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Research Paper

In Vitro Vetting for Phosphate Solubilizing Bacteria (PSB) from Healthy Plants Rhizospheric Niches

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A B S T R A C T

Soil samples from rhizospheric niches of Tur, Soya bean, Neem, Gram, Jowar and Bavanchya were collected in sterile polythene bags and brought to the laboratory. All the rhizospheric soil samples were tested for phosphate solubilizing bacteria on Pikovskaya agar by Serial Dilution Method. Among the soil samples tested, rhizospheric niches from the Soya bean displayed highest phosphate solubilizing bacteria, 114 than the other rhizospheric soil sample tested. The rhizospheric niches of Tur, Neem and Bavanchya demonstrated 47, 07, and 02 phosphate solubilizing bacteria. Among all the 170 phosphate solubilizing bacteria isolated from different rhizospheric niches, four isolates, SMD18, SMD36, SMD38 and SMD40 were found qualitatively produce more than 5 mm zone of solubilization on Pikovskaya's agar plates after 9 days incubation. Among the 4 bacterial isolates, two bacterial isolates, namely SMD 38 and SMD 40 when quantitatively analysed, showed maximum P solubilization on 7th day as 444 µg/ml and 421 µg/ml respectively in PKV broth supplemented with tri – calcium phosphate. While in comparison to these isolates, RRR18 and SMD36 isolates shown less phosphate solubilization as 409 and 400 respectively, when quantitatively analysed. By 16S rRNA sequencing and Phylogenetic analysis identified RRR18 as *Sporolactobacillus laevolacticus* RRR18, SMD36 as *Sporolactobacillus laevolacticus* SMD36, SMD38 as *Sporolactobacillus laevolacticus* SMD38 and SMD 40 as *Sporolactobacillus laevolacticus* SMD40.

***Key words*:** Rhizospheric soil, Pikovskaya agar, Phosphate solubilizing bacteria

Phosphorous being one of the major growth-limiting macronutrients required for proper plant growth, particularly in tropical areas, due to its low availability in the soil [1-2]. Soluble Phosphorous plays an important role in virtually all major metabolic processes in plants including photosynthesis, energy transfer, signal transduction, macromolecular biosynthesis, and respiration [3]. Phosphorous also needed for the development of roots, strengthening the stalks and stems, formation of flowers and seeds, crop maturity and quality of crop, energy production, storage and transfer reactions, root growth, cell division and enlargement, N fixation in legumes, resistance to plant diseases [4], transformation of sugar to starch, and transporting of the genetic traits [5].

Although Phosphorous is abundant in soils in both inorganic and organic forms, it is the second most important macronutrient required by the plants, next to nitrogen as it is in an unavailable form for root uptake. The availability of soluble forms of Phosphorous for plants in the soils is limited because of its fixation as insoluble phosphates of iron, aluminum, and calcium in the soil [6]. Soil Phosphorous dynamics is characterized by physicochemical (sorption-desorption) and biological (immobilization-mineralization) processes.

So, to cope up the requirement of Phosphorous, traditionally the soil phosphorus deficiency is addressed by the application of phosphorus fertilizers. However, the majority of the applied fertilizer phosphorus is not available to plants and the addition of inorganic fertilizers in excess of the amount that is commonly employed to overcome this effect can lead to environmental problems such as, groundwater contamination waterway eutrophication and soil fertility depletion. Large amount of P applied as fertilizer enters in the soil as immobile pools through precipitation reaction with highly reactive Al^{3+} and Fe^{3+} in acidic, and Ca^{2+} in calcareous or normal soils [7]. It is therefore of great interest to investigate management strategies that can improve phosphorus fertilization efficiency, increase crop yields and reduce environmental pollution caused by phosphorous loss from the soil.

Soil microorganisms enhance plant nutrient acquisition through a wide range of biological processes including the transformation of insoluble soil nutrients. Some are capable of solubilizing and mineralizing insoluble soil phosphorous for the growth of plants. Apart from chemical fertilization, microbial P-solubilization and mineralization is the only possible way to increase plant available phosphorous. In the natural environment numerous microorganisms in the soil and rhizosphere are effective at releasing phosphorus from total

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soil phosphorus through solubilization and mineralization [8]. This group of microorganisms are referred to as Phosphorus Solubilizing Microorganisms (PSM). Many species of soil fungi and bacteria can solubilize phosphorus *in vitro* and some of them can mobilize phosphorus in plants. PSM increases the bioavailability of soil insoluble phosphorus for plant use [9]. They solubilize insoluble inorganic (mineral) phosphorus and mineralize insoluble organic phosphorus.

