

Original Research Article

<http://dx.doi.org/10.20546/ijcmas.2016.507.033>

Effect of Phosphate Solubilising Bacteria *Bacillus* PSB24 on Growth of Tomato Plants

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ABSTRACT

Keywords

Phosphate solubilising bacteria, PSB inoculants, morphological growth parameters, fresh weight, dry weight.

Article Info

Accepted:

12 June 2016

Available Online:

10 July 2016

Soils contain substantial reserves of total phosphorus but majority is present in the form of insoluble phosphates and hence cannot be utilized by the plants. To overcome this P deficiency in soils, P fertilizers are applied. However, after application, a considerable amount of P is rapidly transformed into less available forms by forming a complex with Al or Fe in acid soils or Ca in calcareous soils before plant roots had a chance to absorb it. Thus the fixation and precipitation of P cause P deficiency, and in turn, restrict the growth of crops severely. Phosphate solubilizing bacteria (PSB) are ubiquitous in soils and capable of hydrolyzing organic and inorganic phosphorus from insoluble compounds and converting to the soluble forms. Use of PSB as inoculants increases P uptake by plants and thus play a role in plant phosphorus nutrition by enhancing its availability to plants and improving their growth and yield. In the present study, tomato plants were inoculated with culture isolate *Bacillus* PSB 24 and various morphological growth characters were analyzed at different time intervals. The culture inoculum of *Bacillus* PSB24 caused an increase in growth parameters over control and showed better growth in shoot as well as root and an enhancement in both root and shoot dry and fresh weight in tomato plants.

Introduction

Phosphorus is one of the major essential macronutrients required for biological growth and development of plants. Phosphorous is associated with many vital functions and is responsible for several physiological and biochemical plant activities such as utilization of sugar and starch, photosynthesis and transporting of genetic traits. It promotes early root formation, plant growth and it improves the quality of fruits, vegetables and grains and is vital to seed formation (Deepika Divya Kadirir *et al.*, 2013).

A majority of agricultural soils contain large reserves of phosphorus, of which a considerable part is accumulated as consequence of regular applications of P fertilizers (Katiyar and Goel, 2004). However, only a small part of phosphorus is utilized by plants and the rest is easily precipitated and converted into insoluble fixed forms and thus cannot be utilized by the plants (Vassileva *et al.*, 1998; Rodriguez and Fraga, 1999). Inadequate P nutrition interferes with protein synthesis and the synthesis of nucleic acids. Plants suffering

from P deficiency are thus retarded in their growth. The formation of seeds and fruits is especially depressed in plants suffering P deficiency. Thus P deficiency in crops leads to low yields as well as poor quality fruits and seeds. To replenish the soils with Phosphorus, chemical P fertilizers are being used as the best means to circumvent P deficiency in different agro-ecosystems. But their use is always limited due to its increasing cost. Moreover, an excess application of P fertilizer to crop land leads to pollution due to soil erosion and runoff water containing large amounts of soluble P.

Soils generally contain substantial reserves of total phosphorus and Organic phosphorus constitutes a large proportion of the total phosphorus. However, plants can utilize P only in soluble inorganic form. Phosphate solubilising microorganisms render these insoluble phosphates into soluble form. A large number of heterotrophic and autotrophic microorganisms including bacteria, fungi, and cyanobacteria, are reported to solubilize insoluble phosphate forms e.g. hydroxyapatite, tricalcium phosphate, and rock phosphate (Roy choudhury and Kaushik, 1989). But bacteria form the largest group which bring about mineralization of organic phosphorus compounds and are known as 'Phosphate Solubilizing Bacteria' (Asea, 1988; Das *et al.*, 2003).

