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# Variation in the Phosphate Solubilizing Bacteria from Virgin and the Agricultural Soils of Punjab

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## Abstract

Bacteria with phosphorus (P) solubilization potential are considered vital in promoting bioavailability of phosphorus in soil. The present study was conducted to isolate and study the variation of phosphate solubilizing potential of bacteria isolated from virgin and agricultural soils. Total 30 isolates from virgin soil and 4 isolates from agricultural soil which retained their activity on repeated subculturing were selected. Among the isolates, there was insignificant difference in the total bacterial count from virgin and agricultural soils, however, a significant difference was found in the phosphate solubilizing bacteria (PSB) count and their P solubiling potential. Soil organic matter and available P content were correlated with PSB count. The mean solubilization index (SI) was higher from the isolates from virgin soils. Equal distribution method was employed to categorize the bacterial isolates into low, medium, and high P solubilizers which depicted $H \geq 89.44$ and $L \leq 68$. Among all the isolates, 23.53% were high P solubilizers (P-89.44–110.88 µg/ml), 55.88% were medium P solubilizers (P- 68–89.44 µg/ml), and 20.58% isolates produced low soluble P (46.56–68 µg/ml). Analysis of the data showed that all the isolates categorized under high P solubilizers belonged to the virgin soil. The isolates were characterized based upon biochemical characterization and belonged to Pseudomonadaceae, Enterobacteriaceae, Bacillaceae, Paenibacillaceae, Micrococcaceae, Burkholderiaceae, Flavobacteriaceae, and Streptococcaceae families. 16 sRNA sequencing of the two isolates showing maximum P solubilization were characterized as *Enterobacter hormaechi*. However, they differ appreciably in their P solubilization at different temperatures.

## Introduction

Recycling of soil phosphorous (P) residual has a great potential to be used as source of P to the plants. Menezes-Blackburn [1] performed meta-analysis of the available soil P exploring various opportunities that can be directed towards recycling of the 'P bank legacy' for its better agronomic use. Crop rotation, manure and compost amendments, amendments with immobilized phytases, selective breeding programs, and genetic modifications are some of the practices followed to increase P availability to crops [2, 3]. The use of soil thriving bacterial genera, such as *Pseudomonas, Enterobacter, Aeromonas, Klebsiella, Mycobacterium, Acetobacter, Corynebacterium, Gluconacetobacter, Achromobacter, Erwinia, Escherichia, Ralstonia, Flavobacterium, Serratia, Bacilli, Rhizobium, Agrobacterium*, is a highly efficient method that can be employed to solubilize unavailable P [4]. Also, these phosphate solubilizing bacteria (PSB) augment the plant growth by various direct and indirect mechanisms. Antioxidants produced by PSB help plant in inactivation of reactive oxygen species (ROS) and enhances its tolerance to abiotic and biotic stress conditions [5].

Unfortunately, input intensive green revolution has negatively impacted the soil microbial community causing the stagnation and decline in yield [6]. Land use patterns significantly affect the soil quality by controlling the microbial structure and activity [7]. The intensive use of fertilizers has reduced the microbial counts in soil altering its natural flora [8]. There has been continuous decrease in the major taxa of complex network of useful root associated rhizospheric microbes due to agriculture intensification [9]. On contrary, the virgin soil has been considered to be more productive due to better diversity of microbes (fungi, bacteria,

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nematodes, actinomycetes, and viruses). The viable and actively reproducing microbial population has been found to be higher in the virgin soil that is directly linked to better soil quality. The conversion of virgin soil to agricultural soil has directly been linked to loss of soil microbial biodiversity both in terms of quantity and their activity [10].

PSB has been isolated from diverse environments ranging from rhizospheric and pneumatophoric zones of mangroves [11], trans-himalayan, cold desert [12], volcanic ash-derived soils [13], grasslands [14], ocean, sea, river, and lake environments [15], semiarid coastal lagoon [16] and desert [17]. Diversity and occurrence of PSB in agricultural soils with different chemical characteristics have been studied in different parts of the world [18]. Few studies have been performed in virgin or uncultivated soils [19, 20].

However, to best of our knowledge, no comparative study has been performed till date showing occurrence of PSB in agricultural and virgin soils. This study was planned with the aim to ascertain the prevalence of PSB in agricultural and virgin soils of Punjab. We also attempted to find out the relationship between the occurrence of PSB and the soil type and their ability to perform in temperature stress conditions.

