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Effect of Phosphorous Mobilizing and N2 Fixing Bacteria on Growth of Plant

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A B S T R A C T

The aim of our study is to clarify inoculating a soil with Bacillus and Rhizobium bacteria would stimulate the enzymatic cleavage organic phosphorus and nitrogen compound in the rhizosphere, promoting plant growth. Adding four treatment: 1) Viable Bacillus and Rhizobium. 2) Heat treated Rhizobium and Bacillus. 3) Bacterial mix with Rhizobium and Bacillus .4) unselectively cultivated soil bacteria. We had done rhizobox experiment with greenhouse effect and plant were grown under low phosphorous and nitrogen condition. A plant growth promotion effect with improved P and N2 observed in both viable and bacterial mix Rhizobium and Bacillus treatment. Inoculation of bacteria increased microbial phosphate and nitrogen activity in rhizosphere. Plant growth promotion is primarily associated with increased microbial phosphorus activity(PA) and nitrogen activity (NA) in soil.

Keywords: Phosphorus-mobilizing bacteria, Nitrogen Fixing bacteria, Plant growth promotingbacteria, SolanumLycopersicon, Bacillus and Rhizobium

1. Introduction

It is well known that rhizosphere processes are necessary for plant P acquisition, the processes underlying growth promotion by beneficial microorganism are even not well understood. Since organic P and N2 are often the dominant forms P and N in soil; and may constitute up to 90% of the total P and N2 in soil. For conversion of organic P into plant available form, the P mineralization is a pre-requisite. The extracellular Phosphates produced by microorganism and plants catalyzes mineralization of P. Microorganism produces acid as well as alkaline phosphatases, whereas plants produce only acid phosphatases. Microbial and plant P acquisition occur in different zones of rhizosphere. Plant uptake of P occurs mostly at root tips and in the proximal elongation zone, whereas microbial P uptake is highest in the root hair zone. For conversion of organic N2 in plant available form, the N2 mineralization is pre-requisite. Microbial P and N2 comprise the major share of phosphatase and Nitrogen in soil, contributing significantly to the P and N2 of plant. Phosphorous mobilizing bacteria (PMB) and Nitrogen fixing bacteria are beneficial bacteria that effectively mobilize P and N2 through solubilizationof sorbed P pool and N2 pools; and mineralization of organic P compounds which are otherwise not readily available in plants. Application of PMB and N2 fixing bacteria to soils can therefore be a promising approach for improving P fertilization efficiency in agriculture.

In several studies it has be documented that the targeted application of high concentration of PMB such as representative from Bacillus, and fungi in soil limited in P availability for plants result in effect of plant growth. Whether future use ofPMB and N2 fixing bacteria can improve P& N2 nutrition of arable crops and vegetables remains to be tested. Three various microbial-driven functional mechanisms are currentlybeing investigated. First, the added PMB might releases the phosphatases by catalysing the hydrolysis of organic P compound. Second,the PMB secretes organic acid that reduces by secreting organic acids which would reduce rhizospherepH, and other Organic acids like bicarbonates,carboxylates, and other anions function as exchange

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ligand. The PMB may solubilise bound organic P into easily available phosphates. Third, added PMB may interact synergistically with other beneficial indigenous microbes, like mycorrhizal fungi or N2-fixing bacteria optimizing P mobilization in soil. Althoughthe role of PMB during P solubilization has been investigated, the importance of enzymatic cleavage of organic Presources by PMB, especially under P-limited conditions, has been less well studied. Due to addition of N2 fixing bacteria which provide symbiotic association with plant root increase the efficiency of N2 fixation by speeding N2 cycle. Since the formulation of the commercial products may also affect microbial P mineralization, we selected *Bacillus* and *Rhizobium*as the model organism, omitting any formulation.Whereas the addition of viable cells of *Bacillus*&*Rhizobium* should clarify direct mechanisms (e.g., PMB for enzyme production), due to the addition of heat killed PMB and N2 fixing bacteria should allow testing indirect mechanisms (e.g., via endogenous microorganisms). Plant growth promoting effects of the PMB and N2 fixing bacteria due to increased microbial activity by addition of living soil bacteria, an inoculation treatment using a mix of soil bacterial isolates were also tested. The following hypotheses were tested. (1) The bacterial strain was successfully isolated that result in plant growth promoting effect. (2) The growth effect of viable Bacillus and rhizobium under low P and N2 condition in soil is based on enhanced PA and N_2A leading to enhanced P and N_2 availability in soil and increased uptake by plants.(3) Both the bacterial strain dominates colonization of rhizosphere that leads to distinct zone of enriched alkaline/acid PA and NA to a shift in microbial community composition.

