Regulation of Life Cycle in Epiphytic Brown Alga, *Dictyosiphon foeniculaceus*[*,**]

By

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Introduction

The life histories of brown algae have been studied for over one hundred years. Since the latter half of the 19 th century a large number of papers have been published by many investigators. In the early stages of these investigations, KUCKUCK (1899), ROSENVINGE (1893, 1899), SAUVAGEAU (1897), and others, contributed toward life cycle studies of brown algae by field observation and using elementary culture methods. First of all, the occurrence of the alternation of generations in brown algae (*Zanardinia,* Cutleriales) was recognized by REINKE (1878). Then, the life cycle of *Sacchorhiza,* Laminariales, which shows the heteromorphic alternation of generations between the macroscopic sporophyte and the microscopic gametophyte was clarified in culture, and the life cycle of brown algae was first interpreted correctly by SAUVAGEAU (1915). Also, since the early part of the 20 th century, the cytological study of brown algae has been carried out and the alternation of nuclear phases became clear due to the work of YAMANOUCHI (1912, 1913).

The system of classification of Phaeophyta based mainly on the life cycle was proposed by KYLIN (1933). This used a number of life cycle patterns. The life cycles of brown algae were divided into three types, *Dictyota*-type (isomorphic alternation of generations), *Laminaria*-type (heteromorphic alternation of generations) and *Fucus*-type (no alternation of generations). The life cycle studies progressed by the advancement of culture methods and the improvement of culture media. Recent results from life cycle studies have been reviewed by SCAGEL (1966), RUSSELL (1973), WYNNE and LOISEAUX (1976) and PEDERSEN (1981). There are many examples of these life cycle patterns, however it is now recognized that some patterns are excluded from KYLIN's system, for instance the proposal for a

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Scytosiphon-type of life cycle by NAKAMURA (1972) and the exceptional pattern in the system of a life cycle given by SAKAI and SAGA (1981).

There are some unsolved problems in the life cycle studies for example pleo- or poly-morphism, incomplete life cycles, and geographical variations of life cycles.

Examples of these are often observed in the members of Ectocarpales, Chordariales and Dictyosiphonales, especially the members of epiphytic algae. These phenomena may appear owing to their own genetic character or to the use of different culture techniques. In epiphytic brown algae, pleo- or poly-morphic phenomena are known in several species of Myrionemataceae (LOISEAUX 1968), *Feldmannia-Acinetospora* (KNOEPFFLER-PEGUY 1970), *Myriotrichia* (PEDERSEN 1978), and incomplete life cycles are known in *Coilodesme* (WYNNE 1972), several species of Ectocarpales, Chordariales, or Dictyosiphonales (SAGA and SAKAI, unpublished). Moreover, geographical variations of life cycle are known in *Spongonema* (SAUVAGEAU 1928, WILLIAMS 1964), *Litosiphon* (KYLIN 1933, DANGEARD 1966, NYGREN 1975), *Elachista* (KYLIN 1937, KOEMAN and CORTEL-BREEMAN 1976).

There are many epiphytic species whose life cycles are still unknown. This may be due to the difficulty of identification of species and problems with accurate isolation of strains. Moreover, the life cycles of epiphytic algae are affected by their host, as the example of the relation between epiphytic *Janczewskia* and its specific host *Laurencia* shows (NONOMURA 1979, NONOMURA and WEST 1981).

For the resolution of the aforementioned problems, life cycles must be studied under carefully regulated condition using up-to-date culture techniques, *e. g.* pure culture or tissue culture. The purpose of the present study is to clarify the life cycle of epiphytic brown alga and to artificially regulate the life cycle. The results obtained in the present study will aid the advance of life cycle studies of brown algae, and also be useful for improving the classification system of Phaeophyta. In addition, these achivements will make a significant contribution to basic understanding of plant development and the establishment of a new experimental system for studying plant morphogenesis.

It is common to both plant and animal systems that cell division or cell differentiation occupies a important position in the process of morphogenesis. However, the way of tissue construction is drastically different between plant and animal, that is, the heaping system was introduced in the former, on the other hand the flowing system by cell movement was introduced in the latter. Such a mode of plant development was caused by the existence of the cell wall in plant cells. Therefore, special attention must be paid to the direction and dimention of growth in order to understand plant morphogenesis.

In Pteridophyta, the factors regulating growth direction have been investigated in some gametophytes (KATO 1964), and the relationship between the direction of formation of the cell plate and the light quality has also been known in detail (WADA and FURUYA 1971). In Phaeophyta, the growth dimension of *Petalonia* and *Scytosiphon* (LÜNING and DRING 1973) and *Analipus* (SAGA 1977) was also regulated by light quality.

On the other hand, in those plants having heteromorphic alternation of generations, growth dimensions varied according to generations. For example, microscopic gametophytes of laminarian species grow one-dimensionally to give minute thalli, and their macroscopic sporophyte grows three-dimensionally into large thalli. It is a remarkable phenomenon that two generations of the same species show dissimilar shape just like they were different species. I am investigating the morphogenetic mechanisms which regulate the determination of generation. Algal life cycles were controlled by various environmental factors, *e. g.* physical, chemical or biological factors, and life cycles of epiphytic algae were affected mainly by their specific host community. The physiology of these algae should be studied in pure culture using a defined medium.

The life cycle is composed of some genetically determined developmental processes and it is regulated by various environmental factors. The alternation of generations phenomenon has occupied an important position in life cycle studies, and this is likely to continue. The alternation of generations needs to be reexamined in a morphogenetic context.

The purpose of the studies is clarification of the factors regulating the life cycle of the epiphytic marine brown alga, *Dictyosiphon foeniculaceus*. For this purpose, following procedures were performed: clarification of the life cycle under unialgal and axenic conditions; identification of macrothallus inducing substance (MIS); identification of plurilocular sporangium inducing substance (PSIS); and a nutritional study on the inducing substances.

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I Life Cycle

The life history of *Dictyosiphon foeniculaceus* (HUDSON) GREVILLE was reported by SAUVAGEAU (1917), ARASAKI (1949), PEDERSEN (1984) and PETERS and MÜLLER (1985). According to their results, life history of this species was the heteromorphic alternation of generations between macroscopic sporophyte bearing unilocular sporangia and microscopic gametophyte bearing plurilocular gametangia. Their culture studies were done without an

environmental control. Moreover, in their studies, the life cycle of this species could not be completed in the laboratory.

According to the recent culture studies on the life cycles of the members of the order Dictyosiphonales, there are many life cycle patterns and their life cycles are apt to transform to correspond to a geographical distribution or culture conditions (SAKAI and SAGA 1981). Therefore, it is important to clarify the life cycle and its regulation for members of this order. An exact culture study of *Dictyosiphon foeniculaceus*, which is the type species of this order, has been needed for a long time.

The present study was carried out to clarify the complete life cycle of *Dictyosiphon foeniculaceus* by a carefully regulated culture study.

Materials and methods

The materials used in this study were collected from the usual host, *Scytosiphon lomentaria* growing on the beach of Charatsunai, Muroran, Hokkaido from April to July in 1976–1979. The thalli of the alga bore many unilocular reproductive organs and the culture studies were started by using zoids derived from these organs.

For the culture study, the collected materials were wiped with a clean gauze to remove diatoms and other microorganisms, and they were rinsed several times with autoclaved seawater. These cleaned materials were stored in the refrigerator at 5℃ for more than 24 hours. For the inoculation of the culture, one fertile fragment of them was placed in a petri dish containing sterilized seawater. After a few minutes numerous zoids were liberated. They were washed several times in sterilized seawater by the micropipette method, and pipetted onto a glass slide unialgally. After they had settled, each slide was transferred into a glass vessel (6.5×8.0cm) containing 180m*l* of PESI medium (Table 1, slightly modified PROVASOLI's ES medium, TATEWAKI 1966). Occasionally cultures were examined in the following way. One of the cleaned zoids obtained by the aforementioned method was inoculated in a test tube containing 10 m*l* of PESI medium by the micropipette method. The culture medium was renewed monthly. Cultures were grown in an incubator illuminated with cool white fluorecent lamps (2,000 1x) under the following sets of temperature-

Table 1 Composition of stock solution for PESI medium[a,b].

Distilled water	100 m*l*	P II metals[c]	25 m*l*
$NaNO_3$	350 mg	KI	0.1 mg
Na_2-glycerophosphate	50 mg	Tris	500 mg
Fe (as EDTA; 1:1 mol)	2.5 mg	pH	7.8

[a] TATEWAKI 1966.

[b] For PESI medium add 2 m*l* of PESI stock solution to 100 m*l* autoclaved seawater.

[c] 1 m*l* of P II metals contains: Na_2 EDTA 1 mg, Fe (as Cl^-) 0.01 mg, B (as H_3BO_3) 0.2 mg, Mn (as Cl^-) 0.04 mg, Zn (as Cl^-) 0.005 mg, Co (as Cl^-) 0.001 mg.

photoperiod:

	Temperature (℃)	Photoperiod (hr light-hr. dark)
Set 1	5	14-10
Set 2	5	10-14
Set 3	10	14-10
Set 4	10	10-14
Set 5	14	14-10
Set 6	14	10-14
Set 7	18	14-10
Set 8	18	10-14
Set 9	22	14-10
Set 10	22	10-14

For the crossing experiment, the field-collected and cultured materials were used. This experiment was set up between the zoids liberated from reproductive organs on various thalli obtained by the above described culture conditions, and about 40 strains were used for each experiment. The sexual union was examined by checking for aggregation or fusion of zoids.

For the cytological study, the thalli of various developmental stages in the life cycle were fixed in the fixative of 1:3 acetic acid: ethanol and stained by application of the aceto-iron-haematoxylin-chloral hydrate method (WHITTMANN 1965). The materials used in this study were 50 field-collected individuals and more than 20 cultured strains in every stage.

