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Algal-Fungal Interactions in the Marine Ecosystem: Symbiosis to Parasitism

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A wide array of partnership exists between algae and fungi. These range from loose commensal association between algae and fungi as in primitive lichens, obligate symbiotic association termed mycophycobioses between the systemic marine fungi and the macroalgae, parasitism where the fungi are pathogens causing disease in the host and saprobic association where fungi grow on senescent to moribund algae. Among these, the fungal parasites are relatively fewer in number than those reported as parasites in terrestrial plants and are limited in their geographical distribution due to their range of host specificity. Fungal pathogens of fresh water phytoplankton play an important role in governing their periodicity in lakes. However, we do not know whether this holds good for the marine environment too. Fungal pathogens associated with green, brown, red algae and phytoplankton around the coast of India are described here with an emphasis on different kinds of algal-fungal relationships. The observation on seasonal occurrence of a few fungal pathogens, host specificity and changes in physiology of host algae are discussed here.

Keywords: algae, fungal pathogens, phytoplankton, parasitism, symbiosis

Marine algae, which include phytoplankton and macroalgae, are a diverse group of organisms ranging in size from unicellular to highly complex giant kelps. These are distributed in coastal and open oceans and are the major primary producers in the marine ecosystem. They play an important role in coastal detrital and herbivore food webs. They are the major contributors of particulate organic carbon (POC) and dissolved organic carbon (DOC) which in turn sustains a host of microorganisms in these habitats. The growth and distribution of phytoplankton and macroalgae is determined by nutrient availability, water motion and force, light and salinity (Littler & Littler, 1985). Besides these, the other determinant factors are the grazers and parasites.

Among parasites, fungi are the most dominant ones. There is a symbiotic association of fungi with algae in lichens where both the partners benefit. On the

extreme end this association is called mycophycobiosis where an obligate symbiotic association exists between systemic fungi and marine macro algae. Marine crustose and foliose lichens are commonly found growing on rocks in the coasts (Kohlmeyer and Kohlmeyer, 1979). Fungi associated with algae without causing any visual harmful effects are also reported (Testrake and Aldrich, 1984). However, fungi occurring as pathogens with various algae have received the most attention (Sparrow, 1960; Kohlmeyer and Kohlmeyer, 1979). Among these, a majority of work has been carried out with fungal pathogens of freshwater phytoplankton (Sparrow, 1968; Canter, 1979, Karling, 1981). Several reports on aquatic phycomycetous fungi were published from India (Karling, 1978; Dayal & Kiran, 1980; Chaudhry and Agarwal, 1980; Hasija and Khan, 1985). Professor J.S.Karling's participation as a marine mycologist with UNESCO-sponsored International Indian Ocean Expedition in 1963 resulted in reporting several new aquatic fungi from India (Karling, 1966). Although fungal pathogens of algae and phytoplankton in the marine environment were reported from several parts of the world during this period, not much attention was paid to the interactions between the host and the pathogen (Andrews & Goff, 1985). Unfortunately, the scenario has not improved much even after 50 years. There are several reasons for this situation. Knowledge of algal and fungal system combined with field-oriented work and labor intensive search for fungal pathogens of algae deter many researchers from this field. Studies on fungal pathogens of higher plants and especially crop plants in the terrestrial ecosystem have advanced tremendously in the last 50 years after the advent of molecular biological methods. Intensive cultivation of crop plants leads to serious outbreak of diseases on land but a similar situation may not arise in marine environment except for species which are cultivated in aquaculture system (Andrews, 1976). A few such diseases are however reported in cultivated species of algae (Kazama, 1979). However, in natural stands of vegetation in the sea the incidence of diseases might be as common as on land. A systematic survey of algal diseases of the Indian coast was carried out at the National Institute of Oceanography, Goa. The methodologies developed in algal pathology and algal-fungal interactions in different groups of algae are presented in this synthesis.

DETECTION AND ETIOLOGY OF DISEASES

Detection of disease in algae is not a very easy task as is possible with land plants. A basic understanding in morphology of algae is a must for this purpose. Micro and macroalgae need to be collected separately for further microscopic examination and isolation of associated causal organisms in the laboratory. The disease symptoms may be the result of abiotic or biotic agents. The etiology of diseases if any, may be recorded digitally or by photomicrography. For identifying the biotic agents, the following procedures may be used. These methods are recommended basically for studies on fungal pathogens.

Detection and/or isolation of fungi

- a) Freshly collected algae and phytoplankton are examined under microscope for the presence of fungi. Staining with lactophenol-cotton blue helps to a certain extent in detecting fungal structures. Staining with optical brightner calcofluor or wheat-germ agglutinin (WGA) conjugated with fluorescein isothiocyanate (FITC) followed by observation under an epifluorescence microscope is reported for visualization of fungal parasites on phytoplankton (Müller and Sengbusch, 1983).
- b) The algae and phytoplankton may be incubated in sterile seawater in Petri dishes in diffuse sunlight. The seawater needs to be changed daily to avoid bacterial growth. A solution of antibiotics such as streptomycin and penicillin may also help in checking bacterial growth. Microscopic examination of these algae help in detecting fungal structures that are either epibiotic or endobiotic.
- c) Macroalgae may be incubated in moist chambers for a few days followed by microscopic examination for the presence of fungi.
- d) Pieces of surface-sterilized algae are directly plated on mycological agar media prepared with sea water and supplemented with antibiotics. Sodium hypochlorite or ethanol is recommended for surface-sterilization of algae. The concentrations of surface sterilizing agent and time of sterilization need to be standardized for different types of algae. Filamentous fungi growing out of the algal pieces are picked and subcultured on culture media and purified.
- e) Pieces of surface-sterilized algae are homogenized in seawater and used for dilution plating on mycological agar media prepared with sea water and supplemented with antibiotic solution.
- f) Phytoplankton and algae are baited with pine pollen or crushed sesame seeds for isolation of associated thraustochytrids and chytrids (Gaertner, 1979).
- g) For obligate fungi, dual cultures of phytoplankton and its pathogens are maintained (Raghukumar, 1978). For physiological studies on such interactions the phytoplankton cultures are to be axenic in which the obligate fungal parasites are maintained.

