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CYANONEWS

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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.

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.

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A new COMPUTER NETWORK has been organized, reports JOHN ERIKSSON, with the purpose of promoting informal and rapid exchange of information between people in different parts of the world working on subjects related to TOXIC CYANOBACTERIA and CYANOBACTERIAL TOXINS. Specifically, the organizers envision the following services:

a. Distribution of meeting announcements.
b. Updated directory of people working in the field.
c. List of published papers, updated at regular intervals.
d. Information on papers in press.
e. Exchange of technical information on methods and equipment.

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All subsequent contributions should be sent to either (bitnet/earn) cyan-tox@grearn or (internet) cyan-tox@grearn.bitnet. If you experience problems subscribing, contact JMeriluoto@FinAbo or TLanaras@FinAbo.

Two errors crept into the recently distributed DIRECTORY OF CYANOBACTERIOLOGISTS:
The electronic mail addresses for C. Sybesma should be Z23001@BBRBFU01.Bitnet
and for Jeff Elhai should be 21417BBS@MSU.Bitnet

"CRC HANDBOOK OF SYMBIOTIC CYANOBACTERIA", edited by Amar Rai, will soon be available from CRC Press. The book has chapters devoted to cyanobacterial associations with fungi, bryophytes, Azolla, cycads, and Gunnera, including physiology, biochemistry, and molecular biology of the cyanobiont. Applied aspects are also discussed, such as the use of Azolla as a nitrogen source. The handbook (catalog no.3275) is available November, 1989, from CRC Press, Inc., 2000 Corporate Blvd., N.W., Boca Raton, Florida 33431 U.S.A. (Tel) 800-272-7737. 272 pages, $139.95 (within U.S.), $165.00 (outside U.S.).

LUBOMIR KOVÁCIK sent in a paper (written with Dieter Mollenhauer) of interest to students of the history of our chosen field. Entitled "Who was who in cyanophyte research", the paper offers brief biographies of our predecessors, with the stated goal "to keep the memory green of all those that contributed to the knowledge of Cyanophytes". The first installment presents sketches (words and pictures) of Agardh (father and son), Lyngbye, Bornet, Thuret, and many others. Here's an opportunity to give faces to those genus and species names.

MEETINGS

A EUROPEAN WORKSHOP ON THE MOLECULAR BIOLOGY OF CYANOBACTERIA will be held in Dourdan (25 km from Paris) from May 13 to May 16, 1990. One of its main goals is to gather together young European scientists interested in that field. It will be a small informal meeting. The number of participants will be limited to about 70. For further information, contact without delay: Nicole Tandeau de Marsac, Physiologie Microbienne, Institut Pasteur, 28 rue du Dr. Roux, F-75724 Paris cedex 15, FRANCE. Tel: (1) 45 68 84 15. Fax: (1) 43 06 98 35. Electronic mail: CYANO@PASTEUR.Bitnet.

Organizers of the IXth INTERNATIONAL CONGRESS ON PHOTOSYNTHESIS have already sent out the first circular, describing the meeting to be held in Nagoya, Japan, August 30 - September 4, 1992 (three years from now, no misprint). Several satellite meetings will take place in Tokyo, Osaka, and elsewhere. The 11th International Congress on Photobiology will be held the following week in Kyoto. To receive the second circular (to be sent in the Fall of 1991), contact Norio Murata, IXth International Congress on Photosynthesis, National Institute for Basic Biology, Okazaki 444, Japan.

POSITIONS WANTED

CONTACT: R.S. Shantha Kumar Hopper, Department of Microbiology, School of Biological Sciences, Madurai Kamaraj University, Madurai, 625 021, INDIA.
POSITION SOUGHT: Post-doctoral research.
Ph.D: 1986 (U. Madras), "Studies on Blue-Green Algae (Cyanobacteria) from Several Rice Fields of Kerala State, India" [see synopsis in this issue].
EXPERIENCE: Six years research experience on the taxonomy and physiology of cyanobacteria.
AVAILABLE: Immediately.