Application of phosphate-solubilizing bacteria increases soil fertility due to their ability to convert insoluble P to soluble P by releasing organic acids, chelation and ion exchange [10]. Soil bacteria that have been reported to mobilize poorly available phosphorus via solubilization and mineralization include *Pseudomonas spp.*, *Agrobacterium spp.* and *Bacillus circulans* [11]. Other phosphorus solubilizing and mineralizing bacteria include various strains of *Azotobacter* [12], *Bacillus* [13-14], *Burkholderia* [15], *Enterobacter*, *Erwinia* [16], *Kushneria* [9], *Paenibacillus* [17], *Rhizobium* [18], *Rhodococcus*, *Serratia*, *Bradyrhizobium*, *Salmonella*, *Sinomonas*, and *Thiobacillus* [14]. The present investigation mainly focuses on (i) *in vitro* vetting for Phosphate Solubilizing Bacteria from rhizospheric niches of different healthy plants, (ii) qualitative and quantitative estimation of the phosphate solubilizing efficiency.

MATERIALS AND METHODS

Chemicals

The chemicals used during the study were acquired from M/S Hi-media, Mumbai, Glaxo Ltd., Mumbai, Sigma Aldrich, USA. Analytical/Guaranteed (AR/GR) grade chemicals and double glass-distilled water was used.

Collection of Rhizospheric Niches soil sample

Soil samples from the rhizospheric niches of healthy plants viz. Tur, Soya bean, Neem, Jowar, Gram and Bavanchya were collected near the Purna City (Plate 1). For this purpose, the plants were uprooted carefully, shoots were cut off and roots along with rhizosphere soils were brought to the laboratory in sterile polythene bags. The soil samples were processed immediately for the isolation of Phosphate solubilizing bacteria.

Plate 1 Collection of soil sample from different Rhizospheric niches of plants

Isolation of Phosphate Solubilizing Bacteria (PSB)

Phosphate Solubilizing Bacteria (PSB) was isolated from the rhizospheric soil samples by dilution plate technique using Pikovskaya's medium [19] containing tri-calcium phosphate (TCP) [20].

One gram each of the soil sample was transferred to 9 ml sterile dilution blank under aseptic conditions and serial dilutions were made. Appropriate soil dilutions were plated on Pikovskaya's agar medium by spread plate technique and incubated at 30 ± 1ºC for 2-3 days. The colonies forming halo

zone of clearance (Pikovskaya's medium) around them were counted as P - Solubilizers. All the bacterial colonies exhibiting halo zones were selected, tentatively named, purified and maintained on nutrient agar slants for further studies.

Estimation of Phosphate Solubilization Efficiency

The phosphate solubilization efficiency of the phosphate solubilizing bacteria was assessed by qualitatively and quantitatively.

Qualitative estimation of Phosphate Solubilization efficiency

For qualitative assessment of Phosphate solubilization efficiency, pure cultures of phosphate solubilizing bacteria were spot inoculated on the plates containing Pikovskaya's agar [20]. The plates were incubated at 28±1°C and halo zone around colonies were recorded at regular interval up to 10 days. The phosphate solubilizing abilities of the isolates to solubilize TCP on Pikovskaya's agar media were determined in terms of solubilization index (SI). Phosphate solubilization index was calculated by measuring the colony diameter and the halo zone diameter using the following formula of [21].

$$\text{Phosphate Solubilization Index (SI)} = \frac{\text{(Colony diameter + Halo zone diameter)}}{\text{Colony diameter}}$$

Quantitative estimation of Phosphate Solubilizing efficiency

The quantitative assessment of solubilized Phosphate by bacterial isolates was done by Chloromolybdic Acid Method [25].

Chloromolybdic acid Method

The phosphate solubilizing activity of the selected bacterial isolates was determined quantitatively in liquid medium by following Chloromolybdic Acid Method [20, 22] (Jackson 1973, Kaur 2014).

