Impact of *Pseudomonas pseudoalcaligenes* on growth of *Solanum lycopersicum* and *Oryza sativa*

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**ABSTRACT**

Plant macro-micro nutrients are essential elements for healthy plant growth and thereby food supplies. Depletion of plant nutrients may lead to severe agricultural as well as environmental problems, such as insufficient crop yield. A soil in many agricultural areas suffers from most micronutrient deficiencies. Soil microbial activity in rhizosphere may influence the growth of higher plants by various processes. *Solanum lycopersicum* and *Oryza sativa* plants were inoculated with siderophore producing *Pseudomonas pseudoalcaligenes* improve plants efficiency in nutrients acquisition. Differences in vascular tissue development and number and diameter of xylem vessels observed as the result of application of siderophore producing microorganism *Pseudomonas pseudoalcaligenes* in anatomy of plants. *Pseudomonas pseudoalcaligenes* also showed biocontrol activity against pathogenic fungi, *Alternaria* sp., *Fusarium oxysporum*, and *Fusarium* sp. The present observation showed that *Pseudomonas pseudoalcaligenes* is capable of cope out the acquisition problem of micronutrient in plants.

**Keywords:** Siderophore, *Fusarium oxysporum*, Micronutrient uptake, Biocontrol, Plant growth

1. **INTRODUCTION**

Sustainable agriculture is essential to a healthy adequate food supply. Plants need the right combination of nutrients to live, grow and reproduce. When plants suffer from malnutrition, they show symptoms of being unhealthy. Too little or too much of any one nutrient can cause problems. The productivity of agriculturally important plants is often limited by the availability of essential mineral nutrients in the soil. The rhizosphere is an incredibly complex environment, harbors a multitude of microorganisms which play a crucial role in maintaining an adequate supply of plant nutrients for plant growth [1].

Siderophore producing microorganisms enhance nutrient uptake by production of iron chelating agents called siderophore. Siderophore is a metabolic product of microorganism which binds iron and facilitates its transport from the environment into the microbial cell. [2] Plants produce various type of phytosiderophore, but it is not effective to take up its own siderophore as compare to microbial siderophore. Therefore, providing plants with accessible forms of iron is necessary when it is scant or unavailable in soils [3]. Siderophores low molecular chelators have high affinity to Fe3+, but they can also make complexes with other metal ions such as Zn, Cu, Mn, Cd, Cr, Ni and Al.
Siderophore producing microorganisms can also play a pivotal role in uptake of micronutrients by modifying the root morphology, resulting in greater root surface area for the uptake of nutrients within the soil, and also protect crops against disease [4].

Phytopathogenic microbes have an immense impact on agricultural productivity, greatly reducing crop yields and sometimes causing total crop loss. Every year, severe global economic losses to agricultural crops are encountered due to plant diseases caused by more than sixty pathogens leading to the loss of 30% crop yield [5]. Biocontrol through siderophore-mediated competition for iron have merged as a sustainable approach for integrated plant disease management [6-10]. Effective options include employing the pathogen’s natural enemies as biological control agents.

Starvation is the most common cause of death for microorganisms, so that competition for limiting nutrients results in biological control of fungal phytopathogens [11]. In most filamentous fungi, iron uptake is essential for viability and under iron starvation; most fungi excrete low-molecular-weight ferric-iron specific chelators, termed siderophores, to mobilize environmental iron [12]. Some microorganisms produce highly efficient siderophores that chelate iron and stop the growth of other fungi [13]. Fusarium oxysporum by competing for both rhizosphere colonization and nutrients, with biocontrol becoming more effective as the nutrient concentration decreases. This mechanism depends on the role of siderophores as competitors for Fe in order to reduce the Fe availability for the phytopathogens [14].

The present study is intended to focus on the emergence of agriculturally important microorganisms to develop an ideal agricultural system through efficient utilization of nutrients and to investigate bio-control activities of Pseudomonas pseudoalcaligenes against various soil borne plant pathogens including Alternaria sp., Fusarium oxysporum, and Fusarium sp.

2. MATERIALS AND METHODS

2.1. Isolation of Siderophile producing microorganism

Pseudomonas pseudoalcaligenes was isolated from the rhizospheric soil of pigeon pea (Cajanus cajan) plant and identified by 16S rDNA ribotyping as is discussed in Gamit and Tank et al., 2014 [15].

Siderophore production by Pseudomonas pseudoalcaligenes was tested qualitatively by Chrome Azural S (CAS) plate assay [16].

