment Analysis

The spectrum from the extraction of the water soluble pigments in phosphate buffer was determined for the green layer (Figure 3). Peaks were seen at 613 nm (phycocyanin) and 676 nm (Chlorophyll a). After extraction of the green layer with acetone/methanol, the spectrum showed a peak at 617 (phycocyanin) and at 664 nm (Figure 4). The latter peak shifted to 656 nm after acidification, indicating the presence of chlorophyll a (Figure 5). Similarly, spectra from the methanol/acetone extraction of the pink layer both before (Figure 6) and after acidification (Figure 7) were obtained. They showed the presence of bacteriochlorophyll a (771 nm), chlorophyll a (656 nm) and phycocyanin (604 nm). The spectra in methanol/acetone were very similar between the two layers, however the ratio of chl a to bchl a decreased in the pink layer extraction.

V. In Situ Hybridization

The attempt to remove the chlorophyll a from the cyanobacterial cells by extraction in methanol and acetone (K. Hanselmann, pers. com.) failed to remove background fluorescence. This fact made it very difficult to distinguish cyanobacterial fluorescence from that of the fluorescent dye rhodamine used to label the probes. Additionally, the universal probe did not label all the cells that stained with coumarin dye. Further work on this problem would be necessary.

VI. 16S rDNA Analysis

PCR amplification products were sized by electrophoresis on agarose gels (for example Figure 8) and several were cloned (Table 1). The clones were checked by restriction digest with BamHI to determine if an insert of the correct size was present. This was determined by electrophoresis on an agarose gel (for example see Figure 9). In this way, we obtained 13 clones which were deemed suitable for sequencing. Seven clones were used as templates for sequencing and partial sequences obtained.

For one cyanobacterial clone (clone 20), two sequencing reactions were performed; one with M13Rev primer giving an ITS sequence (Fig. 14 e) and one with M13For primer giving the 5' end of the 16S rRNA sequence. A similarity search with BLAST showed this latter sequence to be most closely related (93.2% for 309 bp) to a small filamentous species *Phormidium minutum* D5 (see Figure 10 for alignment). To calculate this percentage of similarity, insertions and deletions were not taken into account because the quality of the sequence was not very good and most of the present indels are probably an artifact. In the distance tree in Figure 11, the bootstrap percentage for the grouping of the two sequences is 58 %, indicating a low statistical support. The sequence obtained for the second cyanobacterial clone (clone 6) corresponded to the 3' end of the 16S rRNA and was most closely related (95.4% for 431 bp) to *Phormidium* sp. VRUC 135 (Figure 12). In the distance tree of Figure 13, the bootstrap percentage is 79 %. Additionally, a sequence most closely related to the chloroplast sequence from the diatom *Skeletonema costatum* was obtained using the cyanobacterial specific primers.

Four heterotrophic bacterial sequences were obtained (Figure 14 a-d). One sequence was found to be most closely related to the cytophagales, *Microscilla aggregans* by a BLAST search. *Microscilla* spp. are commonly found in association with marine plant material as agar decomposers (Reichenbach, 1992). This sequence (Figure 14 a) was aligned with cytophagales sequences in the databank of Dr. Bruce Paster (which unfortunately did not contain *Microscilla* sp.) with the 'rna1' program (Figure 15) and a distance tree constructed by the neighbor-joining method (Figure 16). Additionally, two separate 16S sequences (starting from different ends of the molecule) showed similarity to *Desulphorhopalus vacuolatus* (Figure 14 b, c). One of these (clone 9) was aligned with sulfate reducers in Dr. Paster's databank to generate an alignment (Figure 17) and distance tree (Figure 18). The close relation of clone 9 to a whole group of sulfate reducing bacteria strongly suggests that this represents a SRB sequence. We also obtained a sequence that appeared most closely related to *Frankia* sp. following the BLAST analysis. However, this relationship is not significant because only 81% identity across 144 bp was found. Unless further sequence information is obtained, the phylogenetic affiliation of this sequence remains indeterminate, but can be hypothesized to be an actinomycete.

Discussion:

Sampling

We were surprised at the variability in thickness and diversity of the layers even within a single mat at 10-15 cm distance. We had planned to take several cores from a defined area (about 1 m²) but the pink layer exhibited such a spatial heterogeneity that we decided to scrape the layers from about 10 dm². This probably reflects the highly dynamic nature of such mats, in response to changing environmental parameters. In addition, no clear-cut peach layer was observed under the pink layer, in contradiction to the findings of Nicholson et al. (1987).

Microscopic observations

Contrary to the observations of Nicholson et al. (1987), we found many bundle-forming *Microcoleus* in the green layer of the mat. The "lamination" of the mat was not a perfect one, as we observed cyanobacteria in the pink layer. They were a minor component of the community but nevertheless, the species diversity appeared to be quite great and was different from the one observed in the green layer. Many of these species (e.g. *Spirulina*, *Oscillatoria*) are motile and could migrate up and down in response to environmental factors.

Pigment analysis

From the spectra we obtained, we could not infer the presence of chlorophyll b and c containing organisms like green sulfur bacteria of the genus *Prosthecochloris*. This is in contradiction with previous results from Pierson et al. (1987, 1990). However, these organisms might have been in too low concentrations to be detected. Alternatively this may reflect the spatial and temporal heterogeneity of the mats. Whereas the presence of phycocyanin is conspicuous in both layers, it is surprising not to find phycoerythrin-containing cyanobacteria underneath the layer of phycocyanin-containing ones.

16S rRNA Analysis

Our sample contained a lot of sand grains and it was not easy to separate the cells from the sediment. We performed serial washing of tufts of filaments and thus restricted our analysis to cells associated with these filaments for practical reasons. Therefore, we might have missed the cells attached to sand grains. Additionally, the success of the lysis method depended on several parameters. The ratio of Gene Releaser to TE buffer and the use of the thermocycler instead of microwave to lyse the cells were key factors. The Gene

Releaser method is a mild lysis method, which is probably not be able to efficiently liberate nucleic acids from all taxa in an environmental sample. This may bias our analysis. However, we did not have the time to work out a mechanical lysis method, like using a beadbeater. This would have probably been more efficient to break cells with thick cell walls. On the other hand, we obtained the 16S rRNA sequence of a Gram-positive bacterium.

In our first cloning experiments, we simply purified the PCR products on a column to get rid of the PCR-reagents. As a result, the transformation yield was quite high (several hundred colonies per plate) but our plasmid preparations gave a low percentage of clones with inserts of the expected size. However, the clones which were selected always contained 16S rRNA. In the second cloning experiment, we first submitted the PCR product to electrophoretic separation, cut out the band of the expected size and extracted the DNA on a minicolumn. Only a few white colonies were obtained after transformation and the BamHI digest gave ambiguous results. The bands corresponding to the pUC18 vector size were weaker than the band corresponding to the insert size. These clones were not sequenced and thus the uncertainty remains.

The two cyanobacterial sequences obtained showed similarity to filamentous, phycoerythrin-containing strains with a narrow diameter (~ 2 µm). *Phormidium minutum* is a marine, epiphytic cyanobacterium found in the Balearic islands (Spain). *Phormidium* VRUC 135 was isolated from frescoes in the palace of emperor Nero in Rome, a very low light environment. During our microscopic observations we frequently have observed such narrow filamentous cyanobacteria but their small size hindered precise characterization. Additionally, the presence of sand grains causes the mounts to be too thick for precise focusing. It is noteworthy that one such small blue-green *Phormidium* was observed growing in the sulfide shake tubes inoculated with a green layer sample.

We also retrieved a sequence with high similarity to a *Microscilla* sp. from the green layer. The presence of this sequence, while not anticipated, is logical as this marine glider is commonly isolated from marine benthic algae. Once the sequence was obtained, we looked back at the mat samples which had been stored at 4° C to see if we could identify any such thin, filamentous gliders. Indeed, we found thin filaments that may represent this member of the community, however, perhaps due to cold storage, no gliding motility was observed. Enrichments might yield better results in the future.

The presence of sulfate-reducer sequences in the pink layer sediments also accords with our expectations. Due to the diurnal tidal flux, high levels of sulfate are present in this layer, which is anoxic. Additionally, the phototrophic organisms present in this layer consume sulfide and produce sulfate which may be used by sulfate reducing bacteria. This

"sulfur cycling" would benefit both types of organisms. At night, even the green layer probably becomes anoxic. Therefore, it would be interesting to sample the mat before sunrise and make enrichments for anaerobic bacteria.

The fact that we found a somewhat low (81%) similarity in one of the sequences to *Frankia* sp. suggests that at least, this probably represents some type of actinomycete. The presence of a Gram-positive organism is encouraging, considering the problems we had with the lysis procedure, as the cell walls of Gram-positive organisms are often more recalcitrant to lysis. *Frankia* sp. are known to be involved in symbiotic relationships with plants as well as existing in a free-living state, so perhaps this sequence represents a symbiotic member of the marsh grass community.

The numbers of nucleotides which could be used in the analysis is obviously too small to give a correct picture. The trees obtained in Fig. 14 and 16 differed from previously published cyanobacterial trees where longer sequences (700 to 1500 bp) were used. Depending on the proportion of conserved and variable sites in the blocks of sequence determined, the trees obtained seem to vary substantially.

We are aware that retrieving DNA sequences does not indicate metabolic activity of the corresponding organisms. It is even possible that some DNA was extracted from dead cells or existed as free DNA bound to a mineral matrix. In order to characterize metabolically active organisms, RNA sequences may be more appropriate because there is a relation between translational activity and the ribosome content of cells. Alternatively, sequences from metabolic genes could be used, like the *nifH* gene which encodes the Fe protein of the nitrogenase complex. This gene was amplified directly from a marine cyanobacterial mat in an intertidal lagoonal region of North Carolina. The results suggested that heterotrophic nitrogen-fixing bacteria, anaerobes and aerobes were quite abundant in the mat. A few sequences appeared related to *Azotobacter* and *Klebsiella* spp., but also to clusters containing *Chromatium*, *Desulfovibrio*, and *Clostridium* (Zehr et al. 1995). A possible caveat of this approach is that the possession of a gene does not mean that the gene is actually expressed. To prove this, the mRNA should be used as template for the amplification, but this is probably quite difficult because of the low copy-number and instability of mRNA.

Our original intention was to get clonal isolates from the enrichments and to use them as templates for 16S rRNA amplification. This would have allowed us to compare the diversity of sequences of cultivated versus environmental samples. Moreover, specific 16S rRNA probes could have been designed to perform in situ hybridization. The latter method has the advantage that the binding of the probe depends on the ribosomal content and thus, on the metabolic activity of the cells. Anyway, this would not have been possible for the

cyanobacteria using a rhodamine-labelled probe. However, it is possible that a different fluorochrome could still be visible against the background of naturally red-fluorescing cyanobacteria.

