BioResource Now !

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Introduction to Resource Center 〈NO. 43〉

Collection, Preservation, and Future Tasks of Various Algal Resources

Masanobu Kawachi, Head
Biodiversity Resource Conservation Section, Center for Environmental Biology and Ecosystem Studies, National Institute for Environmental Studies

Various Algae

Algae are living organisms that are characterized by "oxygenic photosynthesis." All living organisms that perform "oxygenic photosynthesis," except terrestrial plants such as mosses, ferns, and seed plants, belong to the category of algae. Even terrestrial plants that differentiated from algae have evolved from algae belonging to the class Charophyceae (National BioResource Project [NBRP]-Algae conserves 81 species and 256 strains). Algae do live in normal waterscape environments, e.g., lakes, mashes, coasts, and oceans, as well as in extreme environments, e.g., hot springs, snow, ice, high-salinity waters, and arid zones. Many algae are known to coexist with other living organisms such as corals and lichens. Various living organisms, including prokaryotes, eukaryotes, unicellular organisms, and multicellular marine plants, that possess complicated systems and life histories, belong to the class algae. The NBRP- Algae collects, conserves, and distributes various algae resources.

Structure of NBRP-Algae

The National Institute for Environmental Studies (NIES), a central resource center, collects, conserves, and distributes resources of micro algae, endangered algae, and protists that are phylogenetically close to algae. Kobe University, an allocation resource center, is in charge of collecting, conserving, and distributing macro algae. The University of Tsukuba, another allocation resource center, is newly in charge of collecting algae resources with high utility values and preparing additional information such as classification information. Hokkaido University, a third allocation resource center, is in charge of backing up important strains.

What We Learned from Disastrous Earthquakes

The Great East Japan Earthquake that occurred on March 11, 2011, greatly affected algae conservation facilities of the NIES. Infrastructures such as power, water, and gas supply systems could not be used for a long time, and operations, including subcultures, were difficult to perform. Lighting and temperature that are required for algal cultures could not be controlled. While being anxious about the restoration of infrastructures, we must adopt urgent measures, e.g., moving stock cultures to sunny places during daytime. The temperature in a liquid nitrogen refrigerator, in which frozen cultures were kept, could be maintained within a safety zone even during a long-term power outage, and frozen cultures were not affected by the earthquake. A lesson learnt from the Great Hanshin-Awaji Earthquake is that frozen cultures have been mutually stored in Kobe University and the NIES since 2008. This mutual storage system was a great relief to us. These 2 earthquake disasters taught us that risk diversification is important to prevent the loss of precious resources.

Characteristics of Algae from the Viewpoint of Phylogenetic Diversity

Recent molecular phylogenetic studies revealed that algae are composed of groups, which greatly differ from each other in their phylogenetic profiles. At present, eukaryotes are composed of at least 9 groups (Fig. 1).

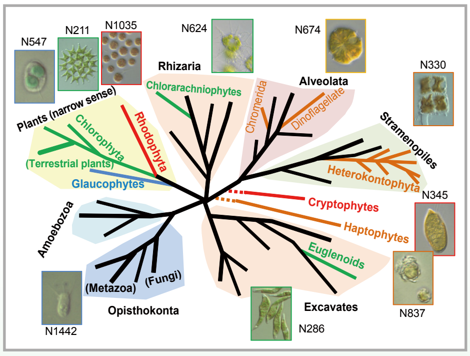

Fig.1: Phyletic relationship among eukaryotes; modifications based on Baldauf (2003). Colored branches indicate algae and terrestrial plants. The numbers attached to the photographs are NIES strain numbers.

Many eukaryotes are microorganisms. The presence of algae has been confirmed in all of these groups, except Amoebozoa and Opisthokonta. In this typical dendrogram, colored branches of algae are interspersed. The reason for this interspersion is considered to be that chloroplasts have been independently acquired in each group. Algae that are able to perform photosynthesis greatly differ from each other in their evolution and phylogenetic profiles.

The differences among algae appear as variations in their cytoarchitecture and physiological, biochemical, and biological characteristics. The diversity of algae, which extends over different classes of eukaryotes, is the reason why algae are attractive as research resources.

Resources for Elucidating the Mechanism underlying Evolution and Formation of Chloroplasts

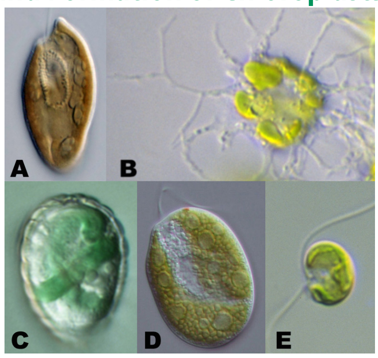

Fig. 2: Cryptophytes (A, provided by Ms. Mayumi Sato), Chlorarachniophytes (B, NIES-624), *Paulinella chromatophora* (C), *Hatena arenicola* (D, provided by Ms. Haruyo Yamaguchi), and *Nephroselmis* (E, NIES-483).

