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STATUS AND PHOSPHORUS SOLUBILIZATION POTENTIAL OF BACTERIA AND ARBUSCULAR MYCORRHIZAL FUNGI ISOLATED FROM VARIOUS LOCATIONS OF KHYBER PAKHTUNKHWA PROVINCE

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Abstract

The soils of Pakistan are alkaline calcareous in nature and its high pH makes phosphorus (P) unavailable for plants uptake. Chemical sources of P fertilizers are a costly and detrimental practice. Therefore, investigations were conducted to determine the native status of phosphate solubilizing bacteria (PSB) and arbuscular mycorrhizal fungi (AMF) in three different zones of Khyber Pakhtunkhwa province of Pakistan. In order to select the efficient PSB strains for solubility enhancement of P from rock phosphate (RP), rhizosphere soil samples were collected from irrigated and rainfed fields of maize, sorghum, pastures and vegetables. Population density of PSB ranged from 1.7×10^7 to 2.7×10^8 CFU g^{-1} rhizosphere soil. The bacterial strains *Coccus, Streptoccocus* and *Bacillus* sp. were identified on the basis of their microscopic, phenotypic and morphological characters. Most of the AM fungal spores identified were belonging to *Glomus mosseae* and *Glomus intradices*. A range of 02-35 spores per 20 g air dried soil were recorded.The PSB strains such as *Coccus* DIM7, *Streptococcus* PIM6 and *Bacillus* sp. PIS7 solubilized more P from RP than any other strain in both of the liquid and solid medium. Results show that areas under investigations are rich in P solubilizing micro flora providing a rich source for inoculum production. Moreover, the PSB strains have the capability to solubilize P from RP that can be used as biofertilizers for optimum crop production.

Key words: Phosphate solubilizing bacteria, AM fungi, Phosphorus, Rock phosphate, Crops and Locations.

Introduction

Phosphorus (P) is one of the major essential macronutrients for plants, which is applied to the soil in the form of phosphatic fertilizers. However, a large portion of applied P is rapidly immobilized, rendering it unavailable to plants (Goldstein, 1986). The plants can recover only 10-30% of P from the soil, while the remainingis accumulated and fixed by the formation of complexes with iron (Fe) and aluminum (Al) in acidicsoils or with calcium (Ca) and magnesium (Mg) in alkaline soils (Holford, 1997; Yang *et al*., 2010). The use of microorganisms is an important practice in agriculture for improving nutrients availability for plants (Freitas *et al*., 2007). The bioavailability of P to plant roots and increasing P-mobilization in soil can be improved by soil microorganisms. The ability of these microbes to convert insoluble forms of P to a soluble form is important for the promotionplant growth and productivity (Richardson, 2001). With regard to the plant health and promotion, the commonly found endophytes such as bacteria and fungi are very important (Nair and Padmavathy, 2014). The bioavailability and solubility of inorganic and soil bound P can be improved by using beneficial microbes such as phosphate solubilizing bacteria (PSB) (Khan *et al*., 2007). Many PSB strains have been isolated at the beginning of the last century, including, *Agrobacterium Bacillus, Pseudomonas, Erwinia, Serratia, Flavobacterium, Enterobacter, Micrococcus, Azotobacter, Bradyrhizobium, Salmonella, Alcaligenes, Chromobacterium, Arthrobacter, Streptomyces, Thiobacillus*, and *Escherichia* (Zhao, 2001).These bacteria have the capability of colonizing in the plant rhizosphere andthus play an active role in promoting plant growth, therefore these bacteria are also termed as plant growth promoting rhizobacteria (PGPR) (Hayat *et al*., 2010, Kang *et al*., 2012). Plant roots and its rhizosphere provide food, energy, shelter, and biological diversity to PSB (Mc Millan, 2007). PSB promote crop productivity, resistance to diseases, protection against insect damages, inoculants production (Bashan *et al*., 2000) and stability of ecological environment (Rodriguez *et al*., 2006).The Arbuscular mycorrhizal fungi (AMF) account for 5–50% of soil microbial biomass and are mostly found in cultivated soils (Wahid *et al*., 2016;Olsson *et al*., 1999). It has been found that about 80% of all terrestrial plants establish their mutualistic association with agricultural, horticultural and hardwood crop species (Pozo and Azcón-Aguilar, 2007). Colonization of roots by AMF has been shown to improve growth and productivity of several field crops (Cavagnaro *et al*., 2006; Pasqualini *et al*., 2007). Vesicular arbuscular mycorrhizal fungi occur widely diverse environmental conditions and are found in association with a number of leguminous and forage crop (Islam & Ayanaba, 1981; Carrenho *et al*., 2001; Chen *et al*., 2005., Souchie *et al*., 2006). Different vegetables species also contain varying spore densities in their rhizospheric soils ranging from 65-215 per 100g air dried soil. Various types of VAM spores with colours like dark, reddish, brown and yellowish with various shapes such as oval, spherical and irregular are present in the rhizospheric soils of several vegetable crops species (Akond *et al*., 2008). It has also been found that the AM fungi hyphae, spores, and glomalin-related soil protein were more abundant underneath shrub canopies, but bare land shrub interspaces had similar amounts of viable propagules, spore diversity, and spore community composition compared to canopies. The soil organic matter, phosphorus concentration, climate, local and regional scale abundance and community composition are strongly correlated with variation in AMF densities (Chaudhary *et al*., 2014).