Some of the most important bacteria recognized as PSB belong to the genera *Pseudomonas*, *Rhizobium*, *Bacillus*, *Burkholderia*, *Achromobacter*, *Agrobacterium*, *Micrococcus*, *Aerobacter*, *Flavobacterium*, *Acinetobacter*, *Serratia*, *Erwinia* and *Pantoea* etc. (Rodriguez and Fraga, 1999; Torres *et al.*, 2008; Peix *et al.*, 2009; Satyanarayana and Johri, 2005; Hameeda *et al.*, 2008; Castagno *et al.*, 2011; Goraknath Gupta *et al.*, 2013)

Diverse groups of organisms in soil employ variety of solubilization reactions to release soluble phosphorus from insoluble phosphates (Illmer *et al.*, 1995; Singh and Kapoor, 1998; Ahmad Ali Khan *et al.*, 2009). The principal mechanism of mineral phosphate solubilization is the production of organic acids and acid phosphatases which play a major role in the mineralization of phosphorous in the soil (Surange *et al.*, 1995; Dutton and Evans, 1996; Nahas, 1996; Hilda and Fraga, 2000; Shankar *et al.*, 2013). PSB are non specific in the sense that they can be used on all plants. Under appropriate conditions, PSB can enhance plant development and promote the yield of several agricultural important crops in different soils and climatic regions. Many factors combined together produce positive effects on crop yield especially for vegetables and cereals. The tomato (*Lycopersicum esculentum*) is an important vegetable crop worldwide. Tomatoes, aside from being tasty and nutritious, they are a good source of vitamins A and C and lycopene content and many other nutrients. Hence, this crop is gaining importance both in developing and developed countries and efforts are being made for the quality and quantity production of this commodity (Mahajan and Singh, 2006; Ramakrishnan and Selvakumar, 2012).

The research work was carried out to isolate and screen phosphate solubilizing bacteria (PSB) from Rhizosphere soil samples of different field crops in Telangana, India. The selected bacterial isolate was identified as *Bacillus* PSB24. The present paper discusses the inoculation of the culture isolate into Tomato plants and its effects on various growth parameters of the plants.

Methodology

Rhizosphere soil samples of Wheat, Maize, Jowar, Cotton, Beans, Red Gram and Bengal

Gram crops were collected using sterile techniques from various fields of Telangana, India and used for experimental analyses.

Isolation and screening of PSB

The Soil samples were serially diluted and spread plated on Pikovskayas agar (PVK) media and incubated at 30⁰ C for 3–5 days. Colonies were selected on the basis of phosphate solubilization as indicated by clear halo around the bacterial colonies. Solubilization Efficiency (SE %) was calculated and those showing more than 50% SE were selected and further checked for their solubilisation potential *in vitro*. The phosphorous solubilization potential of the selected PSB isolates was tested by estimating available phosphorous in the Pikovskaya's broth medium amended with tricalcium phosphate as a substrate. According to quantitative analysis results, PSB 24 was the best phosphate solubilising bacteria among the various isolates obtained (Sreedevi, 2015).

Identification of PSB24

The bacterial isolate PSB 24 was characterized for its morphological and biochemical characters as per the Bergey's manual. The isolate was subjected to identify morphological characters by Gram staining (Gram nature, shape & arrangement) and Schaeffer Fulton (spore) staining methods. A Hi assorted Biochemical Kit (Hi Media) was used to study the biochemical characters of the organism. The organism was isolated and purified and pure culture was inoculated into nutrient broth till it showed absorbance of 1.0 OD at 620 nm. Kit was opened aseptically. Each well was inoculated with 50µl of the inoculum by surface inoculation method. Temperature was maintained at 37°C for 24 - 48 hours and the results were checked and compared with standard

interpretation chart

Effect of PSB on Plant Growth and Development

Pot experiments were designed and carried out to evaluate the potential of the isolate *Bacillus* PSB24 on growth and development of tomato plants. The growth promoting activity of *Bacillus* PSB24 was quantified by measuring the effect of the bacterium on the growth parameters like root and shoot elongation, fresh and dry weight of the developed Tomato plants.

Seedlings preparation: The seeds of tomato var. VNR-3357 were obtained from Local market, Hyderabad. Tomato seeds were surface sterilized and were sown in seedling tray and watered.

