MYCORRHIZA NETWORK

MYCORRHIZA NEWS

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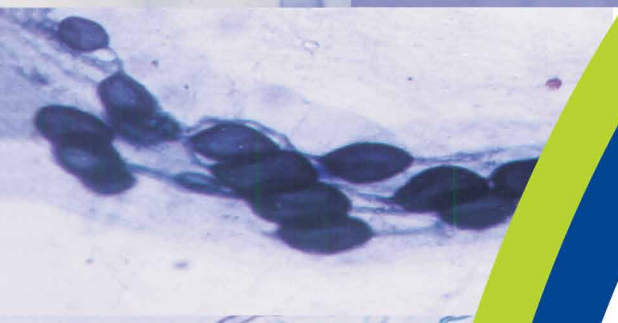

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TERI's Mycorrhiza Network

TERI's Mycorrhiza Network is primarily responsible for establishing the MIC (Mycorrhiza Information Centre), the CMCC (Centre for Mycorrhiza Culture Collection), and publishing Mycorrhiza News. The Network helps scientists carry out research in mycorrhiza and promotes communication among mycorrhiza scientists.

Mycorrhiza News

The Mycorrhiza News provides a forum for dissemination of scientific information on mycorrhiza research and activities; publishes state-of-the-art papers from eminent scientists; notes on important breakthroughs; brief accounts of new approaches and techniques; publishes papers complied from its RIZA database; provides information on forthcoming events on mycorrhiza and related subjects; lists important research references published during the quarter; and highlights the activities of the CMCC.

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Research finding papers

Interaction of Chitin-Degrading Soil Actinomycetes with Mycorrhizal Colonization of Chilli Roots

*Dhruba Das, Pinki Pal, and Sujata Chaudhuri**

Introduction

Among the microbial groups, actinomycete bacteria and arbuscular mycorrhizal (AM) fungi are known to promote activities, which can improve agricultural developments (Barea *et al*. 2005). Arbuscular mycorrhizae are necessary for the management of soil fertility and crop production. Their survival in soil and effectiveness in bringing about a positive plant growth response is strongly influenced by microorganisms in the mycorhizosphere. Actinomycetes are one of the components of the microbial populations existing in soil. They belong to an extensive and diverse group of gram-positive, aerobic, mycelial bacteria that play important ecological role in soil nutrient cycling and participate in the decomposition of various complex compounds (chitin, pectins, cellulose) by using them as carbon and energy sources. Chitynolytic actinomycetes play the role of biological agents of plant protection by hydrolyzing the fungal cell wall. They also influence the mycorrhizal population present in the soil. The aim of the present work is to analyse the effect of such chitinolyic activity on mycorrhizal colonization in the roots of higher plants. Six chitin decomposing *Streptomyces* strains were isolated from jute field soil and their interaction with *Glomus mosseae* with reference to colonization in chilli roots and the spore population in the soil was studied.

Material and Methods

Soil samples were collected from jute fields at 10–15 cm depths. The soil was pretreated and actinomycetes were isolated by pour plate method on Starch Casein Nitrate Agar (SCNA) media. The isolates were purified in Glycerol Arginine Agar (GAA) slants. They were identified by microscopic characteristics and biochemical tests. All the isolates were grown in petri dishes containing SCNA media with colloidal chitin to study the chitinolytic enzyme production (Chen and Lee 1994). After 14 days of incubation at 28°C, width of the zone of chitin hydrolysis was measured. Of the 13 strains isolated from soil, six exhibited chitinase activity and were identified to belong to the genus *Streptomyces*. The strains were designated as KuAc 1–6. Another *Streptomyces* strain, which did not show chitinase activity, was designated as KuAc7 and was selected for this study. All the seven strains were grown separately in glycerol arginine broth medium for 15 days at 28°C. The biomass was harvested at the end of the incubation period. The harvested biomass of each *Streptomyces* strain was mixed with sterilized soil as 0.1g biomass/200g of soil. The soil was kept moist and covered with a cellophane sheet for 10 days. *Glomus mosseae* was mixed with this soil seven days before sowing chilli seedlings (25g of infected roots of *Zea mays* L + external mycelium and spores/ kg of soil).

