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Yoshiaki Hara*, Isao Inouye*, Mayumi Erata*, Hisayoshi Nozaki** and Makoto M. Watanabe**: Report of International Phycology Forum (IPF) in Tsukuba; Phycological Sciences —Today and Tomorrow—

International Conference Room in University of Tsukuba, 23th to 27th August, 1993

This forum was planned and organized by members of the Organizing Committee of the IPF listed below and sponsered by the Ministry of Education, Science and Culture, Japan, University of Tsukuba, Tsukuba EXPO'85 Memorial Foundation, Global Environmental Forum and several private companies. It was also carried out as a satelite symposium under the auspices of the Organizing Committee of the XV International Botanical Congress, Tokyo, the Phycologcal Society of Japan and Japanese Society of Marine Biotechology. Current topics selected from the basic and applied phycology were published and discussed in 6 symposia (36 invited papers); Mangrove ecosystem and algae (S1-S11), Algae as constituents of the carbon cycle and global environment (S12-S15), Algae as resouces for technology and industry (S16-S20), Algae as a model experimental system for plant sciences (S21-S26), Biodiversity and phylogeny of algae —Morphylogy and molecular aspects—(S27-S31) and Algal blooms in eutrophicated waters (S32-S36), memorial lectures in honor of Professors Tamiya and Avron (2 invited papers), a special seminar open to the public entitled "*Microcystis* blooms in Lake Kasumigaura." (5 invited papers) and a poster session (40 contributed papers). All of the abstracts are given in this report. Participants totalled 220 including 46 from 18 different overseas countries. A field trip to Lake Kasumigaura including a visit to the Microbial Culture Collection of the National Institute for Environmental Studies and JOEL Ltd., Tsukuba Branch was also made as an excursion of this forum.

Abstract

S-1: MANGROVE MACRO-ALGAL STUDIES IN AUSTRALIA: A REVIEW. *R. J. KING*. (SCHOOL OF BIOLOGICAL SCIENCE, UNIVERSITY OF NEW SOUTH WALES, SYDNEY, AUSTRALIA)

Until the 1980s mangrove macroalgae in Australia were dealt with only indirectly in taxonomic works or listed in the works of Dr Erika Post. Subsequently there have been several publications on the mangrove algal floras of particular regions in temperate Australia. Most recently we have completed a survey of the entire coast with particular attention to the phycologically poorly known tropical coasts. Results of that survey are discussed here.

In addition there has been a range of studies on the morphology, anatomy, ultrastructure and taxonomy of *Bostrychia, Caloglossa* and *Stictosiphonia*. Particular emphasis has been placed on physiology of mangrove algae especially in relation to salt tolerance.

S-2: MORPHOLOGY AND TAXONOMY OF *CALOGLOSSA* SPECIES (DELESSERIACEAE, PHODOPHYTA) FROM AUSTRALIA. *J. TANAKA* (NATIONAL SCIENCE MUSEUM, TOKYO, JAPAN) & N. YAZAKI (YAMANASHI UNIV., YAMANASHI, JAPAN)

Caloglossa is a most common genus occurring in brackish waters, especially in mangrove areas in the tropical to temperate regions all over the world. Vegetative and reproductive plants of the following taxa of *Caloglossa* were collected from mangrove areas in Australia: 1) *C. leprieurii* var. *hookeri* ♂ (male), ♀ (female and carposporophyte), ⊕ (tetrasporophyte), 2) *C. leprieurii* f. *leprieurii* with ♂, ♀, ⊕, 3) *C. adnata* with ♂, 4) *C.*

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ogasawaraensis with ♂, ⊕, 5) *C. stipitata* with ♂, ♀, ⊕, 6) *Caloglossa* sp. with ♂, ♀, ⊕. They can be distinguished by the following vegetative characteristics: 1) degree of the constriction at the node, 2) width and shape of the blade and 3) structure of the secondary branches and the rhizoids.

All these taxa have the common reproductive features as follows;

1) A carpogonial branch is consisted of four cells including a carpogonium, two hypogynous cells and a supporting cell.
2) One group of strile cells is produced from a supporting cell.
3) One cystocarp is produced on a midrib of the upper part of the terminal branch.
4) Most cells of the gonimoblasts produce many carpospores.
5) Spermatangia are transformed from spermatangial mother cells which are produced by the divisions of cortical cells.
6) Tetrasporangia arise from the middle layer, not from the cortical cells, of the fertile thallus parts.
7) Cover cells of tetrasporangia are not well developed.

The last taxon (*Caloglossa* sp.) collected in Daintree River, Queensland is similar to *C. leprieurii* f. *continua* on its gross morphology, but it can be distinguished by the narrower blades, the fewer rhizoids issuing from the nodes and the widened tips of the fertile female blades.

S-3: BIOGEOGRAPHIC RELATIONSHIPS OF *BOSTRYCHIA RADICANS* & *B. MORITZIANA* (RHODOPHYTA): HYBRIDIZATION & DNA SEQUENCING.

J. A. WEST (UNIVERSITY OF MELBOURNE) & G. ZUCCARELLO (UNIVERSITY OF CALIFORNIA, BERKELEY)

We compare the results of classical hybridization with those from molecular biology (DNA sequencing) using culture isolates, from many geographic regions, of *B. radicans*, that has polysiphonous laterals, and *B. moritziana*, which has partially monosiphonous laterals. In Pacific Mexico all 15 isolates (from over a 3000 km distribution) are intercompatible even though field specimens range from completely polysiphonous isolates to isolates with monosiphonous laterals. In world-wide crossings, various degrees of genetic isolation are observable and certain hybridization patterns are evident. Genetic isolation includes: pseudocystocarp formation, poor germination of carpospores, poor growth of tetrasporophyte and low viability of tetraspores. Positive hybridization (based on the formation of cystocarps and carpospore release) os isolates from Pacific Mexico is seen with most isolates from Venezuela even though these populations have been isolated by the isthmus of Panama for at least 1.5 million years. A few NE USA isolates interbreed with Pacific Mexico and Venezuela isolates which may reflect longer genetic separation of these populations. Isolates of *B. radicans* from Brazil hybridize with isolates from Peru although not all isolates from Brazil are intercompatible. In the western Pacific no intercompatible isolates of *B. radicans* were observed. However, *B. moritziana* isolates (with compound monosiphonous branches) from Australia and South Africa are intercompatible. Sequencing of chloroplast DNA (Rubisco spacer) indicates that *B. radicans* falls into one group from the northern hemisphere: Pacific Mexico and NE USA, and another group from the southern hemisphere: Brazil, Peru and Australia. This DNA-based phylogeny generally supports the hybridization patterns observed.

S-4: MANGROVE-ASSOCIATED BOSTRYCHIETUM IN KENYA.

COPPEJANS E. & DE SCHRYVER T. (UNIV. GENT, BELGIUM)

This research was carried out in September 1989 in Gazi Bay as a follow-up of a previous work (COPPEJANS & GALLIN 1989). For this study 367 pneumatophores of *Sonneratia* and *Avicennia* as well as rhizophores of *Rhizophora* were collected along 7 transects at right angles to the tides and at increasing distnace from the sea. This resulted in a data-matrix of 1092 relevés. Species composition was analyzed and cover estimates made

along 5 cm zones of these aerial roots. Species composition was high: 36 species of which 14 Chlorophyta, 2 Phaeophyta and 18 Rhodophyta. The most frequent taxa were *Bostrychia tenella, Catenella caespitosa, Caloglossa leprieurii* and *Murrayella periclados*. A strong decline in species diversity and total cover is found along the transects towards the upper tidal range. Epiphytic growth was maximal in the 5-15 cm above ground level, but the zone with maximal cover tends to be lower with increasing distance from low tide (desiccation effect). Strong exposure to direct sunlight is reflected by lower cover estimates and a shift in species composition. This sciaphilous character is prominent among the red algae; *Ulvaria oxysperma* on the contrary has its optimal development in the sun-exposed regions. Most of the algal species do not show a preference for a mangrove species, except for *Bostrychia* and *Ulvaria*: the first one shows a better development on harder substrates such as rhizophores and old *Sonneratia*-pneumatophores and is absent from the softer, young or dead pneumatophores. *Ulvaria* on the other hand prefers these soft substrates.

The maximal dry weight of the Bostrychietum was found close to the open sea and was 352 g/m^2 on *Sonneratia* (with 189 pnph/m^2) and 170 g/m^2 in nearby *Avicennia* plot (with 521 pnph/m^2). The biomass declined upstream, but also direct sunlight results in a lower biomass.

S-5: MORPHOLOGICAL COMPARISON AND HYBRIDIZATION EXPERIMENTS IN THE THREE INFRASPECIFIC TAXA OF *CALOGLOSSA LEPRIEURII* (CERAMIALES, RHODOPHYCEAE).

M. KAMIYA, Y. HARA (UNIV. OF TSUKUBA, JAPAN) AND J. TANAKA (NAT. SCIENCE MUSEUM, JAPAN)

Members of the genus *Caloglossa* constitute the benthic algal community in mangrove forests growing in tropical and subtropical regions. In this genus, *C. leprieurii* is distributed worldwide and shows many morphological variations. Several varieties and formae of this species can be distinguished from their morphological features; blade width, blade constriction at the node, number of secondary proliferous branches and rhizoids forming bundles or not (Post 1936). The existence of reproductive isolation among these taxa and the evaluation of their taxonomic status have not previously been examined.

In this study, three infraspecific taxa of *C. leprieurii* followed by Post, *C. leprieurii* var. *leprieurii* f. *leprieurii*, var. *leprieurii* f. *continua* and var. *hookeri*, were reexamined; morphological comparison of natural and/or cultured plants collected from the mangrove areas or estuaries in Japan, Singapore, Australia and Pakistan. Hybridization experiments were also performed to investigate the potential reproductive isolation among these three taxa.

In f. *leprieurii* and f. *continua*, there are less than 4 secondary proliferous branches at the node and the rhizoids do not form bundles, whereas var. *hookeri* has 4 or more proliferous branches and produces bundles of the rhizoids at the base. The blade constriction at the node of var. *hookeri* is remarkably deeper than those of f. *leprieurii* and *continua*. *C. leprieurii* f. *continua* can be distinguished from f. *leprieurii* by the degree of the constriction at the node and the blade width in the widest part at internode; 0.3-1.0 mm (mean 0.47 mm) and 0.5-1.7 mm (mean 1.07 mm), respectively.

Isolated strains of f. *leprieurii* from one habitat in Australia, f. *continua* from three habitats in Japan and var. *hookeri* from six habitats in Japan and Australia were used for hybridization experiments. Any crosses between the different taxa were unsuccessful. The formation of cystocarps, release and germination of carpospores were observed in any cross between members of the same taxon, though the clear reproductive isolation was recognized between Japanese and Australian populations of var. *hookeri*.

These three taxa were confirmed to be distinguishable by comparison of their morphological features, and they were also genetically incompatible. These results suggest that

these infraspecific characters used by Post (1936) should be attributed to interspecific differences, and at the same time that these varieties and formae of *C. leprieurii* sould be assigned to distinct species.

S-6: MANGROVE RED ALGAE: THEIR ACCLIMATION TO ENVIRONMENTAL FACTORS. *U. KARSTEN* (MARINE BOTANY, UNIV. OF BREMEN, GERMANY)

The macroalgal flora of mangroves is dominated by members of the rhodophycean genera *Bostrychia, Stictosiphonia, Caloglossa* (Ceramiales) and *Catenella* (Gigartinales). These algae grow in the eulittoral zone as epiphytes on the pneumatophores, trunks and prop roots. Here the species experience large fluctuations in salinity, irradiance, temperature, nutrient levels and desiccation due to the tidal flows. *Bostrychia* synthesizes and accumulates high concentrations of the hexitols D-sorbitol and D-dulcitol, which are otherwise uncommon in the red algae. *Stictosiphonia* contains only D-sorbitol and *Caloglossa* D-mannitol. In *Catenella* floridoside has been found, and this heteroside is more typical for the Rhodophyta. All these compounds are strongly involved in osmotic acclimation of the corresponding genera, i.e. their concentrations significantly increase with increasing salinities and vice versa. The euryhaline nature of the mangrove algae is also reflected by growth and photosynthesis over a wide range of salinities. In addition, most of these species require very low irradiances for growth and photosynthesis and can, hence, be characterized as typical "shade-plants".

S-7: THE EFFECT OF THE SALINITY ON THE PHYSIOLOGY OF *CALOGLOSSA LEPRIEURII* (CERAMIALES, RHODOPHYTA). *ANIKA S. MOSTAERT* AND ROBERT J. KING (SCHOOL OF BIOLOGICAL SCIENCE, UNIVERSITY OF NEW SOUTH WALES, AUSTRALIA)

The red alga *Caloglossa leprieurii* (Montagne) J. Agardh occurs in the eulittoral zone of marine, estuarine and freshwater habitats on a variety of solid substrata and is frequently associated with mangroves. Populations of this species are subjected to emersion-immersion cycles due to tides and thus experience changing salinities. Ecophysiological investigations in response to salinity were conducted on populations from a marine, estuarine and freshwater location within the Sydney region (Australia).

All populations exhibited a broad salinity tolerance with respect to cell viability. Growth rates of algae from the three localities were measured at a range of salinities (0-125‰) and maximum growth rates were in the range 5-35‰.

Large water fluxes resulting in pronounced cell wall swelling without plasmoysis is the initial response of *Caloglossa leprieurii* following hypersaline stress. Osmotic adjustment is achieved in part by the production of compatible solutes and changes in internal ion concentration. Under hypersaline conditions this alga synthesizes and accumulates high concentrations of the polyol D-mannitol. The effects of hyposaline and hypersaline treatments on the intracellular inorganic ion concentrations for the marine, estuarine and freshwater habitats will be presented.

Different physiological response patterns between the 3 populations have been found indicating the evolution of ecotypes.

S-8: UNICELLULAR RED ALGAE IN MANGROVES AND RELATED ENVIRONMENTS: THEIR TAXONOMY AND DISTRIBUTION. *Y. HARA*, K. ISHIDA, A. TSUNAKAWA (UNIV. OF TSUKUBA) AND N. HATAKEYAMA (NIPPON ROCHE)

Mangrove forests serve as severe habitats for benthic microalgal organisms which are exposed to water of various salinity and/or desiccated conditions. Despite this, microalgae with some physiological and morphological adaptations to these conditions can survive and grow well there. For these microalgae, the mangrove forests offer interception of intensive sunshine by their canopies, eutrophic conditions due to inflow of nutritious sub-

stances from adjacent areas and both stable daily and annual temperature.

Previously, we reported that many unicellular algae have been found from Japanese and other mangroves after enrichment culture of soil samples. Of these, unicellular red algae were characteristic in inhabiting the mangal environments that were surveyed. They constituted a regular component but were not found to be major or dominant in any habitats.

In the International Research Project of Mangrove Algae (1991-1993), we tried to survey species compositions and new taxa of unicellular reds from various mangroves and related environments, and to analyze the physiological growth rates under different salinities and morphological properties possiblly adapted to their own habitats, using the culture strains originally isolated and gathered from Culture Collections.

Through the project, we have already collected many strains including members often making psuedofilaments; *Porphyridium* (4 species), *Rhodella* (3 spp.), *Dixoniella* (1 sp.), *Rhodospora* (1 sp.), *Chroothece* (1 sp.), *Cyanidium* (1 sp.), *Asterocytis* (1 sp.) and *Flintiella* (1 sp.).

From results obtained, we could distinguish the patterns of their growth under different salinities which are equivalent to 0-100% seawater into three referred to freshwater, euryhaline and marine types. We also recognized that all of the algae enable to inhabit mangrove areas showed only the euryhaline type without exceptions. They commonly possessed motile activity correlating to phototaxis with gliding or amoeboid movement. Almost all are benthic and also possess eyespot like particles in the chloroplast and gelatinous thick walls with stalks. It seemed that these physiological and morphological properties could be significant adaptations to the mangal environments. The algae showing the freshwater or marine types of growth pattern could not be found inside mangrove forests, even though they could be isolated nearby the mangrove forests. They lacked motile activity and eyespot like particles. The members of unicellular reds isolated from mangrove forests should be one of major components of the mangrove microalgal flora, however, not be specific. Because they have also been found in other related environments such as estuaries without mangroves, seashores, salt marshes and surface soils in greenhouses.

S-9: A CHLORARACHNIOPHYTAN ALGA FROM SOUTH BAJA CALIFORNIA.

K. ISHIDA AND Y. HARA (UNIV. OF TSUKUBA, JAPAN)

Chlorarachniophytes have attracted attention as another elvolutionary chaemera remaining a nucleomorph contrasting with cryptomonads. For understanding the symbiosis and companionship between eukaryotic host and eukaryotic endocytobiont as well as their phylogenetic origins, it is urgent to analyze their genes from the viewpoint of molecular evolution or systematics. In addition, further basic information concerned with the taxa, habits and distributions are required.

After two species (*Chlorarachnion reptans* and *Cryptochlora perforans*) have been described to be found from sea shores in tropical region, we tried to find out new taxa of this algal group and to establish the culture strains from samples collected in subtropical and tropical regions, using enrichment culture techniques. Several taxa distinguishable from the described ones were collected there, however, we had no experience to observe them from samples of mangal forests. This is one of our special interests to know whether algae of this group were eventually found out from mangrove forests, when our research project of mangrove algae was started.

Through the expeditions of North Australia, South Baja California and others, we have often observed algae of this group in samples of soils and water rinsing the surface of seaweeds. They could not appear in any kinds of samples from mangrove forests, even though we could obtain them from the samples collected nearby. it is obvious that they were certainly prevented from expanding their distribution into those areas. This is a

further significant problem to understanding their distributions.