## Materials and Methods

### Site Description and Soil Sampling

Soil samples were collected from the cultivated (sites having a long history of P fertilizer usage) and virgin soils (native areas covering native grasses and tress) from different sites distributed cross the sub-mountainous zone and central zone of Punjab, India, during the period of June – September, 2019. From each zone two geographically diverse districts were selected (Table 1) for the study. For sub-mountainous zone, soil samples were collected from areas in Mohali (30°53′ N, 76°38′E) and Gurdaspur (32° 2′ 30.9948′′ N, 75° 24′ 19.2024′′ E). From central zones, soil samples were collected from Nawanshahr (31.1167° N, 76.1333° E) and Ludhiana (30.9010° N, 75.8573° E). From each district, two soil samples of virgin soil and two samples of agricultural soil were collected. Each sample was collected in triplicates and mixed to form a composite sample. The soils in these regions are classified as alkaline with medium to high P content (Punjab State Council for Science & Technology, 2015). Rhizospheric soil adhering to root hair was removed from the depth of 15 cm and collected in sterile plastic vials and stored at °C until use. One part of each sample was used to isolate the PSB and other part was analyzed for physiochemical characterization of soil like soil texture, pH, electrical conductivity (EC), organic matter (OM), P and potassium (K). Physiochemical characterization of the soil was performed at Department of Soil Science, Punjab Agricultural University, Ludhiana using the method described by Okalebo et al. [21]. All experiments were performed in triplicates.

**Table 1** Total Bacterial count and total PSB count in soils from different areas of Punjab

| S.no | District | Total number of colonies ($10^7$) | PSB CFU ($10^7$) | % PSB |
|---|---|---|---|---|
| Virgin soil | | | | |
| 1 | Nawanshahr S1 | 3.24 ± 4.55 | 0.367 ± 0.47 | 1.13 ± 0.13 |
| 2 | Nawanshahr S2 | 4.45 ± 3.68 | 0.167 ± 1.25 | 0.30 ± 0.28 |
| 3 | Ludhiana S1 | 2.75 ± 4.11 | 0.367 ± 2.05 | 1.32 ± 0.73 |
| 4 | Ludhiana S2 | 2.19 ± 4.50 | 0.3 ± 0.82 | 1.37 ± 0.38 |
| 5 | Gurdaspur S1 | 1.75 ± 3.68 | 0.167 ± 0.47 | 0.95 ± 0.27 |
| 6 | Gurdaspur S1 | 1.97 ± 3.68 | 0.467 ± 0.47 | 2.35 ± 0.20 |
| 7 | Mohali S 1 | 5.51 ± 8.60 | 0.833 ± 0.94 | 1.51 ± 0.19 |
| 8 | Mohali S 2 | 6.20 ± 9.09 | 0.133 ± 0.47 | 0.21 ± 0.08 |
| | Mean | 3.50 | 0.35 | 1.14 |
| | Range | 1.75–6.20 | 0.133–0.833 | 0.30–2.35 |
| Agricultural soil | | | | |
| 1 | Nawanshahr S1 | 3.23 ± 9.20 | 0.01 ± 0.00 | 0.31 ± 0.01 |
| 2 | Nawanshahr S2 | 3.37 ± 13.20 | 0 ± 0.00 | 0.00 ± 0.00 |
| 3 | Ludhiana S1 | 3.14 ± 7.59 | 0 ± 0.00 | 0.00 ± 0.00 |
| 4 | Ludhiana S2 | 3.21 ± 48.36 | 0.01 ± 0.00 | 0.32 ± 0.04 |
| 5 | Gurdaspur S1 | 2.18 ± 8.06 | 0.01 ± 0.00 | 0.45 ± 0.02 |
| 6 | Gurdaspur S1 | 1.84 ± 4.99 | 0 ± 0.00 | 0.00 ± 0.00 |
| 7 | Mohali S1 | 5.81 ± 5.79 | 0.67 ± 0.47 | 0.11 ± 0.08 |
| 8 | Mohali S2 | 4.88 ± 3.09 | 1.33 ± 0.47 | 0.27 ± 0.10 |
| | Mean | 3.45 | 0.25 | 1.46 |
| | Range | 1.84–4.88 | 0.00–1.33 | 0.00–0.45 |

S1 and S2 represents sample 1 and sample 2

Values are Mean ± S.D of three independent estimations

### Isolation and Enumeration of Phosphate Solubilizing Bacteria

Bacteria with inorganic phosphate solubilizing potential were isolated and enumerated using the Pikovskaya (HiMedia, Mumbai) medium [22]. Under aseptic conditions, one gram of soil sample was taken and homogenized in 9 ml of $dH_2O$ and serially diluted upto $10^6$. Each aliquot was spread plated on Pikovskaya agar and incubated for 48 to 168 h at 28 °C. The total bacterial count (TBC) and PSB count was enumerated and expressed as colony forming unit. The enumeration data were collected in triplicates. The bacterial colonies with clear zones were selected and repeatedly sub cultured to get potential strains that do not lose their PS potential on repeated sub culturing on the same medium. Potential isolates were preserved in sterile 20% glycerol at − 80 °C.

### Determination of Phosphate Solubilizing Index

Phosphate solubilization index was calculated by inoculating 10 µl of 24 h old culture containing approximately $10^6$ CFU/ml in center of Pikovskaya agar plates supplemented with TCP. To minimize the chances of error, each sample was inoculated in triplicates. The plates were incubated at 28 ºC. The diameter of colony and the halo zone diameter were measured at 48 h, 96 h and 168 h and the solubilization index were calculated using the following formula: Phosphate Solubilization Index: (Colony diameter + Halo diameter) / Colony diameter.

