

# CYANO NEWS

Volume 5 Number 1

May 1989

CYANO NEWS - 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.

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:

- \* Improved closed system for microalgae production
- \* In vitro plasmid replication
- \* Gene replacement:
  - In a heterotrophic *Anabaena*
  - Positive selection for double recombination
- \* Meetings
- \* Post-doc openings

BULLETIN BOARD\*BULLETIN BOARD\*BULLETIN BOARD\*BULLETIN BOARD\*BULLETIN BOARD\*BULLETIN BOARD\*BULLETIN BOARD\*

## MEETINGS

It was necessary to cancel the INTERNATIONAL SYMPOSIUM ON CYANOBACTERIAL RESEARCH planned for April, owing to the loss of funding from the major symposium sponsor.

The Society for Industrial Microbiology will hold its annual meeting August 12-18, 1989, in Seattle, Washington, U.S.A. Of special interest is a symposium entitled "MICROALGAE: FROM LABORATORY TO COMMERCIAL REALITY". The lineup for the symposium is: John Benemann (Microalgae products and production: an overview), David Kyle (Production of specialty lipids), Blaine Metting (Microalgae applications in agriculture), Dan Anderson (Commercial mass culture of microalgae), and L. Brown (Applications of genetics to microalgae production). Contact Ann Kulback, SIM Headquarters, P.O. Box 12534, Arlington, VA 22209-8534, U.S.A. (Tel): 703-941-5373.

"RECENT ADVANCES IN ALGAL BIOTECHNOLOGY" is the topic of the 5th International Conference of the Society of Applied Algology. The meeting will be held January 28 - February 2, 1990 in Tiberias, Israel. Main themes include: technology of algal biomass production, products from algae and their use, genetics and cell biology, environmental limitations and growth physiology, and new technologies (harvesting and reactor design). A conference package is available for US \$440 (double occupancy), including registration, room, and most meals. Deadline for abstracts and payment at the package rate is October 15, 1989. Contact: Conference Secretariat, Algology Conference, Melia Te'um, POB 8388, Jerusalem 91082, Israel. (Tel) 972-6-792950, (Telex) 6703 JRTIB, (Fax) 972-2-790453.

The 5th International Symposium on NITROGEN FIXATION WITH NON-LEGUMES will be held in Florence from September 10-14, 1990. The scientific program will include papers invited lectures on the following main topics: (1) Free-living diazotrophs, (2) Root-associated diazotrophs, (3) Nitrogen-fixing photosynthetic microorganisms, and (4) Diazotrophic actinomycetes. Contact: M. Vincenzini - Istituto di Microbiologia Agraria e Tecnica, P.le delle Cascine, 27 - I - 50144 Firenze, ITALY.



...the amino acid sequence for the predicted protein shares homology with previously determined Type II cytidine-modifying methylases. However, all known Type II methylases are encoded by a single gene, while expression of *M.AquI* requires two genes. The conserved regions are divided between these two genes. Type I methylases also require expression of two genes, and C.K. speculates that cyanobacteria may have diverged from the line leading to Gram negative and Gram positive bacteria before the appearance of the distinct types of restriction/modification systems that we observe today.

#### PATTERN OF PROTEIN SYNTHESIS RELATED TO SALT TOLERANCE

SHREE KUMAR APTE reports on recent work he and Arvind Bhagwat completed, aimed at understanding mechanisms underlying salt tolerance in cyanobacteria. They compared patterns of protein synthesis in two strains of *Anabaena*: strain L-31, a salt-sensitive freshwater strain, and *A. tolurosa*, a salt-tolerant strain isolated from brackish water. With both strains, conditions of salt stress altered the pattern of protein synthesis, and with both the degree of alteration depended on the salt concentration. Saturation occurred well below the 50% lethal dose of salt. However, the two strains differed in important respects. With strain L-31, salt-stress-induced proteins appeared only transiently after exposure to high salt, but with *A. tolurosa*, such proteins persisted throughout the period of stress. Furthermore, the induced proteins of strain L-31 were mostly confined to the cytoplasmic fraction, but those of *A. tolurosa* were found in significant number in the membrane fraction as well.

