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Enhancement of spore germination of *Glomus fasciculatum* by bacterial cell free extracts

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Spore germination of *G. fasciculatum* (Thaxter sensu Gerdmann) Gerdmann and Trappe was stimulated by cell free extracts of non symbiotic nitrogen fixers like *Azotobacter chroococcum* Beijerinck, *Azospirillum brasilense* Cd and *A. lipoferum* (Beijerinck) Tarrand Kreig and Dobereiner. Cell free extracts of non nitrogen fixing bacteria did not show significant increase in spore germination except *Pseudomonas putida* and *P. fluorescence*, whereas all the three diazotrophs contributed significant enhancement in spore germination.

Beneficial response of crop plants to inoculation with vesicular arbuscular mycorrhizal (VAM) fungi is well documented[1]. Synergistic effects of VAM fungi and nitrogen fixing microorganisms on various agricultural crop plants have also been reviewed[2-4]. Since VAM fungi are obligate symbionts, the successful colonization of host plant roots by VAM may be affected by the successful germination of spores. Many factors affect the spore germination of VAM fungi[5-11]. Various spore-associated bacterial genera including *Pseudomonas* and *Corynebacterium* have been reported to stimulate the spore germination in the case of *Glomus versiforme* (Karsten) Berch[12]. Present investigation has been undertaken with a view to explore the influence of cell free extracts of bacteria on spore germination of VAM fungus, *G. fasciculatum*.

A culture of *G. fasciculatum* (Thaxter sensu Gerdmann) Gerdmann and Trappe was obtained from ICRISAT, Hyderabad, India and maintained as a pure culture in pots containing sterilized soil and sand (1:1) using sudan grass (*Sorghum bicolor* var. *sudanese*) as a host. Spores were collected by wet sieving and decanting the soil[13] from 10 different pots and were mixed to form a composite sample. The spores that are formed singly in soil were selected for this study. They were pale yellow to pale yellow-brown in colour with a thick walled subtending hypha and had a spore wall structure of three walls[14]. The spores were surface sterilized in chloramine-T (2 % w/v) for 20 min and rinsed 3-4 times in sterilized water.

Three diazotrophic bacteria, viz., *Azotobacter chroococcum* (strain 1) grown on N-free sucrose medium, *Azospirillum brasilense* (strain S 1) and *A. lipoferum* (strain AL-5) grown on N-free malate[15] medium isolated from the rhizosphere soil and roots of one month old sudan grass plants inoculated with *G. fasciculatum* were taken for present studies. Six other non-nitrogen-fixing bacterial strains were also considered to compare the effect of diazotrophs on spore germination. These bacerial species included *Escherichia coli* which was grown on eosin methylene blue medium, *Bacillus megatherium* grown on sucrose mineral medium, *Pseudomonas* sp., *P. putida, P. fluoresence* grown on King's medium and *Enterobacter* sp. grown on Mac Conkey medium. *Pseudomonas putida* was obtained from Dr R G Linderman, Corvallis, Oregon, USA. The organisms were grown on respective media (200 ml) in 750 ml conical flasks. Six replicates were maintained in each case. The cell free extracts were obtained by passing the log phase cultures through bacteria proof filters (0.22 μm).

For germination studies, 100-150 surface sterilized spores of *G. fasciculatum* were carefully transferred with a sterile micropipette into petridishes (8 cm diam.) containing 1 % agar (bacto) dissolved in water and 0.1 % cell free extracts of different organisms under a stereomicroscope in a clean incoulation chamber and incubated at 25° ± 1°C. Percent germination was recorded after 24 hr intervals for 15 days. Uninoculated media and autoclaved culture extracts served as controls. The experiment was a completely randomized design, each treatment replicated 6 times i.e. 6 plates each having 20 spores. The data were analysed statistically using analysis of variance[16].

A spore was considered germinated if a germ tube longer than 60 μm was present[5]. The spores germinated through the attachment hypha. Although some hyphal germ tubes might have been broken during sieving this did not often occur near the hyphal attachment where hyphal diameter is greatest.

The results (Table 1) indicated that there was no considerable increase in the percent germination of *G. fasciculatum* spores in the cell free extracts of various non-nitrogen-fixing bacteria except *Pseudomonas putida* and *P. fluorescence*, which significantly enhanced spore germination as compared to the contr-

Table 1—Percent germination of *G. fasciculatum* in cell free extracts of different bacteria
[Values are mean ± SE of 6 replications of 20 spores each]

Bacteria	% spore germination		
	Cell free extract	Autoclaved extract	Uninoculated medium
Escherichia coli	22.5 ± 2.8	18.5 ± 2.5	18.0 ± 2.8
Bacillus megatherium	25.7 ± 4.5	22.5 ± 3.2	20.5 ± 2.5
Pseudomonas sp.	25.5 ± 4.2	23.2 ± 3.5	25.5 ± 3.2
Pseudomonas putida	40.5 ± 5.2*	30.7 ± 4.2	25.5 ± 3.2
Pseudomonas fluoresence	35.8 ± 4.8*	26.5 ± 3.2	25.5 ± 3.2
Enterobacter sp.	25.8 ± 3.5	22.5 ± 3.5	20.5 ± 2.5

CD at 5 % = 9.2
*Significant increase over corresponding control

ols. This may be due to the growth promoting substances and siderophores producing capability of these two species. An early infection of VAM to subterranean clover (*Trifolium subterraneum* L.) due to dual inoculation with *Pseudomonas putida* has been reported[17].

