Production of chitinase by some aquatic fungi*

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Wadhwani, K., Chatterjee, S. & Srivastava, S.K. 1992. Production of chitinase by some aquatic fungi. *Geophytology* **21** : 103-104 .

The paper deals with the chitinase activity in four fungi belonging to different groups: *Helicomyces* and *Papulaspora* (Aeroaquatic hyphomycetes), *Cunninghamella echinulata* (Zygomycotina) and Trichoderma viride (Deuteromycotina) isolated consistently from aquatic habitat for three consecutive years. The production of chitinase ranged from 13.2% to 18.3% after 40 days, maximum being in *T. viride* (18.30%) followed by *C. echinulata* (15.70%), *Papulaspora* sp. (14.0%) and *Helicomyces* sp. (13.2%). Chitinase production decreased after 30 days in aeroaquatic fungi, while in *C. echinulata* and *T. viride* it showed further increase upto 40 days. The implications have been discussed.

Key-words - Chitinase activity, aquatic fungi.

INTRODUCTION

Fungal populations in aquatic habitat exhibit high biochemical diversity ranging from generalized degraders to specialized ones. The stability in succession in any ecosystem depends upon the production of both exo-and endo-enzymes. In the aquatic habitats, the fungal populations not only encounter plants but a variety of invertebrates, which may provide an additional substrate for fungal communities.

Parasites and saprophytes need certain enzymes to utilize the substrate from which they draw nutrition. Cellulose, pectin and chitin are the most common structural polysaccharides in large parts of plant and animal kingdoms. Degradation of these substances is necessary by micro-organism to obtain nutrition. There are reports on the ability of many bacteria and actinomycetes (Veldkamp, 1955; Ames et al., 1989) to degrade chitin. The mycorrhizal fungi produce higher fungal biomass in the presence of chitin (Leake & Read, 1990). The chitin in gut and on gills of crustaceous and aquatic insects is soft and permeable and may be forming a suitable substratum for fungi occurring in aquatic habitat. Production of chitinase was studied in four fungi, *Helicomyces, Papulaspora, Cunninghamella echinulata* and *Trichoderma viride,* which were encountered abundantly in aquatic habitat for three consecutive years (1987-1989).

MATERIAL AND METHOD

Species of *Helicomyces* and *Papulaspora* isolated by moist chamber technique (Webster, 1981) are aeroaquatic hyphomycetes occurring in mycelial form from decaying blackened leaves and twigs at the bottom of stagnant water bodies. *Cunninghamella echinulata* is a mucorale, with high cellulolytic capability, and *Trichoderma viride* which is regarded as a typical terrestrial fungus and highly cellulolytic has been isolated consistently from aquatic habitats.

The chitinase activity of four fungi was estimated following the method of Norkhans (1963). The culture filtrates (of blended mycelium from peptone medium incubated at 28 ± 2°C) were tested turbidimetrically for chitinase production after 10, 20, 30 and 40 days. The assay medium consisted of 3 ml. of 0.045 M phosphate buffer solution (pH 6.5) containing 0.53 mg/ml of chitin and 0.06 ml 1% merthiolate. Two glass pearls were added to each tube. At zero time each tube was supplied with 3 ml culture filtrate (pH adjusted). The tubes were sealed with cellotape and kept shaking continuously at 30°C during during all tests. The percent chitinase activity was measured as decrease in extinction of chitin solution at 610 nm in a colorimeter.

OBSERVATIONS

The percent chitinase activity of four fungi viz. *Helicomyces* sp., *Papulaspora* sp., *Cunninghamella echinulata* and *Trichoderma viride* upto 40 days at 28 ± 2°C is presented in Table 1. It is evident that all the four fungi could produce measureable amount of the enzyme chitinase as early as 10 days of incubation. The table reveals that there was a continuous increase in the production of the enzyme upto 30 days by all the fungi, being maximum by *Trichoderma viride* (17.7%) followed by *Helicomyces* sp. (16.9%), *Cunninghamella echinulata* (15.4%) and

*Paper presented at the Birbal Sahni Birth Centenary Palaeobotanical Conference, Lucknow, November 20-22, 1991.