Preparation of Sand Soil Mixture and Sterilization: Red soil and sand were mixed in 2:1 ratio to give a favourable medium for the growth of root system. The sand soil mixture was moistened with water and sterilized in the autoclave at 121°C for 2 hours to destroy the various bacteria and other organisms and their spores. After this process, the sand soil mixture was aerated overnight.

Evaluation of Phosphate Solubilising Isolates on Plant Growth: An amount of 5 kg sand soil mixture was placed into plastic pots as control. In experimental pots, 24 hours old bacterial culture was mixed with sterile distilled water in 1:1 ratio prior to application in soil. Twenty day old seedlings were transplanted into control as well as experimental pots. Three replicates of pots with each treatment and control were used for each experiment. The pots were watered every 2–3 days to maintain the moisture content. Sterile water was slowly added over the topsoil in each pot. At the end of the experimental period, the plants were

harvested, measured, and analyzed and data recorded from time to time.

Observation of the Tomato plant growth (Morphometric Analysis): Plant height i.e. shoot length and root length; fresh weight and dry weight were observed and recorded at regular intervals of 30, 45, 60 and 90 days.

Shoot length and root length (cm): After the treatment period, three plants of each control and experimental pots were uprooted without any damage to the seedlings or plants and were thoroughly washed with tap water in order to remove soil and debris particle. The height of the shoot was measured and expressed in cm scale. The root length was also measured with the help of scale in uprooted plants (both treated and control).

Fresh Weight of Roots and Shoots (g): The plants were removed gently from the soil without disturbing the root system and then the roots were washed with tap water to remove the soil particles. The roots and shoots were weighed separately using electrical balance and expressed in grams.

Dry Weight of Shoot and Root (g): The plants were uprooted gently without disturbing the root system and then roots were washed with tap water to remove the soil particles. The fresh shoot and root from each treatment and control were cut into pieces and kept in an oven at 80°C for 24-72 hours. The dried samples were weighed using electrical balance and then shoot and root dry weight was recorded separately.

Results and Discussion

Isolation, screening and Identification of efficient PSB

In the present investigation, eighteen Rhizosphere soil samples were collected

from various field crops in Telangana, India and used for isolation by plate assay method. PSB colonies were isolated based on phosphate solubilization on PVK agar media plates and those showing more than 50% Solubilisation Efficiency were further screened by estimating their solubilisation potential *in vitro*. Among the various isolates PSB 24 showed highest amount of solubilised phosphate concentration and thus was considered as efficient PSB (Fig. 1). PSB 24 was subjected to various morphological and biochemical tests and based on the results, the bacterial isolate was identified as *Bacillus sp.* and was named as *Bacillus* PSB 24 (Table-1).

Evaluation of *Bacillus* PSB 24 on Tomato Plant Growth

In our study, tomato plants were inoculated with *Bacillus* PSB 24 to induce plant growth and quantify its growth promoting activity. After 30, 45, 60 and 90 days, the plants were removed and the following morphological growth characters like plant height in terms of shoot length (cm), root length (cm), fresh weight of root (g), fresh weight of shoot (g), dry weight of shoot and root (g) were analyzed. It is represented in the Table 2.

In the present study, the culture inoculum of *Bacillus* PSB24 caused an increase in growth parameters like shoot length, root length, fresh weight and dry weight. It showed better growth in shoot as well as root and there was an enhancement in both root and shoot dry and fresh weight in tomato plants. Thus the bacterial inoculum used for the present study showed significant differences between the treated plants and untreated control plants (Figure 2). These findings in the present study are also supported by a number of previous studies (Sundara *et al.*, 2002; Shen *et al.*, 2004; Turan *et al.*, 2006).