PROVING KOCH'S POSTULATES

Fulfillment of four criteria under Koch's postulates are essential to prove the causal organism as disease causing agent in nature. These are: 1) association of the organism constantly in all the diseased specimens, 2) isolation of the disease-causing organism and culturing it, 3) inoculating the isolated organism on healthy host and duplication of natural disease symptoms, 4) re-isolation of the causal organism from experimentally inoculated host and comparing it with the initially isolated organism (Andrews and Goff, 1985). However, it is difficult to fulfill all the above mentioned criteria for the obligate fungi which cannot be cultured using the routine laboratory

media. Maintaining dual culture of the host with the parasite and healthy host also helps to partially fulfill these postulates.

RANGE OF ALGAL-FUNGAL INTERACTIONS

The fungi associated with algae show a wide range of host-parasite interactions and these fall into 3 main categories:

- a) biotrophic association where the host exhibits mild or no symptoms. The fungi grow on the surface of algae (epibiotic) or inside (endobiotic) and draw nourishment from their host without destroying them. Most of these are obligate parasites and are till date uncultured in artificial culture media.
- b) biotrophic association where the host exhibit severe disease symptoms. These are also obligate parasites causing total destruction of host organelles and occupy the entire cell of the host, the zoospores released seek new healthy cells for further infection and thus repeat the cycle. These obligate parasites are also uncultured.
- c) necrotrophic fungi grow on partially senescent host and cause further destruction of the host cells.

The following examples of fungal parasites are chosen to represent these three ranges of parasitism occurring in different phyla of algae.

FUNGAL PARASITES OF THE MARINE GREEN ALGAE CHLOROPHYTA

A number of fungal parasites belonging to the group Chytridiomycota were reported as parasites in green algae (Johnson and Sparrow, 1961). Among the green algae, the filamentous alga *Cladophora* species collected from the Indian coast was reported to harbour a range of fungal parasites (Raghukumar and Raghukumar, 1994). Among these, the polycentric chytrid, *Coenomyces* sp was found to be always associated with healthy alga (Table 1). The alga appeared healthy and no morphologically visible symptoms of fungal infection were seen. On incubation in sea water, groups of fungal sporangia were seen emerging out of algal filaments. When the algal filaments were bleached and dead, the fungus also appeared to die with it.

Sporangia of *Olpidium rostriferum* were also found to be associated with *Cladophora frascatii* without causing any externally distinguishable morphological changes in the host. On further incubation of the alga in sea water, chloroplasts of infected cells turn golden brown and accumulate around the fungal sporangia (Figs. 1, 2). The fungal sporangia release zoospores outside the algal filament through long discharge tubes which penetrate through the host cell

Table 1 — Fungal pathogens in the green algae (Chlorophyta) reported from India

S. No.	Algal host	Fungus	Group	Location	Reference
1	<i>Cladophora repens</i> , <i>C. fascicularis</i> , <i>Rhizoclonium</i> sp.	<i>Coenomyces</i> sp.	Chytridiomycota	Goa, Veraval (Gujarat), Lakshadweep Islands	Raghukumar 1986a
2.	<i>Cladophora expansa</i> , <i>C. fascicularis</i> , <i>C. frescatti</i> , <i>Rhizoclonium</i> sp	<i>Olpidium rostriferum</i>	Chytridiomycota	Mandapam (Tamil Nadu), Goa, Lakshadweep Islands, Veraval	Raghukumar 1986a, 1987a
3.	<i>Cladophora frescatti</i> , <i>C. gracilaris</i>	<i>Sirolpidium bryopsidis</i>	Oomycota	Goa, Lakshadweep Islands, Tuticorin (Tamil Nadu)	Raghukumar 1986a, 1987a
4.	<i>Chaetomorpha media</i> , <i>Valoniopsis</i> sp.	<i>Pontisma lagenidioides</i>	Oomycota	Goa, Mandapam, Veraval	Raghukumar 1987a, Raghukumar & Raghukumar, 1994
5.	<i>Cladophora</i> sp, <i>C. frescatti</i> , <i>C. repens</i> , <i>Chaetomorpha media</i> , <i>Valoniopsis pachynema</i> , <i>Rhizoclonium</i> sp.	<i>Labyrinthula</i> spp.	Labyrinthulamycota	Goa, Lakshadweep Islands, Mandapam, Veraval	Raghukumar, 1986a, 1987a & b, Raghukumar & Raghukumar, 1994

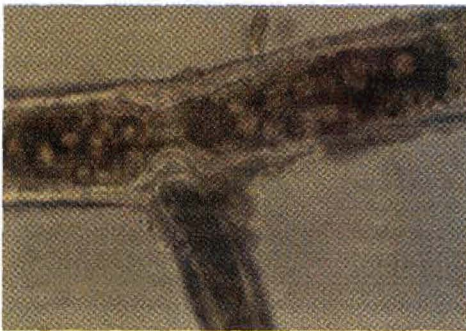


Fig. 1 — The green alga *Cladophora frescattii* infected with *Olpidium rostriferum*. Note the breakdown of chlorophyll in the host.