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ACCUMULATION OF GLUCOSYLGLYCEROL IN SALT-STRESSED MICROCYSTIS FIRMA

ULRICH SCHIEWER tells of the results of experiments performed in his labs, with coworkers N. Erdmann, M. Hagemann, C. Berg, and S.Fulda. Microcystis firma, like most other cyanobacteria, is unable to adapt to higher salt concentrations in the dark. This is caused by the limited glycogen pool, which is depleted before a sufficient amount of glucosylglycerol has been synthesized from the compound. Glucosylglycerol is an osmoprotectant found thus far only in cyanobacteria. In the light, the salt-dependent accumulation of glucosylglycerol is characterized by two features: negligible turnover in salt-adapted cells, and small but continuous leakage into the medium.

PLASMID TO MEASURE PROMOTER STRENGTHS IN SYNECHOCYSTIS PCC 6803

FRANK CHAUVAT describes a vector-host system for testing promoters in the cyanobacterium Synechocystis PCC 6803 that he and Fabrice Ferino constructed. It relies on a small E. coli promoter-probe plasmid, pFF11, which has four unique restriction sites in a polylinker upstream from a gene encoding chloramphenicol acetyl transferase (CAT). This plasmid can transform PCC 6803 and persists by recombining with a resident plasmid through a cloned region of homology. Transformants do not have detectable CAT activity unless DNA has been inserted into the polylinker. This system was used to measure the activity of several heterologous promoters in PCC 6803. Results obtained with the lambda P_R promoter and the cI_{857} repressor gene demonstrate that these elements can be used for high level and tightly regulated gene expression in PCC 6803.

PLEIOTROPIC MUTANT IMPAIRED IN AMMONIUM-DEPENDENT REGULATION ISOLATED

ENRIQUE FLORES and other coworkers (M.A. Vega, F. Madueño, and A. Herrero) have isolated several mutants from Synechococcus R2 simultaneously impaired in several activities normally subject to repression by ammonium. The mutants are impaired in the nitrate assimilation system (i.e., nitrate reductase, nitrite reductase, and the 48-KDa cytoplasmic membrane protein involved in nitrate transport), in glutamine synthetase, and in methylammonium transport. All of these proteins or activities are subject to ammonium repression in Synechococcus. The mutants, however, do not respond to the nitrogen status of the medium (NH_4^+, NO_3^-, or no nitrogen source), exhibiting levels similar to those of the wild type grown on ammonium. Therefore, the gene altered in these mutants appears to be required for the derepression of ammonium-repressible enzymes in Synechococcus. The regulatory gene has just been cloned by complementation and is being characterized.

PHYSIOLOGY OF ISOLATES FROM RICE FIELDS STUDIED

R.S. SHANTHA KUMAR HOPPER passed on a summary of the work that made up his Ph.D. thesis entitled "Studies on blue-green algae (cyanobacteria) from several rice fields of Kerala State (India)". The goal of his work was to lay the groundwork for the exploitation as fertilizer of nitrogen-fixing strains native to southern India. 161 taxa of cyanobacteria were identified in rice fields from all districts of Kerala, but only twelve taxa are widely distributed amongst the fields. Soils of the rice fields of Kerala have two distinctive ecological characteristics: low pH and fluctuating salinity. Over 50 cyanobacterial isolates were screened for their tolerance to salt and low pH. Certain isolates were very sensitive to high salinity, excreting ammonium and losing phycobilin pigments to the medium after a short exposure. Three strains were chosen for further study: A halotolerant isolate, Oscillatoria sancta (Kütz) Gomont, and two sensitive strains, Oscillatoria salina Biswas and Nostoc piscinale Kützing ex Born. et Flah. Several physiological properties, including growth, photosynthesis, pigment content, carbohydrate content, and (in the case of N. piscinale) nitrogenase activity, were determined in response to various levels of salt. Several salts were tested.

