

CyanoNews

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CYANO NEWS

Volume 7 Number 1 April 1991

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.

SUBSCRIPTIONS - \$8 or equivalent/year. (See last page)

CONTRIBUTIONS - Expected every couple of years: a new result, an upcoming meeting or a summary of a past meeting, a post-doctoral opening, a new publication, a request for strains, a change of life... something. See last page for addresses you can send news to.

HOW TO FIND OUT MORE ABOUT SOMETHING YOU READ HERE - Contact the person whose name is capitalized in the news item. Addresses are given at the end of the issue. Also, a Directory of Cyanobacteriologists is distributed every two years. If you need one, write to Jeff Elhai (see last page of newsletter).

INSTRUCTIONS TO AUTHORS - Send news.

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PHOTOSYNTHESIS

- * Blue-light pulse induces heterotrophic growth by *Synechocystis* PCC 6803
- * PS I genes expressed in *E.coli*
- * Mutants hint at chromophore attachment mechanism
- * DNA-binding factor requires green light

SYSTEMATICS

- * Rare branching strains rediscovered
- * Codon usage yields *nif* phylogeny

MOLECULAR TOOLS

- * Shuttle vector made for *Microcystis*
- * Restriction:
 - by *Nostoc*, characterized
 - by *Agmenellum*, thwarted with novel trick

NITROGEN FIXATION

- * *nif* gene found on cyanophage

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The AMERICAN SOCIETY FOR PHOTOBIOLOGY will hold its annual meeting June 22-26, 1991, in San Antonio, Texas, U.S.A.

Richard Burke, Jr, American Society for Photobiology, 8000 Westpark Dr., Suite 130, McLean, VA 22201. (Tel) 703-790-1745. (Fax) 703-790-9063.

The AUSTRALIAN SOCIETY OF MICROBIOLOGY will hold its annual meeting in Gold Coast, Queensland, June 30 - July 5, 1991.

D. Moriarty, Chairman, CSIRO, PO Box 120, Cleveland 4163, Australia. (Fax) 61-7-2862582

The 13th NORTH AMERICAN SYMBIOTIC NITROGEN FIXATION CONFERENCE will be held in Banff, Alberta, Canada, August 25-30, 1991.

L. Nelson, Plant Biotechnology Institute, Saskatoon, SK, Canada. (Tel) 306-975-5583.
or: C.van Kessel, Dept. of Soil Science, Univ. of Saskatchewan, Saskatoon, SK, Canada. (Tel) 306-966-6854, (FAX) 306-966-6881.

The AGROTECH INTERNATIONAL EXHIBITION OF AGRICULTURAL TECHNOLOGIES AND PRODUCTS will be held in Moscow, USSR, August 20-26, 1991.

Globe International KG, P.O. Box 800349, Cologne, Germany

The INTERNATIONAL CONFERENCE ON BIOLOGICAL CONTROL IN TROPICAL AGRICULTURE will be held in Malacca, Malaysia, August 27-30, 1991.

Ahmad Anwar Ismail, MARDI, P.O. Box 12301, Kuala Lumpur 50774, Malaysia.

A conference on BIOTECHNOLOGIES AND ENVIRONMENT FOR A SUSTAINABLE DEVELOPMENT will be held in Montreal, Quebec, Canada, August 23-26, 1991.

Diane Chalifour, University of Montreal, C.P. 6128, succursale A, Montreal, Quebec, Canada H3C 3J7.

The INTERNATIONAL SYMBIOSIS CONGRESS will be held November 17-22, 1991, in Jerusalem, Israel
International Symbiosis Congress, P.O. Box 50006, Tel Aviv 61500, Israel. (Tel) 972-3-654571.
(Fax) 972-3-655674.

The Biomedical Centre (BMC) at Uppsala University and the Swedish University of Agricultural Sciences is offering a course in ALGAL BIOTECHNOLOGY, with emphasis on tissue culture and nitrogen/carbon metabolism. Electron microscopy, Western blot, and immunogold cytochemistry are some of the techniques that will be presented. The course, intended for graduate students, will be held July 1-8, 1991. There is no registration fee. Application deadline May 1, 1991.

Marianne Pedersén, Department of Physiological Botany, Uppsala University, Box 540, S-751 21 Uppsala, Sweden. Phone: +46 18 18 28 17. FAX: +46 18 55 98 55.

