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1990

CyanoNews (Vol. 6, No. 1, March 1990)

Jeff Elhai

Virginia Commonwealth University, elhaij@vcu.edu

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CYANONEWS

Volume 6 Number 1

March 1989

CYANONEWS - a newsletter intended to provide cyanobacteriologists with a forum for rapid informal communication, unavailable through journals. Everything you read in this newsletter is contributed by readers like yourself. Published occasionally (about three times per year).

SUBSCRIPTION RATE - one communication every two years or so (your address label shows the date of your last communication). A communication might be a new result, news of an interesting meeting, a post-doctoral opening, a request for strains, a new article, even confirmation of your address!

WHERE TO SEND CONTRIBUTIONS - See the last page.

HOW TO GET ON THE MAILING LIST - See the last page.

INSTRUCTIONS TO AUTHORS - Send news.

HOW TO FIND OUT MORE ABOUT SOMETHING YOU READ HERE - The name of the correspondent for each item in this newsletter is capitalized, so you know who to write to for more information. The correspondent's address appears at the end of the newsletter.

INSIDE:

- * Spotlight on biotechnology
- * Mass cultivation
- * Cyanobacterial DNA isolation
- * Sites of e⁻ carrier proteins
- * Nitrogen fixation genes
 - Phylogeny
 - New genes
 - Pattern of expression
- * Meetings, Jobs

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The Vth CHINESE CONGRESS OF ALGOLOGY will be held June 25 - 30, 1990 in Nanjing, Peoples' Republic of China. For more information, contact Chao-Tsi Tseng, Centre of Marine Sciences, Dept. of Biology, Nanjing University, P.R. CHINA, (Tel) 637551-2551.

A COURSE ON ALGAL BIOTECHNOLOGY will be offered Aug 31 - Sept 7, 1990, with an emphasis on tissue culture and nitrogen/carbon metabolism and the use of marine macroalgae and cyanobacteria in biotechnology. The following methods will be used during the course: electron microscopy, immunogold cytochemistry, Western blots, and cultivation techniques. There is no registration fee for academic participants, so costs will be limited to travel and accommodations. Contact by June 1, 1990: Marianne Pedersén, Department of Physiological Botany, University of Uppsala, Box 540, S-751 21 Uppsala, SWEDEN, (Tel) +46 18 182800, (Fax) +46 18 559885.

Computer users will thank Bob Knox for compiling an extensive DIRECTORY OF BITNET ADDRESSES, comprised of researches with an interest in photosynthesis. If you want to get a copy of the directory, contact Bob at RSKN@UORVM. If you want to add yourself in the directory, then include in your message the answers to the following questions:

How often do you check your mail?

- (1) About once per week
- (2) between once per week and once per day
- (3) at least once daily.

Does anyone else use the same address?

If so, include the name of the addressee in the header!

POSITION AVAILABLE

CONTACT: Murray Badger, Plant Environmental Biology group, Research School of Biological Sciences, Australian National University, PO Box 475, Canberra City, ACT, 2601, AUSTRALIA.
(Tel) 062-493741. (Fax) 062-489995.

RESEARCH: Operation of CO₂-concentrating mechanisms. In particular, the role of the carboxysome in cyanobacterial photosynthesis. Initial research will involve analysis of already isolated DNA fragments that complement mutants impaired in carboxysome function, and isolation of related sequences with a view to understanding the processes of assembly, regulation by CO₂, and the detailed functioning of the carboxysome. Further objectives relate to active inorganic carbon transport mechanisms in cyanobacteria and eukaryotic algae.

REQUIREMENTS: PhD in appropriate field. Training in molecular biology and biochemistry desirable.

SALARY: A\$29,388 to A\$32,599, depending on qualifications.

START: 30 June 1990

NEWS*

IMPROVED MASS CULTIVATION OF MICROALGAE AND CYANOBACTERIA

MARIO TREDICI has recently devised and patented a vertical alveolar panel (VAP) for mass cultivation of microalgae and cyanobacteria. The main characteristics of the VAP photobioreactor are:

- 1) has high surface to volume ratio (about 85 m⁻¹);
- 2) may be oriented at any angle to the sun's rays;
- 3) effective mixing and O₂ removal is achieved by air bubbling, which minimizes the shear to which the organism is exposed.