### The Quantitative Estimation of Soluble Phosphorous

The in vitro quantitative analysis of soluble phosphorous was determined according to the method given by Bray and Kurtz [23] with slight modifications. Pikovskaya's broth (50 ml) was inoculated with culture of 0.5 McFarland standard grown in the same media. Triplicate flasks were inoculated for each isolate and incubated at 28 °C at static conditions. For the quantification of available soluble phosphorus, 2 ml of sample was taken aseptically at different time intervals, i.e., 48 h, 96 h, and 168 h and centrifuged at 11410 g for 10 min. The cell free supernatant was used to quantify the amount of soluble P released by bacteria by phospho-molybdate blue color method. The quantification of soluble P of the two isolates showing highest solubilization after 72 h of inoculation was done at different temperature (22 ºC, 28 ºC, 35 ºC, 42 ºC) to test their activity at temperature stress conditions.

### Characterization and Identification of the Isolates

For the identification of bacteria, various biochemical tests were performed according to the Bergey's Manual of systematic bacteriology [24] and the results were interpreted using the ABIS 7 online software. One isolate each from virgin soil and agricultural soil producing highest soluble P after 72 h of incubation were further identified using the 16S ribosomal DNA (rRNA) sequencing methods. The bacterial culture of Optical density 0.5 was used for extraction of genomic DNA using the method described by [25]. The extracted DNA (67.4 ng/µl) was amplified along with 10 pM of each of 16S forward primer (5′-GGATGAGCCCGCGGCCTA-3′) and 16S reverse primer (5′-CGGTGTGTACAAGGCCCGG-3′) primer using high–fidelity PCR polymerase. After 35 cycles of initial denaturation for 3 min, followed by denaturation for 1 min at 94 °C, annealing for 1 min at 55 °C, extension for 2 min at 72 °C, the final extension was carried out at 72 °C for 7 min. The PCR product was purified using the gel electrophoresis and sequenced using Big Dye Terminator sequencing machine version 3.1 (ABI 3130 Genetic Analyzer). The sequences were further aligned with reference 16S rRNA sequences in the NCBI database using the BLAST program and analyzed to identify the bacteria and its closest neighbors using MEGA7 software [26].

### Statistical Analysis

The data were presented as Mean ± SD of three replicates. Mean values were subjected to Tukey's test using SPSS version 16.0. Equal distribution method was employed to categorize the isolates as low, medium and high P solubilizers by subtracting the minimum value from the maximum value and the difference of the two was divided by 3 to get the three classes with equal interval.

## Results and Discussion

The preliminary isolation of bacteria with phosphate solubilization potential was done using tricalcium phosphate amended Pikovskaya medium because in the alkaline soils inorganic phosphate fractions are precipitated with calcium ions [27]. No significant difference was found in the TBC among the agricultural and virgin soil. The TBC after 24 h of incubation ranged from $1.75–6.20 \times 10^7$ CFU/ g and $1.84–5.81 \times 10^7$ CFU/ g in the virgin and agricultural soil, respectively. However, the virgin soils account for significantly higher proportion of PSB (86%) compared to low of 14% in agricultural soil. The virgin soils accounted for PSB count between $0.133–0.833 \times 10^7$ CFU/ g, compared to a low count of $0.01–0.67 \times 10^7$ CFU/ g of agricultural soil (Table 1). Total six PSB were isolated from agricultural soils (data not shown). Of these, two isolates lost their solubilization potential on repeated subculturing. The four isolates were selected for further studies. No phosphate solubilizing bacteria was isolated from three agricultural soils samples (Table 1). In contrast, total 30 isolates were selected from virgin soil samples which retained their activity on repeated subculturing.

The results showed that the prevalence of differential PSB in these soils can be attributed to differences in the soil properties, soil conditions [28] and application of fertilizers [29, 30]. Previous studies have reported similar results of low

percentage of PSB in agricultural soils [18, 31–33]. Insignificant difference in TBC and high significant difference in PSB among the virgin soil and agricultural soil may be due to the use of inorganic fertilizers that produce no effect on the soil microbial population but affect the microbial activity [28]. The regular addition of fertilizers and chemical agents has resulted in the loss of microbial activities that have resulted in loss in phosphate solubilizing phenotype [30].