To our knowledge, no attempts have been made so far to isolate and characterize the native status of PSB and AMF from rhizosphere soils of irrigated, rainfed crops, vegetables and pastures of different agro ecological zones of the country and to investigate the role of different isolates of PSB for P solubility from RP qualitatively and quantitatively. In this regard the present investigations were carried out to study the population density of PSB and AMF to find out potential inoculants for solubility enhancement of P from RP.

Materials and Methods

Collection of rhizosphere soil samples: The rhizosphere soil samples were collected from different agro ecological zones (Chitral, Peshawar and Dera Ismail Khan) of Khyber Pakhtunkhwa province of Pakistan to determine the native status of phosphate solubilizing bacteria (PSB) and Arbuscular mycorrhizal fungi (AMF) and to select potential PSB strains for solubility enhancement of P from RP. In total 30 samples (10 from each location) were collected from the entire region including 3 different maize and sorghum samples from irrigated and rainfed fields. The rhizosphere soils of tomato (*Solanum lycopersicom*), chilli (*Capicsicum* sp.), eggplant (*Solnum melongena*), salad (*Lactuca sativa*), pumpkin (*Lofia sativa*), onion (*Allium cepa*), spinach (*Spinacia oleracea*) and lady finger (*Abelomoschus esculentus*) were collected from vegetable farmlands and that of common cocklebur (*Xanthium Strumarium*), bermuda grass (*Cynodon dactylon*), barnyard grass (*Echinochloa Crusgalli*), common cattail (*Typha Latifolia*), and prosopas spp. (*Prosopis Juliflora*) were collected from pastures. The plants were uprooted with whole root system along with adhering soil particles at maturity stages. The samples were kept in aseptic bags andstored at 4°C in Soil Biology and Biochemistry laboratory, Land Research Resource Institute (LRRI), National Agriculture Research Center (NARC), Islamabad. Physical and chemical properties of the soils were analyzed for texture, soil pH, lime, organic matter, N and P contents. At Peshawar and Dera Ismail Khan (DIK) the soil texture was silty clay loam and alkaline in nature while at Chitral it was sandy to silty clay and slightly acidic in nature. The soils under investigations were also low in total N (less than 1%) and AB-DTPA extractable P (2-4 mg kg^{-1}). Moreover, the soils at Peshawar and D.I. Khan were strongly calcareous in nature with 18% lime and less than 1% organic matter content, while at Chitral the organic matter content was 1.1% (Table 1).

Isolation and purification of phosphate solubilizing bacteria (PSB): The different types of PSB were isolated from each rhizosphere soil sample by using the serial dilution method (Johnson & Curl, 1972). Initially, rhizosphere soil suspensions were prepared for the 10 folds serial dilutions. 0.1 ml of each dilution was taken and spread on LB agar media (Miniatis *et al*., 1982) and kept in incubator for 48 h. The viable bacteria were enumerated by using colony forming unit (CFU) count method. The concentrations of PSB in the original sample were calculated by using the formula as described by James (1978).

$$\text{Colony forming unit (CFU) g}^{-1}\text{ soil} = \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Volume of inoculums}}$$

The prominent colonies were picked and purified on Pikovskaya's agarmedia (containing 5 g tricalcium phosphate) and incubated at 28°C for 7 days by the method of Pikovskaya (1948). In all the 30 rhizosphere soil samples ofmaize and sorghum irrigated and rainfed fields, vegetables and pastures the bacterial strains *Coccus, Streptoccocus* and *Bacillus* sp. were identified on the basis of their microscopic, phenotypic and morphological characters by the procedure of Goenadi *et al*. (2000). For Gram staining purpose pure bacterial slides of all the isolated strains were prepared by the procedure of Vincent (1970).

