ISOLATION & SCREENING OF PSB FROM RHIZOSPHERE SOIL OF DIFFERENT CROP PLANTS

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Abstract: Phosphate solubilizing Bacteria (PSB) are a group of beneficial bacteria capable of hydrolyzing organic and inorganic phosphorus from insoluble compounds. Use of PSB as inoculants increases P uptake by plants and thusplay a role in plant phosphorus nutrition by enhancing its availability to plants. The present study was undertaken to isolate highly efficient PSB from Rhizosphere soil samples collected from different field crops. Rhizosphere soil samples were obtained and used for isolation by plate assay method on Pikovskaya's agar medium (PVK). PSB colonies were isolated based on phosphate solubilization on PVK agar media plates. Solubilization Efficiency (SE %) was then 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 Pikovaskaya's broth medium amended with tricalcium phosphate as a substrate. A significant variation in the capacity to solubilize phosphorous by the PSB isolates was noted whichranged from 3.563 to 47.74 µg/ml.Among the various isolatesPSB 24 showed highest amount of solubilized phosphate concentration and thus was considered as efficient PSB.

Keywords: Pikovaskaya's agar,Phosphorus solubilisationefficiency, PSB, Rhizosphere soil,

Introduction: All natural soils contain vast populations of microorganisms- bacteria, actinomycetes, fungi, algae and protozoa, majority being bacteria. A considerable number of bacterial species are considered to be beneficial to higher plants. They can be used as biofertilizers or control agents for agriculture improvement [1]. Application of these microbes as inoculants enhances an abundant population of active and effective microorganisms in the rhizosphere region which in turn increases plant ability to uptake more nutrients. Phosphate Solubilising Microorganisms (PSMs) play an important role in supplementing phosphorus to the plants and thus enhance plant growth and crop yield.

Phosphorus (P) is an important plant nutrient, next only to nitrogen and classed along with nitrogen and potassium as a major plant nutrient element. Phosphorus is an essential nutrient for plant development and growth making up about 0.2 % of plant dry weight. 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 [2].

A majority of agricultural soils contain large reserves of phosphorus. Organic phosphorus compounds such as Inositol phosphate (soil phytate), phosphomonoesters, phosphodiesters including phospholipids and nucleic acids constitute a large proportion of the total phosphorus in many soils. However, plants can only utilize P in soluble inorganic form. Also, a large portion of the soluble inorganic phosphate is accumulated as a consequence of regular applications of P fertilizers but are rendered insoluble [3},[4]. Phosphate anions are extremely reactive and may be precipitated with highly reactive Al^{3+} and Fe^{3+} in acidic, and with Ca^{2+} in calcareous or normal soils [5], [6]. In these forms, P is highly insoluble and unavailable to plants. Thus, a greater part of soil phosphorus, approximately 95–99% is present in the form of insoluble phosphates and hence cannot be utilized by the plants [7].

Soil Microorganisms are capable of transforming soil P to the forms available to plant. They enhance the P availability to plants by mineralizing organic P in soil and by solubilising precipitated phosphates [8]-[12]. This group of beneficial bacteria capable of hydrolyzing organic and inorganic phosphorus from insoluble compounds and converting to the soluble form are called as Phosphate solubilizing Bacteria (PSB).Some of the PSB are *Bacillus brevis*, *Bacillus licheniformis*, *Pseudomonas cepacia*, *Serratiamarcescens*, *Xanthomonas* spp., *Flavobacterium*spp.etc. [13],[14].

Diverse groups of organisms in soil employ variety of solubilization reactions to release soluble phosphorus from insoluble phosphates [15-17]. Thus 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 [18-22]. Organic acids most frequently Gluconic acid and 2-ketogluconic acidsynthesized by the microorganisms are associated with phosphate solubilizing ability[23-25]. Other organic acids such as glycolic, oxalic, malonic and succinic acid have also been identified among phosphate solubilizers. Chelating substances and inorganic acids such as sulphuric, nitric and carbonic acid are considered as other mechanisms for phosphate solubilization. However the effectiveness and their contribution to P release in soils seems to be less than organic acid production. PSB have been reported to have the ability to solubilize insoluble inorganic phosphate compounds, such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite and rock phosphate [26].

The PSB are ubiquitous with variation in forms and population in different soils. Population of PSB depends on different soil properties (physical and chemical properties, organic matter, and P content) and cultural activities [27].Larger populations of PSB are found in agricultural and rangeland soils [28]. High proportion of PSB is concentrated in the rhizosphere, and they are metabolically more active than from other sources [29].Although a number of bacteria have been isolated with phosphate solubilising properties, there is still a need to search for and characterize new effective microbial inoculants in order to improve phosphorus nutrition in plants and thus promote plant growth and productivity. Thus the present study was undertaken with the objective of Isolation and screening of highly efficient Phosphate Solubilising Bacteria (PSB) from Rhizosphere soil samples of different field crops.

Methodology:

***Collection of Soil samples*:**Rhizosphere soil samples approximately 200-300g were collected from different field crops such as Wheat, Maize, Jowar, Cotton, Beans, Red Gram and Bengal Gram and transported in plastic Zip lock bags to Laboratory in Hyderabad.