In algae, the presence of chloroplasts that are peculiar to each group has been confirmed. Cryptophytes (NBRP-Algae conserves 21 species and 46 strains) (Fig. 2 A) greatly differ from Chlorarachniophytes (NBRP-Algae conserves 5 species and 6 strains) (Fig. 2B) in their phylogenetic profiles. Remnants of eukaryotic nuclei (nucleomorphs) have been found in chloroplasts of Cryptophytes and Chlorarachniophytes. It is known that chloroplasts in Cryptophytes and Chlorarachniophytes are derived from those in Rhodophyta and Chlorophyta, respectively. It is also known that in a nucleomorph in Cryptophytes, whose genome size is 0.55 Mb, approximately 600 genes code for proteins. The existence of nucleomorphs directly indicates the origin of chloroplasts and the evolution process of organelles.

The alga *Paulinella chromatophora* (NIES-2635) (Fig. 2C) has a chloroplast (it is called cyanelle for differentiation from other chloroplasts). Its origin differs from that of chloroplasts in other algae. It is interesting that the photosynthesis-related genes

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remained in the cyanelle, while they have already shifted to the nuclear genome in other chloroplasts. Therefore, the cyanelle is considered to represent a stage during which the chloroplast shifted to become an organelle.

In various other algae and protists, interesting phenomena such as intracellular symbiosis and kleptoplasty have been observed. For example, *Hatena arenicola* (Fig. 2D), a protist, seems to be a flagellate alga possessing green chloroplasts. However, this protist "incorporates" a free-living prasinophyte alga into its cell, changes the size and shape of the alga, and uses the alga as if it was a chloroplast. This prasinophyte alga was found to be a species that belongs to the genus *Nephroselmis* (NBRP-Algae conserves 5 species and 15 strains) (Fig. 2E). How can *Hatena arenicola* change and control *Nephroselmis* in its cell? Has a part of the genome of *Nephroselmis* already been shifted to that of *Hatena arenicola*? *Hatena arenicola* is an interesting object to elucidate the initial process of chloroplast formation.

Some algae and achromatic protists that are phylogenetically close to algae acquire chloroplasts again after having secondarily lost them. Thus, algae are materials suitable to study the evolution and formation process of an organelle, a chloroplast.

Resources for Study and Practical Use of Photosynthesis

Among algal resources, there are species that have excellent proliferation potency; they proliferate very densely and are easy to handle in experiments.

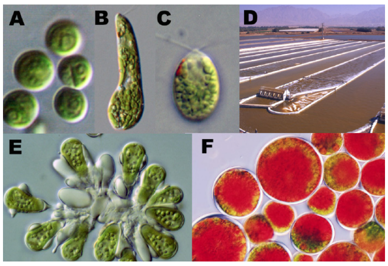


Fig. 3: *Chlorella vulgaris* (A), *Euglena gracilis* (B), *Dunaliella tertiolecta* (C), a farm of *Dunaliella* in Israel (D), *Botryococcus braunii* (E), and *Haematococcus lacustris* (F)

Chlorella vulgaris (NIES-2170) (Fig. 3A) and *Euglena gracilis* (NIES-48) (Fig. 3B) are typical algae that have been used in various research fields, including photosynthesis studies, physiological and biochemical studies, and research and development studies aiming at their practical use. Recently, *Euglena gracilis* has been frequently used in research and development projects aiming at its practical use for biofuels and foods. *Dunaliella tertiolecta* (NIES-2258) (Fig. 3C) is a species of Chlorophyta and has adapted to high-salinity environments. By maintaining a high-salinity environment, mass cultivation of this species can be achieved in open ponds, similar to pure cultures. Although the proliferation potency is not very high, *Botryococcus braunii* (NIES-836) (Fig. 3E) that accumulates oil (which is similar to fuel oil) in its algal biomass and *Haematococcus lacustris* (NIES-144) (Fig. 3F) that produces astaxanthin (which is a substance useful for human health) have often been used in the field of practical research in recent years.

Future Task of NBRP-Algae, No. 1 Serious Dilemma Due to Diversity

Although the diversity of algae is an advantage of algae as resources, it sometimes becomes the cause of worry when conserving resources. In the NIES, of the 2,339 available strains, 786 strains have been cryopreserved and 1,553 strains have been sub-cultured (approximately 2/3 of the available strains). For these sub-cultured strains, 76 types of media and 7 types of containers are used and various temperatures and light conditions are applied. The subculture interval varies according to the strain, and weekly growth inspections and regular sterility tests are indispensable. We have been sub-culturing various strains using the knowledge that has been gained by our experiences over a long time. However, some strains die out almost every year.