One of the strains newly isolated from coasts of South Baja California close to a mangrove forest is presented. Cells are coccoid with multilayered walls and mostly 8-15 μm in diameter. They are multiple with binary or quaternary fissions and share almost all of the whole life cycle. Neither single flagellar zoospores nor amoebae like *Ch. reptans* were found. Several bilobed chloroplasts with a remarkably projecting pyrenoid occupy the cell periphery. The subcellular organization is basically the same as those of the other chlorarachniophytes except for pyrenoid ultrastructure. The pyrenoid is surrounded by two double membranes with cytoplasm of the periplastidal compartment, and a capping vesicle. The distal end of the pyrenoid is characteristically scooped out like a shallow ditch where it is filled with cytoplasm of the periplastidal compartment, instead of a nucleomorph. The nucleomorph is located beside the isthmus of the pyrenoid connected with the chloroplast. These features are closely similar to those of *Lotharella scrobicolata* nom. nud. (=CCMP242), however, the depth of ditch at the distal end of pyrenoid and lacking of amoeboid cells in its life cycle are clearly distinct. It might be appropriate that this alga be assigned to the genus of *Lotharella*, as a new species.

S-10: A TAXONOMIC SURVEY OF DINOFLAGELLATES AND RAPHIDOPHYTES OF MANGROVES. *T. HORIGUCHI* (HOKKAIDO UNIVERSITY, JAPAN)

Species composition of phytoflagellates of various mangroves was investigated and results of taxonomic survey of dinoflagellates and raphidophytes are presented. Study sites were mangroves of various localities in Australia, mangroves in Baja California, Mexico and those in Key West, Florida, U.S.A. When one considers habitats for these flagellates in mangrove ecosystem, three main habitat types can be recognized. These include 1) water column, 2) sand or mud layer in the bottom of river of lagoon, and 3) tide pools. In our survey, habitat types of 2) and 3) were mainly investigated.

So far, 17 species of dinoflagellates and two species of raphidophytes have been found. The dinoflagellates found in various mangroves are members of the genera; *Prorocentrum, Amphidinium, Gymnodinium, Gyrodinium, Peridinium, Scrippsiella, Stylodinium* and *Spiniferodinium*. Most of these species are, however, not specialized mangrove inhabitant. They have also been found in other environments such as sandy beaches or rocky tidal pools of tropical and temperate zones. One species of *Gyrodinium* found in Key West was thought to be a new species. The cell is oval in ventral view and dorsiventrally compressed. Ultrastructural investigation revealed that the dinoflagellate possesses typical dinoflagellate organelles, including dinokaryotic nucleus and a chloroplast with a central pyrenoid. The cell is 17.5-20 μm in length and is one of the smallest members of the genus *Gyrodinium* so far described.

Raphidophytes found in this survey are only two species, viz. *Olisthodiscus luteus* and an underscribed species with an uncertain taxonomic position (Probably, a member of the genus *Heterosigma*). The latter species is characteristic in having both motile and benthic phases in its cell cycle. It produces copious amount of mucilage substance and embeds itself in the mucilaginous matrix. Ultrastructural survey of the new species revealed that it possesses sack-life structures which resembles pusule of dinoflagellates, although function of the organelle is still to be investigated. Preliminary investigations on effects of salinity on growth of these two species revealed that *O. luteus* was able to grow in the salinity range 5 to 55 ppt, while the latter species was able to grown in the salinity range 15 to 45 ppt. The significance of regular alternation of benthic and motile phases in the latter species is discussed in view of adaptive strategy against salinity change in mangrove environment.

S-11: DINOFLAGELLATES IN A MAN-

GROVE ECOSYSTEM, TWIN CAYS, BELIZE: AN OVERVIEW. *M. A. FAUST* (DEPARTMENT OF BOTANY, NMNH, SMITHSONIAN INSTITUTION, MARYLAND U.S.A)

An overview is presented on the ecology and taxonomy of benthic dinoflagellates in a red mangrove ecosystem, Twin Cay, Belize, Central America. Twin Cays is an undisturbed, diverse, intertidal island within the barrier reef ecosystem of the Northern Hemisphere that extend from the Mexican border in the north to the Gulf of Honduras in the south. The population structure of toxic and non-toxic benthic dinoflagellate species and other taxa are examined in the Lair, a protected embayment considered low turbulence environment and high in organic matter. On the water surface patches of detritus increases during the day and sinks out of sight in the late afternoon. Detritus floats to the surface by visible expanding gas bubbles introducing to the water column organisms previously retained in detrital aggregates at the sediment surface where oxygen, temperature and light may be limited. In this shallow mangrove ecosystem microorganisms were found in aggregates at the sediment-surface and they proliferated as free-living in the water column. As a result, aggregates acted as environmentally important reservoir of microbial diversity, provide environmental conditions for the dominance of different populations. On the water surface patches of detritus was a rich source of benthic phytoplankton and zooplankton and it provided a potential link between increased microbial production and a source of high quality biomass for consumer organisms. Variations in major group of taxa affected by temperature, salinity and oxygen is illustrated in short-term studies. Composition of microalgae present in detritus in time series experiments is documented: dinoflagellates represented 50-85%, diatoms 5-14%, cyanobacteria 8-32% and cysts 2-9% reaching the highest proportion at 14 h. Microalgae were found attached to detritus by means of a stalk, a rigid appendage or embedded in a matrix of mucilage. Toxic dinoflagellates in the detritus were: *Gambierdiscus toxicus, Amphidinium carterae, Ostreopsis lenticularies, Prorocentrum lima, P. mexicanum, P. maculosum* and *P. hoffmannianum*. Zooplankton populations increased with time until 14 h and declined by 17 h. The life cycle strategies of *Prorocentrum lima, P. foraminosum* and *Coolia monotis* (Dinophyceae) in floating detritus are also discussed. Evidence is presented that *P. lima* cells cycle between free-living motile stage and an encysted benthic stage during asexual reproduction for maintenance of epiphytic populations. It is suggested that floating detritus serves as a nursery for benthic dinoflagellates. When dinoflagellates are ciguatoxigenic, floating detritus may take on extreme importance, and undoubtedly contribute to their proliferation in tropical mangrove habitats. On the other hand the continual reintroduction to the water column organisms previously retained in aggregates may be a key factor providing for the prolonged stability of complex biological systems such as mangroves. This study draws attention to the role of floating detritus, its effect on dinoflagellate distributions, including toxic species.

S-12: RUBISCO GENES IN THE HAPTOPHYTA. *S. FUJIWARA*[1], M. SAWADA[1], N. MINAKA[2], M. KAWACHI [3], I. INOUYE[3], AND J. SOMEYA[1] ([1]NAT. INST. OF BIOSCIENCE AND HUMAN-TECHNOLOGY, AIST, JAPAN; [2]NAT. INST. OF AGRO-ENVIRONMENTAL SCIENCES, JAPAN; [3]UNIV. OF TSUKUBA, JAPAN)

Genes for the large (*rbc*L) and small (*rbc*S) subunits of Rubisco from the haptophyte *Pleurochrysis carterae* were isolated and characterized. Southern and Northern blot analyses indicated that these genes are encoded by plastid DNA and cotranscribed, as in the Cryptophyta, Chromophyta, and Rhodophyta studied so far. The genes of *Pleurochrysis* show higher identities with those of the Cryptophyta, Chromophyta, Rhodophyta, and the α (Type I)- and β-purple bacteria than with those of the Chlorophyta or Cyanophyta.

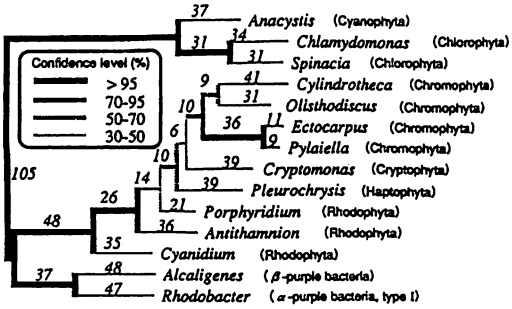

Fig. 1. The most parisimonious phylogenetic tree based on *rbc*L amino acid sequences. A number on a branch, reflected by its relative length, represents the number of amino acid substitutions along the branch using the DELTRAN option of PAUP.

The molecular phylogenetic tree of *rbc*L genes suggests that the plastids of the Haptophyta, Cryptophyta, and Chromophyta originated from those of the Rhodophyta, which agrees with the secondary endosymbiotic theory (Fig. 1). Northern analysis of *Pleurochrysis* demonstrated that a certain amount of the transcript is possibly processed around the 3' end of *rbc*L. When cells were transferred from light to dark for 6 hours, the amount of Rubisco mRNA was almost the same as in continuous light. This suggest that Rubisco mRNA is synthesized and/or stable even in the dark.

To infer the phylogenetic relationships among the Haptophyta, the nucleotide sequences of the *rbc*L genes were determined for six more members of the Haptophyta. The most parsimonious trees suggest that the Haptophyta is a monophyletic group. For the relationships among the Haptophyta, the trees imply that, the member of the order Pavlovales, *Pavlova salina*, is a sister taxon of the relatively reliable monophyletic group composed of other six species of the Haptophyta, which belong to the orders Isochrysidales, Coccosphaerales, and Prymnesiales. The nucleotide sequence of *rbc*L from *Gephyrocapsa oceania* is identical to that from *Emiliania huxleyi* within the examined region. Our trees suggest that *G. oceania* and *E. huxleyi*, both included in the Isochrysidales, are phylogenetically more closely related to the member of the Prymnesiales, *Chrysochromulina hirta*, than to the other member of the Isochrysidales, *Pleurochrysis carterae*. Our findings imply that the orders in the Haptophyceae including the above-mentioned genera should be redefined.

S-13: *EMILIANIA HUXLEYI* (ISOCHRYSIDALES, HAPOTOPHYTA) AS A MODEL SYSTEM FOR THE STUDY OF PERAGIC CALCIFICATION.

P. WEST-BROEK (LEIDEN UNIV., THE NETHERLANDS)

The production of calcium carbonate in the pelagic ocean, and the sinking of this material to the deep ocean (the 'carbonate pump') is thought to influence the global carbon cycle and climate. The bulk ocean tends to be (super) saturated with respect to calcium carbonate and therefore encrustration with this mineral forms a permanent risk to all marine biota. Profusive excretion of the crystallisation inhibitor, slime, prevents this from happening. As a result of this active anti-calcifying activity of the marine biota, spontaneous precipitation is (almost) entirely suppressed in the oceans, and this brings the production of calcium carbonate under more or less stringent biological control. Therefore, calcium carbonate production depends on the evolutionary and ecological success of the calcifying biota rather than on the degree of supersaturation of the ocean water. The diversity of these biota and the variation in their response to environmental conditions then form a fundamental problem in assessing the amount of calcium carbonate produced when conditions change.

To overcome this difficulty, we propose a 'model system' approach, whereby a single representative organism, the coccolithophore *Emiliania huxleyi*, is investigated in detailed interactive experimental and modelling studies. To construct a comprehensive model of the carbonate pump, subsequent research is envisaged on additional representative organisms, but this work is likely to be facilitated by the experience gained with *E. huxleyi*. The model system approach permits (1) an emphasis on the non-linear character of the fluxes;

(2) a focus on the coupling of the carbonate pump with other climatically important phenomena—the organic carbon pump and DMS production; (3) exploitation of the experimental accessibility of the *E. huxleyi* system.

S-14: THE ROLE OF ZOOXANTHELLAE IN THE ENERGY AND NUTRIENT FLUXES IN CORALS. *Z. DUBINSKY.* (BAR-ILAN UNIVERSITY, ISRAEL)

Coral reefs dominate coastal zones in warm, oligotrophic, tropical seas. These ecosystems are unique in the marine realm in their high biomass, primary productivity and species diversity, and as such, represent one of the peaks in the evolution of life on our planet. The corals provide both the structural framework of the system, as well as the energetic basis for its function. Coral reefs line 100 000 km of shores and were widespread as early as the Triassic. This remarkable spatial and temporal success of coral reefs in the "blue deserts" of tropical seas stems from the tightly coupled mutualistic symbiosis between the host animal, the coral, and the endozoic microalgae, the zooxanthellae. In this association the algae supply the system with energy, as photosynthate, while gaining access to the nutrient rich waste products of the hosts metabolism.

Underwater light levels determine the rate of carbon influx into the zooxanthellae at any depth and time, and thereby the energy available for the whole coral symbiotic association. Long term photoacclimation of the zooxanthellae to the time-averaged light regime at which the host coral grows results in optimization of light harvesting and utilization. Most of the photosynthate produced by the algae is translocated to the host and respired by it. However the capability of the zooxanthellae and the coral to retain carbon beyond that required to meet their respiratory needs depends on the availability of limiting nutrients, primarily nitrogen and phosphorus. Unless the ratio of these nutrients to carbon approaches that found in the zooxanthellae and the coral, all of the photoassimilated carbon above that can only be respired or excreted, but cannot support growth. Therefore, the ratio of the flux of these nutrients into the colony to that of the photosynthetically driven carbon flux, will regulate the growth of the zooxanthellae and of the animal.

The sources of nutrients in the waters sorrounding reefs are dissolved inorganic compounds, available in vast quantities, but at extremely low concentrations, and the sparse zooplankton. Nutrients acquired by predation of the coral on zooplankton, and its digestion, are available first to the animal, whereas those absorbed by the zooxanthellae from seawater as inorganic compounds lead first to growth of the algae.

S-15: RECYCLE OF CARBON DIOXIDE BY MEANS OF MICROALGAL PHOTOSYNTHESIS. *H. IKEMOTO*, N. KURANO AND S. MIYACHI (MARINE BIOLOTECHNOLOGY INSTITUTE, JAPAN)

Besides deforestation, carbon dioxide is constantly being pumped into global atmosphere as the result of human activities, and the natural CO_2 sink can no longer accommodate the increasing amount of industrial CO_2 emission. To reduce the absolute amount of CO_2, therefore, we need to create new CO_2 sinks. Even the excessive CO_2 disposed in deep sea does not seem to be confined ultimately. In this sense, the pool of algal cultivation does not largely contribute to cancel the absolute amount of CO_2 increase, either. But, what we may expect is to convert CO_2 into the form of resources for the second use.

As the substitution for photosynthesis of terrestrial plants to absorb atmospheric CO_2, several advantages may be found in microalgae. The rate of photosynthesis is higher, thus it would require smaller area of land. Utilization of biomass may not be limited to fuels or papers. Microalgae with a variety of species, like bacteria, should show a wide range of environmental adaptability.

Taking the limitation of freshwater into account, cultivation of marine microalgae using seawater-based media is strongly suggested.

Scientists in Marine Biotechnology Institute surveyed the western Pacific Ocean in the past three years seeking for new hints on future algal utilization. Although the ocean is known as the well-buffered environment, it did not restrict the findings of a variety of unique strains with respect to tolerance to extreme pH etc. Several pelagic isolates of green algae were shown to grow fast at pHs as low as 3-4, and some thermophilic isolates of red algae from volcanic regions appeared to be extremely acidophilic. These low-pH preferring strains are examined for the tolerance to high concentrations of CO_2, and some of them were shown to grow under vigorous aeration with 20-100% CO_2. These would enable us to collect CO_2 by means of algal photosynthesis from the industrial flue gases which contain extremely high concentrations of CO_2 for ordinary plant growth. Besides, some of the isolates turned to be novel in taxonomic aspect. Starting with the finding of fast-growing marine *Chlorococcum* species, unexpectedly wide distribution of primitive green-colored algae keep us realizing that marine microalgae are still not sufficiently understood.

As a part of efforts to enhance the algal growth per area with crops and forests, high-density cultivation of a marine green alga, *Chlorococcum littorale* was attempted. Current preliminary results of the linear growth rate, 2.5 g (dried cell)/l/day at cell density of 10 g/l, and the maximum cell concentration of 14.4 g/l under 20% CO_2 with ordinary culture system may further be improved by the use of a novel photo-bioreactor which provides sufficient illumination. To deal with the entire amount of CO_2 emitted, e.g. from electric power plants, millions of barrels of high-density algal cultures would still be required in calculation, although the algal productivity in this calculation is far beyond the present agricultural productivity, and worthy of considering for biological recycle of CO_2.

This work was supported by New Energy and Industrial Technology Development Organization (NEDO), Japan.

S-16: *DUNALIELLA SALINA* (VOLVOCALES, CHLOROPHYTA) AS A SOURCE OF BETA-CAROTENE. *L. J. BOROWITZKA* (WESTERN BIOTECHNOLOGY LIMITED, AUSTRALIA)

Since 1986, beta-carotene products extracted from the green micro-alga, *Dunaliella salina*, have filled the growing niche market for natural beta-carotene. Beta-carotene is used as an orange-yellow food colouring, and as a nutritional supplement benefitting human health as a source of provitamin A and as a natural antioxidant. There are four commercial producers of algal beta-carotene, and each uses an algal culture system suited to the local environment. Western Biotechnology uses 70 ha of semiintensive culture ponds for year-round production of algal biomass. Western Biotechnology's product range sold under the Bionova name, is based on vegetable oil suspensions of many concentrations. Recent additions are a dry form of natural beta-carotene suitable for use in tablets and a cold water soluble powder for use in foods.

S-17: INDUSTRIAL UTILIZATION OF BIODIVERSITY AND METABOLIC PLASTICITY OF MICROALGAE IN CLOSED PHOTOREACTORS. PRESENT AND FUTURE. *C. GUDIN* (HELIOSYNTHESE CADARACHE, FRANCE)

Biodiversity of photosynthetic microorganisms (eucaryotic microalgae and procaryotic cyanobacteriae) among $\geqq$27 000 species is available for industrial utilization as we are now able in closed photoreactors to play on the biosynthetic pathways by applying environmental stresses.

Playing on salinity leads to the production of osmoregulators, on water stress to the excretion of polysaccharides, on temperature to the control of polyunsaturated fatty acids ($C_{20:4}$; $C_{20:5}$; $C_{22:6}$) or to the production or thermoresistant enzymes. With the choice of light quality or quantity, we direct the metabolism towards carotenoids or phycobiliproteins depending on the genus we have selected.

