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Phosphate solubilization and multiple plant growth promoting properties of rhizobacteria isolated from chickpea *(Cicer aeritinum L.)* producing areas of Ethiopia

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Chickpea is one of the major legume crops widely grown in Ethiopia. The low availability of phosphorus in soil is among the stresses that constrain the production of this crop in the country. However, there are rhizobacteria capable of solubilizing insoluble forms of phosphorus in soil and make it available to the plant. Thus, this study was aimed at isolation and characterization of phosphate solubilizing bacteria from chickpea rhizosphere. Fifty phosphate solubilizing bacterial strains were isolated from the soil samples, characterized biochemically and identified by 16S rDNA sequences analysis. The results indicate the presence of genera *Acinetobacter, Bacillus*, *Brevibacillus*, *Burkholderia*, *Empedobacter, Enterobacter*, *Pseudomonas*, *Ralstonia, Sphingomonas* and *Stenotrophomonas*. Phosphate solubilizing efficiencies of the strains were analyzed using different insoluble phosphorus sources and the results show that most isolates released a substantial amount of soluble phosphate from tricalcium phosphate, rock phosphate and bone meal. Screening for multiple plant growth promoting attributes showed that 44 and 18% of them were capable of producing indole acetic acid and inhibiting the growth of *Fusarium oxysporum* under *in vitro* conditions, respectively. A direct impact of several strains (*Bacillus flexus* (PSBC17), *Pseudomonas fluorescence* (PSBC33), *Enterobacter* sp. (PSBC35), *Enterobacter sakazaki* (PSBC79) and *Enterobacter* sp. (PSBC81)) on the growth of chickpea in pot culture has been demonstrated by the increase in the number of root nodules, shoot dry matter, nitrogen and phosphorus concentration of shoot. Based on the results, we conclude that chickpea rhizosphere harbor phosphate solubilizing bacteria which are diverse in taxonomy and phosphate solubilizing efficiencies. Thus, consecutive studies should focus on field studies on those strains due to their potentially high importance for the phosphorus nutrition of crops in this area and in this context for the improvement of the sustainability of crop production in the country.

Key words: Plant growth promoting rhizobacteria (PGPR), indole acetic acid (IAA), rhizosphere soil, rock phosphate, bone meal.

INTRODUCTION

Chickpea (*Cicer aeritinum* L.) is one of the major food legume crops grown widely in tropical, sub-tropical and temperate regions of the world. It is also widely grown in Ethiopia over an area of 208,388.6 ha (Central Statistical Agency (CSA), 2011).

This crop is an important source of dietary protein for the majority of the Ethiopian population. In addition, chickpea restores and maintains soil fertility and therefore, grown in rotation with major cereals in traditional low-input agricultural systems. This is due to its ability to fix dinitrogen in association with root nodule bacteria belonging to the genus *Mesorhizobium* (Nour et al., 1994). However, its yield has remained rather low due to several stresses which prevail under Ethiopian edaphoclimatic conditions (Keneni et al., 2011; Jida and Assefa, 2012).

Phosphorus (P) is one of the key macronutrients required for growth and development of plants. Most Ethiopian soils are acidic and mainly characterized by low available P (Beyene, 1982; Mamo et al., 1988). This is partly due to acid mediated P-fixation in the soil. Hence, low available P in soil is one of the major soil-related stresses that limit the production of chickpea and other pulse crops in the country (Tilaye, 1985). The most common method of improving P limitation and hence increasing crop production is application of chemical fertilizer. This is highly limited by its increasing cost and for this reasons its application in chickpea production is particularly low in Ethiopia (Jida and Assefa, 2012) causing an urgent need for economically feasible and ecologically acceptable P-fertilizer sources. Thus, using microbial biofertilizer for improving the availability of P in the rhizosphere of plant represents an attractive and practically applicable alternative to the use of chemical fertilizers (Naik et al., 2008).

Bacteria that colonize the rhizosphere and enhance plant growth are referred to as plant growth promoting rhizobacteria (PGPR). Phosphate solubilization that makes the P available in soil for plant growth is considered as one of the important attributes of PGPR (Chen et al., 2006), since phosphate solubilizing bacteria (PSB) are capable of converting insoluble phosphate to soluble orthophosphate ions which can be taken up and utilized by plants (Pal, 1998; Rodriguez and Fraga 1999; Chen et al., 2006; Vyas and Gulati, 2009). Previous studies already showed the presence of phosphate solubilizing bacteria (PSB) in the rhizosphere of different agriculturally important crops and their pivotal role in converting of insoluble P to the orthophosphate (Pal, 1998; Peix et al., 2001a; Rajapaksha and Senanayake, 2011).

Several studies revealed that rhizobacteria particularly from genera *Agrobacterium, Bacillus, Burkholderia, Enterobacter, Erwinia, Pseudomonas, Rhizobium, Mesorhizobium, Pantoea* and *Serratia* are known to be highly efficient in solubilizing insoluble soil phosphate into available inorganic phosphate (Illmer and Schinner, 1995; Rodriguez and Fraga, 1999; Naik et al., 2008; Castagno et al., 2011). It has also been documented that different species of PSB isolated from Ethiopian soils were able to release phosphate from insoluble P sources such as tri-calcium phosphate, hydroxyl apatite, rock phosphate and old bone (Muleta et al., 2013; Keneni et al., 2010; Jida and Assefa, 2012). Recently, emerging evidences indicated that, besides increased P uptake, the production of phytohormones such as indole acetic acid (IAA) and suppression of different soil borne phytopathogens by PSB could also play an imperative role in plant growth promotion (Peix et al., 2001b; Ponmurugan and Gopi, 2006; Naik et al., 2008; Shahab et al., 2009). Several reports demonstrated that inoculation of PSB which exhibited multiple plant growth promoting activities significantly improved plant growth and yield under glasshouse and field conditions (Peix et al., 2001a; Shahab et al., 2009; Yadav et al., 2010).

In view of their multiple plant growth promoting attributes, PSB could maintain soil quality and health. Therefore, studies committed to isolation, characterization and selection of efficient PSB with different plant growth promoting properties from chickpea rhizosphere soils are highly desirable to design strategies to use those native strains for the development of inoculant technologies for organic agriculture. Despite the benefits that PSB could present to legume cultivation, the information about indigenous rhizobacterial strains with these properties from the rhizosphere of chickpea in Ethiopian soils is very limited. Thus, the aim of this study was to isolate and characterize phosphate solubilizing bacteria from chickpea rhizosphere and evaluate their plant growth promoting activities under glasshouse conditions.

MATERIALS AND METHODS

Study sites and soil samples collection

Soil samples were collected from 36 chickpea grown fields found in Amhara, Oromia and Tigray Regional States of Ethiopia (Figure 1) with altitude range from 1,526 (Alamata) to 2,840 masl (Sheno) in October, 2009 (Table 1). About 3 kg of soil samples were excavated from 15 to 20 cm depth from each site, collected into sterile

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Figure 1. Soil sample collection sites.

polyethylene bags and carefully transported to Applied Microbiology Laboratory, Addis Ababa University. The soil samples were stored at 4°C until further analysis.