The bialgal culture with *Scytosiphon* was established by the following procedures. A glass tube (10×1.8 cm) was closed at the bottom by a glass filter (Whatman, GF-C) and a Erlenmeyer flask (12×8 cm) was prepared as a culture vessel. One fragment of the *Scytosiphon* thallus cut into differing lengths, was enclosed into the tube containing 10 m*l* of PESI medium. The contents of the tube was inoculated with the *Dictyosiphon* and added to the Erlenmeyer flask which contained 100 m*l* of PESI medium. The other culture conditions were similar to ones described above.

Results

Fertile plants collected from the field were cylindrical and branched either alternate or occasionally opposite, 5-20 cm long, covered with a dense growth of fine hair (Pl. I A). Superficial cells were small, rounded or angular and containing several discoid chromatophores per cell (Pl. I B-C). Unilocular reproductive organs were spherical or ellipsoidal, 30-50 μm diameter, scattered, and submerged in the cortical tissue (Pl. I B-C).

Zoids from unilocular organs were pear-shaped or ovoid, 4.8−8.5×3.3−5.3 μm in size, laterally biflagellated and contained one chromatophore and eyespot.

Development of spores from unilocular sporangia in field-collected plants

The zoids liberated from unilocular reproductive organs did not fuse with each other but they settled and developed on a glass slide. Knowing that the zoids could develop without a copulation into the next stages, they should be called spores (Pl. I D). The settled spores immediately became spherical and measured about 6 μm diameter (Pl. I E).

Since the preliminary examination indicated that the basic developmental process of the spores did not vary with the culture conditions, the development of them was observed in set 3. In set 3, within 12–24 hours after the inoculation, the settled spores began to germinate by pushing out germ tubes (Pl. I F). The germ tubes elongated without protoplast movement, and then they divided at the base of the germ tube within 2 days (Pl. I G). By successive transverse cell divisions, the germlings became single filaments (Pl. I H), and within 5 days these filaments began to form branched prostrate thalli and their cells were 4.5–8.0 μm in breadth and 1–7 times as long in length, and contained 1–3 discoid or plate-like chromatophores per cell. The phaeophycean hairs were not observed in these prostrate thalli (Pl. I I). Within 10 days, colonies of the prostrate thalli had the diameter about 100 μm (Pl. I J). Within 20 days, successive growth of the prostrate thalli occurred (Pl. I K, L), and they produced plurilocular reproductive organs (Pl. I M).

These organs were 5.0–7.5 μm breadth, 40–120 μm long, mostly uniseriate, rarely biseriate and comprised 20–60 compartments (Pl. I N). Within 1 month, successive growth of the prostrate thalli occurred and the germlings developed into about 1 mm diameter prostrate colonies, and within 2 months, they had the diameter about 3 mm. Erect thalli and unilocular sporangia were never observed through this culture study.

Table 2 Growth and formation of thalli and formation of plurilocular sporangia under various culture conditions.

Temperature (℃)	Daylength	Growth of PT	Formation of PT-P	Formation of ET
5	SD	+	+	−
	LD	+	+	−
10	SD	++	++	−
	LD	++	+	−
14	SD	++	++	−
	LD	++	+	−
18	SD	+++	++	−
	LD	+++	+	−
22	SD	++	+++	−
	LD	++	++	−

+++ very abundant; ++ abundant; + moderate; ± few; − absent; ET=erect thallus; PT=prostrate thallus; PT-P=plurilocular sporangia on prostrate thallus.

Under the other culture condition examined, all developmental processes of the spores pursued the same course, but the rate of the growh of prostrate thalli and the formation of plurilocular reproductive organs varied with the culture conditions. The effect of the controlled environmental factors on the growth of the prostrate thalli and the formation of the plurilocular reproductive organs and the erect thalli are summarized in Table 2.

Development of spores from plurilocular sporangia produced on cultured prostrate thalli

The zoids from the plurilocular reproductive organs of fertile prostrate thalli cultured under 14℃ −LD conditions were pear-shaped or ovoid, 3.8−7.3×3.0−4.5 μm in size, slightly smaller than the spores of unilocular sporangia (Pl. II A), laterally biflagellated, and contained one chromatophore and eyespot. The developmental process of the spores under various conditions was identical with the result of the culture study of spores from the unilocular sporangia (Pl. II B-F).

Erect thallus formation in bialgal culture with *Scytosiphon* thallus

The developmental process of the germlings under various conditions of bialgal cultures was similar to the unialgal culture within ten days after the inoculation. Within 15 days the germlings began to produce the phaeophycean hairs and the erect thalli (Pl. III A), within 1 month successive production and elongation of the erect thalli occurred and unilocular sporangia were produced in these thalli (Pl. III B-D), and within 2 months the erect thalli developed into 1-2 cm in length (Pl. III E).

The effect of the bialgal culture with *Scytosiphon* on the erect thallus under various culture conditions are summarized in Table 3.

Table 3 Formation of macrothalli under condition of bialgal culture with *Scytosiphon*.

Temperature (℃)	Daylength	Length of thallus (cm) 5	3	2	1
5	SD	+	+	+	+
	LD	+	+	+	+
10	SD	++	++	++	++
	LD	+++	+++	+++	+++
14	SD	++	++	++	++
	LD	+++	+++	+++	+++
18	SD	+	+	+	+
	LD	++	++	++	++
22	SD	−	−	−	−
	LD	±	±	±	±

+++ very abundant; ++ abundant; + moderate; ± few; − absent.

Crossing experiment

The materials used in this experiment and the results obtained are shown in Table 4. The experiment was carried out on all sets, but no aggregation or fusion was observed.

Table 4 Crossing experiments of zoids from various culture conditions.

Material	Aggregation or fusion
Zoids from same U	−
Zoids from U of same ET	−
Zoids from U of different ET	−
Zoids from same P	−
Zoids from P of same PT	−
Zoids from P of different PT	−

+ fusion; − no fusion; ET=erect thalli; PT=prostrate thalli; P=plurilocular sporangia; U=unilocular sporangia.

Cytological study

The chromosome numbers of specific stages in the life cycle were observed. The eight stages observed (cf. the caption for Pl. IV) were as follows: 2 stages in the field-collected plants; 2 stages in developmental process of spores from unilocular sporangia produced on field-collected plants; and 4 stages in the developmental process of spores from plurilocular sporangia produced on cultured prostrate thalli.

As a results of these observations, 8 chromosomes were recognized in each cell of each stage, and neither meiosis nor vegetative diploidization was observed (Pl. IV A-H).

Discussion

According to the results of this study, it is clear that the prostrate thalli of *Dictyosiphon foeniculaceus* produced the plurilocular sporangia. Therefore these thalli are recognized as a generation. On the other hand, the erect thalli which were formed in bialgal culture with *Scytosiphon* thallus produced unilocular sporangia. Therefore, these thalli are also recognized as a macrothallus generation. The life cycle of this species is the heteromorphic alternation of generations between the macrothallus bearing unilocular sporangia and the microthallus bearing plurilocular sporangia (Figs. 1, 2). The spores from both reproductive organs of this species, which were characterized by a crossing experiment, have a potential to follow the same developmental process.

The life cycle of this species was influenced by environmental factors. The macrothallus never appeared in the unialgal culture, but could appear when the organisms were set up under bialgal culture with *Scytosiphon* thallus. It was proved that the formation of them was influenced by the specific host *Scytosiphon*. It should be noted that the *Scytosiphon* thallus used in this study was not completely clean and they seemed to have epiphytic microorganisms (micro-algae, fungi, bacteria, etc.). With the present case, there are some problems

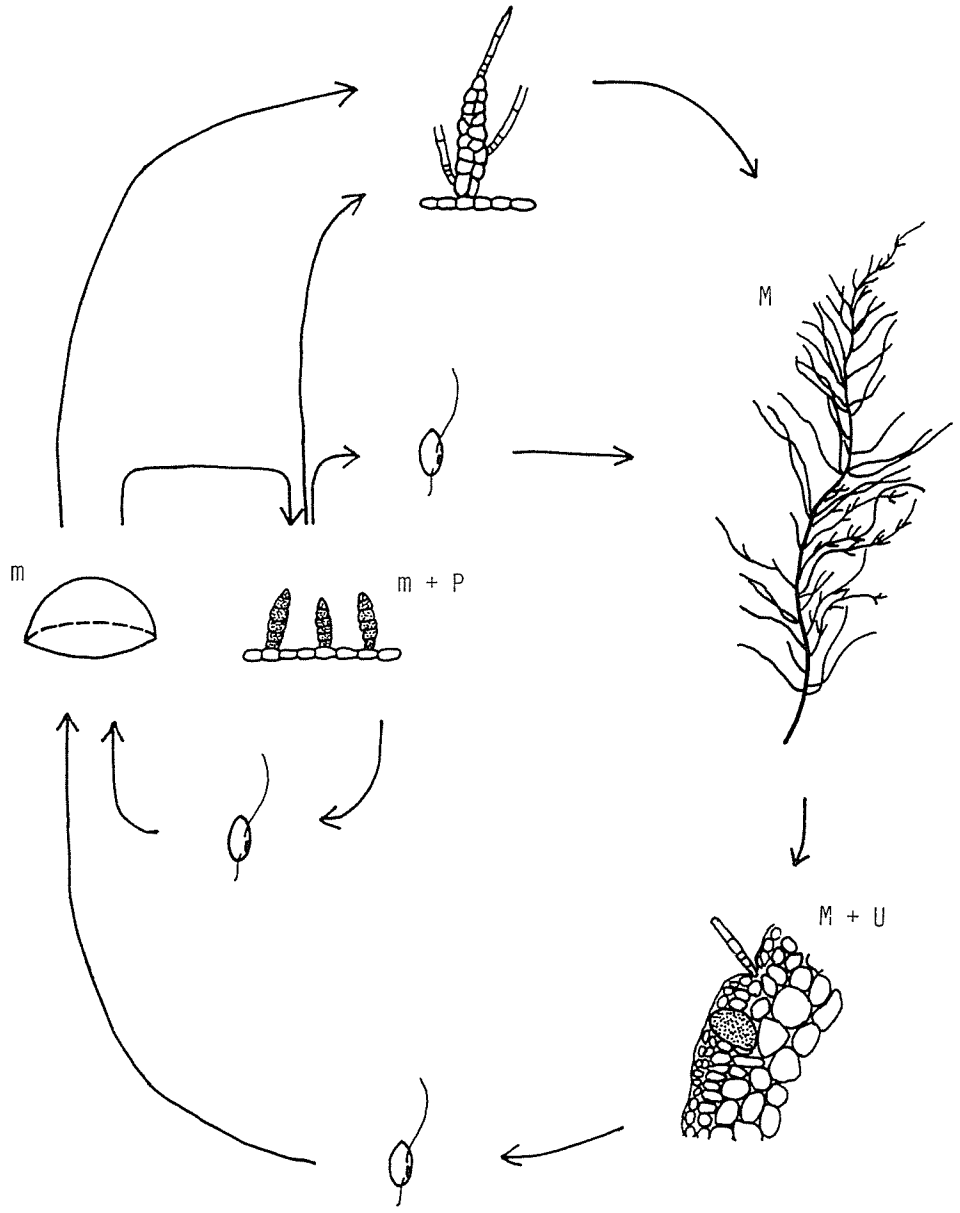

Fig. 1 Diagram summarizing the life cycle of *Dictyosiphon foeniculaceus* (M=macrothallus; m=microthallus; U=unilocular sporangia; P=plurilocular sporangia).

