1996

CyanoNews (Vol. 12, No. 1, January 1996)

Jeff Elhai
Virginia Commonwealth University, elhaij@vcu.edu

CYANONEWS

Volume 12 Number 1 January 1996

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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.

SUBSCRIPTIONS - $10/year for hard copy version. No charge for electronic version. See last page for details.

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 - Each news item contains, prominently displayed, the name of a contact person. A Directory of Cyanobacteriologists is distributed every two years or on request.
INSTRUCTIONS TO AUTHORS - Send news.

Cyanonews has been placed on-line to assist in the proliferation of this important document.
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The Web

For those of you who have experienced the World Wide Web, no explanation is necessary. For those of you who have not, no explanation is possible... nonetheless, here's an attempt. Imagine an encyclopedia that is continuously and instantaneously being updated by its readers strewn throughout the world. Each entry, or homepage, is a collection of pertinent text, graphic images, and sounds deemed appropriate by its creator, plus instant connections to other entries. You can visit a homepage and consume or even download items of interest or you can surf from connection to connection, often finding yourself far afield from where you started.

There are already several homepages of interest to cyanobacteriologists. Ben Long (La Trobe U.) is perhaps furthest along in a homepage devoted to cyanobacteriology. The focus of his homepage is TOXIC CYANOBACTERIA and currently includes:

1. A short introduction to microcystins, including diagrams
2. A description of Microcystis aeruginosa,including a scanning EM picture
3. Preliminary information on protein phosphatases
4. Information on Cyano-Tox, a discussion group on toxic cyanobacteria and their toxins
5. Access to Cyanonews, including back issues and the Directory of Cyanobacteriologists
6. Links to some other relevant homepages

The site is still under construction (and probably always will be), and Ben is anxious to receive feedback, submissions, and suggestions for additions. In particular, he would like contributions that update or correct any errors on the existing page and information from those who work on other cyanobacterial toxins (or secondary metabolites). Even contributions that are only tangentially related to toxic cyanobacteria are welcome, as the more information available on any topic will make the page much more useful. Some people have indicated eagerness to see pictures of their favorite cyanos. Any pictures (in GIF format) are welcome! Ben is also interested to make contact with someone well versed in HTML, the language of making web pages. The page may also be used as a bulletin board for up and coming events. Please send them in!

CONTACT: Ben Long, TEL: 61 3 9479 2771, FAX: 61 3 9479 1188 WEB: http://Luff.Latrobe.Edu.Au/~BotBML/Cyanotox.Html E-MAIL: BotBML@Luff.Latrobe.Edu.Au

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Mark Schneegurt (Purdue U.) is initiating a GENERAL CYANOBACTERIOLOGY web site, which should be up and running the middle of January, 1996. It will

eventually contain a searchable bibliography of cyanobacteria-related articles. Anyone with items that would be of interest -- e.g., lists of gene names, sequenced genes, etc. Mark is especially interested in protocols and teaching materials. Items can be sent to him by E-mail or FTP.
CONTACT: Mark Schneegurt E-MAIL: MSchnee@Bilbo.Bio.Purdue.Edu WEB: (to be announced)
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The University of Arizona, Dept. of Chemistry and Biochemistry has put together a site devoted to matters of PHOTOSYNTHESIS. WEB:
http://aspin.asu.edu/provider/photosyn/
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The University of Antwerp runs a site that collects RRNA SEQUENCES. WEB:
http://www-rrna.uia.ac.be/
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Matters Arising
A summer course on the ORGANIZATION AND ASSEMBLY OF THE PHOTOSYNTHETIC APPARATUS will be held 12-23 May 1996 at the Weizmann Institute, Rehovot, Israel, open to graduate and post-graduate students. The course is intended to aid students from either a biochemical or biophysical background comprehend the current state of the field and its methodological basis. The registration fee of $200 will cover full board and tuition. Since there is a limitation of 25 participants only, those interested should apply in writing as soon as possible. The application should include a resume of studies, personal details (full name, nationality, date of birth, gender) and address details (mailing address, fax, e-mail etc.). Include also a brief (not more than 1 page) resume of present work, list of publications (if available), and letters of recommendation (preferably from at least two mentors).
CONTACT: Shmuel Malkin, Biochemistry Department, Weizmann Institute of Science, Rehovot, 76100, ISRAEL. E-MAIL: BCMalkin@Weizmann.Weizmann.Ac.IL
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A book entitled Detection Methods for Cyanobacterial Toxins is now available from CRC Press. The book is based on presentations at a meeting of the same name held in Bath, September 1993. The 202 page book, edited by Geoff Codd and others sells for U.S.$89.95.
CONTACT: CRC Press, Inc., 2000 Corporate Blvd., N.W., Boca Raton, FL 33431-9868 U.S.A. TEL: 407-994-0555
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Some readers may not be aware that The Molecular Biology of Cyanobacteria, edited by Don Bryant and recently reviewed [Haselkorn R (1995) Science 269:1121], is just the first volume of a series, entitled ADVANCES IN PHOTOSYNTHESIS. The series, from Kluwer Academic Publishers, is intended to provide a multidisciplinary approach to the topic, spanning the range from macromolecular structure to whole

plant physiology. Volume 2 was recently released: Anoxygenic Photosynthetic Bacteria, edited by Robert Blankenship, Michael Madigan, and Carl Bauer. Future volumes include:

Oxygenic Photosynthesis: The Light Reactions (Don Ort and Charles Yocum)

Environmental Stress and Photosynthesis (Neil Baker)

Physical Methods in Photosynthesis Research (Jan Amesz and Arnold Hoff)

Lipids in Photosynthesis: Structure, Function and Genetics (Paul Siegenthaler and Norio Murata)

If you have an idea for an additional volume, then by all means pass it on to Govindjee, the series editor, at:

CONTACT: (with ideas for additional volumes) Govindjee, Department of Plant Biology, University of Illinois, 505 South Goodwin Avenue, 265 Morrill Hall, Urbana, IL 61801-3707 TEL: 217-333-1794, FAX: 217-244-7246, E-MAIL: Gov@Pop.Life.Uiuc.Edu

CONTACT: (inquiries about published books) Kluwer Academic Publishers: E-MAIL: Services@Wkap.NL

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MEETINGS

The Jacques Monod Conference on Synthesis and Function of Photosynthetic Complexes will be held in Aussois, France, 25-29 March, 1996. Four major themes will be discussed: Expression of genes involved in photosynthesis, transport and targeting of chloroplast proteins, structure of photosynthetic complexes, and function of photosynthetic components. Interested scientists may apply by sending their CV, a one page summary of their research interests, and a list of their most relevant publications to:

CONTACT: J.D. Rochaix, University of Geneva, Department of Molecular Biology, 30, Quai Ernest Ansermet, CH-1211 Geneva 4, SWITZERLAND. E-MAIL: Rochaix@Sc2a.Unige.Ch

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Participants in a two-day meeting entitled MOLECULAR TO GLOBAL PHOTOSYNTHESIS will discuss factors that govern the efficiency of solar energy conversion by oxygenic photosynthetic organisms at all levels, ranging from primary charge separation and carbon fixation to biomass production and global gas and energy balance. The meeting will be held 28-29 March 1996, at Imperial College, London, UK.