ADAPTATION OF PLANKTIC CYANOBACTERIA TO LIGHT AND NITROGEN SUPPLY

J.-G. KOHL offers two instructive examples of how physiological properties of a strain are in accord with the needs imposed by the strains particular ecological niche. An approach to estimate ecologically relevant traits of cyanobacteria and algae was recently summarized (Kohl and Nicklisch, 1988).

Planktic cyanobacteria within shallow waters are exposed to very short cycles of light supply (on the order of minutes) due to Langmuir-circulation. Growth studies with isolated strains of Limnothrix (formerly Oscillatoria) redekei and O. agardhii under different light regimes showed very high light-use efficiency maintained even with extremely short daylight periods (3 hrs light, 21 hrs dark). The initial increments of growth vs. light were 0.021 and 0.013 $J^{-1}cm^2$ for L. redekei and O. agardhii respectively. However, maximum specific growth rates were lowered in a species-specific manner: 0.78 d^{-1} for L. redekei and 0.58 d^{-1} for O. agardhii, both grown 12 hrs light, 12 hrs dark; 0.22 d^{-1} for both strains grown 3 hrs light, 21 hrs dark. The maximum specific growth rates achieved under continuous light were approximated by supplying cultures illuminated 3 hrs, 6 hrs, or 12 hrs per 24 hr period with short light/dark cycles (5 min/15 min or 15 min/15 min). These traits mark these species as well adapted to conditions found in lakes that are well mixed by turbulence and have steep vertical light attenuation. (Nicklisch and Kohl, in press)

Some Anabaena species (e.g., Anabaena solitaria, Anabaena lemmermannii) differ from more typical heterocystous strains (e.g., Aphanizomenon flos-aquae and Aphanizomenon gracile) by maintaining heterocysts even if ammonium or nitrate is supplied in ecologically relevant concentrations (10 mM). The nitrogenase activity of both groups, however, is suppressed under these conditions. These different regulatory behaviors may be interpretted as attempts to minimize costly heterocyst differentiation in response to a nitrogen supply that is continuous (for the Aphanizomenon strains) or changing (for the Anabaena strains). Neither the maximum specific growth rate nor the efficiency of light utilization by these planktic species are strongly affected by the shortage of combined nitrogen. Therefore, these species are able to stabilize effectively primary productivity during nitrogen limitation, even when subjected simultaneously to light limitation (Kohl et al., in press).

The Third International Workshop on the Molecular Biology of cyanobacteria took place in Toronto this past July 27 - July 29. It was quite a trick to fit about seventy posters and talks into the five sessions, but organizer John Coleman made this and many other feats possible. Since even his talents could not fit the meeting into the space of this newsletter, only a taste of it follows.

Photosynthesis (physical aspects)

33 kDa manganese stabilizing protein (MSP): Two groups told of their efforts to perform site-directed mutagenesis on the manganese stabilizing protein (MSP), encoded by *woxA* (also known as *psbI* and *psbO*). Rozita Rosli has used secondary structure analysis to identify potentially important functional regions in the MSP of *Anacystis nidulans* R2. Aspartic acid residues 251 and 253 of the MSP were changed by saturation mutagenesis, and the modified *woxA* genes were cloned behind a temperature-sensitive promoter and introduced into *A. nidulans*. Some of these strains exhibited altered growth rates and color differences relative to wild-type when the modified *woxA* genes were expressed (at elevated temperatures). Rob Burnap has analyzed cadmium resistant mutants, using molecular modelling, to identify putative metal binding sites in the MSP of *Synechocystis* PCC 6803. He described two constructions: (1) replacement of *woxA* with either a kanamycin or spectinomycin cartridge, which is being used to delete *woxA* from the PCC 6803 chromosome; and (2) insertion of a kanamycin cartridge after *woxA*, which will be used to introduce mutagenized *woxA* genes into the PCC 6803 chromosome.

cytochrome b_{559}: Himadri Pakrasi described chromosomal deletions of the *psbEFIJ* gene cluster from *Synechocystis* PCC 6803. Deletion of this cluster eliminated PSII activity and resulted in the additional loss of D1 and D2 from the thylakoids. Characterization of directed single site mutations in the beta- and alpha-subunits of this protein are in progress.