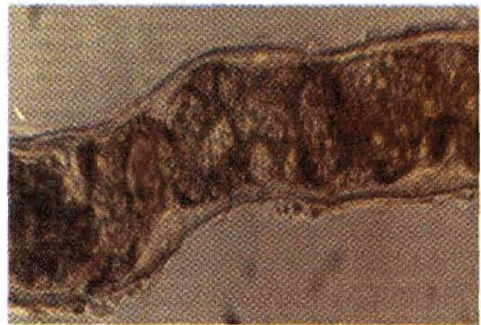


Fig. 2 — An advanced stage of the infection in *Cladophora frescattii*.

wall. Another green alga, *Rhizoclonium* sp. also harboured both the above mentioned fungi without displaying any symptoms of disease externally. Huth and Gaertner (1973) reported a host specific obligate parasite *Rhizophyidium sphaerocarpum* growing on *Rhizoclonium* sp growing in the esturine environment in Northern Germany. The filamentous alga *Codium mucronatum* is infected by an ascomycetous fungus *Pontogeneia*. The only symptom of infection is dark colored fruiting bodies in or on the algal thallus (Kohlmeyer, 1975)

Cladophora frascatii and *Rhizoclonium* spp. also regularly harbored *Sirolopidium bryopsisidis*, an oomycetous fungus displaying browning of terminal and subterminal cells of branches. Only the infected cells were filled with fungal sporangia and the neighboring cells appeared green and turgid (Raghukumar, 1986a).

Chaetomorpha media, another green filamentous alga from western and eastern coasts of the South Indian peninsula showed infection by an oomycetous fungus *Pontisma lagenidioides* (Raghukumar 1987a and b). The infected cells appear brownish and the infection spreads from the tip downwards of the algal filament on incubation in sea water in the laboratory (Fig. 3). On longer incubation, filaments turned completely brown and the plant loses its turgidity (Fig. 4). The chloroplasts in healthy cells of the alga are tightly packed initially. As the infection spreads, they aggregate around fungal sporangia and turn brown (Figs. 5, 6). Later the brown pigmented bodies disappeared completely leaving the empty fungal sporangia with their persistent zoospore discharge tubes. Microcosm studies showed that the fungal infection resulted in loss in weight of the alga, chlorophyll *a* and *b*, total carbohydrate and protein with consequent increase in phaeopigment concentrations (Raghukumar 1987b, Raghukumar and Chandramohan, 1988). Reduced chlorophyll content was also reported in *Cladophora glomerata* infected by the filamentous fungus *Acremonium kiliense* (Bott and Rogenmuser, 1980).

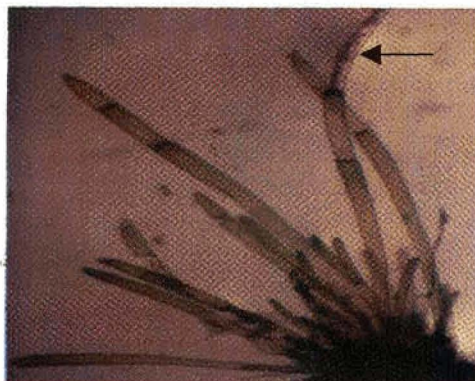


Fig. 3 — The terminal portion of the green alga *Chaetomorpha media* showing infection by *Pontisma lagenidioides* (arrow). The sample was freshly collected from Anjuna beach in Goa.

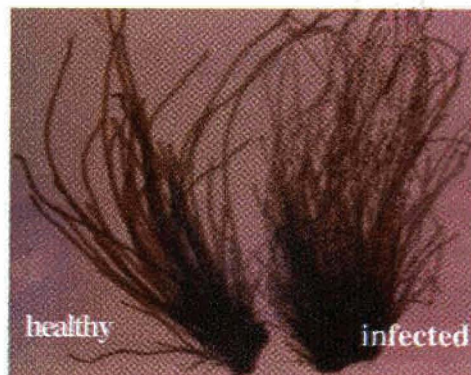


Fig. 4 — *Chaetomorpha media* after 7 days incubation in sterile sea water is totally infected.

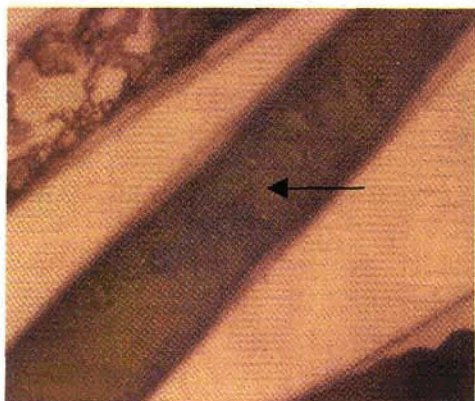


Fig. 5 — Growth of the pseudomycelium of *Pontisma lagenidioides* (arrow) growing inside the host cell.



Fig. 6 — An advanced stage of the infection where the host chloroplast is totally destroyed and host cell is filled with the pseudomycelium of the fungus.