One-month old seedlings of *Capsicum annum* L. were sown into plastic pots (4" height) containing sterilized soil + the actinomycete + *G. mosseae*. A

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control without actinomycete was also maintained. All treatments were replicated thrice. Feeder roots and soil samples were collected randomly from each treatment, 30 days after sowing of the seedlings. AM fungal infestation, and spore population in the treated soil and control was recorded. Roots were stained with trypan blue (Phillips and Hayman 1970) to determine the degree of mycorrhizal infection in the roots (Trouvelot *et al.* 1986). Spore population of *G. mosseae* in different soil treatments was determined by conventional wet-sieving process.

Result and Discussion

Results are presented in Table 1. The strain KuAc6 showed the highest chitinase enzyme activity amongst the 13 strains of *Streptomyces* isolated. In general, it was found that chitinolytic streptomycetes have reduced the percentage of mycorrhizal colonization of *G. mosseae* in the roots and also the spore population in the soil. Maximum inhibition in root colonization (43.75 per cent) was observed when treated with the strain KuAc6, while the maximum reduction in spore density was recorded to be 75 spores/100g of dry soil. KuAc1 was also found to be significantly inhibitory to *G.mosseae* when compared to the other strains. Interaction with KuAc7 was found to be stimulatory to mycorrhizal colonization, though there was no significant change in the AM fungus spore density in the soil compared with that in the control treatment.

Arbuscular mycorrhizae and rhizosphere microorganisms are known to influence their mutual development and exert combined effects on plant growth. Mycorrhizae affect specific groups of bacteria and actinomycetes in rhizosphere soil as well as in rhizosplane and vice-versa. Mutual antagonism was observed between the AM fungus and the *Streptomyces* isolate (Krishna *et al.* 1982). Meyer and Linderman (1986) showed a decrease in the populations of both *Streptomyces* spp. and chitinase-producing actinomycetes in the rhizosphere, but no such decrease was observed on the rhizoplane of mycorrhizal plants.

Golińska and Dahm (2011) recorded that most *Streptomyces* strains in the soil produce chitinase, which catalyze the degradation of chitin, the main component of the pathogenic fungal cell wall. This activity was about four times higher in the presence of fungi, such as *Fusarium oxysporum* and *Rhizoctonia solani*. The cell walls of fungi, including AM fungi, contain chitin, thus these organisms provide one of the major substrate for chitin degrading soil actinomycetes (Williams and Robinson 1981, Williams *et al.* 1984).

In the present investigation, an antagonistic relationship was reported with all the strains of chitinolytic streptomycetes and a stimulatory effect was also observed with the non-chitinolytic *Streptomyces* strain. Anatogonism was more prominent with strains exhibiting higher chitinase activity, viz., KuAc6 and KuAc1. Such stimulation was also reported by Franco-Correaa *et al.* (2010). They observed that the AM mycelial growth was improved by the phosphate solubilizing actinomycete strains. These strains also stimulated AM fungal spore germination in soil, which resulted in enhanced plant growth and nitrogen acquisition.

Table 1: Effect of different isolates on AM infestation in chilli roots and soil spore

Population Strains	Colony Morphology	Chitin Clear Zone (mm)	Percentage of AM Fungi Infestation in Roots	Percentage Inhibition of AM Colonization	No. of Spores/ 100g of Dry Soil
KuAc1	White colony with flexuous spore chain, spore surface smooth	4.1	52	-28.0	210
KuAc2	Creamy white colony with coils and spirals, spore surface warty	2.5	71	-11.25	237
KuAc3	Silver colony with flexuous spore chain, spiny spore surface	2.3	76	-5.0	234
KuAc4	Light brown colony with whorls of spores, spore surface smooth	2.8	68	-15.0	225
KuAc5	Brown colony with short spore chains, spore surface warty	3.1	64	-20.0	220
KuAc6	Light chocolate colony with chocolate coloured spore, straight spore chains, surface smooth	5.02	35	-43.75	178
KuAc7	Creamy white colony with open loops, spore surface hairy	0	85	+6.25	248
Control			80	--	253

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Occurrence of Ectomycorrhizal Fungi in Different Forests of Odisha

