Isolation from the *Sorghum bicolor* Mycorrhizosphere of a Bacterium Compatible with Arbuscular Mycorrhiza Development and Antagonistic towards Soilborne Fungal Pathogens

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A gram-positive bacterium with antagonistic activity towards soilborne fungal pathogens has been isolated from the mycorrhizosphere of *Sorghum bicolor* inoculated with *Glomus mosseae*. It has been identified as *Paenibacillus* sp. strain B2 based on its analytical profile index and on 16S ribosomal DNA analysis. Besides having antagonistic activity, this bacterium stimulates mycorrhization.

In recent years, several types of microorganisms have been reported to be associated with the rhizospheres of different host plants colonized by arbuscular mycorrhizal (AM) fungi. These have been identified as associative N₂-fixing bacteria (13), plant growth-promoting rhizobacteria (17), phosphate-solubilizing bacteria (15), and antagonists of plant pathogens (4). AM fungi are a ubiquitous component of most agroecosystems and play an important role in key rhizosphere processes (14), including plant protection against soilborne diseases (1, 5). Associated microorganisms may complement mycorrhizal activities, particularly in biological control in agricultural systems. However, few data on the compatibility between these microorganisms and AM fungi are available. There are reports that biocontrol agents, like gram-negative *Pseudomonas* strains, do not have inhibitory effects on AM formation (2, 11). We report the isolation and identification of a gram-positive bacterium from the mycorrhizosphere of *Sorghum*, and describe its activity towards root pathogens and AM fungi in vitro and in vivo.

Eight morphologically different bacteria (B1 to B8) were isolated from the mycorrhizosphere of sorghum plants (*Sorghum bicolor* L. var. Esquirol) inoculated with the AM fungus *Glomus mosseae* (Nicol et Gerd) Gerdemann et Trappe (BEG 12) (obtained from the Banque Européenne des Glomales [1a]). Seeds of sorghum were surface sterilized (30 min in 7% calcium hypochlorite with a few drops of Tween 20, followed by 30 min in 4% chloramine-T), and sporecarps of *G. mosseae* were surface sterilized as previously described (3). Plants were grown in a sterilized clay loam soil-calcinized clay (Oil Dry Type III; OIL·DRI, Limited, Wisbech, United Kingdom) mixture (1:1) in sterile Sunbags (catalog no. 7026; Sigma) under constant conditions (22 to 24°C, 16-h photoperiod, 300 μmol of photons/m²/s, and 70% relative humidity) for 12 weeks. All the components (soil-clay mixture sporecarps, seeds, and water) were extensively checked for the absence of contaminants by incubation in malt agar medium. Bacteria were isolated from the growth substrate of *G. mosseae*-inoculated plants according to the method described by Zuberer (18) and were selected by colony characteristics: shape, size, edge morphology, surface, and pigment. No bacteria were obtained from the growth substrate of plants not inoculated with *G. mosseae* sporocarps.

Bacteria were screened in vitro for their antagonistic activity towards *Phytophthora parasitica* isolate 204 by measuring the radial colony growth of the fungus and the zoosporangium production in the absence and presence of the bacteria according to the methods of Dal-Soo et al. (6). Zoospore germination on Millipore filters left unsaturated or saturated with the filtered supernatant of a B2 culture was evaluated, and the in vivo antagonism of the bacteria in terms of plant growth and the percentage of necrosed roots, in the presence or absence of *G. mosseae*, was assessed as described by Cordier et al. (5). AM development was determined according to the method of Trouvelot et al. (16). In vitro experiments were performed with five replicates for each experiment and were repeated at least twice. A randomized block design was used for in vivo growth room experiments, with seven replicates per treatment. Percentages were arcsine transformed prior to analysis. All data were analyzed by one-way analysis of variance and the Newman-Keuls test at a P value of <0.05. Results are given for one representative experiment.

