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The University of Toronto Culture Collection of Algae and Cyanobacteria (UTCC): a Canadian phycological resource centre

by

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With 1 figure and 1 table

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Abstract: The University of Toronto Culture Collection of Algae and Cyanobacteria (UTCC, http://www.botany.utoronto.ca/utcc/index.html), established in 1987, is unique in Canada. Prior to the opening of this facility, no service culture collection of freshwater algae and cyanobacteria existed in the country, even though Canada has extensive freshwater systems with diverse algal and cyanobacterial communities. Of the 6000 species of algae and cyanobacteria recorded from freshwater and marine systems in Canada, only a few hundred were being maintained in the Northeast Pacific Culture Collection of marine phytoplankton, and in research and teaching collections. The UTCC was established in response to the need for a facility that would serve as a phycological resource centre focusing on freshwater species, particularly those that are from areas of environmental concern or that are causes of environmental problems. The facility currently maintains 434 isolates, of which 308 are microalgae, 115 are cyanobacteria and 11 are aquatic vascular plants. Most of these isolates originated in central Canada; about 70% are unique to the UTCC and approximately 30% are axenic. The UTCC provides research-quality cultures and related services e.g. custom-isolation, technical training and Safe-Deposit to users in academic, government and commercial laboratories worldwide. The Natural Sciences and Engineering Research Council of Canada provides funding for basic maintenance of the collection and user fees cover additional costs. Future directions for the UTCC include expansion and diversification of the collection, cryopreservation, purification and further taxonomic verification of as many isolates as possible.

Introduction

The algal and cyanobacterial resources of Canada are extensive in both the freshwater and marine systems, as the country has close to 2 million lakes, 131, 650 nautical

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miles of coastline and a wide range of ecological habitats and climatic zones. The biodiversity of these systems has begun to be explored and to date there have been about 6000 species of algae recorded from Canadian waters (Mosquin et al. 1995). A recent 5-year study of the St. Lawrence River and Gulf of St. Lawrence by Environment Canada recorded 1074 species of algae in this large and economically important system (Deshaye et al. 2000). A recent study of the Rideau River, a major waterway in south central Canada, listed 300 species of algae (Poulin & Hamilton 2002). There are many environmentally sensitive areas in the country and the need for study and preservation of the species of algae and cyanobacteria is significant, particularly in areas that are affected by human activities. Culture collections can play an important role in conserving our valuable native species.

Status of algal culture collections in Canada

Although the biodiversity of the algal resources of Canada is great, the algae have been greatly under-represented in the collections preserving our native species. In 1986, the Ministry of State for Science and Technology published a directory of Canadian culture collections (Weldon et al. 1986). In the 140 collections listed there were just 9 that contained any algae; most of these were small teaching collections with just a few species and many of these isolates were from culture collections outside Canada. The only significant collection specializing in algae was a service collection of marine phytoplankton, the Northeast Pacific Culture Collection (NEPCC), which at that time had 340 isolates.

Since the 1960s, the University of British Columbia (UBC) has maintained the NEPCC, which was initiated and directed by Dr. F.J.R. "Max" Taylor, a world expert in dinoflagellates. The collection is diverse and currently has about 240 isolates of marine phytoplankton representing most of the algal classes. The emphasis is on dinoflagellates, diatoms and microflagellates, particularly from the northeast Pacific (Acreman 1986). The NEPCC is a member, No. 535, of the World Federation for Culture Collections and is now part of an amalgamated collection, the Canadian Centre for Culture of Microorganisms (CCCM), a facility of the Botany Department at UBC (http://www.botany.ubc.ca/cccm). The CCCM also includes a collection of about 100 strains of freshwater algae, including some species isolated from British Columbia in western Canada. The CCCM also maintains a fungal culture collection containing hundreds of unique strains that were formerly the collection of Dr. Robert Bandoni.

Since 1986 when the first Directory of Canadian Culture Collections was published, some of the collections, including the NEPCC, have been downsized and some have been lost due to lack of funding, change in research priorities or retirement of the curator. A subsequent directory compiled in 1994 listed only 5 collections maintaining algae, including the NEPCC and the University of Toronto Culture Collection of Algae and Cyanobacteria (Miller & Babcock 1994). According to my recent inquiries there are currently several small, specialized research collections across Canada in university and government laboratories that are preserving some of the biodiversity of algae and cyanobacteria (Table 1). Typically, phycological teaching collections in

Table 1. List of some of the research culture collections of algae and cyanobacteria held in academic and government laboratories in Canada (not including the service collection CCCM which is described in the text).