2.2. Bacterization of seeds

Bacterization of tomato (Solanum lycopersicum) and rice (Oryza sativa) seeds was performed according to Russel and Khalid [17, 18]. Seeds were surface-disinfected by dipping in 95% ethanol and 0.2% (w/v) HgCl₂ solutions and rinsed thoroughly with sterilized water. Sterilized seeds were treated for 20 min with siderophore (90% units) rich broth of Pseudomonas pseudoalcaligenes (10⁸ cells mL⁻¹) grown in SM (Succinic acid Medium) for 72 h then the seeds were removed and allowed to dry.

2.3. Pot experiment

Treated seeds were sown in pot containing sterile soil. Water was added in equal quantity in the pots as per daily requirement and effect of siderophore producing Pseudomonas pseudoalcaligenes on Tomato and Rice plants germination and plants growth observed with respect to control after 10 days and 90 days. The treatment was arranged in a randomized block design with three replicates for each treatment.

2.4. Plant analysis

Plants harvested and carefully separated into roots, shoots. The plants root and shoot were oven-dried at 60°C for 48 h. The ground fine powder digested with nitric acid APHA standards Method 3030 G [19] and used for the analysis of micro-nutrients Fe, Cu, Mn, Zn, Co, Ni [Agilent Technologies (200series AA) Flame Atomic Absorption Spectroscopy (FAAS)].

2.5. Statistical analysis

In all the studies, statistical procedures were applied to analyze the data using IBM SPSS statistics 21 Premium x86.

2.6. Antagonism in vitro

Antagonistic properties of Pseudomonas pseudoalcaligenes against plants pathogenic fungi, Fusarium oxysporum, Fusarium udum and Alternaria alternata were tested on PDA plates using a dual culture technique [20]. Five day old mycelia discs (6 mm diameter) were placed in the 2 cm² from edge of PDA plates. Exponential phase cultures of Pseudomonas pseudoalcaligenes were streaked 2 cm opposite from the fungal disc and incubated at 30°C for 5 days. Growth inhibition was calculated by
measuring the distance between the edge of bacterial and fungal colonies as compared to the control (without bacteria). The zone of inhibition was recorded using the formula:

\[
\text{Inhibition (\%) = \frac{(C-T)}{C} \times 100,}
\]

Where “C” is the maximum growth of the fungal mycelia under control conditions and “T” is fungal mycelia growth in dual culture.

2.7 Anatomical Study

Anatomy of plant was done with 10 day grown plant to observe for the development of the vascular bundles and number and diameter of xylem vessels as the result of application of siderophore producing microorganism.

2.8 Effect of Bacterization on anatomical behavior of plants

*Pseudomonas pseudoalcaligenes* effective siderophile producing isolate treated plants, *Solanum lycopersicum* and *Oryza sativa* were investigated by means of anatomical analysis under Carl-Zeiss Trinocular Research Fluorescence Microscope Model Axio SCOPE-AL.

3. RESULTS AND DISCUSSION

3.1 Identification of Siderophore producing microorganism

Phylogenetic analysis based on 16S rRNA gene sequences revealed that isolated strain shared > 99 \% sequence similarity with that of the gene sequences available at the nucleotide data base. As shown in Fig. 1 the 10 strains grouped together with different type strains and closest hit strains of *Pseudomonas pseudoalcaligenes* (Accession nos. KF581137) and formed a separate cluster to other closely-related strains.

3.2 Effect of Bacterization on plants growth.

*Pseudomonas pseudoalcaligenes* was highly effective in promoting the root length and root dry weight of Tomato (*Solanum lycopersicum*) and Rice (*Oryza sativa*). In addition to improving roots length, it also effect on shoot growth by production of siderophore. A significant effect was observed on root and shoot growth of both plants, compare to control. The total chlorophyll content of *Solanum lycopersicum* and *Oryza sativa* was also significantly enhanced, compare to control. (Figure: 2, 3 & Table 1)

3.3 Measurement of micronutrients in plant root and shoot

Tomato (*Solanum lycopersicum*) and Rice (*Oryza sativa*) plants were dried and ground and the digested samples were used for analysis of micronutrients Fe, Cu, Mn, Zn, Co, Ni, concentration with an atomic absorption spectrophotometer. PGPR play an important role in solubilization of nutrients from soil and enhancing their availability to plants [21]. In our study, *Pseudomonas pseudoalcaligenes* inoculation also significantly increased root uptake of all micronutrients led to an enhancement in the plant root and shoot growth (Fig: 4, 5& Table 2, 3).