Two POSTDOCTORAL POSITIONS and one GRADUATE RESEARCH FELLOWSHIP are available August, 1991, for research in the structure, function, and assembly of photosynthetic membranes in cyanobacteria. One postdoctoral position will study the structure and function of Photosystem II chlorophyll-protein complexes, including a complex that is controlled by the level of iron availability. The second postdoctoral position and the graduate fellow will analyze gene regulation in nitrogen-fixing, unicellular cyanobacteria. This project will be performed as part of a NASA Center in Bioregenerative Life Support and will provide an opportunity to interact with scientists who are interested in the application of plant biotechnology for the space program. The projects involve such techniques as gene cloning, site-directed mutagenesis, and membrane biochemistry. Experience in either photosynthesis or molecular biology is desirable. Salaries are competitive. Predoctoral applicants should send a brief letter of inquiry. Post doctoral applicants should send a curriculum vitae, three letters of reference, and college transcripts.

Louis Sherman, Professor and Head, Department of Biological Sciences, Lilly Hall of Life Sciences, Purdue University, West Lafayette, IN 47907-1392 U.S.A. (FAX) 317-494-0876.

A new CYANOBACTERIAL DIRECTORY and DIRECTORY OF E-MAIL ADDRESSES has been compiled.

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We note with sadness the passing of MOSHE SHILO, who died on June 27, 1990.

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MASS CULTURES OF CYANOBACTERIA FIGHT CANCER

TOM ALLNUTT tells us about cyanobacterial activities at Martek Corporation. They are currently using cyanobacteria to produce labeled biomass, using stable isotopes (C-13 and N-15), and to produce phycobiliproteins in bulk. They are also a sub-contractor for the National Cancer Institute for scale-up production of cyanobacterial strains that have been shown to produce compounds with anti-cancer or anti-AIDS activity, concentrating at present on *Lyngbya lagerhemii*, *Phormidium tenue*, and *Aphanocapsa musicola*. Martek is always looking for unique compounds that cyanobacteria might economically produce.

Unfortunately, we are told by GREG PATTERSON, due to the 10% blanket budget cuts imposed at the U.S. National Cancer Institute, the recompetition for the blue-green cultivation contract has been cancelled. The original contract funded the collection of over a thousand cyanobacterial strains by workers at University of Hawaii for the purpose of identifying compounds active against cancer and AIDS.

NEW RESTRICTION BARRIERS FOUND, THWARTED BY NOVEL TRICK

DON BRYANT reports that after much anguish JIANHUI ZHOU in his laboratory made the realization that there is a second, previously unrecognized restriction activity in *Synechococcus* PCC 7002 (*Agmanellum quadruplicatum*) in addition to *AquI*. The specificity of the activity is not known, but they have overcome the problem in a way that might be generally applicable to other systems. They use sonicated extracts of PCC 7002, in the presence of 10 mM EDTA and 80 μ M S-adenosylmethionine, to methylate DNA prior to transformation. DNA that yields no transformants without methylation can readily transform *Synechococcus* after the procedure. The extracts can be stored for 7 to 14 days at -80° C.

DUANE MOSER is investigating restriction by *Nostoc* PCC 7121 as a potential barrier to transformation. He has partially purified one endonuclease from the organism and found that it is an isoschizomer of *AsuI*.

CYANOPHAGE MAY TEACH *NOSTOC* TO FIX NITROGEN

F. KAMILOVA and her colleagues have recently shown that at least one of the genes encoding nitrogenase is present in the DNA of temperate cyanophages of the NP-1T series and in the DNA of *Nostoc* sp. N39 lysogenized by these phages. The probe used (a 6.2 kb *EcoRI* fragment from *Klebsiella pneumoniae*) did not detect *nif* sequences in uninfected *Nostoc* sp. N39.

RARE BRANCHING CYANOBACTERIA REDISCOVERED

LUCIEN HOFFMAN announces the rediscovery of rare branching cyanobacteria. Many of the true branching cyanobacteria (section V of Rippka et al. [J Gen Microbiol (1989) 111:1-61]; *Stigonematales* of algologists), the morphologically most differentiated prokaryotes, are very rare and often found only in biotopes with very specialized ecological conditions. Only two genera have been recharacterized from axenic cultures. Recently two of these rare cyanobacteria, the genera *Loriella* and *Mastigocladopsis*, were rediscovered. *Loriella osteophila*, described in 1892 on the basis of material growing on human skulls in Papua New Guinea and not observed since, was rediscovered on limestone in the same region [Hoffmann, Br Phycol J (1990) 25:391-395]. This genus is characterized by dichotomous branching, a rare feature in cyanobacteria. *Mastigocladopsis jogensis*, also not observed since its first description in India in 1946, was rediscovered in Corsica [Hoffmann, Crypt Algal (1990) 11:219-224]. This genus is characterized by having lateral heterocysts, a characteristic observed in very few cyanobacteria. The fact that these two species were so far not found in the well studied temperate regions of Europe and North America indicates that they may have a very limited geographical distribution. These findings also show that taxonomists should continue to work with the old floras, because many of the cyanobacterial genera have not yet been cultured. Neglecting these genera would mean that the morphologically most differentiated cyanobacteria would not be considered.

