PROCEEDINGS OF THE SYMPOSIUM ON CULTURE COLLECTION OF ALGAE

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Cover: Green alga, *Characiochloris sasae* Nozaki taken by Dr. H. Nozaki

Editor's Preface

Over the last 10 years, microalgae have become a major focus of attention in environmental science and also other fields of science for two major reasons. Firstly, microalgae are not only associated with water pollution and purification, but also with global environmental protection; they can utilize the sun's energy to fix carbon dioxcide and are consequently responsible for half of the "bio-production"on earth. Secondly, many microalgae have specific metabolic capabilities including nitrogen fixation, hydrogen evolution and oil accumulation, as well as those with high protein production capability; consequently, they have attracted the attention of experts from the fields of agriculture, energy development and food manufacture. However, basic research into the collection, isolation,characterization and preservation of microalgae had been neglected relative to that of bacteria and fungi, although this research contributes to the systematic accumulation of data, and hence, the advancement of the phycology-related fields mentioned above.

In April, 1989, we received a proposal suggesting collaboration between algal culture collections from The Culture Collection of Algae and Protozoa (CCAP), Institute of Freshwater Ecology, UK. In July of the same year, this proposal was placed on the list of possible areas of future contact and co-operation for further examination at the Anglo-Japanese Science and Technology Co-operation talks. In 1991, we were awarded "The Bilateral International Joint Research Special Coordination Fund for Promoting Science and Technology" from the Science and Technology Agency, Japan, for initiating collaboration between Japanese algal culture collections and CCAP.

As a first step in the collaboration, we held a symposium on "Culture Collection of Algae: Its Roles in Basic and Applied Phycology" at National Institute for Environmental Studies in Tsukuba, Japan on February 15, 1991. Its principal objectives were to exchange the information and opinions on many areas of mutual interest including taxonomy, the developement of long-term preservation techniques, construction of database of culture strains, and construction of international joint ownership of important culture strains of algae. On the last point, there were two problems which we need to overcome; 1) we have different policys on distribution of the strains, and 2) the selling price of strains are quite different between the culture collections. These problems will undoubtedly be resolved by future cooperation.

This volume contains seven papers on taxonomy, culture collections, the data activities of the World Federation for Culture Collection of Microorganisms (WFCC), and the abstract of a special lecture on sexual bipolarity of microalgae presented by Dr. Ichimura, IAM, University of Tokyo. The symposium organizer would like to thank the chairmen of the symposium, Prof. M. Chihara, The Japanese Red Cross College of Nursing, and Prof. K. Sugahara, Kinki University and the members of Environmen-

tal Microbiology Laboratory and Microbial Culture Collection, NIES who assisted with the symposium. Furthermore without the supports of STA, Global Environmental Forum (GEF) and The Japan Federation for Culture Collection of Microorganisms (JFCC), the symposium could not have been held. I express my thanks for their support.

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CONTENTS

The Algal Classes

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Abstract

Classification of the algae into classes is discussed. A system comprising 11 divisions with two prokaryotic and 20 eukaryotic classes is suggested. Current knowledge suggests that the eukaryotic algae are not a natural taxonomic group but may be derived from as many as 7 or 8 different groups of heterotrophic organisms (animals).

Key words: algal classes, classification, phylogeny

Introduction

The algae comprise a large and very diverse assemblage of primitive plants. Most researchers now agree that the algae, the fungi, the animals, and the protozoa do not represent natural taxonomic groups, but rather groups of organisms with similar modes of nutrition (heterotrophy, autotrophy, etc.). The term alga is generally used for both pro- and eukaryotic autotrophs, the former comprising only two divisions, Cyanophyta (blue-green algae) and Prochlorophyta (no vernacular name), while the eukaryotic algae may be classified into a smaller or larger number of divisions and classes, according to the different ideas and backgrounds of the different authors. In the present contribution I will present a classification of the algae into 11 divisions and 22 classes. All the classes of eukaryotic algae had, I now believe, heterotrophic (phagotrophic) ancestors, but few of these have been identified, and the idea of endosymbiotic origins of chloroplasts and mitochondria has on the whole had very little impact on algal classification. The present paper is not an exhaustive description of the algal classes, but merely a brief contribution indicating the "state-of-the-art".

The Prokaryotic Algae, Divisions Cyanophyta And Prochlorophyta

Chlorophyll a-containing prokaryotes may be classified into the two classes mentioned in the introduction, blue-green algae and prochlorophytes. The blue-green algae utilize chlorophyll a and phycobilins in photosynthesis, while the 3 known genera of prochlorophytes (*Prochloron, Prochlorothrix* and *Prochlorococcus*) utilize divinyl chlorophyll a and divinyl chlorophyll b (Jeffrey 1991, and pers. comm.).

The prochlorophytes are notably diverse in ecology - *Prochloron* is a marine symbiont or epiphyte, *Prochlorothrix* a filamentous freshwater alga very similar to certain blue-green algae, and *Prochlorococcum* a marine picoplankton organism. Molecular data show the three genera to be rather distantly related (Palenik and Haselkorn 1991), indicating that the prochlorophytes, like the blue-green algae, is an old group. It may turn out to be polyphyletic.

The Eukaryotic Algae

In most classifications of algae, the red algae are considered to be one of the most primitive groups, due to the complete lack of flagella and the similarity between red algal chloroplasts and blue-green algae. There is some indication, however, that the lack of flagella, in red algae and in certain fungi, is a derived feature, and rRNA data now indicate that the most primitive extant eukaryotes are flagellated protozoa, classified by Cavalier-Smith (1991) into the Archezoa. All other algal classes include flagellates or flagellated reproductive stages. The groups will be discussed separately below.

Division Rhodophyta (Red Algae), With A Single Class *Rhodophyceae*

The red algae form a well-defined group and it is nearly universally accepted that they constitute a single class, Rhodophyceae. Rhodophyceae is a descriptive name based on rhodos, red. Equally valid is the typified but little used class name Bangiophyceae, based on the genus *Bangia*. The chloroplasts of red algae are characterized by single thylakoids, with phycobiliproteins attached to the outer surfaces of the thylakoids. The chloroplast is surrounded by only two membranes. The chloroplasts are morphologically so similar to protoplasts of blue-green algae that a symbiotic origin of the chloroplasts appears likely. This indicates that the presently unknown ancestor of the red algae was a heterotrophic eukaryote, which took up a blue-green algal cell by phagocytosis (as food) and stored the cell in a food vacuole. The inner chloroplast membrane of red algae thus represents the plasmamembrane of the blue-

green algal endosymbiont, whilst the outer membrane is the vacuole produced by the host. To be able to engulf the blue-green alga the progenitor of the red algae was probably wall-less , i. e. it was a naked, heterotrophic, phagotrophic unicell. It may have possessed flagella, since present evidence from RNA nucleotide sequencing (using 16S-like rRNA) indicates that red algae are relatively advanced, and evolved from flagellate ancestors long after the appearance of the first flagellated eukaryote (Bhattacharya et al. 1990). All signs of flagella subsequently disappeared. Contrary to this, Hori and Osawa (1986, 1987) suggested, based on 5S rRNA nucleotide sequences, that red algae represent the first divergence from the eukaryotic line of descent, i. e. they evolved before the evolution of eukaryotic flagella. A search for present-day heterotrophic organisms related to the ancestors of red algae should include all known heterotrophic and phagotrophic unicells (flagellates or amoebae) with flat mitochondrial cristae.

Division Cryptophyta, With The Single Class Cryptophyceae

The finding of a separate DNA-containing compartment, the nucleomorph, within the outer membranes of the cryptomonad algal chloroplast has been of major importance in explaining the origin of algal chloroplasts in general. It is now widely accepted that the chloroplast of cryptomonads and the nucleomorph represent an entire eukaryotic cell, taken up by the host but subsequently used for photosynthesis. The host was therefore probably a phagotrophic apoplastidic flagellate. Its phylogenetic affinity remains uncertain. The flagellation of cryptomonads is unique in the presence of two rows of bipartite hairs on the long flagellum, and a single row on the other (some variation was noted by Kugrens et al. 1987). The origin of the hairs is similar to the origin of the heterokont tripartite hairs, indicating that these may be homologous structures. Cryptomonads are otherwise very unlike heterokont protists, differing in nearly all details of the flagellar apparatus internal structure (transition region, flagellar root structure) and in having flat rather than tubular mitochondrial cristae. The ancestor of the cryptomonads was probably a heterotrophic biflagellate with flat mitochondrial cristae. The function of the gullet-furrow-vestibulum system of cryptomonads is presently obscure, but following the line of thoughts presented above this system may initially have been involved in food uptake. The plate systems covering the cells of cryptomonads are apparently absent in the gullet and thus not an obstacle to phagotrophic food uptake. It may therefore have been present also in the ancestral cryptomonad. The colourless cryptomonad *Crytomonas paramecium* contains leucoplasts, probably representing reduced chloroplasts.

Division Dinophyta, With The Single Class Dinophyceae

Approximately half the known dinoflagellates are without chloroplasts and lack all remains of such organelles, indicating that the oldest dinoflagellates may have been heterotrophic flagellates. They were probably more or less wall (theca) less, since this would facilitate food uptake by phagocytosis. The presence of a theca cannot be excluded, however, considering that some thecate dinoflagellates are able to ingest other cells. The very great diversity of dinoflagellate chloroplasts is explained by uptake of a large variety of other eukaryotes: green algae (*Lepidodinium*, Watanabe et al. 1990), haptophytes (*Gyrodinium*, Tangen and Björnland 1981), cryptomonads (Wilcox and Wedemayer 1985), etc. The phylogenetic relationships of dinoflagellates remain somewhat uncertain. They differ in practically all features of the flagellar apparatus from other eukaryotes examined, but many more heterotrophic flagellates with two flagella and tubular mitochondrial cristae need to be studied and assessed as possible ancestors of dinoflagellates. In rRNA studies dinoflagellates group with ciliates and the Apicomplexa group of parasitic protozoa (Sogin et al. 1991; Wolthers 1991), suggesting a common, but ancient ancestor.

Dinoflagellates are usually classified into a single class. *Noctiluca*, with its unique mitosis, may prove to represent a class of its own. The Syndiniophyceae has been suggested as a separate class for a number of symbiotic dinoflagellates (Loeblich 1976), but this idea has received little support.

Division Heterokontophyta, Division Haptophyta

The heterokonts comprise a large assemblage of morphologically diverse organisms, from chrysophycean flagellates to branched filamentous xanthophytes and parenchymatous brown algae in which the thallus may be very complex: members of the Laminariales even possess sieve-like tissue resembling that of higher plants.

The heterokonts form a well-defined group, characterized by the presence of tripartite hairs on the front flagellum. The movements of this flagellum pull the cells forward in the water or, in sessile forms, draw water and food particles towards the cell. The cells always contain mitochondria with tubular cristae. The heterokont algae may be grouped into the phylum Heterokontophyta, but in a protistan classification should include also the heterokont water molds (Oomycetes, Hyphochytriomycetes), the labyrinthulids and the thraustochytrids. Together these may be classified under the (oldest) name Heterokontae. There are several problems with defining class levels in the heterokont algae, however. The Chrysophyceae may be used in a broad sense, i. e. including Mallomonadales, silicoflagellates, pedinellids, etc. or some of the latter may be given class rank: Synurophyceae, Dictyochophyceae (probably in-

cluding the pedinellids) and perhaps others.

The Raphidophyceae represents a distinct class of its own, characterized by the very complex internal structure of the flagellar apparatus (Vesk and Moestrup 1987). Members of this class show some similarity to the naked form of silicoflagellates (Moestrup and Thomsen 1990) which differs, however, in the presence of flagellar wings, paraxial flagellar structures, and in the structure of the flagellar root system.

The class Xanthophyceae (for which the typified name Tribophyceae has been suggested) is mainly defined on chloroplast features, and this class is in need of additional detailed studies. Using 16S-like RNA sequences Ariztia et al. (1991) found the xanthophyte *Tribonema* to group with the brown algae, a finding supported by similarities of the flagellar apparatus in other members of these groups (Moestrup 1970).

The diatoms and the Eustigmatophyceae are well-defined classes, while the distinction between brown algae (Phaeophyceae or the little-used typified name Fucophyceae) and the Chrysophyceae is presently somewhat uncertain. O'Kelly (1989) considered the Chrysomeridaceae, an apparently advanced group of chrysophytes, to contain the ancestors of the brown algae. They differ from a brown algae by lacking plasmodesmata, alginic acid, unilocular sporangia and physodes. Characteristic of heterokont algae is the presence of four membranes around the chloroplast, the outer membrane being continuous with the outer nuclear membrane, although in cells with many chloroplasts this connection is often lost (for an interesting exception, see *Pseudodichotomosiphon*, Hori et al. 1979). Thus the connection is known to be present in some members of the Eustigmatophyceae, but lacking in others (Hibberd 1980). The heterokonts share the presence of four chloroplast membranes with the Haptophyceae (= Prymnesiophyceae) of the division Haptophyta. Most haptophytes are characterized by iso- or anisokont flagella which lack tripartite hairs and, in many species, by the presence of a *haptonema*. It appears likely that the two groups have a common ancestor, probably a colourless phagotrophic flagellate. The chloroplast represents the remains of a eukaryotic cell ingested by phagocytosis but retained in a food vacuole in the perinuclear space. Colourless organisms like *Cafeteria* or *Pseudobodo* of the Chrysophyceae (Fenchel and Patterson 1988), or *Balaniger* of the Haptophyceae (Thomsen 1986), may represent the ancestral, chloroplast-lacking state.

Division Euglenophyta, With The Single Class Euglenophyceae

The Euglenophyta occupies an isolated position among the algae. It shares the presence of both chlorophyll a and b with the green algae, but most other features are different. The euglenoids are apparently unrelated or very distantly related to other algae. Ultrastructural and molecular data indicate, however, that the euglenoids are

related to bodonids and trypanosomes. These groups form a natural assemblage ("Euglenozoa"), in which the primitive condition was probably a heterotrophic flagellate, which engulfed a green alga or a green algal chloroplast subsequently stored in a food vacuole. Montegut-Felkner and Triemer (1991) and Triemer and Farmer (1991) have suggested that an organism similar to *Petalomonas cantuscygni* may represent the ancestral condition of the "Euglenozoa".

Division Chlorarachniophyta, With The Single Class *Chlorarachniophyceae*

The Chlorarachniophyta also occupies an isolated position within the algae. Very few species are known and the presence of a reduced nucleus (*nucleomorph*) associated with the chloroplast is a parallel to the situation in the cryptomonads. As presently understood, the chlorarachniophytes were originally heterotrophic flagellates or amoebae which by phagotrophy engulfed a whole eukaryote, perhaps a prasinophyte (Hatakeyama et al. 1991).

Division Chlorophyta, Division Glaucophyta

Finally the large division Chlorophyta, the green algae. There is presently no consensus regarding the number of green algal classes. Table 1 represents a rather conservative view, with two primitive classes, the Pedinophyceae and the Prasinophyceae, and three more advanced classes. The Ulvophyceae are mainly marine, the Chlorophyceae freshwater organisms. Members of the Charophyceae are related to the algae which gave rise to the land plants. Van den Hoek et al. (1988) suggested a system of 7 or 8 green algal classes, one of which, the Ulvophyceae, is now being split into several additional classes (C. van den Hoek, pers. comm., see also Zechman 1991) which all share the lack of both a phragmoplast and a phycoplast and show the apparently primitive state of an anticlockwise orientation of the flagellar basal bodies. The ancestral condition of the Chlorophyta may be reflected in the class Glaucophyceae of the division Glaucophyta (Moestrup 1982), in which the few known members contain cyanelles rather than true chloroplasts. The ancestors of the green algae were probably heterotrophs which in one branch - the glaucophytes - took up blue-green algae that were later transformed into cyanelles, while in the chlorophytes the ancestors took up a chlorophyll a and b containing prokaryote perhaps related to one of the present-day prochlorophytes. In the classification of green algae by van den Hoek et al. (1988) the class level approaches the order level of previous classifications. This reflects an inflationary tendency in modern classifications, the old order level is gradually being replaced with the class level.

Table 1. CLASSIFICATION OF ALGAE

Prokaryotic algae:

Division	**Class**
Cyanophyta (Cyanobacteria)	Cyanophyceae (blue-green algae)
Prochlorophyta	Prochlorophyceae

Eukaryotic algae:

Division	**Class**
Rhodophyta	Rhodophyceae (red algae)
Cryptophyta	Cryptophyceae
Dinophyta	Dinophyceae (dinoflagellates)
Heterokontophyta	Chrysophyceae Synurophyceae Raphidophyceae Xanthophyceae Phaeophyceae (brown algae) Bacillariophyceae (diatoms) Eustigmatophyceae Dictyochophyceae (silicoflagellates, pedinellids)
Haptophyta	Haptophyceae
Euglenophyta	Euglenophyceae (euglenoids)
Glaucophyta	Glaucophyceae
Chlorophyta	Prasinophyceae Pedinophyceae Ulvophyceae Chlorophyceae Charophyceae
Chlorarachniophyta	Chlorarachniophyceae

Related to dinoflagellates: ciliates, apicomplexa.
Related to euglenoids: bodonids, trypanosomes, Diplonema.
Related to Heterokontophyta: some phycomycetes, labyrinthulids, thraustochytrids.
Ancestral eukaryotic group (?): Archezoa.

Conclusion

Classification of the algae at the class level is still unstable, but many differences may be explained by a general devaluation at all levels of classification, from division to species level.

