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Occurrence of nitrogen-fixing Azospirillum in vesicular-arbuscular mycorrhizal fungi

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Abstract

Nitrogenase activity measured by acetylene reduction, was detected when surface-sterilized spores of vesicular-arbuscular (VA) mycorrhizal fungi(*Glomus fasciculatum, G. intraradices, G. scientillans, G. mosseae, Gigaspora gilmorei* and *Endogone dusii*) were inoculated into nitrogen-free liquid medium containing malic acid and incubated under microaerophilic conditions (99% N_2 + 1% 0_2) at 30°C. *Azospirillum* species were isolated from the nitrogenase-active cultures.

Introduction

Through the years there have been reports of bacteria isolated from fungal spores. Several heterotrophic, fermentative and anaerobic bacteria from the lumen of *Glomus macrocarpus* growing in the rhizospheres of *Ficus bengelensis* and *Acacia* sp. have been reported (Varma *et al.*, 1981). Occurrence of *Klebsiella* sp.,. *Clostridium* sp. and *Streptococcus lactis* from the lumen of *Glomus microcarpus* extracted from the rhizospheres of certain xerophytes has been documented (Singh and Varma, 1985). Nitrogen-fixing (acetylene-reducing) bacteria associated with the ectomycorrhizae of Douglas-fir (*Pseudotsuga menziesii*) have recently been demonstrated (Li and Hung, 1987). Nitrogen-fixing azospirilla were isolated from within the sporocarps of the ectomycorrhizal fungi like *Hebeloma crustuliniforme, Laccaria laccata* and *Rhizopogon vinicolor* (Li and Castellano, 1987).

This study explores the possible association of nitrogen-fixing *Azospirrillum* sp. with the spores of VA-mycorrhizal fungi.

Materials and methods

Spores of *Glomus fasciculatum, G. intraradices, G. scientillans, G. mosseae, Gigaspora gilmorei* and *Endogone dusii* were isolated from the rhizosphere soil of maize infected with the respective VAM fungi by the standard wet sieving and decanting technique (Gerdeman and Nicholson, 1963). They were rinsed with tap water in 70% alcohol followed by immersion in 1% chloramine -T (J T Baker Chemical Co., Phillipsburg, NJ)* for 1 h shaken frequently, and rinsed in two changes of sterile distilled water (Döbereiner, 1980). About 50–75 surface-sterilized spores of VAM fungi were transferred into 8 ml of nitrogen-free semi-solid sodium malate medium (Döbereiner and Day, 1976) in 20 ml capacity screw-capped tubes and incubated at 30°C for a week. Six replicates were maintained in each case. The formation of white pellicel 2–4 mm below the surface of the medium was noticed with all the cultures.

* Use of trade names does not imply endorsement or approval by the USDA Forest Service.

Acetylene was injected into each tube to 10% of the total gas volume. Tubes without added acetylene served as controls. After 24 h, 0.1 ml gaseous sample from each tube was removed and analyzed for ethylene and acetylene with a Hewlett-Packard 5830A gas chromatograph fitted with 2 m × 2.1 mm, 80–100 mesh Porapack R column with oven temperature at 70°C, and flow rate of the nitrogen carrier gas was adjusted to 40 ml per min.

Acetylene-reducing bacteria from the mixed cultures were purified by repeated streaking on N-free sodium malate medium supplemented with 0.002% yeast extract (Barber and Evans, 1975). To test the nitrogenase activity of isolated bacteria, as measured by acetylene reduction an aqueous suspension of each bacterial isolate was inoculated into screw-capped tubes that contained N-free sodium malate medium supplemented with yeast extract (0.002%). The tubes were incubated under micro-aerophilic conditions (99% N_2 + 1% 0_2) at 30°C for 3 days. Acetylene was injected into each tube. The formation of ethylene was determined as previously described.

Bacterial cells in the tubes were harvested and washed with cold 5% trichloro-acetic acid. Cell protein was solubilized with 0.5 *N* NaOH in a boiling water bath for 10 min (Agarwal and Keister, 1983) and measured by the modified Lowry method (Markwell *et al.*, 1978).

Results and discussion

Bacteria isolated from the surface-sterilized spores of *Glomus fasciculatum, G. intraradices, G. scientillans, G. mosseae, Gigaspora gilmorei* and *Endogone dusii* showed characteristic spirillar movement and grew well in nutrient and trypticase soy agar media. The cells are long and slightly curved rods with poly-β-hydroxy-butyrate (PHB) granules inside and showed a distinctive pellicle formation with acetylene reduction activity in N-free sodium malate semi-solid medium under microaerophilic conditions (Table 1). These characteristics are typical of the genus Azospirillum (Krieg and Döbereiner, 1984). The Azospirillum isolated from *Glomus* species, in general had significantly more nitrogenase activity than that isolated from either *Gigaspora gilmorei* or *Endogone dusii*.

Table 1. Nitrogenase activities of *Azospirillum* sp. isolated from surface-sterilised spores of different vesicular-arbuscular (VA) mycorrhizal fungi[1]

VAM spores	Nitrogenase activity (nmoles ethylene produced/ mg protein/h)
Glomus fasciculatum	125b
G. intraradices	119b
G. scientillans	105b
G. mosseae	110b
Gigaspora gilmorei	55a
Endogone dusii	34a

[a] Data are means of six replicates. Treatment means not sharing a common letter differ significantly at 5% level of significance (analysis of variance). The critical difference at 5% level is 22.52.

The implications of nitrogen-fixing azospirilla from the surface-sterilized spores of VAM fungi is not known, although many fungi do utilize large amounts of nitrogen in producing sporocarps and spores. Fixation of atmospheric nitrogen by associated bacteria could perhaps satisfy such demands to certain extent (Cowling and Merrill, 1966). Although the methods adopted to isolate nitrogen-fixing azospirilla from thoroughly surface-sterilized spores of VAM-fungi, there is a possibility of association of few other bacteria which may not be of much ecological significance. However, further studies on the association of bacteria other than nitrogen-fixing azospirilla and their contribution to nitrogen fixation either directly or indirectly needs further investigation.

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