Solubility of P from RP by PSB in solid and liquid media: The capability of PSB strains (*Coccus, Streptococcus* and *Bacillus* sp.) isolated from all maize and sorghum irrigated and rainfed field of different locations were investigated for solubility enhancement of P from Hazara RP (23% P) in an incubation experiment. The solid medium of Pikovskaya's agar was used in this experiment in the form of a complete randomized design (CRD) having 11 treatments and 3 replications. Each of the pure PSB strain was placed on Pikovskaya's agar plates. RP (5.0 g) used instead of tricalcium P in the media preparation in combination with other ingredients as given in section (Gupta *et al*., 1994). The pH of the media was adjusted at 7.0 ± 0.2 and incubated at 28°C for 7 days. The plates were then used to examine the capability of PSB to solubilize insoluble P compound qualitatively to form a clear light zone surrounding colonies. The solubility index (SI) was measured using following formula as given by Edi-Premono *et al*. (1996).

$$\text{SI} = \frac{\text{Colony diameter} + \text{Halozone diameter}}{\text{Colony diameter}}$$

Table 1. Physico-chemical properties and climatic conditions ofareas under investigations.

Parameters	Peshawar	D. I. Khan	Chitral
Soil texture	Silty clay loam	Silty clay loam	sandy to silty clay
Soil pH	8.4	8.2	7.6
Organic matter (%)	0.8	0.7	1.1
N (%)	0.075	0.082	0.093
P (mg kg^{-1})	3.8	4.5	5
Altitude (m)	359	173	1500
Average temperature (min-mix ^{o}C)	25-40	28-48	15-33
	4-18	4-26	0-15
Precipitation per year (mm)	420	270	800
Relative humidity (%)	46-76	42-79	22-83

For solubility of P from RP in liquid media, three to four loops of pure bacterial colonies were inoculated in 100 ml of the liquid Pikovskaya's broth culture (pH 7.0) in a 250 ml flask. Initially, the flasks were autoclaved for 20 minutes at 121°C and 15 psi. The 0.25 g L^{-1} of rock phosphate as a source of P was supplemented to each flask and incubated for seven days (24°C) on rotary shaker at 200 rpm. After incubation, the broth cultures were centrifuged at 10000 rpm for 15 minutes to remove insoluble materials and bacterial cells. The supernatant from the flask was mixed with 100 ml distilled water and its pH was noted. Quantitatively, soluble P was determined with the blue colour of phospho molybdate by spectrophotometer (Murphy and Riley, 1962). The soluble P by bacterial isolates was quantified from the standard curve. The treatments combination were including as 1) Control 2) Rock phosphate (RP) 3).RP + *Coccus* CIM7 (Chitral irrigated maize) 4) RP + *Coccus* CIS7 (Chitral irrigated sorghum)5).RP + *Bacillus* CUM6 (Chitral rainfed maize) 6) RP + *Streptococcus* PIM6 (Peshawar irrigated maize) 7)RP + *Bacillus* PIS7 (Peshawar irrigated sorghum) 8)RP + *Coccus* PUM6 (Peshawar rainfed maize) 9) RP + *Coccus* DIM7 (D. I. Khan irrigated maize) 10) RP + *Streptococcus* DIS7 (D. I. Khan irrigated sorghum) and 11) RP + *Bacillus* DUM7 (D. I. Khan rainfed maize).

Based on the results of qualitative and quantitative study, the 3 types of the most efficient PSB strains (*Coccus* DIM7, *Streptococcus* PIM6 and *Bacillus* sp. PIS7) were selected and stored in the laboratory of Soil Biology and Biochemistry, LLRI, NARC, Islamabad at 4°C for further research.

Isolation of Arbuscular mycorrhiza fungi (AMF): The arbuscular mycorrhizal fungi spores were isolated from rhizosphere soil by wet-sieving and decanting techniques as described by Gerdeman and Nicolson, 1963. Initially, 20 g rhizosphere soil was mixed with 200 ml distilled water in a beaker and after 10 minutes it was passed through different size of sieves. The ingredients of the last sieve were poured into a falcon tube containing 25 ml sugar solution. After centrifugation at 2200 rpm for three minutes, the solution from the tubes was passed again from the minimum sieve and then collected in the petri plates for observing different AM fungal spores under microscope. The isolated spores from the soil sample were identified according to their morphological characteristics including shape, size, color, distinct wall layer, attached hyphae and surface orientation of spores as described by Schenck & Perez (1990).