Acknowledgements

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Figure 1 (adapted from Nicholson et al., 1987)

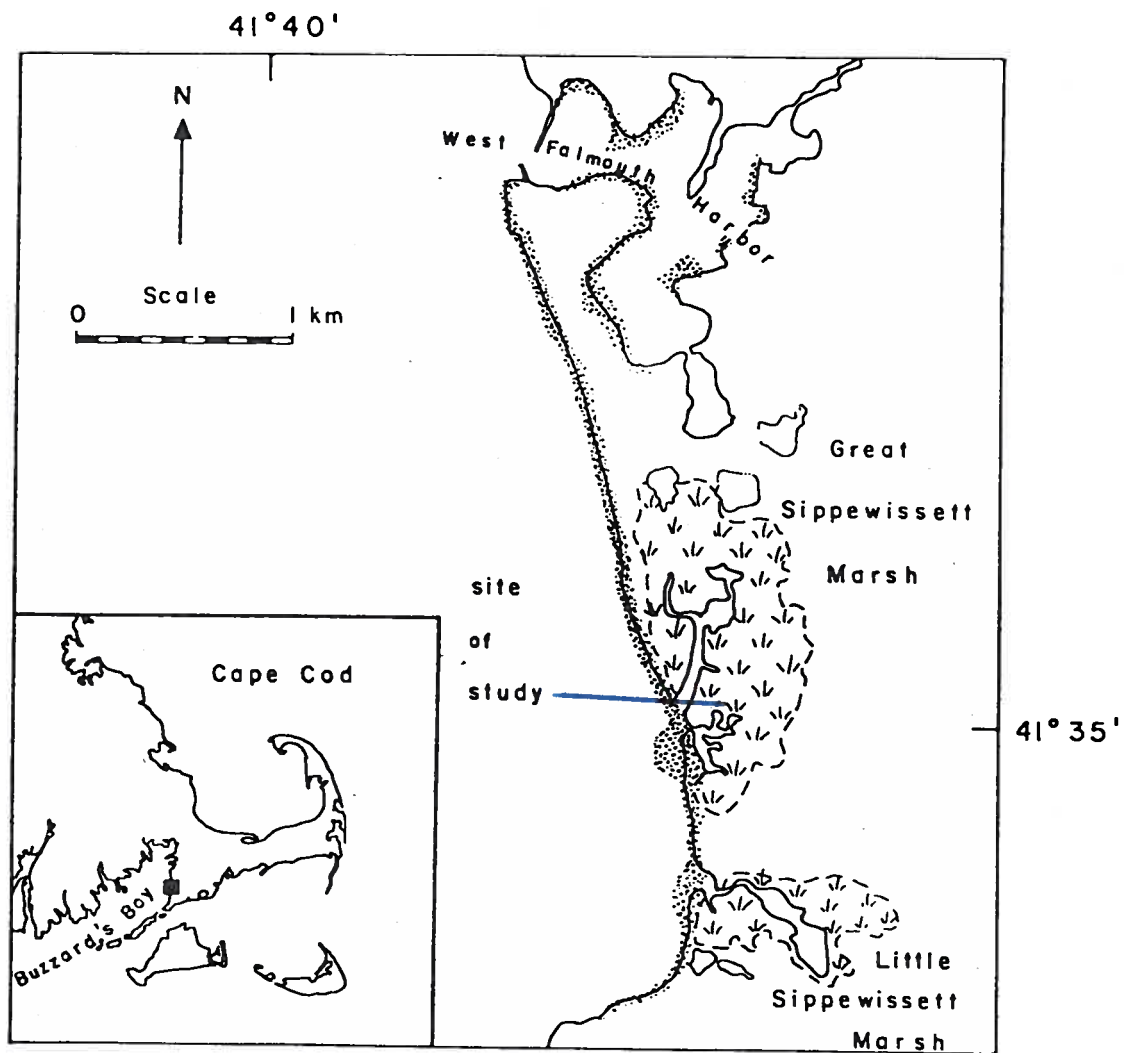
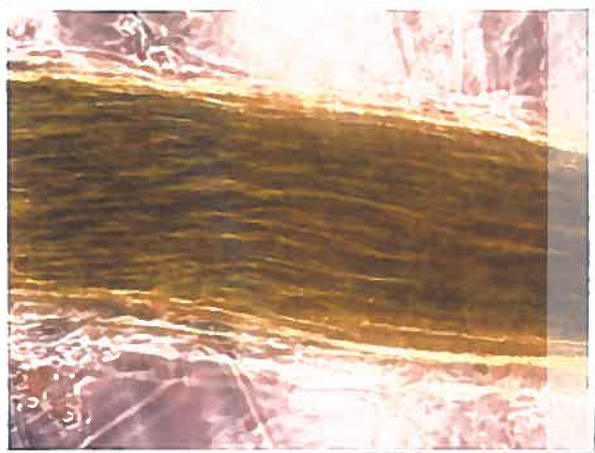


Fig. 1. Location of the microbial mat which was studied in Great Sippewissett Salt Marsh, Cape Cod, Massachusetts. The salt marsh is located on the eastern side of Buzzard's Bay.

Figure 2:

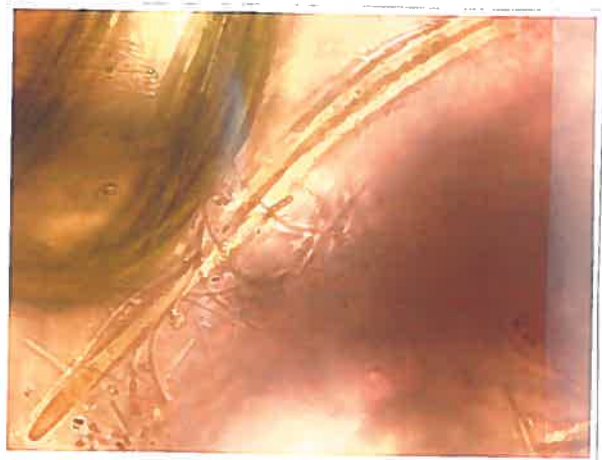


a) *Microcoleus* sp. from environmental sample - green layer

b) *Thiopedia* sp. from pink layer of environmental sample

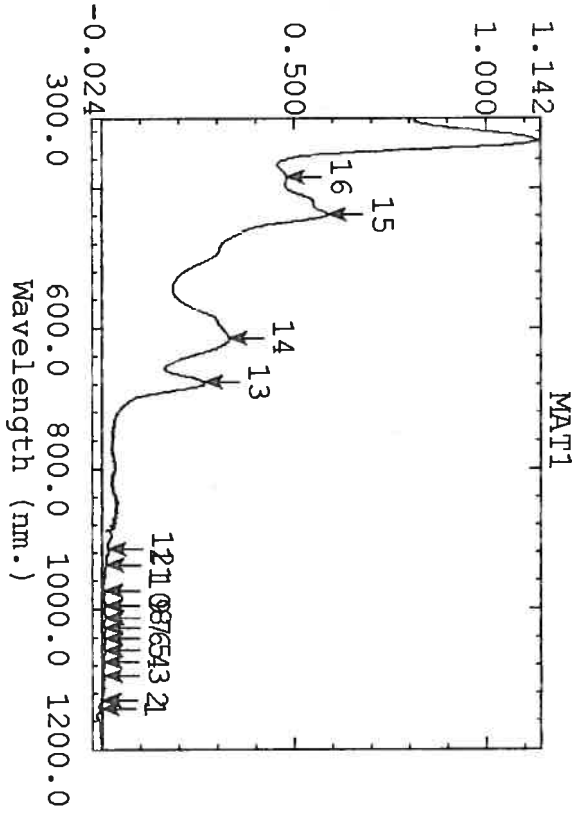


c) Mixed sample from pink layer of environmental sample



d) Mixed sample from cyanobacterial enrichment

Figure 3



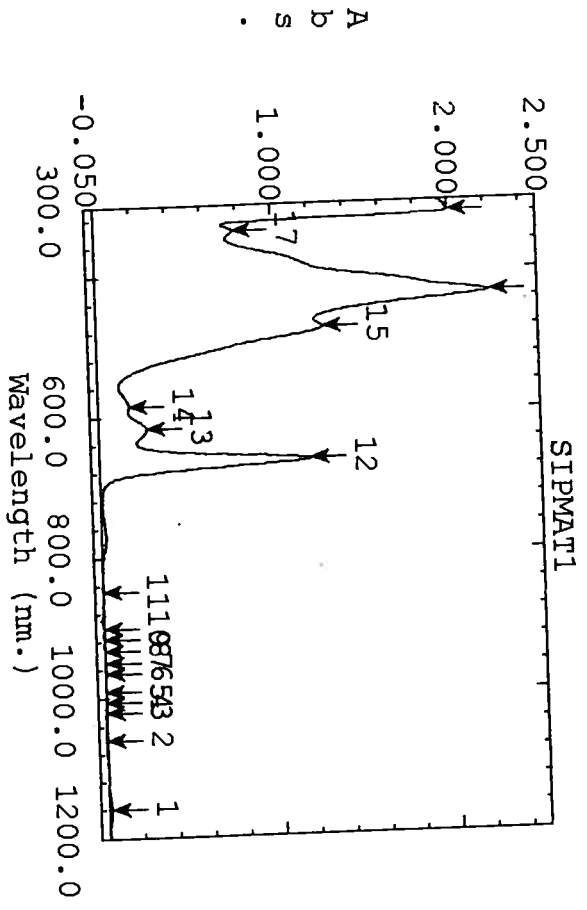
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4	1075.00	0.0077
5	1058.00	0.0090
6	1041.00	0.0096
7	1026.00	0.0103
8	1012.00	0.0108
9	995.00	0.0101
10	974.00	0.0080
11	937.00	0.0119
12	915.00	0.0176
13	676.00	0.2669
14	613.00	0.3325
15	437.00	0.5882
16	384.00	0.4801
17	332.00	1.1305



No.	Wavelength (nm.)	Abs.
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2	1060.00	-0.0043
3	1020.00	-0.0026
4	1006.00	-0.0011
5	991.00	0.0004
6	964.00	0.0034
7	949.00	0.0022
8	933.00	-0.0011
9	916.00	-0.0013
10	901.00	-0.0016
11	849.00	0.0016
12	664.00	1.1967
13	617.00	0.2727
14	584.00	0.1735
15	476.00	1.2789
16	432.00	2.2312
17	336.00	0.7811
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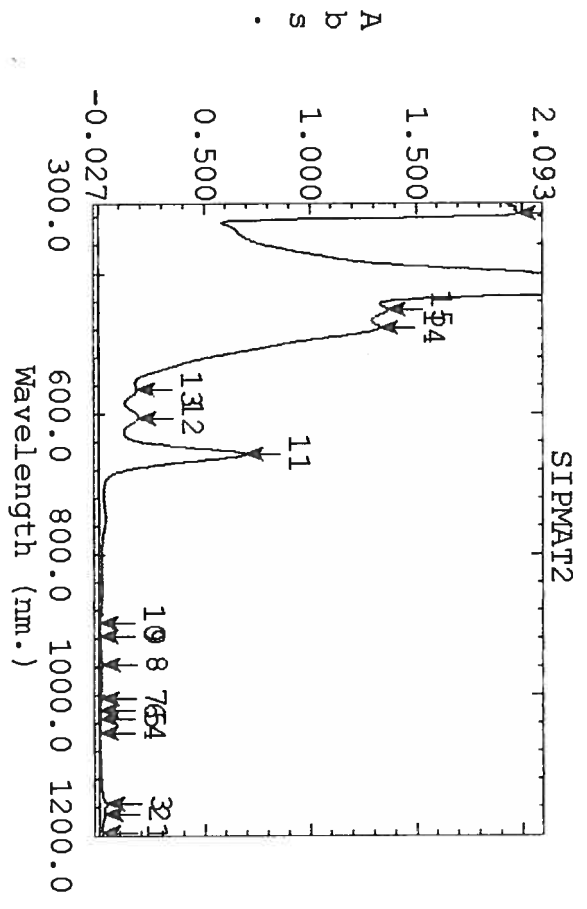
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 green layer Meth/ac 1

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 Sampling Interval: 1.0

A b s .