Isolation and enumeration of phosphate solubilizing bacteria

Each soil sample was air-dried and filled into surface sterilized plastic pots. Chickpea seeds (*Akaki* cultivar) were surface sterilized with 95% ethanol and 3% sodium hypochlorite solution for 10 s and 3 min, respectively. The seeds were rinsed five times with sterilized distilled water and allowed to germinate on water agar (1%) surface for three days at 25°C. Five pre-germinated seeds were planted in each pot. The seedlings were thinned down to three 5 days after emergence (DAE). All pots were situated in the greenhouse and watered every three days.

Forty five days after planting (DAP) chickpea seedlings were selected from each pot and uprooted carefully with their rhizosphere soil. 10 g of rhizospheric soil with adhered roots were transferred to flasks containing sterilized 90 ml saline solution of 0.85% w/v NaCl. The flasks were incubated on a gyratory shaker at 120 rpm for 30 min followed by serial dilution to 10^6. Aliquots (0.1 ml) of the appropriate dilutions were plated on Pikovskaya medium (Pikovskaya, 1948). All plates were incubated at 30°C for 5 days. The number of phosphate solubilizing bacteria found in each soil samples was determined using the plate count method. Colonies with a large halo zone and different morphologies were selected and purified by re-streaking on Pikovskaya (PK) medium. The persistence of their phosphate solubilizing ability was confirmed by three successive subcultures in the same medium. Pure isolates were maintained on PK agar slants at 4°C and 50% (v/v) glycerol at -20°C. Isolates were designated as phosphate solubilizing bacteria from chickpea (PSBC) 01- PSBC135 (Table 1).

Phosphate solubilization efficiency of PSB strains

The PK agar plates were spot inoculated with 10 µl of bacterial culture grown in PK broth to the exponential phase. After incubation at 30°C for 5 days, formation of a clear zone around the spot was checked and their solubilization indices (SI) were calculated according to Edi-Premono et al. (1996). Based on their SI along with the abundance, isolates were selected and used for quantitative analysis of phosphate solubilization efficiency in PK broth. 100 ml of PK broth without phosphorus sources was dispensed in a 250 ml Erlenmeyer flask and supplemented with tricalcium phosphate (TCP), Egyptian rock phosphate (RP) or bone meal (BM) that contained equivalent amount of P as sole P source. The flasks were inoculated with 100 µl bacterial culture grown in PK broth to exponential phase. Uninoculated PK broths supplemented with the aforementioned insoluble P sources were included as controls. The flasks were incubated at room temperature on a gyratory shaker at 120 rpm for 12 days. 5 ml subsamples were withdrawn from each treatment on day 0, 4, 8, and 12 for pH change and soluble P analysis. The sample was centrifuged at 15,000 g for 15 min and the supernatant was used for the analysis. The amount of

Table 1. Abundance and distribution of phosphate solubilizing bacteria in chickpea producing areas of Ethiopia.

Isolation sites	Regional state of isolation site	Altitude of isolation site (masl)	Abundance of PSB (CFU/g) in soil isolation site soil	Number of isolates selected	Isolates obtained from each site
Galessa	A	2017	3.7×10^{4de}	1	PSBC01
Alamata	T	1526	1.5×10^{5bcde}	1	PSBC02
Robe	T	1658	6.9×10^{4cde}	1	PSBC05
Woldya	A	2074	1.3×10^{5bcde}	1	PSBC06
Aja	A	2023	5.6×10^{5a}	2	PSBC07,41
Debre Selam	A	1896	6.1×10^{4cde}	1	PSBC10
Alem Tena	O	1637	5.9×10^{4cde}	1	PSBC13
Chirameda	A	1747	3.5×10^{4de}	1	PSBC14
Sirnka	A	1843	3.6×10^{4de}	2	PSBC15, 16
Angut Michael	A	1850	1.1×10^{5cde}	2	PSBC17,18
Maksagnt	A	1978	6.1×10^{4cde}	2	PSBC28,29
Yetinora	A	2437	7.8×10^{4cde}	2	PSBC30,31
Lalibela	A	2138	4.8×10^{4de}	2	PSBC33,40
Olankomi	O	2378	2.2×10^{5bc}	1	PSBC35
Debre Libanos	O	2594	2.2×10^{5bc}	2	PSBC42,46
Dibulko	A	1992	4.8×10^{4de}	1	PSBC45
Ginchi	O	2378	2.9×10^{3e}	2	PSBC61,62
Ambo	O	2170	4.0×10^{3e}	1	PSBC63
Fiche	O	2748	1.9×10^{5bc}	1	PSBC67
Tikana	T	1942	5.9×10^{4cde}	2	PSBC68,126
Fogera	A	1931	4.8×10^{4de}	1	PSBC69
Alshin	T	2082	3.1×10^{4de}	1	PSBC70
Bilbila	A	2069	3.4×10^{4de}	1	PSBC71
Amber	A	2454	6.0×10^{4cde}	1	PSBC72
Gurura	O	1906	2.3×10^{3e}	2	PSBC74,79
Goro	O	1832	2.9×10^{3e}	1	PSBC81
Teji	O	2065	1.4×10^{5bcde}	2	PSBC86,89
Asgori	O	2078	4.0×10^{3e}	1	PSBC97
Itacha	A	2134	4.6×10^{4de}	1	PSBC99
Ilala	A	1924	6.2×10^{4cde}	1	PSBC108
Goha Tsion	O	2517	4.6×10^{4de}	1	PSBC109
Mojo	O	1774	3.7×10^{4de}	1	PSBC123
Obbi	O	2108	5.9×10^{3ce}	1	PSBC125
Sandafa	O	2554	1.5×10^{5bcde}	2	PSBC131,132
Sheno	O	2840	ND	2	PSBC133,134
Chole	O	2612	4.8×10^{4de}	2	PSBC135

ND, not determined, PSB abundance data are average of triplicates and data in the same column followed by the same letter do not differ significantly at p=0.05 using Duncan's Multiple Range Test (DMRT). A, Amahara; O, Oromia; T, Tigray.

solubilized P was determined following the phospho-molybdate method (Murphy and Riley, 1962) and the amount of solubilized P obtained from the control was deducted from their respective treatments.

Morphological, biochemical and API characterization of the isolates

The isolates were characterized using the following tests: Gram reaction (KOH test) (Gregorson, 1978); Oxidative/Fermentative (O/F) test (Huge and Leifson, 1953); endospore formation test and Analytical Profile Index (API) biochemical test kits assisted by API computer software (bioMèrieux, France) following the manufacturer's instruction. Gram positive, endospore forming rods were identified to species level using API 50CH test kits. Gram negative rods with fermentative reaction in the O/F test were identified using the API 20E test strips while Gram negative rods with oxidative reaction in O/F test were identified by API 20NE test kits. Moreover, isolates were subjected to 16S rDNA sequence analysis based on the result of API identification system.

Identification by 16S rDNA sequence analysis

Genomic DNA extraction

All isolates were streaked on Luria Bertani (LB) agar media and incubated for 24 h at 30°C to get single colonies. A single colony of each isolate was picked using a sterile tooth pick and suspended in 30 µl sterile H_2O in 50 µl reaction tubes. The DNA from Gram negative bacteria was extracted using the thermal denaturation method (Mohran et al., 1998). All tubes were incubated at 95°C for 10 min. The DNA of Gram positive isolates was extracted by consecutive heat and freeing (for 3 min at 65°C followed by freezing for 3 min at -70°C), the cycles were repeated three times. All the tubes were centrifuged at 13,000 *g* for 2 min and stored at 4°C until further analysis.

