The Bioscan 9(4): 1435-1443, 2014
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

# POPULATION AND FUNCTIONAL DIVERSITY OF PHOSPHATE SOLUBILIZING BACTERIA FROM APRICOT (*PRUNUS ARMENIACA*) OF MID AND HIGH REGIONS OF HIMACHAL PRADESH

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**KEYWORDS**
Apricot
P-solubilizing rhizobacteria
Indole acetic acid
Siderophore
Antifungal activity
HCN production

**Received on :**
01.06.2014

**Accepted on :**
16.10.2014

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**ABSTRACT**

Phosphate solubilizing microorganisms play an important role in supplementing phosphorus to the plants by several mechanisms and thus benefit plant growth and development. In this study, isolation, screening and characterization of phosphate solubilizing bacteria (PSB) from different apricot growing sites of mid and high hill regions of Himachal Pradesh were carried out. A total of one hundered and eighty eight bacterial isolates were recovered from the rhizosphere soils of the apricot trees. The cells of all bacterial isolates were rod shaped, motile and gram positive in reaction. Seventy two P-solubilizing bacterial isolates were selected and further characterized for different plant growth promoting activities. Indole acetic acid production was detected in 59.72% isolates, siderophore synthesis in 48.61% isolates, hydrogen cyanide in 5.55% isolates and the per cent growth inhibition against *Dematophora necatrix* was detected in 65.28% PSBs. Overall, 9.60% of PSB isolates from RS and 8.10% from ER showed none of the PGPTs tested. The study on distribution of PGPT-possessing PSBs revealed that they are distributed more within rhizosphere soil than in endorhizosphere according to Shannon-Weaver diversity index. The most efficient strain $AG_{1(3)}$ was identified as *Bacillus subtilis* by 16S rDNA analysis. Results indicated that identification and characterization of soil PSBs for the effective plant growth-promotion broadens the spectrum of phosphate solubilizers available for field.

## INTRODUCTION

Apricot (*Prunus armeniaca*) is a member of family Rosaceae and is one of the important stone fruit crop of mid hills and dry temperate region of India. In India, apricot is being grown commercially in the hills of Himachal Pradesh, Jammu and Kashmir, Uttarakhand and to a limited extent in north-eastern hills between the elevations of 900-2000 meter above mean sea level with a wide climatic adaptability (Loudon, 1838). In Himachal Pradesh, it occupies an area of 3556 ha with an annual production of 2438 metric tonnes in 2011-2012 (Anonymous, 2012) and is one of the important temperate fruit crop on the basis of its consumption and nutritive value. However, there is increasing problems associated with the use of synthetic chemicals in agriculture that include impacts on health and the environment, resistance development in plant pathogens and pests, etc. To resolve these problems, there has been an ever-increasing interest in the use of native and non-native beneficial microorganisms to improve plant health and productivity while ensuring safety for human consumption and protection of the environment (Kushwaha *et al.,* 2013; Das and Singh, 2014).

Great interest towards environmental friendly agricultural practices has been increased in recent years. Therefore, it become necessary to develop biofertilizers derived from indigenous PGPR for use in organic production of apricot (FAO, 2011). Numerous soil bacteria which flourish in the rhizosphere of plants, which may grow in, on, or around plant tissues and stimulate plant growth by a plethora of mechanisms are collectively known as Plant Growth Promoting Rhizobacteria (PGPR).

PGPR are directly involved in increased uptake of nitrogen, synthesis of phytohormones, solubilization of minerals such as phosphorus and production of siderophore chelate ions and make it available to plant root. Among different plant nutrients, phosphorus is most important and it is least mobile element in plant and soil contrary to other macronutrients. Plants acquire phosphorus from soil solution as phosphate anion. It precipitates in soil as orthophosphate or is absorbed by Fe and Al oxides through legend exchange. Phosphorus solubilizing bacteria play role in phosphorus nutrition by enhancing its availability to plants through release from inorganic and organic soil P pools by solubilization and mineralization. Principal mechanism in soil for mineral phosphate solubilization is lowering of soil pH by microbial production of organic acids and mineralization of organic P by acid phosphatase (Sharma *et al.,* 2011). In turn, the plants supply root borne carbon compounds, mainly sugars which can be metabolized for bacterial growth (Deubel *et al.*, 2000).

There are several studies indicating that soil inoculation with phosphate solubilizing bacteria improve solubilization of fixed

soil P and result in higher yield of agricultural crops (Das *et al.,* 2001; Bashan and Holguin, 2002; Zayed *et al.,* 2005). Though much information is available on activity of soil micro-organisms and plant growth promotion for annual crops (Glick *et al.,* 1995; Bashan, 1998), very limited information is available in respect of phosphate solubilizing bacteria (PSB) associated with the perennial crop such as apricot. Thus, objectives of present study was to explore population and functional diversity of PSBs obtained from rhizosphere of *Prunus armeniaca*.