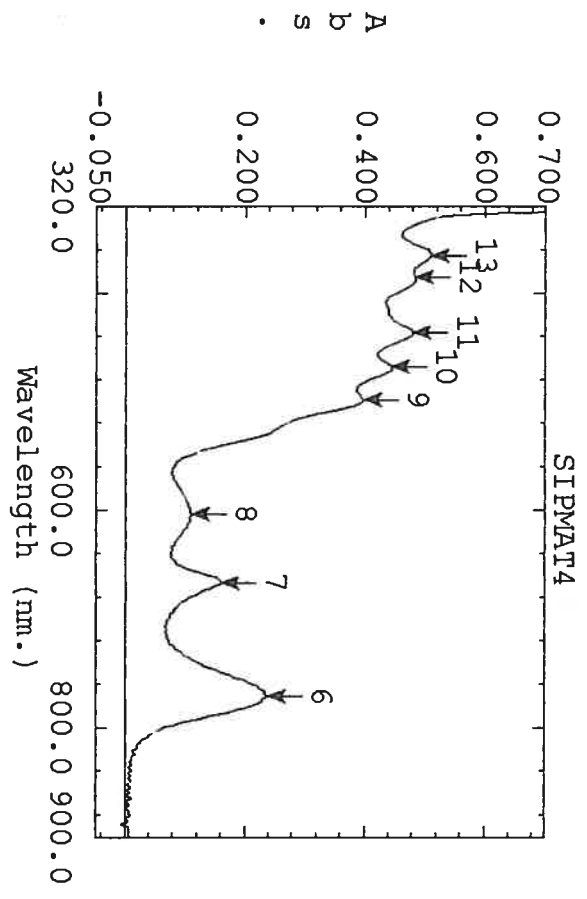
Figure 5



File Name: SIPMAT2
 green layerMeth/Ac + acid
 Created: 08:12 -01/29/80
 Data: Original
 Measuring Mode: Abs.
 Scan Speed: Fast
 Slit Width: 3.0
 Sampling Interval: 1.0

No.	Peak	Wavelength (nm.)	Abs.
1	1	1195.00	0.0138
2	2	1170.00	0.0240
3	3	1154.00	0.0320
4	4	1054.00	0.0022
5	5	1034.00	0.0025
6	6	1021.00	0.0049
7	7	1004.00	0.0066
8	8	956.00	0.0117
9	9	916.00	0.0051
10	10	898.00	0.0048
11	11	656.00	0.6918
12	12	605.00	0.1857
13	13	564.00	0.1785
14	14	477.00	1.3235
15	15	452.00	1.3630
16	16	419.00	3.0176
17	17	315.00	1.9767

Figure 6

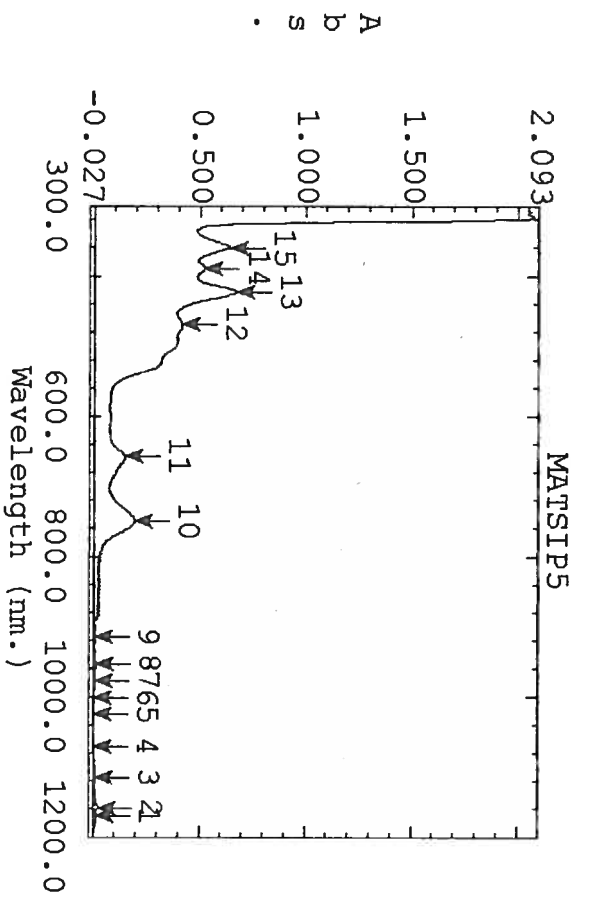


File Name: SIPMAT4
pink layer 1 Meth/Ac

Created: 08:28 01/29/80
Data: Original

Measuring Mode: Abs.
Scan Speed: Fast
Slit Width: 3.0
Sampling Interval: 1.0

No.	Peak Pick	Wavelength (nm.)	Abs.
1		1153.00	0.0068
2		1028.00	0.0017
3		993.00	0.0033
4		960.00	0.0065
5		917.00	0.0097
6		771.00	0.2385
7		667.00	0.1610
8		604.00	0.1102
9		498.00	0.3974
10		468.00	0.4449
11		436.00	0.4812
12		385.00	0.4845
13		365.00	0.5106
14		313.00	1.9937



No.	Wavelength (nm.)	Abs.
1	1170.00	0.0097
2	1158.00	0.0197
3	1114.00	0.0025
4	1070.00	-0.0003
5	1024.00	-0.0006
6	1002.00	-0.0003
7	977.00	0.0037
8	952.00	0.0063
9	915.00	0.0032
10	749.00	0.1906
11	656.00	0.1446
12	468.00	0.4145
13	422.00	0.6680
14	388.00	0.5200
15	359.00	0.6368

File Name: MATSIP5
 green layer 1 Meth/Ac+acid

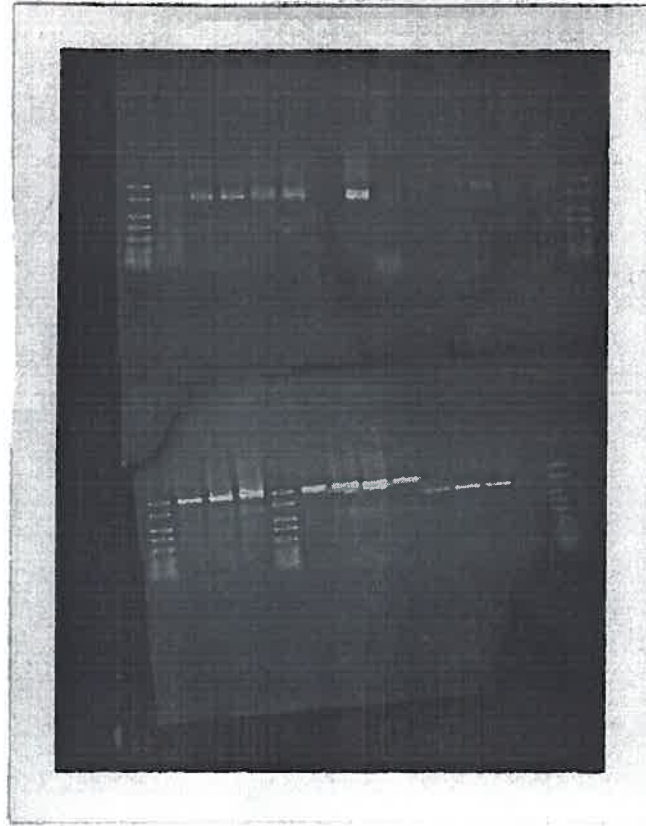
Created: 08:33 01/29/80
 Data: Original

Measuring Mode: Abs.
 Scan Speed: Fast
 Slit Width: 3.0
 Sampling Interval: 1.0

Figure 8 Electrophoresis of PCR products

- 1. PCR marker (Sigma)
- 2. } PCR green layer 1
- 3. }
- 4. }
- 5. } PCR pink layer
- 6. }
- 7. }
- 8. PCR green layer 2
- 9. Blank

Primers: Universal



- 10. } PCR green layer 1
- 11. }
- 12. }
- 13. } PCR pink layer
- 14. }
- 15. PCR marker
- 16. PCR marker
- 17. }
- 18. } PCR green layer 2
- 19. }

Primers: Cyano Specific

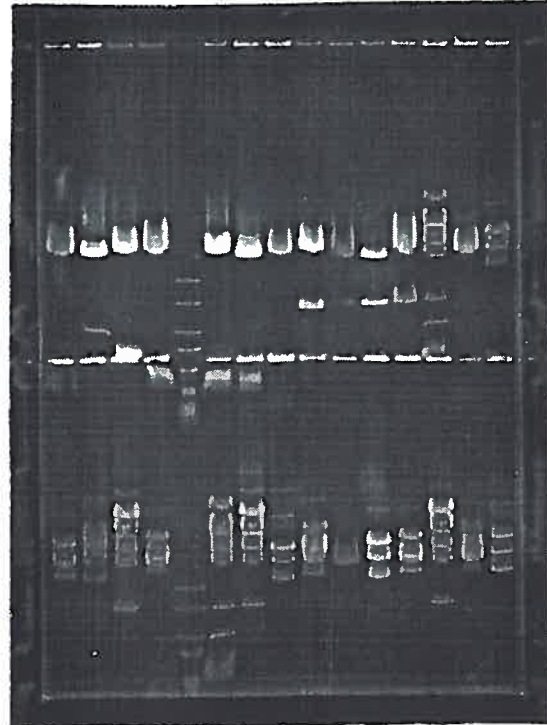
1/10 of the PCR-reaction volume loaded on 1% agarose gel

Figure 9 Electrophoresis of Bam HI digests of plasmid DNA

- 1. plasmid 2
- 2. plasmid 3
- 3. plasmid 4
- 4. plasmid 5
- 5. PCR marker
- 6. plasmid 6
- 7. plasmid 7
- 8. plasmid 8
- 9. plasmid 9
- 10. pUC 18
- 11. plasmid 11
- 12. plasmid 12
- 13. plasmid 13
- 14. plasmid 14
- 15. plasmid 15

green layer 1
Cyano Specific primers

green layer 2
Cyano Specific primers



- 16. plasmid 16
- 17. plasmid 17
- 18. plasmid 18
- 19. plasmid 19
- 20. PCR marker
- 21. plasmid 20
- 22. plasmid 21
- 23. plasmid 22
- 24. plasmid 23
- 25. pUC 18
- 26. plasmid 24
- 27. plasmid 25
- 28. plasmid 26
- 29. plasmid 27
- 30. plasmid 28

green layer 1
Universal primers

green layer 2
Universal primers

pink layer
Universal primers

pink layer
Universal primers

Plasmids 11, 12, 18, 21 and 26 were O.K.