Only one strain, B2, out of the eight isolated showed a significant antagonistic activity towards *P. parasitica* in vitro (Table 1). The antagonistic effect was also obtained with partially purified (by ion-exchange chromatography after heat treatment) media from a B2 bacterial culture, indicating that the strain secretes an antifungal factor (data not shown). The effect of antagonistic B2 bacteria was also tested in vivo on tomato plants grown in the presence or absence of *G. mosseae* and/or the bacterial strain B2 and subsequently inoculated with *P. parasitica*. Root necrosis caused by *P. parasitica* was reduced by 32, 53, and 63% when plants were inoculated with B2, *G. mosseae*, and B2 and *G. mosseae*, respectively (Table 2). The presence of B2 increased the root and shoot fresh weights of the mycorrhizal tomato plants, and together with *G. mosseae* it abolished the negative growth effect of *P. parasitica*. Inoculation with the bacteria also stimulated root colonization by *G. mosseae* (Table 2).

The bacterial strain B2 (gram positive) was characterized by
However, the reference strain *Paenibacillus azotofixans* in 100% of the trees obtained after bootstrap (5976T) did not show any antagonistic activity towards *P. polymyxa* confirmed the high similarity to each other at a 16S rDNA sequence analysis. The occurrence of unidentified antagonistic bacteria in pot cultures of *G. mosseae* was found in pot cultures of *S. bicolor* (Fig. 2).

The occurrence of unidentified antagonistic bacteria in pot cultures of *G. mosseae* on strawberry has recently been reported (4). In the present study, such antagonistic bacteria were found in pot cultures of *S. bicolor* inoculated with spores of *G. mosseae*, one strain of which (B2) strongly inhibited the in vitro mycelial growth of *P. parasitica* since such effects can hinder the completion of the life cycle of the plant pathogen in vivo. In addition to inhibiting the in vitro growth of *P. parasitica*, *Paenibacillus* sp. strain B2 also reduced the hyphal growth of other pathogenic fungi: *Fusarium oxysporum* Foeu1 (35.7%), *Fusarium culmorum* Fcul 1 (35.7%), *Aphanomyces euteiches* sporum 3 (35.0%), and *Rhizoctonia solani* Chaella elegans 84.1 (36.0%), *Pythium* sp. strain OP 4 (36.0%), and *Rhizoctonia solani* AG3 (53.0%). This suggests that this bacterium has a broad spectrum of antagonistic activity. Interestingly, *Paenibacillus* sp. strain B2 stimulates AM fungal root colonization and also appears to be compatible with the germination of AM fungi has a broad spectrum of antagonistic activity. Interestingly, *Paenibacillus* sp. strain B2 stimulates AM fungal root colonization and also appears to be compatible with the germination of AM fungi.

<table>
<thead>
<tr>
<th>Status of B2</th>
<th>Radial mycelium growth (cm) after 6 days</th>
<th>No. of sporangia/culture after 6 days</th>
<th>% Zoospor germation after 6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>5.3a</td>
<td>1,040a</td>
<td>62.5a</td>
</tr>
<tr>
<td>Present</td>
<td>2.1 (75.0)b</td>
<td>0 (100)b</td>
<td>25.9 (58.6)b</td>
</tr>
</tbody>
</table>

* Values in a column followed by the same letter do not differ significantly from each other at a P value of <0.05. Values in parentheses are the percents inhibition.

**TABLE 1. Effect of B2 bacteria on in vitro growth and zoosporangium production of mycelial cultures of *P. parasitica* and effect of filtered culture supernatant of B2 on zoospore germination**