INSTITUTION	RESEARCHER	TYPE OF CULTURES	NUMBER AND SOURCE OF ISOLATES
University of Western Ontario, London, Ontario	Dr. Charles Trick	Marine Raphidophyceae: *Heterosigma akashiwo* (Hada) Hada	~20 isolates mainly from coastal waters of British Columbia Canada
University of Waterloo, Ontario	Dr. Kirsten Müller	Freshwater Rhodophyceae and Chlorophyceae	~90 isolates from Canadian sites)
University of Calgary, Calgary, Alberta	Dr. Susan Watson	Freshwater Chrysophyceae causing odour and taste in drinking	About 25-30 isolates, mostly from Alberta, Canada
University of New Brunswick, Fredericton, New Brunswick	Dr. Gary Saunders	Marine, red algae (Acrochaetiales) and a few brown macroalgae	Fluctuates ~ 40 isolates from Canada
National Research Council in Halifax, Nova Scotia	Dr. Allan Cembella	Marine, toxic dinoflagellates and sdiatoms	~30 isolates from Canada
Freshwater Institute in Winnipeg, Manitoba	Dr. Len Hendzel	Freshwater algae and cyanobacteria	~40 species from Canadian boreal lakes
Fisheries and Oceans Canada, Moncton, New Brunswick	Dr. Steve Bates	Marine diatoms *Pseudo-nitzschia* spp.; some toxic strains	66 clones from ~ Eastern Canada

Canada contain cultures obtained from commercial or service culture collections in the USA and a few local isolates from Canadian sites, and documentation on the cultures is either non-existent or limited. Unfortunately, with the shift away from teaching phycology in many Canadian universities, many of the algal cultures that were maintained in the past have now been discarded.

History and organizational structure of the University of Toronto Culture Collection of Algae and Cyanobacteria (UTCC)

The UTCC is the first service collection of freshwater algae and cyanobacteria in Canada. It was established in 1987 as a joint facility of the Institute for Environmental Studies and the Botany Department in response to the need for such a collection.

Funds were awarded to Drs. Pamela Welbourn (Stokes) and John R. Coleman by the Ontario Ministry of Colleges and Universities for the start-up of the facility. Dr. Stokes became the first director and served in this position until her retirement in 1990. Dr. Czesia Nalewajko, who has also been highly instrumental in initiating and developing the facility, served as director from 1990–1995. Dr. Johan Hellebust was the director of the UTCC from 1996 until 1999 when he retired and Dr. Robert Sheath became the new director, serving until 2001. Our current director is Dr. Brian Colman (York University) who has been contributing to the development of the collection since it opened. I have been privileged to serve as the curator of the collection since the inception of the UTCC.

The collection is a member, Reference No. 605, of the World Federation for Culture Collections and of the United States Federation for Culture Collections. It is funded by a Major Facilities Access grant, now in its 4th year, from the Natural Sciences and Engineering Research Council of Canada (NSERC). There is an official UTCC website at http://www.botany.utoronto.ca/utcc/index.html with links to other major culture collections of algae and cyanobacteria.

The director of the UTCC is also the Principal Investigator on the grant applications and is responsible for major financial and policy decisions. We have an advisory committee that meets annually to review the progress, discuss the budget and advise on the general direction of the development of the facility. Those members who are eligible for NSERC funding are co-applicants on the grant proposals. We are fortunate to have the following members presently serving together with the director on the advisory committee:

Drs. Roberta Fulthorpe, Johan Hellebust and Czesia Nalewajko
(University of Toronto, ON, CANADA),

Drs. Stephanie Guilford, Robert Hecky, Kirsten Müller and Ralph Smith
(University of Waterloo, ON, CANADA),

Dr. Robert Sheath
(CALSTATE University at San Marcos, CA, USA)

Dr. Michael Twiss
(Clarkson University, Potsdam, NY, USA).

The curator is responsible for the day-to-day administrative and technical management of the facility and is the only regular salaried employee.

Goals of the UTCC

The initial goals of the UTCC were to centralize the valuable algal cultures already in existence at the University of Toronto and to serve as a national phycological resource centre with the following main functions:

1. To isolate, culture and preserve algae and cyanobacteria of interest in ecological and biotechnological research, focusing mainly on isolates from Canadian sites and in particular, those from stressed environments, e.g. low or high pH, low temperatures, polluted by heavy metals or other contaminants.