Polymerase chain reaction (PCR) amplification of 16S rDNA and sequencing

2 µl of the extracted DNA was used as a template for PCR amplification. The 25 µl PCR reaction generally contained 0.4 µl 10 mM dNTP, 2.5 µl 10× PCR buffer, 2.5 µl 25 mM $MgCl_2$, 0.2 µl (5 units/µl) Taq polymerase, 1 µl (10 mM) of forward primer 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1 µl (10 mM) of reverse primer 1492r (5'-GGTTACCTTGTTACGACTT-3'). The PCR program included a denaturation step of 5 min at 95°C, followed by 30 cycles of 95°C for 30 s, 50°C for 45 s, 72°C for 1.30 min, and a final extension step of 10 min at 72°C. PCR products were sanger-sequenced using the forward primer 27f at the Institute of Clinical and Molecular Biology, University of Kiel**.** The sequences were edited using Bioedit (http://www.mbio.ncsu.edu/BioEdit/). A BLAST search on the NCBI Gene Bank database (www.ncbi.nlm.nih.gov/) was used to identity of the isolates.

Phylogenetic analysis

Sequence data were multiple aligned using Clustal W and compared with available sequences of bacterial lineage from the NCBI database. A phylogenetic tree was constructed by using Neighbor-Joining method from distance matrices on MEGA4 program (Tamura et al., 2007). The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test is shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004) and were given in units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset.

Screening in vitro antagonistic activity against Fusarium oxysporum

The *in vitro Fusarium oxysporum f.sp. ciceri* (FOC) growth inhibition activity of the PSB isolates was tested using the dual culture technique (Landa et al., 1997). 20 µl of bacterial culture grown in nutrient broth to the exponential phase was equidistantly spotted on the margins of nutrient agar (NA) plates amended with glucose (1.0%) and incubated at 30°C. A 4 to 5 mm diameter agar disc from potato dextrose agar (PDA) cultures of the fungal pathogens was placed at the center of the NA plate inoculated with the bacterial isolate and incubated at 30°C for 5 days. The radii of the fungal colony towards and away from the bacterial colony were measured and *in vitro* mycelia growth inhibition % was calculated according to Idris et al. (2008).

Assay for Indole-3-acetic acid (IAA) production

Isolates were grown in LB broth supplemented with 5 mM L-tryptophan for 48 h and their IAA producing ability was detected according to Bric et al. (1991). Quantitative analysis of IAA was performed using the method of Loper and Scroth (1986). The culture were centrifuged at 10,000 *g* for 15 min and 2 ml supernatant was transferred into a tube to which 100 µl of 10 mM O-phosphoric acid and 4 ml of Salkowaski reagent (1 ml of 0.5M $FeCl_3$ in 50 ml 35% (v/v) $HClO_4$) were added. The mixture was incubated at room temperature for 25 min. The tubes were observed for the development of pink color and their absorbance was measured at 530 nm. The IAA concentration was determined against standard curve constructed from different concentrations of indole-3-acetic acid.

Response of chickpea to the inoculation with PSB isolates under glasshouse conditions

Sterilization, germination, inoculation and planting of chickpea

Bacterial isolates that showed high phosphate solubilization efficiency in PK broth were selected for their *in vivo* growth promotion test under greenhouse conditions. Chickpea seeds were surface sterilized, germinated and transplanted to plastic pots as described before. Prior to seedling transplantation, germinated seedlings were inoculated with PSB culture grown in PK broth and adjusted to 10^8 cells per seed. Inoculated seedlings were transferred to each pot containing 3 kg soil with the following physical and chemical properties (pH: 6.0, total N: 0.025%, organic carbon: 0.975 %, total P: 1667 ppm, available P: 2.99 ppm, available K: 25.33 ppm, cation exchange capacity: 50 $Cmolkg^{-1}$ and loamy texture). The soil sample was analyzed at the laboratory of Addis Ababa Environmental Protection Agency.

The pots were arranged in completely randomized block design and irrigated with distilled sterilized water every three days. Uninoculated P-fertilized (20 mg kg^{-1}as KH_2PO_4) pots were included as positive controls (PC) and uninoculated non P-fertilized pots as negative controls (NC). After sixty days all seedlings were carefully uprooted and washed under gently flowing tap water. The nodules were counted, and shoot dry weight was measured after drying for 48 h at 70°C. Total N and P concentration analysis of the shoot was carried out using the micro-kjeldahl and phospho-molybdate methods (Murphy and Riley, 1962), respectively at the laboratory of Addis Ababa Environmental Protection Agency.

Data analysis

The data sets were statistically analyzed using analysis of variance (ANOVA) and the treatment means were compared relatively to the controls following Duncan's multiple range test (DMRT) (Duncan, 1955). The correlation between different parameters was evaluated using Pearson's correlation coefficient on SPSS V.15 (SPSS Inc.2000).

RESULTS AND DISCUSSION

Isolation, enumeration and identification of PSB isolates

The abundance of PSB ranged from 2.3×10^3 to 5.6×10^5 CFU g^{-1} of rhizosphere soil from Alamata or Sandfa and Gurura respectively (Table 1). The PSB ≥ 3.1×10^4 in most (83%) of the samples, indicating that those

bacteria are generally highly abundant in the chickpea rhizosphere soils collected from the producing areas of Ethiopia. The population of PSB varied among the samples from different sites. This variation might be attributed to many factors such as soil nutrient status, pH, moisture content, organic matter composition and certain soil enzyme activities (Ponmurugan and Gopi, 2006).

Similarly, several studies indicated that the proportions of such bacteria were very high in the rhizosphere of agriculturally important crops, such as maize, sorghum, rice, barley and chickpea, and thus they could play a crucial role in P nutrition of the crops (Ponmurugan and Gopi, 2006; Rajapaksha and Senanayake, 2011).

Fifty PSB isolates with SI ≥1.40 were selected from 36 soil samples (Table 1). All of them were identified to the genus and species level by API biochemical tests and 16S rDNA sequence analysis. The results revealed the presence of diverse groups of phosphate solubilizing bacteria in the rhizosphere of chickpea. They were dominated by Gram negative rhizobacteria which accounted for 86% of the isolates (Table 2). Gram positive isolates were only 14%, indicating that they represent rather a minority among phosphate solubilizing bacteria in the investigated rhizosphere.The rhizospheres of many agriculturally important crops favor more Gram negative rhizobacteria than Gram positive (Muleta et al., 2009). Furthermore, 8 genera were identified using biochemical tests. Those genera were identified as *Burkholderia* (28%), *Pseudomonas* (20%), *Bacillus* (12%), *Enterobacter* (8%), *Stenotrophomonas* (4%), *Brevibacillus* (2%), *Sphingomonas* (2%), and *Ralstonia* (2%). Interestingly, members of the genus *Pseudomonas* were dominated by *P. fluorescence* (80%) while all isolates of the genus *Burkholderia* were identified as *B. cepacia*. The other isolates, however showed a low identification level (<90%) which might be due to the limitations of using biochemical characteristics to identify environmental isolates with a diverse nature.