An image is emerging, however, in which the ancestors of all the major groups of algae were phagotrophs, which enslaved pro- or eukaryotic prey, subsequently tranformed into chloroplasts. A phagotrophic ancestor is likely in red algae, green algae (a prokaryotic prey organism), cryptomonads, dinoflagellates, heterokonts & haptophytes, chlorarachniophytes and euglenoids (a eukaryotic prey organism, or perhaps a chloroplast). It will be one of the goals of future research to identify the relatives of these early ancestors of the algal groups, some of which are probably hidden in that vast and poorly examined assemblage termed protozoa.

References

Ariztia, E. V., Andersen, R. A. and Sogin, M. L. 1991. A new phylogeny for chromophyte algae using 16S-like rRNA sequences from *Mallomonas papillosa* (Synurophyceae) and *Tribonema aequale* (Xanthophyceae). *J. Phycology* **27**:428–436.

Bhattacharya, D., Elwood, H. J., Goff, L. J. and Sogin, M. L. 1990. Phylogeny of *Gracilaria lemaneiformis* (Rhodophyta) based on sequence analysis of its small subunit ribosomal RNA coding region. *J. Phycology* **26**:181–186.

Cavalier-Smith, T. 1991. Archamoebae: the ancestral eukaryotes? *BioSystems* **25**:25–38.

Fenchel, T. and Patterson, D. J. 1988. *Cafeteria roenbergensis* nov. gen., nov. sp., a heterotrophic microflagellate from marine plankton. *Marine Microbial Food Webs* **3**:9–19.

Hatakeyama, N., Sasa, T., Watanabe, M. M. and Takaichi, S. 1991. Structure and pigment composition of *Chlorarachnion* sp. *J. Phycology* **27**, suppl.:29.

Hibberd, D. J. 1980. Eustigmatophytes. In: *Phytoflagellates* (Ed. by E. R. Cox). pp. 319–334. Elsevier/North Holland, N. Y.

Hoek, C. van den, Stam, W. T. and Olsen, J. L. 1988. The emergence of a new chlorophytan system, and Dr. Kornmann's contribution thereto. *Helgoländer Meeresuntersuchungen* **42**:339–383.

Hori, T., Kobara, T. and Chihara, M. 1979. Electron microscope observations on *Pseudodichotomosiphon constrictus* with special reference to the systematic position of the genus. *Jpn. J. Phycology* **27**:183–191.

Hori, H. and Osawa, S. 1986. Evolutionary change in 5S rRNA secondary structure and a phylogenetic tree of 352 5S rRNA species. *BioSystems* **19**:163–172.

Hori, H. and Osawa, S. 1987. Origin and evolution of organisms as deduced from 5S ribosomal RNA sequences. *Molecular Biology and Evolution* **4**:445–472.

Jeffrey, S. W. 1991. New pigments and their impact on algal systematcs. *J. Phycology* **27**,

suppl.:34.

Kugrens, P., Lee, R. E. and Andersen, R. A. 1987. Ultrastructural variations in cryptomonad flagella. *J. Phycology* **23**:511-518.

Loeblich, A. R. III. 1976. Dinoflagellate evolution: speculation and evidence. *J. Protozoology* **23**:13-28.

Moestrup, Ø. 1970. On the fine structrue of the spermatozoids of *Vaucheria sescuplicaria* and on the later stages of spermatogenesis. *J. Mar. Biol. Assoc. UK* **50**:513-523.

Moestrup, Ø. 1982. Flagellar structure in algae: a review, with new observations particularly on the Chrysophyceae, Phaeophyceae (Fucophyceae), Euglenophyceae, and *Reckertia*. *Phycologia* **21**:427-528.

Moestrup, Ø. and Thomsen, H. A. 1990. *Dictyocha speculum* (Silicoflagellata, Dictyochophyceae), studies on armoured and unarmoured stages. *Kongelige Danske Videnskabernes Selskab, Biologiske Skrifter* **37**:1-57.

Montegut-Felkner, A. E. and Triemer, R. E. 1991. *Petalomonas cantuscygni*: a phylogenetic link between the Euglenophyta and the Kinetoplastida based on small subunit rRNA sequence. *J. Phycology* **27**, suppl.:51.

O'Kelly, C. J. 1989. The evolutionary origin of the brown algae: information from studies of motile cell ultrastructure. In: *The Chromophyte Algae* (Ed. by J. C. Green, B. S. C. Leadbeater and W. L. Diver). Systematics Association Special volume 34, pp. 225-278. Clarendon Press, Oxford.

Palenik, B. and Haselkorn, R. 1991. Evolutionary divergence of the prochlorophytes. *J. Phycology* **27**, suppl.:56.

Sogin, M. L., Stickel, S. K., Bibeau, C. and Bhattacharya, D. 1991. Molecular evolution and phylogenetic relationships in the chromophyte algae. *J. Phycology* **27**, suppl.:69.

Tangen, K. and Björnland, T. 1981. Observations on pigments and morphology of *Gyrodinium aureolum* Hulburt, a marine dinoflagellate containing 19'-hexanoyloxyfucoxanthin as the main carotenoid. *J. Plankton Res.* **3**:389-401.

Thomsen, H. A. 1986. A survey of the smallest eucaryotic organisms of the marine phytoplankton. In: *Photosynthetic Picoplankton* (Ed. by T. Platt & K. W. Li William). Can. Bull. Fish. and Aqua. Sci. **214**:121-158.

Triemer, R. E. and Farmer, M. A. 1991. Ultrastructural organization of the heterotrophic euglenids and its evolutionary implication. In: *The Biology of Free-living Heterotrophic Flagellates* (Ed. by D. J. Patterson and J. Larsen). Systematics Association Special volume 43, pp. 185-204. Clarendon Press, Oxford

Vesk, M. and Moestrup, Ø. 1987. The flagellar root system in *Heterosigma akashiwo* (Raphidophyceae). *Protoplasma* **137**:15-28.

Watanabe, M. M., Suda, S., Inouye, I., Sawaguchi, T. and Chihara, M. 1990. *Lepidodinium viride* gen. et sp. nov. (Gymnodiniales, Dinophyta), a green dinoflagellate with a chlorophyll a-and b-containing endosymbiont. *J. Phycology* **26**:741-751.

Wilcox, L. W. and Wedemayer, G.J. 1985. Dinoflagellate with blue-green chloroplasts derived

from an endosymbiotic eukaryote. *Science* **227**:192-194.

Wolthers, J. 1991. The troublesome parasites - molecular and morphological evidence that Apicomplexa belong to the dinoflagellate-ciliate clade. *BioSystems* **25**:75-83.

Zechman, F. W. 1991. A phylogenetic analysis of the Ulvophyceae based on rRNA genes and morphology: implications for character evolution and the evolutionary process. *J. Phycology* **27**, suppl.:80.

Algal Culture Collections And Biotechnology

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Abstract

The primary remit of algal culture collections is identical to that of other collections of microorganisms, that is to act as a depository for a particular group of microorganisms, in this case eukaryotic microalgae and prokaryotic cyanobacteria, effectively acting as a gene bank. The major collections are mostly service collections which are responsible for collecting, maintaining and preserving algae. They also provide viable stable cultures and associated information for a variety of customers including teaching establishments, academic research bodies and industrial organizations.

The concept of algal biotechnology and culture collection involvement in biotechnology is relatively recent, although man has exploited natural populations of microalgae for many generations. It is largely manipulations of natural phenomena that form the basis of current commercial exploitation of microalgae eg. nitrogen fixation by heterocystous cyanobacteria and carotenoid formation by some of the Chlorophyceae. Current algal biotechnological processes/products on a commercial scale are detailed in this paper and some possible future product areas are also discussed. The role of culture collections and the services they could provide biotechnology are also described.

Key words: algae, biotechnology, culture collection.

Introduction

New roles are emerging for culture collections of living microorganisms. The demanding requirements of biotechnologists and industrialists are increasing and impinging particularly on algal collections where until recent years interest in commercial ex-

ploitation of algae has been comparatively slight. With appropriate encouragement from the algal collections in the form of publicity for their organisms and the adequate provision of easily accessible data on their properties, this interest is expected to grow.

There is a large gap between test-tube culture, together with some theoretical knowledge of its properties, and Kg. quantities of a particular product. Problems can occur at any stage of process development, particularly where pilot-scale production process were not fully investigated. It is in this area as well as other more fundimental areas that culture collections could provide a vital service.

Primary Remit Of Algal Culture Collections

The primary remit of all culture collections is to act as a depository of strains. In the case of algal culture collections the range of microorganisms is restricted to the prokaryotic cyanobacteria or blue-green algae and the eukaryotic microalgae. This is not a hard and fast restriction as some collections eg. American Type Culture Collection (ATCC) include a wide variety of microorganisms other than algae and other collections eg. Culture Collection of Algae and Protozoa (CCAP) maintain protozoa and some small thalloid rhodophytes as well as microalgae and cyanobacteria.

Most of the major algal culture collections located in Europe, North America, Australia and Japan (Table 1) act as service collections, performing not only the primary role of being a depository, but also providing cultures for third parties. These collections are charged with the task of collecting from the wild or obtaining cultures from other researchers and purifying the algae to axenic clonal cultures or at least to unialgal cultures. Each organism should then be authenticated and a maintenance/preservation protocol developed prior to accession into the collection. The central core of the primary remit is the maintenance/preservation of the algal cultures under conditions which produce maximum strain stability and will prevent genetic drift, but which allow the culture to remain in a viable state. The provision of viable, stable cultures and associated information to outside bodies/researchers is the end point of the primary remit. This involves the development of an administration which is responsible for the culturing of strains, their packaging and posting as well as the invoicing and other financial and regulatory considerations.

Culture collections also provide a number of other services, including involvement in algal biotechnology. These are discussed in greater detail in the subsequent sections.

Table 1 List of major algal culture collections.

Acronym	Name	Country	No. of cultures lodged
ASIB	Algensammlung am Institute fur Botanik.	Austria	1570
ATCC	American Type Culture Collection.	USA	108
CALU	Collection of Algal Cultures Leningrad Univ.	USSR	600
CAUP	Culture Collection of Algae.	Czechosl.	140
CCALA	Culture Collection of Autotrophic Organisms.	Czechosl.	498
CCAP	Culture Collection of Algae and Protozoa.	UK	1631
CCMP	Provasoli-Guillard Center for Culture of Marine Phytoplankton.	USA	1000
CS	CSIRO Culture Collection of Microalgae.	Australia	300
IAM	Institute of Applied Microbiology.	Japan	500
IPPAS	Culture Collection of Unicellular Algae.	USSR	340
LMS	Carolina Biological Supply Co.	USA	165
MUACC	Murdoch Univ. Algal Culture Collection.	Australia	157
NEPCC	North East Pacific Culture Collection.	Canada	340
NIES	Microbial Culture Collection.	Japan	500
NIVA	Culture Collection of Algae (NIVA).	Norway	260
PCCIP	Pasteur Culture Collection of Cyanobacterial Strains.	France	200
PLYMOUTH	Plymouth Culture Collection.	UK	150
SAG	Sammlung von Algenkulturen.	Germany	1400
SVCC	Sammlung von Conjugaten kulturen.	Germany	400
UTCC	Univ. Toronto Culture Collection.	Canada	148
UTEX	Culture Collection of Algae at the Univ. Texas at Austin.	USA	2089
UWO	Culture Collection, Dept Plant Sciences, Univ. Western Ontario.	Canada	133

Major collections = Collection with >100 cultures lodged.

Secondary Roles Of Algal Culture Collections

Most algal culture collections are associated with universities of research institutes. They tend to be involved in the research programmes of those establishments, not only by the provision of authenticated cultures and advice, but also through the work of individuals connected with the collections, who are generally active research scientists themselves. Areas of research include algal taxonomy, physiology and ecology as well as research into preservation techniques and various aspects of algal biotechnology.

Increasingly culture collections are becoming involved in education. Previously this was largely restricted to research students working on research projects based at collections. Several collections currently run courses which cover algal identification, culturing, basic physiology and preservation techniques. Culture collections in the past tended only to produce catalogues, all other publications being contributions to scientific publications. Over the past ten years this production of material has expanded to cover a variety of educational resource materials including booklets, practical experiment kits and videos.

Another area in which culture collections are involved is as resource and information centres. Virtually all major collections retain much of their information on computer and increasingly the construction of data-bases and net-works is becoming a priority. This could eventually provide a comparable sevice for the research community to those already available for bacteria and fungi.

Commercial Implications Of Microalgae And Cyanobacteria

The most newsworthy effects of algae on commercial operations tend to be negative. The occurrence of algal blooms is becoming increasingly widespread. In fresh waters these are usually due to increased nutrient levels as a result of run-off from agricultural land (Codd 1984). They are often composed of toxic cyanobacteria (Skulberg *et al.* 1984, Leeuwangh *et al.* 1983) and if N_2 fixers are present can lead to subsequent increased eutrophication. In extreme cases toxic cyanobacteria have resulted in animal deaths (Gorham and Carmichael 1979, Lawton and Codd 1990). They may also cause skin irritations and other symptoms in man (Bourke and Hawes 1983, Codd 1984). This has led to considerable public concern and the closure of some reservoirs for both water extraction and recreational purposes, with serious commercial implications. Blooms of toxic marine dinoflagellates can also have severe commercial impacts with blooms of *Gyrodinium aureolum* Hulburt causing caged fish deaths off the southern Norwegian coast in the autumn of 1988 and in earlier years (Dahl and Tangen 1989). Biofouling by algae can have financial repercussions, the blockage of water filters being one example (Palmer 1962).

These deleterious effects are far outweighed by the beneficial role of algae in the natural and managed ecosystems. Oceanic microalgae are the largest group of primary producers and as a result form the base of a vast food-web. It should not be overlooked that microalgae are also major producers of O_2 and removers of atmospheric CO_2. A number of cyanobacteria can fix atmospheric N_2 both in their free-living forms (Bergman *et al.* 1985) and in symbiotic associations (Stewart *et al.* 1983) and thus play an extremely important role in increasing productivity of many higher plants. A commercial usage of microalgae, which verges on algal biotechnology, is their use as bioassay organisms (see Table 2). This usage is becoming increasingly important as ecotoxicity testing becomes a statutory requirement for new products. The final positive commercial implication is biotechnological exploitation of algae and this is discussed in more detail in the following section.

Table 2. Algae used in bioassays.

Bioassay	Test Organism	Reference
Biotin	*Ochromonas danica*	Baker *et al.* 1962
	Amphidinium carteri	Carlucci 1967
Vitamin B_{12}	*Euglena gracilis*	Paker 1977
	Thallasiosira pseudonana	Ryther and Guillard 1962
Ecotoxicity	*Scenedesmus quadricauda*	Klass *et al.* 1974
	Selenastrum capricornutum	Weiss and Helms 1971
	Skeletonema costatum	Kallqvist 1972
	Ankistrodesmus braunii	Trevors 1982
	Asterionella spp.	Lund *et al.* 1975

Algal Biotechnology

Man has exploited natural populations of algae for many genarations, with *Spirulina platensis* being used as a food supplement by both the Aztecs (Farrar 1966) and the people of north-eastern Chad (Dangeard 1940). Algal biotechnology is currently more varied in the range of organisms used and some of the most commercially successful products and processes are discussed below.

Biomass/Health Food

The growth of biomass is the most obvious and technically simple process to accomplish. Mostly the resultant products are used for the health food market (Table 3). The organisms grown tend to be those which grow in extreme environments eg. *Spirulina* and *Dunaliella*, or those with fast growth rates eg. *Chlorella* and *Scenedesmus*. The largest market is in Japan and the Far-east, but in increasingly health conscious Europe and the USA there is a niche market in health food shops. There has been little scientific evidence to explain any actual or perceived beneficial effects from consuming daily doses of algae as tablets, capsules or in extracts. The exception is the evidence which indicates that natural carotenoids, acting as antioxidants can reduce the risk of cancer by "mopping up" free oxygen radicals (Peto *et al.* 1981, Schwartz *et al.* 1988). Some algae also contain high levels of higher unsaturated fatty acids (Borowitzka 1988), which are precursors of prostaglandins (Lehninger 1976) and as such, are valuable for use as food supplements.

Algae have also been used as animal feed and a number of small-scale and pilot-scale processes have been developed (Miernik 1983, Pouliot and De la Noueø 1985, Fallowfield and Garrett 1985). These have largely been used to provide the animals with basic nutrients and in a number of special applications, algae have been added to the basic feed to provide a specific nutrient or pigment eg. zeaxanthin and lutein in chicken feed (Kathrein 1960).

The production systems for all the commercial production processes are photoautotrophic in nature, with the exception of some *Chlorella* production systems which are mixotrophic or which utilize heterotrophically cultivated algae as an inoculum for the production phase (Endo *et al.* 1977, Kawaguchi 1980). A wide variety of systems are used including managed natural lakes (Schlipalius 1990), open-ponds (Moulton *et al.* 1987) and photobioreactors (Robinson pers. comm.).

Table 3. Algae commonly used as health food.

Genera	Product
Spirulina	Biomass, powder, tablet and extracted gamma linoleic acid.
Chlorella	Biomass, powder, tablet and extracts.
Dunaliella	Biomass, powder, capsules and extracted beta-carotene.

Aquaculture

The early developmental stages of molluscs and shrimps have a requirement for microalgae as their primary food source (Walne and Wood 1974, Webb and Chu 1982). The utilization of natural levels of algae is adequate in their natural environment, but in hatcheries, where stock levels are much higher, a variety of algae are cultivated (Table 4). These have been selected for their ease of cultivation and because they fulfil the nutritional requirements of the mollusc spat or shrimp larvae. In most mollusc hatcheries algae are grown up in unialgal cultures and these are then mixed and metered into the tanks containing the molluscs (De Pauw *et al.* 1983). In less intensive systems the algae are cultivated in the presence of the feeding larvae or spat in out-door ponds or tanks utilizing nutrient supplemented seawater (Fujinaga and Kittaka 1975). In some cases these may be covered by a green-house, thus increasing algal productivity (Persoone and Claus 1980).

