

Changes in the Marine Green Alga *Chaetomorpha media* on Infection by a Fungal Pathogen

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Abstract

Biochemical changes in the green filamentous alga *Chaetomorpha media* Kützing infected with a holocarpic endobiotic fungus *Pontisma lagenidioides* Petersen were studied. Healthy plants of *C. media* were inoculated with the infected material under laboratory conditions. The changes in chlorophyll, phaeo-pigments, total phenols, soluble carbohydrate and protein content over a period of 15 days were followed and compared with those of uninfected healthy plants. There was a significant decrease in chlorophyll content, accompanied by an increase in phaeo-pigments in infected plants. This was directly correlated with the level of infection. There was a significant increase in total phenols on infection.

Introduction

One of the common parasitic fungi encountered in Indian waters is *Pontisma lagenidioides* Petersen causing browning disease of the green alga *Chaetomorpha media* Kützing. This zoosporic fungus lives as an obligate, endophytic, biotrophic parasite in *C. media*. The development of the fungus and the disease symptoms have been described in detail earlier (Raghukumar 1987a, and b). Under laboratory conditions, the fungus causes nearly 100% infection of the alga within 10–12 days. As a consequence of this infection, there is a drastic loss of green colour in the alga, accompanied by an increase in the browning of the filaments.

As Andrews (1976) pointed out 'there are many reports of parasitism by fungi on algae, but there are few data on host-parasite interactions, pathogenesis ...'. In the present study, the changes in chlorophyll and phaeopigments over a period of infection were studied. The browning of cells on infection might also result from accumulation of oxidized phenols as has been reported to occur in terrestrial plants (Dickinson and Lucas 1982). The changes in total phenols in healthy and diseased algae were quantified. The

changes in the metabolism of infected plants were studied by estimating total proteins and soluble carbohydrate content of the green alga *C. media*.

Material and Methods

Chaetomorpha media collected from Anjuna beach in Goa, India (15°27'–15°38'N and 73°42'–73°50'E) was not infected with the fungus *Pontisma lagenidioides*. About 5% of plants of *C. media* collected from Baga beach in Goa, showed infection by *P. lagenidioides*. Healthy plants from Anjuna beach, when cultured with the infected material from Baga beach, showed up to 80–90% infection within 10–12 days (Raghukumar 1987b). The procedure for collection, maintenance and inoculation of *C. media* with the fungus and evaluation of disease severity were the same as described earlier (Raghukumar 1987b). In a preliminary experiment it was seen that the growth of the green alga was good both in sterilized seawater from the collection site and enriched seawater medium (McLachlan 1973). Therefore, for all further experiments the algae were maintained in sterile seawater, which was replaced every third day.

Extraction and estimation of chlorophyll

Two replicates were maintained for each experiment. Each replicate contained two plants of similar size. The healthy and infected plants were blotted dry, weighed and immediately extracted in 90% acetone. The plants were stored overnight at 4 °C in the dark in the acetone for extraction of chlorophylls and other pigments. After about 12 h the plants were crushed in the acetone with acid washed sand in a pestle and mortar. The slurry was centrifuged at 8000 g and the volume made up to 20 ml with 90% acetone. Spectrophotometric estimations of chlorophyll *a* and *b* were made following the method of Parsons and Strickland (1963). The results are expressed in mg of chlorophyll per gram wet weight.

The phaeo-pigments in the same samples (acetone extracts) were estimated spectrophotometrically by measuring the extinction at 665 nm and 750 nm, before and after treatment with dilute HCl. The change following acidification is used as a measure of the quantity of phaeo-pigments in the original samples (Parsons *et al.* 1984).

Total phenols were extracted from healthy and infected plants from 0 day to 15 days after incubation. The plants were blotted dry, weighed and crushed in ethyl alcohol with acid washed sand in a pestle and mortar. The slurry was centrifuged at 8000 g and volume made up to 20 ml with absolute ethyl alcohol. Total phenols in the samples were measured using Folin Denis reagent (Swain and Hillis 1959). The results are expressed in μg total phenols per gram wet weight. Catechol (Sigma, USA) dissolved in ethanol was used as reference.