CONTACT: Jim Barber, Wolfson Laboratories, Biochemistry Department, Imperial College, London SW7 2AY, UK. FAX: 171 594 5267, E-MAIL: J.Barber@ic.ac.uk

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The SYMPOSIUM ON MOLECULAR PLANT-MICROBE INTERACTIONS will be held in Knoxville, Tennessee (U.S.A.) 14-19 July, 1996.

CONTACT: Gary Stacey, Director, Center for Legume Research, M409 Walters Life Science Bldg., University of Tennessee, Knoxville, TN 37996-0845, U.S.A., FAX: 615-974-4007, E-MAIL: GStacey@Utkvx.Utk.Edu

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As part of the 50th Anniversary Meeting of the Phycological Society of America (Santa Cruz, California, 14-18 July 1996), a one day symposium will be held on "CYANOBACTERIA AS MODEL SYSTEMS FOR STUDYING BIOLOGICAL PROCESSES". Speakers will include Bob Haselkorn (Development/Nitrogen Fixation), Arthur Grossman (Environmental Stress), Wim Vermaas (photosynthetic protein complexes), John Waterbury (ecological studies), and Susan Golden (circadian rhythms).

CONTACT: (Registration information) Paul Kugrens, PKugrens@Lamar.ColoState.Edu

CONTACT: (Symposium information) Brian Palenik and Bianca Brahamsha, Scripps Institution of Oceanography, U.C.S.D. La Jolla CA 92093-0202 U.S.A. TEL: 619-534-7505, FAX: 619-534-7313, E-MAIL: BPalenik@Ucsd.Edu

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A meeting designed to bring together investigators who apply modern techniques to the study of diverse SYMBIOTIC ASSOCIATIONS is scheduled on 5-8 Sept 1996 in Bar Harbor, Maine.

CONTACT: Scott O'Neill, Dept. of Epidemiology and Public Health, Yale University School of Medicine, P.O. Box 3333, New Haven, CT 06510 U.S.A. TEL: 203-785-3285, FAX: 203-785-4782, E-MAIL: Scott_ONeill@Quickmail.Yale.Edu, WWW: http://Wolbachia.Med.Yale.Edu

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Those who like their nitrogen meetings without competition from Rhizobia might check out the 7th INTERNATIONAL SYMPOSIUM ON BIOLOGICAL NITROGEN FIXATION WITH NON-LEGUMES 16-21 October 1996 in Pakistan.

CONTACT: National Institute for Biotechnology and Genetic Engineering (NIBGE) P.O. Box 577, Jhang Road Faisalabad, PAKISTAN, TEL: 41-65-1471 or 41-651475-79, FAX: 41-65-1472, E-MAIL: Kauser@Nibge.Lke.imran.Pk

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An INTERNATIONAL SYMPOSIUM ON CYANOBACTERIAL BIOTECHNOLOGY will take place 18- 21 Sept 1996 in Tiruchirapalli, India. The emphasis of the meeting will be on environmentally sustainable utilization of cyanobacteria for human welfare.

CONTACT: G. Subramanain, Director, NFMC, Bharathidasan University, Tiruchirapalli - 620 024, INDIA. TEL: 91-431-60352, FAX: 91-431-60245 or 91-431-60320, E-MAIL: bdasan@iitm.ernet.in

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The 11TH AUSTRALIAN NITROGEN FIXATION CONFERENCE will focus on N2-fixing symbioses at its meeting in Perth, Australia, 22-27 September 1996. While

the conference will stress the role of leguminous plants in sustainable agriculture, cyanobacterial symbioses may also find a place.
CONTACT: The Secretary, Australian Society for Nitrogen Fixation, Centre For Legumes in Mediterranean Agriculture, University of Western Australia, Nedlands, W.A. 6907, AUSTRALIA. FAX: 61-9-380-1140, E-MAIL: Asnf@Cyllene.Uwa.Edu.Au

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POSITIONS SOUGHT

POSITION SOUGHT: Post-Doc CONTACT: Sridharan, Govindachary, #6, Sait Colony Ist Street, Egmore, Madras 600008, INDIA, FAX: 00-91-44-8257454 RESEARCH EXPERIENCE AND INTERESTS: 12 years in cyanobacterial photosynthesis, nitrogen fixation, nitrogen assimilation, mutagenesis, selection of herbicide resistant strains, outdoor cultivation of algae, biochemistry and molecular biology POST-DOC EXPERIENCE: 17 months each in India and Israel

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POSITIONS OFFERED

POSITION OFFERED: Post-Doc CONTACT: J. Nugent, Department of Biology, University College London, Gower Street, London WC1E 6BT, U.K. TEL: 0171-380-7098, FAX: 0171-380-7096, E-MAIL: J.Nugent@UCL.Ac.Uk RESEARCH: Join multidisciplinary team to study the biogenesis and functioning of photosystem II in wild-type and mutant strains of Chlamydomonas and cyanobacteria. SALARY: Three year position, within the range UK 14,317 - 17,466 (plus 2,134 London allowance) SEND: CV and names of two referees

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POSITION OFFERED: Post-Doc CONTACT: Jim Golden, Department of Biology, Texas A&M University, College Station, TX 77843-3258 USA. TEL: 409-845-9823, FAX 409-845-2891, E-MAIL: JGolden@Tamu.Edu RESEARCH: Regulation and mechanism of programmed DNA rearrangements during heterocyst differentiation in Anabaena sp. strain PCC 7120 REQUIREMENTS: Expertise in molecular biology, biochemistry, and microbial genetics. Strong preference will be given to individuals with a proven record of quality publication and to those with potential for obtaining independent funding AVAILABLE: immediately SEND: CV and three letters of recommendation