## MATERIALS AND METHODS

### Site characterization and sampling

Rhizosphere soil and roots samples of apricot trees (*Prunus armeniaca*) were collected from six different locations (Nauni, Gaura, Kandaghat and Subathu, Tabo and Recongpeo) of mid hills and high hills region of Himachal Pradesh. Five plants were selected randomly from each site. A total of 60 soil (RS) and root (ER) samples were used for isolation of total bacterial population and PSB population. Samples were immediately stored at 4ºC in plastic bags loosely tied to ensure sufficient aeration and to prevent moisture loss until assaying of bacterial community structure.

### Isolation of RS and ER PSB communities

Rhizobacteria were isolated from the RS and ER samples of *Prunus armeniaca* plants by serial dilution using standard spread plate technique. The serially diluted soil samples were plated on standard Pikovskaya's (PVK) agar medium (pH 7.0) containing tricalcium phosphate (TCP) as sole phosphorus source for selectively screening the bacteria which have the ability to release inorganic phosphate from tricalcium phosphate and incubated at 37ºC for 48h. Populations were expressed as colony forming unit (cfu) per gram of dry soil weight. Endorhizobacteria of *Prunus armeniaca* were isolated using surface sterilization, dilution plating assay (Shishido *et al.,* 1999) and expressed as cfu per gram of root weight. Colonies with clear zones were further purified by replating on agar medium supplemented with TCP. After cultivation, distinct PSB morphotypes were differentiated on the basis of colony morphology, pigmentation and growth rate.

### Quantitative analysis of PSBs in RS and ER of *Prunus armeniaca*

For quantitative analysis, following parameters were considered:

I. Simpson's index of dominance (D)

$$D = \sum P_i^2$$

where $P_i$ is the relative abundance of isolates calculated according to the following equation:

$$P_i = \frac{n_i}{N}$$

Where $n_i$ is the number of individuals with their respective activities and N, is the total amount of individuals considered

II. Shannon–Weaver index of diversity (Hm)

$$H_m = -\sum P_i \ln P_i$$

The diversity of P-solubilizing PGPR in the RS and ER of different sites of two locations was evaluated using diversity indices. Shannon–Weaver index of diversity (Hm) and Simpson's index of dominance (D) are, in general, the measures of diversity that accounts for both richness and proportion of individual (Ventorino *et al.,* 2007).

### Screening for potential plant growth promoting attributes

### Qualitative and quantitative mineral phosphate solubilization

Isolates were screened on Pikovskaya's (PVK) agar plates. Each isolate was inoculated on to the plate and incubated at 37ºC for 48h. The P-solubilization was exhibited with a clear yellow zone formed around the colony. Quantitative estimation of phosphorous (Sundra Rao and Sinha, 1963) was done in PVK broth supplemented with 5.0 $gl^{-1}$ tri-calcium phosphate (TCP) and solubilization of phosphorous was calculated using standard curve of $KH_2PO_4$ (0-10 ppm).

### Siderophore production

The ability of the isolates to produce siderophore(s) was determined using blue agar plates containing chrome azurol S. Each isolate was inoculated on to the plate and incubated at 37ºC for 48 h. Orange halos around the isolate on the blue agar served as indicators of siderophore(s) excretion. Quantitative estimation of siderophores was performed by using CAS liquid assay method (Schwyn and Neilands, 1987).

### Ability to grow on Nitrogen free medium

Each isolate was inoculated on Jensen's medium, incubated at 37°C for 72 h and the colonies showing growth were selected.

### IAA production

For the production of auxins, each isolate was grown in Luria–Bertani broth (amended with 5 mM L-tryptophan, 0.065 % sodium dodecyl sulphate and 1 % glycerol) for 72h at 37ºC under shake conditions. Colorimetric estimation of IAA-like auxins was done using Salkowski reagent (Glick *et al.,* 1995).

### HCN production

For HCN production, isolates were streaked on King's B agar medium with 4.4 g glycine L-1. Filter paper, Whatman no. 1, was cut into uniform strips, 8 cm long and 0.5 cm wide saturated with an alkaline picrate solution (0.5 % picric acid, 0.2 % sodium carbonate; pH 13) and placed inside the lid of a Petri dish. The plates were then sealed airtight with parafilm and incubated at 28ºC for 48 h. Thereafter, a colour change in the sodium picrate present in the filter paper from yellow to reddish brown was considered to be an indication of HCN production (Baker and Schippers, 1987).

### Antifungal activity

Antagonistic activity of the culture filtrate of isolates was studied using agar dilution technique (Warnock, 1989). 72h old culture of bacterial isolates was centrifuged at 15000 rpm for 20 min at 4ºC. Supernatant was filter sterilized using millipore

filter (pore size= 0.22 $\mu$m). Potato dextrose agar plates containing 10% concentration of the filtrate was prepared. Then plates were incorporated with fungal bits (5 mm) of the test pathogens *i.e. Dematophora necatrix* obtained from fungal culture collection centre of Department of Plant Pathology. The plates were incubated at temperature 24 $\pm$ 1ºC for 7 days i.e. when the control plate is filled completely with fungal growth and then colony diameter was measured. Un-inoculated control was kept for comparison of results.