Some strains, which have been sub-cultured, cannot be cryopreserved using current technologies and some can be cryopreserved, even though their survival rates are low. In the future, we will make every effort to examine conditions to improve the survival rates of these strains and to establish an inspection method, by which these strains can be stably cryopreserved, even though their survival rates are low. By exchanging information and technologies with researchers who belong to various conservation facilities in the NBRP, we attempt to develop a new cryopreservation technology.

Future Task of NBRP-Algae, No. 2 Enhancement of Model Organisms

In algae, many interesting phenomena have been observed, e.g., mineralization※1, sex pheromone production, toxin production, and various cell movements. Model organisms used to investigate these biological phenomena and mechanisms must be further enhanced. The NBRP-Algae has also been developing its own model organism using cyanobacteria, which live in many different environments and possess various interesting light-related physiological functions, e.g., photosynthesis, circadian rhythm, and phototaxis. Using 95 species and 706 strains of cyanobacteria conserved by the NBRP, we have been selecting strains useful for molecular-biological research by investigating their genetic modification abilities.

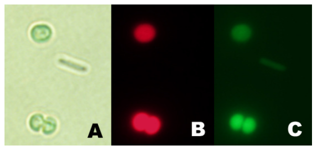


Fig. 4: Cyanobacteria, into which a plasmid containing the *green fluorescent protein (GFP)* gene has been introduced using conjugation with *Escherichia coli:* (A) an optical microscope image, (B) a chlorophyll fluorescence image, and (C) a GFP image. The cell without chlorophyll fluorescence in the center is *Escherichia coli*. These images were provided by Mr. Yohei Shimura.

※1 Mineralization : Extracellular structures composed of minerals have been found in algae, e.g., calcium carbonate in coccolithophorids (Fig. 5A, NIES-2696), siliceous substances in diatoms (Fig. 5B, NIES-2363), and iron oxide + manganese oxide in euglenoids (Fig. 5C, obtained from a natural pond).

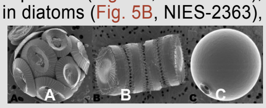
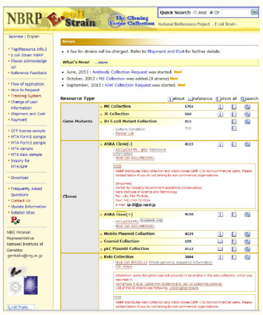


Fig. 5: Mineralization

Database of This Month

Resources Database of *Escherichia coli* "NBRP E.coli Strain"



Number of strains: 22,725
Number of genes: 6,926
Number of papers: 276
(As of February 2013)

DB name : NBRP E.coli Strain
URL: http://www.shigen.nig.ac.jp/ecoli/strain/
Language: Japanese, English
Original contents:
Mutants, clones
Exhaustive mutants (gene-disrupted strains, large deletion mutants, transposon insertion mutants)
Phages, cloning vectors
Features: Various genome-wide mutants are being prepared and can be ordered through the Internet. The entire process from the application procedure to the distribution can be tracked through the Internet.
Because of the cooperation with Profiling of E. coli Chromosome (PEC), a genome database, related strains can be accessed from physical maps.
The description of each collection is substantial.
DB construction group: NBRP E. coli Strain, NBRP Information
Management organization: Genetic Resource Center, NIG
Year of DB publication: 1999 Year of last DB update: 2013

Comment from a practicing developer: Fourteen years have passed since the first publication, and this database is in a stable state now. I, as a developer of this database, am pleased to know that the number of users has been increasing as the number of types of resources has been steadily increasing. We have also been developing the Profiling of E. coli Chromosome (PEC), the development of which was initiated earlier than that of NBRP E. coli Strain. Therefore, by functionally connecting these 2 databases, users can make the most use of their contents.
This resource database seems to be operated by a single server. However, in order to allow users to follow the entire process from the application procedure to the distribution via the Internet, multiple servers are connected with each other in this database. We receive some positive feedback from our users through the many inquiries sent to us via the Contact us link. We will continuously improve this database, so please feel free to contact us.

Contact Address

Genetic Resource Center, National Institute of Genetics
1111 Yata, Mishima-shi, Shizuoka 411-8540, Japan
Tel.: 055-981-6885 (Yamazaki)
E-mail : brnews@shigen.info

Editor's Note

This month, Mr. Kawachi, who has been the representative of the organization of algae resources since the third stage of the NBRP, kindly wrote about the characteristics and attractiveness of algae as living organisms. The diversity is said to be an advantage of algae as resources. However, I understand that to deal with various conservation conditions is a difficult task for the organization that aims to conserve algal resources. A gallery of beautiful algal images can be accessed at www.shigen.nig.ac.jp/algae/ (Y. Y.) .

BioResource Information

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