3.4 Antifungal activities against phytopathogenic fungi

Siderophore producing *Pseudomonas pseudoalcaligenes* was also checked for antagonistic activity against *Fusarium oxysporum* (MTCC 10278), *Fusarium udum* (MTCC 2204) and *Alternaria alternata* (MTCC 9617) on PDA medium plate. Fungi used in these studies were obtained from Microbial Type Culture Collection and Gene Bank (MTCC). Siderophore production in iron stress conditions gives microorganism an additional benefit, ensuing in the elimination of pathogens because of iron starvation [22, 23]. Siderophores produced by isolates chelate available iron and therefore create artificial shortage of iron to the respective phytopathogens thereby limiting their growth [24]. *Pseudomonas pseudoalcaligenes* inhibited the mycelial growth of *F.oxysporum*, *F.udum* and *A. alternate* (Figure:6).

*Pseudomonas pseudoalcaligenes* exhibited the maximum inhibition of mycelial growth of *A. alternata* (32.00 mm) compared with control (65.4 mm). *Pseudomonas pseudoalcaligenes* exhibited 56.7\%, 66.4\% and 51.0\% growth inhibition of *F. oxysporum*, *F. udum* and *A. alternare* respectively (Table: 4). In vitro phytopathogen suppression by *Pseudomonas pseudoalcaligenes* indicated their biocontrol potential. Siderophore rich culture cell free supernatants were found to inhibit the growth of phytopathogenic fungi namely *F. oxysporum*, *F. udum* and *A. alternare*. The presence of siderophoregenic rhizobacteria around root zone of plants is known to protect the plant from phytopathogen infestations by preventing its iron nutrition [10].
**Figure 1.** Phylogenetic analysis based on alignment using the neighbour-joining method for 16S rDNA sequences of (filled yellow color) isolated strain with closest hit strains of *P. pseudoalcaligenes* with BLAST search.

**Figure 2.** Influence of *P. pseudoalcaligenes* inoculation on growth of *Solanum lycopersicum* plant (after 90 Day).
Figure 3. Influence of *P. pseudoalcaligenes* inoculation on growth of *Oryza sativa* plant (after 90 Day).

Figure 4. Interactive effects of siderophore producing microorganism on the uptake of *Solanum lycopersicum* root-shoot A) Fe B) Cu C) Mn D) Zn E) Co F) Ni.
Table 1. Effect of inoculation with siderophore producing microorganisms on growth of *Solanum lycopersicum* and *Oryza sativa*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Plant height (cm)</th>
<th>Root length (cm)</th>
<th>Root fresh weight (g plant-1)</th>
<th>Root dry weight (g plant-1)</th>
<th>Chlorophyll content (mg/g of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Solanum lycopersicum</em></td>
<td></td>
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<tr>
<td>Control</td>
<td>45.76 ± 0.040</td>
<td>6.73 ± 0.026</td>
<td>16.00 ± 0.010</td>
<td>4.426 ± 0.025</td>
<td>1.71 ± 0.015</td>
</tr>
<tr>
<td>Bacterization</td>
<td>76.30 ± 0.167</td>
<td>32.07 ± 0.064</td>
<td>35.05 ± 0.045</td>
<td>27.32 ± 0.020</td>
<td>2.66 ± 0.010</td>
</tr>
<tr>
<td><em>Oryza sativa</em></td>
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</tr>
<tr>
<td>Control</td>
<td>28.87 ± 0.0251</td>
<td>12.23 ± 0.1527</td>
<td>13.68 ± 0.015</td>
<td>3.10 ± 0.005</td>
<td>1.85 ± 0.008</td>
</tr>
<tr>
<td>Bacterization</td>
<td>35.60 ± 0.0064</td>
<td>15.28 ± 0.0115</td>
<td>20.13 ± 0.118</td>
<td>5.81 ± 0.012</td>
<td>2.97 ± 0.017</td>
</tr>
</tbody>
</table>

