Effect of *Pseudomonas aeruginosa* on symbiotic association of *Glomus aggregatum* an Arbuscular Mycorrhizal Fungus

K. Amutha and V. Kokila

Department of Biotechnology, Vels University, Pallavaram, Chennai, Tamilnadu, India

Email: amutharavi40@gmail.com

**Abstract**

Interactions between *Pseudomonas aeruginosa* as biocontrol agent and *Glomus aggregatum*, an Arbuscular mycorrhizal fungus (AMF) were studied. In all the experiments, the association of *Glomus aggregatum* in *Allium cepa* with *Pseudomonas aeruginosa* was studied. Mycorrhizal activity was assessed by calculating the percentage of association of AMF with *Allium cepa*. The symbiotic activity of *Glomus aggregatum* depends on the presence of *Pseudomonas aeruginosa*. Extra and intracellular proteins of *Pseudomonas aeruginosa* tested on the symbiotic association of *Glomus aggregatum* were analyzed.

**Keywords:** *Glomus aggregatum*, *Allium cepa*, *Pseudomonas aeruginosa* and Extracellular protein

**Introduction**

Arbuscular mycorrhizal fungi (AMF) can have a large influence on plant growth by transfer of nutrients and protection of plant against pathogens (Davis *et al.*, 1986). AMF are able to colonize the plant roots and helps in taking up nutrients such as phosphorus from the soil. It has other roles such as soil aggregation, protection of plant against drought stress and soil pathogens and increasing diversity which can be reduced by AMF (Borowicz, 2000). The effectiveness of AMF in biocontrol are dependent on the AM fungal involved as well as the substrate and host plant (*Allium cepa*). *Pseudomonas* is a better biocontrol agent (Cook, 1993). Extra and intracellular proteins of *Pseudomonas aeruginosa* were isolated and tested on symbiotic associations of *Glomus aggregatum*.

Proteins of *Pseudomonas* were identified as a key determinant for root colonization (Yousef-Coronado *et al.*, 2008). Soil microorganism can be used to decrease the input of pesticides, fertilizers and chemicals. *Pseudomonas* are reported to be good root colonizers with the host plant by the formation of the nodules during endosymbiosis and attachment of bacteria to AM fungal hyphae surfaces change in fungal growth (Sen *et al.*, 1996). Beneficial interaction of plant microbe with AMF in the rhizosphere helps for the growth of the plant and makes the soil fertile.

**Materials and methods**

The mixture of roots and rhizosphere soils, clay soil at Maduravoyal near Chennai were collected. Spores were extracted from the collected soil samples by wet sieving and decanting method (Gerdemann
and Nicolson, 1963). AMF spores were identified by Morton and Msiska (1988). The total spore count was calculated by counting the number of spores per kg of soil. The spores were multiplied by using trap culture with Allium cepa as host plant. After 12 days, roots of Allium cepa were stained in tryphan blue. A percentage of mycorrhizal colonization in the roots was assessed by the gridline-intersect method (Giovannetti and Mosse, 1980).

Isolation of Pseudomonas aeruginosa from soil samples

Pseudomonas aeruginosa were isolated from soil samples by the serial dilution method. The Pseudomonas aeruginosa were identified based on physical characterization - Gram staining and the biochemical tests - Catalase test, Oxidase test, Methyl red test, Indole test, Nitrate reduction test, Citrate utilization test outlined in Bergey’s manual of determinative bacteriology (Williams and Wilkins. 1994). Pseudomonas aeruginosa were grown in Pseudomonas agar base medium.

Isolation of protein from Pseudomonas aeruginosa

Extracellular proteins were isolated by Hirose et al. (2000) (Trichloroacetic acid [TCA] / Methanol) precipitation method.

Intracellular protein

Intracellular proteins of Pseudomonas aeruginosa were isolated by the modified method of Jarvis et al. (2003).

Protein profile of Glomus aggregatum associated with Allium cepa as host plant

The isolated extra and intracellular proteins were introduced to trap cultures of Glomus aggregatum. Two weeks after mycorrhizal and non-mycorrhizal protein profile were analysed by estimating proteins by Lowry et al. (1951) and by SDS page analyses (Laemmli et al., 1970).

