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ORIGINAL PAPER

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Arbuscular mycorrhizae and phosphate solubilising bacteria of the rhizosphere of the mangrove ecosystem of Great Nicobar island, India

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Abstract Mangroves form an important ecosystem of Great Nicobar, a continental island in the Bay of Bengal with luxuriant tropical rainforests. The rhizosphere of the mangrove plants of Great Nicobar was investigated for the presence of arbuscular mycorrhizal fungus (AMF) and phosphate solubilising bacteria (PSB). The soils of the Great Nicobar mangroves were silt–clays and were poor in phosphate content. Five species of AMF belonging to the genus *Glomus* were isolated. The %AMF colonization in the mangrove plants was between 0 and 17%, and the presence of AMF in the aerenchymatous cortex suggests that the mangrove plants may be aiding in AMF survival by providing oxygen. Two strains of phosphate solubilising *Pseudomonas aeruginosa* were found in the mangrove soils of Great Nicobar. Phosphate solubilisation by the two isolated strains was almost 70% under in vitro conditions. PSB may play a role in the mangrove ecosystems of Great Nicobar by mobilising insoluble phosphate. The plant roots could pick up the released phosphate directly or with the aid of AMF hyphae.

Keywords Arbuscular mycorrhiza · Phosphate solubilising bacteria · Mangroves · Great Nicobar · *Glomus* · *Pseudomonas aeruginosa*

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Introduction

Mangroves form the dominant interface ecosystems between the land and sea in the tropics (Ong et al. 1995). They are formed on sheltered muddy shores where land is extending seaward by accretion (Richards 1996). The annual input of organic matter in the form of litter is around 8 tonnes/ha (Lugo and Snedaker 1974). Growth in mangrove plant communities is limited primarily by P availability because P is adsorbed or co-precipitated with carbonate compounds (Koch and Snedaker 1997). Soil microbes that can solubilise the bound P into available forms and arbuscular mycorrhizal fungus (AMF) hyphae may aid in the uptake of nutrients like P by extending the depletion zone (Cui and Caldwell 1996). In addition, micro-organisms, such as phosphate solubilisers, free living and associated nitrogen fixers and mycorrhizal fungi, can interact in the rhizosphere soil (Garbaye 1991; Andrade 2004).

The mangroves are inundated with saline water for long hours. The saline and anaerobic conditions make survival of most microbes that are crucial in nutrient mineralization difficult. Although AMF have been reported from mangrove ecosystems (Sengupta and Chaudhuri 1990; Sengupta and Chaudhuri 2002), their survival is inhibited in submerged anaerobic conditions (LeTacon et al. 1983; Brown and Bledsoe 1996).

Mangroves form an important ecosystem of Great Nicobar, an island of the Andaman and Nicobar archipelago in the Bay of Bengal. We report here the presence of AMF and phosphate solubilising bacteria (PSB)—functional micro-organism groups that contribute to P-mobilisation—in the mangrove rhizosphere of Great Nicobar.

Materials and methods

Study location and sample collection

South bay at the south of the Great Nicobar island was selected as the study site where the river Galathea flows into the Indian Ocean. Mangroves at the mouth of the

Galathea were selected for study of AMF populations. The mangroves remain submerged under 25 to 50 cm of brackish water for up to 6 to 8 h—twice a day during high tide.

Five-count quadrats (20×20 m) were laid out at random. Tree species in the quadrats were counted, and their Importance Value Index (IVI) was computed as follows:

IVI=[Relative frequency (RF)÷Relative Density (RD)÷Relative Dominance (RDo)]

$$\mathrm{Rf} = (s_i \div S) \times 100,$$

where s_i is the frequency of the i^{th} species, and S is the frequency of all species,

$$\mathrm{RD} = (n_i \div N) \times 100,$$

where n_i is the density of the i^{th} species, and N is the density of all species.

$$\mathrm{RDo} = (\mathrm{ba}_i \div \mathrm{BA}) \times 100,$$

where ba_i is the basal area of the i^{th} species, and BA is the basal area of all species.

Five rhizosphere samples were collected (maximum depth of 15 cm) at random from each quadrat (the roots from adjacent plants formed a dense entangled web beneath the soil making it difficult to delimit the rhizosphere of individual plants). These samples were analysed for nutrient contents and for AMF spores and bacteria involved in phosphate solubilisation. Roots were collected from five individuals of each species at each quadrat for analyses of %AMF colonization.

Soil analysis

Soil particle sizes were estimated by running a suspension of 50 g soil through a set of seven sieves of mesh sizes 2,000, 1,000, 500, 250, 125, 63 and 31 μm. The fraction of soil particles collected at each sieve was weighed and its percentage calculated. Soils were classified based on the relative proportion of different particle sizes following Brady and Weil (1999).

