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The CSIRO Collection of Living Microalgae: Bioapplications

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The CSIRO Collection of Living Microalgae* is a living bank of microalgae isolated from Australian waters from the tropics to Antarctica. Wide microalgal biodiversity is reflected in a broad range of bioactive compounds that have medical, human health, aquafeed, and energy applications. In the CSIRO Wealth from Oceans National Research Flagship, research on exopolysaccharides (EPS) from microorganisms has revealed new adhesives, with microalgae from the Collection being screened for their EPS production, along with development of known targets of EPS-producing bacteria from the extreme Antarctic environment. Gene discovery in microalgae for biosynthesis of omega-3 long chain polyunsaturated fatty acids (LC-PUFA) that have an impressive range of human health benefits is one focus of the CSIRO Food Futures National Research Flagship. The goal is to introduce the microalgal omega-3 pathway into genetically engineered crop plants, thus ensuring a sustainable source of omega-3 LC-PUFA for the future. Some microalgae are characterised by high oil content. The CSIRO Energy Transformed National Research Flagship is investigating the potential for producing biodiesel from algae. High biomass cultivation of microalgae has the potential not only to produce oil and/or biomass for biofuels, but also to mitigate CO_2 and other greenhouse gases due to their uptake during photosynthesis and growth.

*http://www.cmar.csiro.au/microalgae/

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Vitrification cryopreservation approaches for reacalcitrant micro-algae species

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Cawthron maintains a nationally significant culture collection of micro-algae for New Zealand as well as a number of aquaculture species that are fed to shellfish larvae in hatcheries. Many of the >150 strains in the collection are unique, with properties not found in overseas isolates, and are being studied for commercial and research applications. Cryopreservation has a number of advantages: it is cheaper than ongoing maintenance of cultures and safeguards strains from the risk of becoming contaminated or lost due to genetic drift from serial subculture. A standard cryopreservation method using 10-15% (v/v) DMSO for cryoprotectant and controlled rate cooling has been successfully applied to many of the algae species held but some remain recalcitrant, in particular the larger (>30µm) marine dinoflagellates, for example, *Procentrum lima*. Vitrification is an alternative cryopreservation method to controlled rate cooling and incorporates the pretreatment dehydration of cells or application of concentrated vitreous chemical cocktails so that ice crystal formation is circumvented and a stable 'glass' is formed instead, when treated cells are directly immersed in liquid nitrogen. Critical factors such as dehydration and toxicity are controlled by tailoring pre- and post- liquid nitrogen treatment parameters. Sensitive equipment such as Differential Scanning Calorimeters assist in vitrification protocol optimization by pinpointing thermodynamic events, such as glass transitions (Tg), in vitrified cells. Initial trials examining the dehydration and toxicity parameter thresholds of the selected marine micro-algae have begun and preliminary results will be reported.

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Cyanobacteria isolation and growth at QHFSS

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With increasing emphasis on Cyanobacteria and their impacts on water quality, Queensland Health Forensic and Scientific Services (QHFSS), officially started to develop, a Phycology Culture Facility in early 2007, at the Coopers Plains laboratories, in Brisbane. The purpose of the Facility was to supply high quality, monoclonal phytoplankton material for research projects, (primarily PCR based). Over 40 strains of cyanobacteria are currently maintained at the Facility. An important aspect of the Facility has been developing the capability of isolating environmental strains, using an isolation technique developed on site. Information presented gives a brief outline of the isolation and growth techniques employed at QHFSS to produce a cyanobacterial culture from an environmental sample. Highlighted are important aspects that need to be considered by scientists wishing to culture cyanobacteria, such as suitable sample choice, growth media, isolation techniques and quality assurance. The isolation techniques are relatively simple and can be further simplified as a small scale and informal project, utilising basic, inexpensive equipment readily available in most laboratories or up-scaled, to a facility with dedicated resources and formal projects. The scale and complexity of the culturing facility will depend on the desired end use of the cultures, and the resources and facilities available to a laboratory. This presentation may encourage other institutes to attempt to develop their own small scale culture collections, helping add to the collective knowledge of phytoplankton and their biodiversity.

