CYANONEWS

Volume 12 Number 2 July 1996

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. (See address label for expiration date). 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 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.

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More Electronic Resources for the Cyanobacteriologist

The number of world wide web sites having some relation to cyanobacteria, more than 1700 at last count, has grown far beyond the abilities of anyone to comprehend them all. Most sites, however, merely mention cyanobacteria, either as part of a description of a person's research interests, as part of a class syllabus, etc. Some sites, of more general interest, are given below. The list must be considered only a sampling of what is available.

CCAP SITE: Homepage of the Culture Collection of Algae and Protozoa, centered at the Windermere Laboratory, U.K. Provides a searchable on line description of strains held by the collection [see below]. Also contains recipes for growth media and instructions on how to order strains.
http://wiua.nwi.ac.uk/ccap/ccaphome.html

CDAC SITE: Homepage of the Czechoslovak Database of Algae and Cyanobacteria.
http://www.bdt.org.br/bdt/msdn/ccala/

UCMP BACTERIA SITE: The University of California Museum of Paleontology has an online collection of articles and pictures of bacteria, including many entries on cyanobacterially related subjects.
http://www.ucmp.berkeley.edu/bacteria

PHOTOSYNTHESIS SITE: Arizona State University, Departments of Botany and Chemistry and Biochemistry run a site devoted to photosynthesis. It includes an extensive directory of e-mail addresses and list of upcoming meetings.
http://Photoscience.La.Asu.Edu/Photosyn

CYANOSITE: provides useful protocols and other matters cyanobacterial, including directories of cyanobacteriologists and back issues of this newsletter.
http://WWW-Cyanosite.Bio.Purdue.Edu

CYANOBASE SITE: Information regarding the *Synechocystis* PCC 6803 genome [see NEWS] and much else. Reports analysis of the currently available sequence and permits similarity search with sequences provided by visitors. Includes access to many if not all known cyanobacterial sequences and a search engine to help you find what you want. Displays a circular map of the genome and phylogenetic trees based on several gene sequences. Provides links to many other databases with prokaryotic sequences.
http://www.kazusa.or.jp/cyano/cyano.html

TOXIC CYANOBACTERIA SITE: Houses a bulletin boards for announcements, informational entries (including graphics) on microcystins, anatoxin, nodularin, protein phosphatases, and *Microcystis aeruginosa*, and a debate on the merits/risks of ingesting Super Blue Green Algae (*Aphanizomenon flos-aquae*). Provides links to two related discussion groups: Phycotoxin and Cyano-tox.
http://Luff.Latrobe.Edu.Au/~BotBML/Cyanotox.Html

RRNA SITE: A compilation of ribosomal RNA sequences, including 38 of cyanobacterial origin. Makes available a program to facilitate construction of trees, to align multiple sequences, and to draw secondary structures.
http://www-rrna.uia.ac.be/

CYANOBACTERIA INTERNATIONAL SITE: I haven't figured this one out yet, except to realize that it hasn't anything to do with cyanobacteria as we know them. The site collects literature from Cyanobacteria Publications which, by its own description, conducts "a gyrovague experiment operating upon the chromosomes of beauty and information."
http://www.thing.net/~grist/homecyan.htm

Matters Arising

Peter Wolk wants to find strains that both (i) form akinetes specifically adjacent to heterocysts, and (ii) grow to visible colonies within five days.

CONTACT: Peter Wolk, MSU-PRL Plant Research Laboratory, Michigan State University, East Lansing, MI 48824 U.S.A.
TEL: 1-517-353-2049, FAX: 517-353-9168,
E-MAIL: 22333CPW@Msu.Edu

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Biophysical Techniques in Photosynthesis (ISBN: 0-7923-3642-9), edited by Jan Amesz and Arnold Hoff has just been released by Kluwer Academic Publishers (1996) as Volume 3 in the series "Advances in Photosynthesis". Its 24 chapters encompass spectroscopic and magnetic resonance techniques as well as those aimed at elucidating structure or measuring oxygen evolution.