Chloromolybdic Acid

15 gm Ammonium molybdate was dissolved in about 400 ml of distilled water, filtered and then 400 ml of 10 N HCl was added slowly with rapid stirring. Volume was made to 1000 ml with distilled water and stored in amber glass bottle.

Chlorostannous acid
Stock solution:

$SnCl_2.2H_2O$	10 gm
Con. HCl	25.0ml

$SnCl_2$ crystals were dissolved in Con. HCl and solution was kept in glass bottle under airtight stopper.

Working solution

Fresh working solution was prepared by adding 1.0 ml of the above solution to 132.0 ml of distilled water.

Quantitative assessment P Solubilization by Chloromolybdic acid method

The phosphate solubilizing bacteria were grown in 50 ml nutrient broth for 24 hr. at 30°C on rotary shaking incubator. 0.1 ml of each phosphate solubilizing bacteria was aseptically transferred to 100 ml PKV broth containing 250 ml conical flask. The flasks were incubated 30°C on rotary shaking incubator at 130 rpm. Five ml of culture was taken out in sterile condition at regular interval of 2 days from third day onward and centrifuged at 10000 rpm for 10 min. 500 µl of supernatant was transferred to 50 ml volumetric flask. This was followed by addition of 10.0 ml Chloromolybdic acid. The content of the flask was diluted to 40.0 ml with distilled water. Then 1 ml of Chlorostannous acid was added. After mixing, the volume was made up to 50.0 ml with distilled water. The blue colour intensity of the solution was measured at 600 nm. The soluble P was estimated from standard curve of KH_2PO_4 (100 ppm) drawn against O.D. 600 nm.

Identification of Phosphate solubilizing bacteria

The efficient Phosphate solubilizing bacteria obtained from qualitative and quantitative assessment was identified according to Bergey's Manual of Systematic Bacteriology (1984) by using cultural and biochemical characteristics as well as 16s rRNA sequencing. 16s rRNA sequencing of culture was carried out at Agharkar Research Institute (ARI) Pune, Maharashtra.

RESULTS AND DISCUSSION

Natural solubilization of insoluble phosphates is an important activity displayed by different microorganisms including both bacteria and fungi, known as *phosphate* solubilizing microorganisms (PSM). Bacteria are the principal microorganisms that solubilize mineral phosphate in nature, as compared to other microorganisms [23]. Phosphate solubilizing bacteria (PSB) play an important role in biogeochemical phosphorus cycling in both terrestrial and aquatic environments [24].

Isolation of Phosphate Solubilizing Bacteria (PSB)

In present research, 170 phosphate solubilizing bacteria were isolated from different rhizospheric niches of healthy plants by using serial dilution method on Pikovskaya's (PKV) agar plates (Plate 2). Out of 170, 114 phosphate solubilizing bacteria (PSB) were isolated on Pikovskaya Agar from the Soya bean rhizospheric niches, by using dilution technique, which were far greater than the other rhizospheric niches sample. Similarly, from the rhizospheric niches of Tur, Neem and Bavanchya 47, 07, and 02 phosphate solubilizing bacteria were isolated respectively (Table 1). While from rhizospheric niches of Jowar and Gram, no phosphate solubilizing bacteria were isolated. Use of Pikovskaya's agar medium for isolation of Phosphate Solubilizing Bacteria (PSB) was a simple way to detect PSB through formation of halo zone on agar plate containing tri – calcium phosphate as a sole Phosphorous source [22]. These rhizospheric isolates were tentatively named as SMD 1 to SMD 170.

The ability of microorganisms to solubilize insoluble phosphates in soil and making it available to plant as soluble phosphate is well known fact [25]. Phosphate solubilizing microorganisms includes several Bacteria, Fungi, Actinomycetes, Yeast and Cyanobacteria [26]. The phosphate solubilizing microorganisms can be isolated from different sources such as soil [27], rhizosphere [28], root nodules [29], compost [30], and rock phosphates [31]. These reports support the fact that phosphate solubilizing bacteria can be isolated from rhizospheric niches of the healthy plants.