Carbohydrate content was measured by the phenol sulphuric acid method (Kochert 1978). Carbohydrate analysis was made on healthy and inoculated plants from 0 to 15 days. The samples from replicates were washed thoroughly with sterile seawater and dried at 40 °C for a week and reduced to a powder in a pestle and mortar. 10 mg of this dried powder was extracted in 5% trichloroacetic acid by heating in a waterbath for 3 h at 80 °C–90 °C. The slurry was centrifuged and an aliquot of clear supernatant was used for the estimation of reducing sugars (Dawes 1981).

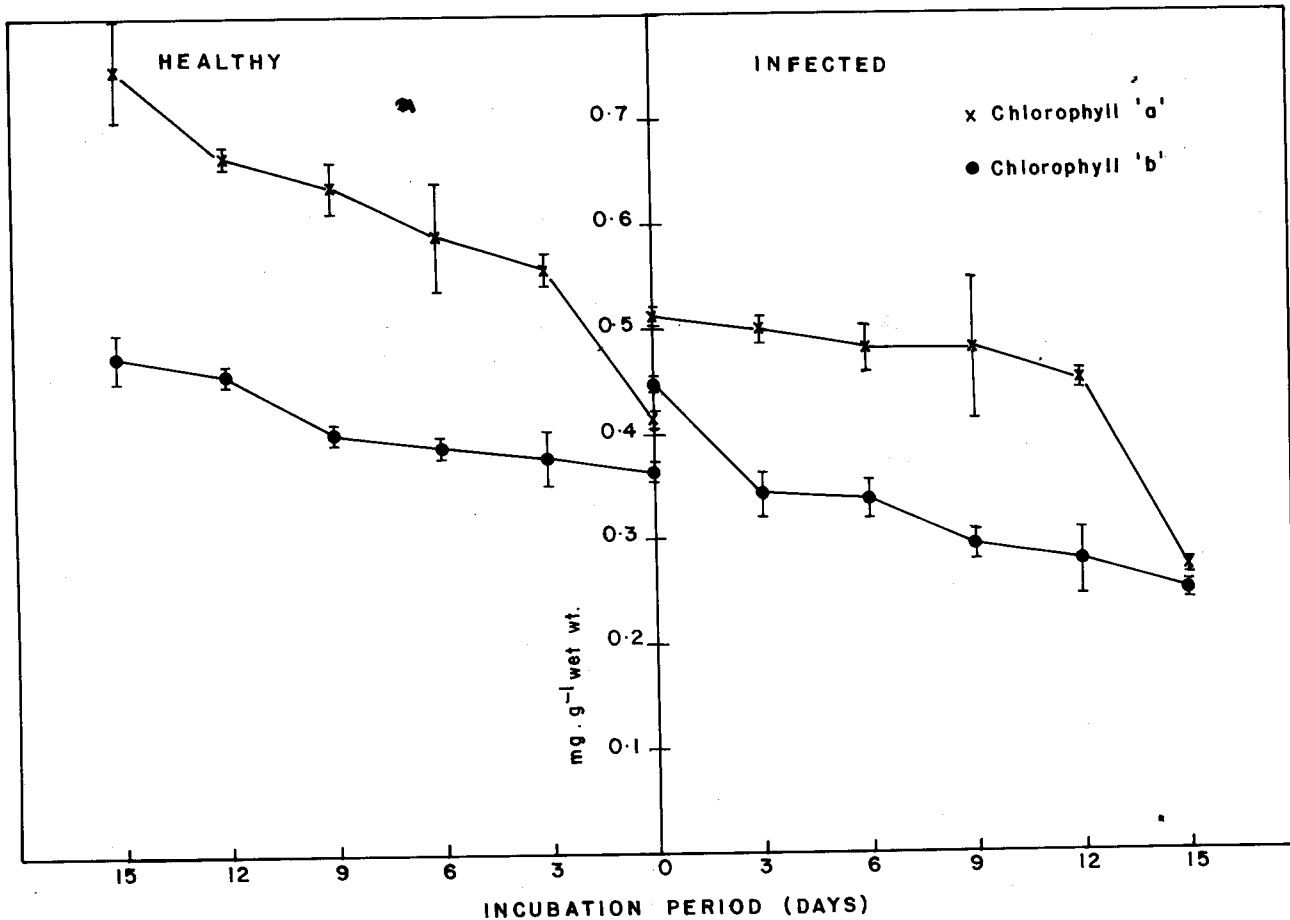


Fig. 1. Chlorophyll 'a' and 'b' content of healthy and inoculated algae from 0–15 days of incubation in sterile seawater. Bar represents standard deviation.

For estimation of proteins, a known amount of dried algal powder (prepared as mentioned above) was digested overnight in 1 N NaOH at room temperature, the slurry was centrifuged and an aliquot of the clear supernatant used for estimation of protein using Folin phenol reagent (Lowry *et al.* 1951).

Results