Figure 10

309 characters

	10	20	30	40	50	60
P.m.	GGGGG-ACAACAGUUGGAAACGACUGCUAAUACCGCAUA--UGGC-----GAGA----G					
M.c.	GAGGG-AC-ACA-UU-G-AACG-CUGC UAAUACCCCAUA--UG-U-----CUAC----G					
	70	80	90	100	110	120
P.m.	CUAAAA-GA-----UUUA-----UUGCCUGAGGAUGAACUCGCGUCUGA					
M.c.	AUGAAA-GA-----UUUUA-----UCGCCUGAGGAUGA-CUCGCGUCUGA					
	130	140	150	160	170	180
P.m.	UUAGCUAGUUGGGG-GUGUAAUGGACUCCCAAGGCGACGAUCAGUAGCUGGUCUGAGAGG					
M.c.	UUAGCUAGUUGUGA-GGGUAAUAGCUCACCAAGGCGACGAUCAGUAGCUGGUCUGAGAGC					
	190	200	210	220	230	240
P.m.	AUGAUCAGCCACACUGGGACUGAGACACGGCCCAGACUCCUACGGGAGGCAGCAGUGGGG					
M.c.	AUGAUCAGCCACACU-GGACUGAGACACGGCCCAGACUC-UAC-GGAGGCAGCAGUGGGG					
	250	260	270	280	290	300
P.m.	AAUUUCCGCAAUGGGGGCAACCCUGACGGAGCAACGCCGCGU-GGGGGAGGAAUGUC-U					
M.c.	AAUUUCCGCAAUGGGGGCAACCCUGACGGAGCAUA-CCGCGU-GCGG-AG-AC-G-C-U					
					
P.m.	GUGGAUUGU					
M.c.	GUGG-UUGU					

P.m. = *Phormidium minutum* D5
M.c. = Mat cyanobacterial clone 20

Distance 0.1

Figure 11

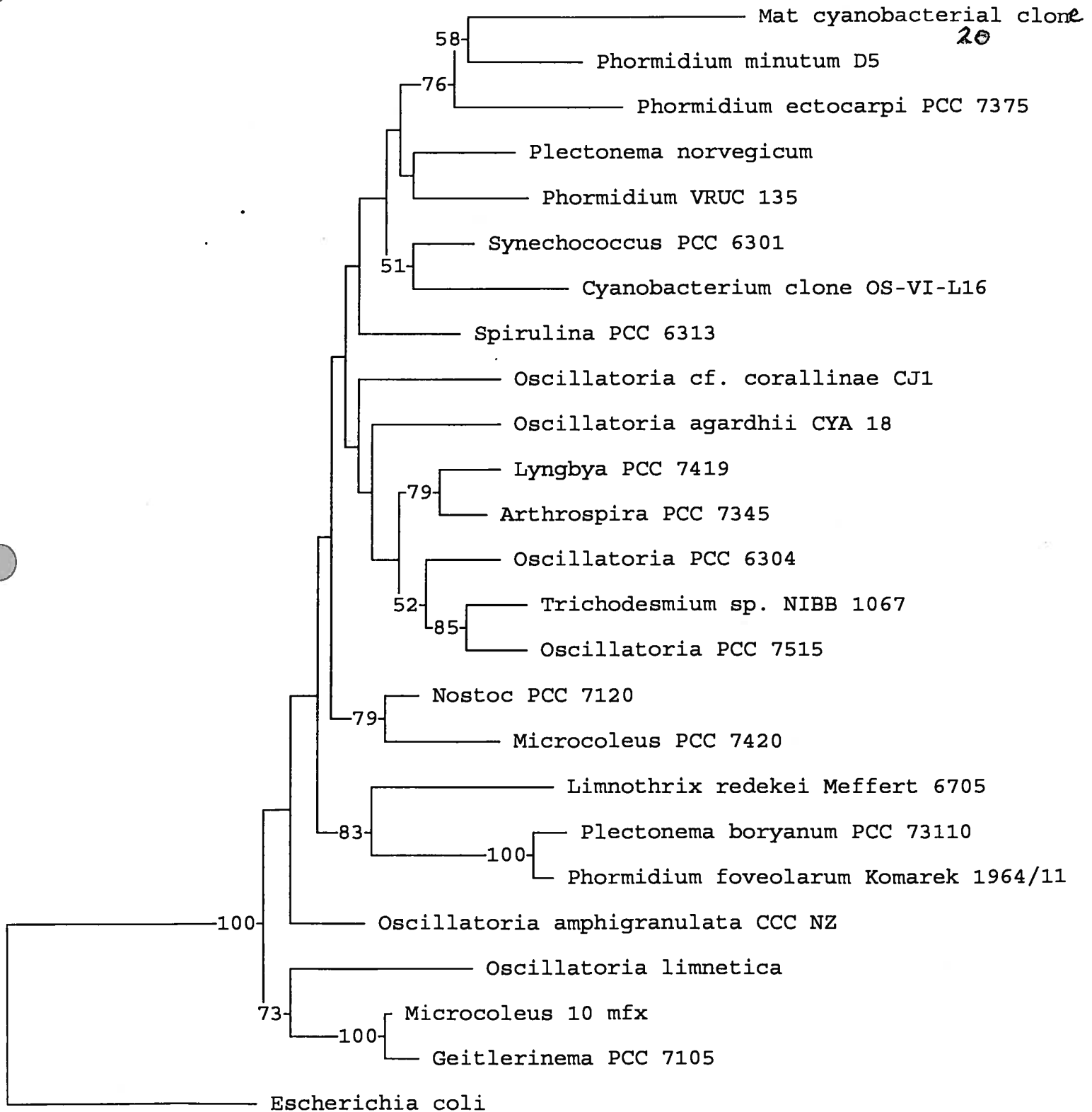


Figure 12

341 characters

```
      10      20      30      40      50      60
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
L.V.  AGGAAGGUGUGGAUGACGUCAAGUCAGCAUGCCCCUACGUCCUGGGCUACACACGUACU
M.6.  AGGAAGGUGUGGAUGACGUCAAGUCAUCAUGCCCCUACGUCCUGGGCUACACACGUACU

      70      80      90      100     110     120
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
L.V.  ACA AUGCUUCGGACAAAGGGUCU-GCGAGCCAGCGA-UGGCAAGCCAAUCCC-AUAAACC
M.6.  ACA AUGCUUCGGACAAAGGG-CA-GCCAGACCGCGA-GGUUGAGCUAAUCCC-AUAAACC

      130     140     150     160     170     180
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
L.V.  GAGGCUCAGUUCAGAUUGCAGGC-UGCAACUCGCCUGCAUGAAGGCGGAAUCGCUAGUAA
M.6.  GAGGCUCAGUUCAGAUUGCAGGC-UGCAACUCGCCUGCAUGAAGGAGAAAUCGCUAGUAA

      190     200     210     220     230     240
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
L.V.  UCGCAGGUCAGC--AUACUGCGGUGAAUACGUUCCCGGGCCUUGUACACACCCGCCCGUCA
M.6.  UCGCAGGUCAGC--AUACUGCGGUGAAUACGUUCCCGGGCCUUGUACACACCCGCCCGUCA

      250     260     270     280     290     300
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
L.V.  CACCAUGGGAGUUGGCCACGCCCGAAGUCGUUAC-UCCAACCA---UUCG-UGGAGGAGG
M.6.  CACCAUGGGAGUUGNCCACGCCCGAAGUCGUUAC-UCCAACC----UUA--GGGAGGAGG

      310     320     330     340
.....|.....|.....|.....|.....|.....|.....|.....|.....|.
L.V.  AUGCCGAAGGCAGGGCUGAUGACUGGGGUGAAGUCGUAACA
M.6.  ACGCCGAAGGCAGGGCUGAUGACUGGGGUGAAGUCGUAACA
```

L.V. = Leptolyngbya VRUC 135

M.6. = Mat clone 6 (green layer)

Figure 14

A) Clone 7 - Microscilla-like

ACGCACAGCTAGCAGGTATTAACtACTAACCTTTCCTCACAACCTGACAGTGCTTTACAACCCGAAGGCCTTCT
TCACACACGCGGCATGGCTANATCACGCTTGCgCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGA
GcTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGATCATCTCTCAAACCAGCTAGGGATCGTCCGCTTGGTGA
GCCATTACCTCACCAACTAGCTAATCCCACCTTGGGCCAATcTAAAGGCGAGAGCCGAAGCCCCCTTGGTCC
GTAGACATTATGCGGTATTAGCCATCGTTTCCAATGGTTGTCCCCCACCTAAAGGCATGTTCCCAAGCATT
CTcACCGTCCGCCGCTCGACATCTTCTAGTAAACTAGAAATGTTAcCGGTCGACTTGCATNATTTAGGCCTg
CCGcCAGCGTTCAATCTGAGCCATGATCAA

B) Clone 8 - SRB-like

GTCGTTACCTTGTACGCTTCACCCAgTAMgAGCATACTTGGAGCTgATcTTcNGAAAGTTNGcTCAACTAcTT
CTGGtANGCCAACCTNCCGTGGTGTGACGgGcNGGTGTGTGcAAGGNCCNGaACGTATTCAgcGAGGCATGTT
GtaTCCACGATTACTACGCGATTCCAACtTcAcTGCGTCGAGTTGCAGACTCCAATCCGGGACTGAGACATGT
TTTACGGGATTTCgTCCTTATCGCTAAGTGGCTGCCCTTGTACATGCCATTGTAGTACGTGTGTAGCCCTGA
TCATAAAGGCCATGAGGACTTGACGTCAT

C) Clone 9 - SRB-like

ATCCGTCAGAGTTTGATCCTGGCTCAGAACGAACGCTGcGCGGCGTGCTTAACACATGCAAGTCGAACGCGA
ACgGTCTCTTCGGAGACTTAGTAGAGTGGCGCACGGGTGAGTAACGCGTAAGTaATCTGCCCTCGCATTCCG
AATAACCCACCGaAAGGTGTNGCTAATAGCKKATACGTCTGaATTAATACCTNT
TATTCAGAGAAAGATAGtCTCTGTTTCAAGCTATTGTGCGAGGAGGAGCTTGCGTACCATTAGCTAGTAGGT
AGGGTAATGGcCTAcCTAGGCAACGATgGTTAGCGcGTCTGAGAGGATGATCCGCCACACTgGA

D) Clone 5 - Actinomycete-like

GTCAGAGTTTGATCCTGGCTCAGGGTGAACGCTGGCGGTGCGCCTAATACATGCAAGTCGAGCGA
GAGCTAGACGAAGTTTACTTTGTTTAGTAAAAGCGGCGGACGAGCGAGTAACACGTAAGCATCTG
CCCCGTACTCAGGGATAACACCATGAAAGTGGTGCTAATACCGGATGGCCCCGAAGGTAAAGATT
TATCGGTACGgGAGGAGCTTGCGgCTTATCAGCTTGTGGTGGGGTAAAGGCCTACCAAGACTATG
ACGAGTAGCTGGTGTGAGAGCATGACCAGCCGCGATGGAACCTGAGACACGGTCCATACTCCTACG
GAG

D) Clone 20 - Spacer region

CTAGGTATCAGNNTAAGNCTTTGTAGCTTGATCTGTTTCTTGGTGGCTCTTTGCTTTTAGAGTCGATAATGTC
TCTCGACACTATCTTCTACCTGCTTTTTTTATCGTTATGTTGTTGTCAAGGTTCTTGCTGAACTCAACATCCAG
CAGCCTAAGCTCTATCTCCTTTTCCAATAACAGTAAAAGGAAATCCTAGGGTGCTTAATTCTAATCCATGGA
GGTAAGCGGACTCGAACCGGTGACATCCTGCTTGCAAAGAGGGCGTTCTACCAACTGAGCTATACCCCCTCT
TTTAGTGGGCCATCCTgGGACTCGAACCCAGGGATCT