Species of *Cladophora*, *Rhizoclonium* and *Chaetomorpha* on incubation in seawater showed presence of *Labyrinthula*, a member of the phylum Labyrinthulomycota growing in healthy as well as senescent and moribund cells. The algal cells were filled with spindle-shaped cells moving on ectoplasmic nets (Fig. 7, 8). On death of the host cells, the fungus appeared to grow on the surface of the algal filament. Species of thraustochytrids were also isolated from these filamentous green algae besides *Codium*, and *Ulva* spp. (Raghukumar 1987a). Haythorn *et al.* (1980) also isolated thraustochytrids from these green algae from British coastal waters.



Fig. 7 — *Labyrinthula* sp. growing inside the green alga *Rhizoclonium* sp.

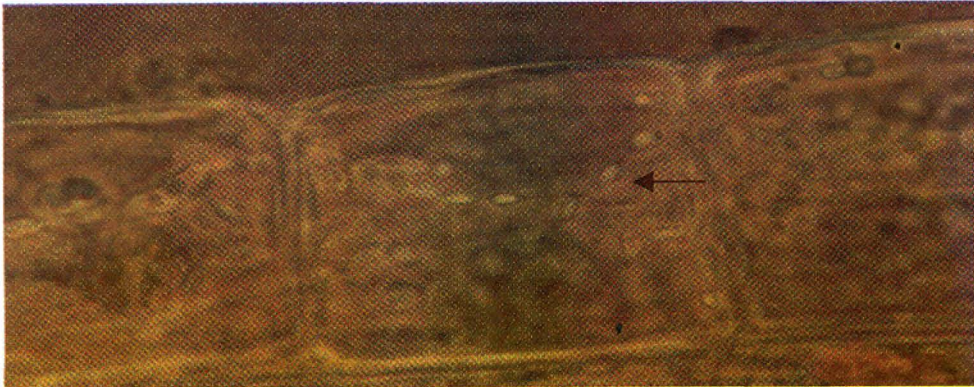


Fig. 8 — *Labyrinthula* sp. growing inside the green alga *Cladophora* sp (arrow).

All the above described fungi except *Labyrinthula* could not be cultured in any natural or synthetic media indicating their obligate nature. On the other hand, the marine chytrid, *Rhizophyidium littoreum* from the siphonaceous alga, *Codium* sp has been cultured successfully on yeast extract-peptone-dextrose medium (Amon, 1984). *Labyrinthula* species could be cultured on modified Vishniac medium (Vishniac, 1955) supplemented with 0.1% cholesterol and also on autoclaved algal filaments (Raghukumar 1986a).

FUNGAL PARASITES OF PHAEOPHYTA

The brown alga *Ectocarpus* sp was reported to harbour an oomycetous fungus *Eurychasma* sp (Johnson and Sparrow, 1961). An epibiotic chytrid *Chytridium polysiphoniae* on the filamentous microalga *Sphacelaria* sp was reported (Table 2) from southern coast of India (Raghukumar, 1987a). A "Olpidium-like" fungus was found to occur commonly in the terminal cells of parent plant of *Sphacelaria* species and in the tetradial propagules (Fig. 9, 10) attached to the host as well as those which were detached (Raghukumar, 1991). The severity of infection increased drastically in January 1989, causing disappearance of this alga for a few months from the rocky shores of Anjuna beach in Goa. As the vegetative propagules were severely infested, this might have brought about abrupt disappearance of this alga from the collection site (Raghukumar, 1991). An ascomycetous fungus *Lindra thalassiae* was isolated from *Sargassum* species from the coast of Goa and the Lakshadweep islands in the Arabian Sea (Sathe-Pathak *et al.* 1993, Sharma *et al.* 1994). *L. thalassiae* grows on air vesicles of *Sargassum* and turns them into dark brown, soft and wrinkled structures raisins and thus the name "raisin disease" (Kohlmeyer & Demoulin, 1981). The disease was reported in Sargasso Sea, with only a few members of plants being affected (Kohlmeyer, 1971). I have isolated this fungus by incubating stalks and vesicles of *Sargassum* in moist chambers but never observed the fungus in fresh samples. A number of ascomycetous fungi as

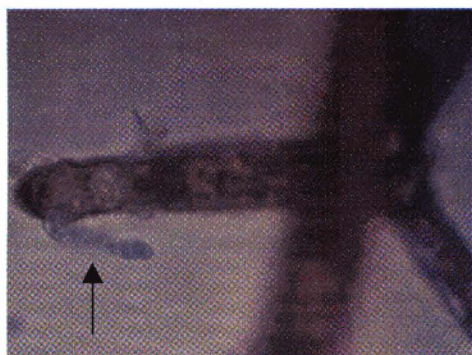


Fig. 9. The detached tetra-radiate propagule of the brown alga *Sphacelaria* sp infected with a "Olpidium-like" fungus. Note the discharge tube of the fungal sporangium (arrow) through which the zoospores escape out of the host cell.



Fig. 10. The *Olpidium*-like fungus growing inside the attached propagule. The sporangial content has cleaved in to zoospores.