The relatively high cost of monospecific algal cultures, \$160-\$200 (Kg dry wt.$^{-1}$) (De Pauw *et al.* 1984), has led to the development of a number of processes for the bulk cultivation and preservation/stabilization of algae. These have the advantage of always insuring that there is a supply of algae. However they have the disadvantage of usually being unialgal and as a result are not usually a full replacement for a mixed algal culture. A number of products are available which are composed of microalgae which have been developed for use in aquaculture (Table 5). In general the algae are stabilized by drying, either freeze-drying or spray-drying. Freezing and the use of chemical anti-oxidants are occasionally employed for the preservation of small amounts of material. Other microalgae including both *Spirulina* and *Chlorella* have

Table 4. Algal genera most commonly used in aquaculture.

Diatoms	Flagellate algae	Non-motile unicellular algae
Chaetoceros	*Tetraselmis*	*Nannochloris*
Skeletonema	*Isochrysis*	*Nannochloropsis*
Thalassiosira	*Pavlova*	*Chlorella*
Cyclotella	*Rhodomonas*	
Phaeodactylum		

Table 5. Products composed of microalgae currently being used in aquaculture.

Product	Company	Alga	Use	Cultivation system
AQUAGRO T-ISO	Martek	*Isochrysis*	Mollusc	Photoautotrophic
AQUAGRO N-EPA	Martek	*Nitzschia*	Mollusc	Photoautotrophic
AQUAGRO NAN.	Martek	*Nannochloropsis*	Mollusc	Photoautotrophic
Celsys 161	CSL	*Tetraselmis*	Mollusc Rotifers	Heterotrophic
Celsys 262	CSL	*Tetraselmis* *Cyclotella*	Shrimp	Heterotrophic

CSL =Cell Systems Ltd.

been used for this application (De Pauw *et al.* 1984). In general they have not proved to be particularly successful or popular.

Biofertilizers And Soil Stabilizers

The application of biofertilizers is widespread in agriculture, with the use of *Azolla*, the small water fern which forms a symbiotic association with *Anabaena azolla* being used for centuries in China (Metting 1988). The utilization of cyanobacteria as "Green" fertilizers, is becoming increasingly common, with a variety of heterocystous cyanobacteria being used (Table 6). The largest usage of cyanobacterial fertilizers is in India, where two million hectares were fertilized in 1979 (Metting 1988). The system used involves the central production of a mixed inoculum of cyanobacteria which is then air-dried and mixed with soil. This material forms dried flakes which are then distributed and used to inoculate $40m^2$ ponds. They in turn are used as an inoculum for the cultivated rice fields. Currently the development of algal biofertilizers for use in temperate environments is being investigated (Metting 1981), and commercial products have been developed, including an agricultural fertilizer produced by Soil Technologies and a lawn and garden fertilizer produced by Cyanotech Corporation.

The use of algae as soil stabilizers/structure enhancers was suggested by Lewin (1977), the efficacy being dependent on the extracellular excretion of mucilage. This improves the soil-crumb structure increasing water retention and reducing the possibil-

Table 6. Genera of Cyanobacteria used as Biofertilizers.

Genera	Reference
Aulosira	Venkataraman 1986
Tolypothrix	Venkataraman 1986
Scytonema	Venkataraman 1986
Nostoc	Venkataraman 1986
Anabaena	Venkataraman 1986
Plectonema	Venkataraman 1986
Aphanothece	Metting 1981

ity of soil erosion. A variety of microalgae and cyanobacteria have been demonstrated to be suitable (Table 7.). They all depend on continual growth of the alga and, as a result, any commercial product needs to be composed of live cells capable of rapid growth. Mixtures of algae are used, the species utilized being dependent on the environment to be seeded. To date, the use of algal soil stabilizers has been on a relatively small scale in comparison to cyanobacterial fertilizers. Two small algal biotechnology companies, R & A Plant Soil Inc. and Soil Technologies Corp. currently produce a variety of microalgae in open-pond systems which they sell in the USA Mid-west.

Fine Chemicals And Pigments

The major disadvantage of using algae as the basis of a production process, other than for simple biomass production, is the cost of production. Despite the obvious potential of apparently free and virtually unlimited energy in the form of sunlight, production costs are usually relatively high. Also, for most products, the percentage of product versus total biomass is usually very low with the exception of a few products including beta-carotene with levels up to 14% (Ben-Amotz *et al.* 1982) and astaxanthin 2–4% (Bubrick In press). This tends to divide the fine chemicals into two groups;

Table 7. Microalgal/Cyanobacterial genera used as soil stabilizers.

Genera	Reference
Chlamydomonas	Lewin 1977
Anabaenopsis	Shainberg & Bar-Or 1990
Microcoleus	Singh 1950
Scytonema	Singh 1950
Aulosira	Roychoudhury *et al.* 1980
Anabaena	Roychoudhury *et al.* 1980
Nostoc	Roychoudhury *et al.* 1980
Tolypothrix	Roychoudhury *et al.* 1980

firstly, those which are produced on a large scale (bulk products) and secondly those which have an extremely high value and are produced for a small specialist market. There is currently a large variety of products produced on a commercial basis by algal biotechnology companies. Some of the major products and producers are listed in Table 8.

The techniques utilized to produce the algae vary from the cultivation of small volumes in laboratory systems through to managed lakes of up to 300 hectares. In the larger scale systems, control of the environment can be a major problem with

Table 8. Fine chemicals and pigments currently produced commercially using algae.

Product	Organism	Major producers.
EPA(20.3)	Various	Martek, R & A Plant Soil Inc.
Biodeuterated lipids	Various	Martek, Chembiotech Ltd.
Biodeuterated oils	Various	Martek.
Biodeuterated aminoacids	Various	Martek.
Deuterated lubricants	Various	Martek.
Restriction enzymes	Various	
Algal toxins	Various	
Beta-carotene	*Dunaliella*	Betatene Ltd., Cyanotech Corporation, Eilat Algae Industry Ltd., Microbio Resources, Vitamins Australia Pharmaceutical & Aquaculture Corp. Ltd., Western Biotech.
Astaxanthin	*Haematococcus*	Microbio Resources.
Phycobiliproteins	*Spirulina*	Cyanotech Corporation, Dia Nippon Ink and Chemical Co., Martek.

potentially disastrous losses in productivity due to climatic perturbations and protozoan grazing. In the main, the most successful products and processes are those which are small scale and therefore easily controlled eg. biodeuterated products, or which depend on the use of extreme environments unpropitious for predators eg. beta-carotene. The future success of algal biotechnology, both technical and commercial, depends not only on the development of bioreactors to increase productivity and control product purity, but also the generation of over-producing strains, either by genetic manipulation, conventional mutagenesis or direct selection techniques.

Pollution Control

The role of microalgae in pollution control is often overlooked. They are however, a key component in many pollution control systems, particularly those which employ ponds or impounds. These can be divided into two catagories; facultative ponds, which are over 1m deep and algae are restricted to the surface waters and high rate oxidation ponds (HROP), which are generally shallow and mechanically agitated. HROP are more commonly used, there the algae not only provide O_2 which is utilized by the oxidative bacteria present, but are also predominantly facultative heterotrophs, (see Table 9. for most-common examples), with up to 50% of their carbon assimilation being by direct heterotrophic nutrition (Abeliovich and Weisman 1978). The yields of algae in these systems can be relatively high eg. 1–2 gL^{-1} (Abeliovich 1980). This has led to the development of a number of pilot-scale processes which combine waste treatment with the production of algal biomass for animal feed (Fallowfield and

Table 9. Algae found in high rate oxidation Ponds (HROP).

Genera	Reference
Euglena	Abeliovich 1986
Chlamydomonas	Abeliovich 1986
Chlorella	Abeliovich 1986
Scenedesmus	Azov *et al.* 1980
Micractinium	Azov *et al.* 1980
Ankistrodesmus	Azov *et al.* 1980

Garrett 1985, Pouliot and De la Noue 1985).

Algae also perform a number of secondary functions in waste-water treatment, including the disinfection of the effluent. They increase the water temperature by converting light to heat, thus increasing the death-rate of temperature sensitive enteric bacteria (Oswald 1988). They increase the effluent pH, by metabolizing bicarbonate and this induces flocculation which increases the sedimentation rate of the effluent being treated. Algae also have negatively charged cell walls. This in conjunction with the raised pH removes heavy metals from the effluent (Oswald *et al.* 1957, Becker 1983). This phenomenon has been used to develop filters which can remove heavy metal ions and refactory organic compounds from industrial effluents (Wilson *et al.* 1991).

Future Product Areas

This paper has so far avoided discussing possible commercial applications/products from microalgae and cyanobacteria. There are however, a large number of metabolites from these groups of organisms which could form the basis of new industries. The most obvious areas are in the production of polysaccharides particularly for use as gelling agents; hydrocarbons for use as chemical feedstock and bioactive metabolites, phamaceuticals and antibiotics. For recent reviews see Vonshak (1988), Borowitzka (1988) and Lincoln *et al.* (1990).

The Role Of Algal Culture Collections In Biotechnology

Culture collections are increasingly becoming involved with commercial aspects of applied algology, with most of the major collections currently required to provide services and information for industrial clients as well as their more traditional role as suppliers of cultures to the academic community. Some of these services are discussed in greater detail below.

The provision of pure/axenic well-documented cultures has been one of the core activities of culture collections. This is increasingly becoming important in biotechnology as the provision of axenic cultures is particularly relevant for those who intend to use mixotrophic or heterotrophic culture systems. The isolation, purification and identification of cultures for commercial customers is occasionally undertaken by collections and the development of media and culture conditions is usually associated with this service. The provision of starter cultures for aquaculture is regularly undertaken by CCAP and this could easily be expanded to provide larger volumes of axenic starter-cultures for other applications.

Collections including CCAP offer a safe depository service for commercially valuable cultures. This ensures that cultures are maintained at a second site, acting as in-

surance against accidental loss of the master stock-cultures held by the customer. This facility allows the owner continual access to their culture but prevents any third party obtaining it. CCAP together with a number of other algal collections is a signatory to the Budapest Treaty (1987) and is an International Depository Authority (IDA). This allows commercial concerns to deposit strains of algae for patent purposes. Again there is restricted access to the cultures lodged. Increasingly as new products and processes utilizing algae are developed, over-producing mutants are being developed, some of which are genetically less stable than the parent wild-type culture. Genetic stabilization by cryopreservation is one possibility. The development of long-term stabilization/preservation techniques, for commercially important strains, is another service collections could provide.

Direct contract research, screening, strain selection, and mutant generation to increase productivity could easily be undertaken for a commercial partner. Furthermore the development/improvement of production processes (culture systems), down-stream processes and product development could be carried out in association with a commercial partner or customer.

Finally culture collections by their nature have a large amount of in-house expertise and are often located in research institutes or universities. This allows them access to a large bank of information which could be used to provide relevant scientific information, literature surveys and paper feasibility studies for commercial customers. Future developments in this area, including the growing interest in algal data-bases, will undoubtedly improve this aspect of the services which algal culture collections currently provide.

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References

Abeliovich, A. 1986. Algae in wastewater oxidation ponds. In: *Handbook of Microalgal Culture.* (Ed. by A. Richmond) pp. 331-338. CRC Press, Boca Raton, Florida.

Abeliovich, A. 1980. Factors limiting algal growth in high rate oxidation ponds. In: *Algae Biomass Production and Use.* (G. Shelef & C. J. Soeder) pp. 205-216. Elsevier/North Holland Biomedical Press, Amsterdam.

Abeliovich, A. and Weisman, D. 1978. The role of heterotrophic nutrition in the growth of the alga *Scenedesmus obliquus* in high rate oxidation ponds. *Appl. envir. Microbiol.* **35**:32-36.

Azov, Y., Shelef, G., Moraine, R. and Levi, A. 1980. Controlling algal genera in high rate wastewater oxidation ponds. In: *Algae Biomass Production and Use.* (Ed. by G. Shelef and C.

J. Soeder) pp. 245-253. Elsevier/North Holland Biomedical Press, Amsterdam.

Baker, H., Frank, O., Pasher, I., Matovitch, B., Aaronson, S., Hutner, H. and Sobotka, H. 1962. A new bioassay method for biotin in blood, serum, urine and tissue. *Analyt. Biochem.* **3**:31-39.

Becker, E. W. 1983. Limitations of heavy metal removal from waste water by means of algae. *Wat. Res.* **17**:458-466.

Ben-Amotz, A., Katz, A. and Avron, M. 1982. Accumulation of β carotene in halotolerant algae; purification and characterization of β -carotene-rich globules from *Dunaliella bardawil* (Chlorophyceae). *J. Phycol.* **18**:529-537.

Bergman, B., Renstrom, E., Hallbom, L. and Codd, G. A. 1985. Effects of aminooxyacetate on glycollate and ammonia release by the cyanobacterium *Anabaena cylindrica. Pl. Physiol., Lancaster* **77**:536-539.

Borowitzka, M. A. 1988. Fats, oils and hydrocarbons. In:*Microalgal Biotechnology*. (Ed. by M. A. Borowitzka and L. J. Borowitzka) pp. 257-287. Cambridge Univ. Press, Cambridge.

Bourke, A. T. C. and Hawes, R. B. 1983. Freshwater cyanobacteria (blue-green algae) and human health. *Med. J. Aus.* **1**:491-492.

Bubrick, P. (In press). Astaxanthin production by *Haematococcus pluvialis*. In: *Recent Advances in Algal Biotechnology*. (Ed. by A. Vonshak and A. Richmond) Elsevier, London.

Carlucci, A. F. 1967. Bioassay of seawater Ⅳ. The determination of the uptake of dissolved biotin from seawater using ^{14}C uptake by cells of *Amphidinium carteri. Can. J. Microbiol.* **13**:979-986.

Codd, G. A. 1984. Toxins of freshwater cyanobacteria. *Microbiol. Sci.* **1**:48-52.

Dahl, E. and Tangen, K. 1989. *Gyrodinium aureolum* bloom along the Norwegian coast in 1988. In: *Toxic Marine Phytoplankton; Proc. 4th int. Conf. toxic mar. Phytoplankton, June 26*-30, Lund, Sweden. (Ed. by E. Graneli *et al.*), pp. 123-131. Elsevier, N. Y.

Dangeard, P. 1940. Sur une algae bleu alimentaire pour l'homme*Arthrospira platensis. Act. Soc. Linn. Bordeaux* **91**:39-41.

De Pauw, N., Morales, J. and Persoone, G. 1984. Mass culture of microalgae in aquaculture systems: progress and constraints. *Hydrobiologia* **116**:121-134.

De Pauw, N., Verboven, J. and Claus, C. 1983. Large scale microalgae production for nursery rearing of marine bivalves. *Aquacultural Enging.* **2**:27-47.

Endo, H., Sanasawa, H. and Nakajima, K. 1977. Studies on *Chlorella regularis*, heterotrophic fast growing strain. II. Mixotrophic growth in relation to light intensity and acetate concentration. *Pl. Cell Physiol.,Tokyo* **18**:199-205.

Fallowfield, H. J. and Garrett, M. K. 1985. The photosynthetic treatment of pig slurry in temperate climatic conditions: A pilot-plant study. *Agric. Wastes* **12**:111-136.

Farrar, W. V. 1966. Tecuuitlatl: a glimpse at Aztec food technology. *Nature, Lond.* **221**:341-342.

Fujinaga, M. and Kittaka, J. 1975. Local and seasonal influences on the large scale production method for penaeid shrimp larvae. *Bull. Jap. Soc. Scient. Fish.* **41**:843.

Gorham, P. R. and Carmichael, W. W. 1979. Phycotoxins from blue-green algae. *Pure appl. Chem.* **32**:165-174.

Kallqvist, T. 1972. Algal assay procedure at NIVA. In: *Proc. Nordic Symp. on Algal Bioassays Oslo 25-26 Oct. 1972.* pp. 5-17. Nordforsk Publ., Helsinki.

Kathrein, H. R. 1960. Method for the production of β-carotene and xanthophylls by the cultivation of green algae. US patent No. 2949700.

Kawaguchi K. 1980. Microalgae production systems in Asia. In: *Algae Biomass Production and Use.* (Ed. by G. Shelef and C. J. Soeder) pp. 25-34. Elsevier/North Holland Biomedical Press, Amsterdam.

Klass, E. Rowe, D. W. and Massiro E. J. 1974. The effects of cadmium on population growth of *Scenedesmus quadricauda. Bull. Environ. Contam. & Toxicol.* **12**:442-445.

Lawton, L. A. and Codd, G. A. 1990. Cyanobacterial toxins and their significance in European waters. In: *New Health Considerations In Water Treatment.* Lecture 6. Inst. Water and Environ. Management, Leeds.

Leeuwangh, P., Kappers, F. I., Dekker, M. and Koerselman, W. 1983. Toxicity of cyanobacteria in Dutch lakes and reservoirs. *Aquat. Toxicol.* **4**:63-72.

Lehninger, A. L. 1976.*Biochemistry.* Worth Publishers Inc., N. Y., v-1104., 687 pl.

Lewin, R.A. 1977. The use of algae as soil conditioners. *Centros de Investigation de Baja California y Scripps Inst. of Oceanographic Trans.* **3**:33-35.