Both the virgin soils and the agricultural soils were found to be alkaline in nature with a mean pH of 8.2 and 8.1, respectively (Fig. 1, Supplementary data). Soil pH is an important factor governing the diversity of rhizospheric bacterial diversity because it directly affects the bacterial growth, reproduction, and interaction with environment [34]. The optimal intracellular pH for most bacterial taxa lies within one pH unit of neutral [35] and therefore any significant variation put stress on bacteria taxa that cannot adapt to varying soil pH. The abundance of PSB is reported to increase with soil pH [34]. However in the present study, soil pH was found to be insignificantly correlated to the PSB count (Table 2). Similarly results were reported by Ndung'u-Magiroi et al. [18] and Abderrazak et al. [33]. The present study is undertaken in alkaline soils, and no significant difference was found in pH of the agricultural and virgin soils,

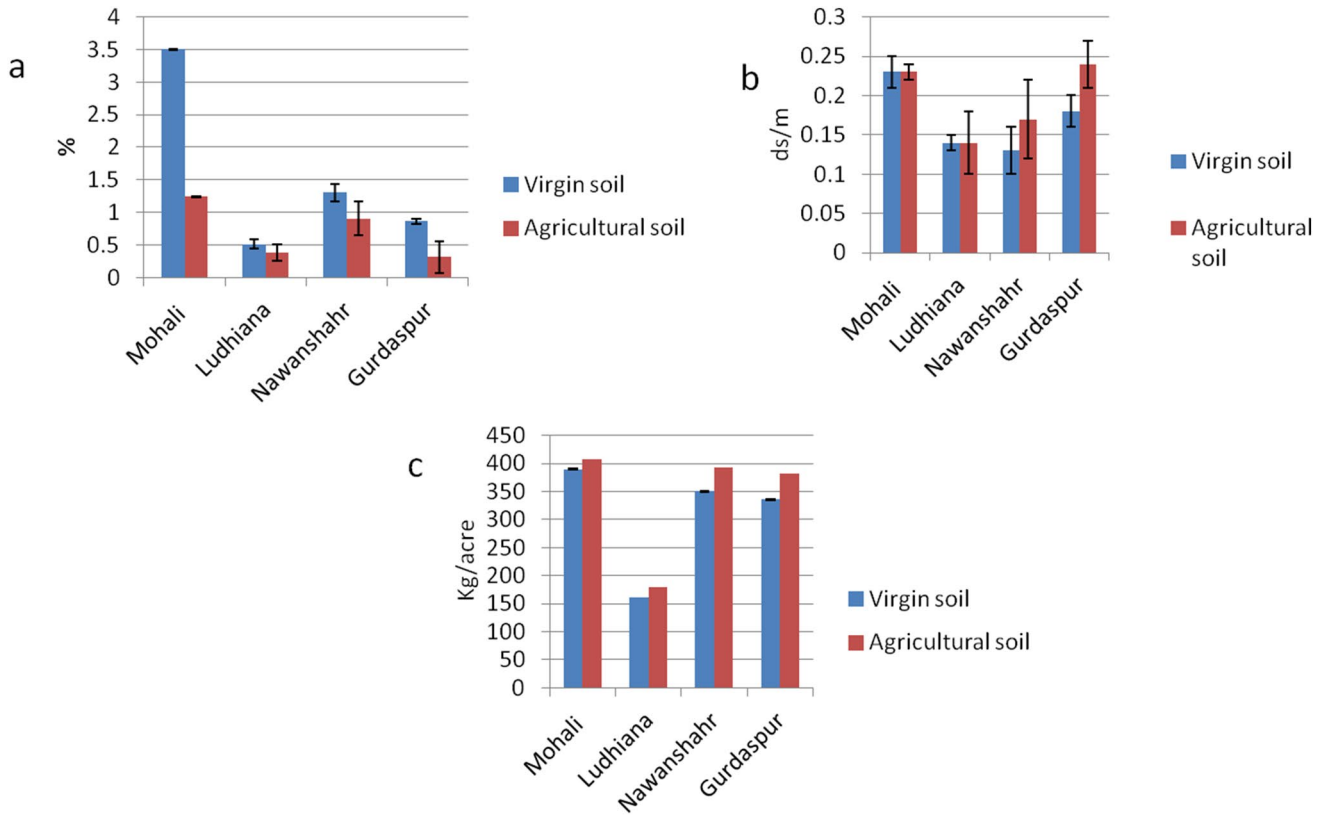


**Fig. 1** Soil Analysis results. a—Organic Carbon (%), b- Electrical conductivity (ds/m), c- Available P (Kg/acre). Values are mean ± S.D of three independent estimations

**Table 2** Multiple correlation between PSB and soil properties

| | PSB | pH | EC | OC | P | K |
|---|---|---|---|---|---|---|
| PSB | | | | | | |
| pH | 0.269 | | | | | |
| EC | −0.021 | 0.173 | | | | |
| OC | 0.692** | 0.394 | 0.379* | | | |
| P | −0.894** | −0.195 | −0.159 | −0.571* | | |
| K | −0.049 | 0.426 | 0.680** | 0.443** | −0.082 | |

*Represents significance at $P < 0.05$

**Represents significance at $P < 0.01$

the difference in number of PSB may be attributed to the fact that imbalanced use of inorganic fertilizers has altered the bacterial composition [36] and functional diversity [34].