*Sushri Shanta Tripathy, Ajay Kumar Mohapatra and Nibha Gupta**

Introduction

Ectomycorrhizal fungi, such as mushrooms, are the important constituent of forest ecosystem growing on the most abundant biomolecules of our biosphere, i.e., cellulose. Both edible and non-edible mushrooms are regarded as macro fungi with a distinctive epigeous or hypogeous fruiting body and are large enough to be seen through naked eyes. Along with their role in nutritional and medicinal aspects, these ectomycorrhizal fungi have been a source of food for tribal poor and act as mycorrhizal partner with the forest trees. Predictive equations of forest mushroom yields and diversity are quite complex with respect to the dynamics that influence the ectomycorrhizal fungal communities. Fungi are key functional components of forest ecosystem (Brown *et al.* 2006) and they have received less attention than animals and plants although they are omnipresent and highly diverse in nature (Pipenbring 2007). Although systematic studies on the agarics of South India (Kerela, Karnataka, and Tamil Nadu) were done extensively for more than three decades back in South India, almost all the work was confined to the Western Ghats over a period of several years (Natarajan *et al.* 2008) .

The Eastern Ghats are isolated hill ranges in Peninsular India (Andhra Pradesh, Odisha, Tamil Nadu, Karnataka), which harbours primarily the tropical moist deciduous vegetation representing species of high economic and medicinal potential. These Ghats lie in 11 30' to 21 0' N latitudes and 77.22' to 85 20' E longitudes. The Eastern Ghats of Odisha starts from North of Similipal in Mayurbhanj district to Malkangiri. Seventeen districts of Odisha comes under the Eastern Ghats including 14 protected areas (13 wild life sanctuaries, one biosphere reserve, one national park, two tiger reserve, and one ramser wetland). According to Champion and Seth (1968), the vegetation of the hill is basically tropical moist deciduous type with many riparian evergreen elements. The average annual rainfall is 1485mm. The maximum temperature goes upto 42 degrees and the minimum temperature falls upto 5 degrees. The relative humidity is normally high during the monsoon and post monsoon months, sometimes being more than 85 per cent, which favours the growth of mushrooms. Many of the hill streams originated from the top of the hills provide multiple habitat to enhance the macro fungal diversity.

Materials and Methods

The study was undertaken from August 2011to November 2013 keeping in view the period of seasonal monsoon. The sampling was conducted every month at regular intervals. The sites selected were the major forest divisions of Odisha including Koraput forest division, Similipal Tiger Biosphere Reserve, Karlapat Sanctuary, Baliguda forest division, and Banei forest division. The major forest divisions are tropical moist deciduous forests except Karlapat Sanctuary (dry deciduous forest) and Similipal Biosphere Reserve (semigreen forest) represented in Figure 1. Macroscopic and microscopic details of the fruiting bodies of the collected mushrooms were noted in the field and identification was done by following the identification chart and literature available. All the mushrooms were stored in dried as well as in formaldehyde solution in the Mushroom Herbarium of the Microbiology division in Regional Plant Resource Centre, Bhubaneswar. In the cases

Figure 1: Study area representing Kandhamal (Baliguda, Kotagarh Sanctuary), Kalahandi (Karlapat Sanctuary), Mayurbhanj (Similipal Biosphere Reserve), Koraput and Sungargarh (Banei) forests of Odisha

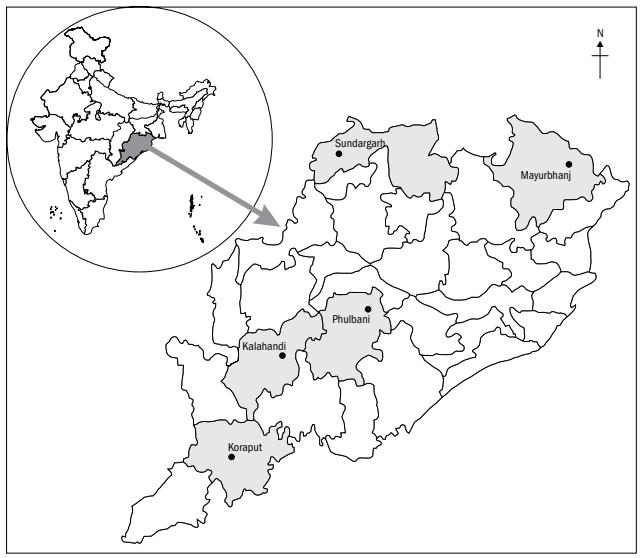

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Table 1: Distribution of ectomycorrhizal fungi enumerated in the six forest sites of Odisha