Statistical analysis: The data collected from all experiments were analysed statistically according to the procedure as given by Jandel Scientific (1991) using Statistic, 2000 package and Least Significant Difference (LSD) test were used for any significant difference among the treatments**.**

Results

Population density of PSB in rhizosphere of plants: Data regarding PSB population density in rhizosphere soils of field crops, vegetables and pastures oflow, medium and high temperature zones (Chitral, Peshawar and Dera Ismail Khan) of Khyber Pakhtunkhwa province of Pakistan are presented in Table 2. The population densities of PSB observed at all these locations were different from each other and ranged from 1.7×10^7 to 2.7×10^8 CFU g^{-1} of soil. At Peshawar region the maximum PSB population density of 2.7×10^8 CFUg^{-1} of soilwas found in the rhizosphere of irrigatedsorghum plants. It was followed by the PSB population of eggplant (2.5×10^8 CFUg^{-1} of soil) in vegetables farms. The lowest PSB population density of 2.1×10^6 CFUg^{-1} of soil was recorded in rhizosphere of *Xanthium strumarium* collected from pastures at Peshawar. Similarly, at D.I. Khan and Chitral districts the population density of PSB was $2.6 \times 10^7$and 2.6×10^8 CFU g^{-1} soilin the rhizosphere of sorghum rainfed and sorghum irrigated soils, respectively. It was followed by rhizosphere of irrigated maize having population density of 3.2×10^7 g^{-1} of soil at Chitral. Moreover the lowest PSB density of 2.6×10^7 CFU g^{-1} of soil was found in rainfed rhizosphere soil of sorghum at D.I. Khan and 2.2×10^6 CFU g^{-1} of soil in the rhizosphere of *Cynodon dactylon* at Chitral (Table 2).

Characteristics of isolated phosphate solubilizing bacteria isolates: The phenotypic and microscopic characteristics of PSB isolated from maize, sorghum, vegetables and pasture plants of Peshawar, D.I Khan and Chitral region is summarized in Table 3. In our study, we observed different phenotypic characteristics of PSB isolated from low, medium and high temperature zones of the province. These isolates were having different elevations such as flate, raised and umbonate, while the prominent colors under investigation were yellow, white, off-white, milky and reddish. The PSB isolated from rhizosphere of maize, sorghum, vegetables and pastures were having different clear margins such as curled, lobate, erose and entire. The prominent shapes of PSB strains were puncti, irregular, circular and filamentous with opaque and translucent opacity. Similarly, during the microscopic examination we also observed the gram staining and cell morphology of PSB. Most isolates of all the regions wereGram –ve and a few were Gram +ve.

Isolation and Identification of arbuscular mycorrhiza fungal spores: The arbuscular mycorrhiza fungal (AMF) spores isolated from rhizosphere soils of crops, vegetables and pastures at Chitral, Peshawar and D.I. Khan are shown in Table 4. The different types of fungal spores isolated from these locations were identified on the basis of their type, shape, color and number of wall layer. Most of the isolated AMF spores identified were of *Glomus mosseae* and *Glomus intradices*. The highest numbers of 35 spores in 20 g soil were observed in sorghum rhizosphere, isolated from irrigated fields of D.I. Khan. It was followed by 26 spores per 20 g soil extracted from rhizosphere of *Capicsicum* sp. at Peshawar. The minimum numbers of 02 spores in 20 g soil were noted in eggplant, isolated from rhizosphere of vegetables farms at Peshawar region.

Table 2. Population density of phosphate solubilizing bacteria in rhizosphere of crops, vegetables and pastures of the areas under investigations.

Locations	Species		Population density (CFU g^{-1} of soil)
Chitral	Crops	Maize (*Zea maize)* irrigated	3.2×10^7
		Maize (*Zea maize)* rainfed	2.3×10^7
		Sorghum (*Sorghum bicolor*) irrigated	2.6×10^8
		Sorghum (*Sorghum bicolor*) rainfed	2.1×10^8
	Vegetables	Onion (*Allium cepa)*	2.7×10^6
		Spinach (*Spinacia oleracea)*	3.4×10^7
		Lady finger *(Abelomoschus esculentus)*	3.0×10^7
	Pastures	Common cocklebur *(Xanthium strumarium)*	1.7×10^7
		Bermuda grass *(Cynodon dactylon)*	2.2×10^6
		Common cattail (*Typha latifolia)*	2.0×10^8
Peshawar	Crops	Maize (*Zea maize)* irrigated	2.1×10^8
		Maize (*Zea maize)* rainfed	2.5×10^8
		Sorghum (*Sorghum bicolor*) irrigated	2.7×10^8
		Sorghum (*Sorghum bicolor*) rainfed	2.4×10^6
	Vegetables	Tomato (*Lycopersicon esculentum)*	3.2×10^7
		Chilli *(Capicsicum* spp.*)*	3.0×107
		Eggplant *(Solnum melongena)*	2.5×10^8
	Pastures	Common cocklebur (*Xanthium strumarium)*	2.1×10^6
		Bermuda grass *(Cynodon dactylon)*	1.8×10^7
		Common cattail (*Typha latifolia)*	2.3×10^7
D.I. Khan	Crops	Maize (*Zea maize)* irrigated	2.4×10^7
		Maize (*Zea maize)* rainfed	1.8×10^7
		Sorghum (*Sorghum bicolor*) irrigated	1.8×10^8
		Sorghum (*Sorghum bicolor)* rainfed	2.6×10^7
	Vegetables	Tomato *(Lycopersicon esculentum)*	2.3×10^7
		Salad (*Lactuca sativa)*	2.1×10^8
		Pumpkin *(Lofia sativa)*	3.2×10^7
	Pastures	Barnyrad grass (*Echinocloa)*	2.2×10^7
		Prosopis spp. (*Prosopis juliflora)*	2.3×10^7
		Common cattail (*Typha latifolia)*	1.5×10^8