Table 2 — Fungal pathogens of algae and phytoplankton reported from India

S. No.	Algal host	Fungus	Group	Location	Reference
Phaeophyta					
1.	<i>Sphacelaria</i> sp	"Olpidium"-like fungus	Chytridiomycota	Goa	Raghukumar, 1990
2.	<i>Sphacelaria</i> sp	<i>Chytridium polysiphoniae</i>	Chytridiomycota	Mandapam	Raghukumar, 1987a
3.	<i>Sargassum</i> sp.	<i>Lindra thalassiae</i>	Ascomycota	Goa, Lakshadweep Islands	Sathe-Pathak <i>et al</i> , 1993; Sharma <i>et al</i> , 1994
Rhodophyta					
1.	<i>Centroceras clavulatum</i>	<i>Chytridium polysiphoniae</i>	Chytridiomycota	Goa, Lakshadweep Islands, Mandapam	Raghukumar 1986b; 1987a
Diatoms (Bacillariophyta)					
1.	<i>Licmophora hyalina</i> ,	<i>Ectrogella perforans</i>	Oomycota	North Sea	Raghukumar, 1978; 1980a & b; 1983
2.	<i>Coscinodiscus</i> sp, <i>Navicula</i> sp, <i>Nitzschia</i> sp, <i>Grammatophora</i> sp, <i>Melosira</i> sp	<i>Ulkenia amoeboides</i>	Labyrinthulomycota	Central Arabian Sea, Mandovi estuary (Goa)	Raghukumar 1986c; 1996

parasites in brown algae from the Pacific and Atlantic Ocean have been reported by Kohlmeyer and Kohlmeyer (1979). These parasitic fungi have been classified by them as a) where the host is externally unaltered b) where discoloration of the host occurs and c) those causing malformations in the host (Kohlmeyer and Kohlmeyer, 1979). The brown alga *Pilayella littoralis* infected by *Eurychasma dicksonii* causes characteristic changes in cell and sporangial morphology so much so that it was misidentified as a different species. Stages in the development of fungal sporangium have been misinterpreted as belonging to the alga (Jenneborg, 1977). The most common fungus on brown algae is *Phycomelaina laminariae* causing "stipe blotch of kelps" in species of *Laminaria*. The parasite forms black circular or oblong patches on the stalks of the hosts. Although the fungus does not kill the host, the infected areas are severely damaged and permit penetration of secondary saprophytic fungi (Kohlmeyer, & Kohlmeyer, 1979). Several genera of brown algae are infected by *Haloguignardia* species causing gall formations on stipes, vesicles and blades of the hosts (Kohlmeyer, 1972).

FUNGAL PARASITES OF RHODOPHYTA

The filamentous red alga *Centroceras clavulatum*, harbors an epiphytic chytrid *Chytridium polysiphoniae* found in the coast of Goa (Fig. 11), the Lakshadweep Island Agatti and the south eastern coast of India (Raghukumar, 1986b, 1987a). The fungus could not be cultured by using pine pollen as bait in sea water nor on killed alga suggesting its obligate parasitic nature. The fungus was restricted to *C. clavulatum* and was not found on *Ceramium* species, another red filamentous alga usually found in the vicinity of the infected *C. clavulatum*. A salinity of 25 psu and temperature of 30°C was found to be optimum to get infection of healthy algae in laboratory experiments (Raghukumar, 1986b). A thraustochytrid *Schizochytrium* sp and a aplanochytrium *Labyrinthuloides minuta* were isolated from living red algae *Centroceras clavulatum* and the brown algae *Sargassum* and *Padina* species in Goa (Raghukumar *et al.*, 1992). Occasionally, *Schizochytrium* sp. was observed to grow densely on live thallus of *Padina tetrastomatica* wherever a cuticular layer was present (Fig. 12). As thraustochytrids are capable of drawing nutrients from their hosts by ectoplasmic net elements, this mode of existence also would expose them to the resistance mechanism of live algae (Raghukumar, 1990). However, where a cuticle is present on the surface of the alga, thraustochytrids might directly grow on this, without having to penetrate the epidermal cells for nutrition, thus avoiding the antagonism of the alga.

A host specific parasite of *Antithamnion floccosum*, reported from Canada was *Olpidiopsis antithamnionis* (Whittick and South, 1972). The red alga, *Palmaria mollis* was reported to get parasitic fungal infection by the oomycetous fungus, *Petersenia palmaria* (Van der Meer and Peuschel, 1985). The infecting fungus produces numerous glistening white blisters on the algal sporelings and apical segments of fronds. The fungus is specific to *P. mollis* and did not infect other red

algae, *P. palmata*, *Gracilaria tikvahiae*, *Chondrus crispus*, *Ceramium rubrum* and *Seirospora seirosperma*. However, subsequently, infection of a high K-carrageenan producing alga *Chondrus crispus* from Nova Scotia, Canada was reported to be infected by *Petersenia pollagaster*, causing white blisters on the tips tips of their fronds (Molina, 1986). Another oomycetous fungus *Pontisma lagenidioides* was reported to occur in *Ceramium* species as a weak parasite or a saprophyte because it thrived best when the alga was under unfavourable conditions (Sparrow, 1960).

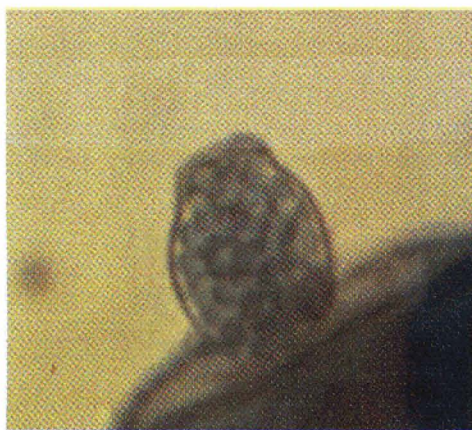


Fig. 11 — An epiphytic chytrid *Chytridium polysiphoniae* growing on the red alga *Centroceras clavulatum*. The alga was stained with lactophenol cotton blue for viewing the parasite. The content of the fungal thallus has cleaved into zoospores which will escape and settle on a fresh host cell.

