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PHOSPHATE SOLUBILIZING BACTERIA ASSOCIATED WITH VEGETABLES ROOTS IN DIFFERENT ECOLOGIES

ALIA[1], AFTAB AFZAL[1]* SHAHIDA N. KHOKHAR[3], BUSHRA JABEEN[2], AND SAEED A. ASAD[4]

[1]*Department of Botany Hazara University, Mansehra, Pakistan*
[2]*Department of Biosciences University of Wah, Wah Cantt. Pakistan*
[3]*LRRI, NARC, Islamabad, Pakistan*
[4]*Department of Environmental Sciences, COMSATS Institute of Information Technology, Abbottabad, Pakistan*
*****Corresponding author's e-mail: aftabafzalkiani@yahoo.com*

Abstract

Forty two isolates were recovered as phosphorus solubilizing bacteria (PSB) from rhizosphere of healthy plants of pea, spinach, lady's finger, French bean, kulfa, cauliflower, turnip, brassica, cucumber, coriander, onion, potato, capsicum, salad, eggplant and field mint from 25 locations in Mansehra district, Taxila area and Islamabad. PSB population ranged from $1.95x10^7$ in lady's finger to $5.33x10^9$ in turnip in Mansehra area. It ranged from $1.9x10^6$ in spinach to $1.3x10^9$ in field mint in Taxila area while in Islamabad up to $8x10^5$ in spinach. Highest Solublization Index (4.25) was found in one isolate from spinach in Mansehra while 10 isolates from 10 vegetables had PSB of Solublization Index in the range of 3.5-4.4 from Taxila area. Solubilization capacity ranged from 5.32-151 μgmL^{-1}. Among the tested isolates, SAFA-2 was found as the best in solubilizing phosphate 151 μgmL^{-1} with the drop in pH from 7.02-3.55. Population in Taxila area was found negatively correlated with clay, phosphorus and organic matter while, positively correlated with soil pH, EC and soil nitrate. The results indicated that soils of the study area inhabit PSB with great potential to be used as bio inoculants.

Introduction

Phosphorus (P) is the second most important plant nutrient after nitrogen (Donahue *et al.*, 1990). Its accessibility is low in soils because of P fixation as insoluble phosphates of iron, Aluminum and Calcium. Deficiency of P is the most important chemical factor restricting plant growth and chemical phosphatic fertilizers are extensively used to get optimum yields. Soluble forms of P fertilizer are easily precipitated as insoluble forms leading to extreme and repeated applications of P fertilizers to cropland.

Phosphorus supply through biological means is a viable substitute as phosphate solubilizing bacteria (PSB), phosphate solubilizing fungi (PSF) and actinomycetes have been reported to be active in conversion of insoluble phosphate to soluble primary and secondary orthophosphate ions (Chabot *et al.,* 1993). Soil microorganisms have great potential in providing soil phosphates for plant growth. Phosphorus biofertilizers can help to increase the accessibility of accumulated phosphates for plant growth by solubilization (Goldstein, 1986; Gyaneshwar *et al.,* 2002). Moreover, microorganisms involved in P solubilization as well as better scavenging of soluble P can enhance plant growth through biological nitrogen fixation (BNF), enhancing the availability of other essential elements and by production of plant growth promoting substances (Gyaneshwar *et al*., 2002). Apart from playing an important role in biogeochemical cycles these soil bacteria have been used for decades in crop production. Due to their colonization in the rhizosphere and active role in promoting plant growth these bacteria are also termed as plant growth promoting rhizobacteria (PGPR) (Hayat *et al*., 2010, Kang *et al*., 2012).

Application of phosphorus along with PSB improved P uptake by plants and yield representing that PSB solubilize phosphates and activate phosphorus in crop plants (Rogers, 1993). In this respect, biofertilization technology has substantially minimized the production costs and evades the environmental hazards at the same time (Galal *et al.*, 2001).

Phosphorus application and bacterial inoculation affected yield of soybean through their effects on phosphorus use effectiveness (Shah, 2001, Khan *et al*., 2011). P-solubilizing *Rhizobium leguminosarum* has been shown to increase the growth of maize and lettuce (Chabot *et al*., 1996). Tandon, (1987) observed that in 10 out of 37 experiments PSB inoculations resulted in 10-15% increases in crop yields. Chabot *et al.,* (1993) found that PSM constituted 26-46% of microbial population of four Quebec soils studied. Khalil (1995) investigated 10 bacteria and 3 fungi being able to solubilize phosphate on the basis of large clear zone on solid media.