**Phylogenetic analysis of PSB**

Total genomic DNA of the most efficient PSB isolate was extracted and purified using the method (Sambrook *et al.,* 1989) and its 16S rRNA genes was amplified by polymerase chain reaction (PCR) using the combination of designed universal primers (forward 5'GCAAGTCGAGC GGACAGA TGGGAGC3') and (reverse5' AACTCTCGTGGTGTG ACGGGCGGTG3'). PCR reaction was carried out in 20 $\mu$L reaction volumes containing ~50ng of template DNA, 20 picomoles of each primers, 0.2 mM dNTPs and 1U Taq polymerase in 1X PCR buffer. Reaction were cycled 35 times as 94ºC for 30 s, 58ºC for 30 s, 72ºC for 1 min 30 s followed by final extension at 72ºC for 10 min. The amplified products of nearly 1500 bp was purified with an Agarose gel DNA purification kit (Real genomic kit, Taiwan, Cat.No. YDF100) and used for sequencing and phylogenetic analysis. The sequencing was done by commercial sequence facility (Xcleris lab. Ahmadabad).

Based on the results of the database searches, sequences were aligned with representative bacterial sequences from the GenBank database using ClustalX and then manually adjusted. Phylogenetic tree was constructed by neighbor-joining algorithm using PHYLIP package (Felsenstain, 1993). The stability of phylogenetic tree was accessed by taking 1000 replications of data set and was analyzed using the programme SEQBOOT, DNADIST, NEIGHBOR and CONSENSE of the PHYLIP package. Tree was viewed with the help of Tree View (Page, 1996).The partial 16S rDNA sequences of the representative PSB isolate were deposited in GenBank, and provided with the accession number (KF560310).

**Statistical analysis**

All the experiments were conducted under statistical framework in triplicates along with equal number of appropriate controls. Appropriate statistical/mathematical tools were utilized as per the objectives and cross-section variation of the data. Dendrograms were also constructed using the unweighted pair-group method with arithmetic mean. All statistical procedures were performed using SPSS 16.0 (Analytical Software).

## RESULTS

**Isolation and enumeration of PSBs from different apricot growing locations around mid and high hills of Himachal Pradesh**

A total of 188 bacterial isolates were isolated from the rhizospheric soil (RS) and endorhizospheric (ER) samples of apricot (*Prunus armeniaca*). Table 1 showed the ability of phosphorous solubilization, siderophore production and growth on N-free medium of 114 rhizospheric soil (RS) and 74 endospheric isolates (ER) associated with apricot trees. Over all, eleven of the (9.60%) of the total one hundred and fourteen isolates from the RS samples and six (8.10%) of total 74 isolates from the ER samples lacked PGP traits.

Percentages of bacterial isolates with binary traits, (Phosphate solubilization and siderophore production) was highest in RS of site Nauni (15.80%) and ER of site Gaura (15.40%). PGPR percentages having growth on N-free medium and phosphate solubilization activity was highest for RS site of Gaura (18.20%) and ER site of Recongpeo (20.00%). Highest percentages of PGPR having binary traits (Siderophore production and nitrogen fixation) was found in RS of Tabo (33.30%) and ER of Subathu (41.70%) (Table 1).

Ratios of PGPR showing triple traits of phosphate solubilization, siderophore production and ability to grow on nitrogen free medium was highest in RS of site Subathu (21.10%) and ER of site Recongpeo (33.30%). In particular, ability to grow on N-free medium was found in majority of endophytes.

Percentages of PGPRs with phosphate solubilization, siderophore production and ability to grow on nitrogen free medium among six sites were decpicted in Fig.1 and 2. Percentages of bacteria having phosphate solubilization activity were arranged in the following descending order Gaura (54.28%) > Recongpeo (50.00%) > Subathu (48.38%) > Nauni (40.62%) > Tabo (28.00%) > Kandaghat (14.81%). From the results it was revealed that only 38.29% (72/188) isolates were able to solubilize TCP in PVK Medium (Table 1).

The bacterial isolates showing siderophore synthesis were arranged in the descending order: Subathu (58.06%) > Recongpeo (52.63%) > Kandaghat (44.44%) > Tabo (44.00%) > Nauni (37.50%) > Gaura (34.28%). The highest siderophore producers were recorded in samples collected from site Subathu and lowest were recorded in Nauni and only 43.61% (82/188) of total isolates were siderophore producers.

Percentages of bacteria having nitrogen fixing activity were arranged in the descending order of Recongpeo (71.05%) > Tabo (64.00%) > Gaura (62.85%) > Subathu (58.06%) > Kandaghat (55.55%) > Nauni (43.75%). From the results it was observed that out of total 188 isolates from mid and high hills of H.P. 57.97% (109/188) isolates were able to grow on nitrogen free medium (Table 1).