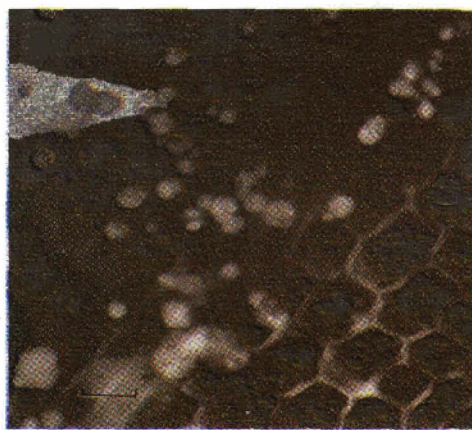


Fig. 12 — A thraustochytrid *Schizochytrium* sp growing on the algal thallus of the brown alga *Padina* sp. A dense growth is visible only where the algal cuticle is intact.

The red alga, *Porphyra* commonly called “nori” is a major commercially cultivated food crop in Japan. The oomycetous fungus, *Pythium porphyrae* causes “wasting disease” in this alga in mariculture facilities (Kazama, 1979). The disease outbreak is quite serious, destroying millions of tons of crop within 2-3 weeks (Andrews, 1976). On infection, small rapidly coalescing lesions appear on the algal fronds (Fuller *et al.*, 1966). Ultrastructural studies revealed that the fungal hyphae penetrate without modifying the host cell wall (Kazama and Fuller, 1970). No sheath membrane or sheath was detected around the infecting hyphae as is typical of parasites that form haustoria for penetrating into host cells. The absence of such a membrane probably makes the fungus a virulent parasite causing total destruction of the host cells. The fungal hyphae grow inter- and intracellularly, the host cells become devoid of contents and rapidly collapse. Damage is restricted to cells actually penetrated by the fungus. The infection process leads to dissolution of the host floridean starch grains leaving only a reticulate network behind (Kazama and Fuller, 1970).

Addepalli and Fujita (2002) demonstrated the role of calcium in the zoospore biology of *P. porphyrae*. Formation and cleavage of zoosporangia into individual zoospores is regulated by the external calcium concentrations. The zoosporangia are not formed in the absence of calcium and in the presence of other mono or divalent cations indicating absolute requirement of calcium for asexual reproduction. Even if zoosporangia are formed in the presence of calcium, sporulation does not occur in its absence. Calcium was found to be involved in the germination of encysted zoospore (Addepalli and Fujita, 2002). This basic information may ultimately help in prevention of infection by *P. porphyrae* in *Porphyra* spp.

The calcareous red algae *Lithophyllum*, *Porolithon* and *Pseudolithophyllum* are infected by an ascomycetous fungus *Lulworthia kniepii*. The fungal mycelium grows in the middle lamellae and destroys the protoplasts of the adjoining cells, whereas the calcified cell walls of the algae are not attacked. The embedded ascocarps and surrounding hyphae appear as dark spots in the light-colored algal fronds (Kohlmeyer and Kohlmeyer, 1979). An ascomycetous fungus, *Chadefaudia marina* causes yellow-green spots with dark ascocarps in the thalli of *Rhodymenia palmata* (Feldmann, 1957). Another common ascomycete in marine algae is *Didymosphaeria danica*. The fungus is reported to be tissue specific and was found restricted to cystocarps of *Chondrus crispus*, causing blackening of the host tissue (Wilson and Knoyle, 1961). Symptoms similar to 'witches' brooms' of terrestrial plants have been observed in the red alga *Ballia callitricha* infected by *Spathulospora calva* wherein the host hair proliferate and enclose the ascocarps of the parasite (Kohlmeyer, 1973).

FUNGAL PARASITES OF BACILLARIOPHYTA

A phycomycetous fungus, *Ectrogella* species are parasites in diatoms and according to Sparrow (1969) *Ectrogella perforans* may infect the marine pinnate diatom species of *Licmophora* to epidemic proportions. Besides *Licmophora*, it infects species of *Synedra*, *Striatella* and *Vorticella* (Sparrow, 1968, 1969). The infected diatom cells become hypertrophied and chlorotic (Figs. 13, 14). Laboratory experiments with *Licmophora hyalina* from the North Sea showed that multiple infections are initiated by zoospores which adhere to the surface (Fig. 14) of the host cells (Raghukumar, 1978). The optimum temperature for the growth of *Licmophora* isolates varied from 15-25°C but the fungal infection was best at 15°C. Infection percentage was higher in light than in dark. Ultrastructural studies on these host-parasite interactions showed that the pathogen enters the host cell by piercing the narrow areolae of the silicified diatom wall (Fig. 15). As the fungal sporangium matures inside the host, the chromoplasts of the host begin to lose their normal color, disintegrate and break down. The cytoplasm of the host appears vacuolated and several fungal sporangia develop inside the host cell (Figs. 16, 17). On maturity of the fungal zoosporangium, the valves of the diatom cells are pushed

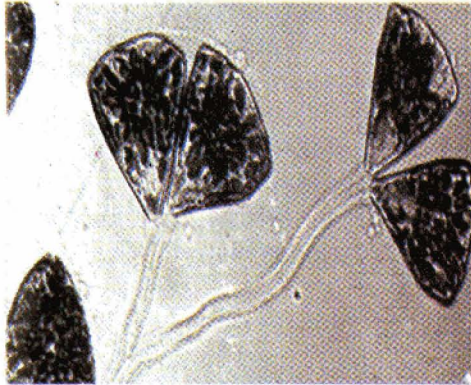


Fig. 13 — Healthy cells of the benthic diatom *Licmophora hyalina*.