To our knowledge, no attempts have been made so far to isolate and characterize the PSB to be used as potential future inoculants for vegetables in Pakistan. Present investigation was designed to study the population density of PSB and find out the potential isolates for future inoculants on the basis of P Solubilization capacity and its relationship with soil characters of different ecologies. Current study would loop out further avenues for researchers interested to commercially produce the PSB based biofertilizers to be effective over a wide range of crops.

Materials and Methods

Soil sample collection: Rhizosphere soil samples were collected from different field crops of Islamabad, Taxila and Mansehra, and brought to lab in polythene bags. Loose soil was removed; roots were separated and placed in 250ml flask containing 100ml distilled water. The flasks were placed on shaker for one hour to prepare rhizosphere soil suspensions. Soil samples were collected at a depth of 0-15cm and kept at room temperature until further analysis was carried out.

Isolation of PSB: PSB were isolated from each sample using dilution plate counting method. Ten folds serial dilutions were prepared from rhizosphere soil suspension. One over ten mL of each dilution was spread on

Pikovskaya's agar medium by using the method of Pikovskaya (1948) containing insoluble Tricalcium phosphate and incubated at 27-30^{o}C for 7 days. Colonies showing halo zones were picked and purified by subculturing on Pikovskaya's (PVK) agar medium for studying the characters of isolates.

Colony morphology: Suspension in sterile water was prepared from each of the purified culture and grown on solid media by spread plate method. The inoculated plates were incubated at 25^{o}C until colonies appeared. Colony morphological characters recorded were color, margins, Colony shape, and elevation as Goenadi *et al.,* (2000).

Microscopic characters: Slides of purified bacterial isolates were prepared for Gram staining reactions. Morphology and Gram staining reaction of isolates were observed under light microscope. Pink colored bacteria were Gram-ve while purple colored were Gram+ ve.

Solubilization index (SI). One over ten mL of each PSB culture preserved in sterile distilled water was placed on Pikovskaya's agar plates containing insoluble tricalcium phosphate 2.5 g, glucose 13g, $(NH_4)_2SO_4$ 0.5 g, NaCl 0.2 g, $MgSO_4.7H_2O$ 0.1 g, KCl 0.2g, Yeast Extract 0.5 g, $MnSO_4$ trace, $FeSO_4.7H_2O$ trace, Agar 15 g, pH adjusted to 7.2 and dissolved in 1000 ml distilled water].culture containing plates were incubated at 28^{o}C for seven days. Solubilization Index was measured using following formula (Edi-Premono *et al.,* 1996).

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

Quantification of phosphorus solubilized by PSB: Phospho-molybdate blue colour method (Murphy & Riley, 1962) was used for determination of available phosphorus. Four to five loops of bacterial culture were inoculated in 100 mL of Pikovskaya s' broth culture with pH adjusted to 7.0 in 250mL flasks and incubated for 7 days on rotary shaker (200 rpm, 24ºC). After incubation, the cultures were centrifuged at 10000 rpm for 15 minutes to remove bacterial cells and other insoluble materials. The supernatant of each sample was decanted, filtered and its pH was recorded.

Biochemical tests of bacterial isolates: Biochemical characters were recorded using API 20 kit (biomerieux, USA). Liquid cultures of isolates were added to the wells of kit following the instructions supplied by the company. The results were verified as prescribed in Bergey's Manual of Determinative Bacteriology (Miwa *et al.,* 2009).

Disc diffusion method (Baeur Kirbyear): The inoculum was prepared by growing the microbe in Pikovskaya's liquid medium. Isolates from the pure and maintained cultures were inoculated and incubated at 28^{o}C till to obtain a population of 10^7mL^{-1} of the culture (Murray *et al*., 2003). One mL of broth culture was inoculated with fine loop on pikov's agar plate and spread. The plates were inverted and incubated at 35^{o}C for 18-24 hours. Streptomycin antibiotic was used in experiments at different concentrations i.e., 500 µgmL^{-1}, 250 µgmL^{-1}, 100 µgmL-1and 50 µgmL^{-1}.

Preparation of soil samples for soil analysis: The collected soil samples were sieved from 2mm mesh size to remove the stones, plant residue and small organisms (earthworms etc.). Then soil samples were air dried, grinded, thoroughly mixed and stored below 4^{o}C until further analysis.

Physio-Chemical analysis of collected soil samples: Soil texture analysis was done by using John *et al.*, (2001) method. All soil samples were analyzed for their pH and ECe by using 1:1(w/v) with the help of method described by McLean (1982) & Rhoades (1982) respectively. The organic matter of all the soil samples was determined by Walkley-Blake method (1934).

