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In-vitro solubilization of waste minerals and morphological characteristics of potassium and phosphorus solubilizing bacteria

Debabrata Nath, Bihari Ram Maurya and Sajiya Khan

Abstract
Soil potassium and phosphorus supplementation relies heavily on the use of chemical fertilizer, which has a considerable negative impact on the environment. There is a growing need to turn back to nature or sustainable agents that promote evergreen agriculture. Among such natural bio-agents, the potassium solubilizing bacteria (KSB), and phosphorus solubilizing bacteria (PSB) which solubilize fixed forms of potassium (K) and phosphorus (P) to plant available K and P vice versa by various mechanisms including acidolysis, chelation, exchange reactions, complexolysis, and production of organic acids are considered one such available viable alternative. In this research screening and characterization of ten KSB and PSB isolates which were evaluated for their ability to solubilize K and P from the waste muscovite (WM), waste biotite (WB), tri-calcium phosphate (TCP) and rock phosphate (RP) by analysing the soluble P and K content after 7, 14, 21 days after incubation (DAI) at 28±2 °C on MABs (Modified Aleksandrov Broths) and Pikovskaya's medium. The Zone of solubilization (cm), Colony size diameter (cm), Solubilization index of PSB40 at 7DAI and 14 DAI 2.83±0.01, 4.05±0.01, 1.26±0.01, 1.50±0.01, 2.61±0.02, 2.81±0.01 respectively. PSB40 showed superiority towards P solubilisation than rest of isolates. Overall, it can be concluded that the diversity of KSB and PSB as bioinoculants to release K and P provides a win-win situation under *in vitro* condition.

Keywords: biotite, muscovite, tricalcium phosphate, rock phosphate, pH dynamics, EC dynamics, potassium solubilization, phosphate solubilization

1. Introduction
Phosphorus (P) and potassium (K) are major essential macronutrients for plant growth and development. These elements play a key role in the synthesis of cells, enzymes, proteins, starch, cellulose, and vitamins. Moreover, K not only participates in nutrient transportation and uptake, but also confers resistance to abiotic and biotic stresses, leading to enhanced production of quality crops and provides resistance to plant diseases (Maqsood *et al*. 2013) [17]. Potassium in soil is present in water-soluble (solution K), exchangeable, non-exchangeable and structural or mineral forms. Potassium from water-soluble and exchangeable pools is directly available for plant uptake there are dynamic equilibrium and kinetic reactions between the different forms of soil K that affect the level of soil solution K at any particular time, and thus, the amount of readily available K for plants. Release of non-exchangeable K to the exchangeable form occurs when levels of exchangeable and solution K are decreased by crop removal and/or leaching and perhaps by large increases in microbial activity (Sparks 1987) [9]. A large portion of total P in the soil is usually present in insoluble forms, which are unavailable for plant uptake (Stevenson and Cole, 1999) [34]. However, a large portion of soluble inorganic phosphate applied to the soil as chemical fertilizer can be rapidly immobilized and become unavailable to plants (Goldstein, 1986) [11] and may result in environment pollution (Singh and Kapoor, 1994). Likewise Potassium is also added to soil in the form of potassic fertilizers. India ranks fourth after USA, China, and Brazil as far as the total consumption of K-fertilizers in the World is concerned (FAI 2007). However, there is no reserve of K-bearing minerals in India for production of commercial K-fertilizers and the whole consumption of K-fertilizers are imported in the form of muriate of potash (KCl) and sulphate of potash (K_2SO_4) which leads to a huge amount of foreign exchange. These necessitate to find an alternate indigenous source of K and P for plant needs and maintain K and P status in soils for sustaining crop production. In this respect, India is fortunate to have the world's largest deposit of mica mines distributed in Munger district of Bihar and Koderma and Giridih districts of Jharkhand and also Rock phosphate in Jhamarkotra in Rajasthan.