Figure 13

Distance 0.1

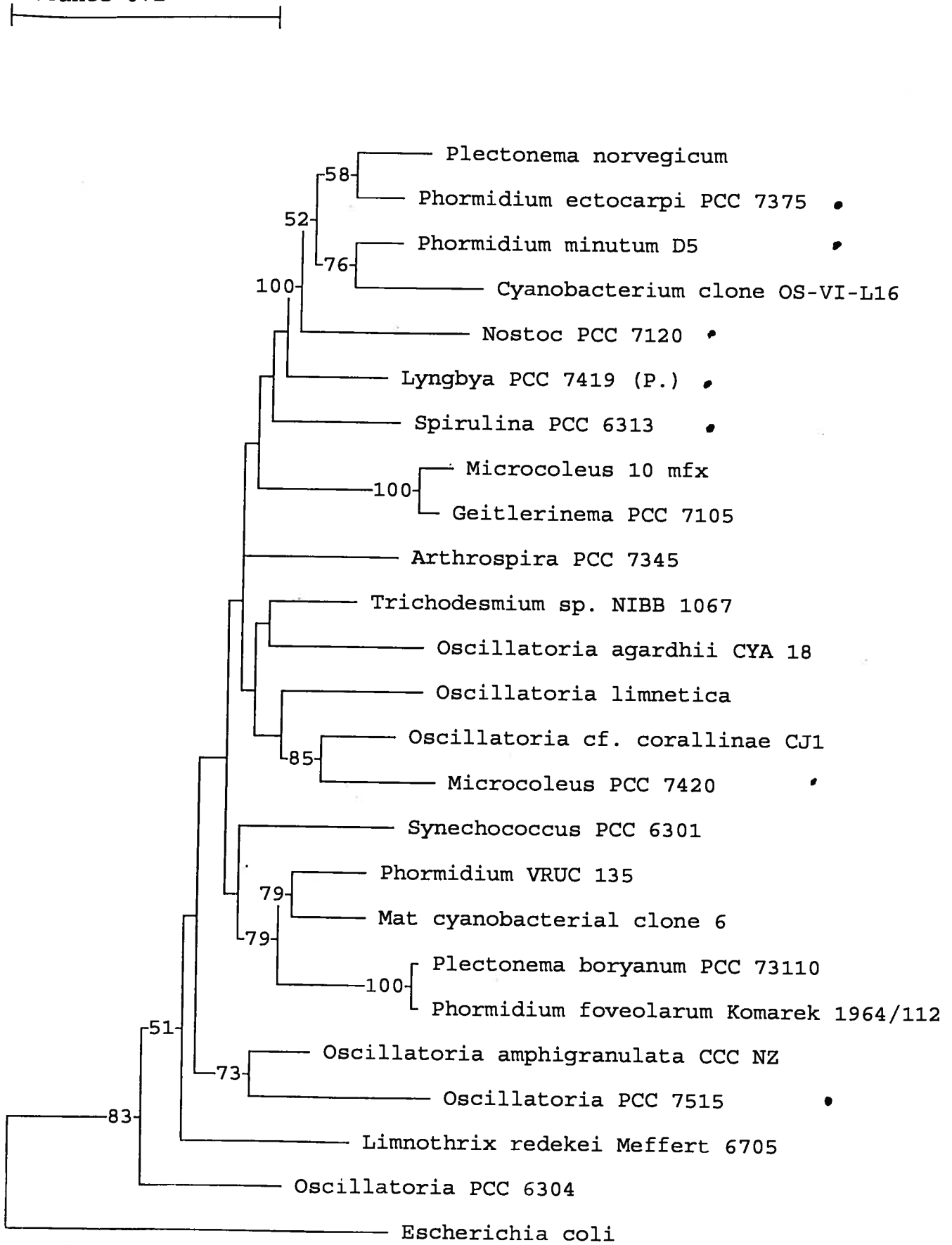


Figure 15 (6 pages)

15:14:06 07-30-1996

MBL Clone #7

Flectobacillus glomeratus ATCC 43844
Vesiculata antarctica ATCC 49675
Cytophaga uliginosa ATCC 14397
Cytophaga lytica ATCC 23178
Flavobacterium gondwanense DSM 5423, ACAM 44
Cytophaga johnsonae ATCC 17061
Capnocytophaga ochracea ATCC 33596 = Holt 25
Capnocytophaga sputigena ATCC 33612 T
Bacteroides fragilis (ATCC 25285 T)?

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Cytophaga johnsonae ATCC 17061
Capnocytophaga ochracea ATCC 33596 = Holt 25
Capnocytophaga sputigena ATCC 33612 T
Bacteroides fragilis (ATCC 25285 T)?

Figure 15 (5 pages)

0 1 0' 2 3 4 5

-----GAGUUUGAUCAUGGCUCAGGAUGAACGCUAGCGGCAGGNCUAACACAUGCAaGUCGAGGGGUAA--CAG

nUACAAUGAA C n C -CA

nUACAAUGAA C n CU -CA

aCGAUGAA C n C U

ACGAUGAA C n CU A U

nUACGAUGAA C n CU -

UAACGAUGAA C n C G -

UAACGAUGAA C n C AG -G

UUACAACGAA C UA CU C UCAGG A

10 20 30 40 50 60 70

5 5' 6 7 8

GGaAGCUUGCUUNCACU--GACGACCGGCACCGGUGAGUAAACGCGUAUACAAUCUACCUCUAACUGGGGGAUAGCCC

. G nnnn . CGCUG C G C n U U A A U

UUGU ACAG . U C G C U U A A U

. U AC . gA . G a C C G C U U

A . G CUUG GC C C G UAC AA

U . GU ACCAGA . G C C G A U AA A

AAGUCU . C . GGCUUUG -A C G U C A A

UU . CUU . C . GG . AAC AGA U C G U C

UU . CCU . C . GG . AAC -AA C G U C AUA

. A U UU GCU G A C C G CU U C U

80 90 100 110 120 130 140 150

8 8' 9 9' 10 10

GNAGAAAUUCGGAUAAUACCCCAUAGUAUAAAGAAUUC-GCAUGUUUUCUUUAUUA AAAU-----UUCG-----

UU GAA G UG C G CAAG . U . nA GA UA

UU GAA G UG C G CAAG . U . A GA UA

. U C UU U CC . UAUUACG CG . A . UA . GG G G . UA

AG U C UU U UUUUA . G CA AAA A G UA

AG U Un U UAG . GYG CAA GA A G UA

. A U UA G U GGACG CAC UR A G CA A

. A U UA G U G . GCG CG U A GC CU

UUC GAAA G C U U . C G A GG A U

160 170 180 190 200 210

7' 11 11' 12

-GUGGUUAGAGAUGAGUAUGCGUCCUAUUAGUUAGUUGGUGAGGUAACGGCUCACCAAGACAGCGAUAGGUAGGGGGUCU

. UC A . A GC n U n U UC UC

. UC A . A GC A U U U UC UC

. -C AG . gn C C U C G G CC

. -C GUA C U A U UA G n CC

. -C A . U C C U A U U G A UC

. AC G . A C C n A U G CA G UC

. G . A U C A C G UAU A U

. G . A U C A nn C G GA A U

. CC A . AG G . G U . C G . U C C UU G . A U

220 230 240 250 260 270 280 290

12' 6' 13 13' 14 14' 4' 15

GAGAGGAUGAUCCCC-ACACUGGUACUGAGACACGGAC CnGACUCCUACGGGAGGCAGCAGUGAGGAAUAUUGGGCAAUG
 GA C CAGACUCCUACGGGAGGCAGCAGUGAGGAAUAUUGGACAAUG
 GA C CAGACUCCUACGGGAGGCAGCAGUGAGGAAUAUUGGACAAUG
 gg C CAGACUCCUACGGGAGGCAGCAGUGAGGAAUAUUGGACAAUG
 GG C CnGACUCCUACGGGAGGCAGCAGUGAGGAAUAUUGGACAAUG
 GA C CAGACUCCUACGGGAGGCAGCAGUGAGGAAUAUUGGACAAUG
 GA C CAGACUCCUACGGGAGGCAGCAGUGAGGAAUAUUGGACAAUG
 qAUG c U CAGACUCCUACGGGAGGCAGCAGUGAGGAAUAUUGGACAAUG
 GAUG C U CAGACUCCUACGGGAGGCAGCAGUGAGGAAUAUUGGACAAUG
 A.G C U A U CAAACUCCUACGGGAGGCAGCAGUGAGGAAUAUUGGACAAUG

300 310 320 330 340 350 360 370

15 15' 3' 16 16' 17

GAGGAGACUCUGACCAGCCAUGC CCGCGUGUAGGAAGAAUGCCCUAUGGGUUGUAAAACUACUUUAUAACAGGAAGAAACA
 GAGGCAACUCUGAUCCAGCCAUGC CCGCGUGUAGGAAGAAUGCCCUAUGGGUUGUAAAACUACUUUAUAACAGGAAGAAACA
 GgCgGGAGCCcGAUCCAGCCAUGCcGcGUGCAGGAAGAAUGCCCUAUGGGUAGUAAAACUGCUUUUAUAACGGGAAGAAAAA
 GAGGACUCUGAUCCAGCCAUGC CCGCGUGCAGGAAGACGGUCCUAUGGAUUGUAAAACUGCUUUUAUAACAGGAAGAAUAA
 GGAGCAAUCCUGAUCCAGCCAUGC CCGnGUGCAGGAAGACUGCCCUAUGGGUUGUAAAACUGCUUUUAUAACGGGAAGAAAAA
 GACGCAAGUCUGAACCAGCCAUGC CCGCGUGCAGGAUGACGGUCCUAUGGAUUGUAAAACUnCnUUUUGUACGAGAAGAAACA
 GUCGGAAGACUGAUCCAGCCAUGC CCGCGUGCAGGAUGACUGCCCUAUGGGUUGUAAAACUGCUUUUAUAAGGGGAAGAAUAA
 GUCGGAAGACUGAUCCAGCCAUGC CCGCGUGCAGGAUGAAGGUcCUaUGGAUUGUAAAACUGCUUUUAUAAGGGGAAGAAUAA
 GGCGCUAGCCUGAACCAGCCAAGUAGCGUGAAGGAUGAAGGCUCUAUGGGUCGUAAAACUUCUUUAUAUAAGAAUAAAG-

380 390 400 410 420 430 440 450

17 17' 18 xxx yy xxxyy 18'

--CUnnUA--UGUA-UACCAG--CUUGACGGUACUGUAAGAAUAAGGACCGGCUnACUCCGUGCCAGCAGCCnnnnnnAU
 --CUGGUA--UGUA-UACCAG--CUUGACGGUACUGUAAGAAUAAGGACCGGCUAACUCCGUGCCAGCAGCCGnnnnAU
 --GGGCUA--CGUG-UAGCCU--ACUGACGGUACCGUAAGAAUAAGGACCGGCUAACUCCGUGCCAGCAGCCGCGGUAUU
 --GACUA--CGUG-UAGUCU--GGUGACGGUACUGUAAGAAUAAGGACCGGCUAACUCCGUGCCAGCAGCCGCGGUAUU
 --GGUUCA--CGUG-UGAACU--GUUGACGGUACCGUAAGAAUAAGGACCGGCUnnnnnnnnnnnnnnnnnnnnnGGUAAU
 --CUCCUA--UGUA-UAGGAG--CUUGACGGUACUGUAAGAAUAAGGACCGGCUAACUCCGUGCCAGCAGCCGCGnnnnAU
 --ng-CUA--CGCG-UaGUUU--GAUGACGGUACCGUAAGAAUAAGCAUCGGCUAACUCCGUGCCAGCAGCCGCGGUnnAU
 --GGAGUA--CGUG-UACUUU--GAUGACGGUACCGUAAGAAUAAGCAUCGGCUAACUCCGUGCCAGCAGCCGCGGUnnAU
 --UGCAGUA--UGUA-UACUGU--UUUGUAUGUAUUAUAUGAAUAAGGAUCGGCUAACUCCGUGCCAGCAGCCGCGGUAUU