Nitrate was estimated colorimetrically (Cataldo et al. 1975) after digesting the samples with sulphuric acid–peroxide (Allen et al. 1974); inorganic P was estimated after treating soils by mixed acid digestion (Allen et al. 1974) followed by analyses with the molybdenum blue method (Chen et al. 1956). Percent organic matter was estimated using the rapid titration method of Walkley and Black (Jackson 1973).

AMF spore isolation and root clearing

AMF spores were isolated from a 500-g soil suspension (from each quadrat) by the wet sieving and decanting method (Gerdemann and Nicholson 1963) and identified following Schenck and Pérez (1990). AMF infection of the roots was localized by the clearing and staining technique (Phillips and Hayman 1970) and free-hand sectioning. The %root colonization was estimated using the formula of Nicolson (1955).

Isolation and determination of phosphate solubilising bacteria

To isolate PSB, a 1-ml aliquot of 10^{-2} dilution of the rhizosphere soil suspension was spread on a plate of Pikovskayas medium (Subba Rao 1988). The formation of a decolourized halo around the cultures indicated the presence of PSB. Single colonies were isolated and identified using standard bacteriological methods.

The isolated PSB were grown in Pikovskayas medium for 15 days at 30°C with shaking at 100 rpm in a New Brunswick Inova 4230 incubator shaker. The cultures were then filtered through a Whatman No. 1 paper to remove the undissolved phosphate and centrifuged at 8,000×g to get a clear solution. To 10 ml of the supernatant, 2.5 ml of Barton's reagent (Subba Rao 1988) was added and the volume made up to 50 ml. The absorbance of the solution was read at 430 nm in a Spectronic 20 spectrophotometer, and the percentage of phosphate solubilisation was calculated as reported by Subba Rao (1988). A medium with no bacterial inoculation served as the control. Five replicates were maintained.

Results

The soils at Great Nicobar mangroves were grey, and, according to soil size fractions (Table 1), they were classified as silt–clays following Brady and Weil (1999). The soils had low levels of nitrate and inorganic P as compared to the average nitrate (21 mg/g soil) and inorganic P (2 mg/g soil) at the riparian and inland forest ecosystems of Great Nicobar. The organic matter content was reasonably higher and similar to other habitat types at Great Nicobar. The low levels of N and P may be a stress factor for the mangrove plants.

The plant community of the mangroves was represented by five species, viz., *Rhizophora mucronata* Lamk., *Rhizophora stylosa* Griff., *Bruguiera gymnorrhiza* (L.)

Table 1 Properties of rhizosphere soils of the mangrove at South Bay, Great Nicobar

%Sand (2000–500 μm)	0.36±0
%Coarse silt (250–125 μm)	13.40±0
%Fine silt (125–63 μm)	41.20±0
%Clay (<31 μm)	45.04±0
Soil type	Silt−clay
Nitrate N–NO_3 (mg/g)	6.91±1.60
Inorganic P (mg/g)	0.86±0.42
Organic matter (%)	6.20±1.16

Table 2 The plant species composition at the mangroves of Great Nicobar and the percentage of AMF colonization of root

Plant	IVI	%AMF colonization of root
B. gymnorrhiza	73.71	17.6±1.81
S. acida	44.10	9.2±1.09
R. mucronata	73.31	7.2±1.30
R. stylosa	39.55	0
N. fruticans	31.62	0

IVI Importance Value Index

Lamk., *Sonneratia acida* Smith and *Nypa fruticans* Wurmb. (Table 2). The average total number of plants in each quadrat was 62.20±6.45. Five species of AMF, all of the genus *Glomus*, were isolated from the mangrove rhizosphere. Two *Glomus* species were identified: *G. fasciculatum* (Thaxter) Gerdemann and Trappe emend. Walker and Koske and *G. magnicaule* Hall; the three unidentified species (Table 3) were labelled as Types (Kothamasi 2000). The average AMF spore population in the soils was 2-spores/g soil.

AMF were present in the cortex and the hypodermal cell layers (Fig. 1a,b). The percentage colonization of AMF in the mangroves of Great Nicobar is presented in Table 2. *B. gymnorrhiza* had the highest percentage AMF colonization. This plant also had the highest IVI.

Two strains of phosphate solubilising *Pseudomonas aeruginosa* (designated GM01 and GM02) were isolated from the rhizosphere soils. Both strains were facultative aerobes and could grow at pH 4; GM01 and GM02 could solubilise up to 71.96±5.17% and 58.15±2.57% phosphate, respectively, under in vitro conditions.

Discussion

AMF are known to protect plants growing under saline stress (Hirrel and Gerdemann 1980; Pond et al. 1984; Pfeiffer and Bloss 1988). However, mangrove plants have developed mechanisms that keep salt out (Scholander 1968; Richards 1996) and are not dependent on AMF for protection from salinity.