CONTACT: Kluwer Academic Publishers, P.O. Box 17, 3300 AA Dordrecht, NETHERLANDS
E-MAIL: Services@Wkap.NL
or Kluwer Academic Publishers, 101 Philip Drive, Norwell, MA 02061, U.S.A. TEL: 1-617-871-6600, FAX: 1-617-871-6528,
E-MAIL: Kluwer@World.Std.Com

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The Proceedings are now available from a workshop entitled TOXIC CYANOBACTERIA: CURRENT STATUS OF RESEARCH AND MANAGEMENT. The workshop, held March, 1994 in Adelaide, Australia, covered various aspects of concern to the researcher and manager of water quality, including monitoring and analysis, health effects and risk assessment, control and removal of toxins, and management issues. The proceedings include contributions from Wayne Carmichael, Geoff Codd, Ian Falconer, Ken-ichi Harada, Gary Jones, Mick Pearson, and Shun-Zhang Yu. The cost of the proceedings is AUS$50 (check should be payable to "Workshop Proceedings").

CONTACT: Australian Water Quality Centre, Private Mail Bag 3, Salisbury, SA, 51 08, Australia; or Brenton Nicholson
Tel: 61-8-259-0246, FAX: 61-8-259-0228,
E-MAIL: Brenton.Nicholson@Sawater.Sa.Gov.Au

Culture Collection Grows with Strains

Culture collections, like the strains they collect, are a product of evolution. The Culture Collection of Algae and Protozoa (CCAP) now maintains about 2000 algae and protozoa, of which more than 200 are cyanobacteria. This collection originated from strains isolated by Ernst Georg Pringsheim and his collaborators, Victor Czurda and Felix Mainx. When Pringsheim moved to England, his collection went with him and was eventually taken over by E.A. George at Cambridge University in 1947. In 1986 the collection was divided, freshwater algae and all protozoa going to the Institute of Freshwater Ecology (IFE) Windermere Laboratory at Ambleside and marine algae going to Dunstaffnage Marine Laboratory (DML) at Oban.

Strains are available for a fee of £21 per 10 ml for academic institutions. The strain list can be searched at the CCAP web site (see above). Alternatively, hard copies of the Catalogue are available for £7.00. A list of freshwater cyanobacterial strains can be obtained by anonymous FTP at the site Cyanonew@Servax.Fiu.Edu (password: Bluegreen). Download CCAP-CB.Asc (pure text).

The CCAP also holds the Fritsch Collection of Illustrations of Freshwater Algae, some 500,000 published figures of taxonomic or floristic significance. They are taken from over 15,000 works and cover over 2,500 genera. A fiche edition has been published.

CONTACT: Culture Collection of Algae and Protozoa, Institute of Freshwater Ecology, Windermere Laboratory, Far Sawrey, Ambleside, Cumbria LA22 0LP, UK. TEL: 44-15394-42468,
FAX: 44-15394-46914, E-MAIL: CCAP@ife.ac.uk,
WEB: http://wiua.nwi.ac.uk/ccap/ccaphome.html
or (for edition of Fritsch illustrations): Inter Documentation Company bv, PO Box 11205, 2301EE Leiden, NETHERLANDS

Meetings

(Anyone wishing to contribute a report on any meeting of cyanobacterial relevance is cordially invited to do so!)

The 1st EUROPEAN PHYCOLOGICAL CONGRESS Cologne will meet 11-18 Aug 1996, Cologne, Germany.

CONTACT: Congress Secretariat, M. Melkonian, Botanical Institute, University of Cologne, Albertus Magnus Platz, D-50923 Cologne, GERMANY. TEL: 49-221-4702475, FAX: 49-221-4705181,
E-MAIL: MMelkon@Biolan.Uni-Koeln.De

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The 9th EUROPEAN BIOENERGETICS CONFERENCE (EBEC) is scheduled for 17-22 Aug 1996 in Louvain-la-Neuve, Belgium. This conference has not previously paid a great deal of attention to cyanobacteria, but this year it will have at least one session, entitled "Archaebacteria, extremophiles and cyanobacteria"

CONTACT: Congress Secretariat, A.-M. Corbisier-Colson, Unite de Genetique, UCL (Universite catholique de Louvain), Place Croix du Sud 4-5, B-1348 Louvain-la-Neuve, BELGIUM.
TEL: 32-10-478261, FAX: 32-10-473742.