Air is bubbled at the bottom of the system, and CO₂ is added at rates regulated by a pH controller. The system is temperature controlled. Four VAP reactors with a surface area of 0.5 to 2.0 m² were constructed from commercially available plexiglas sheets 1.6 cm in thickness and tested for several months both in the laboratory and outdoors. The VAP has proven to be well suited to the outdoor mass culture of cyanobacteria such as *Anabaena azollae* and *Spirulina platensis*, allowing operation at high cell concentrations (up to 20 g l⁻¹) and achieving high biomass productivity (up to 2 g l⁻¹ day⁻¹).

SPOTLIGHT ON CYANOTECHNOLOGY: MARTEK CORPORATION

The vast majority of those who receive and contribute to this newsletter are affiliated with institutes of higher learning. As we spend our days seeking pure understanding of our favorite organisms, we sometimes lose sight of our more practical colleagues who are attempting to harness cyanobacteria for useful purposes. With this in mind, we focus our attention on one commercial enterprise and perhaps in future issues on others as well.

TOM ALLNUT, recently departed from Rutgers University where he studied Photosystem II, describes to us his new home, Martek Corporation. Martek was formed on the premise that closed-culture microalgae technology can be used to produce both new and existing valuable chemical compounds far more efficiently than other production methods. Martek has exploited the natural biochemistry of microalgae to produce useful compounds, such as eicosapentaenoic acid (EPA), the active anti-cholesterol ingredient in fish oil. However, much of their effort is directed towards developing and marketing compounds labeled with stable isotopes. Microalgae, alone among microorganisms, can use water as the sole source of hydrogen, thus growth in D₂O permits almost total substitution of deuterium for hydrogen in every compound they make. Similarly, growth with ¹³C₂O₂ or K¹⁵NO₃ yields compounds greatly enriched in heavy carbon or nitrogen. These compounds may be valuable in molecular structure determination using nuclear magnetic resonance and in health related applications.

Thus far, Martek has concentrated its efforts primarily on eukaryotic algae, growing cyanobacteria only for specialty applications. No cyanobacterial strain has been found yet that grows very well in D₂O. However, genetic techniques are far more advanced with cyanobacteria as compared with eukaryotic algae, and it may soon be feasible to isotopically label overproduced foreign proteins cloned in a cyanobacterial strain. Martek is eager to develop relationships with researchers in academia.

MICROFUGE MINIPREP FOR SYNECHOCYSTIS PCC 6803 CHROMOSOMAL DNA

DEXTER CHISHOLM has passed on a protocol that allows the isolation of moderate amounts of DNA from *Synechocystis* PCC 6803. The procedure allows cells from either plates or liquid culture to be used, and confines all manipulations to 1.5 ml microcentrifuge tubes. Cell lysis is achieved using lysozyme, sarkosyl, and phenol. Polysaccharides are removed by CTAB extraction. He and others have been using this procedure for a few months now with consistent results. The DNA restricts well and supports PCR amplification. The procedure also scales up well. The procedure:

HARVEST CELLS: Spin down 12 ml of culture (OD₇₃₀ of at least 2.0), or scrape a pea-sized glob of cells from a healthy plate. Resuspend in 400 μ l of TES in a microfuge tube.
DIGEST WITH LYSOZYME: Add 100 μ l of lysozyme (@ 50 mg/ml) and incubate for 15 min at 37° (mix occasionally because cells settle out).
LYSE WITH SARKOSYL AND PHENOL: Add 50 μ l 10% sarkosyl, and then add 600 μ l phenol and torture on a rotating wheel for 15 min.
REMOVE DEBRIS: Spin in microfuge for at least 5 min. Transfer supernatant to new tubes.
DIGEST WITH RNase: Add 5 μ l of 2U/ μ l RNase (Boehringer-Mannheim #1119-915). Incubate 15 min at 37°.
PRECIPITATE WITH CTAB: Add 100 μ l of 5M NaCl, 100 μ l of CTAB-NaCl solution, and 600 μ l of chloroform. Extract on wheel for 15 min.
PELLET DNA: Spin for 2 min. to pellet.
RINSE PELLET: Rinse pellet with 70% ethanol. Dry in Speedvac. Resuspend in 100 μ l of TE. Use 10-20 μ l per lane for Southern.

TES solution:

2.5 ml 1M Tris, pH 8.5 (5 mM)
5 ml 5M NaCl (50 mM)
5 ml 500 mM EDTA (5 mM)
Bring volume to 500 ml

CTAB-NaCl Solution:

4.1 g NaCl in 80 ml water (700 mM)
10 g CTAB (10%)
Requires heat to get into solution
Bring volume to 100 ml.