1270 bacteria was isolated on Pikovskaya's agar plate by serial dilution method at 10^{-5} dilutions [20]. Out of these 1270 bacterial isolates only 169 bacteria isolates were observed to be formed a halo zone around the colonies. While Screened rhizospheric soil from potato and tomato for phosphate solubilizing bacteria and revealed that the number of bacteria that created clearing zone in the PKV agar were

greater in the potato sample i.e., seven, Pot1, Pot2, Pot3, Pot4, Pot5, Pot6, and Pot7, compared to the tomato sample i.e., two, Tom1 and Tom2 [32]. Where soil from rhizosphere of vegetables used for isolation of PSB and isolated three strains Rad1, Rad2 Ros2 with high efficiency [33]. The Phosphate solubilizing bacteria from rhizospheric soil of safflower explored by [34] and isolated six efficient PSB strains such RC01, RC02, RC03, RC04, RC05, and RC06 which belongs to the genera *Pseudomonas*, *Sinorhizobium*, *staphylococcus*, *Acinetobactor* and Enterobacter.

Table 1 Isolated PSB from different rhizospheric niches with their Phosphate solubilization index

Rhizospheric isolates	Phosphate Solubilization Index	Rhizospheric isolates	Phosphate Solubilization Index	Rhizospheric isolates	Phosphate Solubilization Index	Rhizospheric isolates	Phosphate Solubilization Index	Rhizospheric isolates	Phosphate Solubilization Index
SMD 1	1	SMD 35	2	SMD 69	1	SMD 103	0	SMD 137	1
SMD 2	2	SMD 36	5	SMD 70	2	SMD 104	1	SMD 138	1
SMD 3	2	SMD 37	5	SMD 71	2	SMD 105	0	SMD 139	1
SMD 4	1	SMD 38	5	SMD 72	2	SMD 106	2	SMD 140	1
SMD 5	1	SMD 39	4	SMD 73	2	SMD 107	1	SMD 141	0
SMD 6	5	SMD 40	5	SMD 74	2	SMD 108	2	SMD 142	0
SMD 7	1	SMD 41	4	SMD 75	2	SMD 109	1	SMD 143	0
SMD 8	1	SMD 42	2	SMD 76	2	SMD 110	0	SMD 144	0
SMD 9	1	SMD 43	1	SMD 77	1	SMD 111	2	SMD 145	0
SMD 10	1	SMD 44	2	SMD 78	1	SMD 112	2	SMD 146	0
SMD 11	2	SMD 45	1	SMD 79	1	SMD 113	0	SMD 147	0
SMD 12	2	SMD 46	1	SMD 80	1	SMD 114	1	SMD 148	0
SMD 13	2	SMD 47	1	SMD 81	2	SMD 115	1	SMD 149	0
SMD 14	2	SMD 48	1	SMD 82	1	SMD 116	2	SMD 150	0
SMD 15	3	SMD 49	1	SMD 83	1	SMD 117	2	SMD 151	0
SMD 16	4	SMD 50	2	SMD 84	1	SMD 118	0	SMD 152	1
SMD 17	1	SMD 51	1	SMD 85	2	SMD 119	1	SMD 153	1
RRR18	5	SMD 52	1	SMD 86	2	SMD 120	2	SMD 154	1
SMD 19	4	SMD 53	4	SMD 87	2	SMD 121	2	SMD 155	1
SMD 20	4	SMD 54	1	SMD 88	1	SMD 122	2	SMD 156	1
SMD 21	1	SMD 55	1	SMD 89	2	SMD 123	2	SMD 157	1
SMD 22	5	SMD 56	1	SMD 90	2	SMD 124	0	SMD 158	2
SMD 23	1	SMD 57	4	SMD 91	2	SMD 125	1	SMD 159	2
SMD 24	1	SMD 58	1	SMD 92	2	SMD 126	1	SMD 160	2
SMD 25	1	SMD 59	1	SMD 93	2	SMD 127	0	SMD 161	1
SMD 26	4	SMD 60	2	SMD 94	1	SMD 128	2	SMD 162	2
SMD 27	2	SMD 61	2	SMD 95	1	SMD 129	2	SMD 163	2
SMD 28	5	SMD 62	1	SMD 96	2	SMD 130	1	SMD 164	1
SMD 29	4	SMD 63	2	SMD 97	2	SMD 131	1	SMD 165	2
SMD 30	5	SMD 64	2	SMD 98	1	SMD 132	1	SMD 166	2
SMD 31	4	SMD 65	2	SMD 99	1	SMD 133	2	SMD 167	2
SMD 32	2	SMD 66	2	SMD 100	1	SMD 134	2	SMD 168	1
SMD 33	4	SMD 67	2	SMD 101	0	SMD 135	0	SMD 169	2
SMD 34	4	SMD 68	1	SMD 102	2	SMD 136	0	SMD 170	0