The 12th INTERNATIONAL CONGRESS ON PHOTOBIOLOGY will be held 1-6 Sept 1996 in Vienna, Austria. The congress will include two sessions, entitled "Antenna systems and Energy Transfer" and "Reaction Centers and Electron Transfer", in which cyanobacteria will be well represented.

CONTACT: Scientific and Administrative Secretariat, Vienna Academy of Postgraduate Medical Education and Research, Alser Strasse 4, A-1090 Vienna, AUSTRIA. TEL: 43-1-405138313,
FAX: 43-1-405138323, E-MAIL: Medacad@via.at

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SYMBIOSIS 96! will bring together investigators who apply modern techniques to the study of diverse symbiotic associations on 5-8 Sept 1996 in Bar Harbor, Maine.

CONTACT: Paul Baumann, Microbiology Section, University of California, Davis CA 95616-9665 TEL: 1-916-752-0272,
FAX: 1-916-752-9014, E-MAIL: PABaumann@UCDavis.Edu
WEB: http://Wolbachia.Med.Yale.Edu

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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A one day conference entitled ALGAL MODELING: PROCESSES AND MANAGEMENT will be held 19 Sept 1996, at the University of Reading, England. Registration is £40 or $60 US. The conference includes talks on modeling algal behavior and the management of algal problems.

CONTACT: Alan Howard, Aquatic Environments Research Centre, Dept Geography, The University of Reading, Reading, RG6 6AB, England, U.K. E-MAIL: A.Howard@Reading.Ac.Uk
WEB: http://www.rdg.ac.uk/geog/conference.html

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The 11TH AUSTRALIAN NITROGEN FIXATION CONFERENCE will focus on N_2-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 in the proceedings.

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

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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MYCOTOXINS AND PHYCOTOXINS will be the focus of a Gordon Conference to be held in North Plymouth, New Hampshire, U.S.A. 22-27 June 1997.

CONTACT: Wanda Haschek-Hock, Dept. of Veterinary Pathobiology, University of Illinois, 2001 S. Lincoln Avenue, Urbana, IL 61801 U.S.A. TEL: 1-217-333-2449, FAX: 1-217-333-7421, E-MAIL: WHaschek@Uiuc.Edu

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For those looking ahead, the IXth INTERNATIONAL SYMPOSIUM ON PHOTOTROPHIC PROKARYOTES is set for 6-12 September 1997, University of Vienna, Austria. The first circular will soon be sent out. You may also get a copy of it by FTP at the site Cyanonew@Servax.Fiu.Edu (password: Bluegreen). Download either ISPP.Doc (an MS-Word document) or ISPP.Asc (pure text). WARNING: Those who have already sent an E-mail request to receive the first circular should send another request, because a hardware error erased your names!

CONTACT: Symposium Secretariat, IXth ISPP Vienna 1997, Institute of Physical Chemistry, University of Vienna, UZA2, Althanstrasse 14, A-1090 Vienna, AUSTRIA.
E-MAIL: Georg.Schmetterer@UniVie.Ac.At

Positions Offered

POSITION OFFERED: Post-Doc
CONTACT: Bridgette Barry, Gortner Laboratory, Department of Biochemistry, University of Minnesota, St. Paul, MN 55108 U.S.A. TEL: 1-612-624-6732, FAX: 1-612-625-5780, E-MAIL: Barry@Molbio.Cbs.Umn.Edu
RESEARCH: Study the structure and function of the photosynthetic water oxidizing complex. Techniques to be employed include infrared and EPR spectroscopy as well as site directed mutagenesis.
REQUIREMENTS: Background in chemistry, biochemistry, or biophysics.
AVAILABLE: Fall 1996.
SEND: CV and three reference letters.