#### ANABAENA AND YEAST GENES WELL MATCHED

Those interested in expressing bacterial genes within yeast might do well to look towards the cyanobacteria, suggest Manjula Mathur and RAKESH TULI. It has been observed that for a given organism, highly expressed genes show a marked bias in codon usage. The better the codon usage of a gene matches that of highly expressed genes, the better it tends to be expressed. One measure of the degree of this correspondence is the Codon Adaption Index (CAI) described by Sharp and Li [(1987) *Nucl.Acids Research* 15:1281-1295]. Our correspondents used this measure to predict the level of expression of *nifHDK* genes, encoding nitrogenase, in the yeast *Saccharomyces cerevisiae*. Of sixteen published sequences for *nifH*, the gene from *Anabaena* PCC 7120 had codon usage most like highly expressed yeast genes. The CAI for the cyanobacterial *nifH* gene is 0.302, while the CAI for *nifH* from other bacteria fall in the range of 0.067 to 0.164. The CAI for the *nifH* gene from *Klebsiella pneumoniae* was only 0.1. This gene is of special interest because the *Klebsiella* genes have been used by different groups to examine transgenic *nif* expression in yeast. The story is much the same for *nifD* and *nifK*: the genes from *Anabaena* have much higher CAI's (0.244 and 0.289) compared to their counterparts from *Klebsiella* (0.07 and 0.066).

#### CLOSED PHOTOBIOREACTOR SHOWS ADVANTAGES IN CYANOBACTERIAL GROWTH

CyanoNews recently sponsored a panel discussion on the mass culture of *Spirulina*. One theme that was reiterated throughout the discussion was the need for improved reactor design, in particular, those making use of closed systems. JOHN BENEMANN sent us a recent report of work he did with K. Miyamoto and O. Wable [*Biotech. Lett.* 10:703-708] describing a closed photobioreactor that appears to have several advantages over older designs in small scale microalgae production. Vertical tubular reactors (VTRs) were constructed from commercially available glass tubes that are mass produced for the fluorescent light bulb industry. A 5 cm (inner diameter) by 2.35 m tube costs \$1.50 and can be adapted to support growth of a 4 liter culture. CO<sub>2</sub>-enriched air was sparged in at the bottom of the tubes, and air escaped from the top. Five cyanobacterial species (*Anabaena* sp., *Nostoc* 29106, *Tolypothrix* sp., *Anacystis* sp., and *Chloroploecopsis* sp.) were tested, as well as species of unicellular diatoms and green algae.

In addition to their low cost, VTRs exhibit several other advantages: (1) the scouring action of bubbles prevent wall growth even after prolonged cultivation, (2) high CO<sub>2</sub> utilization efficiency can be achieved simultaneously with high productivity (this is of particular importance in applications such as the production of isotopically labeled carbon compounds), (3) build-up of oxygen is avoided, (4) productivity is relatively high, and (5) the system is simple and easy to operate.

#### DNA REPLICATION IN CYANOBACTERIA AND CHLOROPLASTS COMPARED

We cyanobacteriologists feel a close kinship with those who study chloroplasts, considering them (chloroplasts) to be cyanobacteria that somewhere took a wrong turn. HENRY DANIELL tells us of his attempt to bridge the evolutionary gap by returning an origin of DNA replication from a chloroplast to a cyanobacterial relative. He has constructed a plasmid, pH407, that carries one of the two unidirec-

trional origins from pea chloroplast on a 4.1 Kb fragment [Meaker et al. (1988), Mol Cell Biol 8:1216-1223]. The plasmid did indeed transform Synechocystis PCC 6803 to chloramphenicol resistance, but it has not been detected in a free-living state. Since the 4.1 Kb fragment also contains portions of the highly conserved genes encoding 16S and 23S rRNA, it would not be surprising if pHD407 had simply integrated into the chromosome by homologous recombination.

Two in vitro systems were used to compare the initiation of DNA replication at the chloroplast origin to that at the origins of the three endogenous plasmids of Synechocystis. Extract from pea chloroplast supported DNA synthesis initiated from the chloroplast origin, but not from Synechocystis DNA. Conversely, extract from Synechocystis supported DNA synthesis initiated from Synechocystis DNA but not from the chloroplast origin. Both systems failed to initiate DNA synthesis from the origin of pBR322. H.D. intends to use these in vitro systems to determine the minimal DNA sequences required to support initiation.