To confirm the biochemical characterization, the 16S rDNA sequence analysis was also employed (Table 2). Based on the abundance of a particular group, biochemical test results and PGP activity 20 isolates were selected for sequence analysis. The presence of biochemically identified genera such as *Pseudomonas, Bacillus, Ralstonia,* and *Burkholderia* have been confirmed while two genera (*Acinetobacter* and *Empedobacter*) have been identified additionally. The disagreements between API biochemical test kits and 16S rDNA analysis results could be due to several reasons such as culture conditions and a diverse nature of environmental isolates.

Gram negative isolates exhibited a higher diversity with regard to the detected genera and species compared to the gram positive isolates. Isolates PSBC13, PSBC67, PSBC68 and PSBC69 showed 99% sequence similarity to *Pseudomonas lini* (EU221401), PGPR isolated from wheat rhizosphere while PSBC41 was found to be 99% similar to *Pseudomonas corrugate* (HQ242748) phosphate solubilizing bacteria. On the other hand, PSBC97 showed 100% sequence similarity with the denitrifying bacterium *Ralstonia pickettii* (HE575958) isolated from soil whereas PSBC02 showed 99% similarity with *R. pickettii* strain QL-A6 (HQ267096) obtained from tomato rhizosphere. Isolate PSBC05 was found to be 99% similar to *Burkholderia phytofirmans* (HQ242761) phosphate solubilizing rhizobacteria isolated from P-rich soil while PSBC07 and PSBC99 showed 99% similarity with *Burkholderia terricola* (FM209484) salt tolerant bacteria isolated from faba bean nodules. Isolates PSBC06 and PSBC89 were found to be 99% similar with *Acinetobacter lwoffii* (FN393792) and *A. lwoffii* (EF204280) obtained from different environmental samples, respectively while PSBC123 showed 99% similarity with *Acinetobacter johnsonii* (EU594557), an endophytic bacteria obtained from sugar beet. Isolates PSBC40 and PSBC63 were found to be 99 and 100% similar with *Empedobacter brevis* (EU293154) isolated from soil, respectively. Several studies indicated that different species from genus *Pseudomonas, Ralstonia, Burkholderia, Flavobacterium* and *Acientobacter* are efficient phosphate solubilizing bacteria (Rodriguez and Fraga, 1999).

Gram positive isolates PSBC31 and PSBC71 were found to be 99% similar with *Bacillus subtilis* (EF656456) endophytic bacteria from wheat and antagonistic to wheat sharp eyespot disease and *B. subtilis* (HM027569) soil-borne PGPR, respectively whereas PSBC28 showed 99% similarity with soil *B. subtilis* (HM027569). Isolate PSBC17 showed 99% similarity with *Bacillus flexus* (JQ660603) obtained from verimcompost. Moreover, the partial 16S rDNA sequences of the isolates were multiple aligned with the nearest neighbors and other relevant bacterial sequences, and their identity and evolutionary history was inferred by constructing phylogenetic tree (Figure 2). The phylogenetic tree showed the clustering of PSBC isolates with their respective genera with high bootstrap support values.

Qualitative analysis of phosphate solubilizing efficiency of the isolates

The SI of each PSB isolate is depicted in Table 1. Phosphate solubilizing efficiency of these isolates was qualitatively determined on PK agar medium using SI as measure of their efficiency and the result showed that there were significant ($p \leq 0.05$) variations among the isolates. The SI of the isolates ranged from 1.40 (PSBC126) to 3.06 (PSBC02). This indicates that PSBC02 was the most efficient phosphate solubilizing bacteria on PK agar medium. Generally, the highest SI was produced by Gram negative isolates indicating that they are more efficient in solubilization of TCP on PK agar medium which has previously been observed

Table 2. Phosphate solubilization index and identification of phosphate solubilizing bacteria isolated from chickpea rhizosphere.

Isolate	SI± SD	Gram reaction	O/F test	Endospore test	API identification	16S rDNA sequence identification
PSBC01	1.7±0.10^{j-n}	-	O	-	*Pseudomonas fluorescence*	ND
PSBC02	3.06±0.42^{a}	-	O	-	UI	*Ralstonia pickettii (JX979097)*
PSBC05	2.25±0.25^{d-f}	-	O	-	UI	*Burkholderia phytofirmans (JX979098)*
PSBC06	1.57±0.15^{k-n}	-	O	-	UI	*Acinetobacter lwoffii (JX979099)*
PSBC07	2.66±0.34^{bc}	-	O	-	*Burkholderia cepacia*	*Burkholderia terricola (JX979100)*
PSBC10	1.54±0.19^{l-n}	-	O	-	*Burkholderia cepacia*	ND
PSBC13	2.28±0.25^{d-f}	-	O	-	*Pseudomonas* sp.	*Pseudomonas lini (JX979123)*
PSBC14	1.44±0.06^{n}		O	-	*Burkholderia cepacia*	ND
PSBC15	2.88±0.18^{ab}	-	O	-	*Stenotrophomonas maltophila*	"
PSBC16	2.83±0.17^{a-c}	+	O	+	*Bacillus* sp.	"
PSBC17	2.53±0.38^{b-e}	+	O	+	*Bacillus* sp.	*Bacillus flexus (JX979101)*
PSBC18	1.94±0.07^{f-j}	-	O	-	*Burkholderia cepacia*	ND
PSBC28	2.85±0.17^{a-c}	+	O	+	*Bacillus* sp.	*Bacillus subtilis (JX979102)*
PSBC29	1.65±0.13^{j-m}	+	O	+	*Bacillus stearothermophilus*	*Bacillus subtilis (JX979103)*
PSBC30	2.48±0.13^{c-e}	-	O	-	*Burkholderia cepacia*	ND
PSBC31	1.91±0.10^{g-k}	+	O	+	*Brevibacillus brevis*	*Bacillus subtilis (JX979104)*
PSBC33	2.20±0.20^{e-g}	-	O	-	*Pseudomonas fluorescence*	ND
PSBC35	2.50±0.10^{c-e}	-	F	-	*Enterobacter* sp.	"
PSBC40	1.90±0.10^{g-l}	-	O	-	UI	*Empedobacter brevis (JX979105)*
PSBC41	1.45±0.04^{n}	-	O	-	*Pseudomonas fluorescence*	*Pseudomonas corrugate (JX979106)*
PSBC42	1.52±0.14^{mn}		O	-	*Burkholderia cepacia*	ND
PSBC45	2.00±0.07^{f-j}	-	O	-	*Burkholderia cepacia*	"
PSBC46	2.12±0.13^{f-h}	-	O	-	*Burkholderia cepacia*	"
PSBC61	2.12±0.12^{f-h}	-	O	-	UI	"
PSBC62	2.06±0.17^{f-i}	-	O	-	UI	"
PSBC63	2.50±0.25^{c-e}	-	O	-	UI	*Empedobacter brevis (JX979107)*
PSBC67	2.07±0.12^{f-i}	-	O	-	*Pseudomonas fluorescence*	*Pseudomonas lini (JX979108)*
PSBC68	1.69±0.27^{j-n}	-	O	-	*Pseudomonas fluorescence*	*Pseudomonas lini (JX979124)*
PSBC69	1.83±0.11^{h-m}	-	O	-	*Pseudomonas aeruginosa*	*Pseudomonas lini (JX979109)*
PSBC70	1.51±0.15^{mn}	-	O	-	*Pseudomonas fluorescence*	ND
PSBC71	2.08±0.29^{f-h}	+	O	+	*Bacillus sp.*	*Bacillus subtilis (JX9110)*
PSBC72	2.07±0.12^{f-i}	-	O	-	*Pseudomonas fluorescence*	ND
PSBC74	2.62±0.13^{b-d}	-	O	-	*Burkholderia cepacia*	"
PSBC79	2.58±0.14^{b-d}	-	F	-	*Enterobacter sakazaki*	„
PSBC81	1.43±0.07^{n}	-	F	-	*Enterobacter* sp.	-
PSBC86	1.95±0.25^{f-j}	-	O	-	*Sphingomonas paucimobils*	-
PSBC89	1.43±0.06^{n}	-	O	-	UI	*Acinetobacter lwoffii (JX979111)*
PSBC97	1.87±0.08^{g-m}	-	O	-	*Ralstonia pickettii*	*Ralstonia pickettii (JX9112)*
PSBC99	1.46±0.05^{n}	-	O	-	*Burkholderia cepacia*	*Burkholderia terricola (JX979113)*
PSBC108	1.52±0.17^{mn}	-	O	-	*Stenotrophomonas maltophila*	ND
PSBC109	1.86±0.13^{g-m}	-	O	-	*Burkholderia cepacia*	-
PSBC123	1.46±0.12^{n}	-	O	-	UI	*Acinetobacter johnsonii (JX979126)*
PSBC125	1.81±0.15^{h-m}		O	-	*Pseudomonas fluorescence*	ND
PSBC126	1.40±0.16^{n}	-	O	-	*Burkholderia cepacia*	-
PSBC131	1.80±0.17^{h-m}	+	O	+	*Bacillus sp.*	-
PSBC132	1.85±0.11^{g-m}	-	O	-	*Burkholderia cepacia*	-
PSBC133	1.66±0.13^{j-n}	-	O	-	UI	-
PSBC134	1.44±0.06^{n}	-	O	-	*Burkholderia cepacia*	-
PSBC135	1.43±0.05^{n}	-	O	-	UI	-