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POSITION OFFERED: Post-Doc
CONTACT: Michael Schaefer, School of Biological Sciences, University of Missouri-Kansas City, 5007 Rockhill Road, Kansas City, MO 64110 U.S.A. TEL: 1-816-235-2573, E-MAIL: MSchaefer@Cctr.Umkc.Edu
RESEARCH: Molecular basis of photoperception and signal transduction involved in complementary chromatic adaptation by Fremyella diplosiphon (*Calothrix* PCC 7601)
REQUIREMENTS: Experience in molecular biology, biochemistry and microbial genetics
AVAILABLE: Immediately
SEND: CV and three letters of recommendation

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POSITION OFFERED: Post-Doc
CONTACT: Fevzi Daldal, University of Pennsylvania, Plant Science Institute, Dept. of Biology, 204 Mudd Building, Philadelphia PA 19104-6019. TEL: 1-215-898-4394, FAX: 1-215-898-8780, E-MAIL: FDaldal@Sas.UPenn.Edu
RESEARCH: Biochemical molecular genetics of cytochromes and cytochrome complexes of photosynthetic bacteria. [See J Bacteriol (1995) 177:608-613; Biochem (1995) 34:15979-16012; Biochem (1994) 33:3120-3127; Chapter 36 of *Anoxygenic Photosynthetic Bacteria*]
REQUIREMENTS: Solid background and experience in either molecular genetics and protein purification techniques or spectroscopy and computer modeling methods; a genuine interest in structure, function biogenesis and regulation of microbial energy transduction complexes.
SEND: CV, description of research accomplishments, and reference letters.

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IGOR BROWN'S Fax number and E-mail address were given erroneously in the last newsletter. They should be:

FAX: 380-482-238320 or 380-482-232463,
E-MAIL: IBrown@Microalgae.Odessa.Ua

JEFF ELHAI, after several years in the southeastern part of the U.S., will be moving up the coast to the University of Richmond the end of this summer. Richmond is about two hours away from Washington, D.C., ideal for visitors.

Dept. of Biology, University of Richmond, Richmond, Virginia 23173, U.S.A. TEL: 1-804-289-8412, FAX: 804-289-8233,
E-MAIL: Cyano@URVax.URich.Edu

FRIEDERICKE KOENIG has moved from J.W. Goethe-Universität to Universität Bremen

Universität Bremen, Fachbereich 2 Biologie/Chemie, Molekulare Pflanzenphysiologie, Leobener Str./NW 2, D-28359 Bremen

We must acknowledge the loss of one of our own, ORSOLA TIBONI, who died 30 March 1996 after a brief illness. Orsola will be remembered for her contribution to the understanding of the molecular biology of cyanobacteria, particularly *Spirulina platensis*.

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Every Base of *Synechocystis* Genome Now Known

Starting in only 1994, Kazusa DNA Research Institute has completed sequencing the entire genome of *Synechocystis* PCC 6803, reports Satoshi Tabata. For the record, the strain has precisely 3,573,470 bp, with an average GC content of 48%. *Synechocystis* has about 3100 likely coding sequences, with two sets of ribosomal RNA gene clusters and 42 potential tRNA genes.

1 Mb of the sequence (from 64% to 92% of the circular map) has already been analyzed and made available electronically through the Kazusa CYANOBASE site (see BULLETIN BOARD). 818 likely coding sequences in this region have been identified based on their lengths or similarity to known genes.

The sequence was reexamined using the program GeneMark, designed to recursively evaluate the probability that an open reading frame (ORF) is indeed a coding sequence, in light of the aggregate of characteristics of other open reading frames in the sequence. The program identified 752 of the 818 previously assigned ORFs, of which 26 were predicted to start at more internal start codons. 50 ORFs not found in the original analysis were predicted by GeneMark to be coding sequences.

Of the 66 ORFs originally identified but not found by GeneMark, 14 showed significant degrees of similarity to known genes, and 10 were found within insertion-sequence-like elements. Perhaps these genes came to *Synechocystis* by horizontal gene transfer, and therefore possess foreign characteristics that would escape the detection algorithm of the program.

Some of this work has been published [Kotani H et al (1994) DNA Res 1:30-307; Kotani H et al (1995) DNA Res 2:133-142; Kaneko et al (1995) DNA Res 2:153-166. Hirosawa et al (1995) DNA Res 2:239-246], and much more can be found on the CYANOBASE web site.