Among 188 bacterial isolates, only seventy two (38.29%) (44 from RS and 28 from ER) were able to create halo zones. From the total population of PSBs, 46 were selected from the mid hill sites and 26 were from high hill of H.P. The colony morphology of the isolates varied from circular to flat, convex, irregular or raised and entire or undulating margins. Microscopic observation showed majority of the isolates to be Gram-positive and rod-shaped bacteria. A total of seventy two P-solubilizing isolates were screened for multifarious PGP traits viz., siderophore, IAA, HCN production, growth on N-free medium and antifungal activity (Fig. 3). Data generated on the basis of PGP traits were subjected to cluster analysis (Fig.3). Dendrogram was constructed using the unweighted pair-group

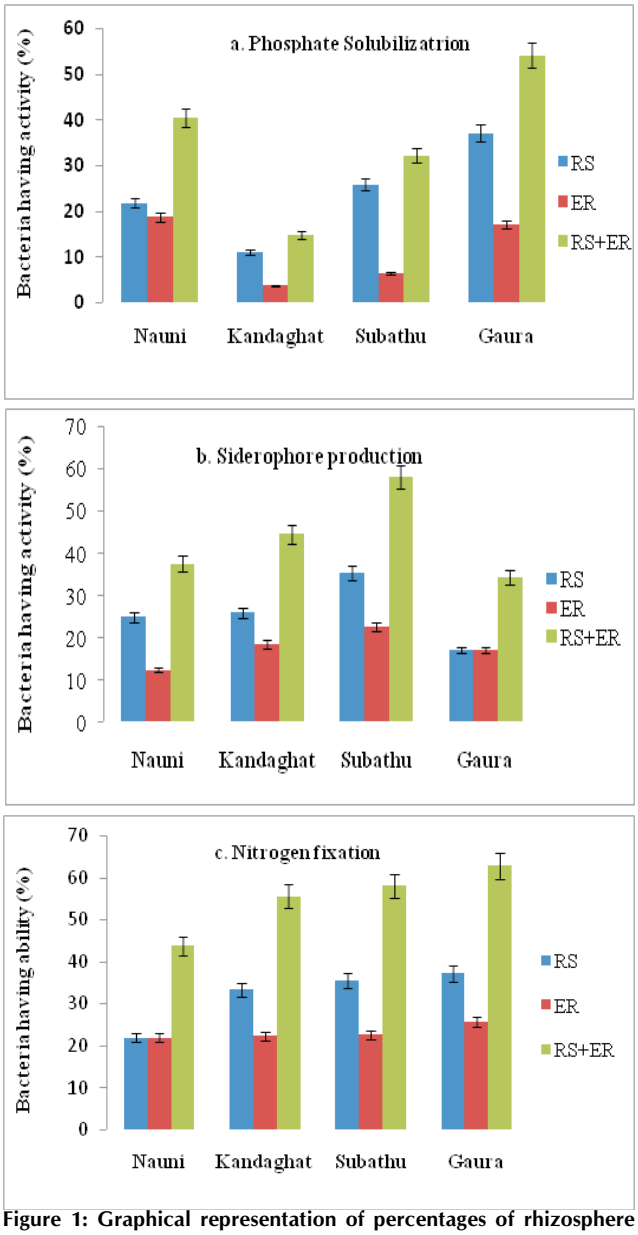


**Figure 1: Graphical representation of percentages of rhizosphere soil and root endophytic bacterial isolates of four different sites of mid hills for plant growth promotion traits: Phosphate solubilization (a); siderophore production (b); Nitrogen fixation(c)**

method with airthmatic mean (UPGMA). According to Fig. 3, IAA production was detected in 59.72% PSB isolates, siderophore synthesis in 48.61% PSB isolates, HCN in 5.55% PSB isolates and % growth inhibition against *Dematophora necatrix* in 65.28% PSB isolates. Maximum per cent solubilization efficiency (%SE) was recorded for isolate $Ap_{2}^{*}{}_{(1)}$ (366.60%). Maximum P-solubilization (245.20 $\mu$g/ml) was found for isolate $AT_{6}^{*}{}_{(2)}$ (380 $\mu$g/ml) followed by isolate $AG_{4\,(2)}$ (366 $\mu$g/mL). Isolate $AG_{1\,(3)}$ showed highest IAA production (62 $\mu$g/mL) and antifungal activity against *Dematophora necatrix* (91%). Among all, $AG_{1(3)}$ was only isolate which showed all PGP traits tested under present study and selected as potential biofertilizer, although it solubilized 200 $\mu$g/ml of phosphorous.