The most distinct feature of this disease was a browning of cells from the tip downwards. On microscopic examination it was evident that by the third day after inoculation the green chloroplasts in infected cells were replaced by granular bodies of brown pigment. This was confirmed by estimation of chlorophylls. The chlorophyll *a* and *b* content decreased on infection (Fig. 1). There was a negative correlation ($r = -0.99$) between chlorophyll *a* content and severity of disease (Fig. 2). There was no remarkable change in spectral characteristics of chlorophyll in diseased plants.

The phaeo-pigments content in the infected plants increased sharply (Fig. 3) as compared to the healthy plants. The increase in phaeo-pigment was correlated with a decrease in chlorophyll *a* content (Fig. 3). When phaeopigments were calculated as percentage of chlorophyll *a* plus phaeo-pigments, it was found to be three times more in infected than in healthy plants. The presence of phaeo-pigments was directly correlated with the percentage of infection and browning of the alga (Fig. 4). The correlation was very significant ($r = 0.9$; $p < 0.05\%$).

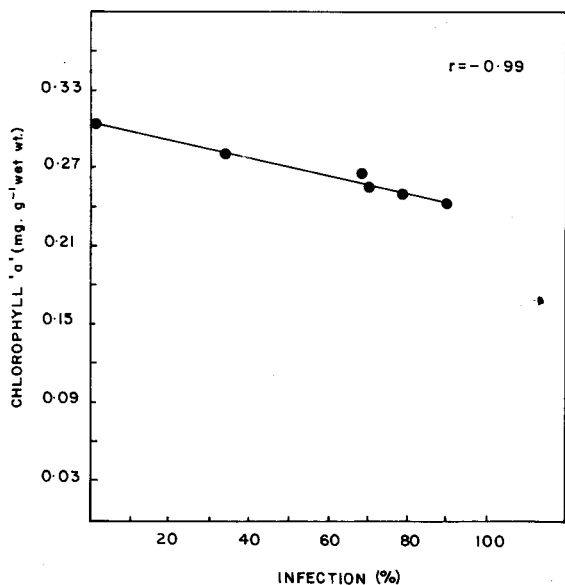


Fig. 2. Negative correlation between percentage of infection and chlorophyll content.

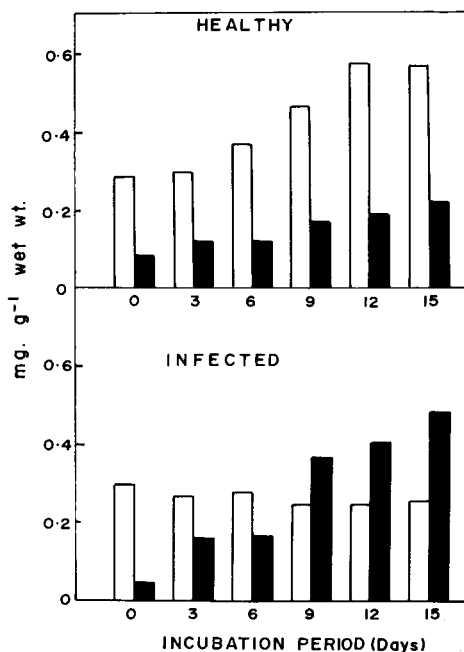


Fig. 3. Chlorophyll *a* and phaeo-pigments in healthy and inoculated algae. □ — Chlorophyll *a*, ■ — Phaeo-pigments.