The forced photosynthetic growth leads to

an excess of dissolved oxygen and consequently to an increase in Vitamin C, Tocopherols and SOD.

Selected Dinoflagellates or certain cyanophycea lead to get some promising tool as specific ion channel agonists or as specific enzyme inhibitors, keeping in mind that Dinoflagellates are still very difficult to grow in mass production.

In spite of the promising molecular biology development, the best results obtained in improving the selected species were due to phenotypic selection: it has been the case for Bêta-carotene with *Dunaliella salina*, polysaccharides with *Porphyridium cruentum*, $C_{20:5}$ with *Isochrysis galbana* and $C_{22:6}$ with *T. Isochrysis*, astaxanthin with *Haematococcus pluvialis*.

If we except the cloning of human SOD in a cyanobacteria, very little has been done till now to fit the potential or real market demand.

The technologies of cultivation control are now ready: tubular or flat reactors, enlighted fermentors or immobilized beds of microalgae on artificial support (polyurethane or other foam, ...). The market is open at least for certain products: phycobiliproteins, polyunsaturated fatty acids, antioxydants, carotenoïds. Need for improved strains and a greater number of new products is now existing. Intelligent screening with physiochemical strategy is needed to extract the best of microalgae biodiversity.

S-18: BIOLOGICAL NITROGEN FIXATION AND HYDROGEN PRODUCTION AND THEIR APPLICATIONS. *A. MITSUI* (INTERNATIONAL RESEARCH CENTER FOR BIOLOGICAL HYDROGEN PHOTOPRODUCTION, RSMAS, UNIVERSITY OF MIAMI, U.S.A)

Hydrogen is a clean fuel alternative to the pollution causing fossil fuels. In order to develop a practical hydrogen energy producing system, hydrogen should be produced by pollution-free methods, without the consumption of existing energy forms. Biological hydrogen photoproduction using solar energy, water (seawater), and photosynthetic microorganisms meets these criteria.

As the first step of biological hydrogen photoproduction, microbial biomass should be produced by pollution-free methods. Among photosynthetic microorganisms, certain species of cyanobacteria and photosynthetic bacteria can grow and produce high biomass using solely atmospheric nitrogen as a nitrogen nutrient source. Ammonia, nitrate and urea pollute the water (seawater). Thus the use of nitrogen fixing photosynthetic microorganisms had an advantage for pollution-free hydrogen production.

Among these high biomass producing, nitrogen-fixing strains, we screened for strains which has extremely high hydrogen production capabilities. The best strain was a marine unicellular cyanobacterium, *Synechococcus* sp. Miami BG043511.

Synchronous culture of this strain under nitrogen fixing conditions was established. Particular phases of the cell cycle were found to produce very high quantities of hydrogen (7 ml. hydrogen per ml. cell suspension per 12 hr. illumination). This is several orders of magnitude higher than other hydrogen producing green algae and cyanobacterial strains.

Hydrogen production by this strain was found to be very unique in that it was not inhibited by oxygen concentrations of 20%. Immobilized cells of this strain produced high amounts of hydrogen for more than a one month period by recycling short photosynthetic processes and long hydrogen producing processes.

These techniques may be applied in the future for developing countries which lack energy sources, as small scale hydrogen production in individual residences. The techniques may also be used by industrial countries using sea floating large scale hydrogen photoproduction facilities in tropical and subtropical areas. Hydrogen produced in these facilities may be shipped to other industrial countries for their use.

In order to make biological hydrogen photoproduction economically feasible, the importance of coupling hydrogen photoproduction

to other production systems such as aquaculture of fish and shellfish, or new medicine production, etc. will be addressed.

S-19: MICROALGAL CO_2-FIXATION AND ITS APPLICATION. *K. MIYAMOTO* (OSAKA UNIVERSITY, JAPAN)

In Japan, more than half of the CO_2 produced comes from electric power plants and industries. The CO_2 from these large point sources can be recovered with relative ease by various established technologies like chemical absorption. The use of photosynthetic microalgae has been proposed in a variety of approaches, because the cultivation of them is able to fix CO_2 and produce energy and chemical compounds. Some high value chemicals have been commercialized and several other products have been studied experimentally with pilot plants. There is wide biochemical and physiological diversity in thousands of microalgal species, and a great variety of chemicals could be produced from this potential source.

Energy from algal biomass is an attractive title, because the advantages of a biomass energy system have been well documented: unlike fossil fuels, biomass is rather uniformly distributed over much of the earth's surface, and would make no net contribution to the CO_2 increase in the atmosphere. Algal biomass is regarded as a low-grade energy because of its high content of moisture. Algae, however, can be used to produce modern gaseous and liquid fuels[1].

We demonstrated a cyanobacterial H_2 production that sustained for up to 5 weeks under natural insolation[2]. Recently, we have shown a biological system that converts sunlight to hydrogen with the aid of microalgae and photosynthetic bacteria[3]. A marine green alga and a marine photosynthetic bacterium both having high H_2 evolution activities have been isolated and characterized with respect to their H_2 metabolism[4,5]. A low H_2 yield in algal fermentation could be overcome by using the photosynthetic bacterium because it produces H_2 from organic compounds secreted by the algal cells. This system exhibited a high conversion yield of 10.5 mol H_2 per mol of starch-glucose[6].

Although there is a frequently discussed problem of the need of large area for capturing solar energy, algal mass culture will be the most promising technology for producing fuels and chemicals in environmentally mild ways. However, the technology of microalgae is still less developed, and thus, in conclusion, it should be supported as sustained, basic research in the fields of molecular biology, physiology, ecology, and bioengineering.

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S-20: MASS PRODUCTION OF MICROALGAE: OPEN VS. CLOSED SYSTEMS. *A. RICHMOND* (MICROALGAL BIOTECHNOLOGY, BLAUSTEIN INST. FOR DESERT RESEARCH, BENGURION UNIVERSITY OF THE NEGEV AT SEDEBOKER, ISRAEL)

Industrial reactors in the world today are nearly without exception all designed as open raceways, i.e. shallow ponds constructed as a loop in which the culture is circulated by a paddle-wheel. This simple mode of cultivation has many disadvantages, the major weakness being the lack of temperature control which greatly decreases the productivity potential. In addition, the light path in an open raceway is relatively long, mandating maintenance of relatively low population densities, i.e. a large volume of water with little biomass. Dilute cultures present many difficulties and result in a high production cost. The overall low productivity of the open raceway, far from the theoretical potential, prompted the development of enclosed

systems, i.e. reactors made of transparent materials in which temperature may be readily controlled and the light path is much shortened. The ratio of culture volume to the irradiated area is greatly improved and the optimal areal density is higher by nearly an order of magnitude, compared to raceway channels. Thus the enclosed reactor has the potential to support a high and steady production of photoautotrophic mass throughout the year, thereby significantly increasing the overall productivity and lowering production costs.

S-21: IMPROVEMENT OF THE ARTIFICIAL CULTURE OF MARINE PELAGIC ALGAE AND ITS APPLICATION TO THE STUDY OF AEROBIC N_2-FIXATION IN THE CYANOPHYTE, *TRICHODESMIUM* SP. NIBB1067. *K. OHKI* (TOKAI UNIVERSITY, JAPAN)

Stable culture of algae in chemically defined media under artificial conditions is necessary not only for physiological, molecular biological or biochemical studies but also for utilization of these organisms as a natural resource. However culture techniques applied for marine pelagic algae have not developed so well compared with those for coastal and fresh-water species. Difficulty of laboratory culture is due, in part, to oligotrophic nature of marine pelagic algae. We have attempted to establish basic culture techniques for marine pelagic algae and found several important strategies: (1) Enriched sea water, such as "f" medium of Guillard and Ryther, was suitable for the initial isolation of cells from their natural habitat, but the nutrient concentrations had to be lowered (1/20 to 1/200 of the original concentration). After isolated cells started to grow, they were transferred to the artificial medium. The nutrient concentrations were increased gradually. (2) Because heavy metals are very toxic for algal growth, contaminated metals in the chemicals (all analytical grade) used were removed with Chelex-100 column, if necessary, and all glassware was soaked in HCl (about 0.1 N). (3) Organic buffers such as tris (hydroxymethyl) aminomethane-HCl (Tris) or glycylglycine had toxic effects for some species. Therefore an inorganic buffer system, combinations of $NaHCO_3$, NaOH and HCl, was used to adjust pH. (4) Some species required higher concentration of Ca^{2+} than coastal species did. (5) Frequent transfer to fresh medium was necessary for some species even before cells had started to grow actively. Using the above techniques, we have succeeded in obtaining stable cultures of more than 50 species of marine pelagic algae. Among them was the N_2-fixing cyanophyte *Trichodesmium* sp. NIBB1067. N_2-fixation in this alga was studied most intensively and found to have an extremely high tolerance to O_2. *Trichodesmium* does not develop heterocysts which are the sites of aerobic N_2-fixation in filamentous cyanophytes: Almost all cells synthesized nitrogenase when this alga was grown in medium free of combined nitrogen. However, the highest N_2-fixation activity was observed in the light when O_2-production by photosynthesis occurred. It was suggested that N_2-fixation and O_2-evolving photosynthesis occurred simultaneously within the same cells. Furthermore, maintenance of active nitrogenase involves light-dependent process: Nitrogenase became inactive when cells were incubated in the dark, and illumination was necessary for activation of the enzyme. Possible mechanisms by which N_2-fixing system is protected from O_2 in *Trichodesmium* will be discussed.

S-22: RHODOPHYTES AS EXPERIMENTAL SYSTEMS FOR TRADITIONAL AND MOLECULAR GENETICS. *J.P. VAN DER MEER*, (NRC INSTITUTE FOR MARINE BIOSCIENCES, CANADA)

Mendelian genetic studies of *Gracilaria tikvahiae* during the 1970's and 1980's provided, for the first time, information on gene transmission in a division not previously examined in this fashion. From a genetic perspective, nothing very novel was discovered. Mutations were found to be inherited either in a typically "Mendelian" fashion, as expected for

genes located on chromosomes in the nucleus, or in a strictly maternal fashion similar to mutations in chloroplast DNA of many terrestrial plants. On the other hand, from a phycological perspective, these same observations brought to light a number of aspects of algal life histories not previously suspected. The genetic observations provided explanations for some long-standing and puzzling observations which had not been clarified by traditional phycological approaches. For example, they explained the origin of diploid gametes that arise regularly on normal diploid tetrasporophytes of *Gracilaria*. Observations on a spontaneous bisexual mutation revealed that loci other than the primary sex-determining locus could influence sexual expression and lead to diploid gametophytes.

This new genetic information on the formation of diploid gametes and gametophytes suggested that *Gracilaria* might be particularly advantageous for examining the biological effects of polyploidy in an organism with isomorphic gametophytic and sporophytic phases. Initial experiments on polyploid production confirmed this thinking and revealed interesting differences between gametophytes and sporophytes. Novel life history information was similarly obtained for species of *Champia, Palmaria, Porphyra* and *Gelidium* by combining phycological and genetic techniques.

In recent years, molecular biology methodologies have complemented, and also largely supplanted, many traditional genetic techniques. Some plant breeding objectives can now be attained more quickly through the use of DNA markers such as those revealed through various polymerase chain reaction (PCR) and blotting techniques. Using the PCR-based RAPD technique (where DNA amplification is initiated through a single primer of arbitrary sequence) to identify hybrids of the monoecious red alga *Gelidium vagum*, we were recently able to demonstrate heterosis for the first time in red algae.

At our institute, application of molecular methods to red algae is providing rich new information in a number of areas. An extensive examination of 18S rDNA sequences representing all of the major groupings of the Rhodophyta was recently completed and is giving some new insights into red algal evolution. Studies on the chloroplast genome of *Porphyra purpurea* through DNA cloning and sequencing is progressing very well. A detailed physical map of the plastid genome has been obtained and approximately 2/3 of the total genes have been mapped. It is evident that the plastid genome of red algae is considerably larger than that of most green algae and terrestrial plants, and that it retains a large number of genes that have been transferred to the nucleus in other lineages. Nuclear genes of two red algae, *Gracilaria* and *Porphyra*, are also being examined, and these studies are yielding significant new information on phase-specific gene expression, gene structure (with respect to introns and exons), and gene regulatory sequences, which will be of fundamental importance to future genetic engineering of these organisms.

S-23: *SPERMATOZOPSIS SIMILIS* (CHLOROPHYTA), A NEW MODEL SYSTEM FOR ALGAL CELL BIOLOGY. *M. MELKONIAN* (UNIVERSITÄT ZU KÖLN, GERMANY)

Spermatozopsis similis Preisig et Melkonian (Chlamydomonadales, Chlorophyta) is a small (4–7 μm), naked biflagellate green alga with a characteristic crescent and helically twisted cell shape (Preisig & Melkonian 1984). Since its first description it has become a model system in several areas of algal cell biology. Its main advantage is that it is covered only by the plasma membrane, no cell wall, glycocalyx or other type of extracellular matrix is present. Since *S. similis* can be grown in mass cultures and its growth characteristics are well characterized, it is well suited for mass isolation of subcellular organelles of a structural and functional integrity previously unattainable.

S. similis has been mainly used to study the cytoskeleton of flagellate green algae and the phototactic apparatus (eyespot apparatus).

Cytoskeletons have been isolated which

retain the peculiar shape of the cells and which can be reactivated in vitro to exhibit several of their in vivo functions, e.g. forward and backward swimming, centrin-mediated basal body reorientations, flagellar shedding, etc. New structural elements of the basal apparatus of green algae have been described from these isolated cytoskeletons, e.g. the rhizosyndesmos, a filamentous structure connecting the plus ends of microtubular flagellar roots. In addition, cytoskeletal proteins new to science have been purified and characterized, like SF-assemblin, the structural protein of the system I fibers (striated microtubule-associated fibers) of flagellate green algae. SF-assemblin represents a member of a new class of cytoskeletal proteins which form segmented coiled coils in vitro and in vivo (Lechtreck & Melkonian 1991, Weber et al. 1993). Basal apparatuses can be purified from the cytoskeletons enabling a detailed study of intrinsic basal body/centriolar proteins.

Intact eyespot apparatuses have been isolated for the first time in green algae using *S. similis* (Kreimer et al. 1991). These apparatuses retain not only the plate of eyespot globules but also the eyespot membranes, i.e. the chloroplast envelope and the plasma membrane overlying the eyespot globules. This preparation has been used to identify the presumptive photoreceptor all-trans retinal within a green algal eyespot (Kreimer et al. 1991b). Such isolated eyespot apparatuses are now used to identify components of the signal transduction chain during green algal phototaxis, e.g. the involvement of protein phosphorylation and G-proteins in signal transduction and amplification.

Future investigations will aim to demonstrate sexuality in taxon, produce a library of mutants and c-DNA.

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S-24: *ACETABULARIA* (DASYCLADALES, CHLOROPHYTA) AS A MODEL SYSTEM TO STUDY THE ROLE OF THE CYTOSKELETON IN ORGANELLE MOTILITY AND MORPHOGENESIS.

D. MENZEL (MAX-PLANCK-INSTITUT FÜR ZELLBIOLOGIE, ROSENHOF, GERMANY)

The giant unicellular green alga *Acetabularia cliftonii* possesses a highly dynamic cytoskeleton which undergoes several dramatic changes in appearance and properties throughout the life cycle. During the first two months of vegetative growth and morphogenesis the cytoskeleton is exclusively built of long parallel arrays of actin bundles which provide the physical basis for bidirectional organelle transport. Microtubules, on the other hand, appear for the first time when the cell enters the reproductive phase and play an important role during cyst morphogenesis. In the course of this process which, in essence, is the compartmentation of a coenocytic cytoplasm into numerous uninucleate units, the actin bundle system first disintegrates and then builds the cytokinetic cleavage system. Each unit gives rise to a walled cyst which represents the gametophytic stage of the alga. The cyst is capable of just one morphological differentiation, namely the formation of a lid. Evidence is presented that microtubules are involved in positioning of the lid. No sign of organelle movement is visible in the cyst up to several months of resting before gametes are formed, although actin is present in the form of scattered bundles. Okadaic acid and calyculin A, both specific inhibitors of protein phosphatases of the type 2A and 1, modulate the appearance of the actin cytoskeleton and the behavior of organelles in each of the stages but most dramatically in the cyst stage. Here, the scattered bundles first begin to laterally associate into long flat bands curving through the cytoplasm. Next, either several of these bands associate into large

circular arrays or individual bands begin to curl up and form tightly packed flat rings or disks. These structures are localized beneath the plane of the plasma membrane. Organelles attach to them and continuous counterclockwise movements of large columns of cytoplasm begin from two hours of treatment onwards up to several days. It is argued that actin or actin-associated components including myosin change their self assembly properties or activity, respectively, as a consequence of a higher level of phosphorylation on serin and threonine residues. This in turn leads to a novel cytoskeletal configuration not programmed by the cells own developmental regime. The identity of the potential targets of protein phosphatases is not known but it has been possible for the first time, to immunocytochemically localize actin-binding proteins such as alpha-actinin, spectrin and tropomyosin, to these actin ring structures. Such proteins could, therefore, be potential candidates responsible for the peculiar changes in the actin assembly and behavior induced by okadaic acid.

S-25: THE CYTOSKELETON OF FLAGELLATE CHROMOPHYTES: RECENT ADVANCES AND FUTURE DIRECTIONS. *P. L. BEECH* (SCHOOL OF BOTANY, UNIVERSITY OF MELBOURNE, AUSTRALIA)

I will review recent advances on the makeup and functions of the protistan cytoskeleton that have come from studies on flagellate chromophytes (especially the Chrysophyceae and Synurophyceae). Such studies include those on the flagellar developmental cycle and the changes in the whole flagellar apparatus during cell division; the secretion, deployment, and manipulation of scales and spines; prey capture and phagotrophy; and the immunological detection of particular proteins in flagellar roots and other cytoskeletal fibres.

I will also nominate areas of special interest for future studies.