#### CONDITIONAL-LETHAL GENE TO SELECT FOR GENE REPLACEMENT IN ANABAENA

Directed gene replacement has long been routine with several unicellular cyanobacteria. It has proven more difficult to replace genes in Anabaena, in part because plasmid DNA introduced by conjugation generally inserts into the chromosome by single recombination, leading to gene duplication, not gene replacement. YUPING CAI has found a simple, effective means of selecting for rare double recombinants. The technique employs the sacB gene from Bacillus subtilis, encoding a secreted levansucrase. When this gene is introduced into Anabaena PCC 7120, the strain becomes sensitive to 5% sucrose in solid media (but grows perfectly well in the absence of sucrose). The sacB gene was inserted into the vector portions of plasmids carrying either an insertionaly inactivated nifD gene (encoding a component of nitrogenase) or hetA gene (required for the differentiation of functional heterocysts). The plasmids were conjugated into Anabaena, and almost all drug-resistant, sucrose-resistant colonies proved to manifest the phenotype expected from gene replacement. Gene replacement was confirmed by Southern hybridization analysis.

In testing the conditional lethality of sacB in Anabaena, a sucrose-resistant strain was recovered in which the sacB gene was interrupted by a foreign sequence. This 1.7-kb sequence is found in several copies in the genome of Anabaena PCC 7120 and appears to function as an insertion sequence.

#### GENETIC MANIPULATION OF A FULLY HETEROTROPHIC CYANOBACTERIUM

Genetic manipulation of photoheterotrophic cyanobacteria has provided important insights into the molecular workings of Photosystem II. Mindful of this, many laboratories have considered applying the same bag of tricks to study Photosystem I. Unfortunately, there has been no report of any transformation or conjugation of a fully heterotrophic cyanobacterium. IRIS MALDENER sends us the welcome news that she has succeeded in introducing DNA into the chromosome of the heterotrophic Anabaena ATCC 29413 (strain FD). Working in collaboration with Wolfgang Lockau and Peter Wolk, she isolated a 5-kb DNA fragment containing a gene from Anabaena ATCC 29413 that encodes a calcium-dependent protease. A cartridge specifying resistance to kanamycin was inserted into this gene, and the resulting plasmid (also containing sacB -- see the previous report) was conjugated into Anabaena ATCC 29413 (strain FD), yielding a large number of kanamycin-resistant colonies. One colony, arbitrarily chosen, was purified and plated on medium containing sucrose to select for double recombinants. All seven colonies that were analyzed by Southern hybridization showed patterns expected from gene replacement. I.M. is now characterizing the mutant, with particular regard to its ability to differentiate heterocysts.

---

PUBLICATIONS\*PUBLICATIONS\*PUBLICATIONS\*PUBLICATIONS\*PUBLICATIONS\*PUBLICATIONS\*PUBLICATIONS\*PUBLICATIONS\*P

---

#### ECOLOGY AND ULTRASTRUCTURE

- Chang DCN, GROBBELAAR N, Coetzee J (1988). SEM observations on cyanobacteria-infected cycad coralloid roots. S Afr J Bot 54:491-495.
- Li WKW, WOOD AM (1988). Vertical distribution of North Atlantic ultraphytoplankton: analysis by flow cytometry and epifluorescence microscopy. Deep-Sea Research 35:1615-1638.
- WOOD AM (1985). Adaptation of photosynthetic apparatus of marine ultraphytoplankton to natural light fields. Nature 316:253-255.

WOOD AM, HURAN PK, MITCHELL K, PHINNEY LA, YENTSCH LM, WATERBURY JB (1985). Discrimination between types of pigments in marine Synechococcus spp. by scanning spectroscopy, epifluorescence microscopy, and flow cytometry. Limnol Oceanogr 30:1303-1315.