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ED CARPENTER is spending a year or two in Washington at the National Science Foundation's Office of Polar Programs. He is still connected to his old lab at State University of Stony Brook.
E-MAIL: ECarpent@Nsf.Gov

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ANDREY DEMIDOV has moved operations from David Andrews lab in East Anglia, U.K., where he worked on polarization spectroscopy in molecular systems with energy transfer. He is now in the U.S. using femtosecond spectroscopy to study primary processes of excitation energy migration and electron transfer in reaction center of photosystem-II.
Physics Department, University of Michigan, 500 E.University, 2071 Randall Lab., Ann Arbor, MI 48109-1120, U.S.A. TEL: 313-763-0998, FAX: 313-665-1345, E-MAIL: ADemidov@UMich.Edu, WEB: http://www.umich.edu/~ademidov

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DIRK GEERTS defended his Ph.D. thesis last June, entitled Genetic modification of photosynthesis in the cyanobacterium Synechococcus sp. PCC7942: gene expression and protein transport. With the demise of the cyanobacterial group at Utrecht (see below), Dirk has taken a post-doc position in Amsterdam, studying integrins and their role in tumorigenesis. Though happy with his position, he laments that his cultures have the wrong color: now red instead of blue-green. On days off and weekends he still teams up with Hans Matthijs (U. Amsterdam) doing physiology experiments on the phycocyanin production in Synechococcus PCC 7942. This offers some compensation.
Division of Cell Biology, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, NETHERLANDS, TEL: 31-20-512.1942, FAX: 31-20-512.1944, E-MAIL: DGeerts@Nki.NL

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MASAHIRO ISHIURA and TAKAO KONDO have both moved from the National Institute of Basic Biology in Okazaki to Nagoya. Both will continue their work on cyanobacterial circadian rhythm.
Department of Biology, Faculty of Science, Nagoya University, Furo- cho, Chikusa-ku, Nagoya, 464-01 JAPAN, TEL: 81-52-789-2495, E-MAIL: ishiura@Bio.Nagoya-U.Ac.Jp

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SVEN JANSON is a new Ph.D., having defended his thesis Cell structure and localizatoin of nitrogenase in some marine and brackish cyanobacteria. For the moment he remains in Birgitta Bergman's laboratory.
Department of Botany, Stockholm University, S-106 91 Stockholm, SWEDEN, TEL: 46-8-16 13 26, FAX: 46-8-16 55 25, E-MAIL: Jansons@Botan.Su.Se

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BART NELISSEN last July defended his doctoral thesis entitled Phylogenetic study of the cyanobacteria on the basis of 16S RRNA gene sequence analysis (See NEWS). He remains at:

Departement Biochemie, Universiteit Antwerpen (U.I.A.), Universiteitsplein 1, B-2610 Wilrijk (Antwerpen), BELGIUM, TEL: 32 3 820 23 05, FAX: 32 3 820 22 48, E-MAIL: Nelissen@Uia.Ua.Ac.Be, WEB: http://www.uia.ac.be/u/nelissen

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YASUYUKI NEMOTO has returned to Japan, having left University of Miami where he had worked in the laboratory of Akira Mitsui on hydrogen production from cyanobacteria.

Dept. Biotechnol., Fac. Technol, Tokyo University of Agriculture and Technology, 2-24-16 Naka-machi, Koganei, Tokyo 184, JAPAN, TEL: 81-423-88-7030, FAX: 81-423-88-7205, E-MAIL: YNemoto@Cc.Tuat.Ac.Jp

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GEORGE OWTTRIM has returned to the fold after a post-doc in Switzerland with Cris Kuhlemeier working on translation initiation factors in tobacco, particularly RNA helicase proteins. He now has a faculty position and intends to exploit his expertise with helicase proteins to their study in cyanobacteria.

Department of Biological Sciences, University of Alberta, Edmonton, Alberta T6G 2E9, CANADA, TEL: 403-492-1803, FAX: 403-492-9234, E-MAIL: G.Owttrim@UAlberta.Ca

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ERIK SODERBACK has returned to Bergitta Bergman's group after a sojourn in England working on regulation of nitrogen fixation genes in the laboratory of Ray Dixon. He has lost little time in recovering his ardor for symbiotic cyanobacteria.

Department of Botany, Stockholm University, S-106 91 Stockholm, SWEDEN, TEL: 46-8 16 38 46, FAX: 46-8 16 55 25, E-MAIL: Soderbac@Botan.Su.Se

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Utrecht Cyanobacterial Group (1975-1995)

We regret to learn of the passing of the Utrecht cyanobacterial group, which accomplished as much as any group in the development of cyanobacterial molecular genetics. Their pioneering work made possible many of the techniques of gene replacement in cyanobacteria that we take for granted today. The group outlived its founder, Gerard van Arkel, by less than a year -- van Arkel died December 1994, four years after his retirement.

The former affiliates of the group have dispersed as follows: PETER WEISBEEK continues to lead the Section Molecular Genetics, which now focuses solely on plant studies. MIES BORRIAS remains in the Section and will extend her work on gene expression to higher plants. GEERT DE VRIEZE, the senior technician, now works with Ben Scheres on root development. ARNAUD BOVY has left to take a post doc position in Wageningen, where he works to improve several cultured plants, including carnation. DIRK GEERTS also has left, taking a post-doc position in the Netherlands Cancer Institute in pursuit of the processes underlying cell adhesion.

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Clue Found to Mystery of Swimming Synechococcus

Several years ago John Waterbury and coworkers [Science (1985) 230:74- 76] reported on the ability of certain marine Synechococcus to swim without apparent benefit of flagella or any other discernible aid. Bianca Brahamsha now tells us she is beginning to get a handle on the question of how they move. Biochemical studies showed that the loss of motility following treatment of cells with proteinase K correlates with the loss of an abundant 120-Kd outer membrane protein. The gene encoding the protein was cloned by a reverse genetics approach, but the sequence gave little indication as to function. After establishing a means of introducing foreign DNA into Synechococcus WH8102, Bianca knocked out the 120-kd outer membrane polypeptide,... and the cells don't swim. They still rotate about their longitudinal axis, but they don't go anywhere, as though they're missing a rudder or something. Of course, this could be an indirect effect -- if they can't make a proper outer membrane, motility components may not be able to insert correctly. Nonetheless, she is excited because for the first time a system is in hand to dissect swimming motility in cyanobacteria.