S-26: VIRUS INFECTIONS IN FILAMENTOUS MARINE BROWN ALGAE. *D. G. MÜLLER* (FAKULTÄT FÜR BIOLOGIE DER UNIVERSITÄT, KONSTANZ, GERMANY)

Field specimens of filamentous brown algae (order Ectocarpales) are frequently found with abnormal reproductive structures, which are symptoms of a virus infection. The following details have been elaborated on laboratory cultures with the host *Ectocarpus siliculosus*:

—Somatic cells of the host are free of symptoms. Virus particles are only formed in vesicles of the host, that are homologous to zoidangia. Upon maturity, these vesicles burst and release one to several million virus particles into the surrounding seawater.

—Virions can only enter the unicellular and unwalled zoids of the host

—The virus genome is transmitted via host mitosis into every cell of the developing new plant.

—Some infected plants can partly restore their fecundity by reducing the expression of symptoms and thus may appear phenotypically "nomal".

—The virus genome is passed to the next generation by mito-spores of such "normalized" host plants.

—During meiosis on a hybrid between a healthy and an infected parent the virus genome segregates in a Mendelian pattern independent of the sex locus, and about 50% of the resulting offspring are free of virus symptoms.

—Virus particles contain at least 13 polypeptides, including two glycoproteins.

—The *Ectocarpus* virus genome is a double-stranded DNA with a size of 320 kbp. It is circular, with regularly interspersed single-stranded sites.

—Parallel studies with viruses in *Feldmannia simplex* and *F. irregularis* show that the brown algal viruses are largely species-specific.

—Cross infections are rare, and only one case of intergeneric transmission has been found (*Ectocarpus* to *Kuckuckia*), where the virulence is maintained. *Ectocarpus* virus that enters *Feldmannia simplex* causes somatic malfor-

mations without production of virus particles. —Field surveys show that virus infections in filamentous brown algae are a world wide phenomenon, and the conclusion seems inescapable, that the host populations and their viruses coexist in a well-coordinated state of pandemy.

S-27: ORIGINS OF CHLOROPLASTS. *R. A. LEWIN* (UNIV. OF CALIFORNIA)

Although the apparently obligate autonomy of chloroplasts has long been recognised, suggestions that they may have evolved as endosymbiotic microbes (prokaryotes) in previously heterotrophic cells (eukaryotes) were generally disregarded as implausible and untestable. However, developments of electron microscopy, fractionation biochemistry and molecular biology, and the discovery of prochlorophytes, have made symbiogenesis of chloroplasts much more plausible. Although phylogenetic trees based on fine-structural, biochemical and sequene data relating chloroplasts to *Prochloron, Prochlorothrix*, various cyanophytes and other plants are not completely concordant, a general consensus strongly supports this theory. Similarities and differences among the various trees will be discussed.

S-28: CURRENT PERSPECTIVES ON THE SYSTEMATICS AND PHYLOGENY OF THE GREEN ALGAE AND THE OTHER GREEN PLANTS. *R. L. CHAPMAN* (LOUISIANA STATE UNIVERSITY, U.S.A)

Relationships among distinctive group of green algae, and betwen green algae and land plants remain as intriguing now as they have been for more than a century. Both organismal characters (i.e., all non-molecular characters including, data on morphology, ultrastructure, life history, and habitat) and molecular data (e.g., RFLPs and gene sequences) provide bases for excluding the Euglenophytes and Chlorarachniophytes from the green algae. Extensive studies of organismal characters have suggested the division of the green algae into two, three, five, seven or more major groups (i.e., classes). Analyses of molecular data (especially nuclear-encoded ribosomal RNA gene sequences) support the concept of a grade of green algae with paraphyletic charophycean green algal lineages (e.g., Charales, Coleochaetales, and Zygnematales). Other green algae (e.g., Ulvophyceae, Chlorophyceae, etc.) form a single clade. The phylogeny inferred from analyses of molecular data is consistent with many aspects of current systematic treatments, but does not support the various proposals for delimiting classes of green algae. It must be noted that such a topology does not include the information derived from organismal data and cannot be considered a synthesis of the "total evidence" available. Although controversial, analyses of combined data sets are justifiable, and must be explored rather than dismissed as unacceptable *a priori*. In many cases, completing a data matrix for organismal data is more difficult than is the generation of a molecular data matrix of comparable size. Nevertheless, because stochastically changing molecules cannot provide the information needed to resolve *ancient rapid radiations*, the potential informational value of episodically changing organismal characters (such as ultrastructural features) remains too great to ignore or cast aside. Analyses of combined data sets for some groups of green algae have been completed, and others, including one for green algae and land plants, are under way. Molecular data for bryophytes and other land plants have been analyzed in combination with the green algal data and strongly support a single origin of land plants. There is no support from the molecular data for the hypothesis that different lineages of bryophytes (viz., mosses, hornworts, and liverworts) evolved from two or three separate green algal lineages. The most derived green algal taxa are, as expected, charophycean algae, but several interesting questions remain unresolved. For example, the relationships among the "conjugating green algae" (i.e., the Zygnematales) are unresolved and even the strongly supported hypothesis of monophyly for this group is

challenged by some of the preliminary data from *Spirogyra* and *Sirogonium*. The phylogenetic position of the Trentepohliales (subaerial filamentous green algae previously classified among the Ulvophyceae, Charophyceae, or Pleurastrophyceae by different investigators) is within the marine green algae (the Ulvophyceae). The strongly supported monophyly of the green algae and land plants (the Chlorobionta *sensu* Bremer) provides a potent argument against including the green algae within a taxonomic grouping of protists. (The research reported was supported in part by NSF grant DEB-9107389).

S-29: CHROMOPHYTES, ANOTHER LINEAGE OF PLANTS. *R. A. ANDERSEN* (BIGELOW LABORATORY FOR OCEAN SCIENCES, U.S.A)

The chromophyte algae are a major lineage of photosynthetic organisms that includes over ten taxonomic classes and an estimated 1 million species. The group, as defined here, includes the Bacillariophyta (Coscinodiscophyceae, Frangilariophyceae, Bacillariophyceae), Chrysophyceae, Dictyochophyceae, Eustigmatophyceae, Haptophyceae (=Prymnesiophyceae), Pedinellophyceae, Pelagophyceae (class. nov.), Phaeophyceae, Raphidophyceae, Synurophyceae, and Xanthophyceae. Also included within this lineage are certain protozoa (e.g., Bicosoecophyceae, Opalinida, some amoebae) and fungi (e.g., Labyrinthulids, Thraustochytrids, Oomycetes). The chromophyte algae occur in all aquatic habitats and in most terrestrial habitats, and they are dominant primary producers in marine habitats (71% of Earth's surface). Only the animals, bacteria and fungi equal or exceed the chromophytes in biodiversity. Due to secondary losses, this diverse assemblage has no characters that occur in all members. Most members contain one or more types of chlorophyll *c*, they harvest photosynthetically active light using carotenoids (especially fucoxanthin or its derivatives), their chloroplasts have three thylakoids per lamella, they have stiff hairs on the immature flagellum, their storage reserves are as low molecular weight β1-3 linked glucans. Other distinguishing characters found in several or many classes include a green autofluorescent substance in one flagellum, homologous flagellar roots, a paraxonemal rod, and a mature flagellum/chloroplast eyespot photoreceptor system. The phylogenetic relationships of the group are undergoing tremendous revision, especially those previously placed in Pascher's Division Chrysophyta and its three Classes, Bacillariophyceae, Chrysophyceae and Xanthophyceae. The Chrysophyceae sensu Pascher included many unrelated algae, and these are now placed in the Dictyochophyceae, Haptophyceae, Pedinellophyceae, Pelagophyceae and Synurophyceae. Now, it appears that the Xanthophyceae are more closely related to the brown seaweeds (Phaeophyceae) than to the other two classes. Also, it appears that the Bacillariophyceae are on a separate evolutionary branch with the Dictyochophyceae, Pedinellophyceae and Pelagophyceae. And, it appears that the Chrysophyceae *sensu stricto* are most closely related to the Synurophyceae and the Eustigmatophyceae. Finally, it appears that the Haptophyceae occupy an evolutionary branch that diverges deeply within the chromophytes. Current information for each class will be summarized. Phylogenetic trees based upon ultrasturcture, pigments and 18S rRNA gene sequences will be presented.

S-30: THE ORIGINS (OR IS IT ORIGIN?) OF CHLOROPLASTS IN CRYPTOMONADS AND CHLORARACHNIOPHYTES. *G. I. McFADDEN* & P. R.GILSON (UNIVERSITY OF MELBOURNE, AUSTRALIA)

Two types of algae, cryptomonads and chlorarachniophytes, are now known to contain photosynthetic eukaryotic endosymbionts. By engulfing and retaining an algal cell, these phagotrophs acquired the ability to photosynthesise. The algal endosymbiont is now integrated into the host cell and has apparently undergone severe reduction. The only remaining features of the endosymbiont are the chloroplast, nucleus, cytoplasm, and plas-

ma membrane. All other cell structures have vanished. We believe that the endosymbiont's nuclear genome and translation machinery are retained because they encode and synthesise proteins essential for the "stolen" chloroplast. Using probes specific for the remnant endosymbiont nuclei, we have identified chromosomes from these "captured" genomes. The endosymbiont nuclear genome is greatly reduced; typically less than 1 megabase. The coding function of these genomes is now being investigated to determine what algal "parts" the phagotrophic protozoans cannibalised for photosynthesis.

Interestingly, the endosymbiont genomes in cryptomonads and chlorarachniophytes have similar karyotypes, each possessing three small chromosomes that carry rRNA genes. The similarity in these karyotypes might be interpreted as meaning that both cryptomonads and chlorarachniophytes contain the same endosymbiont. However, differences in pigment complement, and chloroplast gene sequences (16SrRNA & *rbc*L) suggest that the endosymbionts are different. We believe that chlorarachniophytes and cryptomonads obtained their endosymbionts independently, just after the red and green algae had diverged from a common ancestor. The cryptomonads obtained a red algal-like endosymbiont, while the chlorarachniophytes obtained a green algal/euglenoid-like endosymbiont. The similarities in cryptomonad and chlorarachniophyte nucleomorphs stem from the similarity in the nuclear genomes of these early algal types. According to this hypothesis, the nucleomorph genomes could represent "frozen" examples of very early algal genomes and could tell us much about algal origins.

S-31: HETEROTROPHIC PROTIST PHYLOGENY AND THE ORIGINS OF THE ALGAE. *C. J. O'KELLY* (BIGELOW LABORATORY FOR OCEAN SCIENCES, USA)

At least six, and possibly nine or more, different lineages of eukaryotic algae are now extant. Each began with a different partnership between a phagotrophic host cell and a photosynthetic, prokaryotic or eukaryotic, endosymbiont.

For some algal lineages, the likely nonphotosynthetic antecedents are known. Photosynthetic euglenoids arose from nonphotosynthetic euglenoids, the euglenoids and the kinetoplast flagellates are sister taxa, and the zooflagellate genus *Diplonema* has features suggestive of the euglenoid/kinetoplastid ancestor. Photosynthetic dinoflagellates arose, several times, from nonphotosynthetic dinoflagellates, dinoflagellates are now thought to be sister taxa to ciliates and apicomplexans, and zooflagellates such as *Perkinsus* and *Colpodella* may provide clues to the origin and early diversification of this "alveolate" clade. Cryptomonads may have arisen from a zooflagellate similar to *Goniomonas*. Golden algae may have arisen from origanisms like those now placed in the zooflagellate family Bicosoecidae.

For the other algal groups, including the chlorarachniophytes, prymnesiophytes (if they have a different origin from other golden algae), red algae, green algae and glaucophytes (which may or may not have a single common ancestor), no group of nonphotosynthetic protists has yet been identified that may be regarded, on either structural or molecular evidence, to be the sister taxa of the photosynthetic ones. Discovery and analysis of these antecedents will help resolve major questions about photosynthetic diversity and plastid evolution, in particular whether plastids have one or three primary origins.

The realization that eukaryotic algae are polyphyletic has created major problems for algal taxonomy and nomenclature. Redefinition of higher taxa of eukaryotes, including such fundamental entities as the Plant Kingdom depends on discovery and analysis of the phylogenetic lineages among heterotrophic protists, in particular the free-living zooflagellates. Since relatively few of these organisms are known to science, and relatively few known species have been analyzed by fine structural or molecular methods, there is ample scope for future research.

Studies on a group of protists, informally named jakobids, suggests that most if not all mitochondrial eukaryotes arose from a single group of amitochondrial protists, of which the retortamonads are the extant representatives. These studies also suggest that the three major forms of mitochondrial cristae, discoidal, tubular and flattened, which are often used as a diagnostic feature for protist lineages, arose and were fixed very early in the evolution of mitochondrial eukaryotes. Mitochondrial cristae form may therefore help determine, in ambiguous cases such as the relationship of the protist genus *Katablepharis* to the cryptomonads, whether a heterotrophic protist resembles a photosynthetic one through shared common ancestry or through convergence.

S-32: HARMFUL ALGAL BLOOMS IN WESTERN PACIFIC WATERS. *Y. FUKUYO* (UNIV. OF TOKYO, JAPAN)

Harmful algal blooms are causing serious problems to coastal environment conservation and public health in western Pacific waters. An expansion of the affected area by the blooms has recently been occurring by migration of the causative organisms through natural phenomena such as water current, and by unintended transplantation through human activities such as ship's ballast water and transplanted shellfish for aquaculture.

The planktonic algae causing harmful blooms are subdivided into two groups according to their effects: noxious species which has implication to mass mortality of marine organisms, and toxic species which cause human illness. They differ from each other not only on their consequence, but on their ecological features such as the highest cell concentration and distribution.

Noxious specis often bloom to make red tides, and kill marine organisms after the formation of red tide. They are distributed mainly in highly eutrophic waters into where a large amount of industrial and domestic sewerage flow. Raphidoflagellates *Chattonella antiqua* and *Heterosigma akashiwo* pose serious damage to aquaculture of several kinds of fishes such as yellow-tail fish *Seriola quinqueradiata* in Japan. Total amount of the loss caused by the former species during 1972-1991 reaches 18.5 billion yen. Resting cysts of *C. antiqua* were found widely distributed in the bottom sediments of bloomed area.

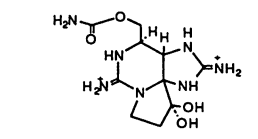
Other troublesome species such as *Gymnodinium mikimotoi* and *Cochlodinium polykrikoides* belong to dinoflagellates. Resting cysts of these species have not been found yet, and consequently they are considered as no cyst-producing species. A small number of planktonic cells of the former species could be found even in non-blooming season in some part of bloomed area.

Three types of consequence by toxic unicellular algae, which belong to Dinophyceae, are found in western Pacific waters: paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), and ciguatera fish poisoning (ciguatera). *Alexandrium tamarense, A. catenella, A. cohorticula* and *Gymnodinium catenatum* are known to bloom and cause PSP in temperate waters, and *Pyrodinium bahamense* in tropical waters, although some of them appear in areas of wide environmental ranges. All of them produce resting cysts, which may work as a good device for survival in unfavorable condition and for expansion of distribution. Toxins responsible for DSP are detected in several species belonging to the genus *Dinophysis* such as *D. fortii* and *D. acuminata* which bloomed in temperate waters. In tropical western Pacific no DSP has been reported, while the toxins are detected in shellfish. Further research is urgently necessary on this problem. Ciguatera causative dinoflagellate *Gambierdiscus toxicus* has been found in Ryukyu Islands, tropical islands of Japan. As the distribution of the species is presumed to be very wide, further research on ciguatera is also important and necessary in tropical countries.

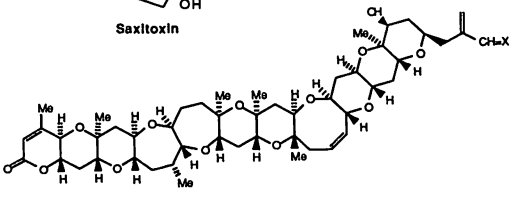
S-33: SOME THOUGHTS ON FACTORS WHICH INFLUENCE THE TOXIGENICITY OF DELETERIOUS MICROALGAE. *Y. SHIMIZU* (DEPARTMENT OF PHARMACOGNOSY AND EN-

VIRONMENTAL HEALTH SCIENCES, THE UNIVERSITY OF RHODE ISLAND, U.S.A)

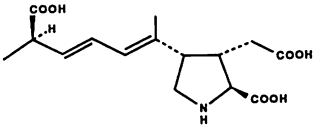
Various physico-chemical or environmental factors are reported to influence the toxin production by dinoflagellates and other deleterious microalgae. One of the most controvercial issues is about the role of bacteria in the toxigenicity. The production of saxitoxin and other paralytic shellfish poisons (PSP) by bacteria isolated from *Alexandrium* spp. and other organisms has been reported by some groups. On the other hand, there seems to be strong evidence that certain strains of the dinoflagellates are inherently toxigenic. This author will report the findings in his laboratory, and discuss possible explanations for these conflicting reports.



Saxitoxin



Brevetoxin B



Domoic Acid

Similar questions also exist regarding the polyether type toxin production by dinoflagellates. The polyether type toxins such as brevetoxins by *Gymnodinium breve* are produced by very unique biosynthetic mechanisms. The unusual biosynthetic pathway seems to be closely related to the peculiar life style and metabolism of the dinoflagellates. Studies have been also made with the amnesic shellfish toxin, domoic acid, production by *Nitzschia* and other diatoms. It was shown that domoic acid is produced by very specific chemovars. The chemovars are also characterized by the production of other secondary metabolites, bacillariolides. It was observed that the extracellular content of domoic acid is phase-dependent and negatively influenced by the presence of bacteria in the culture. Efforts are being made to determine the factors which enhance the production of domoic acid. [Supported by NIH grant, GM 28754]

S-34: TAXONOMY OF WATER-BLOOM FORMING CYANOPROKARYOTES. *J. KOMAREK* (UNIV. SOUTH BOHEMIA, CZECHOSLOVAKIA)

The species delimitation of planktonic cyanoprokaryotic species cannot be solved by a unique scheme. Among picoplanktonic or water-bloom forming cyanoprokaryotes there exist morphologically as well as ecologically very stabilized types, which develop and occur repeatedly under specialized conditions, and which are well defined in spite of their relatedness to similar, but also unambiguously delimited species (*Aphanizomenon flos-aquae, A. klebahnii, Microcystis viridis, Tychonema bourrellyi*, etc.). However, morphologically and biochemically unstable populations also exist, which are extremly variable and diversified into numerous deviations and represent a source of numerous lines and strains (*Microcystis aeruginosa, Limnothrix redekei, Anabaena flos-aquae, Cylindrospermopsis reciborskii*, etc.). In such case we obtain a large set of diversified strains, each slightly different from the other.