Table 3. Phenotypic and microscopic characteristics of PSB strains isolated from rhizosphere of field crops, vegetables and pastures.

Isolates	Elevation	Phenotypic characteristics				Microscopic characteristics	
		Color	Shape/form	Margin	Opacity	Staining	Bacteria shape
Chitral							
Maize irrigated	Raised	Yellow	Puncti form	Entire	Translucent	G-ve	*Coccus*
Maize rainfed	Flate	Off white	Circular	Curled	Translucent	G-ve	*Bacillus*
Sorghum irrigated	Raised	Off white	Puncti form	Entire	Opaque	G-ve	*Coccus*
Sorghum rainfed	Flate	Off white	Circular	Erose	Opaque	G+ve	*Coccus*
Onion	Flate	Milky	Filamentous	Entire	Translucent	G-ve	*Streptococcus*
Spinach	Raised	Off white	Irregular	Curled	Translucent	G-ve	*Bacillus*
Lady finger	Raised	Offwhite	Irregular	Curled	Translucent	G-ve	*Coccus*
Common Cocklebur	Raised	Reddish	Punctiform	Lobate	Opaque	G+ve	*Coccus*
Bermuda grass	Umblicate	Offwhite	Filamentous	Entire	Opaque	G-ve	*Coccus*
Common cattail	Raised	Offwhite	Filamentous	Curled	Opaque	G-ve	*Streptococcus*
Peshawar							
Maize irrigated	Flate	Yellow	Punctiform	Entire	Translucent	G-ve	*Streptococcus*
Maize rainfed	Umblicate	Reddish	Circular	Entire	Opaque	G-ve	*Coccus*
Sorghum irrigated	Raised	Yellow	Punctiform	Curled	Translucent	G-ve	*Bacillus*
Sorghum rainfed	Raised	Offwhite	Circular	Entire	Opaque	G-ve	*Coccus*
Tomato	Flate	Yellow	Punctiform	Entire	Opaque	G-ve	*Bacillus*
Chilli	Raised	Offwhite	Punctiform	Curled	Translucent	G+ve	*Coccus*
Egg plant	Flate	Offwhite	Filamentous	Lobate	Opaque	G-ve	*Bacillus*
Common Cocklebur	Raised	Offwhite	Punctiform	Curled	Translucent	G+ve	*Coccus*
Bermuda grass	Raised	White	Punctiform	Curled	Translucent	G-ve	*Coccus*
Common cattail	Raised	Offwhite	Umbonate	Curled	Opaque	G+ve	*Bacillus*
D.I. Khan							
Maize irrigated	Flate	Offwhite	Punctiform	Erose	Opaque	G-ve	*Coccus*
Maize rainfed	Flate	Yellow	Punctiform	Erose	Translucent	G-ve	*Bacillus*
Sorghum irrigated	Umbonate	Milky	Rhizoid	Curled	Opaque	G-ve	*Streptococcus*
Sorghum rainfed	Flate	Offwhite	Punctiform	Entire	Translucent	G-ve	*Coccus*
Tomato	Flate	Offwhite	Filamentous	Entire	Opaque	G+ve	*Coccus*
Salad	Flate	White	Punctiform	Curled	Translucent	G-ve	*Coccus*
Pumpkin	Umblicat	White	Punctiform	Curled	Opaque	G-ve	*Bacillus*
Barnyard grass	Raised	Offwhite	Punctiform	Entire	Opaque	G-ve	*Bacillus*
Prosopis spp	Raised	Offwhite	Rhizoid	Entire	Opaque	G+ve	*Coccus*
Common cattail	Raised	Offwhite	Rhizoid	Curled	Opaque	G-ve	*Coccus*

Table 4. Morphological characteristics of arbuscularmycorrhiza fungal spores extracted from soil of the areas under investigations.