The OC content of the virgin soils was found to be higher (ranging from 0.51–3.5%) than the OC of agricultural soils (ranging from 0.9 – 1.24%) (Fig. 1a). The soils with high OC was found to have higher number of PSBs, these results are in accordance with the results obtained by Vikram et al. [37]. Strong positive correlation was found between PSB count and organic carbon (r = 0.692). Kumar and Rai [38] also reported a high positive correlation between the OC and PSB abundance. As the PSB are heterotropic bacteria, OC is important for the proliferation, microbial growth, functionality and diversity. The higher availability of OC leads to an increase in microbial biomass, catabolic activity, and in turn microbial abundance [39]. Incidence and abundance of PSB increase with OC [14]. Conversion of uncultivated soils to agricultural land decreases the OC, thus negatively affecting the microbial activity [40].

The total available P content was lower in the virgin soils ranging from 14.30–29.69 kg/acre and higher in the agricultural soils (ranging from 20.44–29.69 kg/acre) of all areas (Fig. 1c). The higher P content in the agricultural soil is due to the excessive application of P fertilizers, 60–70% of which remained unused and get fixed in the soil as unavailable reserves. P availability is directly linked to the incidence of PSB. In the present study, PSB count was found to be significantly negatively correlated with the increasing P reserves of soil (r = −0.894, Table 2). Also, with declining soil P content the incidence of rhizospheric bacteria with PS phenotype increases [14]. Phosphate solubilization is linked to the soil ecosystem. Ndung'u-Magiroi and coworkers [18], found no significant correlation between the PSB and soil available P, likely due to low range of available P in those soils. Gyaneshwar et al. [41], reported that PSB lose in vitro solubilization activity on repeated subculturing. These results attribute to the fact with although there is no variation in rhizospheric bacterial number, PS phenotype significantly vary because under high P stress, they may lose P solubilization ability. Phenotype of P solubilization is repressible in high available P [42].

Very few variations were found in the EC values of the virgin soil and agricultural soil. All the soil samples are non-saline, EC content of the soil samples ranged from 0.13–0.24 ds/m. This type of soil is important for the soil microbial growth and processes. No significant correlation was found between EC, K content and PSB (Table 2). The K content ranged from 160.50– 390 kg/acre of the virgin soils which is lower than the agricultural soils (179.13–407.7Kg/acre). Although K is a limiting factor for crop yield, application of K had no effect microbial diversity and function in alkaline soil [43].

Biochemical analysis showed that all the isolates belonged to ten different families including Pseudomonadaceae (8%), Enterobacteriaceae (26.47%), Bacillaceae (38%), Paenibacillaceae (5.88%), Micrococcaceae(2.9%), Burkholderiaceae (8.82%), Flavobacteriaceae (2.94%) and Streptococcaceae (5.88%) suggesting wide diversity of PSB in the soil. Isolates from agricultural soils belonged to Burkholderiaceae and Enterobacteriaceae family. Biochemical analysis revealed that *Bacillus niacin* (9) and *Klebsiella oxytoca* (5)were the dominant species followed by *Pseudomonas sp.*(2), *Streptococcus sp.* (2), *Enterobacter cloacae* (2), *Enterobacter hormaechei*(2), *Enterobacter cloacae* (2), *Burkholderia sp.*(2). On the other hand, *Bacillus megaterium* (1), *Micrococcus leteus* (1), *Bacillus flexus*(1), *Providenci arettgeri*(1), *Bacillus subtilis*(1), *Paenibacillus thiaminolyticus* (1), *Bacillus endophyticus* (1), *Arthrobacter ramosus* (1), *Paenibacillus polymyxa* (1), *Myroides spp.*(1), *Paraburkholderia tropica*(1) were least abundant species of PSB present in the soil. Earlier studies have also reported bacteria belonging to these families are potential phosphate solubilizers [18, 44].

The screening of the P solubilizing bacteria was done on the basis of solubilization index (SI) shown by the bacteria. The mean of SI after 48 h, 96 h and 168 h of incubation was 1.64, 2.87, 2.32 in virgin soils and 1.68, 2.09, 1.85 in agricultural soils (Fig. 2, Supplementary data). Potential SI was observed in all the isolates on the 2nd day, which increased on the 4th day. However, no further increase in the SI was observed. The mean SI was higher of the isolates from virgin soils; however, no significant variation was found in mean SI of the isolates from virgin soils and agricultural soils (Fig. 4). Decrease in solubilization index by PSB after 72–96 h of incubation is reported earlier by Chakkaravarthy et al. [45], Chen and Liu, [44].