ND, not determined; O, oxidative; F, fermentative; UI, showed low identification level (<90%) by API computer software; +, Gram positive or positive for the test; -, Gram negative or negative for the test; parenthesis: accession number in NCBI Gene bank; means in the same column followed by the same letter do not differ significantly at p=0.05 using Duncan's Multiple Range Test (DMRT)

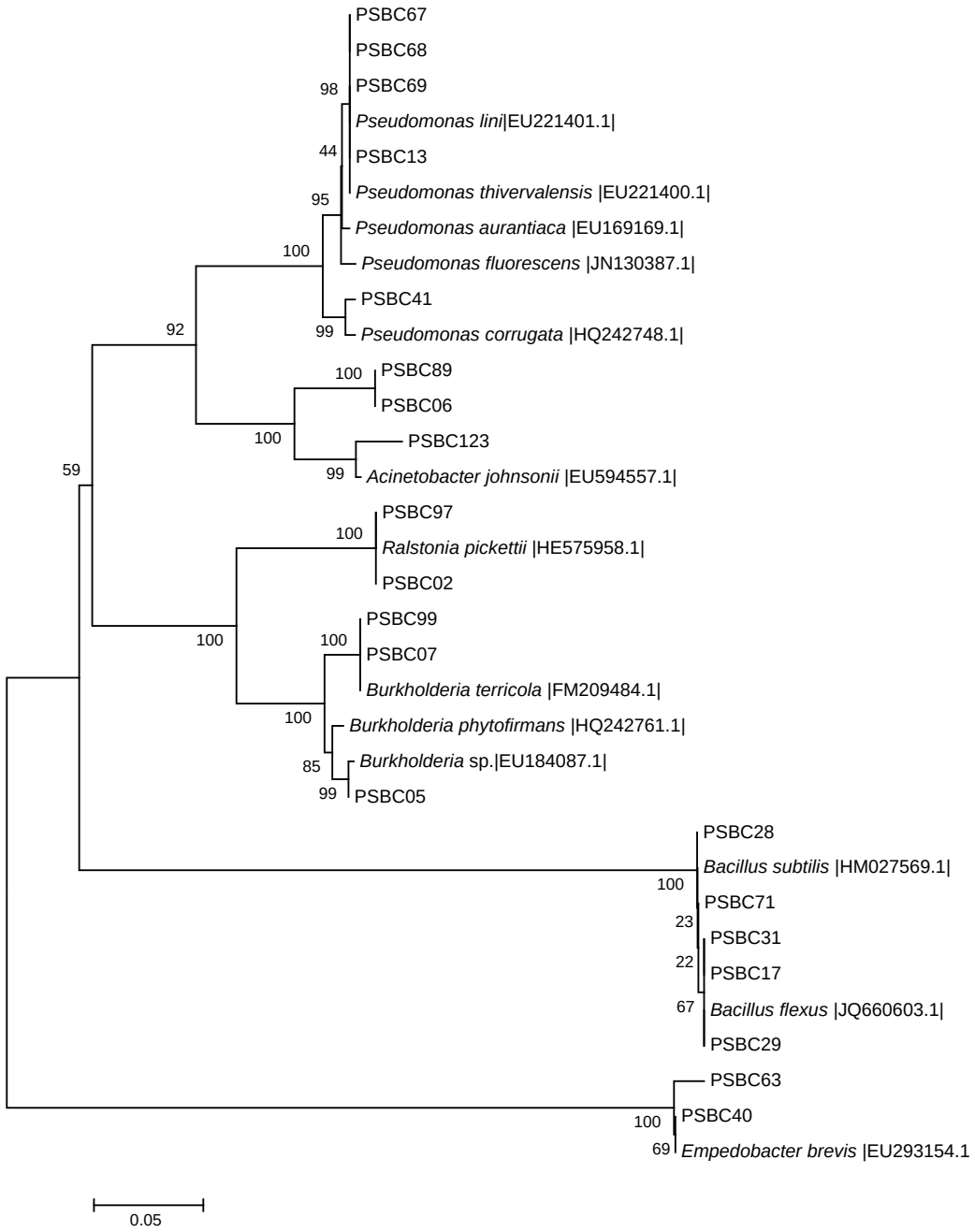

Figure 2. Neighbour-Joining phylogenetic tree of 16S rRNA gene sequence showing the positions of different PSBC strains. Bootstrap values are shown at branching point.

(Muleta et al., 2013).