CONTACT: Bianca Brahamsha, Scripps Institution of Oceanography, Univ. of California-San Diego, La Jolla CA 92093, U.S.A. TEL: 619-534-7505, FAX: 619-534-7313, E-MAIL: BBrahamsha@Ucsd.Edu

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Alternative Alternative Oxidase

Alternative respiration (as defined by cyanide-insensitive oxygen consumption) has long been known in cyanobacteria. Guenter Peschek sent in some results from his group that call into question exactly what we mean by "alternative". Using reverse phase HPLC, his group was able to identify heme B (associated with cytochrome b) and lesser amounts of hemes A (associated with aa3-type cytochrome c oxidase) and O from chlorophyll-free cytoplasmic membranes of both unicellular and filamentous cyanobacteria. Heme O was observed only in cultures grown semi-anaerobically. Surprisingly, monospecific antibodies raised against aa3-type cytochrome oxidase (from Paracoccus denitrificans) and against bo3-type quinol oxidase (from E. coli) both recognized the same band on denaturing gels of cytoplasmic membrane protein from cultures, whether or not grown anaerobically. The band comigrated with subunit-I protein of cyanobacterial cytochrome c oxidase. Perhaps heme A and heme O both combine with the same apoprotein, depending on the oxygen-dependent availability of the two hemes. If so, then "aa3-type" and "o3-type" oxidases may represent not "alternative oxidases" in the usual sense of the word but rather alternative hemes. This work has been recently published [Auer et al (1995) Biochem Mol Biol Internatl 37:1173-1185].

CONTACT: Guenter Peschek, Institute of Physical Chemistry, University of Vienna, Waehringerstrasse 42, A-1090 Wien, AUSTRIA, TEL: 43-1-343616, FAX: 43-1-3104597

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Codon Usage by Synechocystis PCC 6803

Codon Usage Chart

Each codon is followed by a number representing the fraction of instances the amino acid is encoded by the triplet. The second number represents the frequency with which the codon appears on average per 1000 amino acids. The table is based on 10781 codons from sequenced genes of Synechocystis PCC 6803.

CONTACT: Nigel Silman, Biological Sciences, University of Warwick, Coventry, CV4 7AL, U.K., FAX: 203-523701, E-MAIL: Lsrew@Csu.Warwick.Ac.Uk

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Competitive PCR to Quantitate Cyanobacteria

Enumeration of low density cyanobacterial populations is a chancy business, and choices range from the unreliable (plating), to the tedious (microscopy) and horrifically expensive (flow cytometry). Janet Jansson's group recently provided another choice, monitoring luminescence from cyanobacteria tagged with luc, encoding firefly luciferase [Moeller et al (1995) FEMS Microbiol Lett 129:43-50]. Using tagged Synechocystis PCC 6803, they were able to quantitate as few as 4x103 cells per g sediment from a microcosm of Baltic Sea water. Tagged cyanobacteria can also be detected by PCR, taking advantage of the fact that luc is unknown in natural microbial populations, but direct PCR has little quantitative value. Janet now reports that her group has developed a competitive PCR technique that permits quantitation of luc-tagged cyanobacteria in sediment.

The method, soon to be published [Jansson and Lesser (1995) In: Molecular Microbial Ecology Manual, Kluwer Academic Publishers, Dordrecht, Ch. 2.7.4], relies on an internal competitive standard that differs from luc by an additional 35 bp insertion. Otherwise, the target and internal standard are similar and amplified by the same primers during PCR. Using a known concentration of internal standard DNA it is possible to quantitate the original target DNA concentration by the ratio of the amplified products on a gel. The method isn't quite as simple as it sounds, since it is necessary to match the sample to a standard at a comparable concentration, but taking such precautions permitted a very accurate quantitation of luc-tagged Synechocystis in sediment [Moeller and Jansson, manuscript in preparation].

CONTACT: Janet Jansson, Department of Biochemistry, Stockholm University, S-10691 Stockholm, SWEDEN, TEL: 46-8-16-2469, FAX: 46-8-15-3679, E-MAIL: Janet@Biokemi.Su.Se

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Blue-greens on the Rocks

Many of our nonscientific friends know of us vaguely as experts on some sort of algae, and so we are occasionally called upon to identify biological encrustations that we may encounter in daily life. Often we can only respond, lamely, "Looks like green goo to me." Mariona Hernandez-Marine has come to our aid, providing us with a guide to cyanobacteria and algae we might find on buildings and monuments [Ortega-Calvo et al (1995) Sci Total Environ 167:329- 341]. The most commonly encountered cyanobacteria in such places are filamentous species of the genera Phormidium and Microcoleus. We should also point out to our friends that what may appear as very similar blotches of green on the bricks and mortar between them are likely to be quite different communities, owing to a surprising degree of microclimatic variability.

Care must be taken to distinguish such growth from the black sulfated crusts that accumulate on limestone buildings as a result of sulfur dioxide pollution of the air. Even here, however, we must tell our friends that cyanobacteria, particularly of the genus Gloeothece, can thrive on the crusts, despite the presence of toxic compounds, and may provide nutrients for growth of other bacteria.

Some of us may also find ourselves on occasion groping for conversation within dark caves. Here too, Mariona has saved us from a potentially embarrassing assertion, that the grey mat on cave walls could not possibly be due to cyanobacteria. In fact, she tells us, cyanobacteria are amongst the cave's most important epilithic vegetation, despite the very low levels of light. Her description of a calcified cyanophyte of the genus Herpysonema (Mastigocladaceae) has recently been published [Algol Studies (1994) 75:123- 136].

Needless to say, this report only scratches the surface. For further information...

CONTACT: Mariona Hernandez-Marine, Laboratory of Botany, University of Barcelona, E-08028 Barcelona, SPAIN, TEL: 34 3 4024490, FAX: 34 3 4021886, E-MAIL: Hernande@Far.Ub.Es

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Nostoc 6720 unmasked!