Waste mica contains significant amount of K (8-12% K2O) and high grade Rock phosphate contains 20-40% P_2O_5. These minerals can effectively be used as a source of potassium and phosphorus if modified or altered by some suitable chemical or biological means. Some microorganisms in the soil are able to solubilize 'unavailable' forms of K-bearing minerals, such as micas by excreting organic acids which either directly dissolves rock K or chelating silicon ions to bring the K into solution (Bennett *et al*. 1998; Barker *et al*. 1998) [3, 2]. These microorganisms are commonly known as potassium solubilizing bacteria (KSB) or potassium dissolving bacteria or silicate dissolving bacteria. It is also well known that a considerable number of phosphate solubilizing bacteria (PSB) have the ability to solubilize insoluble mineral P by producing various organic acids, siderophores, mineral acids, protons, humic substances, CO2 and H2S, and release soluble P (Cunningham and Kuiack, 1992 [6]; Illmer and Schinner, 1995 [13]; Ivanova *et al*. 2006 [14]; Song *et al*. 2008) [31]. Deficiency in plant-available phosphorus and Potassium is considered to be a major limiting factor to crop production in many Indian agricultural soils. Therefore, it is urgent to explore alternative ways to improve the status of P and K in soils, such as the utilization of biofertilizers. It was shown that KSB increased K availability in soils and increased mineral uptake by plant (Sheng *et al*. 2002, 2003) [25-27]. The use of phosphate and potassium solubilizing bacteria (PSB and KSB) as biofertilization, is a sustainable solution to improve of plant growth, plant nutrition, root growth pattern, plant competitiveness and responses to external stress factors as suggested by (Setiawati and Handayanto 2010 [24], Ekin 2010) [7] Extensive researches have been performed to isolate PSB from field soils and P-deficient soils and to test their phosphate solubilizing activity (PSA). Hence, keep this in mind the objectives of the study were 1) to see the potentiality of KSB in solubilization of K from waste biotite and muscovite. 2) To see the potentiality of PSB in solubilization of Tri-calcium phosphate and Rock phosphate. 3) To see whether KSB can solubilize P from K-minerals and PSB can solubilize K from P-minerals.

2. Materials and Methods

2.1 Minerals

K containing minerals such as waste muscovite and waste biotite were collected from mica mines located at Koderma district of Jharkhand, India. Tri-calcium phosphate from Hi-media of laboratory and low grade rock phosphate from Rajasthan. The waste mica has flake like structure. It was ground to powdered form in a Wiley mill and passed through 2 mm sieve (Meena *et al*. 2014) Chemical Composition of Muscovite and Biotite are enlisted in Table no 1

Table 1: Elemental composition of waste mica (muscovite and biotite).

Minerals	Silica% (SiO_2)	Iron% (Fe_2O_3)	Potash (%) K	Magnesium oxide% MgO	Sodium oxide% NaO	Manganese oxide% MnO	Phosphorus% P	Calcium oxide% CaO
Muscovite	45.10	2.54	9.82	0.61	0.37	Traces	0.022	0.23
Biotite	38.42	16.24	9.70	11.94	0.24	Traces	0.019	0.14

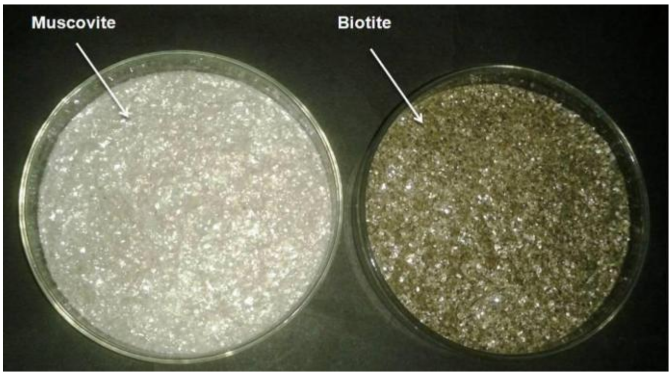

Fig 1: Grinded muscovite and biotite mineral samples

2.2 Bacterial Strain

Five isolates of Potassium Solubilizing Bacteria (KSB) namely KSB40, KSB17, KSB31, KSB94 and KSB105 as well as five isolates of phosphorous solubilizing bacteria (PSB) namely PSB16, PSB27, PSB30, PSB40 and PSB56 were obtained from Soil Microbiology Laboratory, Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, U.P (India)