Treatments	No. of spores (Per 20 g soil)	Shape of spores	Color of wall layer	No. of wall layer
Chitral				
Maize irrigated	25	Globose	Light brown	Single layered
Sorghum irrigated	08	Globose	Bluish	Single layer
Maize rainfed	10	Globose	Bluish	Single layer
Sorghum rainfed	25	Sub-globose	Bluish brown	Double layer
Onion	16	Cylindrical	Bluish brown	Single layered
Spinach	04	Sub-globose	Light brown	Double layer
Lady finger	11	Globose	Yellowish brown	Single layer
Common Cocklebur	08	Globose	Bluish	Single layer
Bermuda grass	03	Sub-globose	Bluish	Single layer
Common cattail	13	Globose	Yellowish brown	Single layer
Peshawar				
Maize irrigated	16	Globose	Yellowish brown	Single layer
Sorghum irrigated	04	Sub-globose	Light brown	Double layer
Maize rainfed	10	Sub-globose	Light brown	Double layer
Sorghum rainfed	13	Globose	Bluish	Single layer
Tomato	11	Globose	Yellowish	Single layer
Chilli	26	Sub-globose	Light brown	Double layer
Egg plant	02	Globose	Bluish	Single layer
Common Cocklebur	08	Globose	Bluish	Single layer
Bermuda grass	13	Sub-globose	Bluish	Single layer
Common cattail	17	Cylindrical	Bluish brown	Single layered
D.I. Khan				
Maize irrigated	18	Globose	Bluish	Single layer
Sorghum irrigated	35	Sub-globose	Light brown	Double layer
Maize rainfed	20	Globose	Yellowish brown	Single layer
Sorghum rainfed	16	Globose	Bluish	Single layer
Tomato	18	Cylindrical	Bluish brown	Single layered
Salad	04	Sub-globose	Light brown	Double layer
Pumpkin	09	Globose	Yellowish brown	Single layer
Barnyard grass	08	Globose	Bluish	Single layer
Prosopis sp.	04	Sub-globose	Bluish	Single layer
Common cattail	14	Globose	Yellowish brown	Single layer

Table 5. Qualitative and quantitative screening of isolated bacterial strains to enhance P solubility from RP.

Bacterial isolates	Solid medium P solubilization index (SI)	Liquid medium P solubility mg L^{-1}	Change in pH from neutral value
Control	0.00 c*	0.000 d*	7.0 a*
RP	0.00 c	218.3 cd	6.6 a
Chitral			
Coccus-CIM7+RP	2.28 ab	427.7 bc	5.2 b
Coccus-CIS7+RP	2.40 ab	394.3 bc	4.4 cd
Bacillus-CUM6+RP	2.30 ab	327.7 c	4.9 bcd
Peshawar			
Streptococcus-PIM6+RP	2.38 ab	894.3 a	4.3 d
Bacillus-PIS7+RP	2.49 ab	527.7 bc	4.5 cd
Coccus-PUM6+RP	2.20 b	417.1 bc	4.4 cd
D.I. Khan			
Coccus-DIM7+RP	2.73 a	661.0 ab	4.4 cd
Streptococcus-DIS7+RP	2.50 ab	361.0 bc	5.0 bc
Bacillus-DUM7+RP	2.25 b	361.0 bc	4.7 bcd
LSD ($p \leq 0.05$)	0.49	316.2	0.65

*Means with different letter (s) in a columns are significantly different at ($p \leq 0.05$)

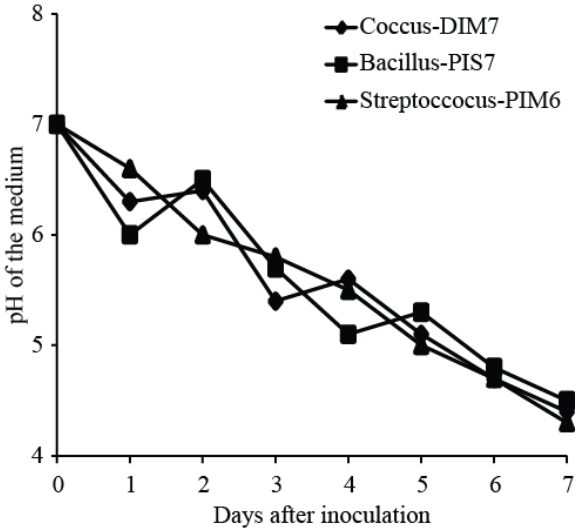

Fig. 1. Change in pH during P solubilization by the three efficient bacterial strains in Pikovskaya's broth media.