To study the efficiency for phosphate solubilization, the quantification of soluble P formed in the supernatant by different isolates from the virgin soils and agricultural soils was tested on seven continuous days of incubation. Content of soluble P formed by the isolates from virgin and agricultural

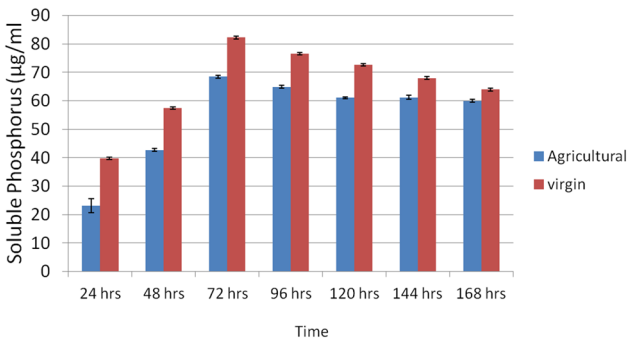


**Fig. 2** Soluble P (mean) at different time intervals

soils was highest on 72 h of incubation with the mean of 82.28 µg/ml and 68.49 µg/ml, respectively (Fig. 2). Equal distribution method was employed to categorize the bacterial isolates into low, medium and high P solubilizers which depicted H ≥ 89.44 and L ≤ 68. Eight isolates (23.53%) were high P solubilizers with soluble P content ranging from 89.44–110.88 µg/ml. Nineteen isolates (55.88%) were medium P solubilizers with soluble P content ranging from 68–89.44 µg/ml and seven isolates (20.58%) produced low soluble P content ranging from 46.56–68 µg/ml. Analysis of the data showed that the all the isolates categorized under high P solubilizers belonged to the virgin soil. The isolates A24 (*Paraburkholderia tropica)*, A27 (*Enterobacter cloacae*), A25 (*Enterobacter hormaechei*) and A28 (*Klebsiella oxytoca*) isolated from agricultural soil lie in medium and low P solubilizing categories. Figure 5 depicts that the mean of soluble P formed was seen to be all time high in the isolates from virgin soils, indicating that they are better P solubilizers than the isolates from agricultural soils. The decrease in amount of soluble P after 72 h of incubation may attribute to high amount of P needed to sustain bacterial growth [46] or due to the accumulation of the chemical compounds due to the bacterial metabolism which causes the reprecipitation of phosphate compound into intermediate phosphatic species, brushite [47]. The secondary solubilization has been earlier reported by Seshadri et al. [48] and Goenadi and Sugiarto, [49].

Among all the isolates, A26 and A27 with maximum phosphate solubilization efficiency after 72 h of incubation were selected for molecular identification. Sequences obtained were deposited in Gen-Bank nucleotide-sequence database under the accession number MN947245and MN865174. Similarity search analysis showed that isolates A26 was identical to *Enterobacter hormaechei subsp. xiangfangensis strain* (99.58%). The next closest homologue was found to be *Enterobacter cancerogenus strain* (Fig. 3) and A27 had highest identity (98.06%) *Enterobacter hormaechei subsp. xiangfangensis strain*. The next closest homologue was found to be *Enterobacter cloacae* strain (97.91%) (Fig. 4). Previous studies have reported genera *Enterobacter* as dominant PSB in soils [33]. *Enterobacter hormaechei* as the potential phosphate solubilizer has earlier been reported by Gupta et al. [50], Mardad et al. [14]. These results indicate that the bacterial composition of agricultural and virgin may be similar, except to the relative abundance of the PSB. Wei et al. [51], also reported that the composition of bacteria from two soil samples with different physiochemical properties was same but their activity vary due to different land use patterns [7].

Results from 16 sRNA sequencing showed that both isolates with highest solubilization efficiency after 72 h of incubation showed maximum homology with *Enterobacter hormaechei*. To study the comparative analysis of P solubilization by these bacteria in relation to their origin of isolation, P solubilization was checked at different temperatures, so as to determine their ability to perform at temperature induced abiotic stress. Both the isolates exhibited active solubilization at 22 °C, 28 °C, 35 °C and 42 °C. In both the isolates, maximum P solubilization was seen at 28 °C with 110.88 µg/ml and 84.70 µg/ml in A26 and A27, respectively. A26 was an active solubilizer of TCA showing P solubilization ranging from 24.07–65.34 µg/