2.3 Morphological characterization of KSB and PSB

The colony characteristics of all screened KSB and PSB strains growing on MAMs and MPMs were studied on the basis of colony size, cell shape, color and margin, size of the plaque and the level of slime production which was examined by touching the colonies with a sterilized inoculation needle and uplifted. Diameters of the zones of solubilization were measured after 7 and 14 days and expressed in centimeters. Solubilization index was calculated through Khande parkar's selection ratio (Ratio=Diameter of zone of clearance/Diameter of colony growth). Length of the thread formed upon testing for slime production was used to classify each isolate as a low, medium or high slime-producer (Zhang and Kong *et al*. 2014) [38].

2.4 Quantitative estimation of K released from waste muscovite/waste biotite

The KSB and PSB isolates were studied for their ability to release K from broth medium supplemented with muscovite/ waste biotite mica 3 gL^{-1}. Two ml of overnight culture of each isolate was inoculated to 100 ml of Aleksandrov broth (Hu *et al*. 2006) in three replicates. Un-inoculated broth was assumed as control all the flasks were incubated at 28±2 °C, flasks were shaken manually twice daily. The amount of K released in the broth was estimated at 7, 14, and 21 days of incubation from triplicates flasks at each stage in comparison with a set of un-inoculated controls. The broth cultures was filtered through whatman No.1 filter paper and taken in a 50 mL volumetric flask and shaken thoroughly. After that solution of the culture was fed to flame photometer using standards and available K content in the supernatant was determined by flame photometry (Sugumaran and Janarthanam 2007) [9]

2.5 Quantitative estimation of released phosphorous from tri-calcium phosphate (TCP) / rock phosphate (RP)

TheKSB and PSB isolates were examined for their ability to release of P from TCP/ RP in broth medium with 5gL^{-1}. Two mL of overnight culture of each isolate was inoculated to 100 ml of Pikovskaya's broth (Modified by Sundara Rao and Sinha 1963) [36] in three replicates. All the inoculated flasks were incubated at 28±2 °C. The amount of P released in the broth was estimated at 7, 14, and 21 days of incubation from triplicate flasks at each stage in comparison with a set of un-inoculated controls. The broth culture was filtered through whatman No.1 filter paper. The available P content in the supernatant was estimated by Phosphomolybdic blue colour method as described by (Jackson 1973) [15].

2.6 Periodical changes in pH and EC of broth
Organic acid production by the isolates is proportional to its incubation time. Organic acids lower down the pH value of broth culture. Broths are filtered through whatman No.1 filter paper and filtrate was taken in the 50 mL beaker. The pH of broth for different isolates was measured with the help of digital pH meter (Chopra and Kanwar 1982) [5].
Same culture filtrate, after estimation of pH was used to determine the EC using digital EC meter (Sparks *et al*. 1996) [32].

2.7 Statistical analysis
Statistical analysis of the data was done by using analysis of Variance (ANOVA), assessed by Duncan's multiple range tests with the help of R 3.3.1 statistical software.

3. Results and Discussion
3.1 Characterization of KSB and PSB isolates
Morphological characteristics of all KSB and PSB isolates are shown in Table 2. Out of 10 isolates seven shown rough margin and rest 3 shown smooth margins. Among all the KSB and PSB isolates, KSB105 and PSB40 has the highest slime production capacity with value 2.80±0.00, 3.92±0.00 and 2.83±0.01, 4.05±0.01 at 7DAI and 14DAI (Table 3). Solubilization index of the isolates ranged from 1.32±0.02 to 2.61±0.02 and 1.98±0.01 to 2.81±0.01 at 7DAI and 14DAI respectively (Table 3).

