THE EFFECTS OF HELPING BACTERIA IN NITROGEN BEANS 
FIXATION AND NODULATION WITH RHIZOBIUM 
LEGUMINOSARUM BV.PHASEOLI

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Abstract

Some batteries settle in the Rhizosphere of legume plants and enhance the performance of ribosome batteries to 
nitrogen fixation and nodulation. In this paper, we used four isolated from two species of Sodonamous containing 
P.putida, P.flourescence Chao, P.Flourescence Tabriz, P.flourescence B119 and Rhizobium leguminosarum 
by.phaseoli. In a factorial experiment with complete accidental blocks base and 5 levels of helping bacteria and two 
Rhizobium levels were inoculated with four repetition of Jamaran418 green bean. At the end, nodulation indexes, 
growth and plant's nitrogen indexes were measured. The results showed that all above mentioned helping bacteria 
enhance the growth and performance of green bean. It should be said that P.putida had 130% effect on the green 
bean nodulation increase along with Rhizobium and it was 83, 63 and 17% about P.Flourescence Tabriz, P. 
florescence Chao and P. florescence B119, respectively. Also, we observed 45, 33, 22 and 8% performance increase 
under the effect of P.putida, P. florescence Chao, P.Flourescence Tabriz and P. florescence B119, respectively.

Keywords: helping bacteria, Rhizobium, green bean, nodulation, Sodonamous

Introduction

The widespread use of chemical fertilizers and pesticides is discussed a lot in today world because of 
their adverse effects on the environment and on the health risks of living. The natural process of 
rhizobacters is a suitable way for plants protection which enhances the growth and protection of plants 
(Delip Koomar, 2001).

Private and public investment has increased to mass production of biologic fertilizers and especially 
microbial ones after the more attention in this area. Rhizobium inoculants are one of the most important 
and widespread of these fertilizers which are used for different legume plants.

Along with the extensive researches in Iran and world to introduce the most efficient ways of these 
bacteria performance, further research is underway to improve the quality of these fertilizers. Some 
evidences show that the bacteria like Sodonamous, Strepto misses and bacilious as a helping one 
enhance the nodulation and fixation performance of Rhizobium bacteria (Ming and Alexander, 1988). The results are patent and the Rhizobium inoculants producers companies access them.

Some PGPR bacteria with synergistic effect on the Rhizobium can enhance the nodulation and fixation of nitrogen in legumes. According to conducted researches, most of the bacteria which are effective in 
this field belong to Phosphate solubilizing microorganisms (Pisney and Azcon, 1987 and Ming 
and Alexander, 1988). Rasi poor and Ali Asgarzade (1381) investigated the role of four phosphates 
solubilizing and isolated bacteria, p.flourescens, P.putida and Areomonas hedrophila with 
Bradyrhizobium japonicum on the soya nodulation and performance and concluded that these bacteria 
enhance the phosphor absorption in soya, comparing with control group. The results show that P.putida 
treatment had the most effect on the soya nodulation, nitrogen fixation and phosphor absorption, 
comparing with the other treatments along with Bradyrhizobium japonicum.

Ali khani and Saleh Rastin (2006) announced that the Phosphate solving bacteria enhance the per 
formance of different rhizubioms in Iran soils.

Rozras et al (2002) had done a farm experiment on the soya and investigated the mutual effects between 
soya bacteria and Sodonamous Piotida phosphate solving bacteria. They reported a significant increase 
in the stem nodulation, Nitrogen fixation and soya...
shoot weight in the inoculation. Also in a farm experiment in India, the effect of phosphate solving bacteria, Sodonamous Steriata on the Beradi Rhizobioum Gaponickom and soya symbiosis and some measures like nodules dry weight and plant dry weight were investigated. Their joint inoculation with Sodonamous Sterila resulted in the significant increase of nodule dry weight and number (Vasole et al, 2002).

Rhizosphere bacteria always do not enhance the nitrogen fixation and nodulation by legumes and sometimes can have negative or neutral effects on these indexes. Pan et al (2002) investigated the effects of two Rhizosphere bacteria (Serratia Proteamawlans and Serratia liguefaciens along with Bradyrhizobium japonicium Strain 5326 on the two soya spices and showed a significant difference of the plant performance treated with Rhizosphere bacteria, but these bacteria had no significant effect on the nitrogen fixation and nodulation. Anjela Bergern et al (2001) had conducted an experiment in Sweden and investigated the effects of P.putida strain A313 symbiosis with Pea symbiotic Rhizobioum bacteria and the results showed the reduction of nitrogen with P.putida strain A313.

We should consider these items more due to increased production of Rhizobium inoculants in the country as a good replacement for nitrogen fertilizers and lack of their efficiency in the farm and do extensive researches to enhance their quality. This study introduces the most effective bacteria between four helping items.