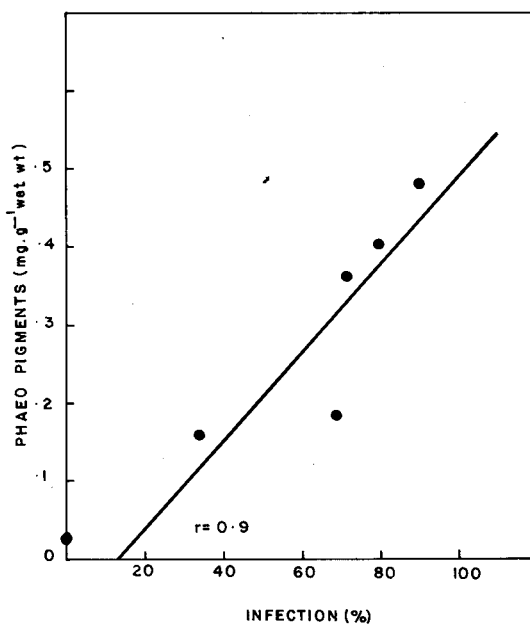


Fig. 4. Positive correlation between percentage of infection and phaeo-pigments.

There was a significant increase in total phenols from 0 to 15 days on infection (Fig. 5).

There was not much change in carbohydrate content on infection. On the whole, carbohydrate content decreased in the plants incubated in sterile seawater (Fig. 6).

The protein content from healthy as well as infected plants decreased on incubation in sterile seawater. However, the decrease in protein content in infected plants was much more notable than in healthy plants (Fig. 6).

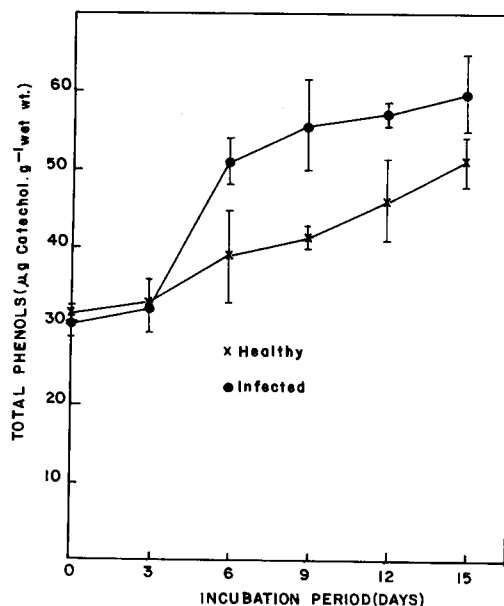


Fig. 5. Total phenols in healthy and inoculated algae. Bar represents standard deviation.