Table 2: Morphological Characteristics of KSB and PSB isolates on modified Aleksandrov and pikovskaya agar media

Isolates	Colour	Margin	Slime Production	Colony elevation	
				Slightly raised	Highly raised
KSB40	Whitish	Rough (Echinulate)	Low	+	-
KSB17	Whitish	Smooth	low	-	+
KSB31	Grey	Rough (Beaded)	Low	+	-
KSB94	Grey	Rough (Beaded)	Moderate	+	-
KSB105	Whitish	Smooth (Filiform)	High	-	+
PSB16	Creamish	Rough (Arborscent)	Moderate	+	-
PSB27	Greyish	Rough	Moderate	+	-
PSB30	White	Smooth	Moderate	+	-
PSB40	Creamish	Rough (Echinulate)	High	+	-
PSB56	Creamish white	Rough	Moderate	+	-

Table 3: Zone of solubilization, colony size, solubilization index of KSB and PSB isolates

Isolates	Zone of solubilization (cm)		Colony size diameter (cm)		Solubilization index	
	7 DAI	14DAI	7 DAI	14DAI	7 DAI	14DAI
KSB40	2.09 ± 0.02	3.22 ± 0.02	1.23 ± 0.00	1.34 ± 0.01	1.45 ± 0.00	2.04 ± 0.00
KSB17	2.14 ± 0.00	3.45 ± 0.00	1.13 ± 0.01	1.42 ± 0.01	1.67 ± 0.01	1.98 ± 0.01
KSB31	2.01 ± 0.01	3.56 ± 0.01	1.21 ± 0.00	1.40 ± 0.00	1.32 ± 0.02	1.78 ± 0.01
KSB94	2.31 ± 0.02	3.12 ± 0.00	1.25 ± 0.00	1.48 ± 0.00	1.98 ± 0.00	2.17 ± 0.02
KSB105	2.80 ± 0.00	3.92 ± 0.00	1.19 ± 0.02	1.54 ± 0.01	2.51 ± 0.00	2.61 ± 0.00
PSB16	2.55 ± 0.01	3.67 ± 0.02	1.24 ± 0.01	1.38 ± 0.01	2.40 ± 0.00	2.69 ± 0.01
PSB27	2.73 ± 0.00	3.74 ± 0.01	1.19 ± 0.00	1.39 ± 0.00	2.41 ± 0.01	2.59 ± 0.02
PSB30	2.61 ± 0.00	3.51 ± 0.02	1.20 ± 0.00	1.43 ± 0.01	2.31 ± 0.01	2.61 ± 0.01
PSB40	2.83 ± 0.01	4.05 ± 0.01	1.26 ± 0.01	1.50 ± 0.01	2.61 ± 0.02	2.81 ± 0.01
PSB56	2.76 ± 0.02	3.85 ± 0.01	1.25 ± 0.01	1.41 ±0.00	2.47 ± 0.00	2.67 ± 0.00

Data are presented as mean±standard error (n=3), Mean followed by similar letter within a column for a particular parameters are not significantly different (P<0.05) level of significance.

3.2 Effect of KSB and PSB on pH, EC and K-mineralize dynamics of Biotite
Initial pH of uninoculated MABs was 7.07 which did not influence the incubation period. However, a slight change in pH values with incubation period was observed (Table no 2). The KSB and PSB isolates characterized in this study might have produced several kinds of organic acids which broke down the mica structure to satisfy their Si+4 and K requirements, bringing them into solution consequently lowering the pH of the inoculated broth.KSB105 and PSB40strain were found to significantly lower pH 6.54±0.10, 6.16±0.05, 5.94±0.02and 6.21±0.11, 5.93±0.06, 5.52±0.12at 7,14 and 21 DAI (p=0.05), respectively, which was significantly lower, as compared to the rest of the KSB and PSB strains (Table 4) depicted in figure 2.Controlslightly decreased pH with incubation time and may be due to production of H+ during the hydrolysis of added Biotite (Girgis, 2006 [10]; Binbin and Bin, 2011) [4]. Electrical Conductivity of the broth increased with incubation. This may be due to the production of organic substances by the KSB and PSB strains with the increasing interval of incubation. Those substances can then solubilize the K-mineral and enhance salt content in broth culture resulting in increases in the EC of the broth (Meena *et al*. 2013 [19]; Maurya *et al*. 2014 [18]; Zhang and Kong, 2014) [38]. Meanwhile, electrical conductivity was significantly influenced by PSB isolates. It was significantly higher with PSB40 with values 0.59±0.01, 0.59±0.02, 0.82±0.08 dSm^{-1}(p=0.05) 7DAI, 14DAI, and 21DAI respectively (Table 4). The EC values of the MABs during the investigation significantly increased as incubation period increased and highest EC was observed at 21 followed by 14 and 7 DAI (Table 4) and also showed in figure 3.This may be due to an increased release of salts as incubation times increased to 7 and 14 DAI (Meena *et al*. 2013 [19]; Maurya *et al*. 2014) [18]. Lowest pH value (5.52) was recorded at 21 DAI by PSB40.And also highest EC 0.82 dSm^{-1} at 21 DAI by