Screening of bacteria for solubility enhancement of P from RP: The capability of PSB strains to solubilize insoluble P from RP qualitatively and quantitatively is depicted in Table 5. The different PSB strains isolated from rhizosphere soil of maize and sorghum crops were inoculated on Pikovskaya's agar plates and their solubilization indexes (SI) were ranged from 0 to 2.73. The maximum SI (2.73, $p<0.001$) was observed by the *Coccus* sp., isolated from maize irrigated areas in D.I. Khan. It was followed by *Bacillus* and *Streptococcus* sp. isolated from maize rainfed in Chitral and irrigated areas in Peshawar with SI values of 2.49 and 2.50, respectively. The lowest SI value of 0 was observed in the treatments of control and RP. There was no PSB inoculation in these treatments and therefore we did not observe any colony and zone. Data regarding P solubility from RP and its effect on pH changes in Pikovskaya liquid medium by different PSB isolates is shown in Table 5. Statistical analysis of the data ($p \leq 0.05$) shows that the amount of P solubilized by PSB isolates were ranged from 218.3 to 894.3 mg L^{-1}, while the pH values decreased from 7.0 to 4.3 during 7 days incubation (Fig. 1). The highest P (894.3 mg L^{-1}) from RP was solubilized by *Streptococcus* sp. isolated from maize irrigated areas of Peshawar with a pH value of 4.3. It was followed by *Coccus* sp. (661.0 mg L^{-1}) isolated from maize irrigated areas of D.I. Khan and *Bacillus* sp. (527.7 mg L^{-1}) isolated from sorghum irrigated areas of Peshawar. The pH values noted in the treatments of *Coccus*-DIM7+RP and *Bacillus*-PIS7+RP for D.I. Khan and Peshawar were 4.4 and 4.5, respectively. Similarly, in control and uninoculated RP treatment the pH values were pH of 7.0 and 6.6, respectively.

Discussion

In this study, the different numbers of PSB and AMF in all the rhizosphere soil samples isolated from plants of the 3 different zones were observed. The average temperature (T°) of Peshawar is higher than Chitral but our findings show the average abundance of PSB population in rhizosphere soilof district Peshawar as compared to Chitral. On other hand, we also recorded low population of PSB in high (T°) zone of D.I. Khan as compared to both Peshawar and Chitral. These results are in agreement with Salcedo *et al*. (2014) who found thatthe growth and colonization of PSB is optimum withsoil fertility, low T° and pH close to neutrality. This difference in the population density of bacteria mayalso be the result of many soil factors such as soil nutrients, moisture, organic matter contents, low molecular weight organic acids and soil enzymatic activities (Ponmurugan & Gopi, 2006). The release of organic acids by PSB (at

low pH) promotes mineral P solubility (Sharma *et al*., 2013).Baby *et al*. (2001) investigated variation in the population density of microbial community in the rhizosphere soil of tea clones and found a significant difference in PSB population level among different tea clones. These results are also in consistency with Alia *et al*. (2013) who observed that the soil factors (especially pH) played a major role in the determination of microbial potential and population density. Several studies have been reported on the isolation of PSB from the rhizosphere soil of different field crop (Chung *et al*., 2005; Reyes *et al*., 2006). Reyes *et al*. (2006) had also found significant differences in PSB population between plant species grown in the same soil type, suggesting that the differences found in the occurrence of PSB might be related to difference in root exudates. These finding are also in line with the report of Pal, (1998) who isolated PSB from the soil samples of forest, pasture, waste land, agricultural and horticultural land and observed potential PSB as varying between 32-60×10^3 CFU g^{-1}soil.We have also shown that all the PSB isolated strains were having different morphological and phenotypic characteristics. The colour, margin, shape and opacity of PSB isolated from the low, medium and high T° zones were nearly similar. De Vos *et al*. (2009) stated that the phenotypic characterizations include morphological, physiological and biochemical properties of the microorganism. Heritage *et al*. (1996) and Rodrı´guez-Dı´az *et al*. (2008) also found that the cell wall composition and the gram reaction is still a valuable diagnostic character for PSB.