Species Name	Similipal Biosphere Reserve	Koraput Forest Division	Karlapat Sanctuary	Belgharand Kotagarh Sanctuary	Banei Forest Division	Chandaka Forest
Amanita caesarea	+	-	-	-	-	-
Amanita craseoderma	-	+	-	-	-	-
Amanita loosii	+	-	+	-	-	-
Amanita pantherina	+	-	+	-	-	-
Amanita vaginata	+	+	+	+	+	-
Boletus aestivalis	+	+	+	+	+	-
Boletus bicolor	+	-	+	+	+	-
Geastrumfimbriatum	-	+	-	+	-	-
Lactariusdeliciosus	+	-	-	-	-	-
Lactariusresimus	+	-	-	-	-	-
Lycoperdonperlatum	-	+	-	-	-	-
Pisolithus arrhizus	-	-	-	-	-	+
Russula aurata	-	-	-	+	-	-
Russula brevipes	+	-	+	+	-	-
Russula lepida	+	+	+	+	+	+
Russula nigricans	+	+	+	+	+	-
Russula vesca	+	+	+	+	+	+
Russula virescens						
Russula xeramphelina	+	-	-	-	-	-
Scleroderma areolatumEhrneb	+	+	-	-	-	-
Strobilomyces floccopus	+	+	+	+	-	-
Suillus luteus	-	-	+	+	-	-
Tuber rufum	-	-	-	-	-	+
Xylobolous subpileatus	+	-	-	-	-	-

where fungi were not identified upto the species level, numbers were given to the specimens. Macrofungi specimens were identified on the basis of their macroscopic characteristics, i.e., habitat, occurrence, association, pileus diameter, context, colour, texture, odour, stipe length, attachment, gill colour, sets of lamellae, ring position, veil characteristics, and volva shape (Atri *et al.* 2003). Microscopic characteristics included ermatocystidia, cuticle, epicutis, hyphae characteristics, gill trama, basidiospores characteristics with different stains, etc.

Results and Discussion

In this inventorization, a total of 24 species of macrofungi, belonging to 12 genera were recorded in the six forest sites (Table 1). About 16 species were enumerated from Similipal Biosphere Reserve under Baripada division,10 species from Koraput forest division, 11 species were recorded from Karlapat Sanctuary under Kalahandi division, 11 species were recorded in Belghar forest division and Kotagarh Sanctuary under Baliguda forest division, 6 species in Banei forest division under Sundargarh division, and 3 species in the campus of Regional Plant Resource Centre, i.e., very closely attached to the Chandaka forest (Table 1). Some species, such as *Russula rosea*, *Russula vesca*, *Russula nigricans,* and *Amanita vaginata,* were common to maximum sites (Figure 2). The species occurring on different substrates varied considerably in all six sites. Most of the macrofungal species were recorded from soil (58.33per cent), whereas 4.16per cent from wood, 37.5 per cent from leaf littered soil (Table 2). This representation shows that the contribution of soil and leaf littered soil are maximum as the major forests are tropical moist deciduous having humid forest environment with dense canopy.

Although the extinction of wild macrofungi species have started tremendously, still it has not been brought into consideration of conservation.

Table 2: Representing lists of ectomycorrhizal fungi along with their season of occurrence, edibility, local name, and habitat collected from different forest divisions of Odisha