PSB40. K-solublization dynamics were significantly influenced by the different KSB and PSB isolates and incubation periods. Control showed much less K mineralization due to the structural disturbance in biotite caused by hydrolysis which resulted in the release of the K in MABs (Liu *et al*. 2012 [16]; Zhang and Kong, 2014) [38]. The ability of all KSB and PSB strains to K-solublization was assessed with Biotite and incubation periods as shown in Table 4. Significantly higher K-solublization was recorded 7.16 µgmL^{-1} by PSB40 at 21DAI.

Table 4: Effect of KSB and PSB on pH, EC and K-solubilize dynamics of Biotite

Isolates	pH dynamics			EC dynamics(dS m^{-1})			K release (µg mL^{-1})		
	7DAI	14DAI	21DAI	7DAI	14DAI	21DAI	7DAI	14DAI	21DAI
Control	7.07± 0.04$_a$	6.94± 0.03$_a$	6.88± 0.03$_a$	0.49 ±0.00d$_e$	0.50 ± 0.01$_{ef}$	0.54 ±0.04$_b$	2.81 ± 0.27$_g$	3.5 ± 0.39$_f$	4.37 ± 0.05$_d$
KSB40	6.95± 0.06$_{ab}$	6.89± 0.06$_a$	6.76± 0.03$_b$	0.54 ±0.02$_b$	0.55 ± 0.01$_{bc}$	0.58 ±0.01$_b$	3.82 ± 0.15$_{ef}$	5.81 ± 0.15$_{abc}$	5.87 ± 0.15$_{abc}$
KSB17	6.60± 0.05$_c$	6.59± 0.02$_b$	6.51± 0.03$_c$	0.53 ± 0.01$_b$	0.54 ± 0.00$_{bcd}$	0.56 ±0.03$_b$	4.45 ± 0.36$_{bcd}$	4.36 ± 0.17$_e$	6.2 ± 0.62$_{abc}$
KSB31	6.58± 0.10$_c$	6.41± 0.03$_c$	6.39± 0.01$_d$	0.48 ± 0.00d$_e$	0.48 ± 0.00$_f$	0.52 ±0.02$_b$	4.18 ± 0.23$_{de}$	5.71 ± 0.28$_{bcd}$	5.67 ± 0.86$_{bc}$
KSB94	6.90± 0.03$_b$	6.83± 0.04$_a$	6.78± 0.04$_b$	0.50 ± 0.00$_{cd}$	0.51 ±0.01$_{def}$	0.52 ±0.04$_b$	3.51 ± 0.29$_f$	5.62 ± 0.29$_{cd}$	5.3 ± 0.87$_{cd}$
KSB105	6.54± 0.10$_c$	6.16± 0.05$_d$	5.94± 0.02$_f$	0.52 ± 0.00$_{bc}$	0.53±0.01$_{cde}$	0.52 ±0.03$_b$	5.01 ± 0.41$_{ab}$	6.26 ± 0.18$_a$	6.53 ± 1.28$_{abc}$
PSB16	6.15± 0.05$_d$	6.19± 0.01$_d$	6.08± 0.06$_e$	0.54 ± 0.01$_b$	0.56 ±0.01$_b$	0.57 ±0.04$_b$	4.81 ± 0.16$_{abc}$	6.16 ± 0.34$_{ab}$	6.8 ± 1.04$_{ab}$
PSB27	6.00± 0.11$_e$	5.95± 0.06$_e$	5.91± 0.02$_f$	0.47 ± 0.00$_e$	0.49 ±0.00$_f$	0.57 ±0.00$_b$	4.52 ± 0.27$_{bcd}$	5.3 ± 0.06$_d$	6.27 ± 0.30$_{abc}$
PSB30	6.60± 0.07$_c$	6.55± 0.03$_b$	6.46± 0.05$_{cd}$	0.49 ± 0.00$_{de}$	0.50 ±0.01$_{ef}$	0.63±0.03$_b$	4.42 ± 0.42$_{cd}$	6.19 ± 0.16$_a$	6.5 ± 0.43$_{abc}$
PSB40	6.21± 0.11$_d$	5.93± 0.06$_e$	5.52± 0.12$_g$	0.59 ± 0.01$_a$	0.59 ±0.02$_a$	0.82 ±0.08$_a$	4.32 ± 0.32$_{cde}$	6.15 ± 0.36$_{ab}$	7.16 ± 0.15$_a$
PSB56	6.00± 0.11$_e$	5.88± 0.18$_e$	5.57± 0.10$_g$	0.55 ± 0.01$_b$	0.55 ±0.00$_{bc}$	0.59 ±0.03$_b$	4.99 ± 0.20$_a$	6.09 ± 0.12$_{ab}$	6.36 ± 0.11$_{abc}$