2. To provide these cultures, related information and services to researchers in universities, government and industry, worldwide and to serve as part of a global network for researchers in algal culture.
3. To serve as a central depository for valuable cultures from other researchers, particularly when the cultures may be endangered due to insufficient technical help, retirement or change in direction of research.
4. To determine the optimal conditions for growth and preservation of the isolates.

Description and maintenance of the UTCC

Initially, the core of the collection was formed from deposits made by Drs. Pamela Welbourn (Stokes), Czesia Nalewajko, Brian Colman, John Coleman and Tom Hutchinson and reflected their research interests. Many of these isolates came from soft-water Ontario lakes that had been subjected to acidification, Lake Ontario, mine tailings ponds in Ontario and sites in the Yukon that were high in arsenic. As a cost-effective measure, the UTCC also maintained valuable strains of algae that had been obtained from other collections e.g. University of Texas Culture Collection (UTEX) and the Culture Collection of Algae and Protozoa (CCAP) and that were being used by the advisory committee members. Many new accessions were made through in-house isolations of algae from Ontario lakes and by deposits from other researchers in Canada. We are continuing to increase the diversity of the collection by isolating new strains particularly from the Great Lakes. About 70% of our isolates are unique to the UTCC; some of our isolates have been requested by other service culture collections and have been deposited in the USA, UK and France.

The number of isolates in the UTCC has grown from about 100 in 1987 to about 434, of which 308 are microalgae, 115 are cyanobacteria and 11 are aquatic vascular plants. Most of the algae and cyanobacteria are from freshwater or soil but there are also a few important marine species in the collection e.g. *Dunaliella tertiolecta* Butcher, *Phaeodactylum tricornutum* Bohlin and *Porphyridium purpureum* (Bory) Drew & Ross. The algal component of the collection is quite diverse with 13 classes, 79 genera and 318 isolates. The cyanobacteria are represented by 21 genera and 115 isolates. Aquatic vascular plants are a minor group with 2 genera, 6 species and 11 isolates. A graph showing the number of genera and species in each class is presented in Figure 1 and a complete list of isolates is found on our website. The taxonomic identification of the species has been based primarily on morphology and growth habits. Background information including site and date of isolation, isolator, depositor and date of deposit is kept on each isolate and is also available on the UTCC website. Some additional data, such as growth conditions and research conducted using the isolates may be obtained from the curator.

The focus of the collection is on isolates from areas of environmental concern or that are useful in environmental research e.g.

- Algae resistant to or tolerant of heavy metals (*Euglena mutabilis* Schmitz, *Euglena gracilis* Klebs, *Scenedesmus acutus* f. *alternans* Hortobagyi and *Stichococcus* spp.)

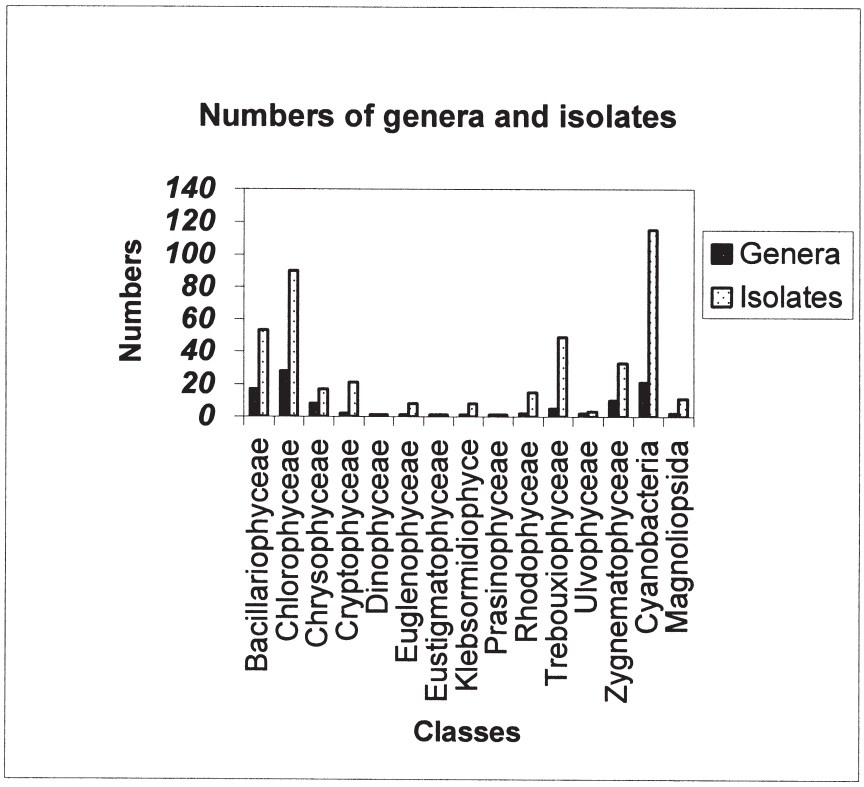

Fig. 1. Numbers of genera and isolates in the various classes of algae, cyanobacteria and aquatic vascular macrophytes in the UTCC.