PATTERNED NIF GENE EXPRESSION BY ANABAENA PCC 7118

Perhaps the world's most famous cyanobacterial species is *Anabaena variabilis*, because of two of its restriction enzymes, AvaI and AvaII, that take their names from a strain of that species. This strain came to us over thirty years ago as a heterocystous, nitrogen-fixing cyanobacterium, but some time since, it spontaneously lost the ability to make heterocysts and fix nitrogen under aerobic conditions. This mutant strain is stored in the Pasteur Culture Collection as *Anabaena* PCC 7118.

Now JEFF ELHAI tells us that the mutant strain is still capable of development in response to nitrogen-deprivation, forming a pattern analogous to that seen in wild-type *Anabaena*. He bases this assertion on several kinds of observations, made with Peter Wolk. First, nitrogen-starved filaments of PCC 7118 break in a nonrandom fashion, yielding fragments of lengths reminiscent of the spacing between heterocysts in wild-type strains. In contrast, sulfur-starved PCC 7118 and nitrogen-starved *Plectonema boryanum* break randomly. Second, phycocyanin-dependent fluorescence is lost in about one in ten cells of nitrogen-starved PCC 7118, producing a regular pattern of nonfluorescent cells. Third, the nonfluorescent cells in general are morphologically distinguishable from their neighbors, although they little resemble proheterocysts. Finally, these morphologically distinct cells are the sites under anaerobic conditions of transcription from the promoter (P_{nifHDK}) of *nifHDK*, which encodes nitrogenase.

A similar phenomenon can be observed with wild-type *Anabaena* PCC 7120, where transcription from P_{nifHDK} is confined to differentiating cells, even under anaerobic conditions, and this result has important implications. First, nitrogen fixation must be confined to differentiated cells, even when the entire filament is subjected to anaerobiosis. Second, the promoter is induced by conditions specific to differentiating cells. It could be that only these cells experience sufficient nitrogen-starvation to induce the promoter. Alternatively, P_{nifHDK} may require for activity a gene product found only in differentiating cells.

LOCALIZATION OF ELECTRON CARRIER PROTEINS IN ANABAENA

AURELIO SERRANO recently returned to Spain from a one-year stay at the University of Konstanz and reports to us some interesting results concerning the cellular localization of the electron carrier proteins ferredoxin-NADP oxidoreductase (FNR) and cytochrome c_{553} (cyt c_{553}). Using antibody directed against FNR, an intense labelling was observed in the thylakoids, whereas no gold particles were located near the cytoplasmic membrane and the centropiasm. In contrast, using antibody directed against cyt c_{553} , a clear labelling appeared associated with the periplasmic area (cytoplasmic membrane and periplasmic space) in both vegetative cells and heterocysts. Some gold particles (about 20-30%) were also associated with the thylakoid membranes. Most of the cellular content of cyt c_{553} of *A. variabilis* is located in the periplasm, as judged by its selective release after treatment Tris-EDTA. This is in agreement with what is known about the small c-type cytochromes of bacteria. Cytochrome c_{553} may act as a donor to cytochrome oxidase, which has recently been identified in the cellular membranes of *A. variabilis*.

NEW GENES IN THE NIF REGION OF ANABAENA

DULAL BORTHAKUR has moved on to browner pastures (he now works on *Rhizobium*), but his parting contribution was an analysis with others in the laboratory of Bob Haselkorn of a newly identified region involved in nitrogen fixation by *Anabaena* PCC 7120. A 1.8-kb transcript, appearing 12 to 18 hours after removal of nitrogen, was found to correspond to DNA 4-kb downstream from *nifHDK*. This region is part of a larger stretch of DNA (about 18-kb) surrounding *nifHDK* that contains at least eight genes known to be induced during the induction of heterocysts. The 1.8-kb region was sequenced, and two open reading frames, ORF1 and ORF2, were identified. ORF2 shows strong sequence similarity to ORF6 in the *nif* gene region of *Azotobacter vinelandii*. A mutant strain was constructed in which ORF1 was interrupted with a drug-resistance cassette. This strain grew very slowly on medium lacking combined nitrogen and possessed only 45% of the acetylene reduction activity of the wild type strain. Thus, ORF1 or ORF2 (or both) is evidently required for efficient nitrogen fixation in *Anabaena*. A complete report of this work will soon appear in *Mol Gen Genet*.