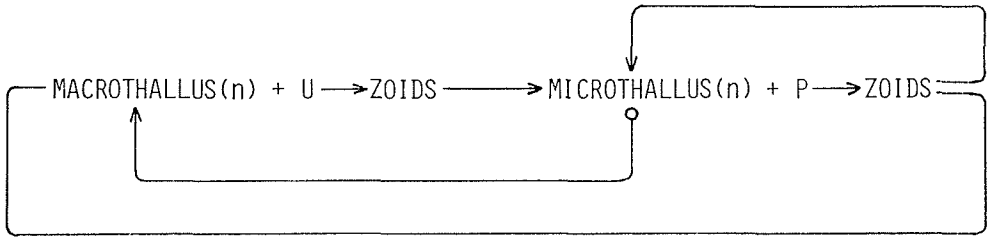

Fig. 2 Diagram summarizing the life cycle of *Dictyosiphon foeniculaceus*. (U=unilocular sporangia ; P=plurilocular sporangia ; ○→=produce new generation by a vegetative way)

whether *Scytosiphon* itself has an effect on macrothallus formation or whether the epiphytic microorganisms exert an effect. It is likely that the formation of the macrothallus was controlled by a certain factor from the *Scytosiphon* community. In the bialgal culture, 10 and 14℃-LD conditions were suitable for macrothallus formation.

The growth of the prostrate thalli and the formation of the plurilocular sporangia varied quantitatively with temperature and day length, as follows: 18℃-SD, LD conditions were suitable for the growth of prostrate thalli, and 22℃-SD conditions were suitable for the formation of plurilocular sporangia. Sometimes plurilocular sporangia did not appear under all the conditions examined in this culture study. It is supposed that such a phenomenon was caused by qualitative variation with the seawater used in this study, but the factor which was concerned in plurilocular sporangium formation is not known.

These pleomorphic life cycle patterns are summarized in Fig. 3. In unialgal culture, the life cycle pattern shows the repetition of the prostrate thallus with or without plurilocular sporangia (Fig. 3 A, A') ; and in bialgal culture with its host, it shows the repetition of macrothallus bearing unilocular sporangia (Fig. 3 B) or the alternation of generations between macrothallus bearing unilocular sporangia and microthallus bearing plurilocular sporangia (Fig. 3 B').

This epiphytic alga appeared on the specific host *Scytosiphon* on the beach of Charatsunai. This species specific phenomenon may be explained by the result of this study that the specific host was indispensable for differentiation of the macrothallus. In an other location, this species appeared on not only *Scytosiphon* but also *Chordaria, Fucus, Pelvetia.* These facts suggest the idea that these algae may supply some factor which affects differentiation of the macrothallus.

Results obtained by the crossing experiment on *Dictyosiphon foeniculaceus* show that the sexual process was not observed at any stages of the life cycle. That is to say the zoids liberated from unilocular and plurilocular organs developed in the same manner as zoospores or parthenogametes. Thus I recognized all reproductive organs of this species associated

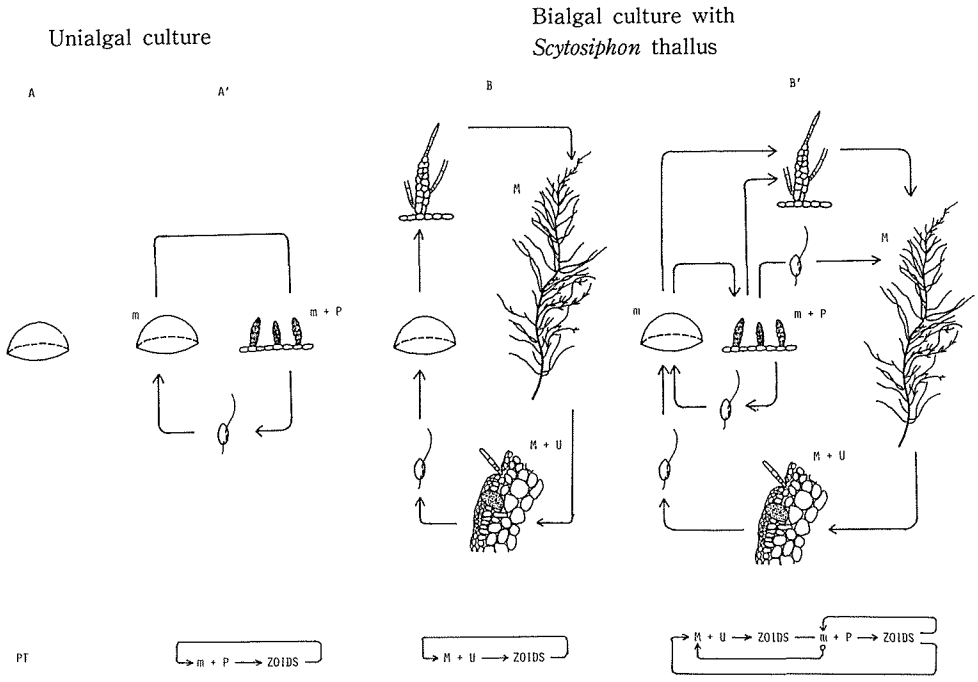

Fig. 3 Life cycle patterns of *Dictyosiphon foeniculaceus* regulated by the host community (M=macrothallus; m=microthallus; U=unilocular sporangium; P=plurilocular sporangium).

with the sporangia. The sexual process of this species had been known in SAUVAGEAU's and ARASAKI's and PETERS and MÜLLER's strains (SAUVAGEAU 1917, ARASAKI 1949, PETERS and MÜLLER 1985). On the contrary, PEDERSEN's strain lacks in sexuality (PEDERSEN 1984). According to the present cytological study on the field collected and cultured thalli, the chromosome number was 8 at all stages of the life cycle. In the case of *Dictyosiphon foeniculaceus* from Muroran, neither meiosis nor vegetative diploidization occurred, and sexual processes were lacking at any stages of the life cycle. A basic haploid chromosome number of Dictyosiphonales has been considered to be 8–13 (COLE 1967). According to these facts, it is clear that nuclear phases of all stages are haploid (n=8). Although there have been a few cytological studies which corresponded to various stages of the life cycle in other species of the order, the present species collected from the beach of Charatsunai seems to be a parthenogenetic species or population. On the other stand point of view, I presume that it is no necessity that the nuclear phases always alter synchronously with the alternation of generations.

In the case of *Ectocarpus,* the germlings could develop into the gametophytes or sporophytes independently of the nuclear phase (MÜLLER 1967). In *Alaria,* the gameto-

phytes or sporophytes had been induced by apospory and apogamy (NAKAHARA and NAKAMURA 1973). Moreover, in the case of *Dictyosiphon foeniculaceus* examined in the present study, successive generation was determined not by nuclear phase but by some environmental factors. According to these facts, it can be considered that the nuclear phase, which seems to have full genetic informations in spite of the change of gene dosage that is shown by the term of haploid or diploid, could not determine the generation or morphological phase. Consequently, the generation is not always determined by nuclear phase, but the phenotypic expression is easily influenced by certain environmental factors which may regulate the gene activation or the enzyme reaction.

In this study, the entire life cycle and its environmental control was established for the first time. Moreover, an interaction between host and epiphyte was clarified. It is considered that the host plays an important role on the determination of generation, and there is a macrothallus inducing factor in the specific host *Scytosiphon* community. On the other hand, it is supposed that a plurilocular sporangium inducing factor is contained in natural seawater. These factors concerning the regulation of life cycle will be clarified by further study.

II Induction of Macrothallus and Plurilocular Sporangium

In chapter 1, the life cycle of *Dictyosiphon foeniculaceus* was clarified by the culture study, but poly- or pleo-morphic phenomena remained as unsolved problems. These appeared in unialgal culture using enriched seawater and are problems to be considered when studying the control of the life cycle. It is supposed that factors inducing macrothallus and plurilocular sporangium participate in the life cycle pattern of the organisms. Pure culture with artificial seawater is important in order to exactly study these factors.

In this chapter, the factors regulating the formation of macrothallus and plurilocular sporangium are studied in axenic culture.

Materials and methods

The material used in this study was an axenic microthallus of *Dictyosiphon foeniculaceus* Strain no. DF-001, obtained by the method described previously (SAGA and SAKAI 1982).

For pure culture in artificial media, 10 aggregates of microthalli which developed into a mass 1.0 mm in diameter were inoculated into one Erlenmeyer flask containing 50 m*l* ASP 12-NTA medium (Table 5, PROVASOLI 1963). The pH was adjusted to 7.8-8.0. The culture medium was changed monthly. The cultures were maintained at 14℃ and illuminated with cool white fluorescent lamps at 2,000 lx with a cycle of 14 hr light and 10 hr darkness.