2. Materials & Methods

Isolation and Staining of PMB (*Bacillus*)

Soil sample was collected from VGS college campus (playground) and serially diluted and plated onto Pikovskaya agar plate (pH7.1) and kept at 37°C for 24-48 hours. After that colonies were formed. After isolation of the colonies, it is then sub-cultured for further use.

Culture mediafor PMB

PMB screening medium contained (g/L) Yeast extract 0.50, Dextrose 10.0, calcium phosphate 5.0, Ammonium sulphate 0.50, Potassium chloride 0.20, Magnesium sulphate 0.10, Manganese sulphate 0.0001, Ferrous sulphate 0.0001, Agar 15.0

Staining of PMB:

For the identification of PMB (*Bacillus*) Gram's staining was done. In Gram's staining 3 stained were used and they are 1. Crystal violet 2.Gram's iodine 3.Safranin. Standard method of Gram's staining was performed.

Isolation of Nitrogen Fixing Bacteria (*Rhizobium):*

The Rhizobium are found in root nodules of plant. We collected root nodules were crushed and in saline sample and then the sample was spread onto yeast extract mannitol agar medium and then it was kept at 37° C for 24-48 hours and then it was identified by Gram's staining.The *Rhizobium* were isolated by using yeast extract mannitol agar (YEMA) medium.For isolation of Rhizobium the plant which is healthy, not broken and which having nodules were selected. They were washed under tap water to remove adhering mud and soil particles, after which they were treated carefully with 5% hydrogen peroxide for surface sterilization. To avoid the sterlant the nodules were repeatedly washed with sterile water, and then treated with 70% ethyl alcohol for about one minute and 0.1% HgCl2 for two minutes. They nodules were washed with sterile water under aseptic conditions and crushed in sterile mortal and pistal. The suspension of the crushed noduleswas made andthe pure culture technique that striking id done and plated on YEMA medium which contains 1%Congo-red dye and incubated at 28±10C for 24 hours. Growth of Rhizobium on YEMA plate was observed after the said incubation period.

Culture Media for *Rhizobium*:

The yeast extract mannitol agar media contained (g/L) Mannitol5.0, Yeast extract 0.5, $MgSO_4.7H_20$ 0.2, NaCl 0.1,$K_2HPO_4$0.5, Na Gluconate 5.0, Agar 15.0.

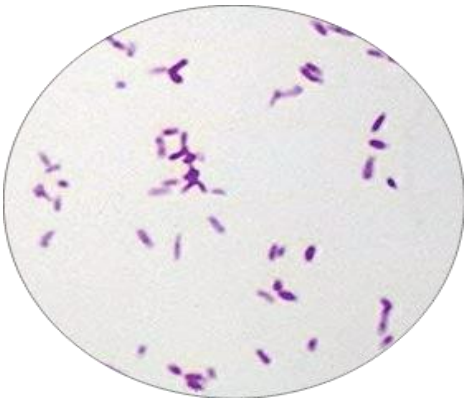
Rhizobox Experiment:

The experiment was performed under low P availability soil conditions using *Bacillus*and *Rhizobium* as the PMB, and tomato (Solanumlycopersicum) as the test plant. We established four treatments to account for the response of plants to heat treated (HT), viable PMB and N_2, Bacterial mix and control which is without any microorganism. Plant growth-promoting effects of the PMB and N_2 fixing bacteriais due to increased microbial activity from having added living soil bacteria, which could affect the P and N2 efficiency of plants, a treatment was performed using unselectively cultivated soil bacteria for inoculation (bacterial mix). To verify the effects of HT and viable PMB on plant growth and nutrition, heat treated Bacillus and Rhizobium were used for inoculation in two different treatments. Details of microorganism cultivation and inoculation are described above. Although the study aimed to determine the effects of PMB and N2 under low plant available P soil conditions, in order to achieve successful germination.