CONTACT: Satoshi Tabata, Kazusa DNA Research Institute, Laboratory of Gene Structure 2, 1532-3 Yana, Kisarazu, Chiba 292, JAPAN.
TEL: 81-438-52-3934, FAX: 81-438-52-3933,
E-MAIL: Tabata@Kazusa.Or.Jp

Toxin Suspect in Mass Killing

Tragedy struck this past spring in a Brazilian hospital. 126 patients undergoing hemodialysis in Caruaru, a city in northeastern Brazil, became ill and, over the course of several weeks, 43 of them died of toxic hepatitis. Much evidence has implicated microcystin, a toxin produced by the cyanobacterium *Microcystis aeruginosa*, as a prime cause of the deaths. If this connection is borne out, it would constitute the first confirmed instances of human fatalities attributed to microcystin poisoning.

Microcystins, potent hepatotoxins that act on protein phosphatases, were found in the liver and serum of affected patients at levels associated with acute or lethal toxicities. Investigation as to the source of the toxin centered on the water source used to feed the hemodialysis unit. Owing to a drought, the hospital had water trucked in from reservoir and treated with chlorine. Chlorination, however, is not effective in destroying microcystin, except with long treatments or at low pH. Indeed, chlorination, by killing any *M. aeruginosa* present, would be expected to liberate microcystin into the water. Microcystin was detected by high performance liquid chromatography in the water source and in the carbon used to filter the water.

Jose Roberto C. Rocha, the physician who broke the story to the cyanobacterial community, voiced the concern that the case in Caruaru may not be unique. Patients on dialysis often show unexplained alterations in liver enzymes, he said, speculating that this might be caused by low levels of microcystin intoxication. A similar case was reported earlier by Filomena Araujo, Evora, Portugal, in which the deaths of dialysis patients may have been due to microcystin. The water supply was drawn from a river heavily contaminated with *Microcystis aeruginosa*.

It is important to note that the case against microcystin is not complete. The water may well have contained other toxins, including pesticides, that have not yet come to light. Wayne Carmichael is continuing a study to pin down the cause. The evidence in favor of some wrongdoing is already convincing enough to officials, however, to prompt action. Three physicians involved in the dialysis have been indicted for manslaughter.

Abstracted from material posted to the Toxic Cyanobacteria Web Site (See BULLETIN BOARD).

Cryopreservation of Cyanobacteria

Cryopreservation refers to the storage of a living organism at ultra-low-temperature such that it can be revived and restored to the same living state as before it was stored. Indefinitely long storage times require that the organism be maintained below the glass transformation temperature of aqueous solutions, approximately -130°C, the temperature at which frozen water no longer sublimes and recrystallizes. Although ultra-cold freezers may stabilize some living cells for weeks or even years, liquid nitrogen is required for longer storage times. Cyanobacteria, as is the case for all living cells, suffer severe osmotic stress and/or ice crystal damage during the freezing and thawing processes. The most effective known ways to minimize these potentially lethal effects are to add a cryoprotective compound to the culture prior to its freezing for storage, and to control the transient cooling and warming rates during preservation.

We have been able to cryopreserve virtually all of the approximately 200 strains of cyanobacteria in the UTEX collection of algae [R.C. Starr and J.A. Zeikus (1993) J Phycol 29 (supp)] located in the Department of Botany at the University of Texas at Austin. This includes unicells, branching and unbranching filamentous species, marine and freshwater species, and those with heterocysts and akinetes. We also have successfully stored several photosynthetic mutants of cyanobacterial species provided by Robert Tabita of Ohio State University and John Golbeck of the University of Nebraska. The required procedures are straightforward and inexpensive, but require attention to a few details. One-hundred percent viability is never expected but viabilities over 50% are typical. High viabilities (i.e. >10%) are especially desirable for mutant strains. Here I will describe procedures that cryopreserve nearly all of cyanobacterial strains we have examined.

Sample preparation: Transfer cyanobacterial liquid culture into a cryovial[1] in preparation for cryopreservation. Alternatively the culture can be grown directly as a lawn on a tiny agar slant prepared within the cryovial [K. Bodas, K.R. Diller, and J.J. Brand (1995) Cryo-Letters 16:267-274]. If the cryovial contains liquid culture, pellet the cells by centrifugation in a clinical centrifuge and discard the supernatant.[2] Add cryoprotective solution containing 1.0 ml of half-strength growth medium (BG-11 works well for virtually all fresh-water cyanobacteria) containing 5% methanol or 8% DMSO to the pelleted cells.[3] Alternatively, if the culture is growing on an agar slant, transfer 1 ml of cryoprotective solution above the slant.[4,5]

[1]Two-ml or 1.8-ml polyethylene or polypropylene cryovials are especially convenient for handling and storage efficiency, although 1-ml and 5-ml cryovials also work well.