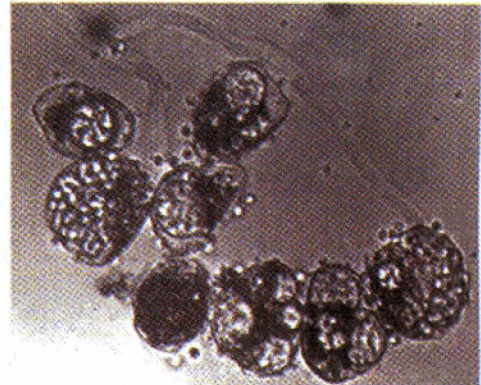


Fig. 14. *Licmophora hyalina* diatom cells infected with the endobiotic obligate fungal pathogen *Ectrogella perforans*. A number of zoospores of the fungus infect the same host cell resulting in development of more than 5-7 sporangia in one diatom cell which makes the host cell hypertrophied.

apart and the fungal discharge tube begins to push out. The zoospores swim out and settle on healthy host surface (Raghukumar 1980a, b). Cell-free filtrates from the cultures of *Licmophora* species resistant to infection by *E. perforans*, when added to a susceptible isolates of the host, resulted in about 80-90% inhibition of infection. The inhibitor factor produced by resistant host cells appeared to be a non-dialysable high-molecular weight substance (Raghukumar, 1983).

Several diatoms collected from the Arabian Sea were found to harbour the thraustochytrid *Ulkenia visurgensis* (Raghukumar 1986b). The protist did not infect healthy cells but was found always in senescent and moribund cultures. A thraustochytrid species *Schizochytrium* was reported as parasite on the diatom *Thalassionema nitzchioides*. It caused disintegration of the diatom cell and the fungus could not be cultured on pine pollen-sea water medium as other thraustochytrids (Gaertner, 1979).



Fig. 15. An infection tube (IT) and an appressorium (A) of the fungal zoospore (arrow) seen in the transmission electron micrograph of the diatom *L. hyalina*. The chloroplast (C) and nucleus (N) of the host cell are intact.

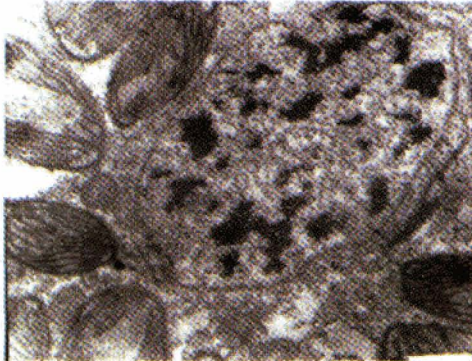


Fig. 16 — An infected diatom cell in the early stage where the host chloroplasts start developing vacuoles (arrow).



Fig. 17 — The cytoplasmic content of the host cell is highly vacuolated and development of fungal sporangia (FS) is seen.

An endobiotic parasite, *Lagenisma coscinodisci* of the marine diatom *Coscinodiscus centralis* from the North Sea was reported by Drebes (1966). The same fungus was reported from the waters off the western Washington coast, USA (Gotelli, 1971). About 13% infection in natural population of *Coscinodiscus* by *L. coscinodisci* in Weser estuary in northern Germany was reported to occur (Chakravarty, 1974). Non-septate mycelium of the fungus is found inside infected diatom cells. The entire fungal cytoplasm gets converted into a single fungal sporangium which produces laterally biflagellate zoospores. The fungal mycelium acts as a discharge tube, passes between girdle bands of the host and the zoospores are released outside the host cell (Drebes, 1966).

PREVENTIVE MEASURES

Unlike terrestrial environment where fungal infections are reported to become epidemic, the infections of algae by fungi in the marine environment remain localized or endemic. As the distribution of algae is highly localized, the fungal infections also remain so. Most of the fungi causing diseases in algae are host specific and this restricts their spread. Thus, these principles act synergistically as natural preventive measures in algal pathology. Fungal infection in aquaculture or mariculture of economically important algae can become epidemic and devastating as was reported in the culture of *Porphyra* in Japan (Fujita, 1990). The *Porphyra* farms consisting of mono species with similar genetic make-up, requires all the precautions to prevent fungal infections. Some of the preventive measures include immersing the *Porphyra* cultivating nets into organic acid, such as citric or phosphoric acid at a pH of about 2 for 5 to 10 min, early harvesting of infected plants, exposure to air or short-term freezing (Woo *et al.* 2002). Long-term cultivation of *Porphyra* of the same genetic

make-up also makes them susceptible to infection by *Pythium porphyrae* (Uppalapati and Fujita, 2001). These authors further reported that the non-cultivated species were relatively resistant.

One of the bacterial isolates obtained from *Porphyra* beds showed promise for biological control of *Pythium* infection (Woo *et al.* 2002). The isolate AP77 exhibited anti-*Pythium* activity and produced an extracellular anti-*Pythium* protein designated SAP. The protein was characterized, its N-terminal amino acids sequenced with an ultimate aim of introducing the gene encoding SAP to produce transgenic *Porphyra* that can resist the infection by *Pythium porphyrae* (Woo *et al.* 2002). The protein SAP was later found to be specific for *P. porphyrae* and the bacterium that produced it was identified to be *Streptomyces* sp (Woo and Kamei, 2003). The protein caused the lysis of fungal hyphae.