SHUTTLE VECTOR CONSTRUCTED FOR *MICROCYSTIS*

SHIRLEY RAPS's laboratory is characterizing a plasmid, pMa025, recently isolated from *Microcystis*. Preliminary results indicate that a hybrid plasmid they have constructed can serve as a shuttle vector between *E. coli* and the strain of *Microcystis* from which pMa025 was isolated.

PS I GENES EXPRESSED IN *E. COLI*

DON BRYANT's laboratory has continued its efforts to express PS I genes from *Synechococcus* PCC 7002 in *E. coli* with the aim of using the gene products in reconstitution experiments. Jindong Zhao has expressed *psaC*, *psaC1*, *psaD*, *psaE*, and *psaF* in *E. coli* (the work on *psaC1* and *psaD* recently appeared in FEBS Lett (1990) 276:175-180). Mutant *PsaC* proteins are being produced for analysis of the roles of iron-sulfur clusters F_A and F_B in electron transport. Wendy Schluchter has completed the sequence of the *petH* gene (encoding FNR = ferridoxin:NADP oxidoreductase) in *Synechococcus* PCC 7002. It predicts a protein of 45kDa, which is the size of the mature protein in *Synechococcus*, as judged by Western blots. The gene is required for viability. Next to *petH* and transcribed in the opposite direction is *ndh5*. Expression of *petH* in *E. coli* would make all proteins available for the reconstitution of the electron transport chain from P_{700} to NADP.

PHYLOGENY OF *NIF* GENES DERIVED FROM CODON USAGE

RAKESH TULI updates us on the analysis he and Manjula Mathur have performed on the genes encoding proteins related to nitrogen fixation (*nif* genes). Previous results were reported in CyanoNews (May '89 and March '90) and have since been published [J Genetics (1990) 69:67-78]. They have developed a method for cluster analysis of relative synonymous codon usage (RSCU). RSCU values groups the *nif* genes from various organisms in a taxon-specific manner. For instance, all the *Azotobacter nif* genes cluster together on the basis of codon usage, while analysis of sequence similarity leads to anomalous groupings. The groupings based on sequence similarity may be indicative of lateral transfer of *nif* genes in some cases [Mathur and Tuli, J Mol Evol (1991) in press].

The *nif* genes of *Anabaena* make a separate group according to RSCU-based cluster analysis. Only in the *nif* genes of *Anabaena* is the GC content at silent and replacement positions similar to each other and fall within the standard deviation for the universal mutation selection equilibrium point [Ep and Sueoka (1988) Proc Natl Acad Sci USA 85:2653-2657]. This finding suggests that the directional mutation pressure is completely counteracted by the selective constraints on cyanobacterial *nif* genes. The *Anabaena* genes show negligible GC/AT pressure and are comprised of codons selected for high translational efficiency (i.e., intermediate codon-anticodon interaction energy [Grosjean and Fiers (1982) Gene 18:199-209]).

ENZYMATIC ATTACHMENT OF CHROMOPHORE TO PHYCOBILIPROTEIN DEDUCED

JIANHUI ZHOU has recently shown that the yellow-green (PC-deficient) phenotype of strain lacking *cpcBA* and mutated in *cpcE* can be suppressed by a plasmid carrying *cpcBA* in which a Tyr of *CpcA* has been replaced by Cys. A similar plasmid carrying wild-type *cpcBA* cannot suppress the phenotype. This result suggests that the attachment of phycocyanobilin is enzymatically accomplished and that an alternative enzyme system can function with the mutant protein (which must be a much better substrate for that system than the wild-type protein).

A site directed mutation (Ser to Cys) in *ApcE* prevents the attachment of the chromophore to that polypeptide, although phycobilisomes are still assembled. Interestingly, a new emission maximum of about 715 nm appears from the intact structures. Perhaps the *ApcE* protein is binding the precursor of peptide-bound phycocyanobilin. The structure of the precursor is unknown, but it is expected to contain one additional double bond, hence would give a red-shifted emission. If true, then it should be possible to use this mutant to study energy transfer and chromophore attachment.