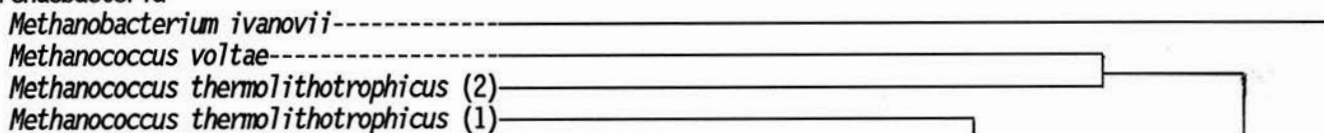
NIF GENE COMPARISON CHALLENGES CONVENTIONAL TAXONOMY

Manjula Mathur and RAKESH TULI offer some interesting taxonomic insights they reached after arranging 27 published nucleotide sequences of *nifH* genes according to their similarity. Hierarchical clustering of sequences, shown on the next page, was performed with no prior assumptions as to ordering, as described by Florence Corpet [*Nucl Acids Res* (1988) 16:10881-10890]. Basically, the greater the percentage of mismatches between two sequences, the greater the horizontal distance separates them in the figure. A penalty of seven mismatches was imposed for every gap introduced to improve an alignment. In general, the clustering of strains based on the similarity of their *nifH* genes corresponds to currently accepted taxonomy, but the exceptions may be instructive. The *nifH3* gene from the Gram-negative bacterium *Azotobacter vinelandii*, encoding a component of an Fe-nitrogenase, does not cluster with genes from other Gram-negative bacteria. Rather it is most similar to an archaeobacterial gene and one of six *nifH* genes from the Gram-positive bacterium *Clostridium pasteurianum*. Perhaps these two similar genes also encode components of an alternative nitrogenase. In contrast, genes encoding subunits of a V-nitrogenase fall within a larger cluster comprised at least in part by subunits of the conventional Mo-nitrogenase. A second anomaly is presented by the positioning of *nifH* from *Frankia* within the cluster of Gram-negative genes. *Frankia* is a Gram-positive bacterium, classified with the Actinomycetes and thus might be expected to cluster more closely with *Clostridium* than with Gram-negative bacteria.

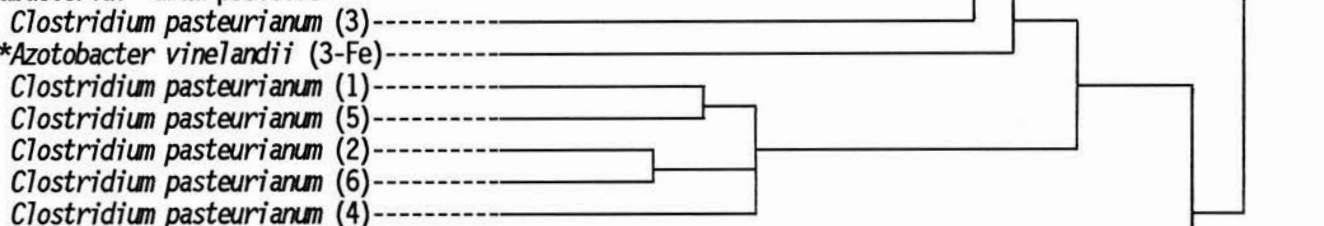
Hierarchical clustering of amino acid sequences derived from *nifH* (not shown) gives substantially the same picture as that based on the nucleotide sequence, except that *Frankia* is grouped closer to *Anabaena* than to other Gram-negative bacteria. Clustering based on ten *nifD* nucleotide sequences (next page) also is in basic agreement. The small number of *nifK* sequences available for comparison (next page) and the lesser degree of sequence conservation makes it difficult to interpret the clustering of these genes.