John Smith has alerted us to the fact (pointed out to him by Terry Thiel) that the cyanobacterium he has been calling Nostoc 6720 is more likely to be a species of Anabaena. He confirmed this by comparing the PCR products amplified from genomic DNA, using HIP sequences [Robinson et al (1995) Nucl Acids Res 23:729-735] as primers (courtesy of Nigel Robinson), with those amplified from DNA from Nostoc MAC and Anabaena PCC 7120. Comparison of the nifH sequence from "Nostoc 6720" with the sequence derived by Martin Mulligan from Anabaena variabilis ATCC 29413 suggests that the former strain is closely related to the latter. In fact, stock records identify the cyanobacterium as Anabaena PCC 7937 (nominally identical to ATCC 29413), and the confusion occurred in 1985 when both cyanobacteria were obtained from the Pasteur collection for work on synchronous akinete germination.

CONTACT: John Smith, Dept. of Biological Sciences, University of Lancaster, Bailrigg, Lancaster LA1 3JC, U.K. TEL: 0524-65201, FAX: 0524-843854, E-MAIL: R.Smith@Lancaster.Ac.Uk

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Peptide Synthetase Genes Found in Toxic Microcystis

Many have yearned for the day when the power of bacterial genetics could be applied to the task of elucidating the biosynthetic pathways leading to toxic peptides made by cyanobacteria. The goal has proven to be elusive, however, and until that day arrives, we will have to rely on clever tricks and deductions. Tom Boerner's group at Humboldt University has used both to clone several peptide synthetase genes from Microcystis aeruginosa and to identify a molecular marker for toxic strains.

They exploited the fact that peptide synthetases studied in Gram- positive bacteria and fungi share two highly conserved adenylate-forming domains. Using primers derived from these domains, the Berlin group obtained four different PCR products, two each from DNA of the toxic M. aeruginosa strains HUB 524 and PCC 7820. All four showed striking sequence similarity to peptide synthetase genes. One of the products hybridized to DNA from three tested toxic strains but not to three tested nontoxic strains. That product was used to identify corresponding sequences from genomic libraries, and the 2982 bp sequence of the region (deposited in the EMBL data base with accession number Z28338) showed extended amino acid sequence similarity to peptide synthetases, particularly to the proline-activating synthetase unit of gramicidin S synthetase from Bacillus brevis.

Their results, soon to appear in FEMS Microbiological Letters, indicate that toxic Microcystis may utilize nonribosomal peptide synthesis of the type used to synthesize other peptide toxins. Furthermore, toxic strains may differ from nontoxic strains in part by the presence of a gene or genes required for the synthesis.

CONTACT: Tom Boerner, Institut fuer Biologie, Humboldt-Universitaet, Invalidenstr. 43, D-10115 Berlin, GERMANY, TEL: 49-30-28 97 26 33, FAX: 49-30-28 97 27 99, E-MAIL: Thomas=Boerner@Rz.Hu-Berlin.De

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Cholera Connected to Blue Green Blooms?

Cholera, once confined largely to the Indian subcontinent, has in the last 200 years become a world disease. The disease has swept through much of humanity in a succession of waves, the last major pandemic initiating in 1961. In between incidents of mass infection, Vibrio cholerae, the causative agent, must reside in some still unknown environmental reservoir. It may be pertinent that the peak incidence of cholera in Bangladesh coincides with the appearance of cyanobacterial blooms. Some have postulated that algae and cyanobacteria may in fact constitute the unknown reservoir [Epstein (1993) BioSystems 31:209], pointing to the ability of V. cholerae to survive long periods in the slime produced by these organisms.

Igor Brown has proposed a different connection between cyanobacteria and V. cholerae. He points out that growth of members of the Vibrionacea is stimulated by induction of sodium cycle energetics [Bakeeva et al (1986) Biochim Biophys Acta 850:466]. Brown's own work has suggested that the growth of cyanobacteria in brackish water is autocatalytic: sodium plus alkalinity stimulate the sodium cycle in cyanobacteria, and the resulting growth increases the alkalinity [Brown et al (1990) Biol Membr 4:2039; unpublished results]. The alkalinization and the accompanying increase in dissolved organic compounds resulting from the bloom may induce the sodium cycle in the Vibrionacea, including V. cholerae. Dissemination of the disease would then occur as water from the bloom is used or dispersed.

Whether or not Brown's suggestion is correct, it is comforting to know that cyanobacteria may participate in matters of utmost importance to humans and their funding agencies, quite apart from the prosaic tasks of maintaining the atmosphere and the food chain.

Igor would love to cooperate with anyone in a position to test his idea.

CONTACT: Igor Brown, Cyanobacter Biol. Res. Lab, Odessa State University, Petr Velikiy St. 2, Odessa 270100, UKRAINE, TEL: 007-0482-68-77-93, FAX: 007-0482-23-82-88, E-MAIL: IBrown@Microalgae.Odessa.Ua

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New Phylogenetic Trees from 16S rRNA

This past summer Bart Nelissen (U. Antwerpen) defended his doctoral thesis entitled Phylogenetic study of the cyanobacteria on the basis of 16S rRNA gene sequence analysis. Nearly complete 16S rRNA sequences of eleven cyanobacteria belonging to different morphological groups were determined from cloned PCR-amplified products to gain a better understanding of cyanobacterial phylogeny. A cyanobacterium-specific oligonucleotide probe was developed to distinguish cyanobacterial 16S rRNA sequences from amplified products originating from contaminating bacteria. Phylogenetic trees were constructed using the cyanobacterial sequences aligned with other previously determined sequences.

The thesis addressed the phylogenetic relationships between filamentous helical cyanobacteria (Spirulina and Arthrospira) and between cyanobacteria and plastids, and the homogeneity of the genera Pseudanabaena and Leptolyngbya. Most of the results have been published [Nelissen et al (1994) Syst Appl Microbiol 17:206-210; Nelissen et al (1995) Mol Biol Evol 12:1166-1173; Nelissen et al (1996) J Mol Evol in press].

CONTACT: Bart Nelissen, Dept. Biochemie, Universiteit Antwerpen (U.I.A.), Universiteitsplein 1, B-2610 Wilrijk (Antwerpen), BELGIUM, TEL: 32-3-820-23-05, FAX: 32-3-820-22-48, E-MAIL: Nelissen@Uia.Ua.Ac.Be

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Meeting Reports

By their very nature, meeting reports are just snapshots of what occurred. A different angle would produce a very different view. The two reports below can only provide a flavor of some of the advances that have taken place.

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Vth Cyanobacterial Molecular Biology Workshop

The Vth Cyanobacterial Molecular Biology Workshop was held again at Asilomar, California, July 21-25. Asilomar has become the permanent home, as the next meeting in 1998 is also scheduled for the site on the Pacific Ocean. Many thanks to Don Bryant and Neil Straus for organizing the meeting.