- Algae and cyanobacteria from extreme environments, e.g. high salt, low pH, low temperature (*Microthamnion* sp., *Dunaliella acidophila* (Kalina) Massjuk and *Planktothrix rubescens* (De Candolle ex Gomont) Anagnostidis & Komárek)
- Filamentous green algae and diatoms from acid-stressed lakes (*Mougeotia, Tabellaria*)
- Algae and cyanobacteria causing odour and taste in freshwaters (*Uroglena, Dinobryon*)
- Toxic cyanobacteria *Microcystis aeruginosa* (Kützing) Lemmermann
- Algae and aquatic vascular macrophytes for ecotoxicity and biocide testing (*Pseudokirchneriella subcapitata* (Korshikov) Hindák (also known as *Selenastrum capricornutum* Printz), *Chlorella vulgaris* Beijerinck, *Ankistrodesmus convolutus* Corda, *Lemna gibba* Linnaeus, *Lemna minor* Linnaeus, *Myriophyllum sibiricum* Komarov and *Myriophyllum spicatum* Linnaeus)

Approximately 30% of the isolates are axenic and we are in the process of purifying more isolates by mechanical manipulations e.g. re-isolation by washing single cells and filaments, streak plating and spray plating (Wiedeman et al. 1964). Methods using phototaxis are being used for flagellated species (Paasche 1971) and motile filamentous cyanobacteria (de Chazal et al. 1992).

All cultures are maintained in defined media by regular serial transfer and are inspected microscopically at each transfer. As the collection is diverse, we need to use many types and variations of media including BG-11, Bold's Basal Medium, CHU-10, ESAW and Modified Acid Medium (MAM). The formulations and references for those commonly used can be found on the UTCC website at http://www.botany.utoronto.ca/utcc/Culture%20Media.html. Occasionally, small amounts of soil extract, representing not more than 5-10% of the medium, may be added in certain cases to serve as a "rescue medium" for some isolates. Where possible, cultures are maintained on agar slants. Liquid media or bi-phasic media (agar slants with an overlay of liquid) are used for those that do not grow well on agar. Sterility testing is conducted regularly on axenic cultures by transfer to medium with organic components such as glucose, yeast extract, soytone and peptone, either in liquid or on agar plates followed by examination using phase or fluorescence microscopy.

Cryopreservation using liquid nitrogen has become an important technique for long-term maintenance of algal cultures to prevent genetic drift and to reduce the risk of loss or contamination (Taylor & Fletcher 1999). We have recently begun to use the 2-step cryopreservation technique i.e. slow cooling at -1 degree C /min. in a -86°C freezer, followed by plunging into the vapour phase of liquid nitrogen. This technique has been used very successfully for cyanobacteria and unicellular green algae.

Physical facilities of the collection

The collection is housed in a large laboratory for the exclusive use of UTCC in the Department of Botany at the University of Toronto. Duplicate sets of cultures are maintained in two environmental growth chambers operated at about 20°C with Day:Night cycles of 12:12 using fluorescent lighting. Cultures requiring lower temperatures are maintained at 10°C in similar conditions. Each growth chamber serves as a backup to the other and this system is critical to the successful operation of the collection. In the cases of new isolates, new deposits and fastidious cultures, a third backup set is maintained in the lab at room temperature under fluorescent lights. We have recently acquired a -86°C freezer and a Custom Biogenic Systems (CBS) 4000 cryopreservation storage unit for the cryopreservation of our strains.

Functions and roles of the collection

The primary functions of the collection are to collect, maintain and preserve isolates of algae and cyanobacteria primarily from Canadian sites, to serve as a depository and to provide these isolates and related information to users in academic, government and commercial laboratories worldwide. Funds awarded to the collection by NSERC cover the cost of basic maintenance but user fees must cover any other expenses.