Data are presented as mean± standard error (n=3), Mean followed by similar letter within a column for a particular parameters are not significantly different (P<0.05) level of significance according to DMRT.

3.3 Effect of KSB and PSB on pH, EC and K-solubilize dynamics of Muscovite.

Initial pH of the MABs with Muscovite was not influenced significantly by KSB and PSB isolates. Although a slight change in pH found which may be due to the dissolution of Muscovite mineral. The lowest pH was observed at 21DAI 5.58 and 5.65 by PSB30 and PSB40 respectively which was significantly much lower than other isolates. Depicted in figure 5.Electrical Conductivity of the broth increased with incubation. This may be due to the production of organic substances by the KSB and PSB strains with the increasing interval of incubation. Particular isolates showed its full capacity to produce organic substances and so they increase soluble salt. Increase in soluble salt mainly depends on potentiality of isolates to solubilize the K present in muscovite. Similar findings were also notice by Parmer & Sindhu (2013) [22] for the solubilization of mica. Electrical conductivity was significantly influenced by PSB isolates. It was significantly higher with PSB40 with values 0.57±0.02, 0.59±0.02, 0.72±0.04 dSm^{-1} (p=0.05) 7DAI, 14DAI,and 21DAI respectively (Table 5) and also depicted in figure 6. K-solublization dynamics of Muscovites were significantly influenced by the different KSB and PSB isolates and incubation periods. Control showed much less K mineralization. Due to the less structural disturbance and more tetrahedral rotation in dioctahedral Muscovite results less K release than trioctahedral Biotite. The maximum K released by PSB40 with 6.80±0.55 µgmL^{-1} at 21 DAI. Sheng *et al.* (2006) reported that solubilization of mineral by microorganisms is due to the production of organic acids like oxalic acid and tartaric acids and also due to production of capsular polysaccharides which helps in dissolution of minerals to release potassium.