Photosynthesis

Pradip Manna (Arizona State U.) reported the involvement of lumenal cytochromes on electron transport in a PSI-LESS MUTANT of Synechocystis PCC 6803. Of particular interest was the important but not essential role of cytochrome c553 in mediating the PSII-dependent flow of electrons to the terminal oxidase. Gaozhong Shen (Pennsylvania State U.) also reported PSII activity in a PSI-less mutant from Synechococcus PCC 7002. His results suggested that PSII-generated electrons can be transferred to NAD through NADH dehydrogenase and that this process seems to be regulated by light. On the PSI side, Shen also described his success in deleting psaC and psaD genes from Synechococcus PCC 7002. Both mutants showed hypersensitivity to light intensity and both mutants showed much lower PSI reaction center content. The construction of these mutants opens a new way to further analyze protein structure and function of PSI.

Jian-Ren Shen (RIKEN) presented evidence that CYTOCHROME C550 is a critical component of PSII located on the lumenal side of thylakoids in Synechocystis PCC 6803. When the gene encoding cytochrome C550 was deleted, a much lower growth rate as a result of slow down of PSII electron transport was observed. Dave Krogmann (Purdue U.) also reported cloning of the petK gene, encoding cytochrome C550. His results indicate that the cytochrome might be involved in H2 production.

Progress in understanding CYTB6/F FUNCTION was presented by Toivo Kallas (U. Wisconsin, Oshkosh). His group evaluated the function of specific amino acids in cytochrome b6 (encoded by petB) and subunit IV (encoded by petD) by introducing site-specific mutations into Synechococcus PCC 7002. Kallas also reported reconstitution of Rieske center from overproduced apoprotein. Functions of low potential cytochrome c were reported by two groups.

The regulation of CHLOROPHYLL-BINDING PROTEIN SYNTHESIS with chlL-mutant was described by Qingfang He (Arizona St. U.). When the chlL gene was deleted, chlorophyll synthesis became totally dependent on light. In darkness, protochlorophyllide accumulated. This mutant should provide a good system to study chlorophyll synthesis and its regulation.

Several presentations focused on CO2 FIXATION. Michael Zianni (Ohio State U.) described the disruption of the rca gene of Anabaena variabilis, encoding RubisCO activase. The resulting strain lacked activase and showed a marked change in RubisCO activity and growth under certain conditions. Dean Price and Dieter Sueltemeyer (both of Austral. Natl. U.) discussed different aspects of the CO2-concentrating mechanism encoded by ccm genes in Synechococcus PCC 7002. The genes have now been cloned. Curiously, a psaE- strain lacking cyclic electron flow around PSI was unable to induce high affinity transport system for bicarbonate under low CO2 conditions. The authors suggested that psaE-dependent cyclic electron flow may be important in energizing or inducing bicarbonate transport.

Physiology

Carol Andersson (Texas A&M U.) presented the results of an international coalition of workers studying the molecular basis of CIRCADIAN RHYTHMS in Synechococcus PCC 7942. Synechococcus and other cyanobacteria remain the only prokaryotes with documented circadian rhythms. Using random transcriptional fusions to luxAB, encoding bacterial luciferase, and an automated image processing system, the group recorded the amplitude and periodicity expression of 6000 reporter fusions. Almost all 800 colonies that were bright enough to monitor showed rhythmic expression of luminescence, suggesting that circadian regulation is surprisingly common. A screen of integrational mutants for colonies defective in the rhythmic expression of psbAI yielded one with a low amplitude phenotype. The affected gene, which turned out to be a member of the sigma-70 family, affected some but not all circadian-regulated genes. Chemical mutagenesis yielded period length mutants that displayed a wider range of periodicity than previously observed in any other organism. Complementation analysis of chemically induced mutants indicates that 80% of these mutations lie in the same region of the chromosome, which may contain the gene(s) for the clock machinery.

Nick Mann (U. Warwick) presented the efforts of his group on the characterization of MEMBRANE ASSOCIATED KINASE activities in cyanobacteria. In Synechocystis PCC 6803, kinase activity is activated by dark or the presence of metabolizable carbon and appears to be related to the switch off of the carbon dioxide concentrating mechanism. The kinase has several targets, and characterization of an 18-kD target by protein sequencing indicated that it was beta-phycocyanin. The phosphorylated phycobiliprotein no longer fluoresces. The physiological role of phycobiliprotein phosphorylation is still obscure but may have to do with regulating energy flow through the photosystems. Characterization of other targets is currently underway.

Participants keenly felt the absence of David Laudenbach (U. Western Ontario), who died suddenly of complications from surgery just a month before the meeting. It was his presence, however, that was evident during the talk on SULFUR-CONTROLLED GENES given in his place by his student Mary Lou Nicholson. She described the isolation and localization of the regulatory gene cysR on a 50-Kb plasmid from Synechococcus PCC 7942. Several open reading frames (ORFs) were also found on this plasmid that are transcriptionally regulated by sulfate deprivation, mediated through CysR. These ORFs where characterized and identified as srpA, srpB, srpC (srp = sulfur regulated plasmid-encoded), and ggt, encoding, respectively, catalase, a Mg transport ATPase, a protein involved in chromate resistance, and gamma-glutamyl transferase (related to glutathione).

Ecology and Evolution

A couple of groups reported their results on responses by cyanobacteria to NUTRIENT DEPRIVATION. Jackie Collier and Brian Palenik (Scripps Inst. Oceanography) described the utilization of urea by a marine Synechococcus. They cloned and sequenced urease genes from several marine cyanobacteria and showed that its expression in Synechococcus WH7805 is not repressed by NO3- and NH4+. Neil Straus (U. Toronto) reported cloning and sequencing of a gene involved in iron repression of some genes. It was 41% similarity to regulatory protein Fur from E. coli and had a putative Fe-binding domain.