**Table 1: Characterization of rhizospheric soil and root endophytic bacterial isolates for plant growth promoting traits**

| Site: | | Sub –Sites: | Number of isolates without PGP activity | Number of isolates with single PGP activity | | | Number of isolates with binary PGP traits | | | Number of isolates with triple PGP traits |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | P | S | N | P+S | P+N | S+N | P+S+N |
| Mid hill | Nauni | RS | 3/19(15.80) | 3/19(15.80) | 3 /19(15.80) | 5/19(26.30) | 3/19(15.80) | 0/19(0.00) | 1/19(5.30) | 1/19(5.30) |
| | | ER | 1/13(7.70) | 4/13(30.80) | 1/13(7.70) | 3/13(23.00) | 0/13(0.00) | 1/13(7.70) | 2/13(15.40) | 1/13(7.70) |
| | Kandaghat | RS | 0/16(0.00) | 0/16(0.00) | 5/16(31.30) | 8/16(50.00) | 2/16(12.50) | 1/16(6.30) | 0/16(0.00) | 0/16(0.00) |
| | | ER | 0/11(0.00) | 0/11(0.00) | 5/11(45.60) | 5/11(45.60) | 0/11(0.00) | 1/11(9.10) | 0/11(0.00) | 0/11(0.00) |
| | Subathu | RS | 2/19(10.50) | 2/19(10.50) | 3/19(15.80) | 3/19(15.80) | 1/19(5.30) | 1/19(5.30) | 3/19(15.80) | 4/19(21.10) |
| | | ER | 1/12(8.30) | 2/12(16.70) | 2/12(16.70) | 2/12(16.70) | 0/12(0.00) | 0/12(0.00) | 5/12(41.70) | 0/12(0.00) |
| | Gaura | RS | 0/22(0.00) | 6/22(27.30) | 1/22(4.50) | 6/22(27.30) | 2/22(9.10) | 4/22(18.20) | 2/22(9.10) | 1/22(4.50) |
| | | ER | 1/13(7.70) | 0/13(0.00) | 1/13(7.70) | 4/13(30.80) | 2/13(15.40) | 2 /13(15.40) | 1/13(7.70) | 2/13(15.40) |
| High Hill Tabo | | RS | 3/15(20.00) | 2/15(13.30) | 0/15(0.00) | 3/15(20.00) | 0/15(0.00) | 0/15(0.00) | 5/15(33.30) | 2/15(13.30) |
| | | ER | 2/10(20.00) | 1/10(10.0) | 0/10(0.00) | 3/10(30.00) | 1/10(10.00) | 0/10(0.00) | 2/10(20.00) | 1/10(10.00) |
| | Recongpeo | RS | 3/23(13.10) | 1/23(4.40) | 1 /23(4.40) | 6/23(26.10) | 1/23(4.40) | 3/23(13.10) | 4/23(17.40) | 4/23(17.40) |
| | | ER | 1/15(6.70) | 1/15(6.70) | 2/15(13.30) | 0/15(0.00) | 1/15(0.00) | 3/15(20.00) | 2/15(13.30) | 5/15(33.30) |
| Sum(%) | RS | | 11/114 (9.60) | 14/114(12.30) | 13/114(11.40) | 31/114(27.10) | 9/114(7.90) | 9/114(7.90) | 15/114(13.50) | 12/114(10.50) |
| | ER | | 6/74 (8.10) | 8/74(10.80) | 11/74(14.90) | 17/74(23.00) | 4/74(5.40) | 7/74(9.50) | 12/74(16.20) | 9/74(12.20) |

P, Phosphate solubilization; S, Siderophore production; N, Nitrogen fixing ability Figure in parentheses are per cent (%) of isolates with plant growth promoting traits (PGP) trait RS, rhizosphere soil; ER, root endosphere

**Table 2: Correlation matrices showing relationship amongst plant growth promoting traits of the bacterial isolates**

| | | P | | | S | | | N | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | RS | ER | RS + ER | RS | ER | RS + ER | RS | ER | RS + ER |
| | RS | 1 | | | | | | | | |
| P | ER | 0.59 | 1 | | | | | | | |
| | RS + ER | 0.90* | 0.90* | 1 | | | | | | |
| | RS | 0.04 | 0.16 | 0.11 | 1 | | | | | |
| S | ER | 0.46 | 0.60 | 0.59 | 0.63 | 1 | | | | |
| | RS + ER | 0.29 | 0.44 | 0.41 | 0.88* | 0.91* | 1 | | | |
| | RS | 0.54 | 0.66 | 0.67 | 0.32 | 0.90* | 0.70 | 1 | | |
| N | ER | 0.80* | 0.87* | 0.94* | 0.21 | 0.80* | 0.58 | 0.85* | 1 | |
| | RS + ER | 0.65 | 0.75* | 0.78* | 0.29 | 0.90* | 0.68 | 0.98* | 0.93* | 1 |

*Correlation is significant at the 5% level of significance; P: Phosphate solubilization; S: Siderophore production; N: Nitrogen fixing ability; RS: Rhizosphere soil; ER: root endosphere