Cytochrome b_6-f complex: Toivo Kallas described his efforts to establish a genetic system for the study of the b_6-f complex. One direction is to express the gene (*petC*) encoding the Rieske Fe-S protein from *Nostoc* PCC 7121/PCC 7906 in *E. coli*, the aim being to combine in vitro this protein with Rieske-depleted complex to obtain catalytically active complex.

Plastocyanin: Linda Briggs reported that plastocyanin is present in *Synechocystis* PCC 6803 in copper-containing medium. She has succeeded in expressing plastocyanin from *Silene pratensis* in *E. coli* as the product of a gene fusion. The product is 18.5 kDa and contains the signal peptide as well as the mature coding sequence.

Photosystem I: Larry Smart has cloned *psaA* and *psaB* (encoding the PSI core proteins) from *Synechocystis* PCC 6803. These genes are present in single copy in the genome and are organized in the arrangement 5'-*psaA*-*psaB*-3'. They appear to be cotranscribed. Shawn Anderson has cloned and sequenced *psaC* (encoding the 9 kDa 2(4Fe-4S)-containing subunit of PSI) from *Synechocystis* PCC 6803. The deduced amino acid sequence is identical to that of tobacco. Analysis of adjacent DNA sequences suggests that the gene arrangement surrounding *psaC* in *Synechocystis* has similarity to that in chloroplast. In chloroplast, *psaC* is preceded by *ndhE* and succeded by *ndhD*, possibly encoding subunits of NADH dehydrogenase. An open reading frame 5' to *psaC* in PCC 6803 is very similar to the maize *ndhE* gene. The correspondence continues downstream from *psaC*, where a small open reading frame shows similarity to the first 20% of *ndhD*, but it extends no further.

(contributed by Edward Bylina)

Gene Regulation

Light-regulated gene expression was the focus of several talks and posters. Both light intensity and wavelength were reported to influence the expression of genes in cyanobacteria.

Sue Golden described some of her group's work on the regulation of the *psbAI*, *psbAII*, and *psbAIII* genes, encoding two forms of the D1 protein of photosystem II in *Synechococcus* PCC 7942. Translational fusions

and immunological assays showed that the expression of psbAI was inversely correlated with light intensity, while the expression of psbAII and psbAIII increased with increasing light intensity. Sylvia Bustos reported on the steady state levels of transcripts from the psbA genes. When Synechococcus was shifted to high light intensity, transcription of psbAII and psbAIII increased, while transcription of psbAI declined. The opposite behavior was seen following a shift to low light intensity. Analysis of the 5' region of psbAII revealed the presence of two transcriptional start sites, separated by about 400 bp.

Robert de Lorimier and Russell Smith presented work concerning the correlation of phycobilisome structure and gene expression with irradiance. Using Agmenellum quadruplicatum PR-6, they found that the ratio of phycocyanin (PC) in the rods to allphycocyanin (AP) in the core varied inversely with light intensity and that this change in the PC/AP polypeptide ration could be directly attributed to changes in the relative steady state levels of mRNA encoding these proteins. Interestingly, the nitrogen source (ammonia vs. nitrate) also influenced the PC/AP ratio, with ammonia producing an increase in relative PC content of 30%. In addition, the abundance of a linker protein, LR33, that is associated with PC increased in parallel with the concentration of PC, but the level of LR33 transcript did not change with light intensity. This raises the possibility that translational or post-translational control mechanisms are involved in determining the concentration of LR33.