Table 2. Effect of with and without siderophore producing microorganism on the microelements uptake in root and shoot of Tomato (*Solanum lycopersicum*) as measured in the pot studies.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Pot experiment</th>
<th><em>P. pseudoalcaligenes</em></th>
<th>Fe</th>
<th>Cu</th>
<th>Mn</th>
<th>Zn</th>
<th>Co</th>
<th>Ni</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Root</em></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>11.5 ± 0.0004</td>
<td>0.04 ± 0.0002</td>
<td>0.75 ± 0.0103</td>
<td>0.38 ± 0.0008</td>
<td>0.04 ± 0.0022</td>
<td>0.06 ± 0.0004</td>
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<tr>
<td>Bacterization</td>
<td>28.4 ± 0.0074</td>
<td>0.14 ± 0.0009</td>
<td>1.46 ± 0.0017</td>
<td>0.63 ± 0.0033</td>
<td>0.08 ± 0.0004</td>
<td>0.08 ± 0.0027</td>
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<tr>
<td><em>Shoot</em></td>
<td></td>
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<tr>
<td>Control</td>
<td>57.0 ± 0.0029</td>
<td>0.14 ± 0.0012</td>
<td>2.62 ± 0.0056</td>
<td>0.45 ± 0.0004</td>
<td>0.26 ± 0.0002</td>
<td>0.47 ± 0.0018</td>
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<tr>
<td>Bacterization</td>
<td>119.1 ± 0.0020</td>
<td>1.04 ± 0.0017</td>
<td>6.51 ± 0.0092</td>
<td>6.80 ± 0.0145</td>
<td>0.43 ± 0.0017</td>
<td>0.80 ± 0.0005</td>
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Table 3. Effect of with and without siderophore producing microorganism on the microelements uptake in root and shoot of Rice (*Oryza sativa*) as measured in the pot studies.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Pot experiment</th>
<th><em>P. pseudoalcaligenes</em></th>
<th>Fe</th>
<th>Cu</th>
<th>Mn</th>
<th>Zn</th>
<th>Co</th>
<th>Ni</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Root</em></td>
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<td></td>
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<tr>
<td>Control</td>
<td>26.62 ± 0.001</td>
<td>0.37 ± 0.0002</td>
<td>0.85 ± 0.0013</td>
<td>0.37 ± 0.0027</td>
<td>0.13 ± 0.0014</td>
<td>0.12 ± 0.0008</td>
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<tr>
<td>Bacterization</td>
<td>74.16 ± 0.0003</td>
<td>0.14 ± 0.0009</td>
<td>13.81 ± 0.0008</td>
<td>0.98 ± 0.0015</td>
<td>0.28 ± 0.0008</td>
<td>0.21 ± 0.0008</td>
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<tr>
<td><em>Shoot</em></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>109.2 ± 0.0010</td>
<td>0.28 ± 0.0000</td>
<td>2.81 ± 0.0005</td>
<td>0.566 ± 0.0007</td>
<td>0.13 ± 0.0011</td>
<td>0.08 ± 0.0009</td>
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<tr>
<td>Bacterization</td>
<td>195.9 ± 0.0004</td>
<td>0.96 ± 0.0000</td>
<td>8.52 ± 0.0008</td>
<td>1.149 ± 0.0002</td>
<td>0.72 ± 0.0003</td>
<td>0.73 ± 0.0005</td>
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</table>
**Figure 5.** Interactive effects of siderophore producing microorganism on the uptake of *Oryza sativa* root-shoot. A) Fe, B) Cu, C) Mn, D) Zn, E) Co, F) Ni.

**Figure 6.** Antifungal activity of siderophore rich supernatant of *Pseudomonas pseudoalcaligenes*. (A) *F. oxysporum*, (B) *F. udum*, (C) *A. alternate*. Growth of the fungal mycelia under control conditions (D) *F. oxysporum*, (E) *F. udum* and (F) *A. alternate*. 
3.5 Effect of Bacterization on anatomical behaviour of plants.

Rice plant anatomy also indicated that treatment given by siderophore producing microorganism very effective in plant growth. In our study young rice treated plant anatomy showed the differences in vascular tissue development when compare to control. In figure – 7 transvers section (T.S) of control stem showing a rolled up leaf blade being enclosed by an older leaf sheath and development of vasculare bundles while treated stem shows well developed vascular bundles. Well-developed vascular tissues and bulliform observed in treated T.S of rice leaf compare to control (Figure 7). Figure 7 (G) and 7 (H) shows anatomy of tomato plant stem. Vascular tissue development in stem and root is well developed in treated tomato plant compare to control.

4. CONCLUSION

There is a growing demand for an economical and feasible approach in agriculture that might be able to provide adequate supply of nutrients for healthy plant growth and thereby food supplies through improvement of the quality and quantity of agricultural products. In vitro Pseudomonas pseudoalcaligenes was found effective for plant growth by providing micronutrients; it also showed good biocontrol potential through siderophore-mediated competition for Iron, emerging as a sustainable approach for integrated plant disease management. So, Pseudomonas pseudoalcaligenes can be implied for eliminating problems associated with the use of chemical fertilizers and pesticides in natural farming and organic agricultural practices.

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Conflicts of Interest
There are no conflicts of interest.

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