Table 2 Qualitative estimation of Phosphate Solubilization Index of selected Rhizospheric isolates after 9 days of incubation

Rhizospheric Isolates	Diameter of Colony + Halo zone (mm)	Diameter of Colony (mm)	Diameter Halo zone (mm)	Phosphate Solubilization Index
RRR18	9	2	7	4.5
SMD 36	10	3	7	3.33
SMD38	10	2	8	5.0
SMD40	10	2	8	5.0

In present study, soil from rhizospheric niches of soya bean displayed greater amount of phosphate solubilizing bacteria on Pikovskaya's agar plate. Out of 170 bacterial isolates, only 9 bacterial isolates designated as SMD6, RRR18, SMD22, SMD28, SMD30, SMD36, SMD37, SMD38 and SMD40 displayed greater efficacy of solubilizing the phosphate provided in the medium. All these finding supports the fact that rhizospheric soil would be great source for isolation of phosphate solubilizing bacteria and also revealed that greater number of PSB were isolated from the rhizospheric soil of soya bean which far better than that of [20, 32, 33, 34].

Estimation of Phosphate Solubilization efficiency

The phosphate solubilization efficiency of the bacterial isolates was assessed by qualitatively and quantitatively.

Qualitative estimation of Phosphate Solubilization efficiency

Qualitative assessment of selected phosphate solubilizing bacterial isolates was completed by the method of [35] and [20] revealed variations in phosphate solubilization efficiency. In total of 170 phosphate solubilizing bacterial isolates from different niches, 4 isolates, RRR18, SMD36, SMD38 and SMD40 were found to show fare more than 5 mm zone of solubilization on Pikovskaya's agar plates after 9 days

incubation (Plate 3). The Phosphate solubilization activity of these isolates of PKV agar plates was ranged between 3.33 to 5.00 (Table 2). 169 phosphate solubilizing bacteria isolated from different rhizospheric niches revealed phosphate solubilization index in range between 1.36 to 3.17 [20].

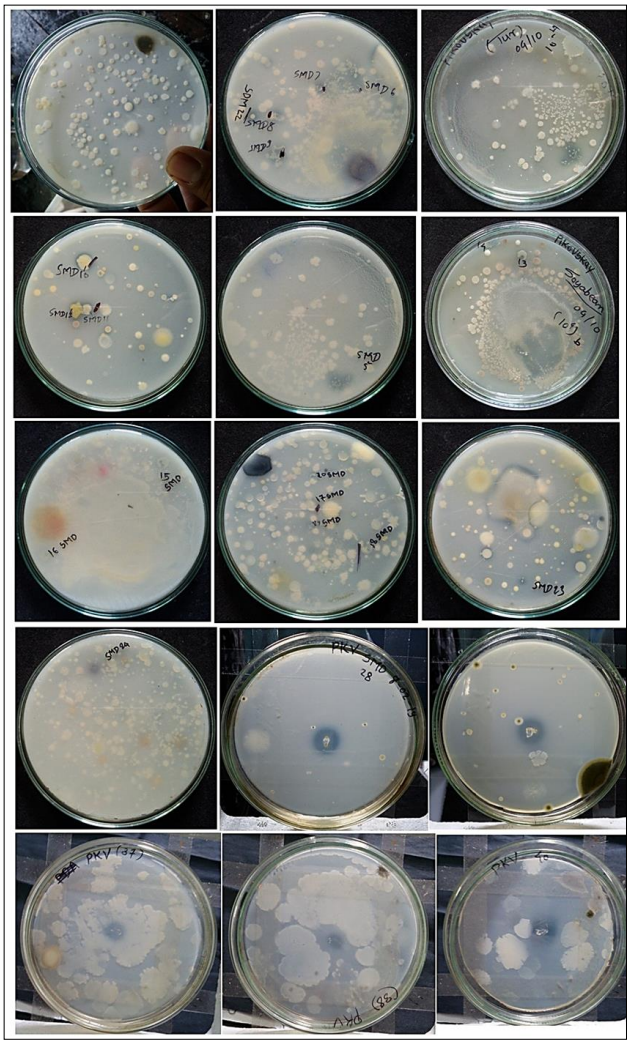

Plate 2 Isolation of Phosphate Solubilizing Bacteria (PSB) with clear halo zone on Pikovskaya's agar plate by Dilution Technique