460 470 480 490 500 510 520 530

18' 2' P18 P19

ACGGAGnGUCcNAGCGUUAUCCGGAAUCAUUGGGUUUAAAGGGUnCGCAGGCGGUCAAUUAAGUCAGAGGUGAAAUCCnG
 ACGGAGGGUCCnAGCGUUAUCCGGAAUCAUUGGGUUUAAAGGGUcNcCAGGCGGUCUAUUAAGUCAGAGGUGAAAUCCCA
 ACGGAGnGUCcNAGCGUUAUCCGGAAUUAUUGGGUUUAAAGGGUCCGUAGGCGGcGCCGAUAAGUCAGGGGUGAAAGUUUG
 ACGGAGnGUCcNAGCGUUAUCCGGAAUUAUUGGGUUUAAAGGGUCCGUAGGCGGCGUGUUAAGUCAGGGGUGAAAGUUUG
 nnGGAGGGUCCAAAGCGUUAUCCGGAAUCAUUGGGUUUAAAGGGUCCGUAGGCGGGAACAUAUCAGUCAGUGGUGAAAGUUUG
 ACGGAGnAUCcNAGCGUUAUCCGGAAUCAUUGGGUUUAAAGGGUCCGUAGGCGGUUUAGUAAGUCAGUGGUGAAAGCCCA
 ACGGAGGAUGCGAGCGUUAUCCGGAAUCAUUGGGUUUAAAGGGUCCGUAGGCGGCUAAUAAGUCAGGGGUGAAAUCGUU
 ACGGAGGAUGCGAGCGUUAUCCGGAAUCAUUGGGUUUAAAGGGUCCGUAGGCGGCGUGUAAGUCAGAGGUGAAAGCGCU
 ACGGAGGAUCCGAGCGUUAUCCGGAAUUAUUGGGUUUAAAGGGAGCGUAGGUGGACUGGUUAAGUCAGUUGUGAAAGUUUG

540 550 560 570 580 590 600 610

P19' 20

UCGCUCAACGACGGnACUGCCUnUGAUACUGGUUGACUUGAGUCAUAUGGAAGUAGAUAGAAUGUGUAGUGUAGCGGUnA
 UAGCUCUAACUAUGGAACUGCCUUGAUACUGGUUGACUUGAGUCAUAUGGAAGUGGAUAGAAUGUGUAGUGUAGCGGUGA
 GUCUCAACUGUAAAUUGCCUUGAUACUGUCgGUCUUGAGUUAUAGUGAAGUUGCCGGAAUAGUAGUGUAGCGGUGA
 GUCUCAACUGUAAAUUGCCUUGAUACUGAUGGUUUGAAUUAUUGAAGUGGUUAGAAUAGUAGUGUAGCGGUGA
 CGGCUCAACCGUAAAUUGCCAUGAUACUGUUGUUCUUGAGUGCUUGUGAAGUGGUUAGAAUAGUAGUGUAGCGGUGA
 UCGCUCAACGGUGGAACGGCCAUGAUACUGCUGAACUUGAAUACUGGGAAAGUAACUAGAAUAGUAGUGUAGCGGUGA
 CAGCUCAACUGAGCAACUGCCUUGAAACUGUUGGUUCUUGAAUGGUUGUGAAGUAGUUGGAAUUGUGUAGUGUAGCGGUGA
 UAGCUCUAACUAAGCAACUGCCUUGAAACUGUCAGUCUUGAAUGAUUGUGAAGUAGUUGGAAUUGUGUAGUGUAGCGGUGA
 CGGCUCAACCGUAAAUUGCAGCUGAUACUGUCAGUCUUGAGUACAGUAGAGGUGGGCGGAAUUCGUGGUGUAGCGGUGA

620 630 640 650 660 670 680 690

20' 19' 2

AAUGCAUAGAGAUUACACAGAAUACCGAUUGCGAAGGCAGnCUACUACGUAUGUACUGACGCUCUAUG-GACnAAAGCGUn
 AAUGCAUAGAUUUACACAGAAUACCGAUUGCGAAGGCAGUCCACUACGUAUGUACUGACGCUGAGG-GACGAAAGCGUG
 AAUGCAUAGAUaUUACAUAGAAACCCGaUUGCGAAGGCAGGUGACUAACUAAUACUGACGCUGaUG-GacnaAAGCGUG
 AAUGCAUAGAUUUACAUAGAAUACCGAUUGCGAAGGCAGAUACUAACA AUUGAUUGACGCUGAUG-GACGAAAGCGUG
 AAUGCAUAGAUUUACUCAGAAUACCGAUUGCGAAGGCAGAUACUAACA AUUCACUGACGCUGAUG-GACGAAAGCGUA
 AAUGCUUAGAUUUACAUGGAAUACCAUUGCGAAGGCAGGUUACUACcnnnnUAUUGACGCUGAUG-GACnAAAGCGUG
 AAUGCUUAGAUUUACAAGAACACCGAUAGCGAAGGCAUUAUUAACA AUUUAUUGACGCUGAUG-GACGAAAGCGUG
 AAUGCUUAGAUUUACAAGAACACCGAUAGCGAAGGCAUUAUUAACA AUUUAUUGACGCUGAUG-GACGAAAGCGUG
 AAUGCUUAGAUUAACAAGAACUCCGAUUGCGAAGGCAGCUCACUGGACUGCAACUGACACUGAUG-CUCGAAAGUGUG

700 710 720 730 740 750 760 770

1 21' P21-1 P21-2 P21

GGGAGCGAACAGGAUUAGAUACCCUnGUnGUCCACGCGGUA AACGAUGGAUACUnGUUGUUGGGC- - - - AUUA- - - - GC
 GGGAGCGAACAGGAUUAGAUACCCUGGUAGUCCACGCGUA AACGAUGGAUACUAGUUGUUGGGA- - - - UUUA- - - - UC
 GGGAGCGAACAGGAUUAGAUACCCUGGUaGUCCACGCcGUA AACGAUGGAUACUAGCUgUCCGGGUC- - - - CUUGa- - - - ggCc
 GGGAGCGAACAGGAUUAGAUACCCUGGUUnGUCCACGCGUA AACGAUGGAUACUAGCUGUUGGGAU- - - - UUCG- - - - AUC
 GGGAGCGAACAGGAUUAGAUACCCUGGUAGUCUACnCCGUA AACGAUGGUUACUAGCUGUUCnGACU- - - - AAUUGC- - - - nGUC
 GGGAGCGAACAGGAUUAGAUACCCUGGUAGUCCACGCGU AACGAUGGAUACUAGCUGUUGGGC- - - - GCAA- - - - GU
 GGGAGCGAACAGGAUUAGAUACCCUGGUAGUCCACGCGU AACGAUGGAUACUAGCUGUUGGGA- - - - GUAA- - - - UC
 GGGAGCGAACAGGAUUAGAUACCCUGGUAGUCCAUUGCUGU AACGAUGGAUACUAGCUGUUGGGA- - - - GCAA- - - - UC
 GGUUAUCAAACAGGAUUAGAUACCCUGGUAGUCCACAGU AACGAUGAAUACUCGCGUUGGCG- - - - AUUAUC- - - - AG

780 790 800 810 820 830 840 8

-2' P21-3 P21-3' P21-1' P18' 22 22' 1' 23

UCAGUGACUnAGCGAAAGUGAUAAAGUAUCCACCUGGGGAGUACGGUCGCAAGACUGAAACUCAAAGGAAUUGACGGGn
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 UGAGUGGCUAAGCGAAAGUGAUAAAGUAUCCACCUGGGGAGUACnUUCGCAAGAAUGAAACUCAAAGGAAUUGACnGn
 UCAGUGGCUAAGCGAAAGUGAUAAAGUAUCCACCUGGGGAGUACGAACGCAAGUUGAAACUCAAAGGAAUUGACGGGGG
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50 860 870 880 890 900 910 920 9

24 25 26 26' 27 28

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cCCGCACAAGCGGUGGAGCAUGUGGUUUAAUUCGAUGaUACGCGAGGAACCUUACCAAGGUUUAAAUGGGAACUGAC-AG
CCCGCACAAGCGGAGAACAUUGGUUUAAUUCGAUGAUACGCGAGGAACCUUACCCGGGCUUAAAUUGCAGUGGAA-UG

30 940 950 960 970 980 990 1000

28 28' 28' 29 30 31 31

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CUUUAGAGAUAGAGUUU---UCUUCGGA---CAGAUUAC-AAGGUGCUGCAUGGUUGUCGUCAGCUCGUGCCGUGAGGU
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AUGUGGAAACAUGUCAG--UGAGCAAUCA--CCGCUGUG-AAGGUGCUGCAUGGUUGUCGUCAGCUCGUGCCGUGAGGU

1010 1020 1030 1040 1050 1060 1070 1080

32 32' 30' 33 34 34'

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CAGGUUAAGUCCUAUAACGAGCGCAACCCUGUUGUAGUUGCCAGCGA-GUCAUG-UCGGGGACUCUAACGnACUG
CAGGUUAAGUCCUAUnACGAGCnCAACCCUUGUUAAGUUAACAGCAC-AUUAUG-GUGGGGACUCUAGCAAGACUG
GUCAGGUUAAGUCCUAUnACGAGCnCAACCCUUGUUAAGUUAACAGCAC-GUAGUG-AUGGGGACUCUAGCAUAACUG
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GUCAGGUUAAGUCCUAUAACGAGCGCAACCCUGUUAAGUUGCUAACGA-GUAAAG-UCGAGCCUCUAAGGGGACUG
GUCAGGUUnAGUCCUAUAACGAGCGCAACCCUGUUAAGUUGCUAACGA-GUCAAG-UCGAGCCUCUAGUGGGGACUG
GUCGGCUUAAGUGCCAUAACGAGCGCAACCCUUAUCUUUAGUUAACUACAG-GUUAUG-CUGAGGACUCUAGAGAGACUG