Hierarchical Clustering of Nucleotide Sequences of *nifH*

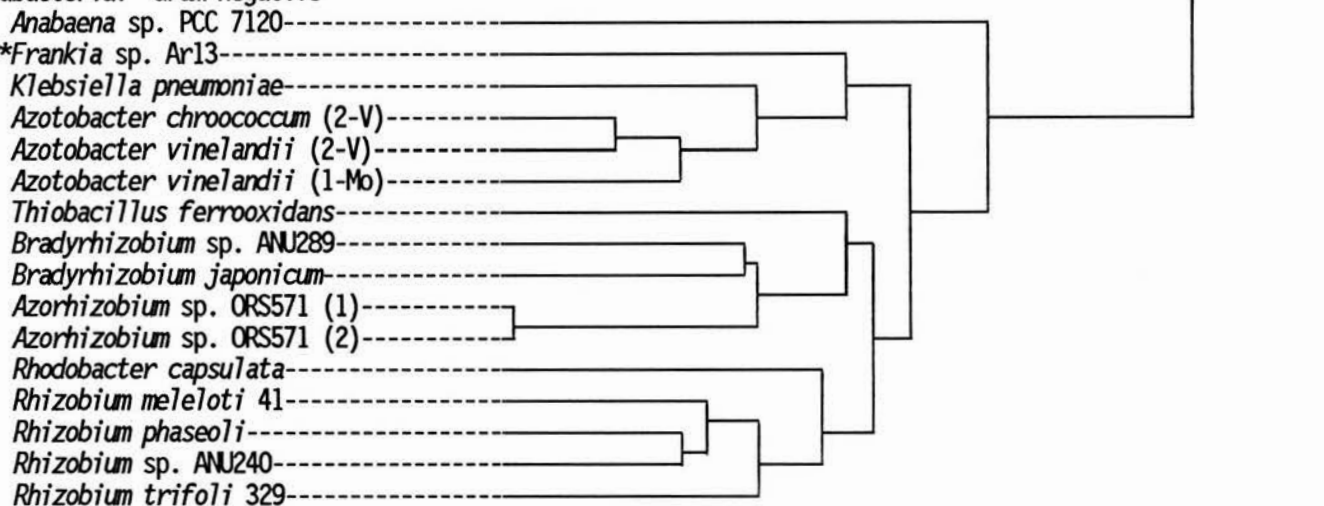
Archaeobacteria



Eubacteria: Gram-positive

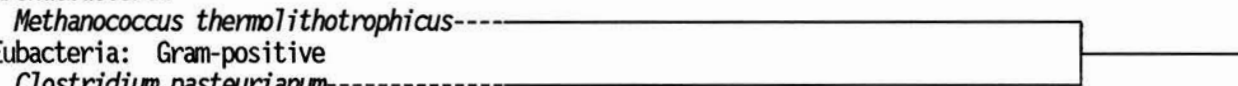


Eubacteria: Gram-negative



Hierarchical Clustering of Nucleotide Sequences of *nifD*

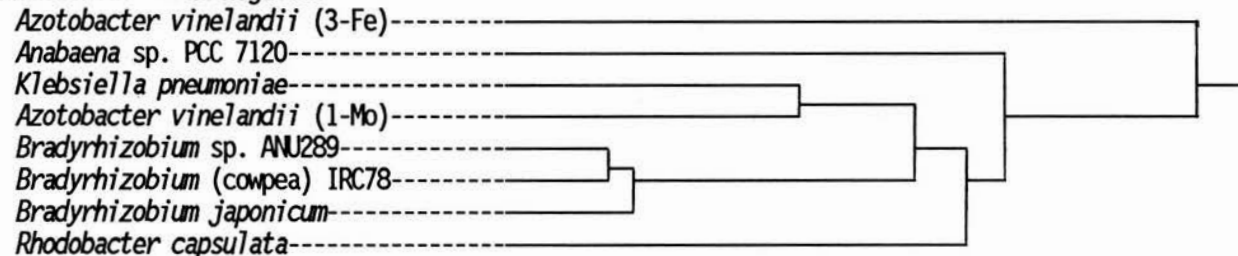
Archaeobacteria



Eubacteria: Gram-positive

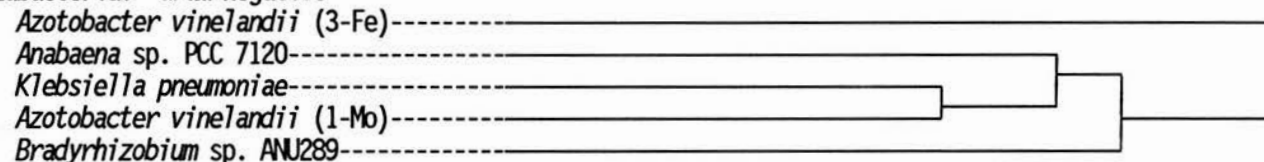


Eubacteria: Gram-negative



Hierarchical Clustering of Nucleotide Sequences of *nifK*

Eubacteria: Gram-negative



**Frankia* is Gram-positive, *Azotobacter* is Gram-negative.

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