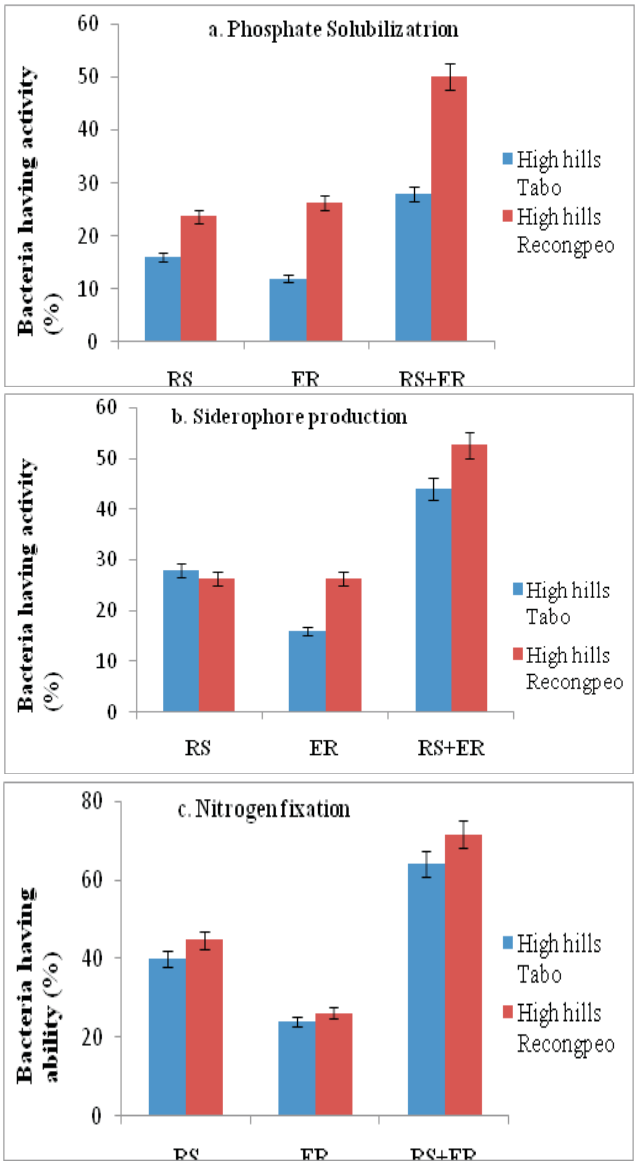


**Figure 2: Graphical representation of percentages of rhizosphere soil and root endophytic bacterial isolates of two different sites of High hills for plant growth promotion traits: Phosphate solubilization (a); siderophore production (b); Nitrogen fixation (c)**

### Correlation Analysis

Table 2 showed the result of the correlation analysis among the P-solubilizing rhizobacteria with multifarious PGPTs. A significant positive correlation was observed between the P-solubilizing bacterial isolates and the isolates with nitrogen fixation and siderophore production. Similarly, a significant positive correlation was observed among the total number of siderophore producing isolates with total number of P-solubilizing and nitrogen fixing bacterial isolates along with the nitrogen fixing bacterial isolates and isolates with P solubilization activity and siderophore production.

### Quantitative analysis of PSBs in RS and ER of *Prunus armeniaca*

Phosphate solubilizing bacteria was considered dominant in RS according to Simpson's index of dominance, which was 0.075 for RS and 0.036 for ER.

The diversity of the two sampling positions (RS and ER) according to the physiological PGPR traits shown by PSB was calculated by the Shannon-Weaver algorithm. The results (data not shown) showed that higher Shannon–Weaver diversity index (1.33) was found in RS as compared to less value of 0.98 in ER.

### Morphological, Biochemical and Molecular Characterization

Among 72 P-solubilizing rhizobacteria, $AG_{1(3)}$ showed marked phosphate solubilization activity along with other PGP traits. Isolate $AG_{1(3)}$ was Gram positive, aerobic, rods present singly, endospore former and was positive for catalase, Voges Proskauer's reaction, citrate utilization, ONPG, nitrate reduction, arginine dehydrolase, gelatin hydrolysis, starch hydrolysis, esculin hydrolysis and could utilize dextrose, fructose, inulin, glycerol, salicin as sole carbon source.

The identification of isolate $AG_{1(3)}$ was confirmed by comparison of 16S rRNA gene sequence in the NCBI database using a BLASTn analysis. Molecular analysis based on 16S rRNA homology of 1375 bp partial sequence confirmed that the isolate $AG_{1(3)}$ belongs to genus *Bacillus*. In the phylogenetic tree (Fig. 4), isolate $AG_{1(3)}$ clustered closely (99%) with to *Bacillus subtilis* (AB38315) Vietnam isolate, isolated from fish sauce and a chinese endophytic isolate *Bacillus subtilis* (EU118756) isolated from leaves of mulberry. The sequence was deposited in NCBI Genbank under accession number KF641185. On the basis of morphological, biochemical and molecular characterisation, the isolate $AG_{1(3)}$ was eventually identified as *Bacillus subtilis* strain CKAT.