AB-DTPA extraction: AB-DTPA method (Soltanpour & Workman, 1979) was used for the determination of macronutrients and micronutrients. Ten grams of soil sample was weighed into 125 mL conical flask. Then 20ml of extract (0.005M DTPA+1.0M NH_4HCO_3) and shaken on reciprocating shaker for 15 minute 180 cycles/min. Extract was obtained by filtering through Whatman No.42 filter paper. This filtrate was used for the determination of various nutrients as described below:

Nitrate-N: One ml of soil extract was transferred to 25mL test tube and 3.0 mL Copper sulfate working solution, 2mL hydrazine sulfate working solution, and 3mL Sodium hydroxide working solution was added. Then it was mixed and heated in water bath at 38°C for 20 minutes. After thorough mixing 3mL of color developing reagent were added to it and kept at room temperature for 20 min. The absorbance was recorded at 540nm on a spectrophotometer (UV Spectronic Genesys 5).

Available phosphorus: A 1.0 mL aliquot of the soil extract was diluted to 10 mL by adding deionized water and 2.5 mL of color developing reagent was added. After 30 min color intensity was measured at 880 nm on a spectrophotometer as detailed in the method used by Olsen & Sommers (1982).

Extractable potassium and sodium: Potassium and Sodium was determined directly from the filtrate by using a flame photometer at 404 nm wavelengths. Standard solutions were made in the extraction with KCl.

Micronutrients: Micronutrients were determined directly from the filtrate by using atomic absorption spectrophotometer (Analyast 700).

Fe, Zn, Cu (ppm) = Fe, Zn, Cu (ppm in extract) x dilution factor (2)

Results and Discussion

Phosphate solubilizing bacteria were isolated from 22 vegetables rhizospheric soil of Mansehra, Islamabad and Taxila. Estimation of population was made from different rhizospheric soil and the isolates studied for their characters of agricultural significance with main focus on their phosphate solubilizing ability/capacity. Population density: Population density of PSB in different rhizosphere soils from vegetable fields of studied area (Tables 1&2) ranged from ($8x10^5$) to ($5.33x10^9$). It was found to be highest in the rhizosphere soil of vegetable i.e., *Bassica rapa* ($5.33x10^9$), *Solanum tuberasum*(2) ($2.31x10^9$) in Mansehra soil,(Table 1) field mint (1.34×10^9), followed by salad (1.45×10^8) and cauliflower (1.20×10^8) in Taxila soil (Table 2) and least in the rhizosphere soil of spinach (8×10^5) in Islamabad soil (Table 1). This variation in the population of phosphobacteria in different ecologies might be attributed to many soil factors such as soil nutrient status, soil pH, moisture content, organic matter and soil enzyme activities.

Table 1. Population density of PSB in rhizosphere soil of vegetables (Mansehra, Islamabad).

S.No.	Local name	Botanical name	Population density $CFUg^{-1}$ of soil
1.	Turnip(1)	*Brassica rapa(1)*	$2.16x10^8$
2.	Turnip(2)	*Brassica rapa(2)*	$1.51x10^8$
3.	Turnip(3)	*Brassica rapa3)*	$1.51x10^8$
4.	Garlic(2)	*Allium sativum(2)*	$8.02\ x10^8$
5.	Coriandar(1)	*Coriandrum sativum(1)*	$3.63\ x10^8$
6.	Coriandar(2)	*Coriandrum sativum(2)*	$8.63x10^7$
7.	Coriandar(3)	*Coriandrum sativum(3)*	$1.7\ x10^8$
8.	Spinich(2)	*Spinacia oleracea(2)*	$3.15\ x10^8$
9.	Spinich(3)	*Spinacia oleracea(3)*	$5.83x10^6$
10.	Karam(1)	*Brassica hybrid(1)*	$3.00\ x10^6$
11.	Karam(2)	*Brassica hybrid (2)*	$6.33\ x10^8$
12.	karam(3)	*Brassica hybrid (3)*	$1.08\ x10^8$
13.	French bean	*Phaseolus vulgaris*	$7.98\ x10^8$
14.	Tomato	*Lycopersicon esculentum*	$2.96\ x10^8$
15.	Lady's finger	*Abelomoschus esculentus*	$1.67\ x10^7$
16.	Spinach	*Spinacia oleracea*	$8\ x10^{5*}$
17.	Cauliflower	*Brassica aleracea*	$6.58\ x10^6$
18.	Malva	*Malva neglagta*	$2.25\ x10^6$
19.	Brassica	*Brassica Compestris*	$2\ x10^8$
20.	Jameia	*Eruca sativa*	$2.14\ x10^8$
21.	Turnip	*Bassica rapa*	$5.33x10^{9**}$
22.	Potato (2)	*Solanum tuberosum(2)*	$2.31x10^9$
23.	Pea(ich)	*Pisum sativum*	$1.74\ x10^8$
24.	Potato(ich)	*Solanum tuberosum*	$1.8\ x10^8$
25.	onion(3)	*Allium cepa(3)*	$7.5\ x10^8$
26.	Methi	*Trigonella foenum-graecum*	$1.33\ x10^7$
27.	Garlic1	*Allium sativum(1)*	$6.288\ x10^8$
28.	Garlic3	*Allium sativum(3)*	$3.258\ x10^8$
29.	Spinach(1)	*Spinacia oleracea(1)*	$2.188\ x10^8$
30.	onion(1)	*Allium cepa(1)*	$3.358\ x10^8$
31.	onion(2)	*Allium cepa(2)*	$2.48\ x10^8$
32.	Kulfa	*Portulaca oleracea*	$7.98x10^8$