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Microbial Culture Collection at the National Institute for Environmental Studies (NIES-Collection), playing a role as a core collection of algae in Japan

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The NIES-Collection holds about 2,500 strains that consist of various taxonomic groups including about 300 genera and 600 species. Among them, 2,096 strains are now available. We distributed 600-700 strains per year in recent years. In 2002 the NIES-Collection was selected to a core collection of algae in Japan under the National BioResource Project funded by the Ministry of Education, Culture, Sports, Science and Technology-Japan. Since then, the NIES-Collection has increased its strain number and taxonomic diversity in collaboration with University of Tsukuba and National Science Museum. In addition, as a result of this project, all algal strains maintained in the IAM Collection, which was the oldest algal culture collection in Japan, were transferred to the NIES-Collection until the end of 2006FY when the IAM Collection was closed. The NIES-Collection is characterized by four groups of algal strains: 1) species relevant to environmental issues such as water bloom and red tide, 2) experimental models, 3) taxonomically important species, and 4) endangered Charales and freshwater red algae. For some of them, nuclear and organelle genomes have been analysed. In addition to routine works in the collection, we have intended to increase strain information such as multilocus genotypic information using seven house keeping genes in *Microcystis aeruginosa*, as well as SSU rDNA sequences in morphologically simple cyanobacteria and eukaryotic microalgae. The former is important to manage a large number of strains in the same species and the latter is important to show phylogenetic position of the strains in the culture collection.

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A culture collection of New Zealand alpine algae

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The New Zealand alpine zone contains a wealth of micro-organisms, largely unknown to science. We have established a culture collection of alpine algae collected mostly from Mt Philistine, Arthur's Pass National Park. The collection presently includes 15 chlorophyte species, 14 cyanobacteria, 2 streptophytes, 2 diatoms, 1 xanthophycean, and 1 chrysophycean. In addition to offering new data on algal diversity, the collection has potential for investigations of the distributions of microbes and in testing the extent of their dispersal, and in providing a resource for more applied research. We provide images of selected strains and tentatively assign relationships to isolates elsewhere in the world, deduced using morphological characters and gene sequencing.

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University of Malaya Algae Culture Collections (UMACC): biotechnological and environmental applications

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The University of Malaya Algae Culture Collection (UMACC) comprises more than 200 isolates from diverse habitats, ranging from aero-terrestrial, freshwater, brackish to marine habitats. The collection includes indigenous strains from Malaysia and the Polar Regions. When the UMACC was initiated in 1987, the strains were mainly used for the screening of high-value chemicals (e.g. polyunsaturated fatty acids, carotenoids and phycobiliproteins) and bioremediation of agroindustrial wastewaters. Presently, the cultures of UMACC are used in the the screening for antiviral, antineoplastic and antiinflammatory activity. Some of the algae are being studied for removing colour from textile dyes using suspension and immobilised cultures. The algae are also used for genotoxicity testing of pollutants such as pesticides, heavy metals and textile dyes. The collection of polar algae is very useful for our comparative studies on their response to global warming and UVR stress with tropical and temperate algae. With the increased interest in biofuels, we are also exploring the potential of the UMACC algae for biofuel production. In addition, the UMACC serves as a valuable resource of tropical algae for DNA barcoding initiatives.