This situation must be taken into consideration in all taxonomic evaluations of cyanoprokaryotes. No isolated approach can solve the taxonomic relations between stable and/or variable types. The biochemical criteria (also, e.g., isozyme analyses, G/C ratios, etc.) cannot be applied without reservation either, and show diversity between strains (sometimes isolated from one and the same locality, population).

The resulting recommendation corresponding with the endeavour to classify the cyanoprokaryotic populations in different natural and managed waters and to characterize their role in planktonic biocenosis, is to

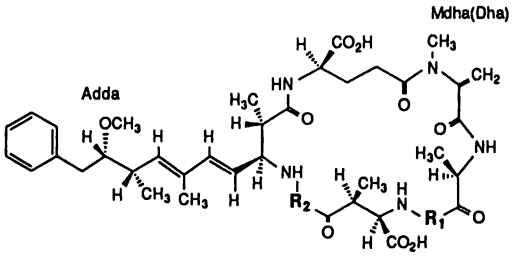
use as wide and complex an evaluation of cyanoprokaryotic populations, as possible, and to define all types, which represent the special ecological and morphological entities in planktonic biotopes.

S-35: CHEMISTRY AND TOXICOLOGY OF MICROCYSTINS AS CYANOBACTERIA HEPATOTOXINS. *K. KAYA* (NATIONAL INSTITUTE FOR ENVIRONMENTAL STUDIES, TSUKUBA, JAPAN)

Eutrophication of freshwater lakes and ponds has reached a fairly advanced stage by urban, agriculture and industrial sources containing phosphorus and nitrogen nutrients, and has caused the occurrence of the bloom-forming by cyanobacteria (Blue-green algae). Blooms of *Microcystis, Anabaena*, and *Oscillatoria* often contain toxins. The toxic blooms were reported as causing death to domestic animals after drinking affected water, and as a potential hazard to human health. The toxic *Microcystis, Anabaena* and *Oscillatoria* produce low-molecular-weight toxins, which have been to be cyclic heptapeptide named "microcystin". In this symposium, we report the current information regarding chemistry and toxicology of microcystins.

Microcystins are monocyclic heptapeptides and contain three D-amino acids (alanine, *erythro*-β-methylaspartic acid and glutamic acid), two L-amino acids (leucine (L)-alanine (A), leucine (L)-arginine (R), tyrosine (Y)-arginine (R) or arginine (R)-arginine (R)) and two unusual amino acids (N-methyldehydroalanine and 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (Adda)). Microcystin RR is the major toxin of *M. viridis*, also microcystin LR is the major toxin of *M. aeruginosa*.

When mice were given microcystin LR intraperitoneally, the lethal dose was about 100 μg/kg. In mice and rats, microcystin LR is a potent, rapid-acting, direct hepatotoxin, with the immediate cause of death in acute toxicities being hemorrhagic shock and hepatocellular necrosis. The livers weights increased about 150~200% of those of the



Adda: 3-amino-9-methoxy-10-phenyl-2,6,8-trimethyl deca-4,6-dienoic acid, Mdha: N-methyldehydroalanine, R_1: L-Leu, R_2: L-Arg

control mice within 3 hr. The uptake of the toxin is through the bile acid transport system. The toxins in hepatocytes act as an inhibitor of protein phosphatase type 1 and 2A, and induces hyperphosphorylation of cytoskeletal proteins and other functional proteins. These hyperphosphorylated proteins induce marked morphological alterations and tumor promotions. The toxin in hepatocytes also induces the production of prostaglandins and thromboxanes as chemical mediators for inflammation.

Peritoneal macrophages are also affected by the toxin. The toxin induces the productions of interleukin 1 (IL-1), tumor necrosis factor-α (TNFα), prostaglandins and thromboxanes. Especially, TNFα and thromboxane B2 are increased strikingly in blood of mice and rats by the injection of the toxins. These results suggest the toxicity of the microcystins is related closely to inflammatory shock.

S-36: ON THE OCCURRENCE AND CONTROL OF TOXIC CYANOBACTERIAL BLOOMS IN AUSTRALIA. *G. J. JONES* (CSIRO DIVISION OF WATER RESOURCES, AUSTRALIA)

Blooms of toxic cyanobacteria have become common in Australian rivers, reservoirs and lakes during the past decade. It is uncertain whether there has been a real increase in the frequency of blooms or simply an increase in awareness, and in the intensity of water quality monitoring, by government authorities. The most common bloom-forming species are hepatotoxic *Microcystis aeruginosa* in lakes and

reservoirs, *Nodularia spumigena* in brackish waters and estuaries, and neurotoxic *Anabaena circinalis* in rivers (*A. circinalis* is also commonly found in reservoirs, particularly in cooler regions). The neurotoxins found in Australian *A. circinalis* were recently shown to be saxitoxins and gonyautoxins, rather than anatoxin-*a* or anatoxin-*a*(s) as found in other parts of the world. Stock deaths caused by consumption of toxic cyanobacteria in rivers and farm dams are common. There have also been widespread closures of drinking water supplies and recreational waters because of the presence of cyanobacterial blooms. Interim health guidelines have recently been established, with a maximum level of 1 μg microcystin L^{-1} recommended for long-term consumption of drinking water.

It is widely held view amongst Australian water managers and scientists, that toxic cyanobacterial blooms occur because of anthropogenic inputs of phosphorus to surface waters (viz. "cultural eutrophication"). However, it is possible to argue that cyanobacterial blooms are a "natural" consequence of impounding water in a hot, dry country, and not the result of nutrient pollution. There are few economically important water bodies in Australia that are not impounded. Most of Australia's major rivers are regulated by weirs which provide a hydraulic head for irrigation off-take. During the dry summer months river flow may be trivial or non-existent downstream of major irrigation areas. Australian reservoirs are typically dammed river valleys that undergo seasonal temperature stratification. I will discuss the hypothesis, that cyanobacteria tend to dominate the phytoplankton of stratified, impounded waters, irrespective of nutrient availability. Furthermore, the more turbid the water body, the greater the likelihood of cyanobacterial dominance. Australian rivers, particularly those of the Murray-Darling Basin, tend to be high in inorganic clay turbidity. An important factor in assessing this hypothesis is the recognition that a "bloom" of cyanobacteria does not necessarily constitute a high total cyanobacterial biomass in a waterbody. The inherent buoyancy of bloom-forming cyanobacteria, coupled with wind-driven advection, provides a natural concentrating mechanism for the formation of surface "scums". This tends to greatly exaggerate the overall cyanobacterial biomass of the water body.

It is possible to predict the occurrence of cyanobacterial blooms in regulated rivers and reservoirs based on a knowledge of water column turbulence. In rivers, the major factors controlling the onset of blooms are river flow (discharge) rate and depth. Cyanobacteria typically succeed diatoms and green algae as stratification occurs, and their dominance is the result of water column stability not increasing nutrient availability. A major management technique for preventing cyanobacterial blooms, therefore, should be artificial destratification and river flow management, not only amelioration of nutrient inputs.

P-1: PHENOLOGY OF ESTUARINE ALGAE ON THE KIDOGAWA RIVER MOUTH, CHIBA PREFECTURE, CENTRAL JAPAN.

M. YOSHIZAKI (TOHO UNIV. JAPAN), T. FUJITA (NIHON UNIV. NARASHINO HIGH SCHOOL, JAPAN), T. HATOGAI (CHIBA PREFECTURAL GENERAL EDUCATION CENTER, JAPAN) & K. IURA (NARASHINO CITY HALL, JAPAN)

The Kidogawa river, whose source is near Narita Air Port, flows through Kujyukuri into the Pacific Ocean. At its mouth area, the tidal range is so great that the river shows its bed at the lowest tide and is filled with water at the highest tide. There are no mangroves around here. The water temperature rose up to 29°C in the summer and in the winter fell down to 7°C. As many as 16 species of macro algae (*Bangia atropurpurea, Blidingia marginata, Bostrychia simpliciuscula, Caloglossa leprieurii, Ca. ogasawaraensis, Cladophora opaca, Cl.* sp. *Enteromorpha linza, E. prolifera, Gracilaria asiatica, Monostroma latissima, Porphyra suborbiculata, Rhizoclonium riparium, Ulothrix flacca,*

Ulva pertusa and *Ulospora penicilliformis*) grew in the range which covers 1 km course up the river mouth, and we closely observed how they distributed and their seasonal succession. Among these species, *Ca. leprieurii, Ca. ogasawaraensis* and *Bo. simpliciuscula* could be seen throughout the year. *Caloglossa* grew in the wide range from the mouth to the upper stream, while *Bostrychia* grew only in the reed-grown marshes. We found the tetrasporophyte of *Caloglossa* of two species all the year round, and form of gametophyte from June to December. By contrast, as for *Bostrychia*, we could find neither its tetrasporophyte nor its gametophyte.

P-2: LIGHT-HARVESTING OF MARINE STRAINS OF *SYNECHOCOCCUS* (CHROOCOCCALES, CYANOPHYTE) UNDER LIGHT ENVIRONMENTS NEAR THE BOTTOM OF EUPHOTIC ZONE IN OPEN OCEAN. *T. IKEYA* (NAT. INST. FOR BASIC BIOLOGY, UNIV. OF TOKYO, JAPAN), K. OHKI* (NAT. INST. FOR BASIC BIOLOGY, JAPAN), M. TAKAHASHI (UNIV. OF TOKYO, JAPAN) & Y. FUJITA (NAT. INST. FOR BASIC BIOLOGY, JAPAN). *PRESENT ADDRESS: TOKAI UNIV., JAPAN.

Photosynthesis of marine *Synechococcus* isolated from waters of Kuroshio and Gulf stream was examined using model light, which simulated blue-green light near the bottom of euphotic zone in open ocean. Although the spectral distribution of light energy is limited around blue-green region near the bottom of euphotic zone in open ocean, photosynthesis was found to be active enough for supporting growth under such a light regime. All strains isolated have a common character that abundance of phycoerythrin is unusually high, and so they can absorb efficiently the blue-green light. Occurrence of phycourobilin as a chromophore of phycoerythrin gives a more advantage in absorption of the blue-green light. Thus, marine *Synechococcus* can perform actively photosynthesis even near the bottom of euphotic zone. Carotenoids, absorbing the blue-green light nearly 50% of total absorption, are effective in light-harvesting for photosystem I. These properties of light-harvesting system of marine *Synechococcus* extends range of living area in open ocean down to the bottom of euphotic zone as found in Kuroshio water.

P-3: CHEMICAL FACTORS LIMITING THE GROWTH OF BLOOMING ALGAE IN JAPANESE COASTAL WATERS (SETO INLAND SEA)—AN ANALYSIS BY ALGAL BIOASSAY. *B. KIMURA*, M. MURAKAMI (SHIMONOSEKI UNIV. FISH., JAPAN), T. NAGAI, T. MANABE (HYOGO PREF. FISH. EXP. STN., JAPAN), T. ETOU, M. KAMIZONO (FUKUOKA PREF. FISH. EXP. STN., JAPAN), AND Y. ISHIDA (KYOTO UNIV., JAPAN)

Chemical factors limiting the growth of blooming algae were studied in Japanese coastal waters by use of algal bioassay method.

In east part of Seto Inland Sea (Harima Nada), where eutrofication level is high (DIN average in 20 m-depth bottom waters during summer period; 7.2 μg-at/l) and bloom of the raphidophyte flagellate *Chattonella antiqua* often occurs, there was no clear-cut correlation between the growth potential of *C. antiqua* and DIN in coastal waters. In 1992, when no bloom of *C. antiqua* was observed, the addition of EDTA to the waters caused remarkable increase in the growth potential of the alga. It was suggested that organic metal chelators were limiting factors for the growth of *C. antiqua* in this area.

In west part of Seto Island Sea (Suo Nada), where eutrofication level is not high as that of Harima Nada (DIN average in 14 m-depth bottom waters during summer period; 1.1 μg-at/l) and bloom of *C. antiqua* occurs less frequently than in Harima Nada, there was a correlation between the growth potential of *C. antiqua* and DIN in the waters. It was suggested that DIN was a major limiting factor for the growth of *C. antiqua* in this area. In

1992, a bloom of the dinoflagellate *Gymnodinium mikimotoi* occurred, accompanied by a sudden appearance of anoxic condition in bottom waters and increase in both in DIN and algal growth potential in these waters.

P-4: A COCCOLITHOPHORID BLOOM IN KAGOSHIMA BAY, SOUTHERN JAPAN. *T. HIWATARI* (JAPAN NUS CO. LTD.), K. ORITA (KAGOSHIMA PREF. FISHER. EXPER. STATION), Y. MITOMI, H. FUKUSHIMA (TOKAI UNIV.) & T. AKANO (KANSAI ELECTRIC POWER CO. INC. JAPAN)

A massive algal bloom of the coccolithophorid *Gephyrocapsa oceanica* Kamptner (Haptophyta) developed in innermost bay of Kagoshima Bay at 31°N, 131°E on the southern Japan, in early May 1992. The bloom discoloured the innermost bay a milky blue-green, which was clearly detectable in the visible band by the USA satellite NOAA-11. The bloom, which reached cell densities of 1.3×10^4 cells/ml, persisted for half a month.

P-5: EFFECT OF CO_2 CONCENTRATION ON CALCIFICATION IN THE COCCOLITHOPHORIDS. *A. YUZAWA*, T. HIWATARI (JAPAN NUS CO. LTD.), M. OKAZAKI (TOKYO GAKUGEI UNIV.), M. YAMAMOTO, T. AKANO & M. KIYOHARA (KANSAI ELECTRIC POWER CO. INC. JAPAN)

To estimate the relationship between CO_2 concentration (0.036, 0.2, 0.5% in air) and calcification, measured as inorganic carbon of coccoliths, *Gephyrocapsa oceanica* and *Pleurochrysis haptonemofera* were culured by aeration at high CO_2 concentrations. In both *G. oceanica* and *P. haptonemofera*, the growth rate and calcification rate at 0.2%, 0.5% CO_2 were lower than those at 0.036% CO_2 bacause the pH values at 0.2%, 0.5% CO_2 decreased rapidly. In an experiment where the pH was maintained around 8.2, growth rate and calcification rate were highest at 0.2% CO_2 in *G. oceanica* and at 0.5% CO_2 in *P. haptonemofera*. These experiments indicated that by controlling the pH, the increase in CO_2 led to enhanced calcification in *G. oceanica* and *P. haptonemofera*.

P-6: MECHANISM FOR ACQUISITION OF DISSOLVED INORGANIC CARBON FOR PHOTOSYNTHETIC CO_2 FIXATION AND CALCIFICATION BY *EMILIANIA HUXLEYI* (PRYMNESIOPHYTA), *K. SEKINO* & Y. SHIRAIWA (NIIGATA UNIV., JAPAN)

Photosynthesis of marine unicellular calcareous alga *Emiliania huxleyi* was found to be sensitive to environmental oxygen and to show low affinity (apparent $K_{0.5} = 5.5$ mM) for dissolved inorganic carbon (DIC), even the alga was grown under the bubbling of ordinary air. Carbonic anhydrase activity was not detected in cell homogenates and the activity of the DIC-concentrating mechanism measured using the silicone-oil-layer-filtering-centrifugation technique was low in comparison with cyanobacteria and green algae.

When only CO_2 was added as substrate, it was used mainly for photosynthetic CO_2 fixation via internal DIC accumulation (CO_2-pool), but not for the calcification. In contrast, HCO_3^- was mainly utilized for calcification via internal DIC accumulation HCO_3^--pool), but not directly used for the fixation. The CO_2-pool was several times larger than the HCO_3^--pool, but not used for the calcification. The HCO_3^--pool was dominantly used for the calcification and also significantly used for the fixation.

These two-way systems for the acquisition of external DIC seems to play an important role for increasing total amount of DIC utilization in this alga.

P-7: RATES OF PHOTOSYNTHESIS AND CALCIUM CARBONATE FORMATION IN THE COCCOLITHOPHORID ALGAE *PLEUROCHRYSIS CARTERAE* AND *EMILIANIA HUXLEYI* (HAPTOPHYTA). *M. OKAZAKI,* C. EZAWA, N. ICHIMURA (TOKYO GAKUGEI UNIV., JAPAN), H. TAKANO & T. MATSUNAGA (TOKYO UNIV. OF AGRICULTURE

AND TECHNOLOTY, JAPAN)

The rates of CO_2 fixation by photosynthesis and $CaCO_3$ deposition by coccolith formation in *Pleurochrysis carterae* and *Emiliania huxleyi* were determined to evaluate the effects of both processes on pCO_2 in seawater. Each process has an opposite effect on the pCO_2 change. Both rates were determined by measuring the change in dissolved inorganic carbon and in titration alkalinity.

In *P. carterae* at logarithmic phase of growth, the rate of photosynthesis and $CaCO_3$ deposition at 6 klux, 22 C were 3400 ng and 170 ng C/hr/10^6 cells, respectively. In the dark, the rates of respiration and $CaCO_3$ deposition were 800 ng and 20 ng C/hr/10^6 cells, respectively. In logarithmically growing *E. huxleyi*, photosynthetic and calcification rates were 320 ng and 280 ng C/hr/10^6 cells, respectively. Thus, during logarithmic growth, carbon fixation rate by photosynthesis was higher than that by $CaCO_3$ deposition. Therefore it is not expected that the pCO_2 seawater increases in the light.