**= Highest value *= Lowest value

Table 2. Population density of PSB in rhizosphere soil of vegetables (Taxila).

S.No.	Local/English name	Botanical name	Population density CFUg^{-1} of soil
1.	Spinach	*Spinacia oleracea*	$1.90\times10^{6*}$
2.	Salad	*Lactuca sativa*	1.54×10^{8}
3.	Field mint	*Mentha arvensis*	$1.34\times10^{9**}$
4.	Cucumber	*Cucumis sativus*	1.60×10^{7}
5.	Cauliflower	*Brassica oleracea*	1.20×10^{8}
6.	Egg plant	*Solnum melongena*	9.90×10^{7}
7.	Potato	*Solanum tuberosum*	9.5×10^{7}
8.	Coriander	*Coriandrum sativum*	1.02×10^{8}
9.	Pepper	*Capsicum annuum*	9.1×10^{7}
10.	Onion	*Allium cepa*	8.3×10^{7}
11.	Spinach	*Spinacia oleracea*	$1.90\times10^{6*}$
12.	Salad	*Lactuca sativa*	1.54×10^{8}
13.	Field mint	*Mentha arvensis*	$1.34\times10^{9**}$
14.	Cucumber	*Cucumis sativus*	1.60×10^{7}
15.	Cauliflower	*Brassica oleracea*	1.20×10^{8}
16.	Egg plant	*Solnum melongena*	9.90×10^{7}
17.	Potato	*Solanum tuberosum*	9.5×10^{7}
18.	Coriander	*Coriandrum sativum*	1.02×10^{8}
19.	Pepper	*Capsicum annuum*	9.1×10^{7}
20.	Onion	*Allium cepa*	8.3×10^{7}
21.	Spinach	*Spinacia oleracea*	$1.90\times10^{6*}$
22.	Salad	*Lactuca sativa*	1.54×10^{8}
23.	Field mint	*Mentha arvensis*	$1.34\times10^{9**}$
24.	Cucumber	*Cucumis sativus*	1.60×10^{7}
25.	Cauliflower	*Brassica oleracea*	1.20×10^{8}
26.	Egg plant	*Solnum melongena*	9.90×10^{7}
27.	Potato	*Solanum tuberosum*	9.5×10^{7}
28.	Coriander	*Coriandrum sativum*	1.02×10^{8}
29.	Pepper	*Capsicum annuum*	9.1×10^{7}
30.	Onion	*Allium cepa*	8.3×10^{7}
31.	Spinach	*Spinacia oleracea*	$1.90\times10^{6*}$
32.	Salad	*Lactuca sativa*	1.54×10^{8}
33.	Field mint	*Mentha arvensis*	$1.34\times10^{9**}$
34.	Cucumber	*Cucumis sativus*	1.60×10^{7}
35.	Cauliflower	*Brassica oleracea*	1.20×10^{8}
36.	Egg plant	*Solnum melongena*	9.90×10^{7}
37.	Potato	*Solanum tuberosum*	9.5×10^{7}
38.	Coriander	*Coriandrum sativum*	1.02×10^{8}
39.	Pepper	*Capsicum annuum*	9.1×10^{7}
40.	Onion	*Allium cepa*	8.3×10^{7}