Materials and Methods

Proliferation of used bacteria

In this experiment, we used P.florescens B119 (H2), P.flouresence Chao (H1) (Tehran Plant Pests and Diseases Research Institute), P.flourescence Tabriz (H3) (Tabriz university soil biology laboratory) and one isolated from P.putida (H4) (Tabriz isolated) as the helping bacteria and control ones (H0) along with leguminosarum bv.phaseoli Rhizobium. King B culture media was used to Sodonamous proliferation and YEMB was for Rhizobioum proliferation.

Those culture media were liquid. Sodonamous flasks were kept for 2 days in shaker incubator in 28 °C and 120 rpm until to bacteria growth and Rhizobioum flasks were in this condition for 3 days.

We used turbidimetry and Mac Farland to identify the number of bacteria in the suspension.

Microbial carrier preparation

First, we milled processed vermiculite and passed it from 100 micrometer sieve. Its 50 g was transported to each flask and was sterilized.

Microbial suspension inoculation on the carrier

12 ml from each microbial suspension with clear population was inserted in flasks containing material and were stirred. The carrier material got wet but kept the powder tissue and another flask containing 12ml sterile culture media was considered as control treatment.

Select and prepare the soil for the greenhouse experiment

We used the soil from agriculture faculty earth in Karkaj. They were placed in plastic pots after passing from 4mm sieve and were sterilized in autoclave for 2 hours.

Preparation treatments and planting pot

After determining the required amount of soil phosphorus and potassium fertilizer recommendations based on soil test and the beans, and calculate the required amount of fertilizer was applied.

To determine the amount of nitrogen-fixing symbiosis with Rhizobium method, no nitrogen was added to the soil in the pot. Green bean seeds were soaked in distilled water for 2 hours and then were placed in 95% ethanol for 30 seconds and immediately after that were put in 0/1% acidity Mercuric chloride for 15 minutes. Then it was put in a solution of 1% sodium hypochlorite for 30 minutes, until the seeds surface to be disinfected. Then it was washed 5 times with sterile distilled water and was put in Petries containing water-agar for 3 days. To create the same condition for all of the pots, they were moved every 2 days. Plants until the pods and seeds were grown for 75 days.

Experimental design and statistical analysis

In a factorial experiment with complete accidental blocks base and 5 levels of helping bacteria and two Rhizobium levels were inoculated with four repetition of Jamaran418 green bean. At the end,
nodulation indexes, growth and plant’s nitrogen indexes were measured. We used variance analysis and comparing their means via MSTATC. Comparing the means was done with Don Ken test in 5% probability. The graphs were drawn by Excel.

Vitro biochemical tests

Sperber culture media was prepared to measure the quantity ability of bacteria for the dissolution of phosphate. The culture media was sterilized in 1/5 pressure atmosphere for 20 minutes and 121 °C in autoclave. The medium was distributed in sterile petri dishes. The bacteria were grown on solid medium using a sterile wooden toothpick into each petri was spot on. After putting in 48 hours of incubation, colony diameter and the diameter of the clear zone around it indicates that the dissolved phosphate was measured.

Measurement potential of siderophore production by bacteria

We used Chrome azurol-s culture media for this purpose (Bernhard et al, 1987).

10 ml of abovementioned media and 90 ml of King’s B were sterilized in autoclave and then Chrome azurol-s was added to the culture media and the final material was aired in the Petries. After 48 hours, colony diameter and the diameter was measured and the ratio of the diameter to the colony diameter (Bernhard et al, 1987).

Germination and seedling growth of helpful bacteria in the presence of green beans and Rhizobium

First, the bacteria suspensions were prepared.

0/8% water-agar was transferred in the petri dishes after sterile. The culture media was sterilized in 1/5 pressure atmosphere for 20 minutes and 121 °C in autoclave. The medium was distributed in sterile petri dishes. The bacteria were grown on solid medium using a sterile wooden toothpick into each petri was spot on.

The time required for maximum germination/ %

Maximum number of germinated seeds= Germination rate (% per day)

Results and Discussion

Physical and chemical analysis of soil used in pot culture experiment is described in Table 1.

Number, fresh weight and dry weight of nodules

Mean comparison shows that all helping bacteria except P.flourescens B119 increased the bean nodulation. P.putida had the more number of nodules, fresh weight and dry weight in the bean.

P.putida had the nitrogen percent and the least percent belonged to the control treatment without bacteria.

Shoot weight

The treatments with Rhizobioum and helping bacteria had the driest weight. P.putida with R.I. bv.phaseoli created the driest weight and wet one of shoot. Figures 2 and 3, the difference in the level of 5%, were significantly different from other treatments and control.