Scientific Name	Habitat	Growing Time	Local Name	Edibility
Amanita caesarea (scop.) Pers.	On soil	Aug	Haladia Manda	Edible
Amanita craseoderma (Bas.)	On soil	July–Aug		Poisonous
Amanita loosii(Bull.) Fr.	On soil	Aug	Dhala Manda	Edible
Amanita pantherina (DC. ex Fr.) Krombh.	On soil	Aug	-	Poisonous
Amanita Vaginata(Bull.) Lam.	On soil	July–Sept	-	Edible
Boletus aestivalis(Paulet) Fr.	On soil	Aug	-	Inedible
Boletus bicolor(Raddi)	On soil	Aug–Sept	-	Inedible
Geastrum fimbriatum (Fr.)	On soil	Aug	-	Inedible
Lactariusdeliciosus (L.) Gray	On leaf littered soil	Aug	-	Edible
Lactarius resimus (Fr.)	On leaf littered soil	Aug	-	Edible
*Lycoperdon perlatum (*Pers)	On soil	July	-	Edible in young
Pisolithus sp. (Scop.)	On soil	July	-	Inedible
Russula aurata (With.) Fr. Version	On leaf littered soil	July-Aug	-	Edible
Russula brevipes Pk	On leaf littered soil	July-Sept	Dhala Kukuda	Edible
Russula lepida Fr. Harter Zinnobertäubling	On leaf littered soil	July-Sept	Patara Chattu	Edible
Russula nigricans(Bull. ex Mérat)	On leaf littered soil	July-Aug	Koila Chattu	Edible
Russula vesca Fr. Fleischroter Speisetäubling	On leaf littered soil	July-Aug	Patara Chattu	Edible
Russula virescens(Schaeff.) Fr.	On leaf littered soil	July-Sept	Patara Chattu	Edible
Russula xeramphelina(Schaeff.) Fr.	On leaf littered soil	July-Aug	-	Edible
Scleroderma areolatum Ehrneb	On soil	July	-	Poisonoous
Strobilomyces floccopus(Smithand Thiers)	On soil	July-Sept	-	Inedible
Suillus luteus(L.: Fries) Gray	On soil	Aug	-	Edible
Tuber rufum (Pico)Var.	On soil	July-Aug	Rutka Chattu	Edible
Xylobolous subpileatus (Bert. and Curt.)	On wood trunk	July- Oct	-	Inedible

Figure 2: Field photograph of ectomycorrhizal fungi collected from different forest divisions of Odisha

Amanita loosii

Russula lepida

Amanita caeserea

Lactarius sp.

Russula brevipes

Amanita pantherina

S. floccopus

Geastrum fimbriatum

Tuber rufum

Many trees having mycorrhizal association with wild macrofungi are being cut down due to human interference, thereby leading to an unfavourable condition for the mushrooms to grow in their proper habitat.

Acknowledgements

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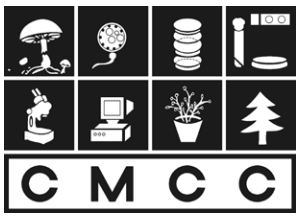

Centre for Mycorrhizal Culture Collection

Biochemical Characterization of AMF

*Maunata Ghorui**, Reena Singh***, and Alok Adholeya*#

Arbuscular Mycorrhizal Fungus (AMF) is an obligatory symbiotic association between the roots of the 90 per cent of the vascular plants and some non-vascular plants, such as mosses (Smith and Read 1997), and the members of an ancient phylum of soil fungi, the Glomeromycota, order Glomales. The arbuscular mycorrhizal symbiosis is thought to have existed 450 million years ago. It is considered to be the world's most abundant mutualism. It is a highly interdependent mutualistic relationship where the fungus provides mineral nutrients to the host plant and in return obtains photosynthetically derived carbon compounds (Harley 1989, Harley and Smith 1983). In addition to the enhanced nutrient uptake, AMF provides resistance to plant pests, improves water relations, increases growth and yield, enhances salt tolerance, etc. The plant-fungal partnership is also responsible for massive global nutrient transfer, global carbon sequestration, and soil stabilization (Parniske 2008). AMF are found in a wide range of habitats, including deserts, lowland tropical rainforests, high latitudes and altitudes, and aquatic ecosystems.

More than 220 species of AMF have been described so far, displaying the enormous diversity; the diversity that is visible in morphology, physiology, and functionality. To conserve and exploit this diversity, the Centre for Mycorrhizal Culture Collection (CMCC) was established in 1993 with seed support from the Department of Biotechnology, Government of India. CMCC is a Mycorrhizal Bioresources Centre that conserves mycorrhizal biodiversity by means of collection, propagation, isolation, characterization, and maintenance of cultures under in-situ conditions. This mycorrhizal culture reservoir stocks and maintains cultures from different agro-ecological zones both nationally and internationally. It has an impressive collection of 912 trap isolates and nearly 2800 monosporal cultures grown from the traps. The bank has an expertise in purification of culture by morphotaxonomic characterization with the purpose of generating pure cultures (monosporal). It also provides substantiation services using different conventional and modern tools such as, morphological characterization, molecular characterization, and biochemical characterization.