**= Highest value *= Lowest value

Solubilization and quantification of phosphorus

Solubilization index (SI) and associated characters: Isolation of PSB was made on Pikov's agar. Thirty two bacterial isolates were inoculated on Pikovskaya's agar plates to evaluate solubilization index. Solubilization index (SI) of PSB isolates ranged from 1.8 to 5.0 in the present work (Tables 3&4). Similar observation has been reported by several workers (Kim *et al.,* 1998; Kumar & Narula, 1999; Guar, 1990; Rashid *et al*., 2004). In another research *Pseudomonas* strain (54RB) exhibited a phosphorus solubilization index of 4.1 (Afzal *et al.,* 2010). The isolate CUFA-4 (From Taxila Table 4) showed highest SI i.e., 5.0 followed by isolate PPM (ich) 4.6 (Table 3), while the other isolate EGFB-1 and TBM1 (Table 3) showed the lowest of SI 1.8. According to Farzana *et al.,* (2009)**,** Six out of 15 rhizobacterial isolates were able to form clear zone around colonies, an indication of calcium phosphate Solubilization. Highest clear zone of 2.03 was observed in *Erwinia cypripedii* UPMSP10 and smallest SI was formed by *Pseudomonas fuscovaginae* UPMSP20 i.e. 0.86. The SI in present work is greater than this finding. The present data reflect that highly efficient P-solubilizers inhabit the rhizosphere soil of vegetable crops in Taxila followed by Mansehra. This may be attributed to soil factors especially pH which is acidic to neutral in Mansehra and neutral to basic in Taxila and soil P concentration which is higher in Mansehra and as compared to Taxila (Data not presented).

Quantification of phosphorus solubilized in liquid culture and change in pH: The phosphate solubilizing ability of PSB isolate as estimated in liquid medium indicated that all of the 32 isolates were able to solubilize tricalcium phosphate effectively. The overall amount of P, solubilized by these isolates ranged from 5.32-151ugmL^{-1}(Table 3&4). Among thirty two isolates, SAFA-2 (From Taxila) was found with maximum ability to solubilize phosphate (151 µg mL^{-1}) with the drop in pH from 7.02-3.55 (Table 4). This finding is well supported by the Chen *et al.,* (2006), where the strains *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) phosphorus in broth medium. According to Samiran *et al.,* (2010) *Bacillus sp.* TRSB16 consistently showed high rates of solubilization of Ca_3 $(PO_4)_2$ (144 µgmL^{-1}), relatively low solubilization of Ca_3 $(PO_4)_2$ (71 µgmL^{-1}) was observed in case of *Arthrobacter sp* TRSB10.

Table 3. Amount of phosphorus solubilized (µgmL^{-1}) on solid and liquid media and pH change by PSB isolates from Mansehra area.

S.No.	Isolate	Solubilization index (SI)	P-Solubilization (µgmL^{-1})	pH change from neutral
1.	KMP	2.2	5.32*	6.1*
2.	PSM2	2.3	48.63	5.9
3.	SPI	2.3	17.73	5.4
4.	LAM	2.2	45.72	5.6
5.	FPM	2.9	43.83	5.4
6.	GAM1	2.4	61.82	4.7
7.	GAM2	2.6	69.36	5.2
8.	TBM1	1.8*	65.03	4.8
9.	TBM3	2.4	65.01	4.9
10.	SS1	2.6	54.22	5
11.	SS2	2.7	55.66	5.1
12.	KBM1	4.1	61.52	5.7
13.	CCM2	2.8	63.33	5.5
14.	PPM(M)	4.6**	60.67	5.5
15.	CCM3	2.4	62.89	5.7
16.	SSM3	3.2	79.97	5.4
17.	TBM2	2.5	77.90	5.3
18.	KBM3	3.7	85.06**	5.6
19.	CCM1	3.4	83.81	5.6
20.	CBM	3.8	82.95	5.6
21.	PSM(ich)	2.9	79.95	4.6**

**= Highest value*= Lowest value and in case of pH it is maximum decrease and minimum decrease respectively

Table 4. Amount of phosphorus solubilized (µgmL^{-1}) on solid and liquid medium and pH change by PSB isolates from Taxila area.

Isolates	Solubilization index (SI)	P. Solubilization	Change in pH from neutral
SPFA-1	2.25	140	4.11
SAFA-2	2.31	151 **	3.55**
FMFA-3	2.22	148	3.72
CUFA-4	5.00**	148	3.65
CFFA-5	2.21*	144	3.84
EGFB-1	2.22	100	4.01
POFB-2	2.38	130	3.74
COFB-3	2.33	144	3.83
CAFB-4	2.5	90	4.26
ONFB-5	2.3	77 *	4.43*

**= Highest value *= Lowest value and in case of pH it is maximum decrease and minimum decrease respectively

Physiological and biochemical studies of PSB isolates: As shown in Table 6 isolates exhibited different colours with entire and lobate margins. They varied in gram staining properties. Cell shape varied from long to short rods from isolate to isolate.

Intrinsic antibiotic resistance (IAR) of selected PSB isolates: IAR is used to mark the genetically controlled isolates. Isolate CUFA-4 showed sensitivity against streptomycin at concentration of 500µgmL^{-1}and 250µgmL^{-1} but, was resistant at concentration of 100µgmL^{-1}and 50µgmL-1 (Table 5). This observation is supported by the findings of Samina *et al.,* (2006a). They reported that antibiotic resistance pattern of *Azospirillium lipoferum* N7 and *Azospirillium brasilense* N8 were resistant to gentamycin and streptomycin up to 100µgmL^{-1} and 50µg mL^{-1}.