Lincoln, R. A., Strubinski, K. and Walker, J. M. 1990. Biologically active compounds from diatoms. *Diatom Res.* **3**:337-349.

Lund, J. W. G. Jaworski, G. H. M. and Butterwick, C. 1975. Algal bioassay of water from Blelham Tarn, English Lake district and the growth of planktonic diatoms. *Arch. Hydrobiol.*, suppl. **49**:49-69.

Metting, B. 1981. The systematics and ecology of soil algae. *Bot. Rev.* **147**:195-312.

Metting, B. 1988. Micro -algae in agriculture. In: *Microalgal Biotechnology.* (Ed. by M. A. Borowitzka and L. J. Borowitzka) pp. 228-304. Cambridge Univ. Press, Cambridge.

Miernik, A. 1983. The quality of protein from algae used in the purification of wastewater from the nitrogen fertilizer industry. *Acta Microbiol. Pol.* **3**:269-274.

Moulton, T. P., Borowitzka L. J. and Vincent D. J. 1987. The mass culture of *Dunaliella salina* for β-carotene: from pilot plant to production plant. *Hydrobiologia* **151**:99-105.

Uswald, W. J. 1988. Micro-algae and waste-water treatment. In *Microalgal Biotechnology.* (Ed. by M. A. Borowitzka and L. J. Borowitzka) pp.305-328. Cambridge Univ. Press, Cambridge.

Uswald, W. J., Goluke C. G. and Gee H. K. 1957. *Wastewater reclimation through production of algae.* Univ. California Water Resources Center, Contrib. 22.

Palmer, C. M. 1962. Algae in water supplies in Ohio. *Ohio J. Sci.* **62**:223-244.

Parker, M. 1977. Vitamin B_{12} in lake Washington USA concentration and uptake. *Limnol. Oceanogr.* **22**:527-538.

Persoone, G. and Claus, C. 1980. Mass culture of algae: a bottleneck in nursery culturing of

mollusc. In: *Algae Biomass Production and Use.* (Ed. by G. Shelef and C. J. Soeder) pp. 265-285. Elsevier/North Holland Biomedical Press, Amsterdam.

Peto, R., Doll, R., Buckley, J. D. and Sporn, M. B. 1981. Can dietary beta-carotene materially reduce human cancer rates? *Nature, Lond.* **290**:201-208.

Pouliot, Y. and De la Noue, J. 1985. Development of a pilot-scale facility for wastewater treatment and microalgal production. *Rev. Francaise des science de l'eau* **4**:207-222.

Roychoudhury, P., Krishnamurti, G. S. R. and Venkataraman G. S. 1980. Effects of algal inoculation on soil aggregation in rice soils. *Phykos* **19**:224-227.

Ryther, J. H. and Guillard, R. R. L. 1962. Studies of marine planktonic diatoms. II Use of *Cyclotella nana* Hustedt for bioassay of vitamin B_{12} in seawater. *Can. J. Microbiol.* **8**:437-445.

Schlipalius, L. 1990. β-carotene from algae. *Food Australia* **41**:742-745.

Schwartz, J., Shuklar, G., Ried, S. and Trickler, D. 1988. Prevention of experimental oral cancer by extracts of *Spirulina-Dunaliella* algae. *Nutr. Cancer* **11**:127-134

Shainberg, I. and Bar-Or., Y. 1990. Stabilization of fine-grained soils by a cyanobacteria flocculant. *Proc. 5th Int. Conf. Soc. Appl. Algology.* L-21.

Singh, R. N. 1950. Reclaimation of"Tsar" lands in India through blue-green algae. *Nature, Lond.* **165**:325-326.

Skulberg, O. M., Codd G. A . and Carmichael W. W. 1984. Toxic blue -green algal blooms in Europe: a growing problem. *Ambio* **13**:244-247.

Stewart, W. D. P., Rowell, P. and Rai, A. N. 1983. Cyanobacterial-eukaryotic plant symbioses. *Ann. Microbiol. (Inst. Pasteur)* **134B**:205-228.

Trevors, J. T. 1982. A comparison of methods for assessing toxicant effects on algal growth. *Biotechnol. Letts* **4**:243-246.

Venkataraman, L. V. 1986. Blue -green algae as biofertilizer. In: *CRC Handbook of Microalgal Mass Culture.* pp. 455-472. CRC Press Inc., Boca Raton Florida.

Vonshak, A .1988. *Porphyridium.* In: *Microalgal Biotechnology.* (Ed. by M. A. Borowitzka and L. J. Borowitzka) pp. 122-134. Cambridge Univ. Press, Cambridge.

Walne, P. R. and Wood, P. C. 1974. A review of some shellfish research undertaken at.the fisheries laboratories in 1973. *Shellfish Inf. Leafl. MAFF* **33**:17-35.

Webb, K. L. and Chu, F. L. E. 1982. Phytoplankton as a food for bivalve larvae. In: *Proceedings of the Second International Conference on Aquaculture Nutrition.* (Ed. by G. D. Pruder, C. J. Langton and D. E. Conklin) pp. 272-291. Louisiana State Univ. Press, Baton Rouge.

Weiss, C. M. and Helms, R. W. 1971. The inter laboratory precision test. An eight laboratory evaluation of the provisional algal assay procedure bottle test. *EPA water quality office project 16010DQT.* Univ. N Carolina/EPA, Chapel Hill.

Wilson, M. M., Jackson, M. M. and Edyvean, R. G. J. 1991. The use of algae to decolourize and remove metal ions from industrial effluents. *Br. Phycol. J.***26**:99.

IAM Culture Collection And Strategies For CO_2 Problem

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Abstract

The Microbial and Microalgal Research Center, Institute of Applied Microbiology (IAM), University of Tokyo houses a culture collection which currently collects and retains microorganisms including algae. The activities of the algal collection are not only to maintain and distribute algal strains but also to play a role as an information center for algal researchers. To perform these services effectively, it is useful to be involved in basic research within the culture collection. In IAM, the effects of CO_2 concentration on microalgae have been investigated.

Key words: culture collection; database; training course; basic research; long-term preservation; CO_2 concentration.

Introduction

Along with the development of human activities, natural environments including forests and grass land have been destroyed, and many species of animals and plants have become extinct. The status is undoubtedly the same for microorganisms. However, unlike animals and plants, ecological and taxonomic information on microalgae are still very poor and there are so many species which have not been identified. Thus it is obviously important to collect microorganisms, and to retain them in a viable state. However, it is less efficient and may be counter productive if a large number of researchers keep microorgainsms indiscriminately. In this respect, it is more efficient if culture collections collect and maintain the strains, and distribute the organisms as required.

There are several established algal culture collections around the world, but their finances, staff members and official systems are not always stable. This can be particularly relevant to a private culture collection which is organized by a professor in a laboratory, it will inevitably to be endangered if subjects of studies change on the pro-

fessor's retirement. The Microbial and Microalgal Research Center (MMRC), Institute of Applied Microbiology (IAM), University of Tokyo is specifically organized as a culture collection center in the research institute. We would like to introduce the activities of the IAM culture collection and consider the general roles of culture collections in this paper.

History

The algal culture collection was commenced by Prof. A. Watanabe in 1957 in IAM and was the first algal collection in Japan (Watanabe 1960; Shimoyama and Miyachi 1988). The work of the collection of microalgae and cyanobacteria including isolation, identification and maintenance have been taken over by a few number of staff within the research laboratory (Watanabe 1960; Iizuka 1966, 1968; Ichimura and Ito 1977). The algal strains were distributed for scientific and technological studies and education. Since the laboratory was not primarily organized as a culture collection but for research on biosynthesis of plants, the successors of the above were not the specialists for microalgae.

There had also been a bacterial culture collection in another laboratory in IAM. By the efforts of Prof. S. Miyachi, the two culture collections were united in 1989. The integrated microbial (bacteria, fungi and yeast) and microalgal collection has been funded for ten years by the Ministry of Education, Science and Culture. There are 5 staff in MMRC, two of whom are allocated to the algal section at present.

Activities

Activities of IAM algal culture collection can be summarized as follows. (1) Collection, maintenance and distribution of strains. (2) Collection and supply of information associated with the strains. (3) Provision of educational services to researchers studying algae. (4) Research directly related to the culture collection such as long-term preservation of algae. (5) Basic research in phycology. Point (1) is the basic and most important task of the collection but the next two points are also requested by various researchers. Research not only on culture methods but also on the other basic fields are necessary to provide the first three services.

1. Collection, maintenance and distribution of algal strains.

We retain 578 strains of microalgae and cyanobacteria at present which are from 8 classes (Cyanophyceae, Rhodophyceae, Euglenophyceae, Chrysophyceae, Xanthophyceae, Bacillariophyceae, Eustigmatophyceae and Chlorophyceae). They are mostly fresh water strains of green algae and cyanobacteria. Strains are maintained by serial transfer on slants of agar (1.5%), in soft agar (0.15%) or in liquid medium.

The transfer intervals are different range from 2 weeks to 1 year depending on the organism. The algae are examined by microscopy to ascertain their conditions especially with respect to contamination. Since most of the strains had been transferred without checking for a long period, some were found to be labeled with wrong names. In order to replace with correctly labelled algae and to add valuable strains, we currently obtain cultures mainly by exchange between culture collections.

On request, the strains are distributed to domestic universities, companies and other institutions and also to foreign countries for scientific and technological studies and education. The number of strains distributed in 1990 (fiscal year) was 297. These requests for strains were mostly from scientists in universities, but orders from researchers in companies have increased over the last two years.

2. Database of the strains

Information on the algal strains maintained has been recollected over two years, and is incorporated into the database. This will be published as the catalogue of the IAM culture collection and will include bacteria, fungi, yeast as well as algae. It contains the history and characteristics of each strain, composition of its medium and the condition for preservation as well as relevant references. Researchers in universities and in institutes of companies ask us often further information on the algal strains as well as general questions on algae over the telephone or while visiting IAM.

3. Training course

Researchers who are beginning to study algae in universities or companies not only need to obtain information on algae but also to acquire the basic expermental techniques. To respond to their requests, a training course on basic algal techniques is held once a year, in cooperation with Dr. Y. Hara in University of Tsukuba and members of his laboratory. About 20 participants on each course learn the basic methods of isolation, identification and culture of algae. It is also a good opportunity for them to exchange information and perspectives on algal research with each other.

4. Studies on long-term preservation

At present, all the algal strains are maintained by serial transfer. The development of long-term preservation methods will reduce the time spent to maintaining the algal strains, but the most important benefit is to minimize the risk of contamination and of incorrect labeling of the algae. Survival of cyanobacteria after freezing to -80℃ as well as -196℃ is under investigation with a number of strains, and most tested have been able to grow after thawing. We plan to adopt the thechnique for the routine preservation of strains in our collection.

5. Effects of CO_2 concentration on algal photosynthesis

Basic research is also important in culture collections, because it increases the information available on the strains. Researchers who have been studying algae can arrange the reported data and incorporate it into the database of strains. Without research in the collection, the full potential of the collection can not be realised.

One of us has been working on the effects of CO_2 concentration on microalgae. This research was initiated by the question why microalgae grown under ordinary air show a high affinity for CO_2 in photosynthesis. Nobody can answer the question completely yet, however the currently available information has recently been reviewed by Tsuzuki and Miyachi (1989, 1991). This research will hopefully lead us to a solution of the global warming problem.

Conclusion

Maintenance of algal species is indispensable not only for science but also for mankind. Many algal species have been retained in laboratories as a result of personal interest. Even culture collections in universities may not be stable after the retirement of their founding professors. Public or government authorized culture collections are necessary for preserving algal strains. We think the roles of a culture collection are not only to maintain species safely but also to support the development of algal research. To provide these services, it is important for the collection to communicate with other culture collections and to proceed with basic research.

References

Ichimura, T. and Ito, T. 1977. Preservation methods of microalgae(I). In: *Preservation Methods of Microorganisms* (Ed. by T. Nei) pp. 355-373. University of Tokyo Press, Tokyo. (Japanese).

Iizuka, H. 1966. Algae. In: *Catalogue of Cultures. Additional Edition*. pp. 218-230. The Japanese Federation of Culture Collectons of Microorganisms, Tokyo.

Iizuka, H. 1968. Algae. In: *Catalogue of Cultures. Additional Edition*. pp. 197-200. The Japanese Federation of Culture Collections of Microorganisms, Tokyo.

Shimoyama, N. and Miyachi, S. 1988. IAM algal collection. *Proceedings of Sixth International Congress of Culture Collections*. pp. 72-73. World Federation for Culture Collections.

Tsuzuki, M. and Miyachi, S. 1989. The function of carbonic anhydrase in aquatic photosynthesis. *Aquatic Botany* 34:85-104.

Tsuzuki, M. and Miyachi, S. 1991. CO_2 syndrome in *Chlorella*. *Can. J. Bot.* 69, 1003-1007.

Watanabe, A. 1960. List of algal strains in collection at the Institute of Applied Microbiology, University of Tokyo. *J. Gen. Appl. Microbiol.* 6:283-292.

NIES-Microbial Culture Collection At The National Institute For Environmental Studies: Cryopreservation And Database Of Culture Strains Of Microalgae

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Abstract

The NIES-Collection, the Microbial Culture Collection at the National Institute for Environmental Studies, was founded as the first collection center of environmentally important microorganisms in 1983. In this collection, microalgae isolated by Japanese researchers over the last 30 years have been collected and preserved most actively.

The collection has four main activities; Firstly, it collects, characterizes, preserves and distributes cultures of microorganisms. Secondly, it carries out studies on the development of long-term preservation methods of microbial strains. Thirdly, it performs studies on taxonomy, physiology and biochemistry of microbial strains. Fourthly, it is responsible for constructing a database of microbial strains to meet the requirements from researchers. Among the 4 areas of activity, cryopreservation and the database on algal strains are introduced in this paper.

Key words: culture collection, cryopreservation, database, microalgae, NIES

NIES-Collection

The NIES-Collection,the Microbial Culture Collection at the National Institute for Environmental Studies, was founded as the first collection center of environmentally important microorganisms in 1983.

Although microalgae, bacteria and protozoa related to environmental problems

will be preserved in this collection in future, microalgae isolated by Japanese researchers during the last 30 years have been collected and preserved most actively as the first step. The specific name, source, conditions of cultivation and preservation, purity, morphological and physiological characteristics, and the environmental characteristics, of all the strains collected have been re-examined, and the revised data processed using a personal computer.

The first list of microalgae maintained in the NIES-Collection (Watanabe and Kasai 1985) listed 262 strains, together with details of the NIES-Collection facilities, organization and the fundamental pattern of research. Since then, as the result of our studies on many strains isolated by us and those donated by colleagues, a considerable number of new algal strains have been added and the supplementary lists (Watanabe and Kasai 1986, 1987), the second list (Watanabe and Kasai 1988) and the third list (Watanabe and Satake 1991) were published. The total number of strains of algae in the NIES-Collection is now 518 (Table 1).

The algal strains maintained were classified into 11 classes, Cyanophyceae, Rhodophyceae, Cryptophyceae, Chrysophyceae, Bacillaliophyceae, Raphidophyceae, Prymnesiophyceae, Dinophyceae, Prasinophyceae, Chlorophyceae, and Euglenophyceae and into 276 species and 15 varieties.

The NIES-Collection has four main areas of activity; Firstly, it collects, characterizes, preserves and distributes microorganisms. Secondly, it carries out studies on the development of long-term preservation methods of microbial strains. Thirdly, it performs studies on taxonomy, physiology and biochemistry of microbial strains. Fourthly, it is constructing a database of microbial strains to meet the requirements of researchers.

Of the 4 activities, cryopreservation and the database of the algal strains maintained in NIES-Collection are presented in this paper.

Table 1. The number of strains, species and varieties of each algal class maintained in the NIES-Collection

Class	Strains	Species	Varieties
Cyanophyceae	88	46	3
Rhodophyceae	1	1	
Cryptophyceae	14	4	
Dinophyceae	58	40	1
Chrysophyceae	4	4	
Bacillariophyceae	47	28	1
Prymnesiophyceae	3	3	
Raphidophyceae	27	5	
Euglenophyceae	7	5	1
Prasinophyceae	20	12	
Chlorophyceae	249	128	9
Total	518	276	15

Cryopreservation

Normally, the algal strains are maintained by subculturing, that is the transfer of viable cells to fresh growth medium. Although this is a simple method, there is always a possiblity of contamination by other organisms and alteration of the genetic character due to mutation and natural selection. The advantages of cryopreservation of algal cultures are to maintain the original properties of the organisms and to reduce the possibility of contamination.

Since 1983 when the NIES-Collection was founded, research has been carried out to develop cryopreservation methods suitable for our strains. As the first step, the recovery of 275 strains after freezing and immediate thawing was examined using the following protocol:

1) Only cells in the stationary phase of growth were used.

2) As a cryoprotectant, dimethyl sulfoxide (DMSO) was added at the concentrations of 0, 5, 10% (v/v) to the cell suspension.

3) A two step freezing method was used. Cells were initially chilled at the rate of -1℃/min to -30℃, and then chilled rapidly to -196℃.

4) Frozen cells were rapidly thawed in a water bath at 40℃.

5) Once thawed, the cells were innoculated into growth medium and cultured under optimal condition.

Table 2 shows the recovery of 275 strains after freezing and immediate thawing. There were many viable strains of blue-green algae and chlorococcalean green algae on thawing. It is noteworthy that 13 desmid strains also survived. Subsequently, the factors affecting cellular viability following freezing and thawing have been examined in detail, in order to develop succesful cryopreservation methods. In such studies, it was important to decide how to determine post-thaw viability.