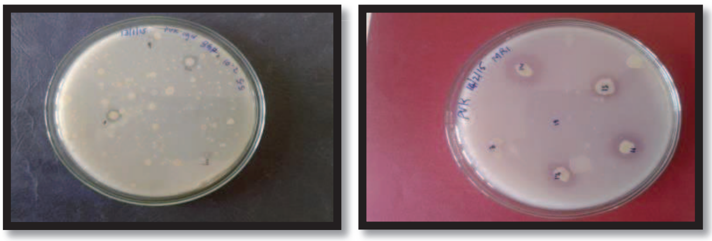
***Isolation and screening of Phosphate Solubilizing Bacteria (Qualitative Analysis)*:** For isolation of phosphate-solubilizing bacteria, sub-samples (10 g) from each soil sample were homogenized in sterile distilled water and serially diluted to 10^{-5}dilution. 100 µl (0.1 ml) of aliquots of each dilution from 10^{-1} to 10^{-5} were spread on Pikovskayas agar (PVK) media plates. The media contained 10 g Glucose, 5 g Ca_3 $(PO_4)_2$, 0.5 g $(NH_4)_2SO_4$, 0.2 g NaCl, 0.1 g $MgSO_4.7H_2O$, 0.2 g KCl, 0.5 g Yeast extract, 0.002 g $MnSO_4.7H_2O$, 0.002 g $FeSO_4.7H_2O$, 20g Agar in 1000ml Distilled water. The pH of the media was adjusted to 7 before autoclaving. Plating was done in duplicate and incubated at 30°C for 3–5 days. Colonies were selected on the basis of phosphate solubilization as indicated by the development of a clear halo around the bacterial colony. The isolated colonies were named and reinoculated on the pikovskayas agar media plates and incubated at 30°C for 3-5 days. The Colony diameter and Halo diameter for each PSB colony was measured using a metric scale and the solubilizing efficiency (SE %) was calculated using the formula,

SE % = (Z-C)/C x 100

Where Z is solubilization zone diameter, C is colony diameter [30].The colonies showing more than 50% of the phosphate solubilizing efficiency were selected and were maintained on nutrient agar slants at 4°C for further studies.

Determination of solubilised phosphate concentration* (Quantitative Analysis)*: The phosphorous solubilization potential of selected strains of phosphate solubilizing bacteria was tested *in vitro* by estimating available phosphorous in the Pikovaskaya's broth medium amended with tricalcium phosphate as a substrate.Quantitative analysis of phosphate solubilization was carried out using Erlenmeyer flasks containing 10 ml of PVK broth medium. After the preparation of PVK medium, pH was adjusted to 7 and sterilized. The flasks were inoculated with 10% (100 µl) of overnight nutrient broth cultures of PSB isolates (OD at 2 - A600). The flasks were incubated at 30°C for 5 days on orbital shaking incubator at 180 rpm. After incubation period, the culture was harvested by centrifugation at 6000 rpm for 15 minutes. The concentration of the solubilised phosphate was estimated in the supernatant as per the Fiske and Subbarow method [31]. The amount of soluble phosphorus by each isolate was determined from the standard curve of KH_2PO_4.

Results and Discussion: Eighteen Rhizosphere soil samples were collected from various field crops and tested. Soil samples were serially diluted and plated on PVK agar media and checked for phosphorus solubilisation. A total of 66 bacterial colonies were considered as PSB isolates as indicated by the clear zones of hydrolysis around them (Fig.1). Solubilisation efficiency was calculated and 42 of them showed more than 50% efficiency (Table 1). The amount of Phosphate solubilised by these isolates in PVK broth was determined and according to quantitative analysis, PSB 24 was the best phosphate solubilizer by giving 47.74 µg/ml of available phosphorus (Fig.2).



(a) (b)

Fig 1: Isolation and screening of PSB from Rhizosphere soil samples on PVK agar plates

Table 1: Solubilization efficiency and P-concentration Solubilized by Selected Strains in PVK Broth

PSB Isolates	SE (%)	P solubilized (µg/ml)
PSB1	100	33.48
PSB 2	150	6.82
PSB3	66	31.93
PSB4	150	26.815
PSB5	150	27.9
PSB6	150	40.92
PSB7	71	18.6
PSB8	100	11.78
PSB9	71	7.13
PSB 10	133	19.33
PSB11	150	13.3
PSB 12	75	12.4
PSB13	75	14.26
PSB 14	200	40.3
PSB15	100	40.92
PSB16	125	44.64
PSB17	200	31
PSB 18	133	15.81
PSB19	233	31
PSB20	200	14.45
PSB21	233	12.71
PSB22	280	10.54
PSB23	57	19.53
PSB24	180	47.74
PSB25	120	42.315
PSB 26	100	46.5
PSB27	66.6	14.26
PSB 28	71	38.13
PSB29	62.5	23.56
PSB 30	200	11.625
PSB31	200	15.5
PSB32	133	3.565
PSB 33	100	23.56
PSB34	57	4.96
PSB35	89	4.03
PSB 36	114	8.525
PSB37	71.4	4.96
PSB38	133.3	18.29
PSB 39	66.66	19.53
PSB40	100	3.565
PSB41	200	5.27
PSB42	60	4.185

Conclusion: Converting soil insoluble phosphates (both organic and inorganic) to a form available for plants is a necessary goal to achieve sustainable agricultural production. PSB are an important means in achieving this goal for improved plant growth and yield. The present work was carried out to isolate highly efficient PSB isolates from Rhizosphere soil samples of different field crops. Among the various PSB isolates obtained, PSB 24 was found to be effective phosphate solubiliser. This isolate can be tested for its plant growth-promoting activity and its potential to be developed as inoculum and applied as biofertiliser. However, further studies have to be conducted to analyse and assess the effectiveness of this isolate on plant growth promotion.

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