3. Result and Discussion

Isolation and screening of PMB and N2:

The aim of present investigation was to isolate bacterial strain that provide N2 and organic P for the growth of plant. All isolate were initially checked for their viability onto their respective agar medium. Among these, those isolates who shows longer viability these isolates were selected for the experiment. For the identification of PMB and N2 colony characteristic, Gram's staining and the Microscopic observation was done.



Bacillus:

Bacillus is a Gram-positive Bacteria as a group are common soil organisms. Bacillus species are Gram positive mesophilic, aerobic heterotrophic bacteria which produce heat-resistant endospore. The enrichment and isolation of Bacillus is very 0simple -a sample soil sample which reach in bacillus is heated to kill non-spore-forming mesophiles, and then plated on pikovaskaya media and incubated at 37^0C.In Gram's staining *Bacillus* appears violet in colour.

Fig.1: Gram's staining of *Bacillusspp*

Rhizobium:

Rhizobium is a of Gram-negative soil bacteria that fix nitrogen in soil. Rhizobium species form an end symbiotic nitrogen-fixing association with roots of legumes and Parasponia.The Rhizobium bacteria observed in plant cells within root nodules, in root nodule they convert atmospheric nitrogen into ammonia using the enzyme nitrogenase enzyme and then provide organic nitrogenous compounds such as glutamine or ureides to the plant. The plant, in turn, provides the bacteria with organic compounds made by photosynthesis. This is mutual relationships of all of the rhizobia, and Rhizobium is of these a typical example.

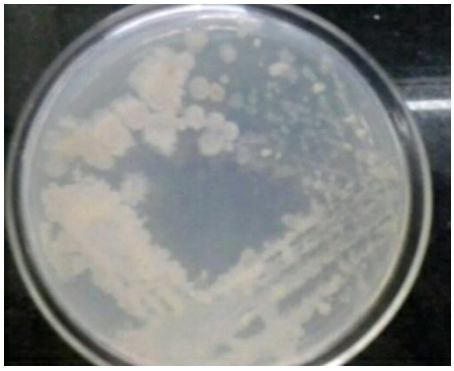


Fig.2: Isolated colonies of *Rhizobium*

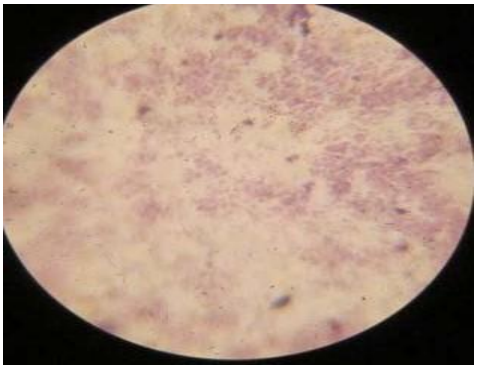


Fig.3: Gram's staining of *Rhizobium*

Plant properties:

Stem diameter, leaf number and area, shoot height and P deficiency symptoms were recorded in control plant. After inoculation of the bacterial culture the stem number, leaf number and the shoot as well as the root height increased. Various parameter of growth was checked after the bacterial inoculation.