To assess cellular viability, some indicator of cell division, such as colony formation in or on agar or an increase in the cell number is the most reliable evidence of

Table 2. Survivals after freezing and immediate thawing (after Watanabe et al. 1988)

Algae	Strains examinaed	Survived strains	Dead strains
Cyanophyceae	63	56	7
Rhodophyceae	1	1	0
Cryptophyceae	2	0	2
Dinophyceae	5	0	5
Raphidophyceae	12	0	12
Chlorophyceae			
Chlorococcales	39	36	3
Volvocales	32	6	26
Conjugales	99	13	86
Prasinophyceae	4	0	4
Euglenophyceae	6	0	6

survival. In Morris's agar assay system (Morris 1976), the percentage viability (V) was calculated using the following equation:

$$V=b/a \times 100$$

where a is total number of colonies on agar before treatment, and b total number of colonies on agar after treatment. In our method using liquid culture, the percentage viability was calculated using the following expression:

$$V=(d-1)/(c-1) \times 100$$

where c is the 4 day's increasing rate of cell number before treatment, and d is the 4 day's increasing rate of cell number after treatment.

Table 3 shows the comparison of viabilities measured by Morris's and our methods. In the case of *Chlorella vulgaris*, viabilities measured using Morris's method and our method are 88.4% and 93.2%, respectively. In *Selenastrum capricor-cornutum*, viabilities measured using Morris's and our methods are 91.8% and 86.0%, respectively. We can say that there is no significant differences between viabilities calculated by the two methods. However, our method is also suitable for strains that do not form colonies in or on agar. In addition, although the FDA (fluorescein di-acetate) staining method is commonly used for determinating cell viability in higher plants, there was no relationship between viabilities estimated by FDA and our methods (Fig. 1).

Using our method, it is possible to maintain some algal strains by freezing, with a high a percentage viability. For example, the viabilities of *Pediastrum duplex* var. *duplex* (NIES-213) and *Scenedesmus acutus* (NIES-95) after 6 years storage in liquid nitrogen are shown in Table 4. *P. duplex* var. *duplex* has viability levels of over 90% after 6 years storages. In this case, 5% (v/v) DMSO was added as a cryoprotectant and the two step freezing method used. *S. acutus* also has high levels of recovery with levels of over 70% post-thaw viability after 6 years storages. In this case, 10% (v/v) DMSO was added and the two step freezing method was used.

I think that many factors, such as growth temperature, age of culture, medium

Table 3. Comparison of viabilities measured by Morris' and Watanabe's methods (after Watanabe et al. 1989)

Species	Mean	Standard dev.
Chlorella vulgaris		
Morris' method	88.4%	4.6
Watanabe's method	93.2%	4.7
Selenastrum capricornutum		
Morris' method	91.8%	10.8
Watanabe's method	86.0%	10.4

5% (v/v) DMSO was added and the two step freezing method used.

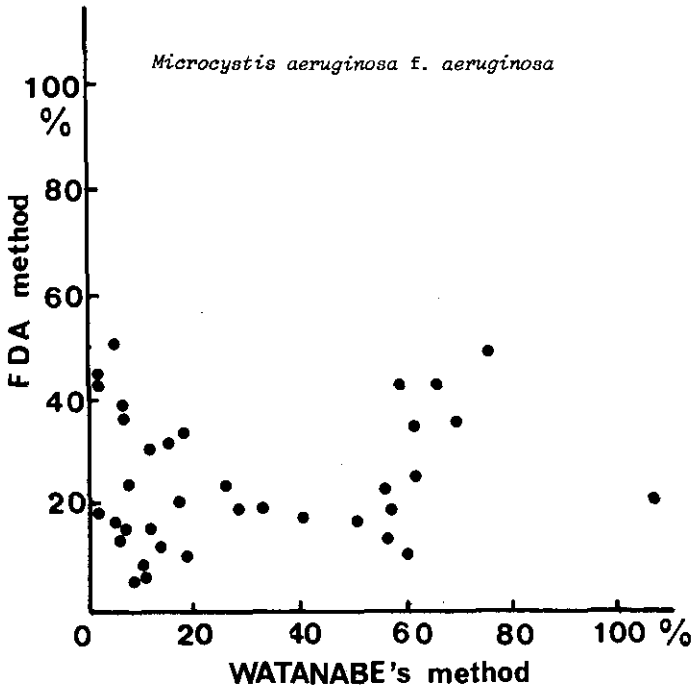

Fig. 1. Comparison of viabilities measured by FDA and Watanabe's methods.

Table 4. Viabilities of *Pediastrum duplex* var. *duplex* (NIES-213) and *Scenedesmus acutus* (NIES-95) on thawing after up to six year storage in a frozen state.

Species	Viability (%)
P. duplex var. *duplex*	
Immediate thawing	90.0
After 1 year	89.6
After 4 year	102.0
After 6 year	94.5
S. acutus	
Immediate thawing	73.0
After 1 year	78.6
After 4 year	78.0
After 6 year	70.2

5% or 10% DMSO was added and two step freezing method used.

composition and post-thaw culture conditions as well as type and concentration of cryoprotectants, rate of cooling and warming and the final temperature attained, all contributed to the post-thaw viability level of cryopreserved cells. In the NIES - Collection, each of these factor is being examined in detail to develop a successful cryopreservation method for esch species or strain. The greatest problem, however, is that there are few scientists working in the field of algal cryopreservation.

Database

The number of strains in the NIES-Collection and the volume of associated data will continue to increase. In order to keep a large quantity of data available, and to avert careless and unforseen mistakes, or data hoarding, we have been developing a Microbial Culture Collection Data Processing System (MCC system) which uses a personal computer.

Although at the present time many fundamental functions are still at the developmental stage, the MCC system can be efficiently utilized in the transferring of strains, and listing of the strain entries and strain data. We are considering the possibility of recording the strain data into a vehicle such as disk pack compatible with a personal computer and offering it to researchers with the appropriate software. The technical possibility of making a disk form with the convenient and unique characterisitics which cannot be converted into a printed form is currently being explored.

Fig. 2 shows the composition of the hardware of the NIES computer system. A fixed disk of 10Mbytes was prepared for recording all the data associated with up to a maximum of 3,000 strains. Fig. 3 shows the software of the NIES computer system. The language used is N88-BASIC (86). There are seven functions in the main menu. Once the user completes any given task, the computer automatically returns to the main menu. A basic outline of each of the functions is given below.

Data editor: Through the data editor, the inputting or revision of each of the 50 items of strain data (Table 5) can be performed. The contents of these items are classified into five categories: 1) the literal data, 2) the numerical data, 3) the items selected from the menu, 4) the date, and 5) other special items. Most of the 50 items correspond to a category from 1 to 4, except for such information as the subculture interval, references etc. Hence the input of all 50 items can be carried out using about ten processing patterns. The range in value of each item is checked carefully to prevent the input of non-existant data, values outside the limits, or other extraordinary data. The data were processed as much as possible by selection from the menu to reduce the record area, and prevent the oversight of data due to misspelling.

Output of the original register of strain data: All the strain data contained in the system is displayed according to a fixed format.

Output of search lists: There are 13 kinds of search lists in the system for conducting conditional searches. For example, there is a search function which lists all the strains scheduled for subculturing, as well as the medium and location. As the number of strains in the NIES-Collection increases, the system will prove to be an indispensable time saving device for subculturing.

Program for making up the list: Strain data from the master file is edited in a fixed format. Once editing is complete, the system prints out the list of strains, index and references.

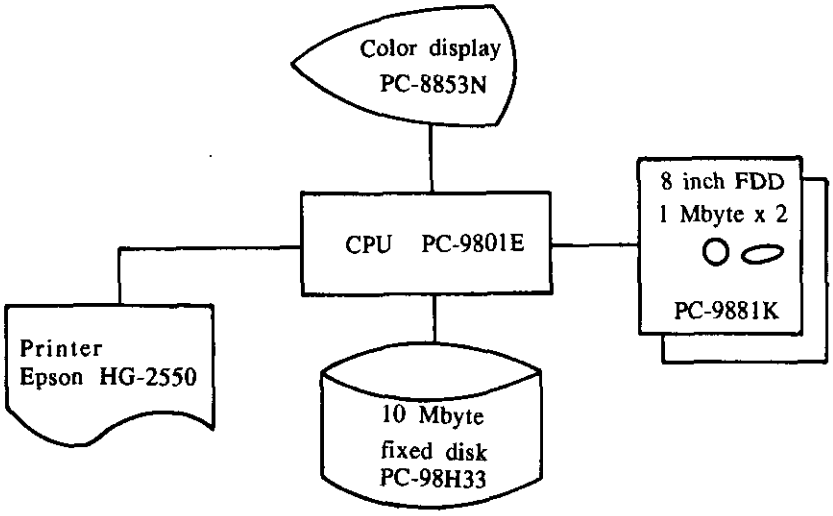

Fig. 2. Hardware components of the MCC system

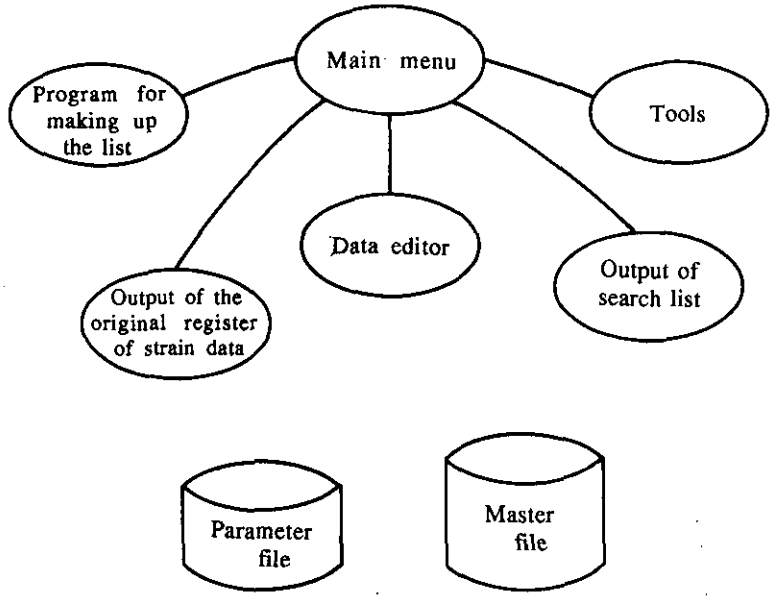

Fig. 3. Software components of the MCC system

Table 5. Strain data

No.	Message	Aux. message
1	Strain number	1 to 3000
2	Genus	Strings Max. 30
3	Species	Strings Max. 40
4	Author name	Strings Max. 40
5	Class	Select ONE from 1 to 24
6	Order	Strings Max. 20
7	Synonym	Strings Max. 110
8	Locality	Strings Max. 40
9	Habitat	Strings Max. 40
10	Collection date	19YYMMDD
11	Collector	Strings Max. 20
12	Isolated from	Select ONE
13	Isolation date	19YYMMDD
14	Isolator	Strings Max. 20
15	Isolation objective	Select ONE
16	Physical separation	Select ONE
17	Treatment	1 to 6 Combination
18	Identified by	Strings Max. 20
19	Purified by	Strings Max. 20
20	State	1 to 7 Combination
21	Bacteria check medium Name	1 to 11 Combination
22	Bacteria check medium Phase	1 to 3 Combination
23	Medium Name	Select from 1 to 73
24	Medium Phase	1 to 4 Combination
25	Preculture Temperature	-9 to 99 [℃]
26	Preculture Light Inten.	0 to 999999 [lx]
27	Preculture L. D. cycle	0 to 24 and L/D
28	Preculture Duration	0 to 31 and D. M. Y
29	Mainte. Succ. transfer Duration	0 to 999 and D. M. Y
30	Mainte. Succ. transfer Org. date	19YYMMDD (day<=28)
31	Place	Select from 1 to 31
32	Mainte. Succ. transfer Temperature	-9 to 99 [℃]
33	Mainte. Succ. transfer Light Inten.	0 to 999999 [lx]
34	Mainte. Succ. transfer L. D. cycle	0 to 24 and L/D
35	Cryoprotectant	1 to 5 Combination
36	Cooling rate	1 to 2 Combination
37	Thawing rate	0 to 99 [℃]
38	Freeze-storage temperature	-999 to 0
39	Freeze-storage time	1 to 999 and D. M. Y
40	Freeze-viability	0 to 100 [%]
41	Freeze-drying	Select 1 or 2
42	Environmental Characteristics	1 to 13 Combination
43	Physiol. and ecol. Characteristics	1 to 36 Combination
44	Miscellaneous Characteristics	1 to 22 Combination
45	Deposited by	Strings Max. 40
46	Deposition date	19YYMMDD
47	Other culture collection	Strings Max. 30
48	Personal code	Strings Max. 40
49	Other informations	Strings Max. 240
50	Reference	Number Combination

Various tool: Various simple function programs are assembled to maintain the MCC system.

Master file: Strain data is recorded in the master file, which has a memory capacity of 1 to 1.5 Kbytes per strain. This file can only be edited by using the previously introduced data editor.

Parameter file: The essential parameters for controlling the MCC system, such as the format, the range in value of each item, the field length, and a table of the menu are concentrated in the parameter file. This maintains simplicity and invites successive expansion of the entire system. Users first select and set the parameters required for controlling the programs with in the system, and then initiate the operations. As the file is improved, the program will be able to perform further functions. Most of the improvements and the expansion of the system can be achieved by the manipulation of the contents of this file.

References

Morris G. J. 1976. The cryopreservation of *Chlorella* 1. Interactions of rate of colling, protective additive and warming rate. *Arch. Microbiol.* 107:57-62

Watanabe, M. M. and Kasai, F. 1985. *NIES-Collection List of Strains First Edition 1985 Microalgae*. Microbial Culture Collection, The National Institute for Environmental Studies, Tsukuba. pp. 116

Watanabe, M. M. and Kasai, F. 1986. *Supplement to NIES-Collection List of Strains First Edition 1985 Microalgae*. Microbial Culture Collection, The National Institute for Environmental Studies, Tsukuba. pp. 25

Watanabe, M. M. and Kasai, F. 1987. *Second Supplement to NIES-Collection List of Strains First Edition 1985 Microalgae*. Microbial Culture Collection, The National Institute for Environmental Studies, Tsukuba. pp. 25

Watanabe, M. M. and Kasai, F. 1988. *NIES-Collection List of Strains Second Edition 1988 Microalgae and Protozoa*. Microbial Culture Collection, The National Institute for Environmental Studies, Tsukuba. pp. 148

Watanabe, M. M., and Kasai, F., Shimizu, A., Sawaguchi, T., Suda, S. and Niiyama, Y. 1988. The Biology of Algal Culture Collection. *Jpn. J. Freezing & Drying* 34: 119 -124 (in Japanese)

Watanabe, M. M. 1989. Maintenance of Algae. *Jpn. J.Freezing & Drying* 35: 77-81 (in Japanese)

Watanabe, M. M. and Satake, K. N. 1991. *NIES-Collection List of Strains Third Edition 1991 Microalgae and Protozoa*. Microbial Culture Collection, The National Institute for Environmental Studies, Tsukuba. pp. 163

UTEX-Culture Collection Of Algae At The University Of Texas At Austin

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ABSTRACT

In 1953 a Culture Collection of Algae was established at Indiana University where it remained until 1976 when it was moved to the University of Texas at Austin. Since 1960 the major support of the Collection has been from the National Science Foundation; additional support comes from the sale of cultures and from the University of Texas. Over 2000 strains of algae are maintained, of which the majority are freshwater in origin. Distribution of the cultures is without restriction to commercial and academic researchers. The distribution pattern over the last 5 years is given by State and Country.

Key word: freshwater algae, algal cultures, UTEX

Introduction And Historical Perspective

Prior to the 1950's the field of Phycology was for the most part dominated by morphologists and taxonomists content to depend on the whims of nature to provide them with material for study. In the late 1940's and early 1950's questions were beginning to be asked of the algae as regards their nutrition, pigmentation, sexual and asexual reproduction, etc. To answer these questions it was necessary to turn to algal cultures which could be grown in the laboratory at will. Many of the techniques which these experimental phycologists used were not new; Professor E. G. Pringsheim and a relatively small group of others had excellent success in growing algae in the laboratory. In 1928 Professor Pringsheim had published a short list of algal species which he and his students had isolated into pure culture in his laboratory at the German University of Prague. For the next 10 years the Prague laboratory was a center of algal cultivation and a culture collection had been established. With the advent of World War II, Professor Pringsheim left Czechoslovakia for England, where with the

interest and help of Professor Felix Fritsch a second collection of algae was established in the Botany school at Cambridge University.

The Cambridge Collection, based on a nucleus of cultures that Professor Pringsheim had brought with him from Prague, continued to enlarge, and under the tutelage of Professor Pringsheim a number of research students worked for varying periods of time. I was among those so privileged. Following my return to the United States, I joined the faculty of the Department of Botany at Indiana University in 1952, and in 1953 organized a culture collection of algae to serve especially the scientific community in the United States.

The Culture Collection of Algae at Indiana University was begun with a nucleus of cultures obtained from the Culture Collection of Algae and Protozoa at Cambridge University. Funds for material and technical assistance were given by the Lilly Research Laboratories of Indianapolis during 1953-55 and by the Charles F. Kettering Foundation of Yellow Springs, Ohio, during 1955-59. Since 1960 the Collection has been supported in part by grants from the National Science Foundation, in the early years from the Genetics Program and later from the Biological Research Resources Program. Income from the sale of cultures has been used since the beginning to cover the purchase of glassware, chemicals shipping containers, the postal charges, and items of equipment needed for the operation of the Collection.