Quantitative analysis of phosphates solubilization efficiency of the PSB isolates

The quantitative analysis of insoluble P sources solubilization efficiency of selected isolates was carried out in PK broth using TCP, RP and BM as sole P sources. All tested isolates released a larger amount of soluble phosphates into the medium until the last sampling day (Table 3). The amount of soluble P was found to vary significantly between the isolates and sampling days. After 12 days of incubation, the highest amount of P was released by PSBC35 (379 µg ml^{-1}), indicating that this isolate is the most efficient TCP solubilizer (Table 3). On the other hand, PSBC29 was found to be poor in solubilization of TCP as it released

Table 3. TCP and rock phosphate solubilization efficiency of PSB isolates from chickpea rhizosphere.

Isolate	$Ca_3(PO4)_2$						Rock phosphate						Bone meal					
	4th day		8th day		12th day		4th day		8th day		12th day		4th day		8th day		12th day	
	pH	P (µg ml^{-1}) ±SE	pH	P (µg ml^{-1}) ±SE	pH	P (µg ml^{-1}) ±SE	pH	P (µg ml^{-1}) ±SE	pH	P (µg ml^{-1}) ±SE	pH	P (µg ml^{-1}) ±SE	pH	P (µg ml^{-1}) ±SE	pH	P (µg ml^{-1}) ±SE	pH	P (µg ml^{-1}) ±SE
PSBC05	5.2	13±8^{c}	5.8	174±9^{b-d}	6.3	145±12^{c}	4.2	28±1^{b}	4.7	42±1^{c}	4.9	44±2b	-	-	-	-	-	-
PSBC17	4.8	26±10^{c}	4.9	200±12^{b-d}	5.9	207±9^{b}	5.1	14±1^{cd}	5.3	53±2^{a}	5.6	56±1^{a}	6.0	155±1^{b}	6.1	161±6^{c}	6.2	162±3^{c}
PSBC28	4.8	30±4^{c}	5.7	172±44^{b-d}	5.9	161±53^{c}	4.1	10±1^{d}	4.2	7±1^{h}	4.5	7±1^{f}	-	-	-	-	-	-
PSBC29	4.9	15±6^{c}	4.7	70±20^{e}	5.9	137±44^{c}	4.6	14±1^{cd}	5.0	20±1	5.1	14±3^{e}	-	-	-	-	-	-
PSBC31	5.0	21±7^{c}	5.3	148±42^{c-e}	5.9	148±58^{c}	5.8	12±4^{cd}	5.8	15±1^{f}	5.8	15±1^{e}	-	-	-	-	-	-
PSBC33	4.6	41±4^{c}	5.1	295±39^{a}	5.8	315±50^{a}	4.1	36±3^{a}	4.1	38±3^{d}	4.9	38±1^{c}	6.1	119±7^{d}	6.1	115±1^{e}	6.1	120±4^{e}
PSBC35	5.4	120±32^{b}	5.8	263±42^{a}	6.1	379±54^{a}	4.9	31±3^{ab}	5.8	46±4^{b}	6.1	57±3^{a}	6.1	124±2^{c}	6.1	131±3^{d}	6.3	139±1^{d}
PSBC79	5.6	171±70^{a}	5.8	238±44^{ac}	6.2	346±28^{a}	4.3	32±2^{ab}	4.6	34±3^{e}	5.0	33±3^{d}	5.0	33±11^{f}	5.5	182±8^{b}	5.7	195±1^{b}
PSBC81	4.9	48±10^{c}	5.2	193±12^{b-d}	6.1	299±48a	4.1	35±9^{a}	4.7	38±1^{d}	5.0	35±4^{cd}	6.0	211±5^{a}	6.4	217±3^{a}	6.5	232±1^{a}
PSBC97	5.0	53±14^{c}	5.6	110±42^{c-e}	6.0	143±37c	6.9	17±1^{c}	5.6	12±1^{g}	6.1	15±1^{e}	4.6	66±2^{e}	5.0	87±9^{f}	4.8	102±4^{f}

SE, standard error; TCP, tricalcium phosphate, the data is average of triplicates. Numbers in the same column followed by the same letter do not differ significantly at p= 0.05 by Duncan's Multiple Range Test (DMRT).

only 137 µg ml^{-1} of soluble P after 12 days. However, a slight decline was found for PSBC5 and PSBC31 after the 8th day of incubation. During solubilization the isolates showed a sharp decline of pH from neutral to acidic until the 4th day of incubation (Table 3). The acidic pH ranged from 4.8 (PSBC33) to 5.6 (PSBC79). After the 4th day all isolates except PSBC29 exhibited progressively increasing pH of the growth medium until the 12th day of incubation. The correlation analysis showed that there was statistically significant inverse relation between the concentration of soluble P and concomitant drop in pH of the growth medium (r = -0.82), suggesting that acid production is the mechanism of TCP solubilization.

Similarly, selected isolates were tested for their rock phosphate solubilization ability. The amount of soluble phosphorus released and corresponding pH change of the medium varied among isolates and sampling dates (Table 3). The highest amount of soluble P was 57 µg ml^{-1} which was released by PSBC35, while the least was 7 µg ml^{-1} recorded for PSBC28 on 12th day of incubation. The amount of soluble P was increased gradually up to the 12th days of incubation for most isolates (PSBC17, PSBC5, PSBC33 and PSBC31) while the other isolates showed a slight decrease. There were sharp drops of pH from 7.0 to acidic pH values during RP solubilization. The lowest pH was noted on 4th day for all isolates except PSBC97. Then, the pH of the growth medium increased gradually until the 12th day. The correlation analysis showed that there was an inverse relation (r = -0.34) between soluble P and an associated drop in pH of the culture medium suggesting that acidification of the growth medium as mechanism of rock phosphate solubilization.

The amount of soluble P released from the finely ground old bone (BM) and corresponding pH drop of the medium by the PSBC isolates is shown in Table 3. All tested isolates showed a very high BM solubilization ability with different levels of efficiency. There was a significant variation among isolates in terms P solubilization and concomitant pH change of the media. Isolate PSBC81 was more efficient in solubilizing BM (233 µg ml^{-1}) than the other isolates, followed by PSBC79 (195 µg ml^{-1}). The concentration of soluble P increased progressively with days of incubation. Similar to other P sources, a drop in pH was observed over the course of BM solubilization in all isolates. On the 4th day drops in pH ranging from 4.6 to 6.1 were noted for isolates PSBC97 and PSBC33, respectively. Then, pH of the medium was increased gradually until the last sampling day. A positive correlation (r = 0.27) was observed between the concentration of soluble P and decrease in pH which was,

however, not statistically significant. This is in line with the result of the previous study (Keneni et al., 2010) suggesting that mechanisms other than acid production were involved in the dissolution of BM. Hence, further studies on the solubilization mechanisms of BM are highly recommended.

All PSB isolates recovered from chickpea rhizosphere soils showed considerably higher solubilization ability of different insoluble inorganic phosphates in broth culture. In general, the highest amount of P was released from TCP followed by BM after 12 days of incubation. The lowest dissolution and sharpest drop in pH was obtained during solubilization of RP. Likewise, a previous study (Keneni et al., 2010) showed that phosphate solubilizing microorganisms mobilized more P from insoluble inorganic salts than from naturally occurring phosphate sources such as rocks and bone meal which are made of apatite.

