

Microbial Culture Collection at the National Institute for Environmental Studies, Tsukuba, Japan

by Masanobu Kawachi and Mary-Hélène Noël



MCC-NIES team (from left to right): Ishimoto (*Charales* curator), Yumoto (curator, DNA), Mori (curator, cryopreservation), Kawachi (head), Matsui (assistant curator), Noël (curator, projects), Niitsuma (assistant curator, DNA), Sato (curator, flow cytometry), Shimura (Post-Doc, cyanobacteria), Fujii (secretary).

History and characteristics of the MCC-NIES collection

The Microbial Culture Collection at the National Institute for Environmental Studies (MCC-NIES), located in Tsukuba, Japan, was founded as an “environmental study-oriented” culture collection in 1983 when eutrophication of lakes and rivers, and air and water pollution were severe in Japan. The MCC-NIES started with *ca.* 250 strains mainly of red-tide-forming algae (*Chattonella antiqua* and *Heterosigma akashiwo*) and water-bloom-forming cyanobacteria (*Microcystis aeruginosa*). Although the MCC-NIES is still characterized by such types of strains, the collection now holds almost all eukaryotic lineages and a diversity of cyanobacteria, too. At present the collection includes 18 phyla, 51 classes, 354 genera, 718 species and 2,356 strains.

Most of the MCC-NIES strains have been directly deposited by researchers, but some have been deposited from exchanges with other collections. Around 80% of the MCC-NIES strains were originally sourced from Japan, giving the collection a high level of specificity.

This is also the only culture collection holding major endangered macroalgae. The collection, which started *ex situ* conservation of endangered algae in Japan, has been in operation since the mid-1990s. In the list of endangered Japanese wildlife (the red list) compiled by the Ministry of Environment of Japan in 2007, 116 taxa (species and varieties) of algae are listed as extinct, extinct in the wild, or as endangered in Japan. At present, the MCC-NIES holds *ca.* 300 strains of these endangered algae, including *Charales* and freshwater red algae. The collection is

partially supported by the Time Capsule Project conducted by the Ministry of Environment of Japan since 2002.

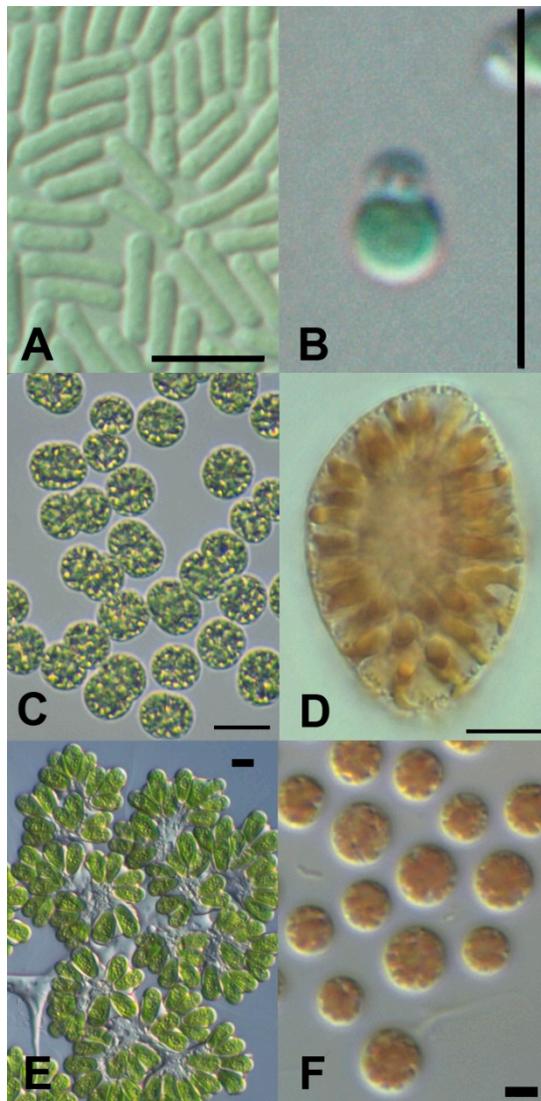
In 2002, the MCC-NIES was selected as the core repository for algae in the National BioResource Project (NBRP) conducted by the Ministry of Education, Culture, Sports, Science and Technology of Japan (NBRP: <http://www.nbrp.jp>). In this framework, more than 200 strains of *Microcystis* and *Anabaena*, collected from representative eutrophic lakes all over Japan, were deposited by the National Science Museum along with phylogenetically diverse strains of microalgae and protozoa deposited by the University of Tsukuba. In addition, more than 300 strains of cyanobacteria and eukaryotic microalgae maintained at the IAM Collection (University of Tokyo) were transferred to the MCC-NIES up until the end of FY 2006, when the IAM Collection was closed.



Charales strains maintained in MCC-NIES.