P-8: EFFECT OF SEAWATER CONCENTRATION ON THE PHOTOSYNTHETIC ACTIVITY IN SOME SEAWEEDS GROWING IN THE INTERTIDAL ZONE. *N. KATAYAMA* (TOKYO GAKUGEI UNIV., JAPAN) & Y. YOKOHAMA (UNIV. OF TSUKUBA, JAPAN)

The effect of a progressive seawater dilution on *Dictyota dichotoma, Ishige sinicola* (brown algae), *Chondrus occellatus, Pachymeniopsis lanceolata, Schizymenia dubyi* (red algae) was examined with reference to its effect on their photosynthetic activity. The photosynthetic rates of these seaweeds were measured at 20°C during a stepwise decrease in seawater concentration which should resemble their *in situ* conditions. In each case the photosynthetic rate decreased gradually as the seawater concentration fell until completely stopping in distilled water (DW). After measuring the photosynthetic rates in DW, the fronds of these seaweeds were reimmersed in normal seawater. While the photosynthetic rate in *I. sinicola* recovered completely with reimmersion, the rates in *C. occellatus* and *P. lanceolata* recovered only partially. Scarecely any recovery of the rate in *D. dichotoma* and *S. dubyi* was observed. The seaweeds examined in the present study except *I. sinicola* grow within the lower eulittoral zone and upper sublittoral zone. The photosynthetic activity in these seaweeds is shown to be more sensitive to the seawater concentration fall than that in the seaweeds growing at the higher level in the eulittoral zone.

P-9: EFFECTS OF TEMPERATURE ON THE ANTARCTIC GREEN ALGA, *CHLORELLA VULGARIS* (CHLOROPHYTA). *H. NAGASHIMA* (SCIENCE UNIV. OF TOKYO), S. OHTANI (SHIMANE UNIV.) & H. MOMOSE (SCIENCE UNIV. OF TOKYO)

Unicellular green alga, *Chlorella vulgaris* SO-26 was isolated from a wet moss surface, near Showa Station, Antarctica, where the temperature ranged from 20°C in summer to about −30°C in winter. The alga SO-26 and a mesophilic alga, *C. vulgaris* IAM C-133 were pre-cultured at 20°C under fluorescent light at about 22 μmol/m^2/s. Strain SO-26 could photosynthesize between 0 and 35°C, with an optimum at 20°C. Strain C-133 could photosynthesize between 5 and 45°C, with an optimum at 35°C. When both strains were pre-cultured at 10°C, their optimal temperatures of photosynthesis shifted downward by about 5°C. After two weeks of culturing, strain SO-26 could grow between 5 and 30°C, while strain C-133 could grow between 10 and 40°C, a higher temperature range than that of strain SO-26. When strain SO-26 was heated to 40°C for 30 min, it lost its photosynthetic activity at 20°C, while strain C-133 retained its photosynthetic activity after the 45°C treatment for 30 min. After treating both strains at −20°C for 2 hours, only strain SO-26 retained its photosynthetic activity. These results show that the Antarctic alga, *Chlorella vulgaris* SO-26, has psychro- and freeze-tolerant properties and that the alga is well adaptable to Antarctic

environments.

P-10: POSSIBLE INVOLVEMENT OF A NITRATE-INDUCIBLE PLASMA MEMBRANE PROTEIN TO NITRATE UPTAKE OF *HETEROSIGMA AKASHIWO* (RAPHIDOPHYCEAE). *N. MIYAGI* & T. FUJII (UNIV. OF TSUKUBA, JAPAN)

Nitrate uptake of *Heterosigma akashiwo*, a marine raphidophycean unicellular alga, was measured using $^{15}NO_3^-$. Nitrate uptake occurred in nitrate-grown cells, but not in ammonium-grown cells. Nitrate uptake was completely lost within 1 day after the trasfer of nitrate-grown cells to ammonium-containing medium, and began to increase 3 hours after the transfer of ammonium-grown cells to nitrate-containing medium. Even when nitrate reductase activity was inhibited using sodium tungstate, nitrate uptake was not inhibited.

Highly purified plasma membranes were isolated by the silica microbead method. Polypeptides on the membranes were analyzed by SDS-PAGE and immunostaining. A major polypeptide with a molecular mass of 26 kDa appeared and disappeared in harmony with the rise and fall of nitrate uptake rate. This polypeptide appeared 3 hours after the transfer of ammonium-grown cells to nitrate-containing medium, and disappeared within 1 day after the transfer of nitrate-grown cells to ammonium-containing medium.

From these, nitrate uptake of *Heterosigma akashiwo* is nitrate-inducible, and a 26 kDa plasma membrane protein would be concerned with nitrate uptake.

P-11: ANALYSIS OF MODE OF A ACTION OF SEX PHEROMONE, PROTOPLAST-RELEASE-INDUCING PROTEIN, IN *CLOSTERIUM PERACEROSUM-STRIGOSUM-LITTORALE* COMPLEX (CHLOROPHYTA). *H. SEKIMOTO* & T. FUJII (UNIVERSITY OF TSUKUBA, JAPAN)

When mating-type minus (mt^-) and plus (mt^+) cells of *Closterium* were mixed together in a mating medium, both cells released protoplasts, this release being the first step in the process of conjugation. Release of protoplasts by mt^- cells also proceeded without pairing when mt^- cells were placed in a medium in which mt^- and mt^+ cells had previously been cultured together. A protein with the ability to induce the release of protoplasts was purified and it was designated as protoplast-release-inducing protein (PR-IP). PR-IP had an apparent M_r of 95 kDa on gel filtration and consisted of two glycopolypeptides of M_r 42 and 19 kDa.

Induction of release of protoplasts was dependent upon the duration of pre-incubation before the treatment with PR-IP and a low density of cells at the pre-culture stage had a significant stimulatory effect on the induction by PR-IP. Based on these results, we suggested that the pre-culture of mt^- cells at a low cell-density under continuous light was very important for the conversion of vegtetative cells to gametes, which could respond to PR-IP.

When mt^- cells were incubated with biotinylated PR-IP for 6 h, only a M_r 19 kDa polypeptide of was detected in cells by staining with the avidin and biotinylated horseradish peroxidase. The amount of this bound polypeptide increased with increasing doses of PR-IP and reached a maximum at around 10 nM, reflecting the PI activity. The binding proceeded only after an appropriate period of pre-culture in the light, and the polypeptide was competitively displaced by non-biotinylated PR-IP. It appeared that the PR-IP induced the release of protoplast from mt^- cells via binding of a M_r 19 kDa polypeptide to the receptor on cell surface, in a manner anlogous to the binding of peptide hormones to their receptors in animals.

P-12: DETECTION AND CHARACTERIZATION OF A NOVEL SEX PHEROMONE, PROTOPLAST-RELEASE-INDUCING PROTEIN INDUCER, IN *CLOSTERIUM* (CHLOROPHYTA). *T. NOJIRI*, Y. INOKI, T. FUJII & H. SEKIMOTO (UNIVERSITY

OF TSUKUBA, JAPAN)

We had previously purified and characterized a sex pheromone, protoplast-release-inducing protein (PR-IP), which was responsible for the induction of gametic protoplast-release of *Closterium* mating-type minus (mt^-) cells [Planta **182:** 348-354 (1990)]. The PR-IP was released from mating-type plus (mt^+) cells when mt^+ and mt^- cells were cultured together in nitrogen-deficient medium. When mt^+ cells were incubated in the medium, in which mt^- cells were cultured alone, release of PR-IP was also observed. These results indicated the existence of substance(s) which was released from mt^- cells and had an ability to make mt^+ cells release PR-IP. We designated the substance as PR-IP Inducer. The PR-IP Inducer was constitutively released from mt^- cells under the light, and the activity showed heat-lability. The PR-IP Inducer was purified by sequential column chromatographic steps (DEAE-Sepharose CL-6B, hydroxylapatite, Sephacryl S-100 HR and Mono Q columns). Molecular weight of PR-IP Inducer was estimated as 18.7 k by mass-spectrum analysis. We suggest that the PR-IP Inducer is a novel pheromonal protein which plays a role for initial events of sexual communication of this *Closterium peracerosum-strigosum-littorale* complex.

P-13: PLASMA MEMBRANE-BOUND ADENOSINE TRIPHOSPHATASE OF CALCAREOUS RED ALGA *SERRATICARDIA MAXIMA* (CRYPTONEMIALES, RHODOPHYTA). *I. MORI* & M. OKAZAKI (TOKYO GAKUGEI UNIV., JAPAN)

A marine alga *Serraticardia maxima* deposits $CaCO_3$ in the cell walls and its content can reach 85% of the dry weight. Cell respiration was specifically accelerated by up to 50% by addition of Ca^{2+} to 60 mM. This sensitivity to Ca^{2+} was not observed in other non-calcareous red algae. These facts suggested the involvement of a Ca-pump to excrete intracellular Ca^{2+}. We therefore purified *S. maxima* plasma membrane from the microsome fraction by an aqueous two-phase partitioning method. The purified membrane fraction had essentially no activity of marker enzymes for Golgi-body, endoplasmic reticulum or mitochondria such as IDPase, NADPH-cytochrome c reductase or cytochrome c oxidase. Most of the membrane vesicles were stained by a phosphotungstic acid-chromic acid-staining which was specific for plasma membrane. The plasma membrane had high ATPase activity which was inhibited by 20% with 0.1 mM sodium vanadate, but activated by 15% with 50 mM potassium nitrate. The Ca^{2+} increased the ATPase activity at the μM level. This ATPase(s) may be involved in Ca^{2+}-stimulated respiration and $CaCO_3$ deposition in cell walls.

P-14: GLYCOLATE OXIDASE AND GLYCOLATE DEHYDROGENASE IN RED ALGAE (RHODOPHYTA). *K. IWAMOTO* (INST. OF BIOL. SCI., UNIV. OF TSUKUBA, JAPAN) & T. IKAWA (INST. OF BIOL. SCI., UNIV. OF TSUKUBA, JAPAN)

Characteristics of glycolate-oxidizing enzymes in crude extracts of red algal specimens in the Porphyridiales, Compsopogonales, Bangiales, Nemaliales, Gelidiales, Cryptonemiales, Gigartinales, Rhodymeniales and Ceramiales were compared by measuring glycolate-dependent H_2O_2 formation and reduction of cytochrome c (cyt-c) and DCPIP, and by checking cyanide sensitivity and the stereospecificity for D- and L-lactate. Unicellular red algae, such as *Dixoniella, Rhodella* and *Porphyridium* and freshwater alga *Compsopogon* contained glycolate oxidase (GOX) similar to that found in Charophyta and Chromophyta whereas all multicellular seawater red algae contained glycolate dehydrogenase (GDH). Among the GDHs in red algae, only GDH in the Nemaliales showed no glycolate: cyt c reducing activity. GDHs in the Bangiales, Nemaliales, Ceramiales, and Gigartinales were insensitive to cyanide whereas those in the Gelidiales, Cryptonemiales and Rhodymeniales were sensitive to it. The occurrence of GOX and GDH in

rhodophytes seems to have a phylogenetic significance in the algae besides Chlorophyta, and GOX of red algae seems to have a different origin from that of green plants.

P-15: IMMUNOLOGICAL COMPARISONS OF NITRATE REDUCTASES FROM VARIOUS PLANT SOURCES USING MONOCLONAL ANTIBODIES TO NITRATE REDUCTASE FROM THE RED ALGA *PORPHYRA YEZOENSIS* (BANGIALES, RHODOPHYTA). *Y. NAKAMURA* (UNIV. OF TSUKUBA, JAPAN), H. SAJI (NAT. INST. ENVIRON. STUD., JAPAN), N. KONDO (NAT. INST. ENVIRON. STUD., JAPAN) & T. IKAWA (UNIV. OF TSUKUBA, JAPAN)

Assimilaroty nitrate reductases (NR) from eukaryotic plants catalyze the reduction of nitrate to nitrite using NAD(P)H as reducing substrate. They can be distinguished from prokaryotic NRs by the difference of their molecular compositions, prosthetic groups and amino acid sequences. Eukaryotic NRs share common structural properties such as molecular weight, subunit composition and prosthetic groups. Comparison of amino acid sequence data obtained from fungi, green algae and higher plants has also shown the resemblance among these eukaryotic assimilatory NRs. However little is known about NRs from non-chlorophyll ab species, which occupy phylogenically major positions. We have recently developed the purification procedure for NR from the red alga *Porphyra yezoensis* and prepared monoclonal antibodies against it. In this study, cross-reactivities of the antibodies thus obtained to the NRs from Rhodophyta, Cryptophyta, Chromophyta, Chlorophyta and higher plants were investigated.

Twenty two monoclonal antibodies were obtained. Fifteen of them could detect 100 kD NR subunit molecule of *P. yezoensis* and cross-reactivity was examined with these antibodies. NR subunit molecules from *Bangia* and *Porphyra katadae* which belong to the same order with *P. yezoensis* showed the highest cross reactivity. NR subunit molecules from multicellular red algae belonging to different orders from *P. yezoensis* could be detected by 3 to 7 of the antibodies. On the contrary, none of the antibodies could detect NRs from unicellular red algae except for that from *Flintiella*. Generally, the antibodies could not detect NR from plants belonging to different divisions. However some of the NR molecules from Cryptophyta, Chromophyta, Chlorophyta and higher plants were detected by one to 4 of the antibodies. it was revealed by this study that conserved regions in eukaryotic NRs do exist in wide variety of eukaryotic plants.

P-16: CLASS II ALDOLASES IN ALGAE—A COMPARISON WITH THE BACTERIAL ENZYMES. *C. SCHNARRENBERGER*, W. GROSS & B. PELZERREITH (INSTITUTE OF PLANT PHYSIOLOGY AND MICROBIOLOGY, FREE UNIVERSITY OF BERLIN, GERMANY)

Class I and class II aldolases differ in their mode of catalysis, molecular structure, and kinetic properties. Class I aldolases are distributed in animals, higher plants, green algae, some other algae and in a few bacteria. Class II aldolases were found in fungi, some bacteria, all cyanobacteria, in the cytosol of *Euglena gracilis* and in some non-green algae. If comparing class II aldolases from *Euglena gracilis*, *Cyanophora paradoxa*, yeast, cyanobacteria and some additional algae and bacteria, we observed that the class II aldolases of eukaryotes are all inhibited by 8 mM cysteine while the activity of the bacterial enzymes is stimulated. In contrast, 100 mM potassium acetate increases the activity of the eukaryotic enzymes several-fold, whereas the bacterial enzymes are inhibited. In *Cyanophora paradoxa* there are 2 class II aldolases, one in the cytosol and one in the cyanoplasts. The cyanoplastic enzyme is intermediate, i.e. it is inhibited by cysteine as the eukaryotic enzymes, but not stimulated by potassium ions as the bacterial enzymes. The cytosolic enzyme of this alga shows typical features of a eukaryotic aldolase. The data imply that the

eukaryotic class II aldolases have developed their own characteristics in the evolution of algae.

P-17: DISTRIBUTION OF THE HYSTERETIC PROPERTY AND THE REGULATORY SITES IN RUBISCOS AMONG ALGAE H. TOKAI, Y. ENOMOTO[1], J. HAMADA[2], S. FUJIWARA[3], & *A. YOKOTA* (DEPT. AGRIC. CHEM., UNIV. OSAKA PREF., [1]MARINE BIOL. STAT., KOBE UNIV., [2]DEPT. COMMUNITY MED., TOYAMA MED. PHARMACEUT. UNIV., [3]FERMENT. RES. INST., AIST.)

Plant-type RuBisCO from C_3 and C_4 plants is a hysteretic enzyme, and the activity decreases gradually with time for the initial few minutes at less than 1 mM RuBP. This type of RuBisCO has the regulatory sites to bind RuBP and suppress the hysteretic decrease of the activity in the presence of more than 2 mM RuBP. RuBisCO from photosynthetic bacteria, cyanobacteria, and microalgae does not show the hysteretic decrease of the activity nor has the regulatory sites. It can be inferred that an extreme increase in the affinity of the enzyme for CO_2 may have caused RuBisCO to have the plant-type characteristics during evolution of the enzyme. It is of interest to know at which step in evolution of photosynthetic organisms these cgaracteristics were aquired by RuBisCO. To this end, we analyzed the reaction courses of RuBisCOs from Rhodophyta, Chromophyta, and Chlorophyta.

RuBisCOs from *Chara, Cyanidium, Porphyridium*, and *Gelidium* were plant-type. *Ulva* and *Enteromorpha*, which have glycolate dehydrogenase, had the plant-type enzyme. *Chromatium, Chlamydomonas*, and *Euglena* had non-hysteretic RuBisCOs. RuBisCO of a conjugate *Closterium* was an intermediate of these two types. Another conjugate, *Spirogyra*, contained RuBisCO that could not be included in these two categories.

P-18: A NEW SPECIES OF *NEPHROSELMIS* FROM THE WESTERN COAST OF AUSTRALIA (PRASINOPHYCEAE, CHLOROPHYTA). *S. SUDA*, T. OKUDA (NIPPON ROCHE RESEARCH CENTER, JAPAN) & M. M. WATANABE (NATL. INST. FOR ENVIRONMENTAL STUDIES, JAPAN)

A species of *Nephroselmis* is described based on observations of cultured mateiral by light and electron microscopy. Two sand samples were collected from Port Hedland and Hamerin pool, Western Australia, Australia in October 1991. They were kept in a cold room at 5°C until rewetted by a sea water medium. The alga was isolated from the rewetted samples by the pipette washing method; about twenty strains were established and examined from each sample.