Strains COFB-3, CAFB-4, and ONFB-5 were sensitive against streptomycin at concentration of 500 µgmL^{-1}and 250 µgmL^{-1} (Table 5). But the strains COFB-3 and ONFB-5 were sensitive at concentration of 100µgmL^{-1}and resistant at 50 µg mL^{-1}. At high concentration of antibiotic, larger zones were produced and vice versa. This data could help to mark the isolates and identify the inoculated isolates while retrieving back from the soil.

Properties of rhizosphere soil of vegetables: Soil Texture of Mansehra varied from Silt clay loam to silt loam (Data not presented). Electrical conductivity was normal and pH was recorded to be 7.0. The organic matter, potassium and amount of phosphorus was high a characteristic of fertile soil. Texture of Taxila soil varied from silt loam to loam. It had medium fertility level with no indication of salinity. Electrical conductivity was normal and pH neutral with slightly alkaline range. The organic matter and potassium were high and amount of phosphorus was also enough to predict a fertile soil. These results were in agreement with the findings of Samina *et al.,* (2006b). The soil was however deficient in nitrate nitrogen. Micronutrients analysis showed that amount of copper and iron was marginal whilst, zinc was present in high amount.

Correlation between different *in vitro* studied parameters: The PSB population of Taxila isolates was negatively correlated with clay, phosphorus and organic matter and positively correlated with soil pH, electrical conductivity and soil nitrate (Fig. 2). The pH and solubilization was negatively correlated (r= -0.302). The PSB population of Mansehra isolates was negatively correlated with clay, phosphorus and organic matter soil pH, electrical conductivity and soil nitrate (Fig. 1). Clay was strongly correlated with PSB as compared to phosphorus. Positive correlation(r=0.225) between solubilization index and P solubilized, Similar correlation was also reported by Kumar & Narula (1999) & Sadia *et al.,* (2002).

Negative correlation (r= -0.862) between pH and phosphorus solubilization in liquid culture was found in the present study. Similar observations were reported by Maliha *et al.,* (2004), where a significantly higher correlation (r = -0.60) between pH and solubilization index was found, and correlation between pH and p. solubilization in liquid (-0.44). Sadia *et al.,* (2002) reported positive correlation between p. solubilized and SI (0.553) and negative between pH and SI (-0.79) well supporting the result of present study.

Table 5. Intrinsic antibiotic resistance patterns of isolates of Taxila (S=Sensetive, R=Resistant).

Isolates	Streptomycin 500 µgmL^{-1}	Streptomycin 250 µgmL^{-1}	Streptomycin 100 µgmL^{-1}	Streptomycin 50 µgmL^{-1}
CUFA-4	S	S	R	R
COFB-3	S	S	S	R
CAFB-4	S	S	R	R
ONFB-5	S	S	S	SR

Table 6. Cultural characters and cell morphology of isolates (Mansehra, Taxila and Islamabad).