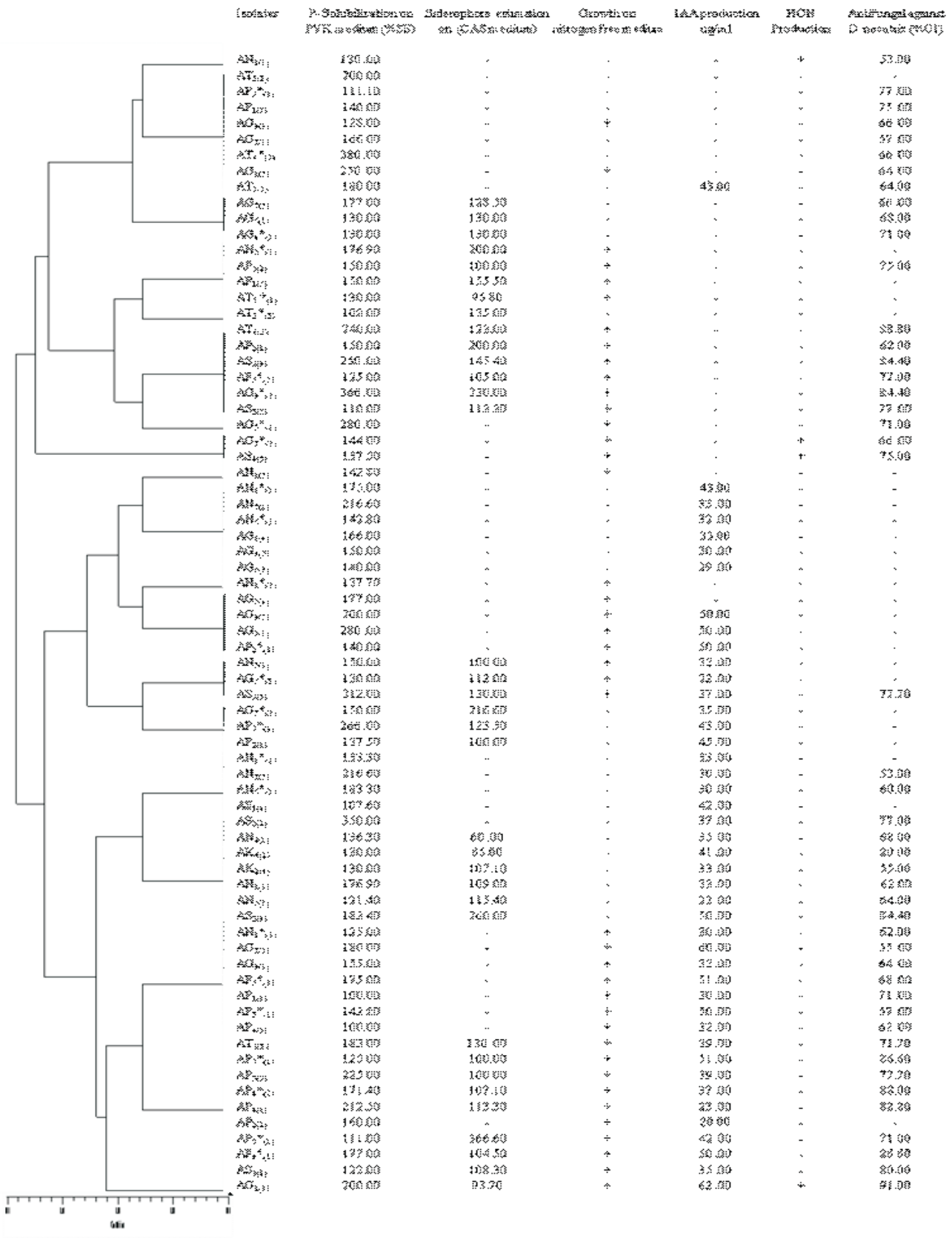


**Figure 3: Dendrogram of P-solubilizing bacterial isolates based on similarity coefficient derived from their multifarious PGP traits**


**Figure 4: Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationship of strain CKS1. The numbers at the nodes indicate the levels of bootstrap support based on data for 1000 replicate**

## DISCUSSION

PGPR colonize plant roots and exert beneficial effects on plant growth and development by a wide variety of mechanisms. To be an efficient PGPR, bacteria must be able to colonize roots because bacteria need to establish itself in the rhizosphere at population densities sufficient to produce beneficial effects. The exact mechanism by which PGPR stimulate plant growth is not clearly established, although several hypotheses such as production of phytohormones, suppression of deleterious organisms, activation of phosphate solubilization and promotion of mineral nutrient uptake are believed to be involved.