The culture in conditioned medium of *Scytosiphon* community was conducted by the following procedures. The conditioned medium was prepared as follows: Ten grams of *Scytosiphon* thalli was flooded with 100 m*l* of ASP medium and then this solution was passed through various filters in pore size, F-1 (passing through nylon mesh, pore size ca. 30 μm),

Table 5 Composition of ASP 12-NTA medium[a] per 100 m*l*, pH 7.8–8.0.

NaCl	2.8 g	PII metals[b]	1 m*l*
KCl	0.07 g	SII metals[c]	1 m*l*
$MgSO_4 \cdot 7H_2O$	0.7 g	Vit. B_{12}	0.02 μg
$MgCl_2 \cdot 6H_2O$	0.4 g	Biotin	0.1 μg
Ca(as Cl^-)	40 mg	Thiamine	10 μg
$NaNO_3$	10 mg	Nitrilotriacetic acid	10 mg
K_3PO_4	1 mg	Tris	0.1 g
Na_2-glycerophosphate	1 mg		
$Na_2SiO_3 \cdot 9H_2O$	15 mg		

[a] PROVASOLI 1963.

[b] 1 m*l* of PII metals contains: Na_2EDTA 1 mg, Fe(as Cl^-) 0.01 mg, B (as H_3BO_3) 0.2 mg, Mn (as Cl^-) 0.04 mg, Zn (as Cl^-) 0.005 mg, Co (as Cl^-) 0.001 mg.

[c] 1m*l* of SII metals contains: Br (as Na salt) 1 mg, Sr (as Cl^-) 0.2 mg, Rb (as Cl^-) 0.02 mg, Li (as Cl^-) 0.02 mg, Mo (as Na salt) 0.05 mg, I (as K salt) 0.001 mg.

Table 6 Composition of ST-3 medium[a].

Seawater	700 m*l*	Liver oxoid (Oxo Ltd)	20 mg
Distilled water	250 m*l*	Vitamin B_{12}	0.1 μg
Soil extract	50 m*l*	Vitamin mix. 8[b]	1 m*l*
$NaNO_3$	50 mg	Carbon source mix. II[c]	20 m*l*
Na_2 glycerophosphate	10 mg	Glycyl-glycine	400 mg
Hy-case (Scheffield Chem.)	20 mg	(Agar	4 g)
Yeast extract	10 mg	pH	7.9

[a] PROVASOLI *et al.* 1957.

[b] 100 m*l* of vitamin mix. 8 contains: Thiamine HCl 20 mg, Nicotinic acid 10 mg, Ca-pantothenate 10 mg, *p*-Aminobenzoic acid 1 mg, Biotine 50 μg, *i*-Inositol 100 mg, Folic acid 250 μg, Vitamin B_{12} 5 μg, Thymine 80 mg, Pyridoxine HCl 4 mg, Pyridoxamine 2HCl 2 mg, Putrescine 2HCl 4 mg, Riboflavin 0.5 mg, Choline-H_2-citrate 50 mg, Orotic acid 26 mg, Folinic acid 20 μg.

[c] 100 m*l* of carbon source mix. II contains: Glycine 100 mg, D, L-Alanine 100 mg, L-Asparagine 100 mg, Sodium acetate 200 mg, Glucose 200 mg, L-Glutamic acid 200 mg.

F-2 (passing through glass filter, Whatman GF-C, pore size 2-3 μm) and F-3 (passing through Millipore filter GS, pore size 0.22 μm). The filtered solution was adjusted at pH 7.8-8.0 and was dissolved in ASP 12-NTA in various concentrations. Other culture methods were similar to the aforementioned methods.

The culture with bacteria epiphytic on *Scytosiphon* was conducted by the following procedures: These epiphytic bacteria were isolated with ST-3 (Table 6, PROVASOLI *et al.* 1957) and ASP-B1 media (Table 7, SAGA and SAKAI 1982) solidified with 1.0-1.5% agar.

Table 7 Composition of ASP-Bl[a] medium per 100 m*l*, pH 7.8–8.0.

NaCl	2.8 g	PII metals[b]	1 m*l*
KCl	0.07 g	SII metals[c]	1 m*l*
$MgSO_4 \cdot 7H_2O$	0.7 g	Vit. B_{12}	0.02 μg
$MgCl_2 \cdot 6H_2O$	0.4 g	Biotin	0.1 μg
Ca(as Cl^-)	40 mg	Thiamine	10 μg
$NaNO_3$	10 mg	Nitrilotriacetic acid	10 mg
K_3PO_4	1 mg	Tris	0.1 g
Na_2-glycerophosphate	1 mg	Bactopepton	0.5 g
$Na_2SiO_3 \cdot 9H_2O$	15 mg	Beef extract	0.2 g
KNO_3	50 mg		

[a] SAGA and SAKAI 1982.

[b] 1 m*l* of PII metals contains: Na_2EDTA 1 mg, Fe (as Cl^-) 0.01 mg, B (as H_3BO_3) 0.2 mg, Mn (as Cl^-) 0.04 mg, Zn (as Cl^-) 0.005 mg, Co (as Cl^-) 0.001 mg.

[c] 1 m*l* of SII metals contains: Br (as Na salt) 1 mg, Sr (as Cl^-) 0.2 mg, Rb (as Cl^-) 0.02 mg, Li (as Cl^-) 0.02 mg, Mo (as Na salt) 0.05 mg, I (as K salt) 0.001 mg.

The bacterial inocula were taken from the F-2 solution and a few drops were put on agar plates (90×20 mm petri dishes containing 20 m*l* medium) with a pipette, and spread over the agar surface with a glass spreader. They were cultured at 20℃ for 1-2 weeks. Then, several strains of bacterial colonies were inoculated into 10 m*l* solidified ASP-B1 medium in test tubes. These strains were mass-cultured on agar plates (90×20 mm petri dishes containing 20 m*l* ASP-B1 medium). The bacteria was suspended in ASP 12-NTA at various cell densities. Other culture methods were similar to the aforementioned methods.

Results

Pure culture in ASP 12-NTA medium

After 1 month from the inoculation, microthalli developed into colonial masses about 3 mm in diameter, but no plurilocular sporangia or erect thalli were produced on them. Successively the culture was maintained for several months, only vegetative growth of microthalli occurred and no reproductive organ or erect thallus was observed.

Culture in conditioned medium

Compositions of each fraction were as follows: F-1 contained microalgae, fungi, protozoa, bacteria; F-2 contained bacteria; and F-3 did not contain any organisms.

The results obtained by this culture study are given in Table 8. In all fractions plurilocular sporangia were not produced, but macrothalli were detected in F-1 and F-2. After all, macrothallus inducing factor may be related to the organisms which were common in F-1 and F-2, namely bacteria.

Table 8 PSIS-activity and MIS-activity in fractionated conditioned medium.

Fraction No. of CM	PSIS-activity	MIS-activity
F-1 (contained micro-algae, fungi, protozoa, bacteria, etc.)	−	+
F-2 (contained bacteria, etc.)	−	+
F-3 (did not contain any organisms)	−	−

+ present; − absent; CM=conditioned medium; PSIS=plurilocular sporangium inducing substance; MIS=macrothallus inducing substance.

Culture with some bacteria

The bacteria obtained by this study showed various colors, e. g. white, pale yellow, yellow, orange, pink. Among them, several yellow bacteria were effective in promoting erect thallus formation. But all bacteria examined were ineffective in promoting plurilocular sporangium formation.

Discussion

The plurilocular sporangia of the microthalli of *Dictyosiphon foeniculaceus* were produced in the enriched seawater medium. Sometimes they did not appear in that medium. This poly- or pleo-morphic phenomenon may have been caused by seasonal fluctuation of the quality of the seawater or where it was collected, as shown in chapter 1. According to the fact that the plurilocular sporangia were not produced in artificial media, it is clear that plurilocular sporangium inducing factor is contained in natural seawater. This factor will be discussed in chapter 3.

In chapter 1, it was already known that macrothallus inducing factor of this organism existed in *Scytosiphon* community. That is to say, the factor is either *Scytosiphon* thallus itself or epiphytic microorganisms on it. In this study, the effective factor was identified, and it was clarified that the factor is produced by the specific yellow bacteria on *Scytosiphon* thallus. This suggests several research questions: Are bacterial filtrates active or not? Is syntrophic growth indispensable or not? These problems will also be solved in chapter 3.

The life cycle regulated by the certain factors under axenic conditions are summarized in Fig. 4.

Studies on pure cultures of macroscopic algae are few. In Phaeophyta, some reports on axenic culture were published, for example *Ectocarpus confervoides* (BOALCK 1961), *E. fasciculatus* (PEDERSEN 1968, 1969), *Litosiphon pusillus* and *Pylaiella littorallis* (PEDERSEN 1969), *Petalonia fascia* (HSIAO 1969), *Desmarestia ligulata* and *D. viridis* (NAKAHARA and TATEWAKI 1971, NAKAHARA 1984) and several members of the Laminariales (DRUEHL and HSIAO 1969, HSIAO 1972, NAKAHARA 1984). But these studies were conducted about one stage of their life cycles. The completion of life cycle under axenic condition has not yet