S.No.	Isolates	Cultural characteristics			Microscopic Characteristics	
		Colony color	Colony shape	Form/Margin	Gram staining	Shape of bacteria
1.	BCM	Yellow	Circular	Entire	(-ive)	Spherical/Cocci
2.	CAFB-4	Yellow	Circular	Entire	(+ive)	Small rods chained/cocci
3.	CBM	Off white	Oval	Entire	(-ive)	Spherical/Cocci
4.	CCM(1)	Off white	Circular	Entire	(-ive)	Rigid/ Spirillum
5.	CCM(2)	Off white	Circular	Entire	(+ive)	Rigid/ Spirillum
6.	CCM(3)	Yellow	Oval	Lobate	(+ive)	Rod shape/ Bacillus
7.	CFFA-5	Off white	Circular	Entire	(-ive)	Long rods, curved
8.	COFB-3	Off white	Oval	Lobate	(-ive)	Shorts to long rods /cocci
9.	CFUA-4	Yellow	Oval	Entire	(+ive)	Small chained rods
10.	EGFB-1	Off white	Oval	Entire	(+ive)	Small to long chained rods
11.	FMFA-3	White	Oval	Entire	(-ive)	Small rods/ cocci
12.	FPM	Yellow	Circular	Entire	(+ive)	Cylindrical/ Bacillus
13.	GAM(2)	Yellow	Circular	Lobate	(-ive)	Rod shape/ Bacillus
14.	GAM(3)	Yellow	Circular	Entire	(-ive)	Rod shape/ Bacillus
15.	JBM	Yellow	Oval	Entire	(+ive)	Rod shape/ Bacillus
16.	KBM(1)	Off White	Oval	Lobate	(-ive)	Helical/Spirochete
17.	KBM(2)	Yellow	Oval	Lobate	(-ive)	Rod shape/ Bacillus
18.	KBM(3)	Yellow	Oval	Lobate	(+ive)	Rod shape/ Bacillus
19.	LAM	Yellow	Circular	Entire	(-ive)	Curvedshape/vibrio
20.	MNM	Off white	Circular	Entire	(-ive)	Rod shape/ Bacillus
21.	MTM	Off white	Circular	Entire	(-ive)	Rod shape/ Bacillus
22.	OAM(1)	Yellow	Circular	Entire	(-ive)	Rod shape/ Bacillus
23.	OAM(1)	Yellow	Circular	Entire	(-ive)	Long oval /chained
24.	OAM(2)	Yellow	Circular	Entire	(-ive)	Long oval /chained
25.	OAM(3)	Yellow	Circular	Entire	(-ive)	Long oval /chained
26.	ONFB-5	White	Circular	Entire	(-ive)	Long oval /chained
27.	POFB-2	Yellow	Oval	Lobate	(-ive)	Short to long rods/curved
28.	PPM(ich)	Yellow	Oval	Lobate	(-ive)	Helical/Spirochete
29.	PSM(2)	Off white	Oval	Lobate	(-ive)	Helical/Spirochete
30.	SAFA-2	Off white	Circular	Entire	(-ive)	Rod shape/chained
31.	SPFA-1	Yellow	Circular	Entire	(-ive)	Small / medium rod
32.	SSI	Yellow	Circular	Entire	(-ive)	Helical/Spirochete
33.	SSM(1)	Yellow	Circular	Entire	(-ive)	Cylindrical/ Bacillus
34.	SSM(2)	Yellow	Circular	Entire	(-ive)	Oval/ Cocci35
35.	SSM(3)	Off White	Oval	Lobate	(-ive)	Helical/Spirochete
36.	TBM	Off White	Oval	Lobate	(+ive)	Rod shape/ Bacillus
37.	TBM(1)	Off White	Convex	Entire	(+ive)	Rod shape/ Bacillus
38.	TBM(2)	Off White	Convex	Entire	(-ive)	Spherical/Cocci
39.	TBM(3)	Off White	Convex	Entire	(+ive)	Rod shape/ Bacillus
40.	TLM	Off White	Convex	Lobate	(-ive)	Spherical/ Cocci