#### PHYSIOLOGY

- APTE SK, Bhagwat AA (1989). Salinity-stress-induced proteins in two nitrogen-fixing Anabaena strains differentially tolerant to salt. J Bacteriol 171:909-915.
- PAERL HW, Bebout BM (1988). Direct measurement of O<sub>2</sub>-depleted microzones in marine Oscillatoria: relation to N<sub>2</sub> fixation. Science 241:442-445.
- Defrancesco N, POTTS M (1988). Cloning of nifH from Nostoc commune UTEX 584 and of a flanking region homologous to part of the Azotobacter vinelandii nifU gene. J Bacteriol 170:3297-3300.
- Peat A, Powell N, POTTS M (1988). Ultrastructural Analysis of the rehydration of desiccated Nostoc commune HUN (cyanobacteria) with particular reference to the immunolabelling of NifH. Protoplasma 146:72-80.
- Thomas J, APTE SK, Reddy BR (1988). Sodium metabolism in cyanobacterial nitrogen fixation and salt tolerance. Gustav Fischer, Stuttgart. In: Nitrogen Fixation (Bothe H, de Bruijn FJ, & Newton WE, eds.) pp.195-201.
- Wealand JL, Myers JA, HIRSCHBERG R (1989). Changes in gene expression during nitrogen starvation in Anabaena variabilis ATCC 29413. J Bacteriol 171:1309-1313.
- HIRSCHBERG R, Wealand JL (1988). Effect of sulfate starvation on gene expression in the cyanobacterium Anabaena variabilis. J Cell Biol 107:353a.
- Xie WQ, Whitton BA, Simon JW, Jäger K, Reed D, POTTS M (1989). Nostoc commune UTEX 584 gene indole phosphate hydrolase activity in Escherichia coli. J Bacteriol 171:708-713.

#### BIOENERGETICS

- VERMAAS WFJ, Rutherford AW, Hansson Ö (1988). Site-directed mutagenesis in photosystem II of cyanobacterium Synechocystis sp. PCC 6803: Donor D is a tyrosine residue in the D2 protein. Proc Natl Acad Sci USA 85:8477-8481.
- Zhao J, BRAND JJ (1988). Sequential effects of sodium depletion on Photosystem II in Synechocystis. Arch Biochem Biophys 264:657-664.
- Riethman HC, Bullerjahn GS, Reddy KJ, SHERMAN LA (1988). Regulation of cyanobacterial pigment-protein composition and organization by environmental factors. Photosynth Res 18:133-161.
- Riethman HC, Mawhinney TP, SHERMAN LA (1988). Characterization of phycobilisome glycoproteins in the cyanobacterium Anacystis nidulans R2. J Bacteriol 170:2433-2440.
- Reddy KJ, Bullerjahn GS, Sherman DM, SHERMAN LA (1988). Cloning, nucleotide sequence and mutagenesis of a gene (irpA) involved in iron-deficient growth of the cyanobacterium Synechococcus sp. PCC 7942. J Bacteriol 170:4466-4476.
- Riethman HC, SHERMAN LA (1988). Purification and characterization of an iron stress-induced chlorophyll-protein from the cyanobacterium Anacystis nidulans R2. Biochim Biophys Acta 935:141-151.
- Riethman HC, SHERMAN LA (1988). Immunological characterization of iron-regulated membrane proteins in the cyanobacterium Anacystis nidulans R2. Plant Physiol 88 497-505.
- Alam J, Curtis S, GLEASON FK, Gerami-Nejad M, Fuchs JA (1989). Isolation, sequence, and expression in Escherichia coli of an unusual thioredoxin gene from the cyanobacterium Anabaena sp. strain PCC 7120. J Bacteriol 171:162-171.

#### GENETICS AND APPLIED CYANOBACTERIOLOGY

- DANIELL H, Torres-Ruiz JA, Inamdar A, McFadden BA (1989). Amplified expression of ribulose biphosphate carboxylase/oxygenase in pBR322-transformants of Anacystis nidulans. Arch Microbiol 151:59-64.
- Jäger K, POTTS M (1988). Distinct fractions of genomic DNA from cyanobacterium Nostoc commune that differ in the degree of methylation. Gene 74:197-201.
- McFadden BA, DANIELL H (1988). Binding, uptake and expression of foreign DNA by cyanobacteria and isolated etioplasts. Photosynth Research 19:23-37.
- Miyamoto K, Wable O, BENEMANN JR (1988). Vertical tubular reactor for microalgae cultivation. Biotech Lett 10:703-708.