In the summer of 1976 I left Indiana to join the faculty of the Department of Botany at the University of Texas at Austin, and arrangements were made for the Collection to be moved to the University of Texas at Austin. A gradual relocation of the algal stocks was accomplished during 1976-77 without any interruption of service to the scientific community which the Collection serves. By June 1977 the Collection was in full operation at its new location in Texas.

Physical Facilities

The Culture Collection of Algae shares common facilities with the teaching and research program in Phycology, in addition to the large specially constructed constant temperature room in which most of the Collection is housed. The facilities and equipment include the following:

a) Large culture room (20 X 20 feet) equipped with both lighted and indirectly lighted shelving for maintenance of algal cultures. Associated with this room is a small office, a transfer room, and space for working with the cultures and preparing them for shipment.

b) An office for the Curator, J. Zeikus, with an adjoining room for the IBM PC-AT purchased by the University for the exclusive use of the Collection. A research laboratory for the Collection adjoins the Curator's office suite.

c) In addition to the main culture room there are two other culture rooms which

provide space for some stocks and for experimental work in the algae.

d) A laboratory equipped with autoclaves, steamer, fine balance, automatic glass still, flash-evaporator, lyophilizer, fraction collector, electric oven, etc. provides approximately 1000 square feet of space for phycological activities.

e) A modern dishwashing facility is operated by the Department of Botany and takes care of all the Collection glassware without charge.

Organizational Structure

The organization of the Collection and the duties of the various individuals are outlined in the following paragraphs:

DIRECTOR-Dr. Richard C. Starr

The Director is responsible for the over-all operations of the Collection and all matters financial.

CURATOR-Dr. Jeff Zeikus

The Curator is responsible for the day-to-day operations of the Collection.

TECHNICIAN-Ms. Liu Yidong

This is a half-time position supported by funds from the NSF grant. Ms. Liu is responsible for curating those cultures maintained on agar slants and for providing transfers to the Curator when he requests them for shipping.

Undergraduate Laboratory Assistants

Two undergraduates, each working 15-20 hours per week, assist in the preparation of glassware and media for the Collection, and in the preparation of the cultures for shipping. In addition they assist in some operations involved in the transfer of those cultures maintained in liquid.

Other Personnel

Others who assist in the operation of the Collection are full-time employees of the Department of Botany, and no charges are made to the Collection for their services. These include all dishwashing, purchasing, accounting procedures including deposits of funds received in payment of cultures, and matters pertaining to appointment and payment of personnel.

General Description Of The Collection

The majority of the cultures in the Collection are of freshwater algae. There are some macroscopic marine forms as well as a number of marine phytoplankters. The greater number of freshwater algal cultures is due first to the fact that freshwater algae have been grown in culture by more investigators for a longer time, but it is also due to the lack of special facilities we would need to maintain those marine forms that require lower temperature. Such marine phytoplankton algae are now available from the Provasoli-Guillard Center in Maine. The large number of mutant stocks of *Chlamydomonas* which require special care and handling are available from the *Chlamydomonas* Genetics Stock Center at Duke University.

The current holdings of the Collection include the following:

DIVISION	CLASS	ORDERS	GENERA	STRAINS
Cyanophyta	Cyanophyceae	3	36	175
Chlorophyta	Chlorophyceae	15	221	1402
	Prasinophyceae	1	9	24
Euglenophyta	Euglenophyceae	3	11	91
Chrysophyta	Bacillariophyceae	2	17	58
	Chrysophyceae	2	6	13
	Eustigmatophyceae	1	5	9
	Xanthophyceae	3	17	42
Chlorarachinophyta	Chlorarachniophyceae	1	1	1
Cryptophyta	Cryptophyceae	1	5	13
Phaeophyta	Phaeophyceae	4	6	15
Pyrrhophyta	Desmophyceae	1	1	7
	Dinophyceae	6	17	41
Rhodophyta	Rhodophyceae			
	Bangiophycidae	4	12	25
	Florideophycidae	6	28	70
	undetermined		1	1
Chloromonadophyta	Chloromonadophyceae	1	3	4
Glaucophyta	Glaucophyceae	1	2	3
Divisio inquirende	-	-	2	2
Totals 11	17	58	415	2016

Acquisition Policy

No attempt is made to actively solicit algal strains from investigators inasmuch as one too often receives the reply that the investigator is not yet finished with his own work on the strain. Professor Pringsheim had the same experience when establishing the Cambridge and Göttingen Collections in Europe. The scientific community has become aware that cultures can be deposited in the Collection without difficulty. Generally we add cultures without hesitation if they have been used in published re-

search inasmuch as this provides the characterization that others will find useful when contemplating use of that particular culture. If the strain represent a new genus or species not in the Collection, or if it has some particularly interesting physiological, sexual, or genetic quality, it is added. The Director reserves the right to have the final decision in the addition of any strain. No strains are added which cannot be distributed without question to any person or organization requesting them. The Collection does not serve as an official patent depository.

Documentation

The Curator maintains as complete a record as possible of the history of each algal strain. Although a minimal amount of information is published in the citation of each strain in the culture catalog, more extensive information is kept in the computer, including all information to the identity of a strain with strains in other collections. The holdings of the Texas Collection are listed in the publication of microorganisms by the World Federaion of Culture Collections.

Strains are usually kept in the Collection under the same names that they had when they were deposited by a researcher. Only when there has been an obvious error will a name be changed and this, of course, is reflected in the strain's history. Users of the strains are urged to identify them in their publications with the UTEX number as well as a specific name. UTEX numbers are never changed; the numbers have no meaning of their own and only refer to its accession.

Quality Control

With competent staff handling the cultures, every effort is made to insure that there are no mix-ups involving the algal strains. I would be the first to admit that the chance for such errors is increased due to the frequency of transfer necessary when cultures are maintained by serial transfer. The best of all worlds would be to preserve all cultures under liquid nitrogen, but this is not possible in most cases. The American Type Culture Collection maintains its limited number of algal cultures in this fashion.

Continuing checks on bacteria-free cultures to see that they have not become contaminated with bacteria are facilitated by the fact that many of the maintenance media contain some substance which will support the growth of bacteria such that a chance contamination can often be detected by periodic observations of the agar slants. Approximately one half of the strains are maintained in bacteria-free condition.

Utilization

A. Services offered to users

The major service of the Collection is to preserve algal strains for future research and to provide these cultures for research to anyone requesting them. No restriction on the use of any culture is made nor are any cultures maintained that have restricted distribution. Patent cultures are not maintained.

In addition to this service, there is provided a great deal of information on the choice of particular organisms for certain purposes, methods of cultivation, references to published research, references to other sources when we cannot supply a culture, etc. Occasionally identifications are provided of material but this is rare. No charge is made to anyone for services other than for the cultures provided.

B. Requests for cultures

On the following pages are lists of the orders and cultures distributed during the period January 1, 1984, through September 1, 1990, showing the states and foreign countries receiving them. The drop in the distributions between 1984 and 1985-87 may well be due to an increase in user fees. This is discussed in more detail in later.

It is not possible to designate whether the cultures were used by students or professionals, or the purpose for which each culture was used. The majority of requests for cultures are routed through Purchasing Departments of the organizations receiving them, and deliveries are too often made to Receiving Departments rather than to individuals.

C. Visitation to the collection

The Collection does not lend itself to visit by scientists for the purpose of working on the algae. Work involving cultures in a long term affair and so the user needs to be in his own laboratory for long term research often requiring special apparatus. Even those who are interested in the taxonomy or morphology of the algae find that they must grow the algae under the proper conditions in order to make observations on which one may base valid conclusions.

User Charges

When the Culture Collection of Algae was started at Indiana University in 1953, a user feet of $1.00 per culture postpaid was set. In those days this income was sufficient to pay expenses other than the personnel costs which were underwritten by the Lilly Research Laboratories at first and then the Charles F. Kettering Foundation. In 1960 the first award was received from the National Science Foundation throught the Genetics Program. The user fee of $1.00 per culture continued until 1969. In that year

USA:	1984		1985		1986	
State	Orders	Cultures	Orders	Cultures	Orders	Cultures
Alabama	11	40	13	35	8	15
Alaska	0	0	0	0	1	1
Arizona	6	51	4	8	6	13
Arkansas	3	14	2	6	1	1
California	58	182	50	132	65	163
Colorado	21	70	8	29	11	42
Connecticut	6	16	4	5	3	3
Delaware	4	13	1	4	1	1
Dist Columbia	1	1	2	12	0	0
Florida	22	70	31	77	11	38
Georgia	4	49	5	36	1	8
Hawaii	13	153	3	17	7	90
Idaho	1	3	0	0	0	0
Illinois	15	52	19	66	18	59
Indiana	15	43	7	15	7	10
Iowa	7	29	5	21	8	22
Kansas	1	4	1	3	1	1
Kentucky	1	1	0	0	0	0
Louisiana	8	30	5	50	14	61
Maine	2	3	2	38	3	7
Maryland	18	54	12	39	12	37
Massachusetts	19	41	14	32	7	10
Michigan	14	52	12	25	13	49
Minnesota	2	3	1	1	3	3
Mississippi	2	23	5	50	5	22
Missouri	13	139	10	32	8	103
Montana	1	1	0	0	0	0
Nebraska	3	11	0	0	2	12
Nevada	2	5	2	5	1	2
New Hampshire	6	46	5	19	14	28
New Jersey	12	43	9	20	10	25
New Mexico	5	41	4	12	4	21
New York	24	114	34	110	29	66
North Carolina	21	63	23	86	23	58
North Dakota	3	7	4	16	2	5
Ohio	23	133	16	109	24	92
Oklahoma	1	2	1	1	8	19
Oregon	2	5	3	9	8	15
Pennsylvania	17	70	8	13	8	21
Puerto Rico	0	0	0	0	0	0
Rhode Island	5	45	4	18	5	47
South Carolina	4	11	2	12	8	43
South Dakota	0	0	0	0	0	0
Tennessee	13	44	9	28	2	4
Texas	29	175	23	57	26	86
Utah	3	10	1	4	1	3
Vermont	1	2	2	4	1	2
Virginia	8	23	4	12	9	19
Washington	23	148	19	35	7	10
West Virginia	1	2	2	2	2	4
Wisconsin	17	35	12	38	19	36
Wyoming	0	0	0	0	0	0
Total USA	**491**	**2172**	**403**	**1343**	**427**	**1377**

Foreign:	1984		1985		1986	
Country	Orders	Cultures	Orders	Cultures	Orders	Cultures
North America						
Canada	31	176	38	179	40	160
Africa						
South Africa	0	0	1	3	1	3
Asia						
China (P. R. C.)	2	121	3	73	1	6
Hong Kong	0	0	1	1	0	0
India	1	3	1	3	0	0
Israel	6	20	6	22	5	6
Japan	12	79	14	84	10	68
Korea	3	20	2	21	0	0
Kuwait	1	6	1	3	0	0
Philippines	1	2	2	2	0	0
Singapore	1	1	2	2	1	6
Thailand	1	1	0	0	0	0
Central America						
Costa Rica	0	0	0	0	0	0
Guatemala	0	0	1	8	0	0
Haiti	0	0	0	0	0	0
Jamaica	1	1	0	0	0	0
Mexico	0	0	1	1	0	0
Trinidad	0	0	0	0	1	2
Europe						
Austria	5	50	7	16	3	5
Belgium	0	0	0	0	0	0
Bulgaria	0	0	0	0	0	0
Czechoslovakia	1	2	1	1	0	0
Denmark	0	0	0	0	0	0
East Germany	0	0	0	0	0	0
Finland	0	0	0	0	0	0
France	4	16	3	5	0	0
Greece	0	0	0	0	0	0
Italy	3	9	1	10	5	53
Netherlands	2	10	4	19	2	2
Portugal	2	2	1	1	2	2
Spain	0	0	1	1	0	0
Sweden	0	0	2	5	1	1
Switzerland	0	0	0	0	1	1
United Kingdom	5	40	3	10	1	1
U. S. S. R.	1	8	0	0	0	0
West Germany	9	36	10	56	4	13
Yugoslovia	0	0	0	0	0	0
South America						
Argentina	1	2	0	0	2	3
Brazil	0	0	1	2	0	0
Chile	2	15	2	6	0	0
Columbia	2	17	1	1	0	0
Ecuador	0	0	0	0	1	2
Peru	0	0	1	4	0	0
South Pacific						
Australia	8	39	6	35	3	10
New Zealand	1	9	2	3	2	8
Total Foreign	**106**	**685**	**119**	**577**	**86**	**352**

USA:	1987		1988		1989	
State	Orders	Cultures	Orders	Cultures	Orders	Cultures
Alabama	8	21	7	12	8	19
Alaska	0	0	0	0	0	0
Arizona	6	10	4	29	3	31
Arkansas	0	0	0	0	3	3
California	50	99	55	135	66	217
Colorado	12	25	13	39	15	37
Connecticut	4	16	2	5	1	2
Delaware	0	0	0	0	1	2
Dist Columbia	0	0	0	0	0	0
Florida	24	66	21	46	38	101
Georgia	1	3	0	0	1	2
Hawaii	7	13	2	5	7	51
Idaho	3	6	1	2	2	3
Illinois	8	48	8	34	7	10
Indiana	5	16	12	63	9	25
Iowa	5	14	7	12	3	5
Kansas	1	1	3	3	2	2
Kentucky	2	14	2	3	5	8
Louisiana	8	18	13	28	16	56
Maine	1	1	2	2	4	18
Maryland	14	28	21	34	32	477
Massachusetts	16	32	8	20	21	38
Michigan	7	16	21	44	25	60
Minnesota	1	2	4	7	8	38
Mississippi	3	17	0	0	0	0
Missouri	16	34	20	95	28	72
Montana	0	0	1	2	3	6
Nebraska	2	4	1	1	0	0
Nevada	0	0	3	9	1	4
New Hampshire	8	19	9	29	14	52
New Jersey	15	40	16	27	13	32
New Mexico	2	11	1	1	0	0
New York	43	147	41	138	44	103
North Carolina	15	39	21	77	21	57
North Dakota	4	13	3	13	3	21
Ohio	21	87	31	105	16	80
Oklahoma	5	5	2	3	5	5
Oregon	5	6	5	10	4	8
Pennsylvania	4	18	8	28	13	39
Puerto Rico	0	0	3	19	1	6
Rhode Island	7	27	7	46	9	40
South Carolina	2	11	8	12	3	3
South Dakota	0	0	0	0	1	3
Tennessee	10	28	12	40	9	19
Texas	30	94	45	148	53	185
Utah	1	1	1	5	2	4
Vermont	2	5	2	3	3	6
Virginia	6	18	8	17	10	33
Washington	17	31	21	62	11	21
West Virginia	2	2	0	0	0	0
Wisconsin	10	38	12	25	17	33
Wyoming	0	0	0	0	1	1
Total USA	**413**	**1144**	**487**	**1438**	**562**	**2038**

Foreign:	1987		1988		1989	
Country	Orders	Cultures	Orders	Cultures	Orders	Cultures
North America						
Canada	20	66	25	249	20	66
Africa						
South Africa	1	2	4	11	1	2
Asia						
China (P. R. C.)	0	0	3	30	1	11
India	0	0	1	4	1	9
Israel	3	5	6	13	4	10
Japan	6	70	16	91	14	83
Korea	0	0	3	8	1	11
Kuwait	0	0	1	3	0	0
Malaysia	0	0	1	4	0	0
Philippines	1	2	0	0	0	0
Saudi Arabia	0	0	1	1	0	0
Singapore	2	2	2	3	0	0
Taiwan	0	0	4	16	3	8
Thailand	0	0	0	0	1	4
Central America						
Costa Rica	0	0	0	0	1	5
Guatemala	1	2	0	0	0	0
Haiti	0	0	0	0	0	0
Jamaica	0	0	0	0	0	0
Mexico	1	3	1	9	1	1
Trinidad	0	0	0	0	0	0
Europe						
Austria	5	26	3	5	8	57
Belgium	0	0	4	9	0	0
Bulgaria	0	0	0	0	1	1
Czechoslovakia	1	11	0	0	0	0
Denmark	0	0	1	1	0	0
East Germany	0	0	1	2	0	0
Finland	0	0	1	2	0	0
France	4	9	0	0	6	13
Greece	0	0	1	3	1	3
Italy	2	4	4	20	7	101
Netherlands	1	1	1	2	0	0
Portugal	1	2	0	0	1	3
Spain	0	0	2	5	0	0
Sweden	1	2	1	1	2	2
Switzerland	0	0	1	8	0	0
United Kingdom	6	12	11	26	4	16
U. S. S. R.	0	0	0	0	1	1
West Germany	7	18	9	29	10	77
Yugoslovia	0	0	2	16	0	0
South America						
Chile	3	36	1	3	1	4
Curacao	1	4	0	0	0	0
Ecuador	1	2	1	4	0	0
South Pacific						
Australia	4	20	13	67	8	54
New Zealand	0	0	1	5	2	8
Total Foreign	**72**	**299**	**126**	**650**	**101**	**552**