<table>
<thead>
<tr>
<th>Content of inoculum (n = 7)</th>
<th>Shoot</th>
<th>Root</th>
<th>% Colonized</th>
<th>AA (%)</th>
<th>% Necrosis by <em>P. parasitica</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>16.07 bc</td>
<td>7.12 c</td>
<td>32.4 b</td>
<td>22.6 a</td>
<td></td>
</tr>
<tr>
<td><em>G. mosseae</em></td>
<td>16.44 b</td>
<td>8.08 b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B2</td>
<td>15.89 bc</td>
<td>7.90 b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>G. mosseae</em> + B2</td>
<td>17.57 a</td>
<td>9.06 a</td>
<td>43.7 a</td>
<td>28.4 a</td>
<td></td>
</tr>
<tr>
<td><em>P. parasitica</em></td>
<td>15.30 c</td>
<td>5.42 d</td>
<td></td>
<td></td>
<td>15.1 a</td>
</tr>
<tr>
<td><em>G. mosseae</em> + <em>P. parasitica</em></td>
<td>16.20 bc</td>
<td>7.18 c</td>
<td>34.2 b</td>
<td>22.7 a</td>
<td>7.2 c</td>
</tr>
<tr>
<td>B2 + <em>P. parasitica</em></td>
<td>15.49 bc</td>
<td>7.10 c</td>
<td></td>
<td></td>
<td>10.3 b</td>
</tr>
<tr>
<td><em>G. mosseae</em> + B2 + <em>P. parasitica</em></td>
<td>17.27 a</td>
<td>8.88 a</td>
<td>45.7 a</td>
<td>31.0 a</td>
<td>5.6 d</td>
</tr>
</tbody>
</table>

* Values in a column followed by the same letter do not differ significantly at a P value of <0.05.

AA, arbuscule abundance.
grown on malt agar medium at 25°C for 2 weeks. 

Parasitica (Phyt) compared with those of another bacterial isolate (B1) and the

A. euteiches

F. oxysporum

Antibes; P. parasitica script. Origins of fungal isolates were as follows:

seeds and to V. Gianinazzi-Pearson for critically reading the manu-

no. AJ011687. The nucleotide se-

quence for isolate B2 was deposited in the EMBL, GenBank, 

ment.

dual bacterial-fungal inoculation for ensuring the production 

while improving AM formation opens the possibility of using

mination and hyphal growth of G. mosseae in vitro (results not 

shown). Similar data have been reported by Barea et al. (2) for 

Pseudomonas strains.

There are several reports about the potential use of AM fungi as biological control agents against soilborne diseases (1, 5, 8, 9). The discovery of a Paenibacillus strain that can act as a 

biological control agent against soilborne fungal diseases while improving AM formation opens the possibility of using dual bacterial-fungal inoculation for ensuring the production of high-value plants in systems compatible with the environ-

ment.

Nucleotide sequence accession number. The nucleotide se-

quence for isolate B2 was deposited in the EMBL, GenBank, and DDBJ nucleotide sequence databases under the accession

no. AJ011687.

We are grateful to Bachar Blal (BIORIZE) for supplying sorghum seeds and to V. Gianinazzi-Pearson for critically reading the manu-

script. Origins of fungal isolates were as follows: P. parasitica, INRA—

Antibes; F. oxysporum, F. culmorum, R. solani, and a Pythium sp., 

INRA—Dijon; A. euteiches and C. elegans, C. Richard (Agriculture 

Canada, Quebec, Canada); and the Tobacco Institute (Bergerac, 

France).

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REFERENCES

1. Azcon-Aguilar, C., and J. M. Barea. 1996. Arbuscular mycorrhizas and bio-


4. Budi, S. W., B. Blal, and S. Gianinazzi. 1999. Surface-sterilization of spora-
carps of Glomus mosseae for studying endomycorrhization in vitro. Mycorrhi-

z-a 6:65–68.


9. Linderman, R. G. 1988. Mycorrhizal interactions with the rhizosphere mi-

10. Liu, R. J. 1995. Effect of vesicular-arbuscular mycorrhizal fungi on Verticil-

lum wilt of cotton. Mycorrhiza 5:293–297.


18. von Alten, H., A. Lindemann, and F. Schonbeck. 1993. Stimulation of vesic-

ular-arbuscular mycorrhiza by fungicides or rhizosphere bacteria. Mycorrhi-


ological and biochemical properties. Soil Science Society of America, Madison, Wis.