Percent germination of *G. fasciculatum* spores was significantly higher in the presence of cell free extracts of *A. chroococcum*, *A. brasilense* and *A. lipoferum* than in the uninoculated media and autoclaved cell free extracts of the organisms tested. Autoclaved extracts produced a significantly high percentage germination than did the uninoculated medium (Table 2). There was no significant ($P = 0.05$) difference among the three diazotrophic bacterial culture extracts.

The rhizosphere is a zone immediately surrounding roots where root exudates are concentrated and soil microbial activity is intense. Root exudates play a significant role in mycorrhiza formation[18-20] by enhancing spore germination as well as certain types of bacterial populations[21]. Mayo *et al.*[12] observed that the hyphae emerging from the spores of *G. versiforme* in the presence of spore associated bacteria are more extensively branched than those from surface disinfected spores.

Besides fixing atmospheric nitrogen, azotobacters are known to synthesize considerable quantities of biologically active substances. Among these are vitamines of B group, indolyl-3-acetic acid and gibberellins[22,23]. Tien *et al.*[24] have reported the production of plant growth substances by *A. brasilense*. The improvement of spore germination of *G. fasciculatum* with cell free extracts of these diazotrophs over that of autoclaved culture extracts may result from the presence of thermolabile substances in the live cell free culture extracts.

Table 2—Percent germination of *G. fasciculatum* spores in cell free extracts of *Azotobacter chroococcum*, *Azospirillum brasilense* and *A. lipoferum*
[Values are mean ± SE of 6 replications of 20 spores each]

Treatment	% Germination
Cell free extract of : *A. chroococcum*	45.5 ± 4.5[a]
A. brasilense	52.7 ± 5.2[a]
A. lipoferum	48.5 ± 4.8[a]
Heat killed extract of : *A. chroococcum*	28.5 ± 2.5[b]
A. brasilense	35.2 ± 4.2[b]
A. lipoferum	29.5 ± 5.6[b]
Uninoculated: *Azotobacter* medium	15.2 ± 3.2[c]
Azospirillum medium	20.5 ± 3.0[c]

Treatments with different letters differ significantly at 5 % level of significance (analysis of variance). The critical difference at 5 % level is 8.25.

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References

1 Mosse B & Hayman D S, in *Tropical mycorrhiza research*, edited by P Mikola (Clarondon Press, Oxford) 1980, 213.
2 Bagyaraj D J, in *V A mycorrhiza*, edited by C L Powell and D J Bagyaraj (CRC Press Inc, Boca Raton, Flordia, USA) 1984, 131.
3 Subbarao N S, in *Current developments in biological nitrogen fixation*, edited by N S Subbarao (Oxford and IBH Publishing Co., New Delhi, India) 1984, 37.
4 Tilak K V B R, *Proc Natl Symp Curr Trends in Soil Biol*, (HAU, Hisar, India) 1985, 219.

5 Daniels B A & Trappe J M, *Mycologia,* 72 (1980) 457.
6 Mosse B, *Trans Br Mycol Soc,* 42 (1959) 273.
7 Mukerji K G, Sabharwal A, Kochar B & Ardey J, in *Progress in microbial ecology,* edited by K G Mukerji, V P Agnihotri and R P Singh (Print House, India, Lucknow) 1984, 429.
8 Schenk N C, Graham S O & Green N E, *Mycologia,* 67 (1985) 1189.
9 Siqueria J O, Sylvia D M, Gibson J & Hubbell D H, *Can J Microbiol,* 37 (1975) 965.
10 Tommerup I C, in *Mycorrhizae in the next decade practical applications and research priorities* (Proc 7th North Amer Conf Mycorrhizae, Gainesville, Florida, USA) 1987, 175.
11 Watrud L S, in *Methods and principles of mycorrhizal research,* edited by N C Schenk (Amer Phytopathol Soc, St Paul, Minnesots) 1982, 81.
12 Mayo K, Davis R E & Hotta J, *Mycologia,* 78 (1986) 426.
13 Gerdemann J W, *Mycologia,* 47 (1955) 619.
14 Walker C & Koske R E, *Mycotaxon,* XXX (1987) 253.
15 Okon Y, Albrecht S L & Burris R H, *Appl Environ Microbiol,* 33 (1977) 85.
16 Yates F, *Imp Bur Soil Sci* (Harpendon, London, Tech Comm) 1937, 35.
17 Meyer J R & Lindermann R G, *Soil Biol Biochem,* 18 (1986) 185.
18 Ratnayake M, Leonard R T & Menge J A, *New Phytol,* 81 (1978) 543.
19 Graham J H, *Mycologia,* 72 (1980) 457.
20 Graham J H, Leonard R T & Menge J A, *Plant Physiol,* 68 91981) 548.
21 Meyer J R & Lindermann R G, *Soil Biol Biochem,* 18 (1986) 191.
22 Azcon R & Barea J M, *Pl Soil,* 43 (1975) 609.
23 Mishustin E N & Shilinkova V K, *Biological fixation of atmospheric nitrogen* (The Pennsylvania State Univ. Press, USA), 1971, 419.
24 Tien T M, Gaskins M H & Hubbell D H, *Appl Environ Microbiol,* 37 (1979) 1016.