CONCLUSION

Host specificity

Some of the algal parasites do show host specificity and this is sometimes linked to their biogeographic distribution. *Olpidium rostriferum* occurred in *Cladophora frescatti*, *C. expansa*, *C. fascicularis* and *Rhizoclonium* species in waters around the southern peninsula of India (Raghukumar 1986b, 1987a) but it was reported to infect zygospores of *Spirogyra* in the Atlantic (Sparrow, 1960). Inoculation of species of *Cladophora* other than that in which it naturally occurred, proved unsuccessful (Raghukumar, 1986b). The fungal pathogen, *Sirolpidium bryopsidis* was reported to infect *Cladophora frescatti* and *C. gracilis* in our waters but Sparrow (1960) reported it to be occurring in another green alga *Bryopsis* besides *Cladophora*. During cross inoculation studies, this fungus did not infect a few other species of *Cladophora*. *Pontisma lagenidioides* originally reported to infect the red alga *Ceramium* (Sparrow, 1960), infected the green algae *Chaetomorpha media* and *Valoniopsis* sp in India (Raghukumar, 1987a) but did not infect *Chaetomorpha linum* in nature or in the laboratory experiments. On the other hand, *Chytridium polysiphoniae* could be cross inoculated from the red alga *Centroceras clavulatum* to a brown alga *Sphacelaria* species and vice versa (Raghukumar, 1991). We are not certain whether these geographically widely distributed species occurring in different hosts are identical. Molecular tools to a certain extent will be able to solve this taxonomic problem.

The species of the green alga *Cladophora* was observed to harbour the maximum number of fungal parasites (Table 1). These fungal pathogens were *Coenomyces* sp., *Olpidium rostriferum*, *Sirolpidium bryopsidis* and *Labyrinthula* sp. In certain localities, the alga harboured at least two pathogens simultaneously (Raghukumar, 1986a). *Labyrinthula* species exhibited a wide range of hosts, behaving as severe pathogen in some and as a weak parasite in others. Fungi belonging to

chytridiomycota and oomycota are mostly associated with green algae whereas the red and brown algae harbour ascomycetous fungi.

As most of these fungal pathogens are obligate parasites, they have not yet been cultured in laboratory media and therefore are to be maintained in dual cultures. Therefore, the physiology of host-parasite interactions and molecular biological studies pose great challenges.

Seasonality of disease incidence

The fungal infections would affect the seasonal occurrence of their host which in turn will dictate the seasonality of the pathogen. During the survey of fungal infections of algae carried out in Goa, we found maximum infection by *Chytridium polysiphoniae* on the red alga *Centroceras clavulatum*; *Coenomyces* species in the green alga *Cladophora repens*; *Olpidium rostriferum* and *Sirolopidium bryopsidis* in *Cladophora frascatii* during south west monsoon period of June-September in Goa (Raghukumar, 1996). High amount of nutrient influx from land and/or the lower salinity conditions possibly favour infections. The biotrophic parasites do not affect the hosts severely and they in turn will be affected seriously when the conditions are unfavourable to their hosts. On the other hand, the biotrophic parasites capable of producing severe disease conditions will be responsible for eradicating their hosts. Spores from such biotrophic parasites may find alternative hosts for survival, if they are not host specific or produce resting stages. The biotrophic parasite, *Ectrogella* species in the marine diatom *Licmophora* species (Sparrow, 1960) and other chytrids in fresh water diatoms (Brunning, 1992) regulate the periodicity of their respective hosts. The necrotrophic parasites occur only in senescent or moribund algae and thus will not affect the periodicity of their host.

Host recognition

The intriguing phenomenon of host recognition in zoospores of these "lower fungi" is not known. This recognition of host surface component and chemotaxis of the zoospores has been studied in terrestrial plant parasites. Extracellular release of soluble substances by healthy algae has been reported (Fogg, 1971). However, in the aquatic milieu, chances of dilution of such factors exist and thus it will be challenging to investigate the lowest threshold value required for recognition in these groups of fungi. The green alga *Chaetomorpha media* growing on wave-beaten rocks on the beaches of Goa invariably harbours the fungus *Pontisma lagenidioides*. *C. media* appears during pre-monsoon (May) and stays till the end of October-November and I have always found this pathogen during this period. It is an obligate parasite and how it survives in the absence of host is not known. Does it switch host during this period or do the zoospores encyst and survive are some of the questions that need to be addressed.

The single celled host-parasite associations offer an excellent opportunity to study the host-parasite interactions at cellular and biochemical levels. Nutrition of

such endoparasites and their effect on host metabolism are important aspects to be considered to understand these associations. As the healthy host cells are challenged by pathogens, their defense reactions in the form of stress proteins, enzymes and other bioactive molecules will be worth investigating. On the whole, research on these organisms have not advanced much after the pioneering work done by Karling, Sparrow (USA), Canter and Lund (UK). As per Sparrow's description they are "minute, elusive but dazzling creatures, here today and gone tomorrow, recalcitrant wherever and whenever the opportunity offers and all in all thoroughly uncooperative" (cited in Canter, 1979). However taming of these organisms will be a challenging task to researchers.

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