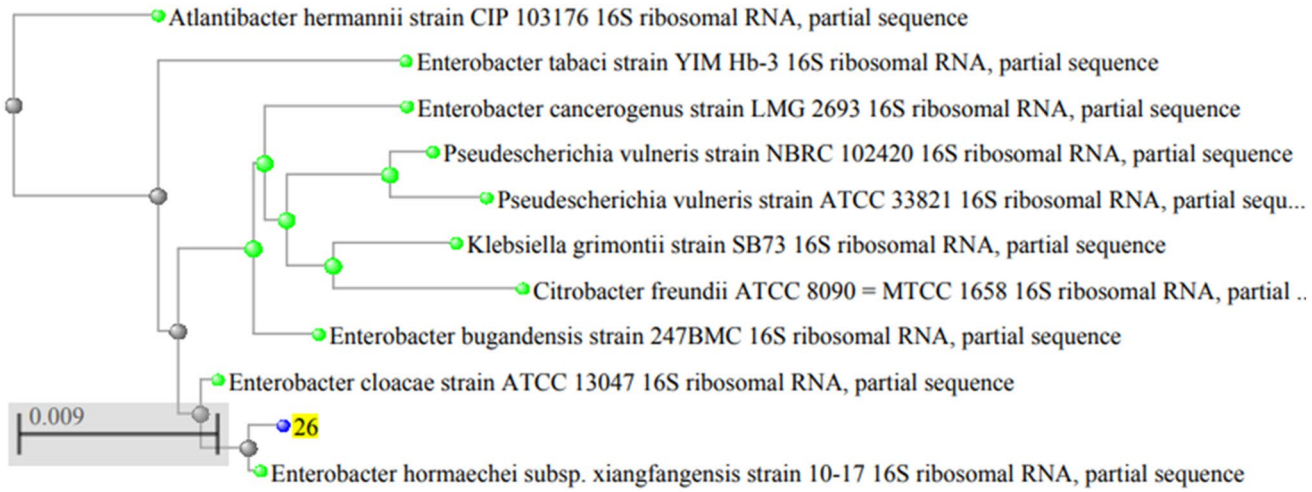


**Fig. 3** Phylogenetic tree based on the 16S rDNA of isolate A26. The tree was constructed by neighbor-joining method

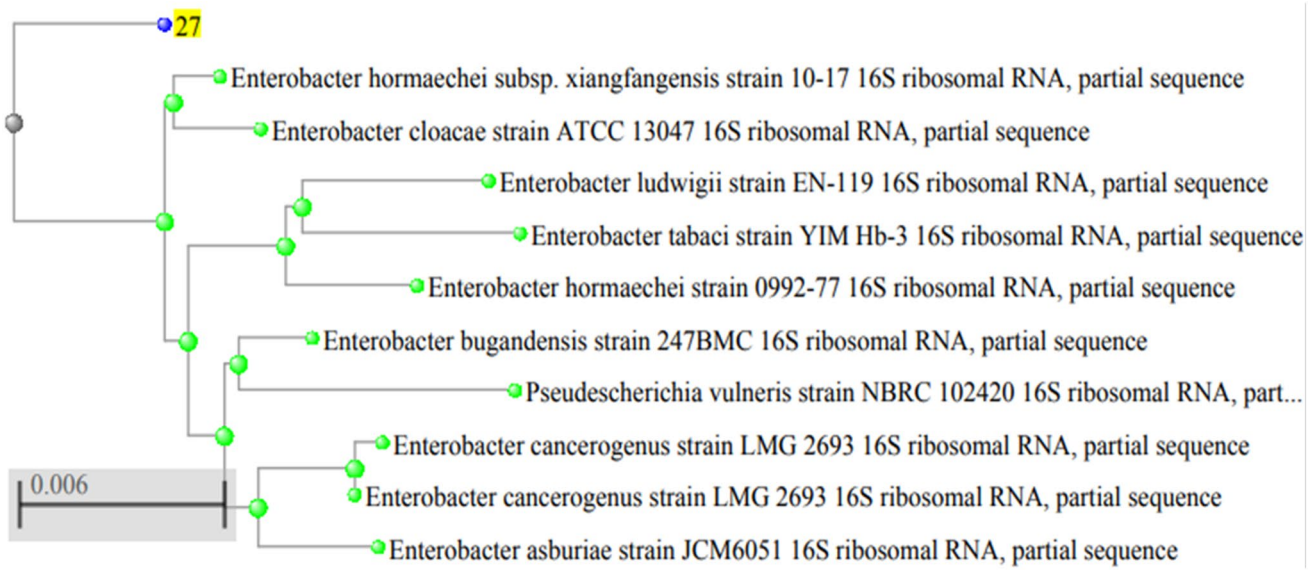


**Fig. 4** Phylogenetic tree based on the 16S rDNA of isolate A27. The tree was constructed by neighbor-joining method

ml, 14.37–72.71 µg/ml, and 24.57–63.09 µg/ml at 42 °C, 35 °C and 22 °C, respectively. A27 showed consistency in its solubilization pattern with a mean of 37.28, 36.76, 66.59 µg/ml at temperature 42 °C, 35 °C and 22 °C, respectively. Jha et al. [52], in a study reported maximum solubilization at 35 °C (Fig. 5). The differences in observation may because of the fact that they isolated the bacteria from desert soil with comparable high temperature. Notably, both the isolates belonged to Genus *Enterobacter* but exhibited variable degree of P solubilization at different temperatures. The isolate from virgin soils A26 was able to perform at temperature stress conditions as compared to isolate from agricultural soil i.e., A27. This may be due to the fact that function of soil microorganism is independently regulated by land use patterns and intensity [53]. Land use intensity can have a pronounced effect on the microbial functionality in rhizosphere [54], indicating the low P solubilization of isolate A27 (isolated from agricultural soil) was due to intensive agricultural practices.