Table.1 Preliminary identification of PSB 24

Identification tests	Results
Morphology	Rod shaped
Gram staining	Gram positive
Spore staining	+
Malonate test	+
Voges proskauer's	-
Citrate test	-
ONPG	+
Nitrate reduction	-
Catalase test	+
Arginine test	+
Sucrose test	-
Mannitol test	-
Glucose test	+
Arabinose test	-
Trehalose test	+

Table.2 Effect of *Bacillus* PSB24 on Tomato plant growth

Days	Treatment	Shoot Length ^a (cm)	Root length ^a (cm)	Fresh weight ^a g/Plant		Dry weight ^a g/Plant	
				Shoot	Root	Shoot	Root
30	Untreated Control	7.3 ±0.208173	5.13 ± 0.202765	0.23 ± 0.012019	0.04± 0	0.09±0	0.01± 0
	Treated with PSB	8.2 ±0.208173	6.43 ± 0.033334	0.283 ± 0.003333	0.083± 0.006667	0.113± 0.006667	0.02± 0.00574
	% Increase	12.32%	25.34%	23.04%	107.5%	25.55%	100%
45	Untreated Control	12.1± 0.208173	8.5± 0.378605	0.637± 0.027285	0.047± 0.003333	0.057± 0.003333	0.023± 0.003333
	Treated with PSB	13.97± 0.185598	8.6± 0.404157	0.997± 0.01453	0.083± 0.003333	0.08± 0	0.04± 0
	% Increase	15.45%	1.17%	56.51%	76.59%	40.35%	73.91%
60	Untreated Control	28.43± 0.551706	9.73± 0.179625	6.32± 0.239928	1.143± 0.107138	0.657± 0.243777	0.05± 0
	Treated with PSB	36.7± 0.230947	11.3± 0.192456	9.36± 0.079548	2.23± 0.103926	0.877± 0.01283	0.1± 0.007698
	% Increase	29.08%	16.13%	48.10%	95.1%	33.48%	100%
90	Untreated Control	59.13333 ± 0.218116	24.83333 ± 0.372081	32.93333 ± 0.487555	1.556667 ± 0.11419	4.453333 ± 0.509306	0.243± 0.01856
	Treated with PSB	70.66667 ± 0.513215	36.76667 ± 0.295099	46.81 ± 0.889145	2.24 ± 0.13087	6.773333 ± 0.16737	0.493± 0.017639
	% Increase	19.50%	48.04%	42.15%	44.51%	52.13%	102%

