

Growth responses of aquatic hyphomycetes to different nitrogen sources

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Various nitrogenous compounds were examined for nitrogen nutrition of four selected aquatic hyphomycetes under controlled laboratory conditions. Potassium nitrate, ammonium sulphate and amino acids viz. alanine, arginine, asparagine, cysteine, proline, phenylalanine, isoleucine, and methionine were tested as nitrogen sources. Nitrates and ammonium ions were found as good nitrogen sources, with preference for the latter. The suitability of amino acids was found to be variable between species. *Tetrachaetum elegans* and *Tetracladium marchalianum* had their maximum growth in asparagine whereas, *Pestalotiopsis submersus* and *Flagellospora penicillioides* grew best in proline. Cysteine was observed as a good source of nitrogen for almost all the fungal isolates used.

Key words: Nitrogen requirements, physiology, nutrition, aquatic fungi.

Because fungi are unable to fix atmospheric nitrogen for their growth, they utilize nitrates and ammonium salts from other sources. Some fungi require organic nitrogen, usually in form of specific amino acids. Aquatic hyphomycetes, which complete their life cycle on submerged decaying leaf litter in running fresh water bodies, are major components of aquatic ecosystem (Ingold 1942, 1975). Ranzoni (1951) investigated nitrogen and vitamin requirements of two aquatic hyphomycetes belonging to the genus *Anguillospora* and reported that they are able to utilize nitrate as a source of nitrogen. Thornton (1963) also reported nitrates and ammonium ions as adequate nitrogen sources for the growth of several aquatic hyphomycetes. Thornton (1965) analysed leaf extracts for amino acids and used these amino acids for comparative nutritional studies in aquatic hyphomycetes. He concluded that a wide range of amino acids and probably of plant proteins in general could supply available nitrogen to many aquatic hyphomycetes.

A great deal of studies on the occurrence and distribution of these fungi has been made in various parts of world (Webster & Descals 1981, Sridhar *et al.* 1992, Marvanová 1997) including Kumaun Himalaya, India (Sati & Tiwari 1997, Sati *et al.* 2002). Also some field studies on physiological factors affecting the growth of aquatic

hyphomycetes were carried out (Suberkropp 1984, Field and Webster 1985, Thompson and Bärlocher 1989). Recently, comprehensive ecological studies on diversity/function relationships in microcosms have been carried out (Bärlocher & Corkum 2003, Dang *et al.* 2005). These studies focused not only on the diversity of aquatic fungi, but also on their function as decomposer organisms.

But there is still no information available on nitrogen nutrition of aquatic hyphomycetes, except from Ranzoni (1951) and Thornton (1963, 1965). In the present work, the growth responses of some aquatic hyphomycetes to eight amino acids and two inorganic nitrogen compounds were studied. The fungal strains were recovered from a stream in the temperate zone of Kumaun Himalaya.

Materials and Methods

Four fungal isolates viz., *Tetrachaetum elegans* Ingold, *Tetracadium marchalianum* De Wildeman, *Flagellospora penicillioides* Ingold, and *Pestalotiopsis submersus* Sati and Tiwari were selected to investigate their nitrogen requirements. These strains were previously deposited in the culture collection of the Kumaun University Mycological Specimens (KUMS), Department of Botany, Kumaun University, Nainital, India. Pure cultures of these isolates were prepared from single spore inoculum following Descals (1997) and maintained at 15 ± 2 °C in Petri dishes containing Malt Extract Agar. The utilization of nitrogen was examined in a basal medium as recommended by Ranzoni (1951), but without yeast extract: 4.0 g glucose, 1.0 g KH_2PO_4 , 0.2 g $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$, 0.02 g FeCl_3 , 1 L distilled water (pH 6.5). The yeast extract was replaced by the following nitrogen containing compounds: biotin and pantothenic acid (each 5 µg/L) were added as growth stimulators; eight amino acids (BDH or CDH Laboratory reagents Bombay, India) were taken separately in such a quantity that the final concentration of nitrogen was 1.0 g per litre of medium. Three replicates were used for each treatment and each fungal isolate. Agar disks (5 mm diameter) with mycelia mat were cut from 15 days old culture plates and transferred into sterilized conical flasks (100 mL cap.) containing 50 mL test medium. The flasks were kept for incubation at $20 \text{ °C} \pm 2 \text{ °C}$ in dark and casually shaken for aeration. After 11 – 12 days of incubation, the pH value of the basal medium decreased from 6.5 to a range between 3.0 and 4.0; it was adjusted to pH 6.5 by adding sterile 4% NaOH. After 15 days, biomass was obtained by filtering off the medium through previously weighed filter papers (Whatman No. 1), which were thoroughly rinsed with distilled water to remove residues of medium from the mycelia. Fungal biomass was then dried for 24 h at 80 °C in an oven and cooled

in a desiccator prior to reweighing. Dry weight of biomass was determined once only after 15 days of incubation because we observed in previous studies that the growth curve of these fungi became plateau within 15 days.