Similarly, during this study we recorded that the maize, sorghum and vegetable of irrigated and rainfed areas at Peshawar and D.I. Khan were dominant in AMF spores of *Glomeraceae* family. It might be the presence of nutrients and predominately clay soils as compared to sandy and silty soils. Moreover, some of the AMF spores in the family *Gigasporaceae* were also observed in sand and silty soils (Yassen *et al*., 2013) isolated from pastures of Chitral, Peshawar and D.I. Khan regions of Khyber Pakhtunkhwa province, Pakistan.This information is also in agreement with Lekberg *et al*. (2007) who found that the AM fungi in the family *Glomeraceae* occurred predominantly in clay soils, whereas AM fungi in the family *Gigasporaceae* dominated in sand soils. In our findings, we have demonstrated the different shapes of AMF spores as globose, sub-globose and cylindrical, while the prominent color of the spores wall were yellowish brown, light brown, bluish and yellow to bluish brown with single and double layers. These results showed similarity with the work of Akond *et al*. (2008) who studied thevarious types of VAM spores with colours like dark, reddish, brown and yellowish with various shapes such as oval, spherical and irregular were found in the rhizosphere of vegetable plants species. It is well known that AM fungi are distributed worldwide, therefore the agricultural management practices and environmental conditions might affect AM fungal communities both qualitatively and quantitatively (Miller *et al*., 1995). Soil tillage practices, crop rotation and fertilization also affects the spore density, extent of mycorrhization, diversity and composition of AM fungi in several terrestrial plants roots of both the tropical and temperate agroecosystems (Jansa *et al*., 2002; Oehl *et al*., 2003). These results were supported by Frank *et al*. (2004) who determined that the formation ofsporesby different AM fungi may not always depend on the abundance and presence of species in the spore community but it may also be the reason of biotic and abiotic conditions. Mohan *et al*. (2005) demonstrated that the different ecological and environmental factors affect the presence and abundance of AMF in the soils and its natural occurrence is closely related tosoil structure, presence of the host plants and favourable environmental conditions.

In this study, we screened potential PSB strains for P solubility from RP qualitatively and quantitatively. All the strains were capable to solubilize P from RP in both the agar and broth medium. Among the 9 strains, the 3 types of most efficient P solubilizing bacterial strains (*Coccus* DIM7, *Streptococcus* PIM6 and *Bacillus* sp. PIS7) were found best in both the assays. These strains efficiently enhanced P solubility from hardly soluble RP and decreased the pH of the medium (Kim *et al*., 1997; Rashid *et al*., 2004). Afzal *et al*. (2010) also found that the *Pseudomonas* strain (54RB) have a P solubilization index of 4.1. According to Farzana *et al*. (2009) 6 out of 15 rhizobacterial isolates formed clear zone around colonies and efficiently solubilized calcium phosphate. Chauhan *et al*. (2013) and Allu *et al*. (2014) found the endophytic bacteria of the genus *Pseudomonas* and *Burkholderia* produce some phytohormones in the soil and thus improve P solubility and plant growth. In the broth culture study, we found an inverse relation between pH and soluble P concentration. The PSB isolates efficiently dropped the pH of the medium and thus increased RP solubility. It may be due to the production of organic acids by these strains that lowers the pH of medium. This inverse relationship between pH and soluble phosphate was also reported earlier by Rashid *et al*. (2004). In another study, Nautiyala *et al*. (1999) reported that the strains isolated from alkaline soil have the potential to solubilize P at the presence of highpH,saltand T°. Alia *et al*. (2013) also found that among the different PSB strains isolated from 3 different localities, SAFA-2 showed higher potential to solubilize P (151 μgmL^{-1}) when pH was dropped from 7.02 to 3.55 (Samiran *et al*., 2010). These results were well supported by Chen *et al*.(2006) who demonstrated that the*Bacillus megaterium* (CC BC 30) and *Rhodococcus erythropolis* (CC BC17) solubilized 140.6 µg P mL^{-1} (pH 4.01) and 151.2µg P mL^{-1} (pH 3.6) P in the broth medium.

Conclusion

It is concluded from the results of the present study that PSB and AMF show a broad range of diversity and variations in rhizosphere soils of field crops, vegetables and pastures of low, medium and high temperature zones (Chitral, Peshawar and Dera Ismail Khan) of Khyber Pakhtunkhwa province of Pakistan. Population density of PSB in areas under investigations were mostly *Coccus*,

Streptococcus and *Bacillus* while the soil spores of AMF were mostly *Glomus mosseae* and *Glomus intradices*. During qualitative and quantitative screening of PSB strains three (*Coccus* DIM7, *Streptococcus* PIM6 and *Bacillus* sp. PIS7) were found best for P solubility from RP. It showed that the isolated PSB strains have the capability to solubilize P from RP and have the potential to be used as bio-fertilizers. Further research is suggested to isolate more efficient AMF and PSB species from soils of different agro ecological zones of Pakistan and to investigate their effects on crop production.

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