1090 1100 1110 1120 1130 1140 1150 11

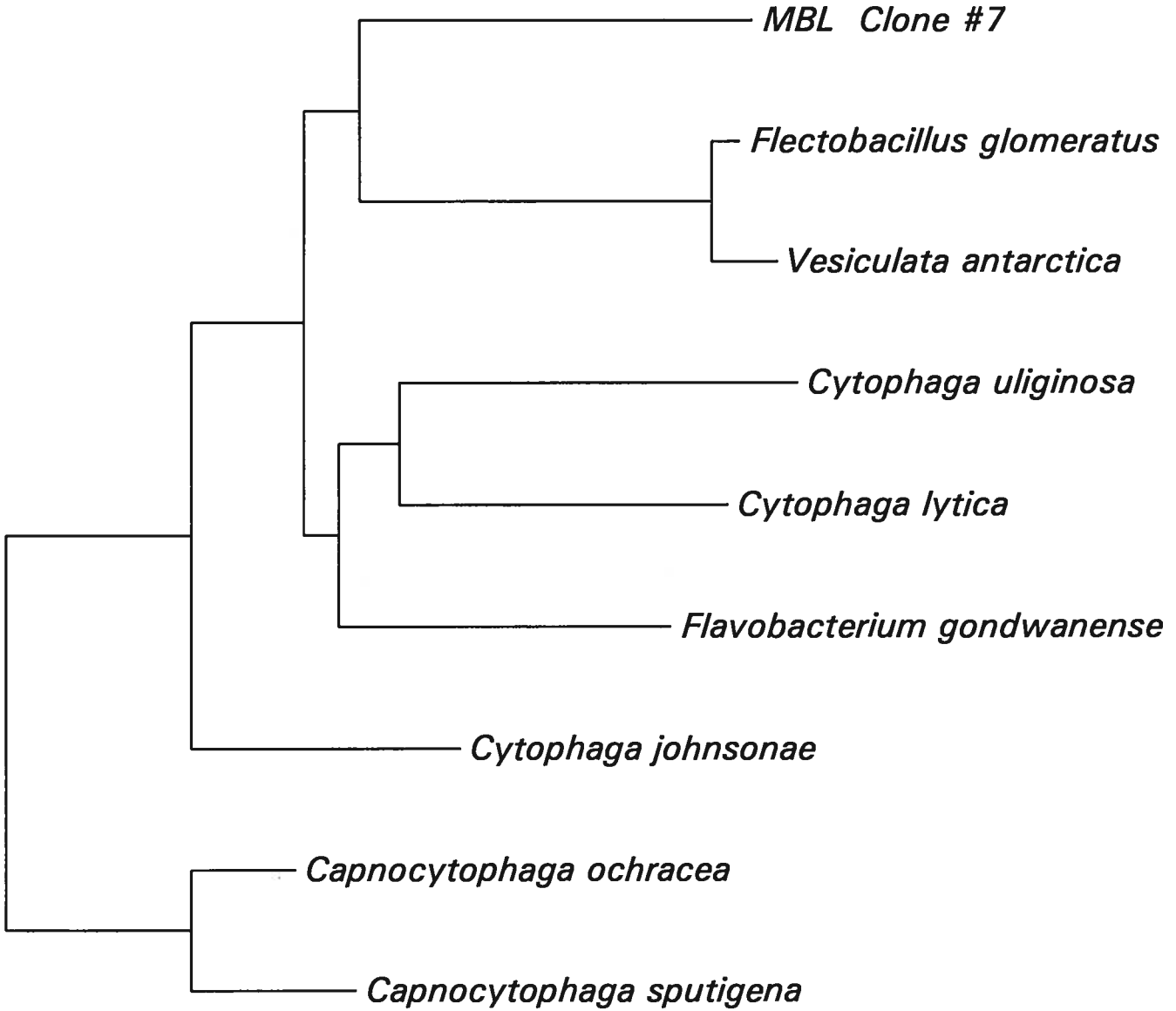
35 35' 33' 29' 27' 25'

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CcGGUG-CAAACCGUGAGGAAGGUGGGGACGACGUCAAUAUCACGGCCCUUACGUCUUGGGCCACACACGUGCUACAA
CCGGUG-CAAACCGUGAGGAAGGUGGGGAUGACGUCAAUAUCACGGCCCUUACGUCUUGGGCnACACACGUGCUACAA
CCAGUG-UAAACUGUGAGGAAGGUGGGGAUGACGUCAAUAUCACGGCCCUUACGUCUUGGGCUACACACGUGCUACAA
CCAGUG-CAAACUGUGAGGAAGGUGGGGAUGACGUCAAUAUCACGGCCCUUACGUCUUGGGCUACACACGUGCUACAA
CCGGUG-CAAACCGUGAGGAAGGUGGGGAUGACGUCAAUAUCACGGCCCUUACGUCUUGGGCUACACACGUGCUACAA
CCGGUG-CAAACCGUGAGGAAGGUGGGGAUGACGUCAAUAUCACGGCCCUUACGUCUUGGGCUACACACGUGCUACAA
CCGUCG-UAGAUGUGAGGAAGGUGGGGAUGACGUCAAUAUCAGCACGGCCCUUACGUCUUGGGGCUACACACGUGUUAACAA

60 1170 1180 1190 1200 1210 1220 1230 12

Figure 16

(% Difference)



15:11:58 07-30-1996

Clone #9 Sulfate reducer Jesse and Annick
Desulfobulbus propionicus ATCC 33891
Desulfobulbus sp. 3pr10 DSM 2058
Desulfobacter postgatei DSM 684
Desulfuromonas acetoxidans DSM 2034
Myxococcus xanthus Published De Wacter
Desulfovibrio desulfuricans El Aghelia Z NCIB 8380
Desulfovibrio vulgaris DSM 644
@Desufobotulus sapovorans@ ATCC 33892

Clone #9 Sulfate reducer Jesse and Annick
Desulfobulbus propionicus ATCC 33891
Desulfobulbus sp. 3pr10 DSM 2058
Desulfobacter postgatei DSM 684
Desulfuromonas acetoxidans DSM 2034
Myxococcus xanthus Published De Wacter
Desulfovibrio desulfuricans El Aghelia Z NCIB 8380
Desulfovibrio vulgaris DSM 644
@Desufobotulus sapovorans@ ATCC 33892

Clone #9 Sulfate reducer Jesse and Annick
Desulfobulbus propionicus ATCC 33891
Desulfobulbus sp. 3pr10 DSM 2058
Desulfobacter postgatei DSM 684
Desulfuromonas acetoxidans DSM 2034
Myxococcus xanthus Published De Wacter
Desulfovibrio desulfuricans El Aghelia Z NCIB 8380
Desulfovibrio vulgaris DSM 644
@Desufobotulus sapovorans@ ATCC 33892

Clone #9 Sulfate reducer Jesse and Annick
Desulfobulbus propionicus ATCC 33891
Desulfobulbus sp. 3pr10 DSM 2058
Desulfobacter postgatei DSM 684
Desulfuromonas acetoxidans DSM 2034
Myxococcus xanthus Published De Wacter
Desulfovibrio desulfuricans El Aghelia Z NCIB 8380
Desulfovibrio vulgaris DSM 644
@Desufobotulus sapovorans@ ATCC 33892

Clone #9 Sulfate reducer Jesse and Annick
Desulfobulbus propionicus ATCC 33891
Desulfobulbus sp. 3pr10 DSM 2058
Desulfobacter postgatei DSM 684
Desulfuromonas acetoxidans DSM 2034
Myxococcus xanthus Published De Wacter
Desulfovibrio desulfuricans El Aghelia Z NCIB 8380
Desulfovibrio vulgaris DSM 644
@Desufobotulus sapovorans@ ATCC 33892

0 1 0' 2 3 4 5

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--AUCCGUCAGAGUUGAUCCUGGCUCAGAACGAACGCUGGCGGCGUGCUUAAACAUGCAAGUCGAACGCGAAC---g
AA·UGG·····n·····U·····U·····n·nn·····A·····A·····G·
UAA·UGG·····n·U·····U·····U·····n·nn·····A·····A·····G·
aA·UGG·····U·····U·····U·····n·nn·····A·····A·····G·
CAAUUGG·····U·····U·····U·····n·nn·····A·····A·····G·
UAAUUGn·····U·····U·····U·····n·nn·····A·····A·····G·
UAA·UGG·····U·····U·····U·····n·nn·····A·····A·····G·
GAA·-GG·····U·····U·····U·····n·nn·····A·····A·····G·

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10 20 30 40 50 60 70

5 5' 6 7 8

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GUCUCUUCGGAGACU--UAGUAGAGUGGCGCACGGGUGAGUAACGCGUAAGUaAUCUGCCCUCGCAUUCGGAUAACCC
-GGGAC·UC·GUC·····G·····A·····A·····GA·C·nn·U·AUG·CU·····UA·
·AU·····AUCC·G·····R·····GA·R·C·n·nAUG·CU·····RC·
·AU·GC·U·C·AU·CC·G·····A·····GA·····U·AAGCCU·G·····UA
·U·····GA·--·AUG·····A·····GGA·····UGAUA·CU·G·····AU
AG-GGGCAACCCU--·····C·····C·····A·····GGA·····UGA·UGC·····A
AYUC·····GAG·C·····C·n·U·····C·····GG·CU·C·····AGA·GA·····AG
-GGA·····YCC--·G·····A·C·····R·····GGA·G·····A·AU·AG·G·····GG
U·nC·····G·GA·····A·····A·····U·····GGA·····A·R·U·A·····AU

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80 90 100 110 120 130 140 150

8 8' 9 9' 10 10

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ACCGaAAGGUGUNGCUAAUAGChAUACGUCUGaAUUA--AUACCUNUUAUUCAGAGAAAGAUAGuCUCUG-UUUCaAGCU
G·····G·RCUA·UACCGG·····UUnUn·CUn·U·CAUAAGU·AUGCA·CR·····G·G·C·····GCA·-·U·····
·····GRCUA·UACCGG·····GUnCUnnUn·U·CAUnAGCG·A·A·AGA·····G·G·C·····A·A·U·····
·····GUAGCUA·UACCGG·····AAGUC·U·····CACAAAG-·AGA·UGAU·····U·C·····UC·GA·····A
·····AA·GCUA·UACCGG·····A·C·CAC·G·····UC·U·GGG·ACAG·G·Ga·····G·C·····UC·GA·····
GU·····A·UGGCUA·UACCGG·····A·C·CACGG·UUC·U·GGAGAC·GAG·GA·····G·G·C·····UA·A·····
UUG·····C·GC·GCUA·UACCG·····AA·C·---·U·UAUn---·GGUU·····GGU·C·····AA·U·U·····
CUG·····C·GUCGCUA·UACCGA·····CUCCG·---·U·U·RA--·Cn·CGG·G·····G·G·C·····C·G·····
CG·····CG·GCUA·UACCGG·····RAUGU·UR·-UUUC·U·GGGGA·G·ACC·····AU·C·····UCA·GA·····A

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160 170 180 190 200 210

7' 11 11' 12

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AUUGUGCGAGGAGGAGCUUGCGUACCAUUAAGCUAGUAGGUAGGGUAAUGGCUAACCUAGGCAACGAUGGUUAGCGcGUCU
·C·CRU·A·····G·UC·····G·····G·····G·····n·····G·····
nC·CRU·R·····G·UC·····U·····G·····n·Gn·····A·····U·····n·····G·····
·····UU·GA·U·····U·····U·····U·····A·····A·····U·····n·····UG·····
·····UAUCA·U·····UCC·····G·····U·····G·····A·····G·····A·····Ug·····U·
·····CACHUUA·U·····UCC·····GC·····C·····U·····CG·····C·····A·····C·····G·····UG·····
·····Chn·UCUn·U·G·CC·CU·····n·U·····n·C·····A·····n·····AG·····Un·U·
·····C·CU·AU·····U·····UCC·····C·····U·····G·····C·····C·····nA·····G·····R·····Gn·····CG·····
U·····UU·····U·····UCC·····C·····U·····CG·····C·····C·····A·····GU·····Gn·····Un·····

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220 230 240 250 260 270 280 290

12' 6' 13 13' 14 14' 4' 15

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·····n·n·····A·····CACUGAAACnCGGGCCnGRCUCnACGGGAGGCnGnGUGAGGAAUnUUGCGCnAUG
·····A·····A·····ACUGGAACACGGUCCAGACUCCUACGGGAGGCAGCAGUGAGGAAUUUUGCGCAAUG
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·····C·A·····GACUGAAACACGGCCnnGACUCCUACGGGAGGCAGCnGUGGGGnAUUAUUGCGCnAUG
·····C·G·····GACUGAAACACGGCCnAGACUCCUACGGGAGGCAGCAGUGGGAUUAUUGCGCAAUG
·····A·····GACUGACACACGGCCnnGACUCCUACGGGAGGCAGCAGUGAGGAAUUUUGCGCAAUG

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300 310 320 330 340 350 360 370

.15 15' 3' 16 16' 17

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380 390 400 410 420 430 440 450

17 17' 18 xxx 18' yy xxxyy 18'

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 UUUGUUGGCUAUUAACCAACAGAAUUGACGGUACCUUAGAGGAAGCaCCGGCUAACUCUCCGUGCCAGCAGCCCGCGUAAU
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 CCACGGUGCUnAU-CAGCCGUnGUCUGACGGUACCUCCAGAGGAAGCACCCGGCUAACUCUCCGUGCCAGCAGCCCGCGUAAU
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460 470 480 490 500 510 520 530