<table>
<thead>
<tr>
<th>pH</th>
<th>ECe(s/cm)</th>
<th>Organic carbon %</th>
<th>Neutral matter %</th>
<th>Moisture of Field capacity %</th>
<th>Nitrogen %</th>
<th>Available Phosphor</th>
<th>Available Potassium</th>
<th>Clay %</th>
<th>Silt %</th>
<th>Sand %</th>
<th>tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>7/56</td>
<td>948</td>
<td>0/6</td>
<td>9/1</td>
<td>15</td>
<td>0/12</td>
<td>5/4</td>
<td>232</td>
<td>24</td>
<td>21</td>
<td>55</td>
<td>Loamy sand</td>
</tr>
</tbody>
</table>

Phosphor:method of Olsen (Na2 CO3,0/5 Molar and PH=8/5) [10]  
Potassium: Ammonium acetate(one normal and PH=7) [10]  
Organic carbon: method of Valkely black [10]
Table 2. Mean comparing the effect of helping bacteria on the nodulation of green bean root

<table>
<thead>
<tr>
<th>Helping bacteria</th>
<th>Phosphate solving</th>
<th>Sydrophore production</th>
<th>Diameter of halo / the colony</th>
<th>Diameter of halo / the colony</th>
<th>Number of Nodule per plant (gram)</th>
<th>Wet weight Nodule per plant (gram)</th>
<th>Dry weight Nodule per plant (gram)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>c0/325</td>
<td>d*19/75</td>
<td></td>
<td></td>
<td>b0/024</td>
<td>c0/325</td>
<td>c0/325</td>
</tr>
<tr>
<td>P. fluorescens B119</td>
<td>cd23</td>
<td></td>
<td>ab0/079</td>
<td>a0/405</td>
<td>ab0/086</td>
<td>ab0/942</td>
<td>ab0/942</td>
</tr>
<tr>
<td>P. fluorescens TABRIZ</td>
<td>ab36/5</td>
<td></td>
<td>a0/122</td>
<td>a1/273</td>
<td>ab0/86</td>
<td>bc0/782</td>
<td>bc0/782</td>
</tr>
<tr>
<td>P. putida</td>
<td>a45/5</td>
<td></td>
<td>bc31/5</td>
<td>ab0/84</td>
<td>P. fluorescens CHAO</td>
<td>bc31/5</td>
<td>bc31/5</td>
</tr>
</tbody>
</table>

Different letters in each column indicate a significant level of 5% is likely.

Table 3. Mean comparing of biochemical tests in helping bacteria

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Phosphate solving</th>
<th>Sydrophore production</th>
<th>Diameter of halo / the colony</th>
<th>Diameter of halo / the colony</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. fluorescens B119</td>
<td>bc2/713</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. fluorescens TABRIZ</td>
<td>b3/585</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. putida</td>
<td>a4/475</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. fluorescens CHAO</td>
<td>c1/974</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Different letters in each column indicate a significant level of 5% is likely.

Figure 1. Rhizobium bacteria and bacterial adjuvant treatment combination effect of nitrogen on shoot.
Figure 2: Rhizobium bacteria and bacterial adjuvant treatment combination effect of nitrogen on shoot weight.

Figure 3: Rhizobium bacteria and bacterial adjuvant treatment combination effect of nitrogen on dry weight.
Weight of 100 seeds per plant

The treatments with Rhizobium and helping bacteria had the driest weight.

P. putida with R. l. bv.phaseoli had the most performance in this field, but had a significant difference with the other bacteria.

The results of biochemical tests on helping bacteria

Siderophore production

Colony diameter and the diameter was measured and the ratio of the diameter to the colony diameter. There was a significant difference between P. flourescens CHAO and the other ones.

Mean comparison shows that all helping bacteria except P. flourescens B119 increased the bean nodulation. P. putida had the more number of nodules, fresh weight and dry weight in the bean.

Discussion

The results show that the helping bacteria with R. l. bv.phaseoli had a significant effect on the most of the traits and enhanced their performances. P. putida and R. l. bv.phaseoli had the most effect on the nodulation, nitrogen percent, phosphor percent and plant performance.

Also, we can say that the solving power of the phosphate by P. putida and P. flourescens TABRIZ enhance the nodulation, nitrogen fixation and green bean performance with R. l. bv.phaseoli. But as the results show P. flourescens B119 has the most power of phosphate solving. Anjela Bergern et al (2001) had conducted an experiment in Sweden and investigated the effects of P. putida strain A313 symbiosis with Pea symbiotic Rhizobioum bacteria and the results showed the reduction of nitrogen with P. putida strain A313. It should be mentioned that the positive effects of the helping bacteria on growth and legumes nodulation is not limited to the abovementioned mechanisms. Because, Sodonamous has a lot of positive effects such as: HCN production, ACC-D aminase activity, oxen production, anti biotic production.

References