^a Mean of triplicate samples

± SEM

% increase values indicate the % increase of treated over the respective control

Fig.1 Concentration of P Solubilized by PSB isolates

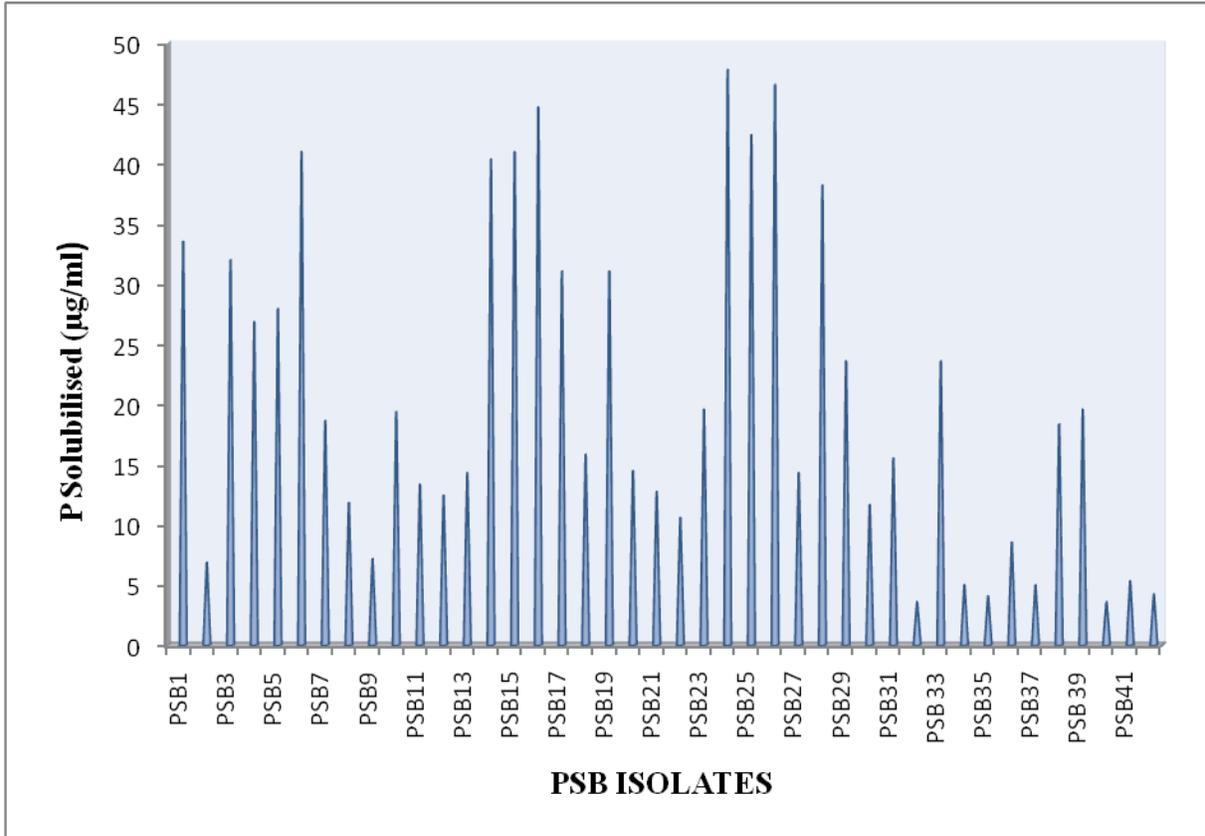


Fig.2 Effect of *Bacillus* PSB 24 on growth and development of tomato plant



Control plant

Treated plant(45 day plants)



Control plant

Treated plant(90 day plants)



45 day plants



90 day plants

There are several reports on plant growth promotion by bacteria that have the ability to solubilise inorganic and/or organic P from soil after their inoculation into soil or plant seeds promoting plant growth and crop production was augmented considerably (Kucey *et al.*, 1989; Bhattacharya *et al.*, 1986). Inoculation of phosphate solubilizing bacteria like *Serratia marcescens*, *pseudomonas fluorescens* and *Bacillus* spp. improved the phosphorous uptake of shoot

and grain in maize and peanut plants (Dey *et al.*, 2004; Singh *et al.*, 2011; Hameeda *et al.*, 2008). Similarly Sachin *et al.*, (2004) showed that *Azotobacter chroococcum* had a positive effect on the growth parameters of bamboo and maize under *In vitro* condition as well as pot experiment. The bacterial inoculums caused significant increase on growth parameters such as seed germination, root and shoot length, dry weight of root and shoot of bamboo and with talc formulation

of *Bacillus sp.* Also Shankar *et al.*, (2013) showed significant increase in various growth parameters such as shoot length root length fresh weight and dry weight of Maize and Chilli plants.

Thus this culture isolate *Bacillus* PSB 24 was found to show positive effect on tomato plants based on their activity. Further this can be developed as a bioinoculant and employed for the growth and development of plants in a more environmental friendly and sustainable manner.

Acknowledgement

The author is thankful to University Grants Commission (UGC) for funding the project and providing the financial support. The author also acknowledges the support of the Principal and the Management of St. Pious X Degree & PG College for providing the necessary facilities to carry out this study.

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How to cite this article:

Sreedevi Sarsan. 2016. Effect of Phosphate Solubilising Bacteria *Bacillus* PSB24 on Growth of Tomato Plants. *Int.J.Curr.Microbiol.App.Sci.* 5(7): 310-320.
doi: <http://dx.doi.org/10.20546/ijcmas.2016.507.033>