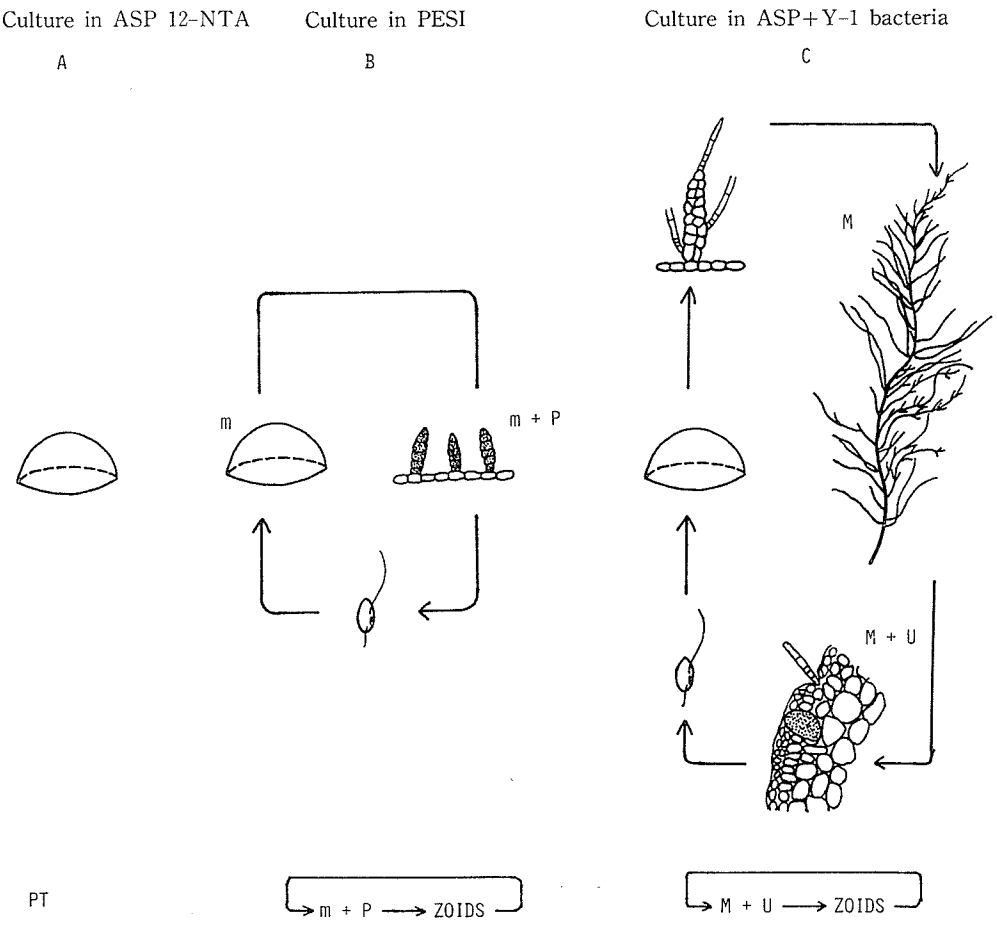

Fig. 4 Life cycle patterns of *Dictyosiphon foeniculaceus* regulated by several factors under axenic conditions (M=macrothallus; m=microthallus; U=unilocular sporangia; P=plurilocular sporangia).

been reported. If the life cycle control factors of *D. foneniculaceus* are identified, at any time the life cycle of this species can go to completion in pure culture.

III Distribution and Characterization of Macrothallus Inducing Substance (MIS) and Plurilocular Sporangium Inducing Substance (PSIS)

Recently, studies on the morphogenesis and growth regulators of marine algae were summarized by PROVASOLI and CARLUCCI (1974) and BUGGELN (1981). According to their reviews, most of these studies have described already-known endo- or exo-genous plant

growth regulators. A few studies on morphogenesis which may be regulated by new growth regulators have been under taken, but the active substances have not been identified yet (PROVASOLI and CARLUCCI 1974). Chemical identification of the growth regulators of algal origin has been needed for a long time, because such a study might be quite rewarding for understanding the control of algal morphogenesis.

In this chapter, plurilocular sporangium or macrothallus inducing substances predicted in chapter 2 will be partially analysed.

Materials and methods

Organisms. The material used in this study was an axenic microthallus of *Dictyosiphon foeniculaceus* Strain no. DF-001 obtained by the method described previously (SAGA and SAKAI 1982).

Media. PESI and ASP 12-NTA medium were used. Active carbon treated PESI medium was also used.

Cell-free extract. Cell-free extracts of various algae and bacteria were prepared as follows: Ten grams of algal thalli was washed several times with deionized water, and macerated for 10 minutes with 100 m*l* 0.1 M Tris buffer (pH 7.0) in a blender. The suspension of thallus fragments was further homogenized with a glass homogenizer for 10 minutes. This solution was centrifuged at 10,000×*g* for 20 minutes, and the supernatant was used as crude extract. In earlier experiments, crude extract was filter-sterilized by passing through Millipore filters of 0.22 μm pore size. However, since it became evident that plurilocular sporangium inducing substance was heat stable, crude extract was subsequently autoclave-sterilized. One gram of bacteria, wet weight (Y-1 strain) was washed several

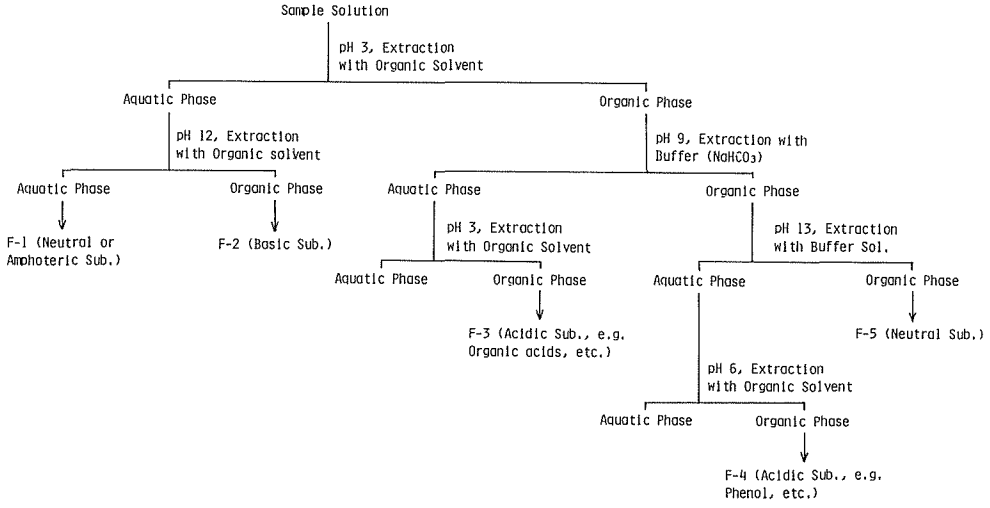

Fig. 5 Flow chart of ethyl acetate extraction method.

times with deionized water, and homogenized with 10 m*l* 0.1 M Tris buffer (pH 7.0) in a Teflon homogenizer for 10 minutes. The other procedures are same as described above. Moreover, malt extract and yeast extract were also examined. One gram of them was dissolved in deionized water and these solutions were used as original ones.

Fractionation of crude extracts. Fractionation of conditioned medium of bacteria (Y-1 strain) and *Neorhodomela* extract was performed followed by an ethyl acetate extraction method (Fig. 5). The volatile fraction (essential oil) of filtrate of bacterial culture was also prepared by the steam evaporation method.

Assay for PSIS- or MIS-activity. Bioassey for plurilocular sporangium inducing substance activity (PSIS-activity) or macrothallus inducing substance activity (MIS-activity) were performed as follows : Ten aggregates of microthalli which developed into a mass 1.0mm in diameter were inoculated in a Erlenmeyer flask containing 45m*l* ASP 12-NTA medium. Five m*l* of sterilized extract were diluted with deionized water to various concentrations and added to culture medium before inoculation of the organisms. The pH was adjusted to 7.8-8.0. The culture medium was renewed monthly. The cultures were maintained at 14℃ and illuminated with cool white fluorescent lamps of 14 hr light and 10 hr darkness. The appearance of plurilocular sporangia or macrothalli was observed after 10-60 days.

Results

Distribution of PSIS-activity. The activities of seawater, activated carbon treated seawater, yeast extract, malt extract and *Neorhodomela* extract were examined. These sample solutions ranging from 10^{-7} to 10^{-1} were added to the basal medium. As shown in Table 9, all the extracts except the activated carbon treated seawater showed the PSIS-activity.

Table 9 Distribution of PSIS-activity.

Solutions	Concentration (%)							
	10	1	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Seawater	+	−	−	−	−	−	−	−
Seawater (active carbon treated)	−	−	−	−	−	−	−	−
Yeast extract	+	+	+	+	−	−	−	−
Malt extract	+	+	+	−	−	−	−	−
Neorhodomela extract	−	+	+	−	−	−	−	−

+ present ; − absent.

Partial characterization of PSIS. To characterize PSIS, *Neorhodomela* extract was used as the source of PSIS, since *Neorhodomela* may actually participates as a inducer of plurilocular sporangia in nature. PSIS was heat-stable and it was dialyzable through

visking tube (Shiramatsu Seisakusho Ltd., TOKYO, JAPAN).

The fractions of crude extract were tested on plurilocular sporangium induction and the results obtained are shown in Table 10. Fraction 1 (water soluble and strongly polarized fraction) was effective in plurilocular sporangium induction.

Table 10 PSIS-activity in fractionated extract.

	Fractions				
	F-1	F-2	F-3	F-4	F-5
PIS-activity	+	−	−	−	−

+ present ; − absent.

Distribution of MIS-activity. The activities of *Dictysiphon* extract, *Scytosiphon* extract, bacteria (Y-1 strain) extract, bacteria (Y-1 strain) culture filtrate, bacto peptone, casamino acid, malt extract, soil extract, trypticase, trypton and yeast extract were examined. These sample solutions ranging from 10^{-6} to 10^{-1} of the original extract or filtrate solutions were added to the basal medium. As shown in Table 11, bacteria (Y-1 strain) extract or filtrate showed MIS-activity.

Table 11 Distribution of MIS-activity.

	Concentration (%)						
Solutions	10	1	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Dictyosiphon extract	−	−	−	−	−	−	−
Scytosiphon extract	−	−	−	−	−	−	−
Bacteria (Y-1) extract	−	−	+	+	−	−	−
Bacteria (Y-1) culture filtrate	−	−	−	−	+	+	+
Bacto-peptone	−	−	−	−	−	−	−
Casamino acid	−	−	−	−	−	−	−
Malt extract	−	−	−	−	−	−	−
Soil extract	−	−	−	−	−	−	−
Trypticase	−	−	−	−	−	−	−
Tryptone	−	−	−	−	−	−	−
Yeast extract	−	−	−	−	−	−	−

+ present ; − absent.