All isolates displayed a significant drop in pH of the culture media during the solubilization of all tested insoluble phosphate sources. Several studies indicated that the drop in pH is due to the production of organic acids during bacterial growth (Chen et al., 2006; Muleta et al., 2013; Vyas and Gulati, 2009). The decreasing pH and the production of organic acids resulted in solubilization of a considerable amount of soluble P (Chen et al., 2006). The results of the present study established a clear relationship between pH drop and P solubilization. These results are consistent with the report of earlier studies (Illmer and Schinner, 1995; Whitelaw, 2000; Naik et al., 2008; Vyas and Gulati, 2009) which showed that solubilization of insoluble phosphate sources were mediated specifically by the decreasing pH of the medium. It has been well documented that mineral phosphate solubilization by phosphate solubilizing bacteria strains results from the release of low molecular weight organic acids (Illmer and Schinner, 1995; Kim et al., 1997; Vyas and Gulati, 2009), probably as an interacting effect of their hydroxyl or carboxyl groups that may chelate the cations bound to the phosphate, thereby converting it into soluble forms (Kpomblekou and Tabatabi, 1994; Kim et al., 1997). Gram negative isolates displayed better performance in solubilization of different insoluble inorganic P sources compared to Gram positive phosphate solubilizing bacterial isolates in the study which is in line with the results of previous studies (Muleta et al., 2013; Tripura et al., 2007). The fluctuations and differences in insoluble P sources solubilization efficiency observed among the isolates could be attributed to differences in the types and the amount of organic acids produced (Vyas and Gulati, 2009). Alternatively, it could also be explained by the differences of in rate of P release and immobilization (Muleta et al., 2013; Prasanna et al., 2011), when cells in the culture immobilize more phosphate for microbial biomass production, the corresponding values decrease, creating such fluctuations (Muleta et al., 2013). From our the observation in this study, a decrease in soluble P and concomitant increase in the pH values of the growth medium indicates re-utilization of available P (Rashid et al., 2004; Tripura et al., 2007). In addition, the excreted organic acids could be reused by the isolates for their own metabolism (Tripura et al., 2007) which is highly associated with an exhaustion of the original carbon source (Rashid et al., 2004) since phosphate solubilization is a complex process depending on many factors such as nutritional, physiological and growth conditions of the culture (Reyes et al., 1999).

The results indicate that PSB isolates native to Ethiopian soils exhibited high potential for RP solubilization. The dissolution of rock phosphate to a significant extent by these indigenous PSB isolates would give the opportunity to utilize rock phosphate deposits found in different parts of the country (Muleta et al., 2013) as cheap P fertilizers. Earlier studies in Ethiopia (Bekele and Hofner, 1993) indicated successful use of rock phosphate under greenhouse conditions. Thus, inoculation of native PSB isolates with superior rock phosphate solubilization ability along with the application of rock phosphate could improve the productivity of chickpea and other crops. Furthermore, the superior BM solubilization of these isolates would also help to use the waste accumulated around the abattoirs found in different urban areas of the country as P fertilizer along with PSB inoculants. This could reduce the dependence on the very expensive chemical fertilizers and enable a shift towards sustainable crop farming.

Multiple PGP characteristics of PSB isolates from chickpea rhizosphere

All PSB isolates were screened for multiple PGP characteristics such as IAA production and inhibition of *F. oxysporum* growth under *in vitro* conditions (Table 4). The results indicate that in addition to their phosphate solubilization activity, the PSB isolates exhibited different PGP properties. Dual culture assay indicated that 18.4% of the isolates inhibited the growth of *F. oxysporum* under *in vitro* conditions (Table 4). These isolates belonged to the genera *Pseudomonas* (44.4%), *Burkholderia* (22.2%) and *Acientobacter* (11.1%). All antagonistic isolates from *Pseudomonas* group were identified as *Pseudomonas fluorescence*. Several studies have reported that phosphate solubilizing bacterial isolates obtained from different agriculturally important crops rhizosphere were antagonistic to different fungal phytopathogens such as *Fusarium* species, *Phytium* species, *Rhizoctonia* species and others under *in vitro* conditions (Peix et al., 2001b; Naik et al., 2008). In Ethiopia, chickpea production has been constrained by wilt and root rot caused by different fungal plant pathogens (Ahmed and Ayalew, 2006). These diseases have been responsible for major yield losses (Ahmed and Ayalew, 2006) and thus isolates which inhibited the growth of the respective pathogens

Table 4. Multiple PGP characteristics of PSB isolated from chickpea rhizosphere.

Isolate	FOC inhibition (%)± SE	IAA (µg ml^{-1}) ± SE
PSBC01	41.2±1.3^{b}	-
PSBC02	-	17.6±0.8^{d}
PSBC05	38.5±1.1^{c}	-
PSBC06	-	12.3±1.1^{f}
PSBC13	-	19.2±1.0^{d}
PSBC14	-	19.7±1.2^{d}
PSBC16	-	21.4±0.2^{d}
PSBC17	-	61.2±1.2^{a}
PSBC28	-	20.5±0.7^{d}
PSBC29	-	16.4±0.8^{e}
PSBC31	-	16.3±0.6^{e}
PSBC33	-	37.7±1.2^{b}
PSBC39		25.8±0.2^{c}
PSBC40	-	22.1±1.4^{d}
PSBC61	-	18.2±0.9^{d}
PSBC62	25.6±1.5^{e}	-
PSBC67	-	19.4±1.4^{d}
PSBC68	40.3±1.8^{b}	13.4±1.3^{ef}
PSBC69	44.2±1.6^{a}	16.8±1.5^{d}
PSBC70	45.8±1.9^{a}	18.7±1.6^{d}
PSBC71	-	19.6±1.3^{d}
PSBC72	-	14.6±0.4^{e}
PSBC79	-	15.5±0.7^{e}
PSBC81	-	58.0±2.0^{a}
PSBC97	-	18.6±1.5^{d}
PSBC123	26.3±2.1^{e}	14.3±0.6^{e}
PSBC125	-	-
PSBC126	-	15.2±1.0^{e}
PSRB134	40.5±1.4^{b}	-
PSRB135	30.3±2.1^{d}	-

IAA, indole acetic acid; SE, standard error; +, positive for the test; -, negative for the test; FOC, *Fusarium oxysporum f.sp. cicero.* Means in the same column followed by the same letter do not differ significantly at p=0.05 using Duncan's Multiple Range Test (DMRT).

would give dual advantage as biofertilizer and biocontrol agents. Consequently, such isolates are highly recommended for greenhouse and field tests.

Further, 49.0% of the PSB isolates were shown to be capable of producing IAA equivalent substance, indicating that this ability is common among rhizosphere bacteria. The majority of these isolates were members of genus *Pseudomonas* (12.3%) and *Bacillus* (27.2%) while all the rest belonged to the genera *Ralstonia, Empedobacter, Acientobacter, Burkholderia* and *Enterobacter*. The highest amount of IAA equivalent substance produced by PSBC17 was 61 µg ml^{-1} whereas the lowest was by PSBC68 (14 µg ml^{-1}) (Table 4). The most capable strains belonged to the genera *Bacillus* and *Enterobacter*. Similarly, several previous studies demonstrated that PSB isolates from different crops rhizosphere were found to exhibit different IAA production ability (Ponmurugan and Gopi, 2006; Vikram et al., 2007; Naik et al., 2008; Shahab et al., 2009; Yadav et al., 2010). These studies indicated that most of the isolates produced considerably higher amount of IAA. The large variation in the amount of IAA produced by different strains has been attributed to the variability in the metabolism of different strains of PSB (Leinhos and Vacek, 1994). Such isolates were found to be excellent in promoting plant growth under greenhouse experiments (Shahab et al., 2009; Yadav et al., 2010). Thus, these isolates could provide additional plant growth promoting activities apart from solubilizing insoluble P sources and hence further studies are required to explore the exact contribution of IAA production in the promotion of plant growth.