The collection includes:

- Evolutionarily important species such as *Mesostigma viride* (NIES-296) and charophytes (NIES-1601);
- Experimental materials that have been well-studied in genomic, genetic, molecular, and physiological terms, such as *Cyanidioschyzon merolae* (NIES-1332/10D), *Chlamydomonas reinhardtii* (NIES-2235/C-9), and *Thermosynechococcus elongatus* (NIES-2133/BP-1);
- Ecologically significant species such as *Prochlorococcus marinus* (NIES-2086) and *Micromonas pusilla* (NIES-1411);
- Harmful algal species such as *Microcystis aeruginosa* (NIES-44) and *Chattonella marina* (NIES-3);
- Commercially useful strains such as *Botryococcus braunii* (NIES-836), *Porphyridium* (NIES-1035) and *Chlorella vulgaris* (NIES-227).



A. *Thermosynechococcus* (NIES-2133), B. *Cyanidioschyzon* (NIES-1332), C. *Microcystis* (NIES-44), D. *Chattonella* (NIES-3), E. *Botryococcus* (NIES-836), F. *Porphyridium* (NIES-1035). Scale bar = 10 μm .

Since the start of the collection, the Committee for Evaluating Microbial Culture Strains has evaluated the NIES strains upon deposition based on a set of criteria. At present, the Committee includes nine researchers at NIES and six supervisors outside NIES. In addition, since 2002 the MCC-NIES has been supervised by the Steering Committee of the NBRP Algae.

Maintenance of strains

About 3/4 of the NIES strains (~ 2,000 strains) are maintained by subculturing under optimal and/or sub-optimal conditions, mostly ranging from 5 to 25°C (37 or 45°C for thermophilic strains) and with a 4 to 50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ photon flux density in a 12-h-light:12-h-dark light regime.



Test tube of *Anabaena*.

The strains are serially transferred at 10-day to 6-month intervals. Specially designed software for the collection allows a flexible gestion of the daily transfers. Maintenance conditions differ with each algal strain and are individually indicated in the catalogue of strains on our web site.

To prevent the loss of strains during maintenance by subculturing, we conduct weekly growth checks. Once a year, we also check axenic strains for the absence of bacteria by using several bacterial check media.

The remaining strains—about 600 including most of the cyanobacterial strains and some of the green and red algal strains—are cryopreserved only, in the vapor phase of liquid nitrogen.



Upper: Subculture of strains in liquid or agar media. Lower: Cryo-preserved strains in liquid nitrogen tanks.

Scientific names and phylogeny

The scientific names of the NIES strains are given primarily by the depositors. However, we have used DNA sequencing (mostly of the 18S rRNA gene) to re-evaluate the strains for which DNA sequence data have not yet been reported. As a result, we have changed the scientific names of the misidentified strains, although we have left their former names as “Formerly identified as.” We have also added “Re-identified at NIES by DNA sequencing” in “Identified by” and, if the original scientific names of these strains were appropriate, we have simply indicated “Confirmed at NIES by DNA sequencing”, with gene names and accession numbers in “Gene data.” We are still re-evaluating the remaining strains.

Dr. Masanobu Kawachi (kawach9i@nies.go.jp) is a microalgae taxonomist working at the National Institute for Environmental Studies based at Tsukuba, Japan. Since 2012, he has been the head of the Microbial Culture Collection. As a Ph.D. student, Masanobu revealed the food capturing role of the haptonema for the haptophyte Chrysochromulina. He spent 6 months at an Antarctica research base, and travelled worldwide to collect microalgae samples from various environments. With co-workers he is involved in the discovery of a new class, the Pinguiphyceae, having specific fatty acid content. Taxonomic revision of Chattonella and toxic cyanobacteria are among the fields of research he is working on. He is currently participating in several research projects: on coral symbionts with global warming concerns, picoplankton diversity with the environmental DNA analysis, and biofuel production from microalgae.

Dr. Mary-Hélène Noël (noel.mary-helene@nies.go.jp) came to the National Institute for Environmental Studies in 1996 as a Post-Doc right after her Ph.D. graduation from the University of Paris XII, CNRS 386 / E.N.S. With a background in marine biogeochemistry, she spent the first 4 years at the Water and Soil Division of NIES, then started to work on the coccolithophorid life cycle at the Biological Division and MCC-NIES. Her participation to the culture collection consists of recovering the strains with growth troubles, adapting strains, and providing advice to customers internationally. She is also involved in research projects on marine picoplankton diversity and life cycle studies.

MCC-NIES services

Most of the strains are available for education, research and development in accordance with the “Agreement for distribution”. The MCC-NIES also provides genomic DNA for worldwide distribution.



Packaging for worldwide distribution of culture strains.

Technical advice and follow-up of the distributed strains are provided to customers. Usual time for delivery of an ordered strain is 10 days as most of the strains are maintained under active growth. For strains kept as cryopreserved samples, the delay is longer since the MCC-NIES first return the culture to active growth before shipping it.

The MCC-NIES accepts the deposit of strains that are environmentally important, as well as those for basic and applied studies. The collection also accepts the deposition of strain types of cyanobacteria and specimen types of eukaryotic microalgae as frozen samples.

Useful information relating to the DNA barcodes, photosynthetic pigments, morphology and other relevant literature are provided for each strain at our web site. The MCC-NIES collection welcomes joint research and collaborations; please contact us at mcc@nies.go.jp. Japanese, English and French are the available languages for any communication with the collection.

A culture collection is “team work”, each step being important for the final state of the cultures (from washing, media preparation to sub-culturing, etc.). We take care of the culture strains as much as possible and hope to share our passion for microalgae.