Characterization of AMF is imperative to document genetic diversity. AM fungi have traditionally been characterized by microscopy based on spore morphology, such as spore size, subtending hyphae, wall layers, reaction with mountants, presence or absence of sporocarp, etc. The identification of AMF based on their morphological characters is subjected to few experts in the field due to sparse spore characters, the ability of species to form dimorphic spores, ambiguous or incomplete species description, and possible spontaneous changes of the spore characters (e.g., colour, size) depending on their age or environmental conditions. Augmentation of molecular characterization techniques should complement the morphological characterization of AMF because inadequately defined characters and ambiguous distinctions between morphologically similar species may not always give precise identification. It is advisable to isolate DNA from a single spore since each spore of AMF is multinucleate, but DNA extraction from a single AMF spore is very precarious.

An amalgamation of biochemical characteristics along with the above techniques leads to the complete and definite characterization of AMF. The cellular fatty acid composition is routinely analysed to identify and distinguish different genera, species, and even strains of bacteria and yeasts.

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Some of the biochemical measures include quantifying:

- **Ergosterol,** which is a fungus-specific membrane component. Ergosterol is broken down rapidly, and thus is an effective measure of viable biomass. However, only a few studies have reported ergosterol in AMF, while most published reports have found negligible concentrations of ergosterol in the Glomeromycota (Beilby 1980, Beilby and Kidby 1980a, Schmitz *et al.* 1991, and Olsson *et al.* 2003).
- **Chitin,** a fungal wall component, but is not an effective means of assessing AMF infection because correlations between chitin levels and other levels of colonization appear to be poor (Schmitz *et al.* 1991).
- **Lipids**, predominantly 16:1ω5c, is a unique molecule having an unusual saturation position that is specific to certain AM fungi and bacteria. This lipid molecule is used as a specific biochemical marker to estimate the biomass and characterize AMF in soil (Frey *et al.* 1992, Olsson 1999). Fatty acid degrades rapidly during metabolism following cell death. So the quantity of fatty acids present is proportional to active biomass (Vestal and White 1989, Zelles 1999).

Due to the limitations in the first two measures, analysis of lipids and their component fatty acids is a promising alternative (Olsson 1999, Olsson *et al.* 2003). The diversity in structure and quantity of fatty acids of 9 to 20 carbon atoms in length has provided characteristic profiles of the species and genera of bacteria and yeasts. This can be used for elucidating relationship between AMF isolates as well. Lipid and fatty acid analysis is used to address an array of questions about taxonomy, interactions among fungal groups, and assessment of biomass within roots and soil.

Lipids are broadly classified into two classes:

- **Neutral lipids (NLFA)—**These are composed entirely of carbon and hydrogen, thus making it non-polar. The most important type of neutral lipid found in vesicles and spores is triacylglycerides (TAG) (Jabaji-Hare *et al.* 1984). Neutral lipids are associated with carbon storage and are found in high concentrations in structures, such as spores and vesicles (Olsson *et al.* 1997).
- **Phospholipids (PLFA)—**Phospholipids have a phosphate group substituted for one of the three fatty acids, thus conferring a charge to the molecule making them polar in nature. Phospholipids form the lipid bilayer of the cellular membranes that provide an effective barrier to the aqueous solutions, thus maintaining the integrity of the cell. As they are significant membrane components, phospholipids remain associated to the biomass.

The carbon derived from the host plants is stored primarily in the form of lipids (Bago *et al.* 2002, Sancholle *et al.* 2001) with small amount of carbohydrates in AMF. Recent work has indicated that hexoses, such as glucose and fructose, are taken up by fungal structures within the root (Amijee and Stribley 1987, Bago *et al.* 2003, Be´card *et al.* 1991, and Bonfante and Perotto 1995). During sporulation, these sugars in the intraradical hyphae directly incorporate hexose into glycogen and trehalose by the incorporation of non-exchangeable hydrogen atoms. Triacylglyceride is the main form of stored carbon in AM fungi (Beilby and Kidby 1980b, Jabaji-Hare 1998). The lipids are found in copiously in spores and vesicles in the roots colonized with AMF. It is this profusion that is exploited as biochemical character for taxonomy as well as quantification of glomalean fungi in host root tissue, i.e., AMF colonization in host plant.