Results and Discussion

Eight amino acids viz., alanine, arginine, asparagine, cysteine, proline, phenylalanine, isoleucine, methionine and two inorganic nitrogen sources, potassium nitrate and ammonium sulphate, were screened for their potential to serve as nitrogen sources for four selected strains of aquatic hyphomycetes. Biomass dry weights after 15 days of incubation of test strains are presented in Tables 1 and 2.

In preliminary experiments in which the yeast extract in the original medium (Ranzoni 1951) was replaced by 1.34 g KNO₃/L, no growth occurred. However, when the basal medium was supplemented with the vitamins biotin and pantothenic acid (each 5 µg/L) as suggested by Thornton (1963), good growth was observed for all isolates. No growth was observed in nitrogen-free controls (without vitamins and amino acids).

Tab. 1. Average biomass [dry weight (DW) ± standard error] of aquatic hyphomycetes after 15 days of incubation with nitrate or ammonium ions as nitrogen sources.

Fungal species	Ammoniumsulphate (DW in mg)	Potassium nitrate (DW in mg)
<i>Tetrachaetum elegans</i> Ingold	27.0 ± 0.4	18.7 ± 0.2
<i>Tetracladium marchalianum</i> De Wildeman	88.3 ± 0.2	75.0 ± 0.4
<i>Pestalotiopsis submersus</i> Sati and Tiwari	254.3 ± 0.5	205.5 ± 0.7
<i>Flagellospora penicillioides</i> Ingold	216.3 ± 0.6	203.0 ± 0.3

Tab. 2. Average biomass [dry weight (DW) ± standard error] of aquatic hyphomycetes after 15 days of incubation with different amino acids as nitrogen sources.

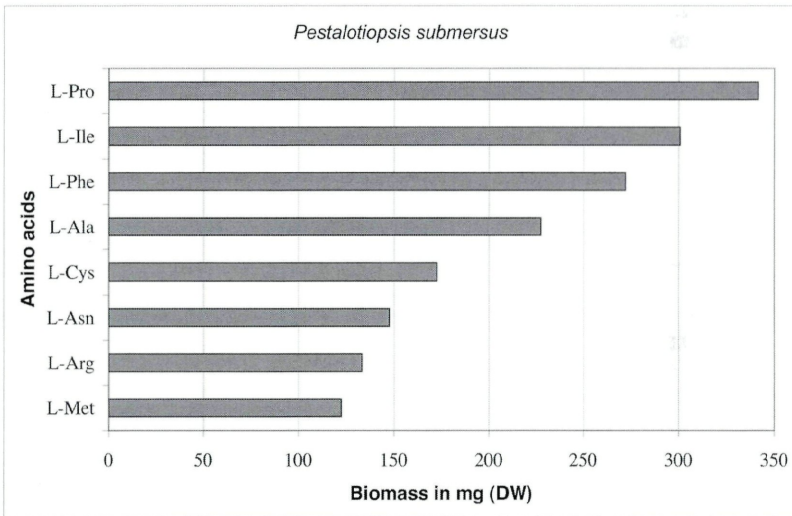
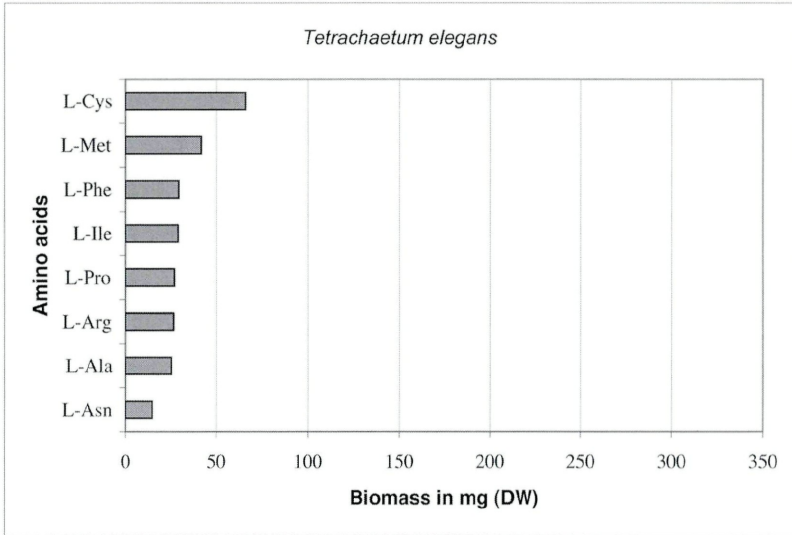
Amino acids	Dry weight of biomass in mg			
	<i>T. elegans</i>	<i>T. marchalianum</i>	<i>P. submersus</i>	<i>F. penicillioides</i>
L-Asparagine	14.67 ± 2.91	271.00 ± 9.02	147.67 ± 18.85	120.33 ± 14.19
L-Alanine	25.33 ± 4.81	316.00 ± 23.07	227.67 ± 39.18	191.33 ± 12.02
L-Arginine	26.67 ± 4.48	245.00 ± 8.74	133.33 ± 8.17	87.67 ± 10.04
L-Cysteine	66.00 ± 6.11	195.00 ± 24.66	172.67 ± 18.48	142.67 ± 15.17
L-Isoleucine	29.00 ± 2.65	64.33 ± 8.25	300.67 ± 6.74	271.67 ± 16.48
L-Methionine	41.67 ± 11.20	147.67 ± 9.17	122.33 ± 22.84	74.67 ± 11.10
L-Phenylalanine	29.33 ± 7.42	83.33 ± 11.02	272.00 ± 29.00	250.00 ± 2.00
L-Proline	27.00 ± 4.51	88.33 ± 11.05	341.67 ± 18.19	292.00 ± 7.02