Jack Meek's group (U. California-Davis) found two transposon-generated mutants of Nostoc ATCC 29133 that have lost the ability to enter into SYMBIOSIS with the hornwort Anthoceros punctatus. Both mutants also are Fox-, i.e. unable to fix nitrogen in the presence of oxygen. It was previously thought that all Fox- mutants would be physiologically complemented by the anaerobic environment of the symbiotic cavity in the plant tissue and, therefore, be Sym+. One of the mutants, UCD307, is defective in heterocyst glycolipid production and the protein encoded by the interrupted ORF shows similarity to polyketide synthesis pathway enzymes and some similarity to HetO and HetQ. Most interesting is the high similarity to fix-23, a locus from Rhizobium meliloti involved in host-symbiont recognition. Perhaps the gene product of the ORF interrupted in UCD307 is involved in production of both heterocyst glycolipid and symbiotic recognition determinants. Both compounds may be synthesized by a common pathway.

Several interesting reports on the MOLECULAR EVOLUTION OF CYANOBACTERIA and chloroplasts appeared in this meeting, with gratifying agreement in their conclusions. Sean Turner (Louisiana St. U.) presented a statistical analysis of small subunit rRNA base composition and concluded that plastids are monophyletic. Nadia Dolganov (Stanford U.) told of the cloning of a gene from Synechococcus PCC 7942 whose product resembles chlorophyll a/b binding protein. This result supports the idea that there was only one original endosymbiosis event. Tanja Gruber (Pennsylvania St. U.) showed a phylogenetic tree based on the amino acid sequences of sigma factors. The tree agrees with Sean's 16S rRNA data. Vickie Stirewalt (Pennsylvania St. U.) reported that their long struggle of sequencing the whole cyanelle genome is finally over. The circular DNA is comprised of 135599 bp with a low G+C content (30.4%). It contains about 192 genes and ORFs and has two inverted repeats. The inverted repeats and gene organization of this genome also supports the idea that all plastids are monophyletic.

The situation is less clear with RBCL. Bob Tabita (Ohio St. U.) presented sequences from an oceanic strain Synechococcus WH7803. The deduced amino acid sequences indicated a close relationship to RbcL from purple bacteria. The importance of this finding in molecular evolution of photosynthetic bacteria remains to be elucidated.

Heterocyst Differentiation

Bob Haselkorn (U. Chicago) gave a talk concerning the role in heterocyst differentiation by Anabaena PCC 7120 of genes that bear similarity to those encoding response regulators and sensor kinases of TWO-COMPONENT REGULATORY SYSTEMS. PCR primers directed at conserved regions in the histidine kinase sensors of other two component systems amplified a series of products, denoted ask, with similarities to phoR, ntrB, pleC, and other sensory kinases. Preliminary results show that askA, which is most similar to sensory kinase phoR (which regulates phosphate deprivation genes), shows no phenotype when inactivated, and askC, most similar to pleC (required by Caulobacter for differentiation), alters heterocyst frequency when inactivated.

Jack Meeks (U. California, Davis) described a mutant (UCD311) evidently defective in the RESPONSE REGULATOR side of a two-component regulatory system. The gene, devR, was found by transposon mutagenesis of Nostoc ATCC 29133. The mutant is unable to fix nitrogen in the presence of oxygen (Fox-) but is symbiotically competent. The DevR gene product represents a different class of response regulators than PatA, a previously characterized gene from Anabaena, and is more closely related by sequence to response regulators CheY and SpoOF (involved in chemotaxis in E. coli and sporulation in Bacillus, respectfully).

Bill Buikema (U. Chicago) discussed results concerning the ROLE AND EXPRESSION OF HETR, a gene required early in heterocyst differentiation, using a fusion of hetR to green fluorescent protein (GFP) to examine cell-specific expression. In wild type Anabaena PCC 7120, a high level of fluorescence from hetR::GFP was seen in well-spaced cells prior to morphologically visible differentiation. When the fusion was placed in hetR, patA, or patB mutant strains, aberrant patterns of fluorescent cells are seen. The use of GFP does carry technical limitations: the protein is toxic and requires oxygen for proper folding. Detection of gene fusions in heterocysts is therefore problematic.

Terry Thiel (U. Missouri, St. Louis) presented a different approach to analyzing the cell-specific gene expression of TWO MOLYBDENUM-DEPENDENT NITROGENASES encoded by gene clusters nif1 and nif2 in Anabaena variabilis ATCC 29413. She and her co-workers utilized C12-fluorescein-beta-D-galacto- side, a substrate for beta-galactosidase, to localize expression of nifH1::lacZ and nifH2::lacZ fusions by fluorescence microscopy. The results indicate that the nif1 gene cluster is regulated developmentally and expressed only in heterocysts while the nif2 genes are expressed in all cells in response to nitrogen limitation and anoxia. Unpatterned expression of nif2 in vegetative cells, and, presumably, ammonia production in all cells did not prevent patterned heterocyst differentiation, suggesting that products of nitrogen fixation may not be involved in pattern formation.

This and other provocative results presented at the meeting prompted an informal roundtable discussion to consider approaches to studying heterocyst pattern formation. The discussion centered on the question of how to determine whether or not a PRE-PATTERN OF CELLS destined to become heterocysts exists in a nitrogen replete filament. Any pattern -- pre- or post-nitrogen stepdown -- that relies on the exchange of signal molecules should be disrupted in a strain that lacks intercellular communication. We should be able to detect in such a strain any intrinsic pattern (i.e. independent of interaction) that may exist. Does such a strain exist? Perhaps, and fluorescent dyes conceivably could be use to test putative communication-less strains.

- Tom Hanson & ZHAO Jindong

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2nd Cyanobacteriology Seminar for PhD Students

In 1994, Konstanze Mez and Beatrix Falch from the University of Zurich, Switzerland, were inspired to arrange a meeting of German-speaking PhD students. The very positive impressions from that meeting led to a second edition this past September in Vienna, Austria. Seventeen young scientists followed the invitation of Wolfgang Gregor, PhD student in the group of Georg Schmetterer, and discussed the results of their present work.

Toxicology and secondary metabolism

Juergen Steiner from the Loeffelhardt group (Wien) presented interesting results concerning the MEMBRANE INSERTION of the nuclear-encoded cytochrome c553 from cyanelles of Cyanophora paradoxa. They isolated the protein and the corresponding nuclear gene. Since the mature protein is located in the thylakoid lumen, it has to traverse three biological membranes (inner and outer envelope membranes, thylakoid membrane) and the peptidoglycan layer before it reaches its final subcellular locale. The transit sequence is composed of two different targeting signals, and this represents the first known bipartite transit sequence of a cyanelle protein.