18' 2' P18 ww 19 P19

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540 550 560 570 580 590 600 610

P19' 20

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620 630 640 650 660 670 680 690

20' zz zzo 19' 2

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700 710 720 730 740 750 760 770

1. 21 21' P21-1 P21-2 P21
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EAGSAAACAGGAUUAGAUACCCUGGUAGUCCACGCCGUAACGAUGUCAACUAGGUGUAGGGGGG-GUUnAU--CCSS
UAGCAAACCGGGAUUAGAUACCCCGGUAGUCCACGCAGUAAAACGUUGUACACUCGGUGUAGCGGGU-AUUAAA--ACCU
GGGAGCAAACAGGAUUAGAUACCCUGGUAGUCCACGCcGUAAAACGAUGGGUACUAGGuGUCGCGGGqU-AUUGAC--CccU
GGGAGCAAACAGGAUUAGAUACCCUGGUAGUCCACGCCGUAACGAUGAGAACUAGGUGUCGUGGGGAG-UUGA--CCCC
GGGAGCAAACAGGAUUAGAUACCCUnGUAGUCCACGCUGUAAAACGAUGGcnnGGUGUCGGGGGG--AUUC--CUUCU
GGGAGCAAACAGGAUUAGAUACCCUnGUAGUCCACGCcGUAAAACGAUGGAUGCUAGGURUCGGGGCC--UUGA--RRCU
GGUAGCAAACAGGAUUAGAUACCCUnGUAGUCCACGCAGUAAAACGUUGAUCACUAGGUGUUGnGGGU--AUUGAC-CCCU
780 790 800 810 820 830 840 8

-2' P21-3 P21-3' P21-1' P18' 22 22' 1' 23
nCUGUGUnGCAGCUAACGCAUUnAGUUnnnnnnnnnnnnAGUACGGnnGCAAGAUUAAAACUCAAGGAAUUGACGGGGn
UCUGUGCCGCAGCURACGCAUURAGUUGACnGCCUGGGGAGUACGGUCGCAAGAUUAAAACUCnAAGnAAUUGRCGGGGG
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GCAGUGCnGAAGCUAACGCAUUAAGUGAUcncGCCUGGGGAGUACGAUCGCAAGAUUAAAACUCAAGGAAUUGACGGGGG
50 860 870 880 890 900 910 920 9

24 25 26 26' 27 28
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CCCRCnAGCGGUGGAGUAUGUGGUUUAAUUCGAUGnnACGCGAAGAACCUUACCCUGGUCUUGACAUCCC-AAGAA-UC
CGCACAAGCGGUGGAGCAUGUGGUUUAAUUCGACGCAACGCGAAGAACCUUACCUGGGCUUGACAUCCC-nAUCG-UA
CCGACAAGCGGUGGAGCAUGUGGUUUAAUUCGACGCAACGCGAAGAACCUUACCnGGUCUUGACAUCCC-CAGAA-UC
nn
nn
nCCGCAAnAAGCGGUnGAGCRUGUGGUUUAAUUCGAnnnAACGCGCAAnnACCUUACCUGGGCUUGAAAUCUG-UGGAA-CU
30 940 950 960 970 980 990 1000

28 28' 28' 29 30 31 31
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UUCUAGAAAUAAGAGAGUG-CUUUUCGGAGAACUUGGUGA-CAGGUCUGCAUGGCUGUCGUCAGCnnnnnnnnnnnnnnnn
UCCCGUAAUUGGGAUAGUG-CCUUCGGG--AGCACAGAGA-CAGGUGCUGCAUGGCUGUCGUCAGCUCGUGUCGUGAGAU
CCUUAUGGAAACAUIUG-GGnCAGUUGGCUnGAUCGGuGA-CAGGUGCUGCAUGGCUGUCGUCAGCUCGUGUCGUGAGAU
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UCCUAGAGAUAGGAGnUGnCCUUCGGGG-nACCAAGAGA-CAGGUGCUGCAUGGCUGUCGUCAGCUCGUGCCGUAAGGU
UUCcCGAAAAGGAAGGnUG-CCUUCGGGG-AAUUCcGAGA-CAGGUGCUGCAUGGCUGUCGUCAGCUCGUGCCGUAAGGU
UGUAUGAAAAGUAUGGGGnnnCCUUCGGGG-AACCGCAAGA-CAGGUGCUGCAUGGCUGUnGUCAGCUCGUGUCGUGAGAU
1010 1020 1030 1040 1050 1060 1070 1080

' 32 32' 30' 33 34 34'
GUUGGUUAAGUCCcnnAACGAGCGCAACCcUUGCCUUAGUUGCCAGCAG-UUCGGC--UGGRCAcUCUAAAAGGGACUG
nn
GUUGGUUAAGUCCAGCAACGAGCGCAACCcUUAUCGUUAGUUGCCAGCAU-UUAAG-AUGGGAACUCUAAAGGAGACUG
GUUGGUUAAGUCCcCAaCGAGCGCAACCcUUGUCUAGUUGCCAUCA--UUAAGU--UGGGCACUCUAAGGGGACUG
GUUGGUUAAGUCCCGCAACGAGCGCAACCcUUGCCUUAGUUGCCAGCAG----GCAAGU----GGAUCUCUAGAGGGACUG
GUUGGUUAAGUCCCGCAACGAGCGCAACCcUUAUUGUCAGUUGCCAUCA-AUAAUG-GUGGnnACUCUGnnnAGnCUG
GUUGGUUAAGUCCCGCAACGAGCGCAACCcUUAUUGCCAGUUGCUACCAG-GUAAUG-CUGGGCACUCUGGUGAGACUG
GUUGGUUnAAGUCCCGCAACGAGCGCAACCcUCGUUCUUCAGUUGCCAGCAC-GUAAAG-GUGGGAACUCUGAAGAUACUG
1090 1100 1110 1120 1130 1140 1150 11

(% Difference)
Figure 18

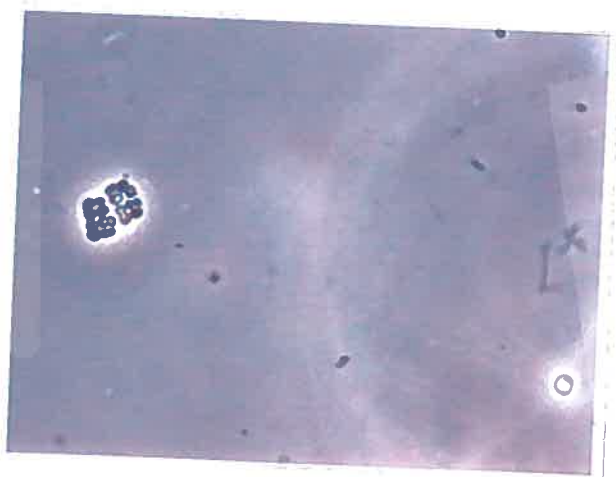
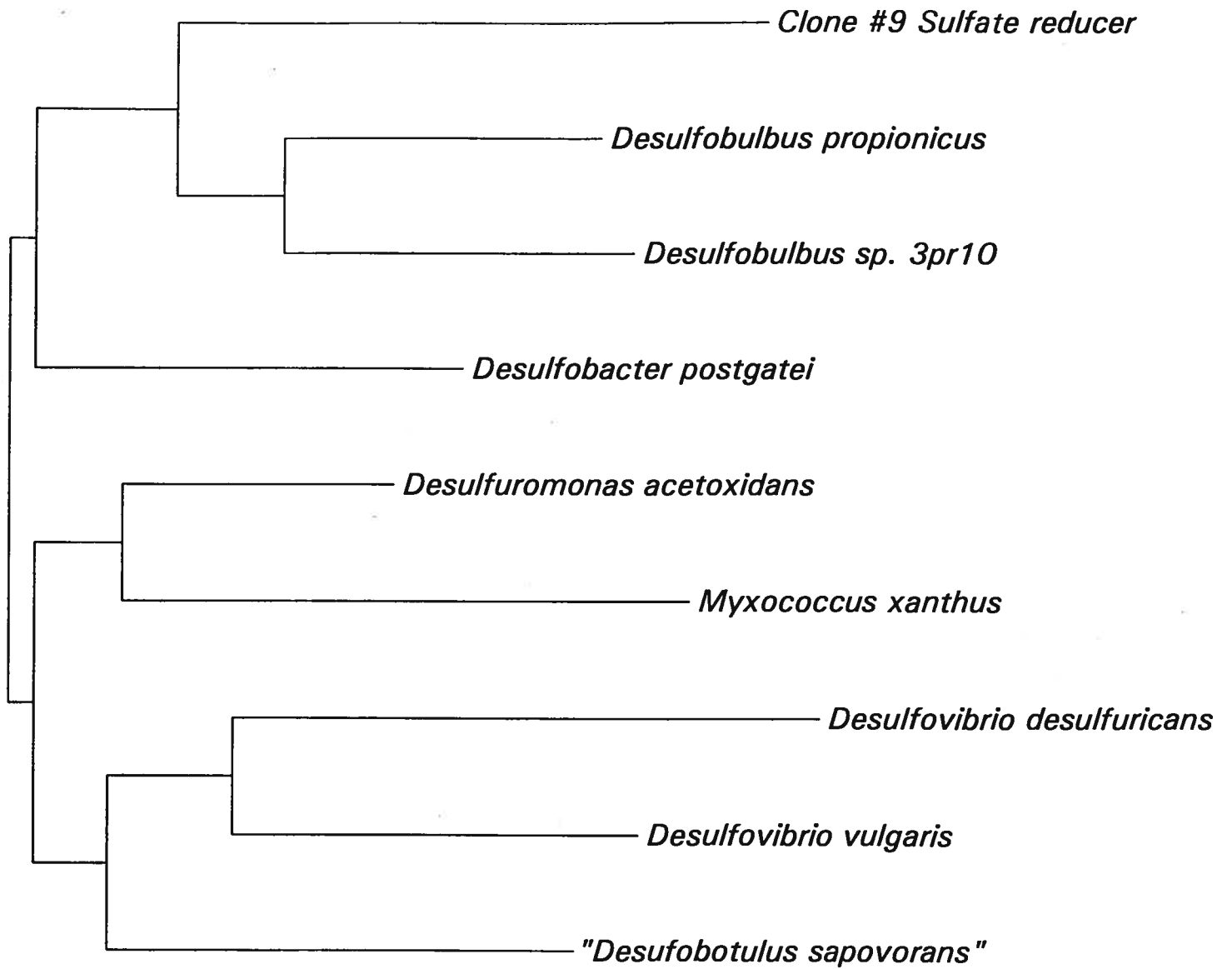
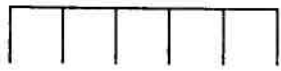


Table 1: Sequenced Clones

Origin	Primers	Clone Number (MD-96)	Insert Size (bp)	Sequence Size (bp)	Putative Identity
Green Layer	Univ. F+R	5	1500	330	Actinomycete
Green Layer	Univ. F+R	6	1500	327	Cyanobacterium
Green Layer	Univ. F+R	7	1500	328	Cytophagales
Pink Layer	Univ. F+R	9	1500	334	SRB
Pink Layer	Univ. F+R	8	1500	323	SRB
Green Layer	Cyano F+R	19	1700	469	Diatom Chloroplast
Green Layer	Cyano F+R	20	2000	327 + 285	Cyanobacterium