The triglycerides are glycerol esters of monocarboxylic fatty acids made up of different, linear, saturated fatty acids, each with an even number of carbon atoms. Triglycerides of fatty acids cannot be analysed directly by gas chromatography (GC), so are derivatized as fatty acid methyl esters. In their free, underivatized form, these highly polar compounds tend to form hydrogen bonds, leading to adsorption issues. Reducing their polarity can make them more amenable for analysis. The polar carboxyl functional groups are first neutralized. This then allows column chemistry to perform separations by boiling point elution, and also by degree of unsaturation, position of unsaturation, and even the cis vs. trans configuration of unsaturation. So they are hydrolyzed and derivatized. The ester bonds are hydrolyzed and the free fatty acids that are formed in the process are converted to the corresponding fatty acid methyl esters (FAMEs). FAMEs are moderately apolar and sufficiently volatile to be determined by GC or GC/MS.

The CMCC bank at TERI has created an extensive fatty acid profile library of about 100 monosporals grown from different trap isolates of Uttarakhand, Haryana, Rajasthan, Madhya Pradesh, and Europe to understand the biochemical biodiversity existing among the different mycorrhiza collected from different regions. The quantitative and qualitative differences in the fatty acid composition in the spores among different isolates were done by generating fatty acid methyl esters (FAME) profiles using gas-liquid chromatography.

The uses of biochemical characterization in AMF are:

Taxonomy and Systematic

In an experiment conducted by Bentivenga and Morton (1994), they examined the fatty acid profiles of eight isolates from Glomales family through four generations with three host plants each. The objective was to check whether the fatty acid composition of the isolates was stable throughout and whether the symbiotic conditions, i.e., the host plant, affect the fatty acid composition. It was found that 16:1ω5c was present abundantly (35–75 per cent of all fatty acids) in all isolates except *Gigaspora* (<2 per cent of total fatty acids), while 18:1ω9c was present abundantly in *Gigaspora* and in small percentage in other strains. The fatty acid profile in spores and roots were same in all isolates with exception being *Gigaspora*. *Gigaspora* sp. consistently had divergent fatty acid compositions from other AMF species. The fatty acid profiles were stable through time and with host plant, suggesting that fatty acids can be an effective taxonomic tool. Fatty acid profiles of all isolates can be separated at species and genera level but were convergent at family levels.

Quantification of AMF

The fatty acid component can be used to enumerate the microbial biomass. The microbial biomass can be quantified by extracting phospholipids and measuring the total phosphate colorimetrically (White *et al.* 1979). There exists a good relationship between total phosphate and other measures of microbial biomass, such as ATP and the rate of DNA synthesis. The composition of fatty acids differ in functional groups, hence the individual fatty acids can be used to detect shifts in microbial community composition or to evaluate the biomass of specific components of the community. Many of the fatty acids are distributed generally but some signature fatty acids are most exclusively associated with the particular functional groups.

To Estimate AMF Biomass by Measuring External Hyphal Production

Fatty acid concentrations have also been used to assess the changes in AMF biomass in response to changes occurring at nutrient levels and soil types. Olsson *et al.* (1997) have measured the hyphal production by *Glomus caledonium*, grown in cucumber, under five levels of phosphorus availability. They found that as the phosphorus concentration increased, both NFLA and PFLA 16:1ω5c levels in the soil declined. They found a strong relationship between hyphal length and PFLA 16:1ω5c (r^2=0.61) and a strong relationship between fungal spore numbers and NFLA 16:1ω5c (r^2=0.82). Van Aarle *et al.* (2003) grew *Plantago lanceolata* with native AMF community on two soils of different pH. He used PFLA 16:1ω5c to estimate AMF biomass and found that hyphal growth occurred in the limestone soil but not in acidic soil.

In less controlled systems, quantifying AMF from PLFA 16:1ω5c can be challenging as this fatty acid is also synthesized by bacteria.