Partial characterization of MIS. To characterize MIS, bacteria (Y-1 strain) culture filtrate was used as the source of MIS. Fractions of culture filtrate were tested for MIS-activity and the following results obtained (Table 12) : Weak activity appeared in Fraction 2, but clear-cut activity was not observed by the ethyl acetate extraction system ; strong activity appeared in volatile fractions prepared by the steam evaporation method.

Table 12 MIS-activity in fractionated culture filtrate.

	Fraction				
	F-1	F-2	F-3	F-4	F-5
MIS-activity	−	±	−	−	−

+ present ; − absent.

Discussion

The present results offer several significant pieces of evidence to suggest that the life cycle of this species was controlled by certain chemical substances.

PSIS which existed in *Neorhodomela*-, malt- and yeast-extracts showed wide distribution, but was absorbed in activated carbon. These results show that PSIS may be a non-specific substance. Since PSIS was heat-stable and dialyzable in a cellulose tube, it is my speculation that PSIS is a relatively low molecular weight substance. And since PSIS flowed in a water soluble and strongly polarized fraction, it is speculated that PSIS was a neutral and amphoteric substance, such as a sugar for instance.

On the other hand, the extract or filtrate of the specific bacteria showed MIS-activity and narrow distribution, but other extracts showed no activity. These results show that MIS may be species specific substance. Since MIS could not be explored by the ethyl acetate system and flowed in highly volatile fraction, it is justifiable to think that MIS was a non-polarized substance and probably a low molecular weight carbohydrate. However weak MIS-activity appeared in Fraction 2 of the ethyl acetate system, and it is not clear whether MIS-activity in Fraction 2 was an artifact caused by contamination. This problem needs to be reexamined carefully.

In this study, the nature of PSIS and MIS were partially elucidated, indicating that the former may be a sugar and the latter may be a carbohydrate. The identification and structure determination of these algal regulators must be done in future. Although it will be very difficult work, the results obtained by such an investigation will bring considerable progress to the study on algal life cycles and the control of developmental processes.

IV Nutritional Study on Induction of Macrothallus and Plurilocular Sporangium*

The life cycle control of *Dictyosiphon foeniculaceus* was investigated through the studies in chapter 1-3. As the results of these studies, physiological outline of the regulating mechanism have been elucidated and algal growth regulators PSIS and MIS will be identified in further work by chemical analysis.

* Abbreviations in chapter 4 : ABA, abscisic acid ; C-AMP, adenosine 2′ : 3′-cyclic monophosphate ; CCC, (2-chloroethyl) trimethylammonium chloride ; 2, 4-D, 2, 4-dichlorophenoxyacetic acid ; GA_3, gibberellic acid A ; TIBA, 2, 3, 5-triiodobenzoic acid.

In this chapter, nutritional studies on the formation of plurilocular sporangium and macrothallus were carried out in order to determine whether PSIS and MIS are common substances.

Materials and methods

Organisms. The material used in this study was an axenic microthallus of *D. foeniculaceus* Strain no. DF-001, obtained by the method described previously (SAGA and SAKAI 1982).

Media and chemicals. ASP 12-NTA medium was used as the basic medium. These chemicals were used: growth regulators, sugars, organic acids, amino acids, alcohols and vitamins.

Culture. Ten aggregates of microthalli which had developed into a mass 1.0 mm in diameter were cultured in an Erlenmeyer flask containing 50 m*l* medium. When necessary, the organisms were once pre-cultured in medium lacking the compound to be tested. The compounds tested were dissolved in the medium at various concentrations* (10^{-7} −1% W/V), adjusted pH 7.8-8.0 and all the solutions except GA_3** solutions were sterilized by autoclaving. The cultures were maintained at 14℃ and illuminated with cool white fluorescent lamps of 14 hr light and 10 hr darkness. The appearance of plurilocular sporangia and macrothalli was observed after 10-60 days.

Results

Growth regulators. Effects of growth regulators listed in Table 13 on plurilocular

Table 13 Effects of growth regulators on formation of plurilocular sporangium or macrothallus.

Compounds	Concentration (% w/v)				
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
ABA	−	−	−	−	−
Adenine	−	−	−	−	−
c-AMP	−	−	−	−	−
CCC	−	−	−	−	−
Cinamic acid	−	−	−	−	−
Coumaric acid	−	−	−	−	−
2, 4-D	−	−	−	−	−
Kinetin	−	−	−	−	−
TIBA	−	−	−	−	−

+ present; − absent.

* Exceptionally concentrations of growth regulators ranged from 10^{-6}-10^{-2} %(w/v) and ones of vitamin mixtures ranged from 10^{-7}-10% (v/v).

** Since GA_3 is heat-sensitive, its solution was sterilized by Millipore filters (pore size 0.22 μm).

sporangium formation or macrothallus formation were tested. As shown in Table 13, all compounds tested were ineffective.

Sugars. Effects of sugars listed in Table 14 on plurilocular sporangium formation or macrothallus formation were tested. As shown in Table 14, only inositol was clearly effective on plurilocular sporangium formation, and all compounds tested were not effective in macrothallus formation.

Table 14 Effects of sugars on formation of plurilocular sporangium or macrothallus.

Compounds	Concentration (% w/v)							
	1	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
Alginic acid	–	–	–	–	–	–	–	–
Arabinose	–	–	–	–	–	–	–	–
Fructose	–	–	–	–	–	–	–	–
Galactose	–	–	–	–	–	–	–	–
Glucose	–	–	–	–	–	–	–	–
Inositol	–	–	–	P	P	P	P	–
Inulin	–	–	–	–	–	–	–	–
Lactose	–	–	–	–	–	–	–	–
Maltose	–	–	–	–	–	–	–	–
Mannose	–	–	–	–	–	–	–	–
Rhamnose	–	–	–	–	–	–	–	–
Sorbitol	–	–	–	–	–	–	–	–
Sucrose	–	–	–	–	–	–	–	–
Xylose	–	–	–	–	–	–	–	–

P=plurilocular sporangia present; M=macrothalli present; – plurilocular sporangia or macrothalli absent.

Organic acids. Effects of organic acids listed in Table 15 on plurilocular sporangium or macrothallus formation were tested. As shown in Table 15, all compounds tested were ineffective.

Amino acids. Effects of amino acids listed in Table 16 on plurilocular sporangium or macrothallus formation were tested. As shown in Table 16, all compounds tested were ineffective.

Alcohols. Effects of alcohols listed in Table 17 on plurilocular sporangium or macrothallus formation were tested. As shown in Table 17, no compounds were effective.

Vitamin mixtures. Effects of vitamin mixture 8M (PROVASOLI *et al.* 1957), vitamin mixture for ASP 12 and vitamin mixture S_3M (PROVASOLI *et al.* 1957) on plurilocular sporangium or macrothallus formation were tested. As shown in Table 18, no compounds were effective.

Table 15 Effects of organic acids on formation of plurilocular sporangium or macrothallus.

Compounds	Concentration (% w/v)							
	1	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
Acetic acid	−	−	−	−	−	−	−	−
cis-Aconitic acid	−	−	−	−	−	−	−	−
Citric acid	−	−	−	−	−	−	−	−
Formic acid	−	−	−	−	−	−	−	−
Fumaric acid	−	−	−	−	−	−	−	−
Glutaric acid	−	−	−	−	−	−	−	−
Glycolic acid	−	−	−	−	−	−	−	−
Glyoxilic acid	−	−	−	−	−	−	−	−
α-Ketoglutaric acid	−	−	−	−	−	−	−	−
Malic acid	−	−	−	−	−	−	−	−
Oxalacetic acid	−	−	−	−	−	−	−	−
Oxalic acid	−	−	−	−	−	−	−	−
Propionic acid	−	−	−	−	−	−	−	−
Pyruvic acid	−	−	−	−	−	−	−	−
Succinic acid	−	−	−	−	−	−	−	−
Sulfamic acid	−	−	−	−	−	−	−	−
Uric acid	−	−	−	−	−	−	−	−
Valelic acid	−	−	−	−	−	−	−	−

+ present; − absent.

Discussion

Recently, algal growth and regulators were reviewed by several authors (FRIES 1973, PROVASOLI and CARLUCCI 1974, AUGIER 1976 a, b, c, 1977 a, b, c, 1978, BUGGELN 1981). According to their reviews, many studies have shown that higher plant hormones have affected growth or development of algae, but few studies have shown that higher plant hormones or specific algal regulators have clearly affected the morphogenesis of algae.

In brown algae, a few papers showed that kinetin or a new cytokinin originated from the *Fucus-Ascophyllum* zone seawater was effective in preserving good growth rate and a normal morphology (PEDERSEN 1968, 1973).

In the present study, inositol induced plurilocular sporangium of *D. foeniculaceus*. In chapter 3, it has been already known that PSIS existed in seawater and algal extract, and that it may be a sugar. Free cyclitols were contained in thalli of 5 classes of algae including Rhodophyceae (CRAIGIE 1974). According to these results, PSIS is inositol or a related substance, and PSIS in seawater may be excreted by certain algae.

Table 16 Effects of amino acids on formation of plurilocular sporangium or macrothallus.

Compounds	Concentration (% w/v)							
	1	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
L-Alanine	−	−	−	−	−	−	−	−
L-Arginine	−	−	−	−	−	−	−	−
L-Asparagine	−	−	−	−	−	−	−	−
L-Cystein	−	−	−	−	−	−	−	−
L-Glutamic acid	−	−	−	−	−	−	−	−
L-Glutamine	−	−	−	−	−	−	−	−
Glycine	−	−	−	−	−	−	−	−
L-Histidine	−	−	−	−	−	−	−	−
L-Hydroxy proline	−	−	−	−	−	−	−	−
L-Isoleucine	−	−	−	−	−	−	−	−
L-Leucine	−	−	−	−	−	−	−	−
L-Lysine	−	−	−	−	−	−	−	−
L-Metionine	−	−	−	−	−	−	−	−
L-Phenylalanine	−	−	−	−	−	−	−	−
L-Proline	−	−	−	−	−	−	−	−
L-Serine	−	−	−	−	−	−	−	−
L-Threonine	−	−	−	−	−	−	−	−
L-Tyrosine	−	−	−	−	−	−	−	−
L-Tryptophane	−	−	−	−	−	−	−	−
L-Valine	−	−	−	−	−	−	−	−

+ present ; − absent.