Similar types of observations were also recorded by [32] where each of the seven isolates from potato and two isolates from tomato shown variation in phosphate solubilization index. The largest clearing zone, created by *Pantoea* sp. Pot 1, had a solubilization index of 1.5 *Pantoea* sp. Pot 5 had smaller index of 1.2. The tomato rhizospheric isolates had significantly smaller indexes compared to Pot1. Phosphate solubilization potential by observing clear zones around the bacterial colony as compared to negative control which gave no zone around the growth of bacteria [33]. The strain Rad1 gave phosphate solubilization index of 2.27 on PVK and 2.5 on NBRIP medium. Strain Rad2 gave good phosphate solubilization index result on NBRIP medium i.e., zone of 2.84 while on PVK medium it gave 2.42. Strain Ros2 gave good phosphate solubilization index result on PVK medium i.e., zone of 2.6 was observed, while on NBRIP medium zone of 2.23 was observed. The PSB strain isolated from the safflower rhizosphere revealed phosphate solubilization index ranged between 2.32 and 4.08 after 6 days incubation [34].

When we compared our findings about the phosphate solubilization index of isolates with that of [20, 33, 34] our findings were greater than these researchers' observations where our isolates displayed phosphate solubilization index ranged between 3.33 to 5.0.

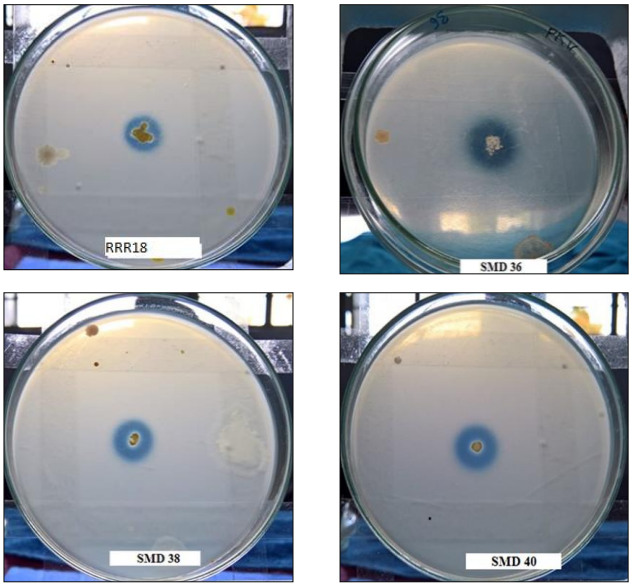

Plate 3 Qualitative estimation of Phosphate Solubilization index of selected Rhizospheric isolates

Quantitative estimation of Phosphate Solubilizing efficiency

Quantitative estimation of phosphate solubilizing efficiency of selected four bacterial isolates was performed by the method of Jackson (1973) and Kaur (2014) in tri – calcium phosphate supplemented PKV broth. Results represented in (Table 3) showed phosphate solubilization activity was increased up to day seven of incubation and maximum solubilization was observed on day seven of incubation in most of the isolates. After seven day, it started to decrease. Out of these 4 selected bacterial isolates, two bacterial isolates, SMD 38 and SMD 40 showed maximum P solubilization in PKV broth supplemented with tri – calcium phosphate.