It is shown in Table 1 that all test strains assimilate both nitrate and ammonium ions but to a varying extent: biomass data indicate higher preference for ammonium ions. Similarly, all examined strains utilized the provided eight amino acids for their growth (Tab. 2). Figures 1–4 show to which extent the different amino acids supported the growth of the four test strains.

Relative growth rates of *Tetrachaetum elegans* were generally very low: maximum growth (average dry weight biomass ≤ 66 mg) was observed when the medium was supplemented with the sulphuric amino acids cysteine or methionine (Fig. 1). Proline followed by Isoleucine and Phenylalanine was found to support maximum growth of *Pestalotiopsis submersus* and *Flagellospora penicillioides* (dry weight biomass between 250 mg and 341 mg), both with an identical preference pattern (Figs. 2–3). But the same three amino acids represented a poor nitrogen source for *T. elegans* and *Tetracladium marchalianum*. For the latter species best mycelial growth (dry weight biomass between 245 mg and 316 mg) was observed with alanine, asparagine, and arginine (Fig. 4). Summarizing these results, it is obvious that a supplementation with suitable amino acids led to distinctly higher biomass yields than those observed with ammonium sulphate and potassium nitrate (Tab. 1 and 2).

Sowden & Ivarson (1959) described the presence of amino acids, among them some of those which are the focus of this study, in older leaf litter and green leaves. Perhaps, this is why the submerged leaf litter in the water of forested streams harbour a good number of aquatic hyphomycetes.