The cells were remarkably right-left flattened and appeared oval to bean-shaped when viewed from their wide side. The cell dimensions were 6.5–8 μm long, 8–11 μm wide, and approximately 3 μm thick. A single parietal chloroplast was pale green to green in color and contained a single conspicuous eyespot in its edge near the base of short flagellum. A conspicuous pyrenoid was located at the bottom of the chloroplast with a few starch plates. Cell devision was of the longitudinal binary fission type as is common in other species. During cultivation, the cells became thick walled spherical cysts having a spiny wall beneath the body scale layers.

This alga possessed four types of body scales. Among them, the outermost layer of long spiny scales were the most distinctive feature. These scales were approximately 1 μm long and consisted of three parts: a basal part consisting of at least six-spines; a middle part that was a slightly bending stick about 40 nm wide; and a short spiny apex bent in the direction opposite to the middle part. This type of scale has never been described. The scale morphology of the genus *Nephroselmis* is one of its important taxonomic characters. Therefore, the present alga is considered to be a new species of *Nephroselmis*.

P-19: BUTTERSCOTCH ON THE ROCKS: A NEW GENUS OF MARINE

CHRYSOPHYTE WITH LARGE, SACCATE COLONIES. *C. S. LOBBAN* (UNIV. OF GUAM, U.S.A), D. HONDA (UNIV. OF TSUKUBA, JAPAN), M. CHIHARA (JAPANESE RED CROSS COLLEGE OF NURSING, JAPAN) & M. SCHEFTER (UNIV. OF GUAM, U.S.A)

Delicate colonies of a novel colonial alga are abundant on reefs on Guam and other tropical Pacific islands. This alga has unique features including the colony morphology, the number of chloroplasts in the vegetative cells, and motile cell production through two successive cell divisions of vegetative cells. The motile cell possesses two unequal, lateral flagella. These features suggest that this alga is a new member of the order Sarcinochrysidales in the class Chrysophyceae. We propose the name *Chrysocystis fragilis* gen. et sp. nov.

P-20: PHYLOGENY OF THE GENUS *ROSSIELLA* (BACILLARIOPHYCEAE). *Y. YANAGISAWA* (GEOLOGICAL SURVEY OF JAPAN, TSUKUBA, 305 JAPAN)

The diatom genus *Rossiella* Desikachary et Maheshwari was investigated by light and scanning electron microscopy to reveal its morphology. Sixteen species are recognized, all of which are fossil, including seven new species and two new combinations. The genus is characterized mainly by colony formation connected by marginal ridges which are often completely fused, as well as by a single rimoportula in the pattern "on per cell" and the presence of an apical pore field at each apex. Such features clearly indicate that the genus can be placed in the family Cymatosiraceae Hasle, von Stosch & Syvertsen.

Phylogenetic relationships are reconstructed based on morphologic characters, stratigraphic data and geographic distribution patterns. Stratigraphic range of each species was determined by examining four deep sea cores in the equatorial Pacific and northwest Pacific. The stratigraphic record is almost complete, and we can determine the exact absolute ages of the appearance and extinction of each species.

The genus is composed of three major intrageneric groups, each of which makes an evolutionary lineage and is well characterized by its own distinctive features and geographical distribution patterns. The first lineage *R. symmetrica* group which comprises five species appeared at about 32 million years ago and became extinct at 6.8 million years ago. The second lineage *R. gombosii* group consisting nine members split off from the foregoing *R. symmetrica* group, and makes a long lineage into the middle Pleistocene (ca. 0.6 million years before present). The third lineage is represent by *R. adaroi* and its related two species. The lineage originated from the *R. gombosii* group and survived to the end of the Pliocene (ca. 1.7 million years before present).

P-21: A NEW SPECIES OF *ENSICULIFERA*? (DINOPHYCEAE) PRODUCING ORGANIC CYST. *S. KOBAYASHI* (TOKYO KYUEI CO., LTD. TECHNICAL CENTER), K, MATSUOKA (DEPARTMENT OF GEOLOGY, FACULTY OF LIBERAL ARTS, NAGASAKI UNIVERSITY) & S. ISO (TOKYO KYUEI CO., LTD. TECHNICAL CENTER)

A new dinoflagellate is found in Imari Bay, Kyushu, West Japan. The cysts of this species are spherical, 23-29 μm in diameter and produce an organic wall. The cyst surface is covered with numerous spines, 5-7 μm long which are randomly distributed. The morphological variation of these spines are relatively wide, from slender and bifurcate to membranous and multifurcate distal extremities. The thecate forms are small and ovoid, 17-25 μm long and 14-21 μm wide. The plate tabulation is Po, X, 4′, 3a, 7″, 5c, 5‴, 2⁗ and 5s. The cingulum consists of five plates, and the Ct has a long slender needle-like projection extending toward apex. Judging from the plate tabulation and the presence of a long spine on the Ct, this species should be attributable to the genus *Ensiculifera*. The organic cyst of this species, however, is differ-

ent from such other species as *E. mexicana* and *E. carinata* producing calcareous cysts. The evidence needs a reconsideration for a taxonomical status of both the genus *Ensiculifera* and the family Calciodinellaceae.

P-22: AUXOSPORE AND INITIAL VALVE STRUCTURES OF *AMPHORA COPULATA* (KÜTZ.) SCHOEM. *ET* ARCH. (NAVICULACEAE, BACILLARIOPHYTA). *T. NAGUMO* (DEPT. BIOL. NIPPON DENTAL UNIV.) & H. KOBAYASI (TOKYO DIATOM INSTITUTE)

Auxospore and initial valve structures of *Amphora copulata* were observed with both a light and an electron microscope. The materials were collected May 19, 1986 from Nabeya-tsutsumi (a little irrigation pond), in the vicinity of Matsumoto City, Nagano Prefecture in Central Japan. The two auxospores are formed perpendicular to apical axes of the gametangial cells. The perizonium consists of transverse and longitudinal bands. The transverse series, of about 40 bands, is laid down centrifugally as the auxospore expands and can be classified into the primary and the secondary perizonial bands. All bands are open hoops. The bands have smooth margins on both sides, and overlap one another from the center to both poles. The longitudinal series includes 5 bands—a wide longitudinally folded central band, with two bands on both sides. The bands overlap one another from the center outwards. The initial epivalve of the new generation is formed on the opposite side from the longitudinal bands series. The epivalve has no external raphe fissures on the valve face. The initial hypovalve formed inside the longitudinal bands series has raphe fissures. The length of the initial valves is about 2.5 times longer than that of the gametangial valves.

P-23: ABNORMAL EXCRESCENCES OF *LITHOPHYLLUM YESSOENSE* FOSLIE (CORALLINALES, RHODOPHYTA). *D. FUJITA* (TOYAMA PREF. FISH. EXP. STN., JAPAN)

Lithophyllum yessoense Foslie is a perennial encrusting coralline species dominant of Isoyake (urchin-dominated barren) areas along the southwestern coast of Hokkaido, and subdominant on the barren areas in Toyama Bay. The thallus of this species is dimerous, composed of primigenous and postigenous cell filaments with secondary pit-connections. On the top surface of cultured plants (at 10°C, 12 : 12 LD, 500 lux, in sea water), two types of abnormal excrescences occurred after two months. One type is a dimerous, leafy excrescence, very similar to a new thallus regenerated from the thallus fracture previously reported by Fujita *et al.* (1992). The leafy excrescence developed from vegetative initials at the edge of dead spots as well as on the healthy thallus surface. The other type is a monomerous, cauliflower-like warty excrescence which is composed of larger cells with few secondary pit-connections. The warty one developed from columner cells below the intercalary vegetative initials, including cells at the bottom of empty conceptacles.

P-24: FLAGELLAR PRODUCTION IN *VOLVOX* (VOLVOCACEAE, CHLOROPHYTA). *H. NOZAKI* (NAT. INST. ENVIRONMENTAL STUDIES, JAPAN)

Flagellar production during daughter colony formation in four sections of *Volvox* was observed by light microscopy. The four *Volvox* taxa exhibited essentially the same mode of flagellar production. Just after inversion or in the later stage of inversion, each protoplast of the embryo bore two short flagella at the anterior end. The two flagella were at first nearly equal in length. Later, however, they became markedly different in length. When a new gelatinous matrix was secreted around the daughter colony, the longer flagellum projected through the matrix whereas the shorter one was almost embedded within the matrix. Therefore, cells of the newly formed daughter colony were uniflagellate apparently. As the daughter colony grew, however, the two flagella became equal in length. In addition, two flagella of markedly

different length in the newly formed daughter colony were examined by electron microscopy for *V.* (sect. *Volvox*) *rousseletii*. Such differential flagellar production has been reported in the volvocacean genera, *Yamagishiella, Eudorina, Platydorina* and *Pleodorina*. However, differential flagellar elongation in the Goniaceae (*Gonium* and *Astrephomene*) differs from that of these volvocacean genera in that only one flagellum at first grows in each protoplast of the newly formed colony. Phylogenetic relationships within the colonial Volvocales are discussed, with special regard to the flagellar production.

P-25: PARTICIPATION OF CALCIUM IN THE LIBERATING SIGNAL OF DAUGHTER CELLS IN THE VEGETATIVE CELL CYCLE OF *CHLAMYDOMONAS REINHARDTII* (VOLVOCALES, CHLOROPHYTA). T. SHIMADA & *Y. MATSUDA* (KOBE UNIVERSITY, JAPAN)

In synchronously growing cultures of *Chlamydomonas reinhardtii*, cells grow during the light period, undergo mitosis and cytokinesis in the dark, and produce 4–16 daughter cells within the original mother cell wall. Upon completion of division, each daughter cell assembles a new wall and then hatches by breaking off the enclosing mother cell wall. This hatching is mediated by a vegetative cell lytic enzyme (VLE). Our recent studies revealed that VLE is a tryptic serine protease with the apparent molecular mass of 120 kDa. Furthermore, this enzyme was found to be stored as an inactive, higher molecular mass precursor (125 kDa) within the newly formed cells and activated at the time of secretion.

To investigate the signalling agents related to the daughter cell liberation, mature sporangia were treated with various chemicals and examined their promotion or inhibition on hatching. The hatching was completely inhibited by the addtion of EDTA and EGTA. Ba^{2+}, procain, and diltiazem also blocked the hatching. By contrast, addtion of Ca^{2+} and A23187, a calcium ionophore, promoted the hatching. These results indicated that Ca^{2+} may play an important role in the liberating signal of daughter cells in *C. reinhardtii*.

P-26: FLUORESCENCE AND IMMUNOELECTRON MICROSCOPIC OBSERVATION ON BEHAVIOUR OF CHLOROPLAST NUCLEOIDS IN THE PYRENOID OF *STICHOCOCCUS BACILLARIS* (CHLOROPHYTA). *Y. KATAHIRA*, S. MIYAMURA (UNIV. OF TSUKUBA, JAPAN), T. NAKANO (HIROSHIMA UNIV., JAPAN) & T. HORI (UNIV. OF TSUKUBA, JAPAN)

The cell of a green alga, *Stichococcus bacillaris*, contains a chloroplast with pyrenoid which is embedded in the matrix of chloroplast. Fluorescence microscopic observation on whole-mounted chloroplast after staining with DAPI suggested that nucleoid-DNA localizes only in the pyrenoid region. But, immunoelectron microscopy of ultrathin sections and fluorescence microscopy of sections of materials embedded in Technovite resin show a number of small patches of DNA in the pyrenoid region. Through binary fission of a chloroplast and pyrenoid these DNA patches are distributed into the daughter chloroplasts and pyrenoids.

P-27: CHLOROPLAST-DNA IN *PINNULARIA* (NAVICULACEAE, BACILLARIOPHYCEAE). *S. MAYAMA* & I. SHIHIRA-ISHIKAWA (TOKYO GAKUGEI UNIVERSITY, JAPAN)

Chloroplast-DNA in diatoms reported by Kuroiwa et al. (1981) and Coleman (1985) is arranged in ring shape around the chloroplast as it is in other chromophyta algae. In the present study, we stained eight *Pinnularia* species with DAPI and observed localization of the chloroplast-DNA. In *P. macilenta, P. rupestris, P. divergens* var. *divergens, P. bogotensis* and *P. microstauron*, the arrangement of the chloroplast-DNA is in the ring shape. However, DAPI-fluorescent dots are evenly distributed throughout the chloroplast in *P. viridis, P. divergens* var. *bacillaris* and *P. sundaensis*. These dots disappear after DNase treatment. TEM observation shows no invagina-

tion of mitochondrion into the chloroplast. The mitochondria are observed only outside of the chloroplast, nemaly, transversely in the alveola of the valve and longitudinally beneath the chloroplast. Therefore, these fluorescent dots are confirmed to be the chloroplast-DNA. The discreet areas of the thylakoids are seen in the cross sections of the chloroplast. These areas seem to correspond with the DNA sites observed by DAPI-staining. Since the dots of the chloroplast-DNA are always observed in *P. viridis, P. divergens* var. *bacillaris* and *P. sundaensis* regardless of season or locality, they seem to be a useful taxonomic criterion.

P-28: PHYLOGENY OF THE PRASINOPHYCEAE BASED ON THE 18SrDNA SEQUENCE DATA. *T. NAKAYAMA* & I. INOUYE (UNIV. OF TSUKUBA, JAPAN)

To estimate phylogenetic relationships of the Prasinophyceae that has been thought to be the most primitive group and ancestral stock of the Chlorobionta, complete sequences of the 18SrDNA were determined and analyzed for six prasinophycean algae: five pyramimonadalean species (*Pterosperma cristatum, Cymbomonas tetramitiformis, Halosphaera* sp., *Pyramimonas parkeae* and *Pyramimonas disomata*) and one species from the Mamiellales, *Mamiella bipyrenoidosa* nom. nud. (Inouye unpubl.). This analysis indicates that *Mamiella* diverged first among these six genera and supports the recent classification proposed based on ultrastructural features. It turns out that *Mamiella* can be treated as an outgroup on analyzing phylogeny of the Pyramimonadales. The analysis of data sets to which other chlorobionts are incorporated suggests the monophyly of the Pyramimonadales. The monophyly of the pyramimonadalean algae is inconsistent with the suggestion from some morphological data.

P-29: BIODEGRADATION OF CYANOBACTERIAL TOXIN MICROCYSTIN LR. *G. J. JONES*, P. T. ORR (CSIRO DIV. OF WATER RESOURCES, AUSTRALIA), D. G. BOURNE, A. JONES, H. DOELLE & R. L. BLAKELEY (UNVERSITY OF QUEENSLAND, AUSTRALIA)

During the past three years, we have undertaken a study of the persistence and biodegradation of the cyanobacterial cyclic heptapeptide toxin microcystin LR. Microcystin LR was found to be extremely persistent in air dried cell scums and in pure water. However, in surface waters microcystin undergoes biodegradation following an induction phase that may last up to three weeks or longer. We have isolated a strain of aquatic bacterium from local river water that degrades microcystin LR. The bacterium is a yellow-pigmented, non-spore forming, gram-negative rod, 2–5 μm in length and 0.5–1 μm in width. API and Biolog tests failed to give satisfactory identification of this strain, although it is most likely a *Pseudomonas* sp. Degradation appears to involve a number of enzymes which act sequentially to first hydrolyse the intact cyclic peptide to form linearised microcystin LR. This is then broken down to smaller peptides and component amino acids. These enzymes are produced constituitively when the strain is grown on PYE media. To date, two intermediate degradation products have been chemically characterised and identified—liner microcystin LR (MW 1012) and the tetrapeptide ADDA-Glu-Mdha-Ala (MW 614). The toxicity of these breakdown products has low ($LD_{100} > 250$ μg/kg mouse) compared with the parent toxin microcystin LR (LD_{100} 50–60 μg/kg mouse). We are now working to clone the gene regulating the key microcystin hydrolase enzyme.

P-30: GROWTH CHARACTERISTICS OF A NEW MARINE *CHLOROCOCCUM* SP. *N. KURANO*, M. KODAMA, M. CHIHARA AND S. MIYACHI (KAMAISHI LAB., MARINE BIOTECHNOLOGY INSTITUTE, JAPAN)

A new species of green alga, *Chlorococcum dorsiventrale*, was isolated from the western Pacific Ocean. *C. dorsiventrale* differed from

C. littorale[1] in its zoospore shape, however, both species showed high growth capability under extremely high CO_2 concentrations (up to 60 to 70%), and could grow quite densely (about 4 g dry wt/l) at 20% CO_2. These characteristics meet the requirement for the biological CO_2 fixation at sites of industrial emission, since its CO_2 concentration is extremely high. The only marine species of the genus *Chlorococcum* previously reported[2], *C. submarinum*, was a highly salinity-tolerant unicellular green alga[3], however, it could not grow at CO_2 concentrations higher than 10%. The effects of other culture conditions on the growth of these strains were also studied.

This work was supported by New Energy and Industrial Technology Development Organization (NEDO).

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P-31: HIGH-LEVEL EXPRESSION OF HUMAN SUPEROXIDE DISMUTASE IN *ANACYSTIS NIDULANS* 6301 (CHROOCOCCALES, CYANOPHYTA).

Y. TAKESHIMA, N. TAKATSUGU, H. INOUE & H. HAGIWARA (HAGIWARA INST. OF HEALTH, JAPAN)

The chemically synthesized gene encoding human CuZn-superoxide dismutase (h-SOD) was expressed in *A. nidulans* 6301 (*Synechococcus* strain PCC6301) under the control of ribulose-1,5-bisphosphate carboxirase/oxigenase (RuBisCO) promoter derived from *A. nidulans* 6301. To investigate the optimal expression of h-SOD gene, five expression cassettes having different sequences and base number between ribosomal binding site and translational initiation site (ATG) were constructed. These expression cassettes were introduced into the shuttle clonig vector, pBAX18, for *A. nidulans* and *Escherichia coli*.

The expression vectors were stably maintained in *A. nidulans* 6301 cells. The sequences around 5′ end of h-SOD gene and length of ribosomal binding site to ATG strongly affected the expression levels of h-SOD gene in *A. nidulans* cells. By means of irradiation of the light, h-SOD expression levels of the transformants increased more than 18-hold. Consequently, the expression levels of the protein reached value of about 3% of total soluble protein. The transformants expressed h-SOD extenuated photo-oxidative damage induced by methyl viologen.