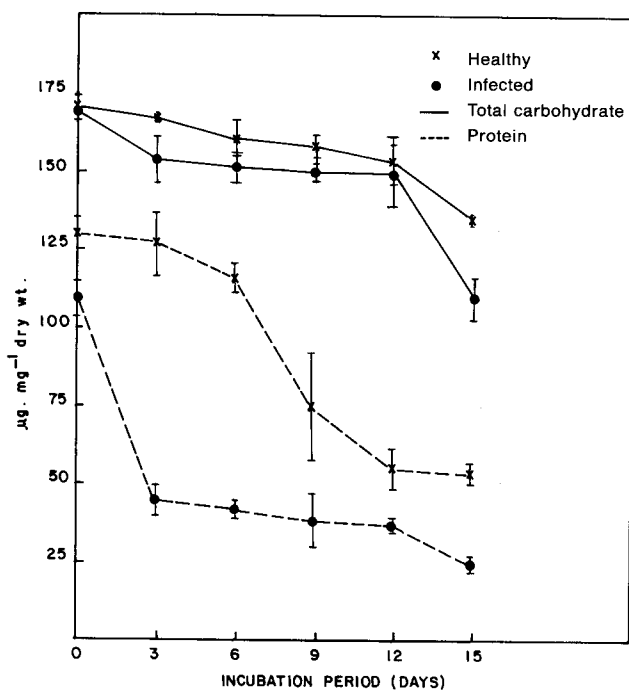


Fig. 6. Carbohydrate and protein content of healthy and inoculated algae from 0 to 15 days. Bar represents standard deviation.

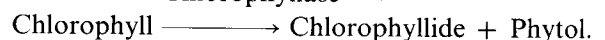
Discussion

Pontisma lagenidioides is an obligatorily biotrophic parasite. The fungus enters the living host cell and obtains energy from it (Lewis 1973). The fungus has a narrow host range and could not be cultured on any artificial medium (Raghukumar 1987b). The mere presence of the developing fungal thallus living

on the content of the host cell, deprives the latter of its energy yielding metabolism, and as a result the host cell dies. In *Spirogyra* sp. a freshwater alga attacked by the chytrid *Micromyces* sp. contraction, discolouration and disintegration of chloroplast take place gradually and eventually the entire contents are reduced to a granular, often brownish mass within which the reproductive structures of chytrid are embedded (see Sparrow 1960). Similarly, the diatom *Licmophora* sp. infected by *Ectrogella* sp. also exhibits gradual disintegration of the chromatophores and accumulation of brown granular material (Raghukumar 1978).

The most striking feature of the infection was the reduction in total chlorophyll content and a marked increase in phaeo-pigments. This coincided with severity of infection and browning of the cells in an infected plant. A reduced chlorophyll content could either be due to the breakdown of chlorophyll or/and to the inhibition of chlorophyll synthesis. In virus-infected higher plants, higher levels of enzyme chlorophyllase have been detected, suggesting that chlorophyll is being degraded by the enzymic reaction (Dickinson and Lucas 1982):

Chlorophyllase



It is possible that similar chlorophyllase may be active in the infected *Chaetomorpha media* but this needs confirmation.

It has been shown that chlorophyll degradation products may constitute a significant fraction of the total green pigments (Lorenzen 1967, Moss 1967). It was noted here that the phaeo-pigments in the infected plants constituted a major portion of the pigments, whereas chlorophyll content was reduced drastically on infection. The presence of about 27% of phaeo-pigments in healthy plants may be due to the presence of dead and senescent cells as has been also observed by Moss (1967).

A reduced chlorophyll content is also reported in *Cladophora glomerata* (L.) Kütz. infected by *Acremonium kiliense* Grütz (Bott and Rogenmuser 1980). *Laminaria* plants infected by an ascomycetous fungus *Phycomelaina* sp. showed limited photosynthetic capacity under saturating light conditions when compared with healthy plants (Schatz and Kursar 1980).

The role of phenols in algae in general has received little attention. Polyphenols in the brown algae *Fucus vesiculosus* L. and *Ascophyllum nodosum* (L.) Le Jolis. play an important role in chemical defense against

the marine herbivorous snail, *Littorina littorea* L. (Geiselman and McConnell 1981). The role of phenols in higher plants is in darkening of host tissue on infection due to accumulation of oxidised phenols. They also play an important role in resistance of plants to infection (Wood 1967). Increase in production of phenols is a general response of plants to infection, irritation or wounds. Increase in total phenols in *Chaetomorpha media* might cause browning of algal filaments or might indicate enhanced metabolism of aromatic compounds on infection. The type of phenolic compounds present in healthy and diseased algae have to be studied in detail to know their role in disease.

A general breakdown and utilization of host storage product is evident from the decrease in total soluble carbohydrates and protein content. They might be utilized by the fungus or might leach out due to altered permeability of the host plasma membrane. Biotrophic pathogens are known to increase the permeability of host cells.

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