Table No. 1: Plant shoot height (cm)

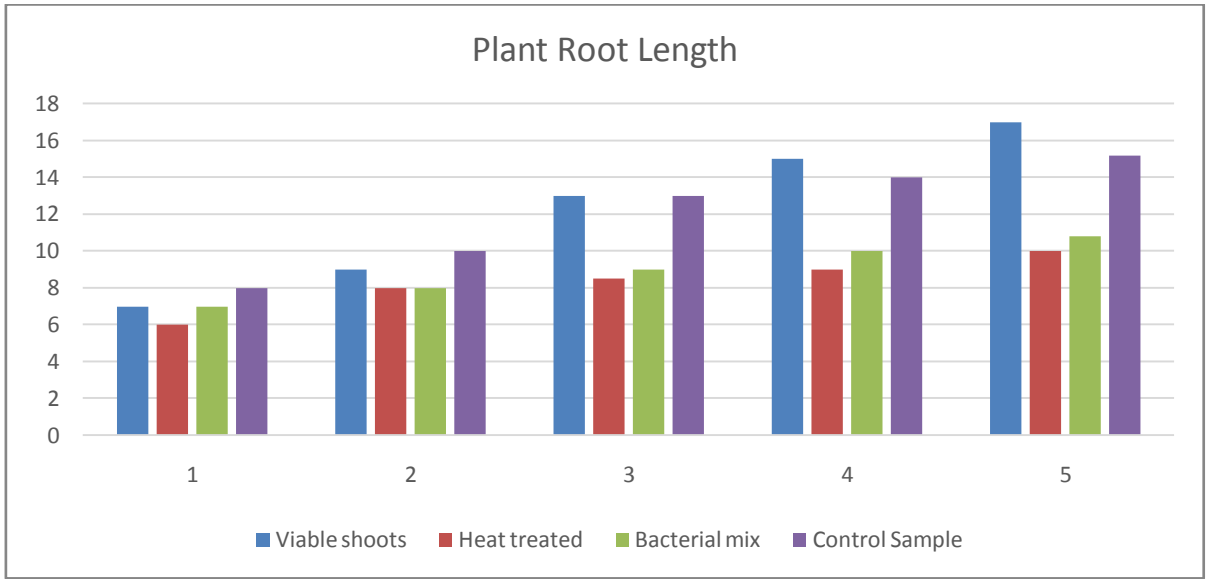
Plant/week	Viable (Shoot)	Heat treated	Bacterial Mix	Control sample
1	18cm	12cm	15cm	11cm
2	21cm	13cm	15cm	12cm
3	23cm	13cm	17cm	13cm
4	25cm	14cm	17cm	13cm
5	27cm	15cm	18cm	14cm

Table No. 2: Depth of Root in soil layer (cm):

Plant/Week	Viable (Root)	Heat Treated	Bacterial Mix	Control Plant
1	7cm	6cm	7cm	8cm
2	9cm	8cm	8cm	10cm
3	13cm	8.5cm	9cm	13cm
4	15cm	9cm	10cm	14cm
5	17cm	10cm	10.8cm	15.2cm

Fig.4: Measuring root height of plant

Graph 1. Shows Plant root length in Centimeter



Soil analysis:

Due to addition of the bacterial strain, it was observed that the fertility of soil was increased. The various parameter of soil was checked in ANALAB soil testing lab. From the report of the ANALAB we conclude that addition of viable bacterial strain there is an increase in soil fertility.

Table No.3: Soil analysis of normal soil with viable strain

Sr. No	Parameter	Result
1	Nitrogen as N	0.25%
2	Phosphorus as P2O5	0.11%
3	Potassium as K2O	0.22%

Table No.4: Soil analysis of normal soil without strain

Sr. No	Parameter	Result
1	Nitrogen as N	0.12%
2	Phosphorus as P2O5	0.4%
3	Potassium as K2O	0.11%

Plant growth:

In comparison to other three treatmenti.e., control, heat treatment, bacterial mix inoculum with bacillus and rhizobium cells resulted in significantly enhanced plant growth. This show by higher stem diameter, leaf number' leaf size shoot diameter size. We observed further trends of increased shoot height and leaf area thesymptoms of p-deficiency are less observed in plant receiving treatment of viable bacillus and rhizobium with comparison bacterial mix the treatment where revelated higher P & N2 uptake in viable treatment. As compared to all three treatment, plant of treatment with viable shows adequate p-supply for tomato plant before flowering its increase the p-uptake compare to other sample.

Fig.5: Plants in Rhizobox with their respective treatments

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