P-32: SIMPLE CULTIVATION METHOD FOR *GRATELOUPIA* (HALYMENIACEAE, RHODOPHYTA) SPECIES BY REGENERATION OF CRUSTS AND THALLI.

M. IIMA (FAC. OF FISHERIES, NAGASAKI UNIV. JAPAN), S. KAWAGUCHI (FAC. OF AGRICULTURE, KYUSHU UNIV. JAPAN) & S. MIGITA (KUMAMOTO PREFECTURAL FISHERIES RESEARCH CENTER, JAPAN)

A simple cultivation method established in the laboratory for four *Grateloupia* species, *G. filicina, G. turuturu, G. acuminata* and *G. kurogii*, has been tested in the sea and found successful.

Released spores of these species develop into basal crusts and these crusts in turn produce erect thalli. In our experiment, the crusts and the erect thalli were cut into pieces with a razor blade, and the fragments were inoculated into a petri-dish. The fragments of basal crusts soon regenerated into new crusts, and those of erect thalli formed filamentous cells (*G. acuminata*) or new blade (*G. kurogii*). This process could be repeated throughout the year. Filamentous cells may be utilized for stock culture.

For cultivation in the sea, the fragments of crusts were inoculated on oyster shells (*G. filicina, G. turuturu* and *G. acuminata*) and synthetic twines of Nori-net (*G. filicina* and G. turuturu) and cultured for about one month.

As the regenerated blades of *G. kurogii* did not attach to the above-mentioned substrata, they were directly inserted into the twines. The oyster shells and the twines with regenerated plants were transferred into the sea from October to December. Many erect thalli developed and grew well into the same or a slightly bigger size than natural plants during a cold season of the year (from December to March).

P-33: DOCOSAHEXAENOIC ACID PRODUCTION BY THE MARINE UNICELLULAR ALGA *ISOCHRYSIS GALBANA* USING A PHOTOBIOREACTOR. J. G. BURGESS, *K. IWAMOTO*, K. SODE AND T. MATSUNAGA (TOKYO UNIVERSITY OF AGRICULTURE AND TECHNOLOGY, JAPAN)

We are interested in the production of useful chemicals from marine microalgae. In this study we have focused on the production of marine microalgae as an alternative source of docosahexaenoic acid (DHA). DHA and other omega-3 fatty acids are important factors in the prevention of several human diseases. The traditional dietary source of these substance has been from fish oils. However the fatty acids originate in the marine microbes upon which the fish feed. We have screened eight species of marine microalga for their ability to produce an important fatty acid, DHA. *Isochrysis galbana* UTEX LB 2307 which produced DHA in the highest quentities (5.4 mg/g), was grown in a new type of closed photobioreactor in which efficient light distribution was achieved using light-diffusing optical fibers. Optimum temperature, light intensity and pH for DHA production were established. The maximum yeild obtained for DHA production was 5.2 mg/L. This amount was 2.4 fold greater than that obtained using conventional culture methods. In addition, we found that the DHA content could be enhanced by low temperature and dark incubation of the culture after growth.

P-34: CONTINUOUS PRODUCTION OF EXTRACELLULAR COCCOLITH PARTICLES BY THE COCCOLITHOPHORID ALGA *PLEUROCHRYSIS CARTERAE*. *H. TAKANO*[2], J. JEON[1], E. MANABE[2], M. OKAZAKI[3] AND T. MATSUNAGA[1] ([1]TOKYO UNIVERSITY OF AGRICULTURE AND TECHNOLOGY, [2]ONODA CEMENT CO., LTD., [3]TOKYO GAKUGEI UNIVERSITY)

Coccolithophorid algae are capable of CO_2 fixation by both photosynthesis and calcification. They produce elaborate calcite structures, coccoliths, which form calcified shells around the cell. Coccolith particles have potential commercial applications.

Continuous production of coccoliths was carried out by continuously recovering them from the growth medium using a nylon mesh module installed in the medium circulation line. The nylon mesh module, a cross-flow filtration unit, was constructed from two halves of a perspex rectangular box clamped together and separated by a nylon mesh membrane (pore size 5 μm). The culture was circulated using a roller pump at flow rate of 800 mL/min. Culture medium containing calcite particles was removed at a rate of 100 mL/h. Re-supply of fresh medium was carried out under constant volume conditions. During continuous culture, coccolith particles are detached from the cell surfaces by optimized air-bubbling. Optimum productivity of ultra-fine coccolith particles was 18 mg/L/day. These results demonstrate the potential of a continuous system for coccolith particle production.

P-35: 2C LEVEL OF DNA CONTENT IN VEGETATIVE CELLS OF SEVERAL CONJUGATOPHYCEAE (CHLOROPHYTA). *J. HAMADA* (TOYAMA MEDICAL AND PHARMACEUTICAL UNIVERSITY, JAPAN) & T. BANDO (KYOTO UNIVERSITY OF EDUCATION, JAPAN)

The measurement of the DNA content (DN-C) by epifluorescence microspectrophotometry after DNA specific dye, 4'6-diamidino-2-phenylindol staining was carried out for the vegetative and gamete cells of *Netri-*

um digitus, Spirogyra sp. and *Zygnema* sp. In these algae, the gametes were able to be distinguished from the vegetative cells from their size, morphology or the location. In *N. digitus*, the mean DN-C of just divided vegetative cells were 2.02C, if that of gametes was postulated to be 1C. The DN-C of vegetative cells in an exponential phase was 2.68C as a mean, suggesting that it was not 1C level (1C to 2C of DN-C), but 2C level (2C to 4C). In *Spirogyra*, the DN-C of just divided vegetative cells in exponentially growing cell population were 1.99C, and the longer size vegetative cells were 2.59C, suggesting 2C level. In *Zygnema*, the DN-C of the vegetative cells in exponentially growing population was 2.93C. These results were coincided with the results obtained in the life cycle of *Closterium ehrenbergii* (Hamada et al 1985, Hamada 1987), where the DN-C of the vegetative cells was not at the 1C level, but 2C level. It also indicates that in Conjugatophyceae, the gametogenesis is the stage where DN-C is reduced by half other than meiosis. In other eight species of Conjugatophyceae, polyploidy or polygenomy was suggested from the survival curve after the irradiation of γ-rays and/or ^{10}B neutron captured beams (more than 70% is α-particles).

P-36: A FLUOROMETRIC METHOD FOR DETECTING CYANOBACTERIA *IN VIVO*. *T. Y. LEE*, T. TAKEUCHI, K. YOKOYAMA, E. TAMIYA, I. KARUBE (RCAST, UNIVERSITY OF TOKYO, JAPAN) & M. TSUZUKI (IMCB, UNIVERSITY OF TOKYO)

A novel method for measuring the concentration of cyanobacteria in phytoplankton water samples has been developed. Phycocyanin, an antenna pigment of cyanobacteria, was excited by a selected wavelength and its fluorescence was detected. The measurement was interfered when eucaryotic algae such as diatoms and green algae were present in the sample. Therefore, chlorophyll *a* fluorescence was also detected, in order to correct the results. A linear relationship between the cell concentration and the fluorescence intensity was obtained in the range of 0.001 to 10 μg phycocyanin/ml for *Microcystis aeruginosa, Phormidinium tenue, Anabaena cylindrica* and *Spirulina plentensis*.

P-37: A TRIAL FOR CONSTRUCTION OF NEW HYDROGEN PRODUCTION SYSTEM USING CYANOBACTERIA. J. YAMADA, TAKAYUKI HOSHINO (UNIV. TSUKUBA), H. NAGAI, M. SHIRAI (UNIV. IBARAKI), M. MIYAKE AND Y. ASADA (NATL. INST. BIOSCI. & HUMAN-TECHNOL.)

Hydrogen production by cyanobacteria and algae have been extensively studied. In these microorganisms, light energy is converted to biochemical energy, and transported to hydrogen producing enzyme, hydrogenase or nitrogenase. However, the energy transport sometimes involves degradation of carbohydrates photosynthetically produced, which reduces the conversion efficiency.

We are trying to construct sustainable and efficient hydrogen production system which is directly dependent on the light energy conversion process. Here, we would like to show i) hydrogen production by coupling of ferredoxin fraction of cyanobacteria with clostridial hydrogenase fraction, ii) light-dependent hydrogen production by cyanobacteria (*Synechococcus* sp.) introduced with clostridial hydrogenase fraction by electroporation, and iii) some genetic transformation systems to breed cyanobacteria to harbor clostridial hydrogenase.

P-38: DNA VARIATION IN THE CYANOBIONT OF *CYCAS REVOLUTA* THUNB. (CYCADACEAE) CORALLOID ROOTS. *F. BRANDIZZI*, M. GRILLI CAIOLA (DEP. BIOLOGY, UNIV. "TOR VERGATA", ROME, ITALY), H. NAGASHIMA (DEP. BIOLOGY, SCIENCE UNIV. TOKYO, JAPAN)

In the *Cycas revoluta* coralloid roots the cyanobiont shows a high variation in the heterocyst morphology and developing status. At the growing tip, heterocysts are in low number, healthy and nitrogen fixing. At the ba-

sis, the number of heterocysts increases and most of them are degenerating or dead.

A study has been carried out to verify the DNA variation in vegetative cells and heterocysts in the different segments of the coralloids. Acridine Orange (AO), a specific fluorochrome staining for total DNA, permitted to verify a thick nucleoid in the middle of the vegetative cells forming globular structures homogeneously fluorescent while the heterocysts showed a diffuse fluorescence with the exception of the polar globes. The amount of the total DNA of intact heterocysts appeared higher than the one of the vegetative cells. Moreover, the amount of DNA of the healty heterocysts was constant in all coralloid root parts while the one of the vegetative cells presented an increase at the basis. The staining with the fluorochrome DAPI, specific to evidence A+T bases, showed higher intensity of fluorescence in the vegetative cells compared to the heterocysts. The heterocysts presented a high loss of DNA during the degenerating phases but the degenerated ones did not present a total loss.

Results suggest a possibility of germination in heterocysts, whereas the DNA increase in the vegetative cells of the oldest parts of the coralloid indicates a probable differentiation of vegetative cells into quiescent forms as akinetes.

P-39: SECONDARY CAROTENOIDS IN *HAEMATOCOCCUS LACUSTRIS* (VOLVOCALES, CHLOROPHYTA). *C. HAGEN* & W. BRAUNE (UNIV. OF JENA, GERMANY)

Research-workers have become increasingly interested in studies on the synthesis of secondary carotenoids (SC) in several organisms. This might be due to rising commercial application of 'algal carotenoids' in food industry and as cancer chemopreventive agents in medicine.

The extrachloroplastic accumulation of astaxanthin (mostly esterified), canthaxanthin, echinenone and other minor xanthophylls in lipid vacuoles of *H. lacustris* accompanies aging processes of aplanospores during formation of resting states. Main factors accelerating biosynthesis of SC are stronger light and limitation of nutrients, in particular of nitrogen sources.

The ability to synthesize SC has often been found in algae living under extreme conditions. The corresponding habitats are characterized by rapid variations of abiotic factors and excessive insolation. Apart from formation of resting cells, protection against 'bleaching' by stronger light is of special importance in these habitats.

To investigate a photoprotective function of SC, several *in vivo* techniques were applied to cell suspensions and single cells of different developmental stages of *H. lacustris* containing different amounts of SC. Results allow to summarize: The main function of SC in *H. lacustris* is protection against primary and secondary injuries induced by excessive insolation.

P-40: FUNCTIONAL ROLES OF THE HAPTONEMA AND SPINE SCALES IN FEEDING PROCESS OF *CHRYSOCHROMULINA SPINIFERA*. *M. KAWACHI* & I. INOUYE (UNIVERSITY OF TSUKUBA, JAPAN)

Chrysochromulina spinifera represents a new type of feeding process .1) Food particles adhere to the spine scales. 2) They move to the haptonemal base and are finally translocated to its tip. 3) Then, an aggregate of food particles is formed there. 4) The aggregate is transported to the surface by haptonemal bend. 5) The aggregate is taken into the food vacuole by phagocytosis. Haptonemal functions of C. spinifera are basically the same as those of C. hirta regarding aggregate formation and its transportation, but the food capturing proceeds in different way, viz., only the spine scales are responsible for food capturing. The location of particle aggregating center is also different between the two species.

The haptonema bends into the fixed direction during food transportation, passing between two flagella, moving along the long fiagellum, and reaches to a particular area of the cell where phagocytosis occurs. Light

and electron microscopic observations indicate that the left flagellum corresponds to the long flagellum, and the food vacuole is always situated some distance bellow it. It is therefore concluded that haptonemal behaviour is constant and closely related to the absolute configuration of intracellular organelles.

Special Program for the 20th Anniversary of University of Tsukuba

ALLOZYME DIVERGENCE OF *MICROCYSTIS* STRAINS FROM LAKE KASUMIGAURA. *T. KATO* AND M. WATANABE (NATIONAL SCIENCE MUSEUM)

As a part of a molecular-taxonomic revision of the genus *Microcystis* (Cyanophyceae), allozyme genotypes at four enzyme loci (IDH, 6PGD, PGI, PGM) were investigated electrophoretically on a total of 168 strains originated from 43 waters in Japan. The obtained data were statistically analyzed according to KATO & DOI's Minimum Replacement Method. As a result, it was revealed that the Japanese strains of *Microcystis* could be classified into five genetic types. This typification is well endorsed, as being shown in the table, by some morphological characteristics (e.g. average cell size, colony form) and toxicity (composition of three cyclic heptapeptide toxins, microcystins-RR, YR and LR). Based on this evidence, we strongly suggest that the five genetic types of *Microcystis* should be regarded as different taxonomic entities.

Taxa	Cell	Colony	Toxicity	Genotype
M. aeruginosa S1-type	small	polymorphic	untoxic	polytypic
M. aeruginosa S2-type	small	polymorphic	toxic	polytypic
M. aeruginosa L-type	large	clathrate	toxic	polytypic
M. viridis	large	cubic	toxic	monotypic
M. wesenbergii	large	pouched	untoxic	monotypic

Massive growth of *Microcystis* (so-called AOKO) at Lake Kasumigaura has recently given rise to severe problems in aquatic environments and drinking water management. For the purpose of promoting further scientific researches and serving for public health, it is now needed to elucidate: (1) which genotypes of *Microcystis* strain inhabit at the lake, (2) whether or not the genotype composition changes seasonally.

Standing this point of view, we started a molecular-phenological study of *Microcystis* at Lake Kasumigaura. The results obtained since 1991 are summarized as follows.

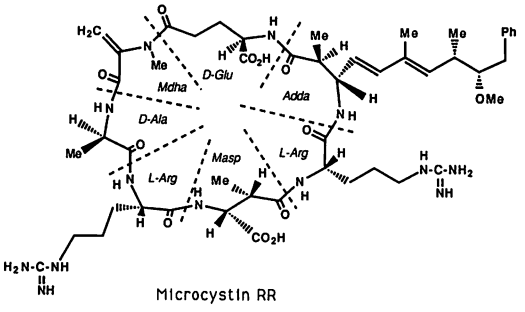
(1) The *Microcystis* flora of Lake Kasumigaura contains at least six different genotypes of *M. aeruginosa*, in addition to *M. viridis* and *M. wesenbergii*.
(2) Among the six genotypes of *M. aeruginosa*, two are attributed to S1-type (untoxic), one to S2-type (slightly toxic) and the other three to L-type (highly toxic).
(3) Genotype composition of the *Microcystis* strains changed in a year, both in 1991 and 1992. However, the patterns of seasonal change are not consistent between the two years.

In the present paper, I would like to discuss about these results with special reference to the toxicity of water-blooms at Lake Kasumigaura.

MICROCYSTINS, POTENT HAEPATOTOXINS PRODUCED BY FRESHWATER CYANOBACTERIA OF *MICROCYSTIS* SPECIES. *T. KUSUMI* (FACULTY OF PHARMACEUTICAL SCIENCES, TOKUSHIMA UNIVERSITY, JAPAN)

The occurrence of water blooms of cyanobacterium (blue-green alga) *Microcystis* in water bodies for public use is becoming a serious problem on the water management and public health, because some causative species are reported to produce toxins. The toxins, potent haepatotoxins, are designated microcystins, and they have been revealed to be strong promoters of liver cancer.

The chemistry and biological aspects of these toxins are discussed in this presentation.



Microcystin RR

BDELLOVIBRIO FEEDING *MICROCYSTIS* (CHROOCOCCALES, CYANOPHYTA).

M. GRILLI CAIOLA (DEP. BIOLOGY, UNIV. "TOR VERGATA", ROME, ITALY) & S. PELLEGRINI (DEP. BIOLOGY, UNIV. MILAN, ITALY)

During water blooms in Lake Varese many cells of *Microcystis aeruginosa* (Kütz) Kütz. contained in their periplasmic space *Bdellovibrio* cells in different developmental stages. The behaviour of *Bdellovibrio* sp. was very similar to that reported for *Bdellovibrio bacteriovorus* Stolp et Starr in *Escherichia coli*.

Bdellovibrio sp. was isolated from water of Lake Varese and its morphology, developmental cycle and activity were compared to *Bdellovibrio bacteriovorus* Stolp et Starr strain 7082 CIP.

Parasitic activity against *Microcystis* of both the *Bdellovibrio* strains was assayed on different *Microcystis* species in cultures.

The *Microcystis* cultures inoculated with *Bdellovibrio* were then collected after different time from inoculation and prepared for the TEM observations. Various cytochemical reactions were made to localize different compounds and to ascertain the metabolic relations between the prey and its parasite.

The results indicate that only *M. aeruginosa* was penetrated and lysed by *Bdellovibrio* and in lower percentage than in the natural populations. No differences have been detected among the *Bdellovibrio* strains. Developmental stages of *Bdellovibrio* in cultured *Microcystis aeruginosa* were similar to those observed in natural populations.

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