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The Cawthron Institute Collection of Micro-algae (CICCM) supports cutting edge research

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The Cawthron Institute Culture Collection of Micro-algae is used by researchers world-wide. Live cultures (many supported by cryopreservation) are supplied to Cawthron researchers working on New Zealand government funded programmes and to approved researchers at other research institutes and universities within New Zealand. Over 10% of all cultures supplied are couriered internationally. Cultures have been used for development of standards for new chemical analytical methods, developing and testing molecular tools, the characterization of novel compounds and determination of their toxicology. They have also been used for the comparative study of 'same' species worldwide. Over the last year a key research programme at Cawthron Institute, dependent on the collection, is the Seafood Safety programme (NZ FRST Contract CAWX0703). Study undertaken in this programme includes research on novel toxin producers and their morphological and molecular identification. For example, in February 2008, analyses were carried out (LC-MS, Cawthron Biotoxin Laboratory) on fresh and frozen oysters collected from Rangaunu Harbour, Northland. The frozen oysters were collected a decade ago and at that time they had tested positive for an unknown biotoxin by mouse bioassay. The recent study focuses on the toxin producer and 20 clonal micro-algal cultures have been isolated from samples collected from Rangaunu Harbour. Other projects include studies of toxins produced by subtropical micro-algae to determine their potential impact on human health and on New Zealand's aquaculture industry in the future, particularly in the light of climate change. The results of these studies are key research outputs supported by the CICCM.

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NIWA Blue-Green Algae Monitoring Service

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NIWA's Algal Monitoring Service was started in Hamilton in 2003 to help organisations monitor a range of water bodies to ensure the safety of recreational and drinking waters. In December 2005, new drinking water standards came into force in New Zealand. They stipulate monitoring of drinking water sources deemed susceptible to blue-green blooms and have set standards to trigger additional monitoring and water treatment. New guidelines are now also being considered for recreational waters. This poster offers an overview of how Algal Services is able to help.

Puwainaphycins J-M isolated from *Calothrix* sp. TISTR 8906

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Up to the present time, a wide range of cyanobacteria (blue-green algae) have been examined for biologically active compounds. Prominent among these compounds are cyanotoxins such as the microcystins, cyclic heptapeptides from freshwater cyanobacteria. We have been screening cell-extracts of over 100 strains of cyanobacteria for antifungal activity against plant pathogenic fungi. The strain of cyanobacterium *Calothrix* sp., TISTR 8906, showed strong activity against *Colletotrichum truncatum* and *Macrophomina phaseolina*. Previously, four major antifungal compounds, puwainaphycins F-I, had been isolated from the cultured cells of TISTR 8906. Further investigation of the strain TISTR 8906, four new compounds have been isolated from the same fraction of puwainaphycins F-I as minor components. From the results of NMR experiments and amino acid analysis, the structures of these compounds (puwainaphycins J-M) were elucidated. The activities of these new compounds have been investigating.

PCR detection and quantification of key microalgal species in oxidation ponds

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With the aim of predicting pond health, we used molecular techniques and microscopy to monitor algal populations in three oxygenation ponds operating in Richmond, New Zealand. Over 2 years, 18 microalgal species were isolated and ribosomal ITS fragments amplified from their DNA. These DNA sequences were used to identify the algae by comparison to sequences in GenBank. Ribosomal fragments were also amplified and cloned from total DNA from pond samples. Species of *Chlorella*, *Scenedesmus, Chlamydomonas, Monoraphidium, Ankistrodesmus, Kolliela, Dunalliela, Cyanobacteria, Phacus* and *Euglena* were identified. The DNA sequences obtained were used to design species-specific primers for a number of microalgae including *Chlorella, Scenedesmus* and *Euglena* species that play a dominant role in pond operation. Species-specific primers were also designed for *Euglena acus* UTEX 1304LB obtained from an overseas algae collection. Species-specific primers were used for quantification of algae species in whole pond samples using SYBR green quantitative PCR (qPCR). *Euglena acus* UTEX 1304LB was used to calibrate our method for qPCR. We found that we could consistently quantify this species when it was spiked into water from real pond samples. Preliminary qPCR for *Euglena acus*, *Chlorella* sp and *Scenedesmus obliquus* in pond samples gave good amplification and reproducibility. Quantitative PCR based on ribosomal sequences has the potential to accurately monitor individual algal species within a complex mix of micro-organisms.