Table 17 Effects of alcohols on formation of plurilocular sporangium or macrothallus.

Compounds	Concentration (% w/v)							
	1	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
n-Amyl alcohol	−	−	−	−	−	−	−	−
n-Butyl alcohol	−	−	−	−	−	−	−	−
Ethyl alcohol	−	−	−	−	−	−	−	−
Glycerol	−	−	−	−	−	−	−	−
n-Propyl alcohol	−	−	−	−	−	−	−	−

+ present ; − absent.

All compounds tested in this study showed no MIS-like function. Since MIS has been shown to be a species specific substance, it may be an uncommon chemical.

Table 18 Effects of vitamin mixtures on formation of plurilocular sporangium or macrothallus.

Compounds	Concentration (% v/v)								
	10	1	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
V *mix* 8M[a]	–	–	–	–	–	–	–	–	–
V *mix* ASP_{12}[b]	–	–	–	–	–	–	–	–	–
V *mix* S_3M[c]	–	–	–	–	–	–	–	–	–

+ present ; – absent

[a] 100 m*l* of vitamin mix. 8M contains: Thiamine HCl 20 mg, Nicotinic acid 10 mg, Ca•pantothenate 10 mg, *p*-Aminobenzoic acid 1 mg, Biotine 50 μg, Folic acid 250 μg, Vitamin B_{12} 5 μg, Thymine 80 mg, Pyridoxine HCl 4 mg, Pyridoxamine 2HCl 2 mg, Putrescine 2HCl 4 mg, Riboflavin 0.5 mg, Choline-H_2-citrate 50 mg, Orotic acid 26 mg, Folinic acid 20 μg.

[b] 100 m*l* of vitamin mix. ASP_{12} contains: vitamin B_{12} 20 μg, Biotin 100 μg, Thyamine 10 mg.

[c] 100 m*l* of vitamin mix. S_3M contains: Thiamine HCl 5 mg, Nicotinic acid 1 mg, Ca pantothenate 10 mg, *p*-Aminobenzoic acid 0.1 mg, Biotin 10 μg, Thymine 30 mg, Folic acid 20 μg.

V General discussion

The life cycle of epiphytic marine alga, *Dictyosiphon foeniculaceus* was clarified by culturing unialgally from generation to generation. The life cycle of this species is the heteromorphic alternation of generations between the macrothallus bearing unilocular sporangia and the microthallus bearing plurilocular sporangia. The important processes in the life cycle of this species which were the formations of reproductive organs and new generations, were affected by the host community. Such a phenomenon is characteristic of epiphytic algae.

The life cycle of this species was also completed by using the axenic artificial medium containing PSIS and MIS. The completion of the life cycle in axenic conditions was established for the first time in seaweeds.

The morphogenesis and reproduction of algae are affected by various environmental factors, for example physical factors (temperature, light quantity, light quality, light period), chemical factors (inorganic substances, organic substances) and biological factors (bacteria, algae). There are some instances where morphogenesis was affected by biological factors (arranged in following order: alga - active substance - related organism) : *Monostroma oxyspermum* - unknown - bacteria, red and brown algae (PROVASOLI and PINTNER 1964) ; *Grinnelia americana* - vitamin B_{12} - bacteria (TSUKIDATE 1970) ; *Ulva lactuca* - unknown - bacteria (PROVASOLI and PINTNER 1972, 1980) ; *Enteromorpha linza* - unknown - bacteria (FRIES 1975) ; *Monostroma* spp. - peptide, saturated hydrocarbon - bacteria, red and brown algae (TATEWAKI and KANEKO 1977). In *Ulva* and

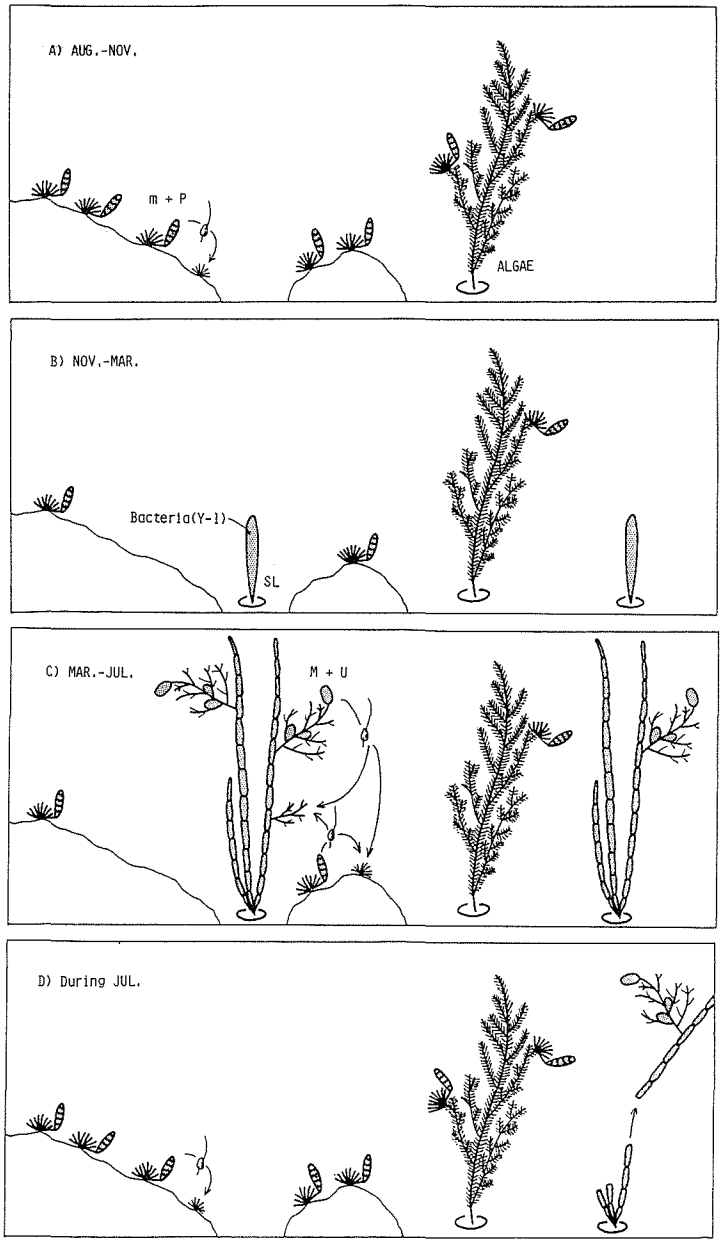

Fig. 6 Hypothesis concerning the life histry of *Dictyosiphon foeniculaceus* (M=macrothallus; m=microthallus; U=unilocular sporangium; P=plurilocular sporangium; SL=*Scytosiphon lomentaria*).

Enteromorpha, syntrophic growth with bacteria seemed indispensable for morphogenesis (PROVASOLI and PINTNER 1964, 1972, 1980, FRIES 1975). Also, *Ectocarpus fasiculatus* required a cytokinin, N^6-(Δ^2-isopentenyl) adenine included in seawater as a morphogenetic substance (PEDERSEN 1973).

In the present studies, two growth regulators were discovered. One is PSIS contained in seawater or some algae and another is MIS secreted from specific yellow bacteria being parasitic on the host, *Scytosiphon.* The former was identified to be a kind of cyclitols, inositol, and the latter was partially characterized to be volatile carbohydrate. Many studies have clarified that life cycles of algae were regulated by inorganic substances, but there have been a few papers suggesting that they were regulated by organic substances. This is the first report on identification of growth regulator of seaweeds (inositol as PSIS). MIS will be identified by further investigation. A discovery of new regulators is expected in other algae.

When discussing the determination of generations in algae we should also consider other information, *e. g.* receptor of regulatory factors, amplification of information, metabolic pattern, and gene activation. I have just begun to study the regulatory mechanisms of life cycle by the application of genetic or biotechnological methods. The present and aforementioned studies will contributed not only to phycology but also to the applied science of seaweeds, in particular for the breeding or cultivation of economically valuable algae.

I suggest that the life history of *Dictyosiphon foeniculaceus* in nature is as follows. The microthalli of this species live on rocks or various algae from summer to winter (August - February). These microthalli mature and bear plurilocular sporangia, and the spores from plurilocular sporangia develop into microthalli. During this period, many microthalli are formed by the repetition of this way of multiplication. On the other hand, thalli of *Scytosiphon,* which are the specific host of the alga, begin to appear from November. The thalli continue growth, and parasitic yellow bacteria which secrete macrothallus inducing substance grow gradually on them. Then spores from plurilocular sporangia of the microthalli adhere to them, and from March the germlings develop into macrothalli, and from April sexual maturation occurs and unilocular sporangia are produced in these macrothalli. When the host, *Scytosiphon,* discharges in July, the macrothalli disappear together, and the life history of this association completes. The aforementioned hypothesis is summarized by Fig. 6. This hypothesis should be reexamined on the basis of accurate ecological study of these organisms in future.

Summary

Epiphytic marine brown alga, *Dictyosiphon foeniculaceus* from Muroran, Hokkaido, was investigated to clarify the entire life cycle, sexual evidence and nuclear phase in laboratory cultures. *Dictyosiphon foeniculaceus* showed heteromorphic alternation of generations

between a macrothallus bearing unilocular sporangia and a microthallus bearing plurilocular sporangia, and the nuclear phases of all stages were haploid (n=8). The sexual process was not observed and the alternation of nuclear phases did not occur. The life cycle of this species was regulated by several environmental factors, especially a chemical substance contained in seawater and the host community.