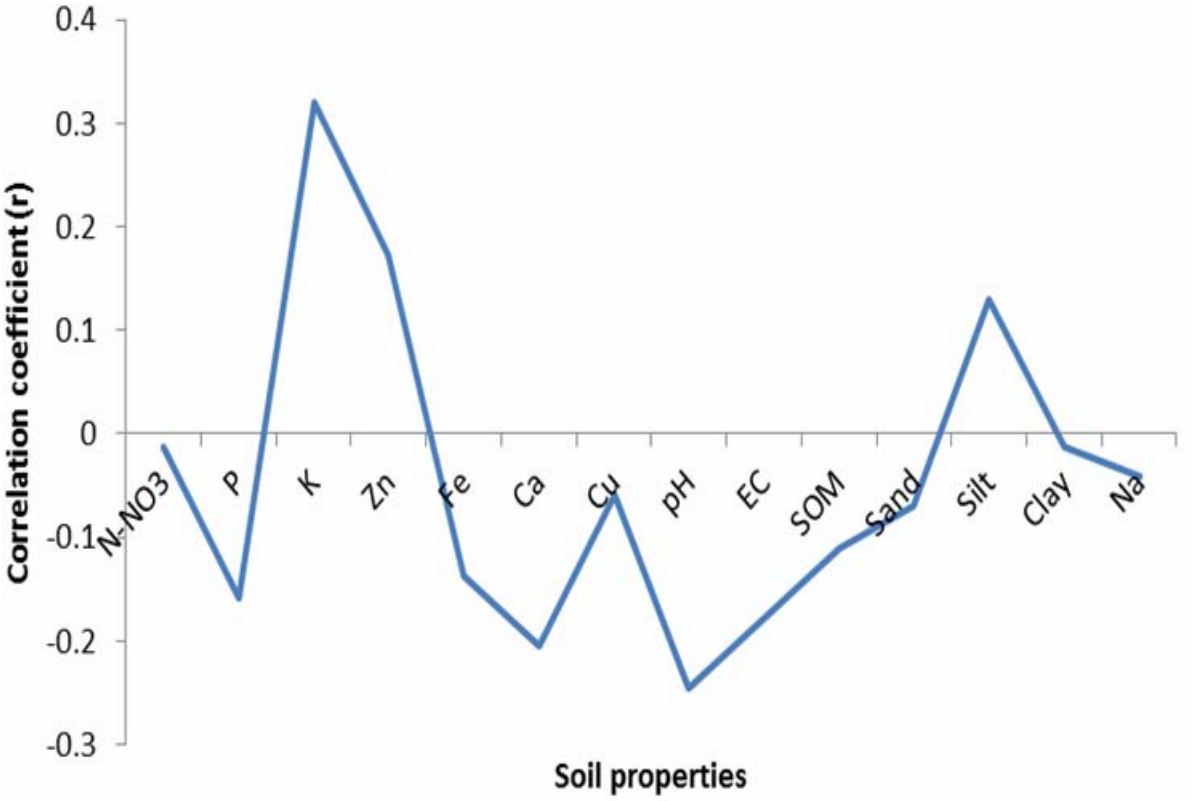

Fig. 1. Correlation between CFU and soil characteristics of Mansehra.

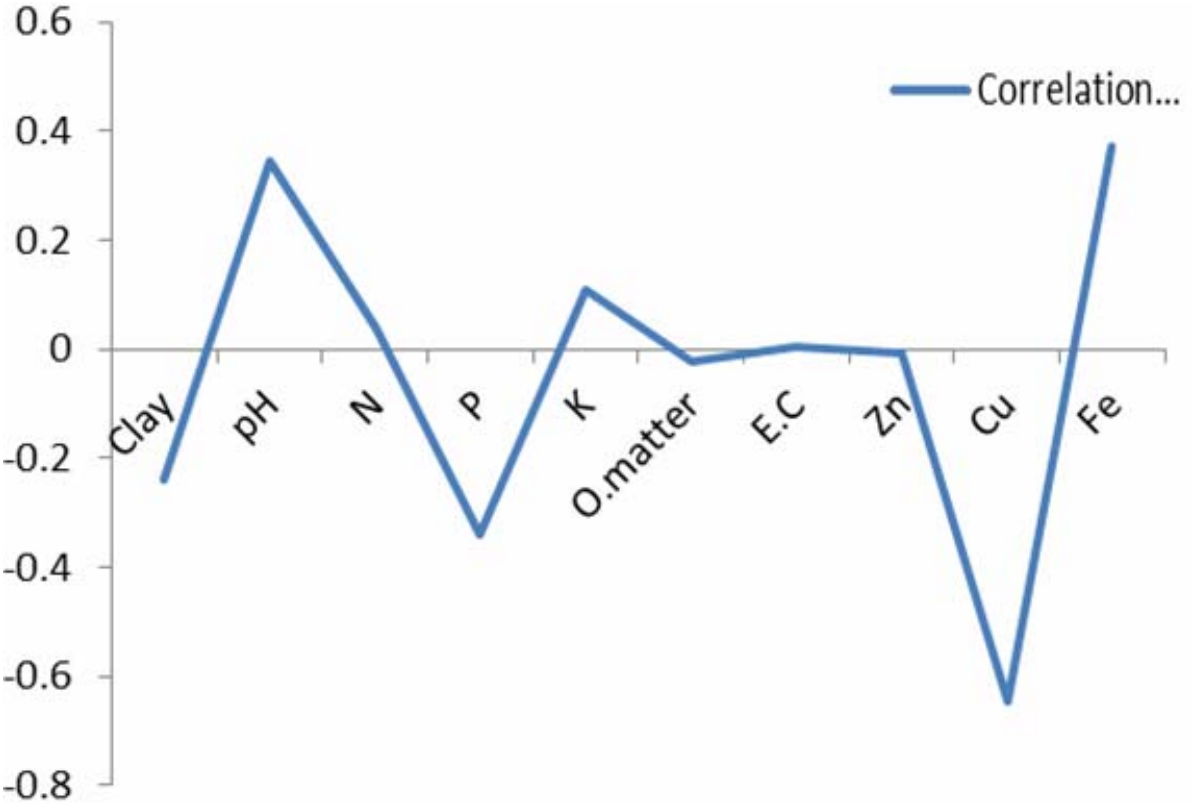

Fig. 2. Correlation between CFU and soil characteristics of Taxil

Conclusions

It was concluded from the present study that PSB exhibited a broad range of variations in soils collected from different areas. Area of Mansehra was found to be rich in population density of micro flora while more potential PSB strains were found in Taxila. Soil factors (especially pH) played major role in determination of microbial potential and population density. Current study also indicated that among all strains isolated from three different localities, SAFA-2 exhibited maximum potential to solubilize phosphate (151 μgml^{-1}) when pH was dropped from 7.02-3.55. Further research should be continued with such efficient PSB isolates. These may be used for inoculum production at commercial scale and their inoculation effect on the plant growth be studied.

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