USA:	1990	
State	Orders	Cultures
Alabama	15	21
Alaska	0	0
Arizona	5	25
Arkansas	1	1
California	56	140
Colorado	13	128
Connecticut	1	1
Delaware	2	4
Dist Columbia	0	0
Florida	16	36
Georgia	2	2
Hawaii	9	25
Idaho	2	3
Illinois	2	7
Indiana	9	28
Iowa	0	0
Kansas	6	7
Kentucky	6	22
Louisiana	14	55
Maine	1	1
Maryland	20	295
Massachusetts	10	29
Michigan	14	36
Minnesota	0	1
Mississippi	3	12
Missouri	25	136
Montana	2	3
Nebraska	1	2
Nevada	0	0
New Hampshire	4	9
New Jersey	19	74
New Mexico	1	1
New York	26	74
North Carolina	14	35
North Dakota	1	4
Ohio	10	43
Oklahoma	0	0
Oregon	2	14
Pennsylvania	16	67
Puerto Rico	0	0
Rhode Island	3	12
South Carolina	5	7
South Dakota	0	0
Tennessee	16	31
Texas	48	163
Utah	0	0
Vermont	2	4
Virginia	5	13
Washington	9	17
West Virginia	0	0
Wisconsin	17	29
Wyoming	1	3
Total USA	**434**	**1620**

Foreign:	1990	
Country	Orders	Cultures
North America		
Canada	22	96
Africa		
South Africa	1	4
Asia		
China(P. R. C.)	1	12
India	1	3
Israel	2	3
Japan	24	103
Korea	0	0
Kuwait	1	3
Malaysia	0	0
Philippines	0	0
Saudi Arabia	0	0
Singapore	0	0
Taiwan	4	14
Thailand	0	0
Central America		
Costa Rica	0	0
Guatemala	0	0
Haiti	0	0
Jamaica	1	30
Mexico	1	3
Europe		
Austria	4	12
Belgium	2	2
Bulgaria	0	0
Czechoslovakia	1	2
Denmark	0	0
East Germany	0	0
Finland	0	0
France	5	10
Greece	1	3
Italy	2	22
Netherlands	2	2
Portugal	0	0
Spain	2	5
Sweden	2	2
Switzerland	1	1
United Kingdom	13	28
U. S. S. R.	0	0
West Germany	7	76
Yugoslovia	0	0
South America		
Chile	3	19
Curacao	0	0
Ecuador	0	0
South Pacific		
Australia	2	12
New Zealand	1	4
Total Foreign	**106**	**471**

Summary to UTEX Cultures Distributed Since January 1, 1984

	1984		1985		1986	
	Orders	Cultures	Orders	Cultures	Orders	Cultures
USA	491	2172	403	1343	427	1377
Foreign	106	685	119	577	86	352
Total	**597**	**2857**	**522**	**1920**	**513**	**1729**

	1987		1988		1989	
	Orders	Cultures	Orders	Cultures	Orders	Cultures
USA	413	1144	487	1438	562	2038
Foreign	72	299	126	650	101	552
Total	**485**	**1443**	**613**	**2088**	**663**	**2590**

	1990 (1/1/90-9/30/90)	
	Orders	Cultures
USA	434	1620
Foreign	106	471
Subtotal	540	2091
*Estimated 1/1-12/31/90	52	590
*Estimated 1990 total	**692**	**2681**

*For the years 1985 through 1989, the period 10/1-12/31 represented 22% of the orders and 22% of the cultures supplied annually. Thus, the projected totals for 1990 includes an estimated 22% of orders and cultures for 10/1/90-12/31/90.

over 12,000 cultures had been sent out, mainly for immediate use in the classroom. This great increase in numbers led to the NSF-approved arrangement that the function of the Collection be restricted in the main providing cultures for research; Carolina Biological Supply Company and Ward's Natural Science Establishment agreed to enlarge their offerings of algae for teaching, using strains that the Collection had found to be most in demand.

In order to accomplish this new mission the Collection raised its user fees to $4.00 for academic customers and $10 for commercial organization. This was slightly higher than the fee charged by the companies that would supply teaching cultures. These fees remained in effect for the next 14 years during which time inflation gradually reduced the buying power until in 1984 it was necessary to increase the fees to $10 per culture to academic institutions and $25 per culture to commercial organizations.

As can be seen in comparing the numbers of cultures distributed in 1984 with those for the next three years, there was a noticeable decline in requests for cultures, but in more recent years this trend has reversed.

Advisory Committee

The following phycologists serve as an Advisory Committee to the Collection:

Dr. Annete Coleman	Dr. Bruce Parker
Brown University	Virginia Polytechnic Inst.

Dr. Lynda Goff
Univ. of Cal., Santa Cruz

Dr. Robert Hoshaw
Univ. of Arizona

Dr. Patricia Walne
Univ. of Tennessee

Advertisement Of The Collection To The Scientific Community

At various times since it was begun in 1953, lists of the culture in the Collection have been published in various scientific journals thereby insuring not only a good distribution to many scientists but also availability to others using the libraries. Reprints are also purchased and distributed free of charge to those requesting them. The last list was published in 1987 as a supplement to the September 1987 issue of the JOURNAL OF PHYCOLOGY. This list included not only a list of the available cultures but lists of maintenance media and references to published research in which many of the algae had been used. The publication was 47 printed pages.

The Phycological Society of America has been a great supporter of the Culture Collection. When the item for publication of a new catalog was removed from an earlier grant proposal to the National Science Foundation, The Executive Council of the Society voted to absorb the approximately $6000 in page charges and publish the catalog as a supplement to the September 1987 number of the JOURNAL OF PHYCOLOGY. This also meant an automatic distribution to more than 2000 members and libraries. A new catalog is planned for 1993 with funds for its publication coming from the income from the sale of cultures.

A continuing advertisement for the Collection comes through the citations in published research. Researchers are urged to identify their materials with the UTEX number and to refer to the Collection as the source of the algae.

Data Activities Of WFCC World Data Center On Microorganisms

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Abstract

The World Federation of Culture Collections (WFCC) relocated its data center "WFCC World Data Center on Microorganisms (WDC)" to the Institute of Physical and Chemical Research (RIKEN) in 1986. Since then WDC/RIKEN has developed databases, one of which is on algal strains retained in culture collections throughout the world. Access routes and search strategy of the database are introduced.

Key words: algae, on-line, databases, culture collections

Introduction

Microbial strains are genetic resources which are invaluable for the development of life sciences. They have been collected, evaluated, preserved and distributed within the scientific community of science and technology. Culture collections, therefore, have been active for many years. However, it is not a feasible scheme to set up one huge culture collections which maintains all the microbial strains ever isolated or developed. Currently there are about 500 culture collections are active in various areas and countries.

WFCC World Data Center on Microorganisms (WDC) is a data center of The World Federation for Culture Collections, one of whose roles is to increase the flow of information between culture collections and users. WDC used to be based in the University of Queensland and was relocated to the Institute of Physical and Chemical Research (RIKEN: Rikagaku Kenkyusho) in 1986. Since then WDC in RIKEN has developed databases on culture collections and microbial strains. Names and contents of the databases are:

ALGAE: a database on algal strains

CCINFO: a database on culture collections
JCM: a database on the strains of JCM
STRAIN: a list of names of strains held
HDB: a database on monoclonal antibodies
IRIS: a bibliographic database on plant

The database ALGAE is introduced in the following.

Access Route

ALGAE is accessible on-line from your laboratories. You are able to use one of networks which are shown in Fig. 1. That is, international packet switching systems (PSS), academic networks which are interconnected by TCP-IP protocols, Microbial Strain Data Networks (MSDN) and commercial value added networks (VAN).

WDC will register you as an authorized user of WDC databases free of charge if you apply. As for the networks, you have to obtain the status of an authorized user from the secretariats of each network. In the case of academic networks, you are advised to consult with the manager of the computer center and/or the local area network (LAN) in your organization. If your LAN is connected to any wide area networks (WAN) then you just type

telnet "IP-address of WDC"

into your terminal or personal computer which is linked to your LAN.

The Database ALGAE

In the database ALGAE, you can search algal strains which are preserved in 58 algal collections in the world. The geographical distribution of the algal collections in ALGAE is:

Argentina	1
Australia	7
Austria	1
Canada	5
Czechoslovakia	3
Egypt	1
Federal Republic of Germany	2
India	2
Indonesia	3
Italy	1
Japan	1

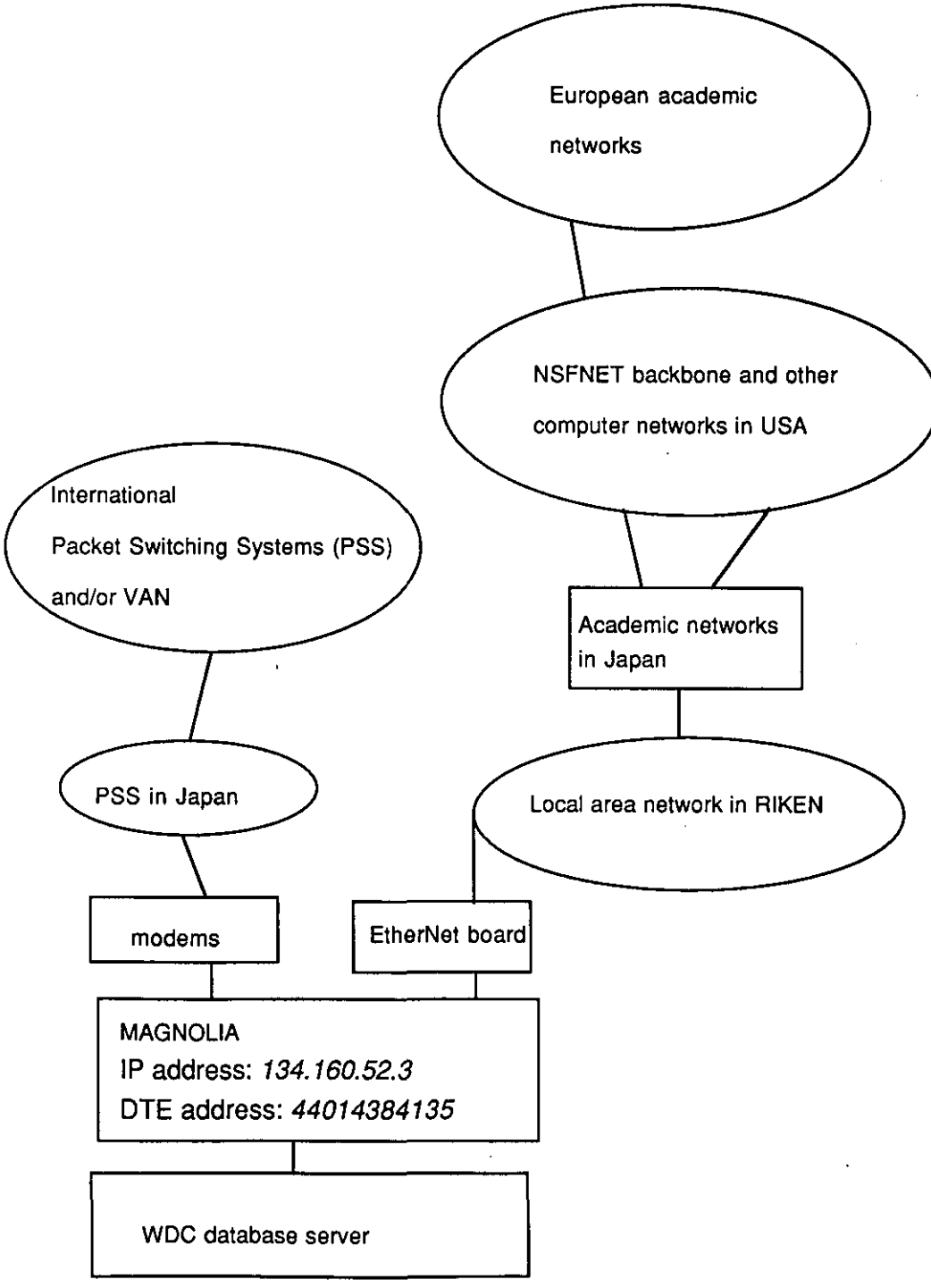

Fig.1. Networks for Accessing WDC system

Jugoslavija	1
Mexico	4
Norway	1
Philippines	3
Russia	5
Senegal	1
Sri Lanka	1
Thailand	1
The People's Republic of China	2
United Kingdom	4
USA	8

Data which you will see in ALGAE is the same as you find in catalogues of the culture collections.

Login To WDC System And Select A Database

WDC databases are provided on an interactive software package called FAIRS-1. The system will ask you a question to which you answer or choose an option. When connection between you and WDC is established, you are asked to type in your user identification code and password after a welcoming message:

```
World Data Center (RIKEN)
login: xyz1234
password: abc9876
```

If your code and password which you sent to WDC by typing were registered by WDC, a main menu of WDC databases will be displayed on your screen and you are asked to select one of options in the menu:

```
====================
 Welcome to WDC (Riken)
====================

 *** WDC MAIN MENU ***

 1. News
 2. Mail
 3. Data Base Search
 4. Profile
```

```
x. logout
Please select [1-4, x] : 3
```

The option "3" is to search one of the databases. It takes a few seconds to activate databases after you type "3":

```
=== Database server system. ===

* * * * * * * * * * * * * * * * * * * *
* * *   Please type ENTER key!   * * *
* * * * * * * * * * * * * * * * * * * *
```

You hit ENTER/RETURN key of your personal computer when the above message is displayed on your screen and you are introduced to WDC databases:

```
 FAIRS-I (V10/L30)
*  〈〈NOTICE from WDC - 'DATABA
--------------
------
--
*  Please select one of the DATABASES in the above
*  by typing        sel NAME-OF-DATABASE
*  like             sel ccinfo

*  Please leave your message in the mailing system
*  of WDC, to 142: CDT0007 on DIALCOM or to
*  r35118@rkna50. riken. go. jp on academic networks.

LISTING OF AVAILABLE DATABASE(S)
ALGAE    CCINFO    HDB    IRIS    STRAIN

RS〉
```

A character string "RS〉" is a prompt from the system which now waits for your command.

The geographical distribution of algal collections in the above were prepared by searching the database CCINFO which is a directory of culture collections of microbial strains. The procedure is:

```
RS〉select ccinfo          〈- to select the database CCINFO
```

RS>show element <- to check names of data elements of CCINFO

LISTING OF 35 ELEMENT(S)

REGISTRATION_NUMBER (REG#)	ACRONYM (ACRO)
CORRESPONDENT (CORR)	FULL_NAME (FULL)
INSTITUTION_NAME (INST)	ADDRESS (ADDR)
COUNTRY (COUN)	MAIL_NUMBERS (MAIL)
HOST_DIRECTOR (HDIR)	HOST_ADDRESS (HOST)
STATUS (STAT)	SPONSORS (SPON)
DIRECTOR (DIRE)	CURATORS (CURA)
NUMBER_OF_STAFFS (STA#)	COMPUTER_SPECIALIST (COMP)
MAIN_SUBJECTS (MAINS)	SUBCOLLECTIONS (SUBC)
CULTURE_HELD (CULT)	
AVAILABILITY_OF_ CULTURES (AVAI)	
CATALOGUE (CATA)	COMPUTERIZED_CATALOGUE (CCAT)
COMPUTER_AVAILABILITY (CAVI)	
PATENT_DEPOSITS (PATE)	STORAGE_SERVICE (STOR)
DISTRIBUTION (DIST)	*IDENTIFICATION (IDEN)*
TRAINING (TRAI)	CONSULTATION (CONS)
OTHER_SERVICES (OSER)	DATA_SUBMITTER (SUBM)
DATE_OF_REPLY (DREP)	DATE_OF_ENTRY (DENT)
SERIAL_NUMBER (SER#)	KEYWORD (KW)

RS>search cult algae@ <- to search collections which hold algal strains (the character "@" is used for truncation)

MASTER SEARCH IN PROGRESS
58 FOUND. $1 SAVED. <- algal collections are 58

RS>and country 'United Kingdom' <- to search algal collections in United Kingdom

```
MASTER SEARCH IN PROGRESS
4 FOUND. $2 SAVED.                    <- four algal
                                         collections are
                                         in UK
```

In the above examples, "select", "search" and "and" are commands. The system always returns to display "RS>" when it completed your order.

Commands For An Interactive Search Of ALGAE

While you are in the option "3: Data Base Search", you are able to select or switch to the database ALGAE anytime when you see the system prompt "RS":

```
RS>sel algae          to select/switch-to
                      the database ALGAE
```

In ALGAE the following data items are implemented:

```
RS>show el                    <- to show a list of elements

LISTING OF 7 ELEMENT (S)

SERIAL_NUMBER (SER#)          SCIENTIFIC_NAME (SN)
AUTHOR (AU)                   CULTURE_COLLECTION (CC)
STRAIN_DESIGNATION (DE)
STRAIN_DATA (SD)              KEYWORD(KW)
```

A command "show" is also useful to get explanation of each item:

```
RS>show el (sn)
LISTING OF SPECIFIED ELEMENT (S)
SCIENTIFIC_NAME (SN)
    HEADER ('Scientific name')
    CHARACTER; VARIED; ADJ (LEFT); OUTPUT; INDEX
    INVERTED; OCC (1); LENGTH (56);

RS>show el (sd)

LISTING OF SPECIFIED ELEMENT (S)
STRAIN DATA (SD)
    HEADER ('Strain data')
```

```
TEXT; OUTPUT; LENGTH (509);
```

The explanation above tells you, for example, "sn" and "sd" stand for a data item "scientific name" and "strain data" respectively.