Papulaspora sp. (14.1%). After 40 days there were two trends, while *Trichoderma viride* and *Cunninghamella echinulata* exhibited an increase of 1.4% and 0.3% in enzymic activity, respectively, *Helicomyces* sp. and *Papulaspora* sp. showed a decrease of 3.6% and 0.1% in the production of enzyme, respectively.

DISCUSSION

The investigations clearly indicate that all the four fungi belonging to Aeroaquatic hyphomycetes *(Helicomyces* and *Papulaspora),* Zygomycetes *(Cunninghamella echinulata)* and Deuteromycete *(Trichoderma viride)* respectively could produce chitinase effectively within 10 days of incubation irrespective of their group in which these have been placed. These studies confirm the observations of Fisher (1979) that blackened leaves which remained submerged carried many species, so there was considerable competition, hence possessed high competitive saprophytic ability and are capable of utilizing the most complex substance like chitin in fresh aquatic habitat. Unestam (1966) while studying the chitinolytic, cellulolytic and pectinolytic activity in-vitro of some parasitic and saprophytic Oomycetes reported that in *Aphanomyces laevis* the chitinolytic activity was markedly stimulated in the presence of chitin in the medium. The fungus often occurred as a saprophyte on animal chitin. Not all the terrestrial fungi could degrade chitin substrate. Baath and Soderstrom (1980) examined the ability of 60 non-mycorrhizal fungi to decompose chitin and reported that only the species of *Mortierella, Verticillium* and *Mucor* had the capability to do so. The chitinolytic capability of fungi belonging to different groups in aquatic ecosystem could be a potent factor contributing to the success of their occurrence, as these could colonize chitin-rich substrates. Saprophytic colonization and decomposition of dead mites in the marine habitat by *Periconia prolifica* has been reported by Kohlmeyer and Kohlmeyer (1979). Additional studies are needed to quantify the contribution of all members of the aquatic community in the decomposition of detritus and detritivores.

Table 1. Per cent chitinase (decrease in extinction of chitin solution) activity of four fungi incubated up to 40 days.

	Helicomyces sp.	*Papulaspora* sp.	*Trichoderma viride*	*Cunninghamella echinulata*
10 days	14.3	12.9	15.2	14.5
20 days	15.1	13.3	16.9	14.8
30 days	16.9	14.1	17.7	15.4
40 days	13.2	14.0	18.3	15.7

REFERENCES

Ames, R.N., Mihara, K.L. & Bayne, H.G. 1989. Chitin decomposing actinomycetes associated with a vesicular-arbuscular mycorrhizal fungus from a calcareous soil. *New Phytologist* **111**: 67-71.

Baath, E. & Soderstrom, B. 1980. Degradation of macro-molecules by microfungi isolated from different podzolic soil horizons. *Can. J. Bot.* **58**: 422-425.

Fisher, P.J. 1979. Colonization of freshly abscissed and decaying leaves by aero-aquatic hyphomycetes. *Trans. Br. Mycol. Soc.* **73** : 99-102.

Kohlmeyer, J. & Kohlmeyer, E. 1979. *Marine Mycology: The higher fungi.* Academic Press, New York and London.

Leake, K.R. & Read, D.J. 1990. Chitin as a nitrogen source for mycorrhizal fungi. *Myco. Res.* **94** : 993-1008.

Norkhans, B. 1963. Influence of some cultural conditions on fungal cellulose production. *Physiol. Plant* **16:** 11-19.

Unestam, T. 1966. Citinolytic, cellulolytic and pectinolytic activity in vitro of some parasitic and saprophytic Oomycetes. *Physiol. Plant* **19:** 15-30.

Veldkamp, H. 1955. A study of the aerobic decomposition of chitin by microorganisms. Mededelingen van de Landbouwh-ageschool te wageningen (Netherland) **55:** 127-174.

Webster, J. 1981. Biology and Ecology of aquatic hyphomycetes. *In.* Wiklow, D.T. & Carroll, G.C. (eds)- *The Fungal Community* : 681-692. Marcel Dekker, New York.