Olaf Neuschaefer-Rube (Konstanz) studies a Synechocystis mutant isolated from the Bodensee that doesn't form normal PHYCOBILISOMES but rather contains a paracrystalline structure made up of phycocyanin and linker protein. Biochemical analysis of the crystal showed the presence not only of phycocyanin alpha and beta, but also of the rod linker LR35 C-PC. A colored polypeptide of 55 kD turned out to be a fusion protein of the rod linker at the n-terminus and a phycocyanin beta subunit. The protein is not predicted by the arrangement of genes in the mutant, indicating that a posttranscriptional event may be responsible for this strange fusion protein.

Stefan Schmitz (Bonn) investigated Anabaena FERREDOXIN-BINDING PROTEINS in E. coli in order to find out if they possess a common binding domain for ferredoxin. All negatively charged and conserved amino acid residues of ferredoxin from

Anabaena were exchanged for neutral residues and the effects on binding to different redox partners were studied. Glu94 was identified as the most important among these residues, and for FNR and nitrite reductase an aromatic residue in position 65 also was essential. After having cloned petF gene (encoding FNR) from Anabaena variabilis, Stefan produced site specific mutations within that protein. He exchanged positively charged residues against neutral ones. Arg153, Lys209, Lys212, and Lys430 turned out to be very important, they are supposed to be lying in a cavern which binds ferredoxin. Such a cavern carrying a lot of positively charged residues could be found in several cyanobacterial nitrite and nitrate reductases.
Markus Geisler (Duesseldorf) characterized the p-type CALCIUM ATPASE from the same strain. The enzyme is localized in the cytoplasmic membrane and is more closely related to eukaryotic ATPases than to bacterial ones.
Josef Niederberger (Institut fur systematik Botanik, Zurich) reported on the TAXONOMY OF TOXIC CYANOBACTERIA in Swiss alpine lakes. He used RAPD-PCR for the classification of 16 toxic and non-toxic strains of Microcystis aeruginosa. The toxicity of the strains had been checked by other groups in a mouse bioassay, with HPLC and in a phosphatase inhibition assay. A phylogenetic tree was derived from the RAPD data, but the toxic strains did not cluster together.
Andrea Nowotny (Institut fur pharm. Biologie, Greifswald) detected ANTIVIRAL ACTIVITY in aqueous extracts from Microcystis waterblooms in the south Baltic Sea. The nature of the substance that inhibits replication of influenza virus A could not be clarified.
Egbert Hoiczyk (Max Planck Institut, Muenchen) related structural details of what may be the motor for GLIDING MOTILITY by cyanobacteria. Electron microscopical studies of the cell walls of strains from three different genera revealed that all species possess identical multilayered cell walls, which are covered with a complex double external layer, the surface of which is formed by a parallel array of helically arranged fibrils. The correlation of these structures with motion and their extracellular location indicates a possible role in gliding motility.
Alfred Hansel (Freiburg) presented his results concerning the major OUTER MEMBRANE PROTEINS of Synechococcus PCC 6301. After purification of the major outer membrane protein complex and its functional characterization, he cloned the gene coding for porin. Its sequence does not show any overall similarity with other porin sequences, but computer analysis indicates that the cyanobacterial porin has the same architecture as other porins. Downstream from this gene lay a second orf that shows great similarity (> 60%) to the porin gene, and reinvestigation of the protein led to the conclusion that Synechococcus has two different major outer membrane proteins, the monomers of which migrate almost identically in SDS gels. It is not yet clear if both function as porins.
-- Alfred Hansel

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AUSTRALIA/NEW ZEALAND
Steve Delaney
Department of Biotechnology,
University of New South Wales P.O. Box 1
Kensington, New South Wales
AUSTRALIA 2033 (Tel) 02-697-2056

AUSTRIA
Georg Schmetterer
Institut fur Physikalische Chemie,
Wahringerstrasse 42, A-1090 Wien
(Tel) 43-1-31367-2555
(EMail) A8422dad@Awiuni11

CANADA
Neil Strauss
Dept. of Botany,
University of Toronto,
Toronto, Ontario M5S 1A1.
(Tel) 416-978-3532/5563
(Fax) 416-978-5878
(E-mail) Straus@Botany.UToronto.Ca

P.R.CHINA
Chao-Tsi Tseng
Centre of Marine Sciences
Dept. of Biology
Nanjing University, Nanjing.

(Tel) 637551-2551
(Fax) 086025-302728

FRANCE
Nicole Tandeau de Marsac
Physiologie Microbienne
Institut Pasteur
29 rue du Dr. Roux
75724 Paris Cedex 15.
(Tel) 567-46-98
(Fax) 40.56.01.25
(EMail) NTMarsac@Pasteur.Fr

GERMANY
Wolfgang Lockau
Biochemie der Pflanzen
Fachbereich Biologie
Humboldt-Universit„t,
Invalidenstr. 42, 10 115 Berlin
(Tel) 30-2897-2686 (Fax) 30-2897-2641

INDIA
Joe Thomas
Biotechnology Division
SPIC Science Foundation
110 Mount Road
Madras 600 032.
(Tel) 432342 (Fax) 432163

ISRAEL
Elisha Tel-Or
Dept. of Agricultural Botany
The Hebrew University
Rehovot 76100
(Tel) 08-481262

ITALY
Mario Tredici
Departimento di Scienze e Tecnologie
Alimentari e Microbiologiche.
Universita degli Studi di Firenze,
P.le.delle Cascine 27 51044 Firenze.

(Tel) 055-352051 (Fax) 055-330431
(E-mail) Tredici@Csma.Fi.Cnr.It

NETHERLANDS
Luuc Mur
Laboratorium voor Microbiologie,
Universiteit voor Amsterdam
Nieuwe Achtergracht 127
1018 WS Amsterdam
(Tel) 31-20-525-7056
(Fax) 31-20-525-5802
(E-mail) A417LMur@Horus.Sara.NL

SCANDANAVIA
Olav Skulberg
Norwegian Institute for Water Research
P.O.box 69
Korsvall, N-0808
Oslo 8 NORWAY.
(Tel) 47 22 185266
(Fax) 47 22 185200

U.K.
Tony Walsby
Dept. of Botany
University of Bristol
Bristol BS8 1UG.
(Tel) 0272-303030

ANYWHERE ELSE
Jeff Elhai
Dept. of Biological Sciences
Florida International University
University Park Campus
Miami FL 33199 USA.
(Tel) 305-348-3584, (Fax)305-348-1986
(E-mail) Cyano@Servax.Fiu.Edu
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