From the present study it can be concluded that the soil's physiochemical components could be the possible reasons behind the low activity of the PSB towards P solubilization in agricultural soils. This points out to the urgent need of adopting the sustainable agricultural practices and adopting organic fertilizers so that soil can regain its organic matter that correlates to the functioning of rhizobacteria. It should be emphasized that soil inorganic P reserves accumulated due to inorganic fertilizers can also inhibit PS potential in the rhizobacteria. Further studies should be focused on the molecular and biochemical mechanisms to understand the loss of the PS potential of the rhizobacteria in response to OM, EC and K. The P solubilization by isolate from virgin soil at different temperature range was found to be higher than the isolate from agricultural soil. The present study opens the new avenues to study the underlining reason behind this attribute.

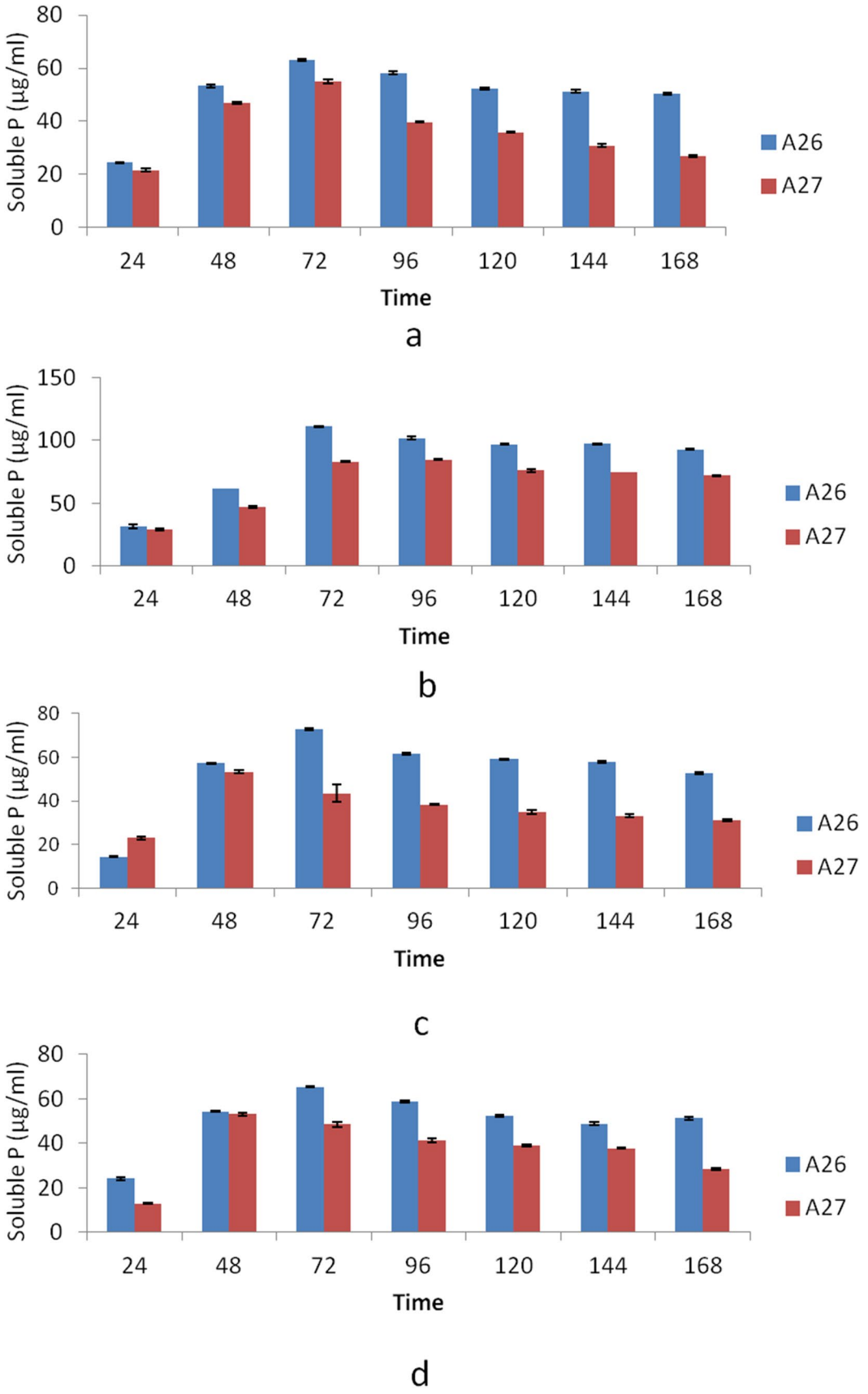


**Fig. 5** Tricalcium phosphate solubilization by isolate A26 and A27 at different temperatures (**a** 22 ºC, **b** 28 ºC, **c** 35 ºC, **d** 42 ºC)

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**Author contributions** The research proposal was designed by Dr. SK, Ms. RK, performed the experimental work and paper writing. Final paper editing and improvement was followed by Dr. SK.

## Compliance with Ethical Standards

**Conflict of interest** Authors declare no conflict of interest (financial and non-financial).

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