One other contribution focused on the regulation of genes encoding phycobilisome components. Nancy Federspiel reported on the characteristics and sequence of two genes from Fremyella diplosiphon that are regulated by green light. These genes, designated cpeC and cpeD, encode phycoerythrin-associated linker proteins and are cotranscribed on a 1930 nucleotide message. A longer transcript, extended 1200 nt at the 3' end, occurs at a lower level. The regulation of this operon parallels that of the operon encoding phycoerythrin (PE). In both cases, transcripts are found at high levels in cells grown in green light and low levels in cells grown in red light. A protein factor is present in cells grown in green light (but not in cells grown in red light) that binds to the PE promoter.

John Brusca also identified a factor that binds to regulated promoters. A factor found in extracts from vegetative cells of Anabaena PCC 7120 bound to the promoters of genes encoding rubisco, glutamine synthetase, and excisase (catalyzing the excision of an 11-kb element in nifD). It did not bind to the promoter of nifHDK, encoding nitrogenase.

Four advances of a technical nature were presented. John Cobley reported on progress in complementing Fremyella diplosiphon mutants defective in chromatic adaptation. He has constructed a cosmid library that can be transferred by conjugation into a mutant of F. diplosiphon. Cosmids have been identified that appear to complement two of the mutants. Teresa Thiel presented a detailed protocol for the electroporation of Anabaena M131 to obtain gene transfer at a frequency of 10^{-3} per viable colony forming unit. This frequency was 100-fold lower if no effort was made to protect the DNA against restriction. Yuping Cai described the use of a conditionally lethal gene, sacB (encoding secretory levansucrase), to select for double recombination events in filamentous cyanobacteria. This technique facilitates the isolation of mutants resulting from insertional mutagenesis. Lamont Anderson showed that the phycocyanin (PC) genes of Synechosystis PCC 6701 could be expressed in a PC-minus mutant of Synechocystis PCC 6803. The foreign genes were incorporated into normal phycobilisomes, utilizing the host linker proteins, which are not normally present in the mutant host.

(contributed by Nancy Federspiel)

Nitrogen Metabolism

Several presentations addressed the question of how heterocyst differentiation is controlled. Jeff Elhai reported evidence that genes encoding nitrogenase are under the control of developmental signals and not regulated merely by local environmental conditions. He used lacZ fusions to monitor the time-course of induction of the nif structural genes under anaerobic conditions and used fusions to luxAB (encoding luciferase) to monitor the localization of expression. Transcription directed by the nifHDK promoter appeared confined to cells morphologically distinguishible from vegetative cells. John Smith looked for heterocyst-specific genes, using polyclonal antibodies directed against either total heterocyst or total

vegetative protein to screen an expression library made from the DNA of Nostoc PCC 6720. His results suggested that many of the genes involved in heterocyst differentiation may experience a quantitative change in the rates of their expression rather than a qualitative turn-on or shut-off.

The search for genes important in the differentiation of heterocysts was approached also by isolating and complementing mutants impaired in nitrogen metabolism. Bill Buikema described several mutants of Anabaena PCC 7120 impaired in their ability to grow on dinitrogen. Two of these could be complemented by a single cosmid, although they differed markedly in phenotype. It is therefore likely that certain genes involved in heterocyst differentiation are closely linked to each other. Doron Holland discussed hetA, a gene required for heterocyst differentiation. Transcription of hetA is induced relatively soon after deprivation of fixed nitrogen. When expression of luciferase was put under the transcriptional control of hetA, light was emitted only from a small number of spatially separated cells.

Enrique Flores also isolated complementable mutants impaired in nitrogen metabolism, with the aim of identifying genes exerting global control over nitrogen assimilation. His mutants, from Anabaena PCC 7120, Anabaena variabilis ATCC 29413, Synechococcus PCC 7942, and Synechocystis PCC 6803, include those impaired in amino acid transport, arginine metabolism, molybdate metabolism, and nitrate metabolism. One class of mutants lost several activities normally subject to repression by ammonium.

Peter Wolk described the construction of a genetic map of Anabaena PCC 7120. This was achieved by restriction mapping and hybridization with gene-specific probes. The strain contains a 6.4 Mb circular chromosome and three large, apparently circular plasmids. Genes encoding components of the photosynthetic apparatus do not lie within a single cluster. He and Chris Bauer independently reported transposition of Tn5 or a derivative into the chromosome of PCC 7120.