Alicia Esteban and Vicki Stirewalt are sequencing *apcEABC* and *apcF* from *Mastigocladus laminosus*. Manuel Glauser has completed sequencing a fragment from the same organism that encodes a portion of *cpcF*, *cpcG1*, *cpcG2*, and *cpcG4*.

ALGAL PLASTID GENOMES SEQUENCED, MAPPED

Perhaps it is presumptuous to admit chloroplast news into this newsletter, but plastids may shed some light on cyanobacterial matters (and they may have some small interest in their own right). MICHAEL REITH has been sequencing the plastid genome of *Porphyra umbilicalis*, a red alga related to the species used to wrap sushi. The red algal plastid genome is larger than that of higher plants (about 185 kb vs. 155 kb) with a smaller inverted repeat (about 5 kb vs. 25 kb). These numbers suggest the potential for approximately 50 kb more of coding capacity in the red algal plastid genome. Indeed, his group has already detected six genes and a possible ORF that aren't encoded on higher plant plastid genomes. For all the genes that they've sequenced or partially sequenced (about 15), the similarity between cyanobacterial and red algal plastid versions is quite high. Pure cyanobacteriologists may be interested in the use of genes from the red algal plastid genome as probes for some genes not encoded on higher plant plastid genomes.

The plastids of *Cyanophora* differs from those of *Porphyra* by the presence of a peptidoglycan envelope and a smaller plastid genome (130 kb). Approximately 60 kb has been sequenced by all laboratories working on *Cyanophora*. Vicki Stirewalt (says DON BRYANT) has continued her work on the plastid genome of *Cyanophora*. She has found genes encoding DnaK (a heat shock protein), GroEL (a chaperonin), Rps11 and Rps13 (ribosomal proteins), and FrxC (a subunit of protochlorophyllide reductase), amongst others. The 16S rRNA sequence has also been completed.

HETEROTROPHIC GROWTH BY SYNECHOCYSTIS REQUIRES BLUE LIGHT

SHAWN ANDERSON and Lee McIntosh report on their study of the heterotrophic growth of *Synechocystis* sp. PCC 6803. A glucose tolerant strain of *Synechocystis* PCC 6803 is unable to grow on glucose in complete darkness unless given a brief daily pulse of light, typically 5 min of 40 $\mu\text{mol m}^{-2}\text{s}^{-1}$ of white light. A doubling time of 36 hours is observed for cultures containing 5 mM glucose that are pulsed with light. The light pulse alone is insufficient for photoautotrophy, as glucose is required and growth yield is dependent upon glucose concentration. A strain of *Synechocystis* PCC 6803 defective in PS II (*psbA*) grows at a rate similar to that of the glucose tolerant strain under light pulsed conditions, indicating that PS II is not required for growth. Only blue light (400 to 500 nm, maximum at 450 nm) is effective to promote growth under these conditions, and this spectral sensitivity precludes energetic contribution from cyclic electron transport around PS I. The required blue light pulse evidently does not support growth via photosynthetic electron transport but rather functions as an environmental signal regulating heterotrophic metabolism, cell division, and/or other photomorphogenic processes. The requirement for pulses of blue light pulse for the growth of *Synechocystis* PCC 6803 has been termed "light-activated heterotrophic growth" (LAHG) to distinguish *Synechocystis* PCC 6803 from those cyanobacteria that can grow heterotrophically under complete darkness (i.e., *Anabaena variabilis* ATCC 29413). A report on this work will appear in the *Journal of Bacteriology*.

GREEN-LIGHT SPECIFIC DNA BINDING FACTOR CROSSES GENERIC BOUNDARIES

Jim Dubbs finished his dissertation in DON BRYANT's laboratory on phycobiliprotein genes in *Pseudanabaena* PCC 7409 and has moved on to work with Jim Barber. In the course of his dissertation work, Jim obtained evidence for a green-light specific DNA binding factor in *Calothrix* PCC 7601 that binds to a small fragment containing the promoter of the *cpeBA* operon. Attempts to identify the precise binding site were unsuccessful, but a region adjacent to the promoter that contains direct repeats is a prime suspect. The DNA binding factor of *Calothrix* also bound strongly to *Pseudanabaena* DNA, and this binding also was green-light specific.

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The FIRST HISPANOAMERICAN MEETING ON BNF (Biological Nitrogen Fixation) RESEARCH (y Reunion Mexicana) will be held 2-5 December 1991 in Mexico. Following the meeting there will be a post-graduate course entitled "Biochemical Aspects of BNF", taught by Lawrence Davis, Kansas State University, USA. Contact:

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