A command "explain" will help you get more information on usage of commands:

```
RS>explain
-------------------------------------------------------------------------------
FAIRS RS HAS FUNCTIONS TO FIND INFORMATION WHICH SATISFIES THE SPECIFIED
   SEARCH CONDITION FROM STORED INFORMATION IN DATABASE AND TO DISPLAY
   THEM.  FOLLOWING COMMANDS ARE PROVIDED.

AND              : TO ADD SEARCH CONDITIONS TO PRECEDING RESULT
BROWSE           : TO DISPLAY INDEX TERMS
CALL             : TO LOAD AND EXECUTE AN USER PROGRAM
CANCEL           : TO CANCEL SAVED SET, SAVED INDEX TERM, CATALOG, LINE IN
                   CATALOG OR TO FREE EXITROUTINE
'CATALOG
   NAME'         : TO EXECUTE CATALOG (SIMILAR TO 'EXEC')
COMMENT          : TO DESCRIBE A COMMENT
CONTROL          : TO SET/RESET THE EXECUTION STATUS OF CATALOG
DEFAULT          : TO GIVE DEFAULT VALUE TO KEYWORD PARAMETER IN
                   CATALOG
DEFINE           : TO PREPARE TO CALL EXIT-ROUTINE DEFINED
EDIT             : TO EDIT ANY COMMANDS IN CATALOG
END              : TO END RS
ENDIF            : TO INDICATE THE END OF NESTED 'IF'
EXEC             : TO EXECUTE CATALOG
EXPLAIN          : TO EXPLAIN COMMAND SYNTAX
FS               : TO CHANGE SCREEN MODE
HELP             : TO HELP CONVERSATION GOING
HISTORY          : TO DISPLAY HISTORY OF CONVERSATION
IF               : TO SET CONDITION TO RUN UNDER COMMANDS IN NESTED 'IF'
INCLUDE          : TO EXECUTE EXTERNAL CATALOG IN USER'S DATASET
INDEX            : TO DISPLAY THE SET IN FORM OF INDEX LIST
'LINE NUM'       : TO REPLACE/INSERT/DELETE A COMMAND LINE BY 'LINE NUM'
ENTER 'EXPLAIN*' FOR MORE EXPLANATION/SYNTAX
NOT              : TO REMOVE RECORDS, SATISFY CONDITIONS, FROM PRECEDING
                   RESULT
```

OPTIONS	: TO SET SOME OPTIONS
OR	: TO ADD RECORDS, SATISFY CONDITIONS, TO PRECEDING RESULT
OUTPUT	: TO DISPLAY SEARCHED RECORDS
PFK	: TO SET COMMAND TO PF KEYS
PROFILE	: TO MARK/FREE THE CATALOG WITH PROFILE ATTRIBUTE FOR SDI
PROMPT	: TO PROMPT TO EDIT COMMAND LINES
QEND	: TO END A QUERY
QSAVE	: TO REGISTER EXECUTED RETRIEVAL COMMANDS TO TEMPORARY CATALOG
RECEIVE	: TO RECEIVE THE RESULT OF SDI VIA THE FILE
RENUMBER	: TO RENUMBER THE LINE NUMBER OF COMMAND LINES IN CATALOG
SAVE	: TO SAVE SET, INDEX TERM OR ANY OTHER INFORMATION
SEARCH	: TO SEARCH RECORDS WHICH SATISFY CONDITIONS
SELECT	: TO SELECT DATABASE TO BE PROCESSED
SHOW	: TO SHOW DATABASES, ELEMENTS, OR ANY OTHER SAVED INFORMATION
SORT	: TO SORT THE SEARCHED RECORDS UNDER THE KEY SPECIFIED
SYNONYM	: TO SET THE USE OF SYNONYM FUNCTIN

FOR MORE INFORMATION ON EACH COMMAND AND ITS SYNTAX, ENTER: EXPLAIN 'COMMAND NAME'.

As you see, FAIRS-I provides commands in English daily used.

An Example Of Searching ALGAE

In this section, a user searched algal strains by use of key words and acronyms of culture collections in the database ALGAE and then locate the culture collections by use of the database CCINFO

RS〉bro kw =freshwater	〈- to browse keyword similar to "freshwater"
BROWSING OF ELEMENT 'KW'	〈- the system show a part of keywords and the number of strains relevant

W-NO.	RECORDS	VALUE
#00001	2	FREIBURG
#00002	2	FREISING
#00003	3	FRENCH
#00004	1	FREQUENTLY
#00005	305	FRESH
* #00006	923	FRESHWATER
#00007	1	FRESNEL
#00008	1	FREZ
#00009	23	FRG
#00010	1	FRIABLE
#00011	2	FRIBOURG

RS〉sea kw freshwater
923 FOUND. $1 SAVED.

〈- to search strains whose keywords include "freshwater" and 923 strains are found to be sotred as a group $1.

RS〉or au freshwater
2518 FOUND. $2 SAVED.

〈- to add strains whose "au" data element includes "fresh-water" to the group $1

RS〉or sd freshwater
2518 FOUND, $3 SAVES.

〈- to add strains whose "sd" data elemetn includes "fresh-water" to the group $2

RS〉and cc utex
1642 FOUND. $4 SAVED.

〈- to find the strains in the culture collection whose acronym is "UTEX"

RS〉out el(all) rec(1:2)

〈- to display data of the first two strains

#1
Serial number 9191
Scientific name
Actinastrum hantzschii
Author (s) 〈gs〉Lagerheim 〈ss〉Lagerh.
〈sy〉Chlorophyceae; freshwater
Culture collection UTEX
Strain designation *LB 605*
Strain data isol. G. Paris 152

#2
Serial number 9208
Scientific name
Amphora veneta
Author (s) ‹gs› Ehrenberg ‹ss› Kuetz.
‹sy› Bacillariophyceae; marine except 2305
Culture collection UTEX
Strain designation 2305
strain data isol. Payne-Howell; Oklahoma, USA;
freshwater (Payne-Howell and Pfiester 1983)

RS›sea $2 and cc ccap ‹- to search the strains of the group $2
816 FOUND. $5 SAVED. preserved in the collection "CCAP"

RS›out el(all) rec(1:2)

#1
Serial number 1765
Scientific name
Anabaena ambigua
Author (s) ‹gs› (Bory) Born. & Flah. ‹ss› Rao
Culture collection CCAP
Strain designation 1403/7
Strain data Mitra; LB; freshwater; M3

#2
Serial number 1766
Scientific name
Anabaena catenula
Author (s) ‹gs› (Bory) Born. & Flah. ‹ss› (Kuetz.)
Born. & Flah.
Culture collection CCAP
Strain designation 1403/1
Strain data Manten; AB; Holland; freshwater; M17

RS›sea $2 and cc nies
NO RECORDS FOUND

```
RS>sea cc nies                    <- to search strains of the group $2 pre-
371 FOUND. $6 SAVES.                served in the collection "NIES"

RS>out el(all) rec(1:2)

#1
Serial number     5984
Scientific name
    Achnanthes longipes
Author (s)        <ss>Agardh
Culture collection     NIES
Strain designation     330
Strain data       Kawazu/Shizuoka (1985-05); Unialgal,
    Clonal, T. Sawaguchi (1985-05-); Culture conditions:
    f/2 (Liquid), 10 C, 2000 lx; Characteristics: Marine;

#2
Serial number     5985
Scientific name
    Achnanthes minutissima
Author (s)        <ss>Kutzing
Culture collection     NIES
Strain designation     71
Strain data       Kosaka River/Akita (1983-04); Axenic,
    Clonal, A. Yuri (1983 -09);
    Identified by: M. Mizuno; Culture conditions: CSi, M Chu
    No. 10 (Liquid), 20 C, 3000 lx; Characteristics:
    Indicator, Freshwater;

RS>sea sn anabae@                 <- to search strains whose name starts
    257 FOUND. $7 SAVED.            with "anabae"

RS>out el(all) rec(1:2)

#1
Serial number     7
Scientific name
Anabaena variabilis <cf.>
Author (s)        <gs> Bory de St. Vincent <ss>Kutz.
Culture collection     ASIB
```

Strain designation S 317
Strain data K. SCHWARZ, 1975, soil, Isle of Lavsa (Jugoslavia)

#2
Serial number 538
Scientific name
Anabaena cylindrica
Author (s) ⟨ss⟩Lemmermann
Culture collection ATCC
Strain designation 29414
Strain data C.P. Wolk*IUCC B381*CU B1446/1c (Anabaena inequalis)*Utrecht P32. Heterocysts (Nature 205: 201-202, 1965; Am. J. Bot. 53: 260-262, 1966; J. Bact. 96: 2138-2143, 1968; ibid., 103: 153-158, 1970; Planta 86: 92-97, 1969; Biochemistry 12: 791-798, 1973; Plant Physiol. 52: 480-483, 1973; J. Cell Biol. 61: 440-453, 1974; Biochem. Biophys. Res. Comm. 67: 501-507, 1975; J. Phycol. 10: 352-355, 1974). (Medium 616 26C under light of 2000 to 3000 lux)

RS⟩sel ccinfo ⟨- to switch the active database from ALGAE to CCINFO and locate culture collections "ASIB" and "ATCC" in the above output

RS⟩sea acronym asib or acronym atcc
2 FOUND. $1 SAVED.

RS⟩out el (corr acronym full address country)

#1
CORRESPONDENT Brandon, Mrs. Bobbie A.
ACRONYM ATCC
FULL NAME American Type Culture Collection
ADDRESS 12301 Parklawn Drive
Rockville
Maryland
20852
COUNTRY USA

#2
CORRESPONDENT Gaertner, Dr. G.
ACRONYM ASIB
FULL NAME Algensammlung am Institut fur Botanik
ADDRESS Sternwartestrasse 15
Innsbruck
A-6020
COUNTRY Austria

RS> end <- to close the interactive session in the option "3: Data Base Search"

FAIRS ENDED
****
*** Please type CTRL + D key ***
****
XWA1040I LOGGED OFF

Electric mail in WDC system

In the option "2: Mail" you are able to send and receive mails among registered users and WDC. In the following example, a user sent a mail to WDC, of which title is "Query" and the text is "Would you please send my order of strain "-----" to CCxxxx? Hideaki". A user has to hit CTRL -key and D to send out a mail. The system will display "EOT" when the mail was successfully delivered.

*** WDC MAIN MENU ***
1. News
2. Mail
3. Data Base Search
4. Profile
x. logout
Please select [1-4, x] : 2

=== MAIL ===
1. Read mail
2. Mail to the WDC
3. Mail to the another user
4. User list
r. Return to the main menu

Please Select [1-4, r] : 2

Mail to WDC
Subject : Query
Would you please send my order of strain "-----" to
CCxxx?
Hideaki
EOT

=== MAIL ===
1. Read mail
2. Mail to the WDC
3. Mail to the another user
4. User list
r. Return to the main menu
Please Select [1-4, r] : r

*** WDC MAIN MENU ***
1. News
2. Mail
3. Data Base Search
4. Profile
x. logout
Please select [1-4, x] : x

NO CARRIER

Conclusion

WDC/RIKEN provides on-line databases on microbial strains and a mailing system. By use of the database, users can effectively search for strains retained in various culture collections all over the world. In addition to the search option, they can directly exchange mail using their personal computers. It is important for scientists carrying out research on algae to recognize the value of electronic processing of information to cooperate and standardize their data format as well as regularly updating their data.

Abstract of Special Lecture On: Sexual Bipolarity As A Basic Concept For Both Homo- And Heterothallism In Microalgae

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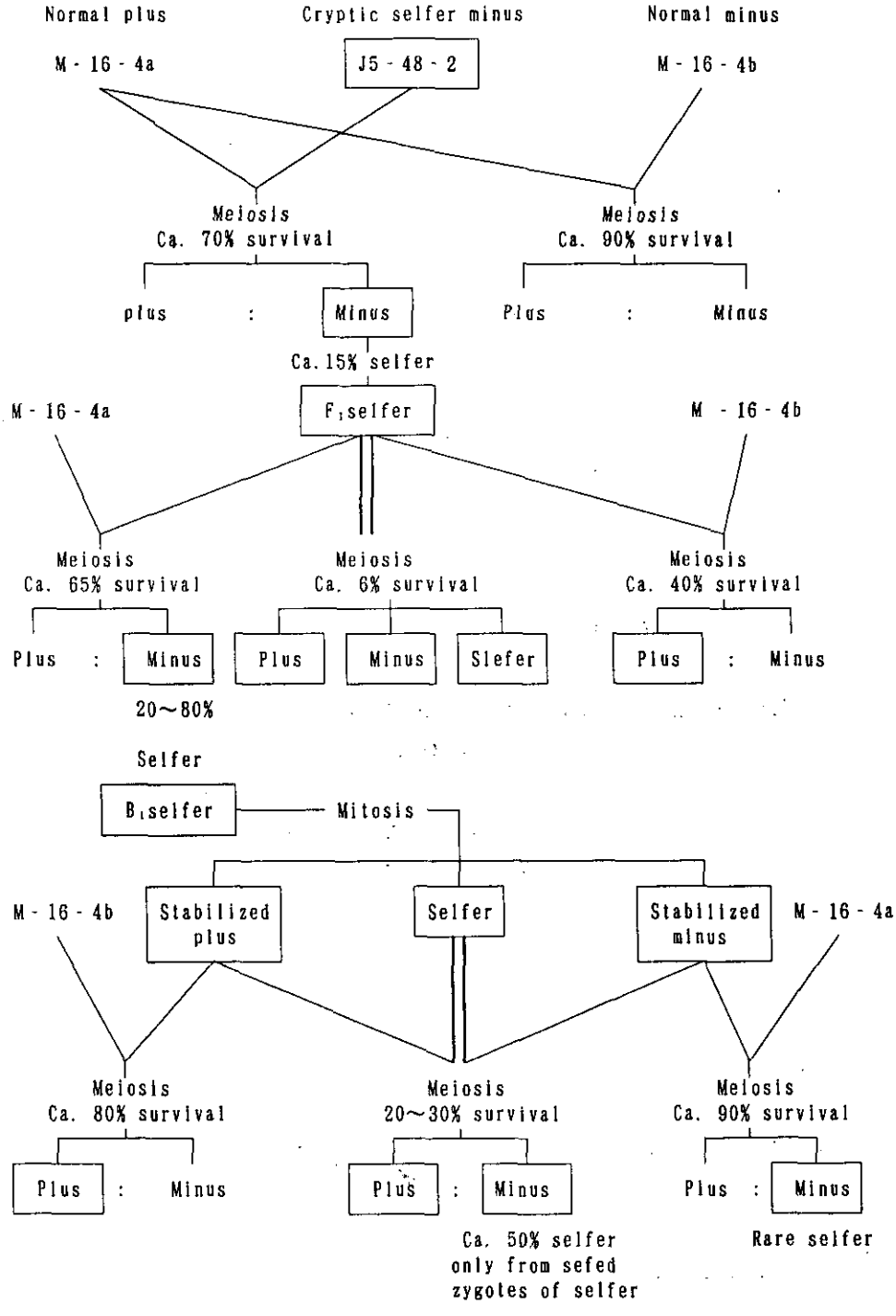
As explicitly argued by Hartmann several decades ago, one basic concept on sexuality may be that there can be no sexual union without sexual differentiation. This sexual bipolarity concept, however, has been challenged by experimental phycologists. Since anisogamous algae produce two types of gametes by definition, it has been a point of controversy whether or not any distinct physiological differentiation can be recognized between copulating gametes in isogamous homothallic algae.

The genus *Chlamydomonas* contains many isogamous species of which some are homothallic, and others heterothallic. Studies on heterothallic species, such as *C. reinhardtii* and *C. moewusii* etc., indicate that the crucial life-cycle events, sexual fusion, spore formation and meiosis, are all controlled by one regulatory supergene, which is known as the mating-type gene. On the other hand, studies on a homothallic species, *C. monoica*, suggest that homothallic species also depend upon the same kind of genetic systems as heterothallic species. In other words, we can expect gametes of opposite mating types in an isogamous homothallic *Chlamydomonas*.

The sexual reproduction of *Closterium* occurs by conjugation of the gametangial cells which are morphologically similar to each other and also to the vegetative cells. The zygote is formed by fusion of the isomorphic gametes, which have migrated equal distances, in the conjugation tube. In this sense, all known species of *Closterium* are isogamous. As in *Chlamydomonas*, both homo- and heterothallism are known in *Closterium*. It has been considered that homo- and heterothallism are two distinct alternative categories, with microalgae classified into either one or the other group. This holds true for most, if not all, strains which we have isolated into clonal cultures from many natural populations. However we obtained an intermediate form of *Closterium ehrenbergii*, namely selfing clones (see Fig.1). Although derived from heterothallic parents, these selfing clones could form zygotes between cells of the same single clone as do homothallic strains. Since they have inherited the mating-type allele from a particular *minus* parent and could be crossed with a *plus* clone, they

should be considered as *minus* and yet, could be crossed with another *minus* clone as well. Selfed zygotes, formed within a single clone, gave rise to only low viability progeny, whereas crossed zygotes, obtained when a selfing clone was mixed with either *plus* or *minus* clone, yielded high viability progeny. Thus, it is clear that a selfing clone can produce both *plus* and *minus* gametes. This was confirmed by splitting and recloning a number of pairs of conjugating gametangial cells. To incorporate all our observations, we propose in a model that selfing in *C. ehrenbergii* may occur by unidirectional mating-type gene conversion from *minus* to *plus*. In addition, we suggest that the mating-type switching mechanisms found in the homothallic yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* may be at work in homothallic strains of *Closterium*. In conclusion, we consider that the sexual bipolarity concept is very important to learn the evolutionary meanings of homo-and heterothallism in microalgae.



=cross, ||=self, :=1 : 1 segregation

Fig. 1 Viabilities and mating type ratios of meiotic products and appearance of selfers in pedigrees of normal *plus* and *minus* and cryptic selfer *minus* dones.

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PROCEEDINGS OF THE SYMPOSIUM ON CULTURE COLLECTION OF ALGAE

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