Root Infection

Conventionally, AMF infection in roots has been measured by staining the roots and then estimating the colonization percentage visually. The method is not only time-consuming but is also subjective. Establishing the relationship between root infection and fatty acid colonization would eliminate the tiresome microscopic evaluation and would establish direct measures of fungal biomass in the roots. Strong correlations have been found between percentage colonization and the PLFA and NLFA concentrations (Olsson *et al.* 1997).

Fatty acid profiles have also been used to distinguish between AMF and pathogenic fungus in roots. Larsen and Bodker (2001) investigated the interactions between *Glomus mossae* and the root pathogenic fungi *Aphanomyces euteiches* in pea roots. They used PFLA 14:0 to quantify pathogen biomass and PFLA 16:1ω5c to quantify AMF. They found that the presence of AMF reduced *Aphanomyces* biomass. Similarly, inoculation with the pathogen reduced AMF biomass as the diseased roots were unable to provide sufficient carbohydrate to the AMF or due to the competition between the pathogenic fungi and AMF for resources.

Indicator of Physiological Status of AMF Using NLFA: PLFA Ratios

In eukaryotes, the ratio of NLFAs to PLFAs can be used as an indicator of physiological status or nutrient status. The NFLAs measure the resources allocated to storage, while PLFAs measure active biomass. The nutrient status affects the carbon storage in AMF by varying nutrient availability to the host. As the level of phosphorus increases in the soil, 16:1ω5c in roots and soil decreases along with the ratio of NFLA to PFLA, thereby indicating reduced carbon storage (Olsson *et al.* 1997). So, under high nutrient availability, plants decrease the allocation of carbon to roots.

Organic carbon availability also alters NLFA:PLFA ratios. The declined ratio suggests that organic compounds increase vegetative structures more than allocation to storage.

The weaknesses of the current approach are:

Quantification and Identification of Fatty Acids

The two key components of fatty acid analysis are peak identification and quantification. Fatty acids can be analysed using GC alone by comparing the peaks with the standards. However, confident identification requires mass spectroscopy (MS). Without MS, identification of unknowns is impossible and peaks may overlap, which means single peak will not correspond to a single fatty acid. This approach is difficult when a large number of fatty acids need to be identified because all fatty acids are not commercially available and it is expensive to have quantitative standard custom made. This internal standard is generally 19:0, a fatty acid that is not naturally found in roots or soil. This standard is added either as fatty acid prior to sample methylation or as FAME prior to GC analysis. The relationship between peak sizes for this internal standard is used to quantify all peaks.

Reporting a fatty acid research can be improved by two ways. First, the GC set up used should be described in sufficient detail for the method to be replicated. Second, the amounts of fatty acid should be reported as total fatty acids percentage. It is impossible to convert total fatty acids to actual amounts.

Signatures Found in Other Components of Microbial Community

16:1ω5c, which is an AMF signature is also a major component of bacterial lipids (e.g., *Flexibacter flexilis* makes upto 50 per cent of the PLFA component). Even under controlled conditions, non-AMF sources of this signature are substantial. A partial solution to this problem is to measure both NLFA and PLFA 16:1ω5c. Only if the two are well correlated or the ratio of NLFA to PLFA is high, one can assume it to be an AMF. When phospholipids breakdown, the phosphate gets cleaved forming diacylglycerol that contributes to NFLA signal (Bääth 2003).

Sample Treatment

Extraction methods can differ greatly in efficiency. Storage of roots or soil prior to analysis can influence fatty acid profiles. For AMF, carbon supplied to the roots as hexose sugars are taken up by intraradical mycelium and are converted into triglycerides prior to transport to the external mycelium (Bago *et al.* 2000). These triglycerides broke down and concentrations decline as the distance from the root increases (Bago *et al.* 2002).

Species, Age, and Nutrient Status

Species vary in fatty acid 16:1ω5c concentration considerably. Even within a single species, fatty acid concentrations can vary substantially. One of the factors is the nutrient availability. Olsen *et al.* (2002) found that the nutrient status affected the fatty acid ratios. At high concentrations of phosphorus, *Glomus intraradices* had lower proportion of unsaturated fatty acids.

The culture condition and age also affects the fatty acid profiles. The relative amounts of same fatty acids vary with age (Stahl and Klug 1996).

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- *Archives of Agronomy and Soil Science*
- *Australasian Plant Pathology*
- *BioEssays*
- *Biological Invasions*
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- *Plant and Soil*
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