The life cycle of *Dictyosiphon foeniculaceus* was regulated by using PSIS (plurilocular sporangium inducing substance) and MIS (macrothallus inducing substance) in pure culture, and characterization of PSIS and MIS was attempted. According to the results obtained, the following factors were clarified. The life cycle of *D. foeniculaceus* was not completed under axenic ASP 12-NTA medium, only vegetative growth of microthalli was observed repeatedly. The PSIS-activity is present in seawater, yeast extract, malt extract and *Neorhodomela* extract, and it appeared as a strong polarized fraction of *Neorhodomela* extract. This indicates that PSIS may be a kind of sugar or a related substance. On the other hand, the MIS-activity was discovered in extract of the parasitic yellow bacteria on *Scytosiphon* thallus and it appeared in a volatile fraction, and this indicates that MIS may be a carbohydrate. By the nutritional study, PSIS is identified with inositol or its chemical analogue and MIS is regarded as an unknown substance. The entire life cycle of *Dictyosiphon foeniculaceus* was completed in axenic artificial medium containing PSIS and MIS for the first time.

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PLATE I

Development of spores from unilocular sporangia in field-collected plants.

A. Fertile plant from field.
B. Unilocular sporangia of fertile plant.
C. Erect thallus of fertile plant.
D. Spore liberated from unilocular sporangium.
E. Settled spore.
F. 24-hour old germling.
G. 2-day old germling.
H. 3-day old germling.
I. 5-day old germling.
J. 10-day old prostrate thallus.
K. 14-day old prostrate thallus.
L. 17-day old prostrate thallus.
M, N. Plurilocular reproductive organs produced on 20-day old prostrate thallus.

Use scale in A for A; scale in B for B, H, I & N; scale in C for C, K & L; scale in D for D-G; scale in J for J & M. Scale A shows 10 cm; scale B & D show 10 μm; scale in C & J show 100 μm.

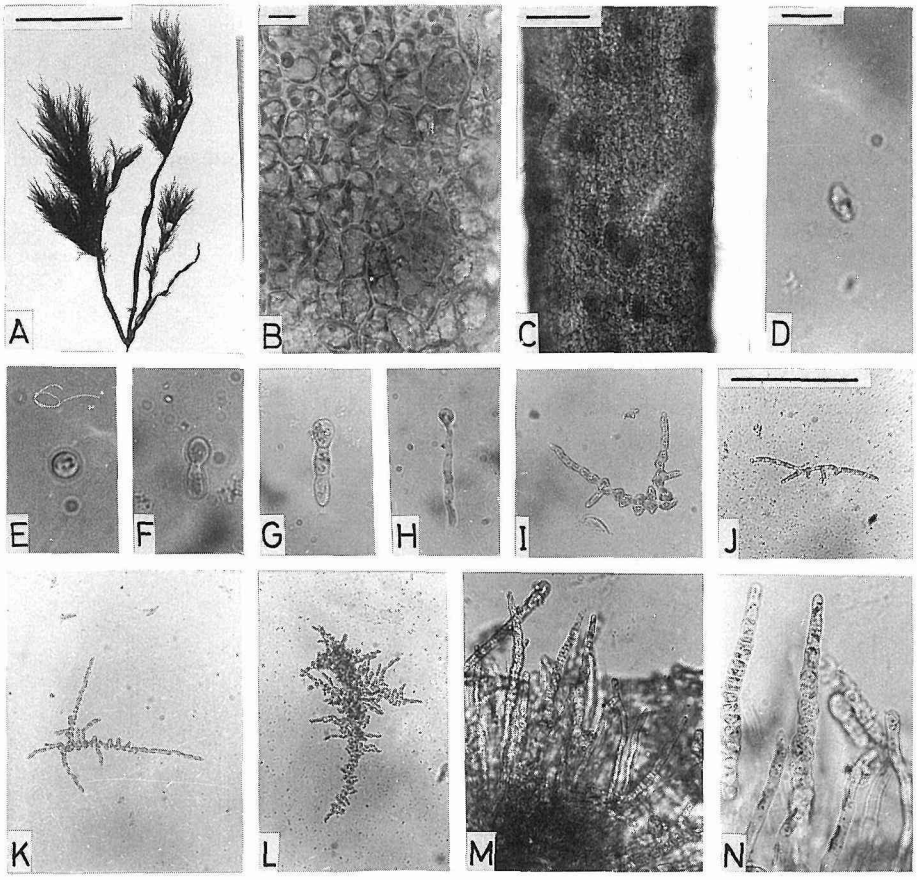

A
B
C
D
E
F
G
H
I
J
K
L
M
N

PLATE II

Development of spores from plurilocular sporangia produced on cultured thalli.

A. Spore liberated from plurilocular sporangium.
B. Settled spore.
C. 3-day old germling.
D. 5-day old germling.
E. 15-day old prostrate thallus.
F. Plurilocular sporangia produced on 20-day old prostrate thallus.

Use scale in A for A & B; scale in C for C & F; scale in E for D, E. Scale A & C show 10 μm; scale E shows 100 μm.

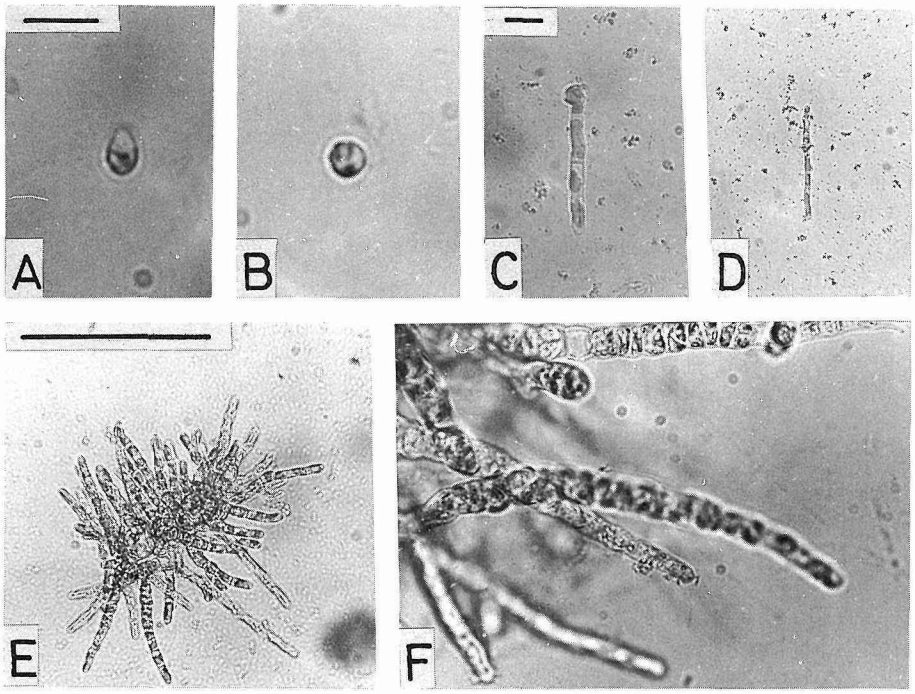

A
B
C
D
E
F

PLATE III

Erect thallus formation in bialgal culture with *Scytosiphon* thallus.

A. 15-day old erect thallus.

B. 1-month old erect thallus.

C, D. Unilocular sporangia produced on 1-month old erect thallus.

E. 2-month old erect thallus.

Scale A & C show 100 μm; scale B shows 1 mm; scale D shows 10 μm; scale E shows 1 cm.

PLATE IV

Chromosomes of *Dictyosiphon foeniculaceus*.

A. Vegetative cell in erect thallus collected from field.

B. Unilocular sporangium in erect thallus collected from field.

C. Vegetative cell in prostrate thallus derived from zoid of unilocular sporangium.

D. Plurilocular sporangium in prostrate thallus derived from zoid of plurilocular sporangium.

E. Vegetative cell in prostrate thallus derived from zoid of plurilocular sporangium.

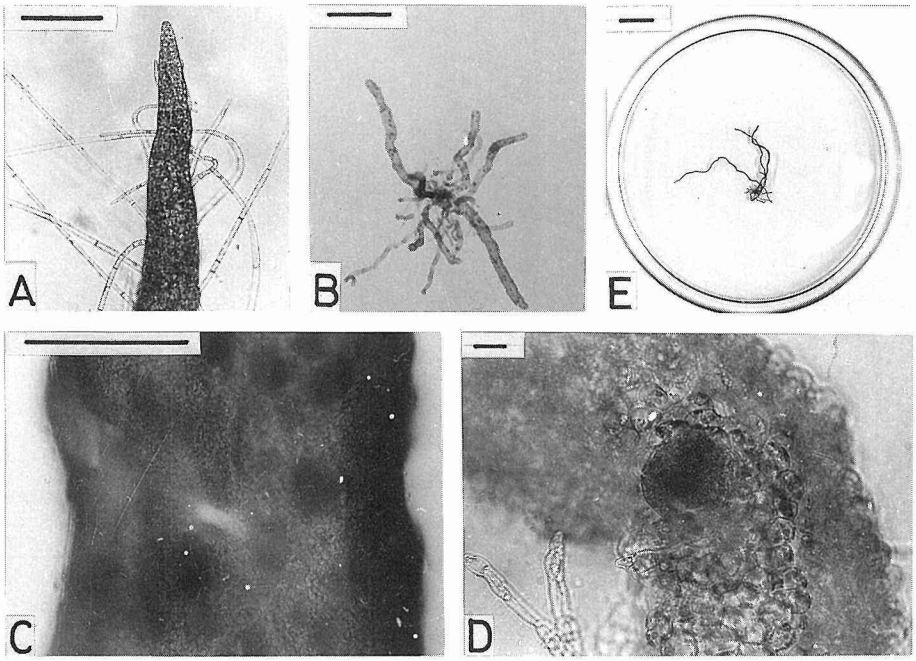
F. Plurilocular sporangium in prostrate thallus derived from zoid of plurilocular sporangium.

G. Vegetative cell in erect thallus derived from zoid of plurilocular sporangium.

H. Unilocular sporangium in erect thallus derived from zoid of plurilocular sporangium.

Use scale in A for A-H. Scale A shows 10 μm.

[SAGA] PLATE III



[SAGA] PLATE IV