Table 3 AMF species isolated at the mangrove rhizosphere of South Bay, Great Nicobar

AM species	Description
Glomus fasciculatum	Spore light yellow, globose to subglobose, 125×95 μ, wall 8 μ, hypha 12 μ at attachment, straight
Glomus magnicaule	Spores reddish brown, subglobose, 135×108 μ, wall 8–10 μ, hypha 35 μ at attachment
Glomus Type 42	Spore reddish brown, subglobose, 118×88 μ, wall 5 μ, two-layered, hypha 18 μ at attachment, pore unoccluded
Glomus Type 46	Golden brown, globose, 75×75 μ, wall 2–5 μ, hypha 10 μ at attachment, funnel-shaped
Glomus Type 48	Spores light yellow, pyriform, 130×112 μ, wall 2–5 μ, hypha 10 μ at attachment

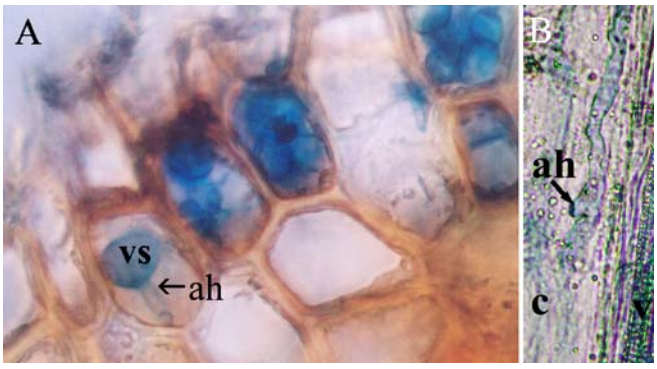

Fig. 1 AMF colonization of the *B. gymnorrhiza* roots. **a** AMF hyphae in the hypodermal cell layer. Vesicles (*vs*) and AMF hypha (*ah*) can be seen. **b** A cleared root whole mount showing AMF hypha (*ah*) adjacent to the vascular cylinder (*v*) in the cortex (*c*) of the root

The mangrove soils of Great Nicobar are fine-textured silt–clays (Table 1). Silt–clays swell on exposure to water and impede air movement (Brady 1990), leading to anaerobic conditions. Anoroxic conditions inhibit mitochondrial development in AMF, and the anaerobic environment of the waterlogged mangroves would make the survival of AMF difficult (LeTacon et al. 1983; Brown and Bledsoe 1996). However, the roots of *Rhizophora*, *Sonneratia* and *Bruguiera* have pneumatophores that facilitate the movement of oxygen to the submerged roots. The submerged roots, like other aquatic plants, have an aerenchymatous cortex, whose tissues, in plants growing in flooded habitats, have been reported to provide oxygen to the roots and permit the colonization and survival of AMF (Cooke et al. 1993; Brown and Bledsoe 1996). The aerenchymatous tissue of mangrove roots of Great Nicobar would permit the movement of surface oxygen both to the submerged roots and the surrounding rhizosphere so as to facilitate the survival of AMF in the anaerobic conditions of the mangrove.

Growth in plant communities is governed by the availability of nutrients like P and N (Read 1990). The mangrove soils of Great Nicobar had high amounts of organic matter and very low P levels (Table 1). This condition is ideal for the formation of the AMF association. The presence of vesicles (Fig. 1a) in the root cells of the mangrove plants suggests that the fungal symbionts may have a role in nutrient uptake as vesicles are nutrient storage organs (Smith and Read 1997).

Two strains of P solubilising *P. aeruginosa* were isolated from the rhizosphere soils of mangroves at Great Nicobar. The two isolated strains did solubilise high amounts of phosphate under in vitro conditions. If these isolates have similar capabilities under in situ conditions, they would be mobilising phosphate in the mangrove rhizosphere of Great Nicobar.

The provision of oxygen and photosynthetic C to the fungi and the latter's ability to take up nutrients under conditions of scarcity could be a reason for formation of AMF association at the mangrove. However, fungi are not very efficient in mobilising nutrients under submerged

conditions (Hackney et al. 2000). If phosphate is made available to the exploring hyphae, they could pick it up and transfer it to the host. Soil PSBs could release phosphate ions that could be picked up by the AMF hyphae or the plant root (Azcon-Aguilar et al. 1986; Toro et al. 1998).

AMF have been reported to function as intermediaries between plants and soil microbes by moving C and other phytoexudates from the plant to the rhizosphere and transferring microbe mobilised nutrients to the plant (Garbaye 1991; Fitter and Garbaye 1994; Toro et al. 1998; Copley 2000). It is possible that in mangroves of Great Nicobar, AMF perhaps have a similar role.

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