Table - 1. Colony Morphology, Physical and Biochemical characterization of bacterial isolates from soil samples

<table>
<thead>
<tr>
<th>S. NO</th>
<th>Cultural Characteristics</th>
<th>Bacterial isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>1.</td>
<td>Colony Morphology</td>
<td>Small, Pigmented circular, Flat, Entire, dry colonies</td>
</tr>
<tr>
<td>2.</td>
<td>Gram’s Staining</td>
<td>Gram negative</td>
</tr>
<tr>
<td>3.</td>
<td>Motility</td>
<td>Active Mobile</td>
</tr>
<tr>
<td>4.</td>
<td>Catalase</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Oxidase</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Methyl Red</td>
<td>-</td>
</tr>
<tr>
<td>7.</td>
<td>Indole</td>
<td>+</td>
</tr>
<tr>
<td>8.</td>
<td>Citrate Test</td>
<td>+</td>
</tr>
<tr>
<td>9.</td>
<td>Nitrate Test</td>
<td>+</td>
</tr>
</tbody>
</table>

+ Positive; − Negative

Fig. – 1. Spores of Glomus aggregatum

Fig. – 2. Pseudomonas aeruginosa isolates from soil samples
Results and Discussion

AMF spore were collected from Maduravoyal near Chennai (per 1 kg of soil). *Glomus aggregatum* spores were identified (Fig. -1.) (www.invam.com). With reference to colonial morphology, physical characterization and biochemical test as outlined in the Bergey’s manual of determinative bacteriology the bacteria were identified as *Pseudomonas aeruginosa* and the results are presented in Table - 1 and Fig. - 2. *Glomus aggregatum* trap cultures were developed in Glass house conditions (Fig. - 3). Tryphan blue stained arbuscules, vesicles, and hyphae were observed in trap

<table>
<thead>
<tr>
<th>Trap plants</th>
<th>Protein (mg/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control plant</td>
<td>1.2</td>
</tr>
<tr>
<td><em>Glomus aggregatum</em> associated with <em>Allium cepa</em></td>
<td>1.6</td>
</tr>
<tr>
<td>Trap culture of <em>Glomus aggregatum</em> treated with extracellular protein of <em>Pseudomonas aeruginosa</em></td>
<td>2.1</td>
</tr>
<tr>
<td>Trap culture of <em>Glomus aggregatum</em> treated with intracellular protein of <em>Pseudomonas aeruginosa</em></td>
<td>1.8</td>
</tr>
</tbody>
</table>

Table – 2: Protein content of Control and Test plant Roots

Fig – 3: Trap Culture *Allium cepa*

C - Control; (b) T1 - *Glomus aggregatum* associated with *Allium cepa* (c) T2 - Trap culture of *Glomus aggregatum* treated with extracellular protein of *Pseudomonas aeruginosa* (d) T3 - Trap culture of *Glomus aggregatum* treated with intracellular protein of *Pseudomonas aeruginosa*

Fig – 4.

(a) Arbuscules (40x) ; (b) *Glomus aggregatum* associated with *Allium cepa* root (100x) ; c) *Glomus aggregatum* treated with extracellular protein of *Pseudomonas aeruginosa* root (40x); (d) *Glomus aggregatum* treated with intracellular protein of *Pseudomonas aeruginosa* root (40x)

Fig – 4. *Glomus aggregatum* associated with *Allium cepa* root stained in tryphan blue

Fig – 4. e. *Glomus aggregatum* associated with *Allium cepa* root stained in tryphan blue
roots of *Allium cepa* (Fig. - 4) and the percentage of *Glomus aggregatum* association was observed (Fig. - 5). Proteins isolated from extra and intracellular proteins of *Pseudomonas aeruginosa* treated with *Glomus aggregatum* associated plants. Protein concentration were calculated and expressed in mg/100 ml and the results are shown in Table – 2 and Fig. – 6. In the control root, the protein band on SDS PAGE gel found to be 58kDa. Similarly the size of the protein from *Glomus aggregatum* associated roots, the proteins from extra and intracellular treated proteins of *Pseudomonas aeruginosa* were 58 kDa, 64 kDa and 41 kDa respectively. In this study, *Glomus aggregatum* has shown very good association with extracellular proteins of *Pseudomonas aeruginosa*. Pseudomonas produce an antifungal compounds, but it is not to be active against AM fungi (Burla et al., 1996). Combinations of AMF associated with bacteria are essential living components of the soil microbiota (Dharma Parkash Bharadwaj et al., 2008). This shows a mutualistic relationship between Pseudomonas and *Glomus aggregatum*.

**Fig. – 5. Percentage of association of *Glomus aggregatum* with *Allium cepa***

S.No 1 - Control (b) S.No 2- *Glomus aggregatum* associated with *Allium cepa* root (c) S.No 3 - Trap culture of *Glomus aggregatum* treated with extracellular protein of *Pseudomonas aeruginosa* (d) S.No 4 - Trap culture of *Glomus aggregatum* treated with intracellular protein of *Pseudomonas aeruginosa*.

**Fig. – 6. Protein profiling of Control and Test Plant Roots by SDS-PAGE***

L1 - Control Plant Root Protein of *Allium cepa*; L2 - *Glomus aggregatum* associated with *Allium cepa*; L3 - Proteins from treated with extracellular protein of *Pseudomonas aeruginosa*; L4- Protein Marker e) L5 - Proteins from treated with intracellular protein of *Pseudomonas aeruginosa*.

**Acknowledgement**

This research was supported by Vels University Research grant.

**References**