Phosphorus is one of the major nutrients, second only to nitrogen in requirement for plants. Most of phosphorus in soil is present in the form of insoluble phosphate cannot be utilized by the plants (Pradhan and sukla, 2005). Soil microorganisms play a key role in soil P dynamics and subsequent availability of phosphate to plants. All the collected samples of rhizosphere soil and rhizome/roots were evaluated for the bacterial population capable P-solubilization, growth on nitrogen free medium and production of siderophore. Rhizobacteria exhibiting all these three plant growth promoting traits were found in all the samples. The results of the present study indicated that in all the sampled sites, the rhizospheric soil bacterial population was observed higher than in the root endophytic bacterial population represented in Fig. 1 and 2. Rhizospheric soil bacterial population was varied between $9.80 \times 10^5$ cfu/g soil to $1.70 \times 10^6$ cfu/g soil as reported for apple crop by Rumberger *et al.,* 2007 and endophytic bacterial population associated with sweet corn and potato was varied from $1 \times 10^2$ and $1 \times 10^6$ cfu/mL as reported by Kobayashi and Palumbo (2000).

Tables 1 depict the comparative variation in the per cent P-solubilizing bacterial population among samples from each site. A total of one hundred and fourteen rhizospheric and seventy four endophytic bacterial isolates were screened for PGP traits from soil and roots associated with apricot trees. In site wise comparision of per cent P-solubilizer for rhizospheric soil and root endosphere, results indicated the percentages of P-solubilizing rhizobacteria having different combination of same or different PGP traits as reported by Mehta *et al.* (2013).

The tested PSB isolates could simultaneously exhibit single, binary and triple PGPR traits, which may promote plant growth directly, indirectly or synergistically, suggesting that the application of PSBs with multifaceted traits for plant growth promoting activity is more beneficial. The percentages of PSB not having PGPTs were relatively higher (Table 1) in RS (9.60%). These results are in accordance with earlier reports having PGPTs were present in high proportions in all the RS samples associated with apple (Mehta *et al.,* 2010). The higher occurrence of P-solubilizers in rhizosphere is of direct significance to the plants as it helps in mobilization of insoluble phosphorus near the root, especially in phosphorus deficient soils.

The ratio of PSB to show binary activities of P-solubilization and siderophore production was 6.91% (13/188) which was much less than the 34.8% (8/23) in the RS of *Carex leiorhyncha* (Koo *et al.,* 2010). The siderophores play important role in the growth of plants with their ability to supply iron (Ramos-Solano *et al.,* 2010). It is also worth mentioning that PSB isolates from RS and ER of apricot also show combination of three PGPTs that is, PGPR ratio 12.10% (19/157) and 16.85% (15/89), probably because more bacterial genes are expressed simultaneously.

In view of the values from the Shannon–Weaver diversity index, the rhizosphere showed higher diversity of PGPT-possessing PSB than the ER, indicating that rhizosphere of apricot may constitute an ideal niche for exploring PSB with diverse mechanisms of plant growth promotion. The low diversity of PGPT-possessing PSB is probably due to undisturbed nature of apricot ER ecosystem that is supposed to be close to an equilibrium state or due to the marked environment fluctuations that take place in the ER caused by the plant's physiological cycle. Our results are in agreement with previous study wherein rhizosphere was good reservoir of bacterial diversity (Mittal and Johri, 2007; Joshi *et al.,* 2011).

The dominance of gram positive, rod shaped rhizobacteria has been reported in both rhizosphere soil and root tissues, by Aranda *et al.,* 2011; Joshi *et al.,* 2011. Our results are in agreement with those investigations that found gram positive, rod shaped bacteria as main composition of rhizosphere and root associated microbial communities in many plant species (Datta *et al.,* 2011).

Further selection of bacterial isolate $AG_{1(3)}$ as potential biofertilizer was done on the basis of similarity coefficient derived from dendrogram generated by the isolates exhibiting various plant growth promoting traits in tendem. The results of the present study indicated that apricot (*Prunus armeniaca)* rhizosphere and endosphere constitute excellent reservoir of bacterial diversity with multifarious plant growth promoting

traits. Variation in plant growth promoting (PGP) traits (P-solubilization, IAA production, growth on nitrogen free medium, siderophore production, HCN production, lytic enzymes and antifungal activity against different fungal pathogens) by bacterial isolates was observed.

In present study, on the basis of partial length 16S rDNA sequencing $AG_{1\ (3)}$ was characterized as *Bacillus subtilis*. The phylogenetic relatedness further validated the strain $AG_{1\ (3)}$ as *Bacillus subtilis*. The dominance of genus *Bacillus* as P-solubilizing bacteria in the rhizosphere of several crops has been reported earlier by Chakraborty *et al.,* 2006; Joshi *et al.,* 2011. They reported *Bacillus* as a dominant group and also a major group of microflora, which lives in close association with various types of agricultural crops (Kaur and Sharma, 2013). $AG_{1\ (3)}$ was characterized as efficient P-solubilizing PGPR by morphologically, physiologically, biochemically, which can further be explored for bioformulation preparation to be used under field conditions for growth promotion of various crops.

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