An extensive range of microorganisms that can solubilize various form of soil bound phosphorous have been reported by [36] and [10] and among them, most prominent are *Bacillus spp.* and *Pseudomonas sp.*

When our results were compared with results of [20], where the phosphate solubilizing bacteria PSB – B6, PSB – B8, PSB – P3, PSB – P7, PSB – P9, PSB – S1, PSB – S2, PSB – S8, PSB – S14, PSB – S15, PSB – MM10, PSB – MM11, PSB – MM16, PSB – SM4, PSB – SM5, PSB – SM9, PSB – SM10, PSB – SF12, PSB – SF15, PSB – SF16, PSB – 1, PSB – 3, PSB – , 4, PSB – 5, PSB – 6, PSB – 7, PSB – 8, PSB – 9, PSB – 10, PSB – 11, PSB – 12, and PSB – 13 showed maximum phosphate solubilization on day five of incubation in PKV broth supplemented with tri – calcium phosphate, as 163, 73, 221, 180, 144, 194, 290, 121, 166, 146, 182, 188, 171, 163, 291, 328, 70, 226, 285, 139, 365, 422, 296, 429, 401, 410, 398, 330, 113, 199, 415 and 413 µg/ml respectively. While our phosphate solubilizing bacteria, RRR18, SMD36, SMD38 and SMD 40, shown maximum solubilization of insoluble phosphate provided in PKV broth on day seven as, 409, 400, 444 and 421 respectively [20].

All the seven isolates for quantitative solubilization in PKV liquid medium. The highest P solubilizing activity was found in *Pantoea* sp. Pot1 (956 mg L^{-1}), while the lowest activity (328 mg L^{-1}) was found in *Enterobacter* sp. Tom2 [32]. Similarly, [33] checked three phosphate solubilizing

strains for P estimation and were compared with the control. Strain Rad1 gave good results in NBRIP medium i.e., 966 µg ml^{-1} while in PVK medium 126.3 µg ml^{-1} was observed. The most promising results were observed by strain Rad2 in NBRIP medium i.e., 1163.1 µg ml^{-1} while in PVK medium 347.4 µg ml^{-1} was observed. Strain Ros2 gave values 955.6 and 648.3 µg ml^{-1} in NBRIP medium and PVK medium, respectively. The phosphate solubilization efficiency six isolates from rhizospheric soil of safflower [34]. The concentrations of soluble P produced by these isolates ranged between 90.9 and 168.5 mg L^{-1} in P solubilization medium. The strain Acinetobacter sp. RC04 showed the highest P solubilization among the strains. When our observations were compared with that of [20-34] Our finding were far better but the findings of [26] with *Pantoea* sp. Pot1 (956 mg L^{-1}), and that of [33] with strain Ros2 (648.3 µg ml^{-1}) far better than us.

Table 3 Quantitative estimation of phosphate solubilization activity of selected bacterial isolates

Rhizospheric isolates	3rd day Soluble P µg/ml	5th day Soluble P µg/ml	7th day Soluble P µg/ml	9th day Soluble P µg/ml	11thday Soluble P µg/ml
RRR 18	365	386	409	399	355
SMD 36	376	397	400	339	303
SMD 38	369	389	444	373	354
SMD 40	372	393	421	365	345

Identification of Phosphate Solubilizing Bacteria

16S rRNA sequencing and Phylogenetic analysis identified RRR18 as *Sporolactobacillus laevolacticus* RRR18, SMD36 as *Sporolactobacillus laevolacticus* SMD36, SMD38 as *Sporolactobacillus laevolacticus* SMD38 and SMD 40 as *Sporolactobacillus laevolacticus* SMD40. The 16S rRNA sequence has been deposited in Genbank of National Center for Biotechnology Information (NCBI), U.S. National Library of Medicine 8600 Rockville Pike, Bethesda MD, 20894 USA with accession no. MT276896 for *Sporolactobacillus laevolacticus* RRR18, MT279508 for *Sporolactobacillus laevolacticus* SMD36, MN853532 for *Sporolactobacillus laevolacticus* SMD38 and MN853531 for *Sporolactobacillus laevolacticus* SMD40.

CONCLUSIONS

The present study is vetting for phosphate solubilizing bacteria from the rhizosphere niches of different healthy plants such as Jowar, gram, soya bean etc. Among these strains, *Sporolactobacillus laevolacticus* RRR18, *Sporolactobacillus laevolacticus* SMD36 *Sporolactobacillus laevolacticus* SMD38 and *Sporolactobacillus laevolacticus* SMD40 showed high performance in phosphate solubilization. This group of rhizospheric bacteria was firstly recognized and recorded as phosphate solubilizing bacteria. Further molecular and biochemical studies of these bacteria will provide efficient ways for incorporating these strains into biofertilizers to promote improved yield of agronomic crops and sustainable agriculture.

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