[2]We have constructed acrylic sleeves that fit into the tube holders of the rotor, positioning the cryovials securely in place within a clinical centrifuge rotor, flush with the top of the tube holders. The cryovials can also be inserted into unmodified tube holders for centrifugation in a clinical centrifuge.

[3]Although glycerol is an effective cryoprotective agent for many bacteria, it is not effective for most cyanobacteria. Methanol at approximately 5% (v/v) is suitable for most strains. However, we have been successful with concentrations of methanol ranging from 2% to 12.5%, and DMSO ranging from 4 to 15 %, depending on the culture. A small fraction of some cultures survive with no added cryoprotective agent.

[4]When the cryoprotective agent is added directly above the culture on an agar slant, the tube is shaken gently prior to freezing, to dislodge some of the cells and ensure that the liquid penetrates through the culture. Cells pelleted from liquid suspension are fully suspended in the cryoprotective solution.

[5]Cells are killed by exposure to bright light when in cryoprotective solution. Keep the culture in subdued room light while handling, and in complete darkness at other times.

Freezing: The cryovial containing the culture in cryoprotective agent at room temperature is inserted into a special "freezing container"[6] which has been pre-chilled to refrigerator temperature. The freezing container is then placed into a -70°C freezer for 2 hours. Then the cryovial is quickly removed from the freezing container, placed into a storage container, and plunged into liquid nitrogen for indefinite storage.[7]

[6]The "Mr. Frosty" freezing container (Nalgene) is satisfactory for nearly all cyanobacteria. It is inexpensive to purchase and holds eighteen 2-ml cryovials simultaneously. Its contents cool at slightly less than 1°C per minute when it is placed into a -70°C freezer.

[7]Sterility is a problem when storing plastic cryovials in liquid nitrogen. Vials equipped with gaskets and those with inside threads seal most tightly, but liquid nitrogen always creeps into some cryovials. This provides a conduit for entry of bacteria, some of which remain viable in bulk liquid nitrogen. Several manufacturers sell heat-shrink tubing that serves as a tight-fitting sleeve around the entire cryovial and lid, thereby eliminating liquid nitrogen leakage. Bacterial contamination can be eliminated also by storage in sealed glass ampoules or by storing plastic cryovials in the nitrogen vapor just above the liquid, although these procedures introduce additional safety and convenience considerations.

Thawing and Recovery: Cultures to be revived are removed from liquid nitrogen storage and warmed rapidly to room temperature.[8] Cells are immediately pelleted by centrifugation of the cryovial,[9,10] and the supernatant is discarded. One ml of fresh growth medium is placed into the vial to suspend the pellet. The cryovial lid is slightly loosened to allow gas exchange, and the contents of the vial are kept in complete darkness for 1-2 days. The culture can then be placed on agar or in liquid growth media under normal growth conditions. The viable cells should begin normal growth within 1 - 2 days in light, although they are especially susceptible to damage by excessive light intensity for the first day or two of illumination.

[8]Warm rapidly by plunging the tightly sealed, still-frozen cryovials into a dish of water at 35°C. An appropriately selected volume of water will cool to approximately 25°C as the cryovial contents are warmed to that same temperature.

[9]Centrifugation of a thawed culture in a cryovial containing an agar slant is best done in an angle rotor that pellets the cells on the agar surface without appreciably altering the position of the agar in the tube.

[10]Cultures of eukaryotic algae especially, and cyanobacteria to some extent, are susceptible to mechanical damage during recovery from storage at low temperature. Cells should be pelleted at the minimum R.C.F. that facilitates pelleting. Excessive agitation should be avoided when suspending the pellet.

Comments welcome!

Jerry J. Brand, Botany Dept., Univ. of Texas at Austin
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TAXONOMY and EVOLUTION

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