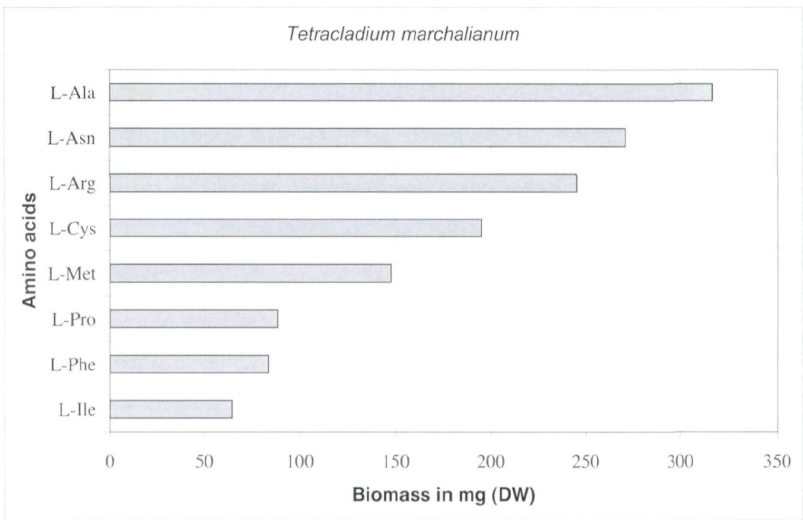
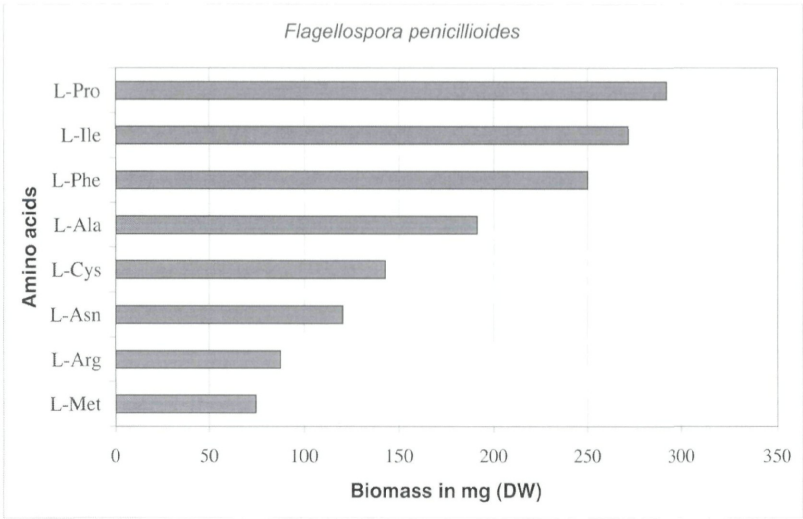
Thresh *et al.* (1949) reported that most streams, which flow on coal measures, millstone grit and sandstone, contained low concentrations of ammonium and nitrate ions, which might be the sources of nitrogen for aquatic hyphomycetes in streams. On perusal of available literature on nitrogen requirements of aquatic 'fungi', it can be concluded that aquatic hyphomycetes utilize inorganic nitrogen leached from the soil into streams more aptly than water moulds (e.g. oomycetes) (Dayal 1961, Thornton 1963, Powell *et al.* 1972, Mer 1982). In a study on water moulds, Dayal (1961) and Powell *et al.* (1972) reported that water moulds are unable to utilize nitrate but utilize ammonium salts or organic acids containing nitrogen. Studying the physiology of water moulds, Mer (1982) recorded that they did not grow with $(\text{NH}_4)_2\text{SO}_4$ or KNO_3 , whereas good growth was observed when organic nitrogen sources were provided. In the present study, all tested aquatic hyphomycetes could assimilate ammonium ions and nitrate ions as a source of nitrogen with a higher preference for ammonium ions. This has also been observed by Thornton (1963).



Figs. 1 – 2. Growth of *Tetrachaetum elegans* (1) and *Pestalotiopsis submersus* (2) with eight different amino acids as single nitrogen sources after 15 days of incubation at 20 °C in a modified Ranzoni's medium.

Ranzoni (1951) considered organic nitrogen and vitamins as essential for the growth of aquatic hyphomycetes and, therefore, supplied his growth medium with yeast extract. But he also recorded that these fungi are able to utilize nitrates.

In the present study the growth responses of *P. submersus*, *Tetrachaetum elegans* and *Tetracladium marchalianum* to two in-



Figs. 3 – 4. Growth of *Flagellospora penicillioides* (3) and *Tetracladium marchalianum* (4) with eight different amino acids as single nitrogen sources after 15 days of incubation at 20 °C in a modified Ranzoni's medium.

organic and eight organic nitrogen sources were tested for the first time. Similar studies were made by Thornton (1965) who observed a maximum biomass yield of *Articulospora tetracladia* and *Flagellospora penicillioides* with aspartic acid and tyrosine, respectively. All hyphomycetes examined by him were found capable of utilizing arginine, asparagine, and other amino acids. Thus, the results of the

present study support the findings of Thornton (1965). The amino acids mentioned before also support maximum growth of water moulds (Mer 1982).

Proline, phenylalanine, methionine, isoleucine, and cysteine were used here for the first time to test the nitrogen nutrition of aquatic hyphomycetes. As already mentioned above, *P. submersus* and *F. penicillioides* grew best with proline, isoleucine, and phenylalanine (descending order) but weakest with methionine (Figs. 2 and 3). According to Mer (1982), these amino acids and alanine were found to be poor sources for the growth of water moulds.

Summarizing the results it can be concluded that nitrogen utilization by aquatic hyphomycetes, which are true fungi belonging to anamorphic Ascomycota or Basidiomycota, distinctly varies from species to species. In contrast to the fungal-like water moulds (Oomycetes), aquatic hyphomycetes are capable of utilizing a wide range of inorganic and organic nitrogen sources, which are leached from leaf litter or the soil into streams. Old leaf litter and green leaves serve as source for both carbon and nitrogen in form of amino acids. The preferential utilization of amino acids by aquatic hyphomycetes (Thornton 1965) may explain their frequent prevalence on decomposing leaf litter.

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