Table 5. Effects of inoculation of PSB strains on growth of Chickpea.

Treatments	Nodule number plant^{-1} ±SE	Shoot dry weight(g) Plant^{-1}±SE	Total Nitrogen (%) of shoot± SE	Phosphorous concentration in shoot(mg/g SDW) ± SE
UIA	25.0±2.6^{d}	0.75±0.02^{f}	1.34±0.07^{d}	1.38±0.059^{c}
P+	32. 7±4.0^{c}	1.09±0.22^{cd}	2.02±0.48^{b}	1.55±0.140^{c}
PSBC17	32. 7±2.5^{c}	0.92±0.02^{def}	1.44±0.05^{d}	1.33±0.051^{d}
PSBC33	33.0±3.6^{c}	0.89±0.06^{ef}	1.63±0.12^{cd}	1.36±0.031^{d}
PSBC35	41. 7±2.6^{b}	1.41±0.16^{ab}	2.48±0.19^{a}	1.75±0.261^{bc}
PSBC81	44.7±2.5^{ab}	1.47±0.15^{a}	2.03±0.02^{bc}	1.42±0.158.5^{c}
PSBC79	32.3±3.2^{c}	0.92±0.03^{def}	2.43±0.15^{ab}	2.01±0.49^{ab}

SE, standard error. The data is average of triplicates. Numbers in the same column followed by the same letter do not differ significantly at p= 0.05 by Duncan's Multiple Range Test (DMRT); SDW, shoot dry weight; UIA, uninoculated control; P+, soluble p fertilizer treated.

Response of chickpea to inoculation PSB with different P sources

The effects of inoculation of PSB isolates on chickpea growth parameters such as the number of nodules, nodule dry weight, shoot dry matter yield and, N and P concentration of shoot were investigated under glasshouse conditions. During this experiment, *B. flexus* (PSBC17), *P. fluorescence* (PSBC33), *Enterobacter sp.* (PSBC35), *Enterobacter sakazaki* (PSBC79) and *Enterobacter sp.* (PSBC81) were used as inoculants (Table 5).

Shoot dry matter yield of the plant increased significantly by inoculation of all isolates except PSBC33 when compared to the uninoculated control. Isolate PSBC81 and PSBC35 were the most effective ones in terms of increasing shoot dry weight of chickpea with an increase in shoot dry weight by 96 and 88%, respectively over the uninoculated control (Table 5). These increases were significantly higher ($p \leq 0.05$) compared to shoot dry weight of P fertilized plants. Though it is difficult to production of growth promoting IAA equivalent substances by the all the isolates used as inoculants contributes to their stimulatory effects on plant growth. Likewise, Yadav et al. (2010) reported that most of PGPR isolates resulted in a significant increase in shoot length, root length and dry matter production of shoot and root of chickpea seedlings under growth chamber conditions.

The number of nodules was one of the parameters measured and used to evaluate the effect of different PSB isolates inoculation. All tested isolates significantly improved nodulation of chickpea over the uninoculated control. PSBC81 was the most efficient in inducing nodulation of chickpea followed by isolate PSBC35, increasing the number of nodules by 78.8 and 66.8%, respectively. These superior increments were also significantly higher than the number of nodules obtained from P fertilized plants. Inoculation of all isolates improved dinitrogen fixation detected as an increase in N concentration in shoot over the uninoculated control. However, only PSBC35, PSBC79 and PSBC81 resulted in a significant increase in N concentration compared to the uninoculated control. The highest concentration of N was obtained in plants inoculated with PSBC35 followed by PSBC79 with N concentrations of 2.48 and 2.43%, respectively.

The study demonstrated that the increase in nodule number is positively correlated with the increase in shoots dry weight (r=0.93) and N content (r=0.70). Peix et al. (2001b) have previously established similar correlation among the growth parameters in common bean inoculated with the phosphate solubilizing strain of *Burkholdera cepacia.* It has been reported that available P deficiency in soils could also have a deleterious effect on symbiotic interaction between rhizobia and legume crops like chickpea, thus affecting its growth and productivity (Gyaneshwar et al., 2002). Several studies have demonstrated that inoculation of seedlings with PSB isolates increased the level of available P in the rhizosphere soil (Sundara et al., 2002; Vyas and Gulati, 2009). In this way, inoculation of chickpea with selected phosphate-solubilizing bacteria improves nodulation and dinitrogen-fixation processes of this legume crop.

Likewise, inoculation effects of PSB isolates on the concentration of P in shoot of chickpea have been shown. Inoculation of PSBC79, PSBC81 and PSBC35 significantly increased the concentration of P in chickpea shoot over the uninoculated control (Table 5). In contrast, the P concentration of PSBC33 and PSBC17 inoculated plants did not vary significantly from uninoculated control plants. The highest concentration (2.01 mg) of P was obtained in PSBC79 inoculated plant whereas the lowest (1.16 mg) was recorded in PSBC33 inoculated plants. Though the effect of PSB inoculation on the level of available P was not determined, results obtained suggests that they improved the availability of soluble P in the rhizosphere soil and thus, the P uptake of the plant. Several studies have revealed that inoculation of PSB isolates increased the available P in soil (Sundara et al., 2002; Vyas and Gulati, 2009) and concomitantly improved P uptake of the plant (Peix et al., 2001a; Peix et al., 2001b; Vikram and Hamzehzarghani, 2008; Castango et

al., 2011).

Conclusion

In general, the results of the study showed that chickpea rhizosphere soils from different producing areas of Ethiopia harbor considerably high numbers of PSB isolates. The study has also demonstrated that those PSB isolates belong to different bacterial genera: *Acinetobacter, Bacillus, Brevibacillus, Burkholderia, Chryseomonas, Enterobacter, Empedobacter, Pseudomonas, Ralstonia, Sphingomonas* and *Stenotrophomonas.* The PSB strains were able to release significantly higher amount of soluble P from different insoluble phosphates sources such as TCP, RP and BM. The results also revealed phylogenetic and biochemical diversity of PSB with multiple plant growth promoting traits. Greenhouse experiment showed that inoculation of chickpea with PSB resulted in significant increase in nodule number, dry matter yield, N and P uptake of the plant. Moreover, the study indicated that these strains are of particular interest for future research and need to be tested in the field under different agroecological conditions. The diversity and PGP characteristics study of PSB from rhizosphere soil will be useful to design strategies to use these strains as inoculants in sustainable agriculture. Thus, consecutive studies should focus on field studies on those strains due to the potentially high importance for the nutrition in this area and in this context for an improvement of the sustainability of land use in Ethiopia.

Conflict of interests

We do not have any conflict of interest.

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