(contributed by Mary Allen)

Physiology and Metabolism

David Laudenbach presented work on genes involved in sulfate utilization by Anacystis nidulans R2. Using the Salmonella typhimurium cysA locus as a heterologous probe, they cloned a 12-kb fragment of the Anacystis genome containing at least five transcripts that are inducible under sulfate deprivation. Insertional inactivation in this region results in inability to take up sulfate, causing cysteine auxotrophy. Sulfate-binding proteins, found in the preiplasmic space, are not required for sulfate transport, but expression of their genes is required for induction of the sulfate transport system. The gene for rhodanase (thiosulfate sulfurtransferase) is normally expressed only in sulfate-starved cells. However, this gene is expressed even in the presence of sulfate in the binding-protein mutants. To interpret these results, it was proposed that the function of the sulfate-binding proteins may be to sense the sulfate status of the cells, rather than to facilitate sulfate uptake.

Elisha Tel-Or described work on osmoregulation in a halodependent Spirulina. These cells grow in 3 M NaCl, actually colonize salt grains, and do not grow below 0.25 M NaCl. The sole cellular osmoregulant is apparently glycinebetaine, and no amino acids, carbohydrates, etc., accumulate. Glycinebetaine, a zwitterion, is also a cellular preservative of several enzymes, and, e.g., protects the conformation of glucose-6-phosphate dehydrogenase. Following a NaCl shock, the cellular glycinebetaine concentration increases over a 48-hour period. However, in the earlier stages of response to salt shock, another salt-protecting mechanism may come into play. Active exclusion of Na^+ begins 12 to 24 hours after transfer of the cells to medium containing high Na^+ concentrations and does not occur in the dark. The early-acting haloprotecting mechanism may be a Na^+-H^+ antiport system, coupled to proton export driven by cytochrome oxidase and a H^+-ATPase. Evidence for such a system includes Na^+-stimulated increased respiration rate and high levels of Na^+-stimulated H^+-ATPase induced by hypersaline growth.

Devorah Friedberg described a role for carboxysome structure in conferring to Synechocystis PCC 7942 (A. nidulans R2) the ability to grow at atmospheric CO_2 concentration. Mutant cells that are unable to grow at atmospheric CO_2 levels contain normal rubisco and CO_2 uptake systems. The mutants were complemented by plasmid-borne DNA from wild-type cells. Rescued plasmids contain an insert that maps to a position 1800 bp upstream from the rubisco gene and bears two open reading frames. Under the electron microscope, the carboxysomes of mutant cells appear aberrant or are absent. Complemented cells have restored car-

boxysome structure. The genes required for growth at atmospheric CO_2 concentration apparently are required for normal carboxysome structure. One possibility is that they encode carboxysome envelope proteins.

Sheldon Broedel presented work on the cloning and expression of the gnd gene of Synechocystis PCC 7942. This gene encodes 6-phosphogluconate dehydrogenase (6PGD), which functions along with glucose-6-phosphate dehydrogenase (G6PD) to convert glycogen-derived glucose-6-phosphate to ribulose-5-phosphate in the oxidative pentose phosphate pathway. The enzyme activity of 6PGD increases four- to six-fold upon transition from exponential to stationary growth phase. Mutants having a disrupted gnd gene are dark sensitive (possibly due to accumulation of toxic concentrations of 6-phosphogluconate), and only 2% of the cells survive 24 hours of darkness. By using gnd mutant cells, the wild-type gene was cloned and its expression studied. Experiments with gnd-lacZ fusion constructs indicate that the level of gnd gene expression increases 4.5-fold upon transition from exponential to stationary growth phase. It was concluded that the increase in 6PGD enzyme is due to increased transcription of the gnd gene during the growth stage transition.

(contributed by Sam Beale)

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