Special services of the UTCC include custom-isolation of algae and cyanobacteria, custom-training on-site or off-site in methods of culture and isolation of these organisms, preparation of specialized media and Safe-Deposit on a confidential basis. Currently, we are not accepting deposits for patent purposes. When time permits, the curator may also provide services in purification and identification of algae and cyanobacteria. Fees apply for all of the above services and information can be obtained from the curator or on the website. The facility also serves as a source of information on culturing algae, cyanobacteria and aquatic vascular plants and is part of a global network assisting scientists in locating appropriate cultures and related information.

The UTCC acts as a depository in accepting valuable cultures from other researchers, particularly when the cultures may be endangered due to insufficient technical help, retirement or change in direction of research. In accepting these deposits we seek to expand the diversity of the collection and have recently added small collections of cyanobacteria isolated from sites in the Canadian Arctic and from effluent ponds associated with pulp and paper mills. We have recently accepted a valuable collection of metal-tolerant green algae formerly maintained by Dr. Brian Whitton in the UK.

The facility plays an important role in the toxicity testing program by providing axenic cultures of several test species of algae and aquatic vascular plants e.g. *Pseudokirchneriella subcapitata, Lemna gibba, Lemna minor* and *Myriophyllum sibiricum*. Cultures and technical information are provided according to the specific requirements of the users. The curator also contributes information and advice on updates to the biological test methods protocols for *Selenastrum capricornutum* and *Lemna minor* published by Environment Canada.

Policies of UTCC

The UTCC accepts isolates for accession into the general collection under certain conditions. It must have no contaminants other than bacteria commonly associated with the species when collected and these should be in low numbers. The geographic site of collection, the date of isolation and if possible, the isolator and identifier for each isolate should be provided prior to deposit. Generally, no restrictions should be placed by the depositor on the distribution of the cultures. The depositor is entitled to receive one culture per year of the deposited isolate, free of charge. We are especially interested in adding additional species isolated from Canada. Priority is given to those that are referred to in publications, represent a species not currently in the collection or that have interesting physiological properties.

When cultures are provided to users, we request that the UTCC strain number be quoted in all publications, that a reprint or notice of publication be sent to us and that the cultures not be redistributed to other users or culture collections without prior agreement with the curator of UTCC. We also request information on the type of research that will be conducted with the strains. Special restrictions are placed on toxin-producing strains. A toxic strain release form must be completed, signed and returned to the curator before the cultures are shipped.

In keeping with our policy of being environmentally responsible, we aim to prevent any possibility of releasing live organisms into our water discharge system and ultimately into Lake Ontario. Cultures to be discarded are heat-killed before disposal by autoclaving or microwaving the old cultures.

Usage of the collection

The UTCC provides approximately 300 cultures annually to users worldwide. To date, the collection has provided cultures and services to more than 500 users in 21 countries. Generally, about 75% of our users are Canadian and 25% are international. In the last 3 fiscal years about 50% of the requests were from academic users, 20% were from users in government institutes and 30% were from commercial users. Most of the academic usage was for research; less than 5% of the cultures were provided for teaching.

Research in the UTCC facility is usually limited to media development, methods of isolation, purification and long-term maintenance. Research programs of our users represent a broad range of interests in the areas of ecology, physiology, taxonomy, cell metabolism, biotechnology and contaminant toxicity testing. Investigations of particular interest include: toxic effects of cyanobacteria, PCR-based methods of detection of microcystin, characterization of microcystin, inorganic carbon transport, effects of trace metal availability on phytoplankton growth, physiology and molecular basis of metal toxicity and tolerance in algae, the impact of UV-B on algae, stress responses of algae and taste and odour problems in drinking water.

Future directions

The provision of pure, axenic cultures with full background information is very important to research in molecular biology, physiology and biotechnology and we aim to increase the numbers of axenic isolates in the UTCC. We have begun using cryopreservation in liquid nitrogen for our cultures and will gradually increase the number of cryopreserved isolates as time permits, to eventually reduce the need for labour-intensive serial transfer. The taxonomic identification of most of our strains has been based on morphology; we would like to collaborate with other culture collections and experts in taxonomy to authenticate the species and to identify more of our strains to species level using molecular techniques where appropriate.

Conclusion

The UTCC has become an important Canadian phycological resource centre not only for cultures of freshwater algae and cyanobacteria but also for related services and information. The facility has increased the accessibility to research quality documented cultures from Canadian sources and is performing a valuable role in preserving the biodiversity of our algal and cyanobacterial resources through isolations and accepting deposits from other scientists.

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