Table 5: Effect of KSB and PSB on pH, EC and K-solubilize dynamics of Muscovite

Isolates	pH dynamics			EC dynamics(dS m^{-1})			K release (µg mL^{-1})		
	7DAI	14DAI	21DAI	7DAI	14DAI	21DAI	7DAI	14DAI	21DAI
Control	7.08 ± 0.04$_a$	6.85 ± 0.06$_a$	6.6 ± 0.03$_a$	0.48 ± 0.00$_{ef}$	0.51 ± 0.01$_{ef}$	0.55 ± 0.02$_{cd}$	2.63 ± 0.73$_b$	2.56 ± 0.05$_f$	3.96 ± 0.20$_e$
KSB40	6.66 ± 0.10$_{bc}$	6.49 ± 0.04$_{bc}$	6.32 ± 0.09$_b$	0.48± 0.01$_b$	0.54 ± 0.01$_{bcd}$	0.57 ± 0.01$_{cd}$	2.46 ± 0.05$_b$	3.03 ± 0.40$_{def}$	4.43 ± 0.05$_{de}$
KSB17	6.40 ± 0.15$_{cde}$	6.28 ± 0.06$_{cd}$	6.18 ± 0.06$_{bc}$	0.52 ± 0.01$_{bc}$	0.53 ± 0.00$_{cd}$	0.56 ± 0.03$_{cd}$	2.30 ± 0.26$_b$	2.63 ± 0.15$_{ef}$	4.90 ± 0.30$_{cd}$
KSB31	6.84 ± 0.05$_{ab}$	6.63 ± 0.05$_{ab}$	6.52 ± 0.08$_a$	0.47± 0.01$_{ef}$	0.48 ± 0.00$_g$	0.52 ± 0.02$_{cd}$	2.50 ± 0.26$_b$	3.20 ± 0.36$_{def}$	5.40 ± 0.10$_c$
KSB94	6.21 ± 0.27$_{ef}$	6.07 ± 0.05$_{def}$	5.89 ± 0.10$_e$	0.50 ± 0.01$_{de}$	0.52 ± 0.01$_{cde}$	0.53 ± 0.04$_{cd}$	2.50 ± 0.17$_b$	3.60 ± 0.75$_{cd}$	5.40 ± 0.26$_c$
KSB105	6.62 ± 0.05$_{bc}$	6.22± 0.36$_{cde}$	6.11 ± 0.02$_{cd}$	0.51 ± 0.01$_{cd}$	0.52± 0.01$_{de}$	0.52 ± 0.02$_d$	2.60± 0.55$_b$	3.30± 0.36$_{de}$	5.23 ± 0.05$_c$
PSB16	6.26 ± 0.17$_{def}$	5.99 ± 0.14$_{ef}$	5.84 ± 0.15$_{ef}$	0.54 ± 0.00$_b$	0.56 ± 0.01$_b$	0.57 ± 0.04$_{cd}$	3.83 ± 1.33$_a$	4.70 ± 0.20$_{ab}$	6.46 ± 0.70$_{ab}$
PSB27	6.10 ± 0.20$_f$	5.98 ± 0.12$_{ef}$	5.79 ± 0.12$_{ef}$	0.47 ± 0.00$_f$	0.49 ± 0.00$_{fg}$	0.57 ± 0.00$_{cd}$	3.46 ± 0.92$_{ab}$	4.06 ± 0.40$_{bc}$	6.60 ± 0.10$_{ab}$
PSB30	6.81 ± 0.12$_{ab}$	5.80 ± 0.14$_f$	5.58 ± 0.07$_g$	0.48 ± 0.01$_{ef}$	0.51 ± 0.02$_{ef}$	0.63± 0.03$_b$	4.00± 0.55$_a$	4.83 ± 0.30$_a$	6.06± 0.32$_a$
PSB40	6.51± 0.13$_{cd}$	6.44 ± 0.11$_{bc}$	5.65 ± 0.21$_{fg}$	0.57 ± 0.02$_a$	0.59 ± 0.02$_a$	0.72 ± 0.04$_a$	3.86 ± 0.40$_a$	5.10 ± 0.50$_a$	6.80± 0.55$_b$
PSB56	6.14 ± 0.17$_{ef}$	6.10 ± 0.20$_{de}$	5.93 ± 0.07$_{de}$	0.54 ± 0.01$_b$	0.54 ± 0.00$_{bc}$	0.58 ± 0.02$_{bc}$	4.60± 0.20$_a$	5.00 ± 0.10$_a$	5.30 ± 0.26$_c$

Data are presented as mean± standard error (n=3), Mean followed by similar letter within a column for a particular parameters are not significantly different (P<0.05) level of significance according to DMRT.

3.4 Effect of KSB and PSB on pH, EC and P-solubilize dynamics of Tri-calcium Phosphate.

Initial pH of un-inoculated pikovskaya broth with TCP was not changed significantly although a slight change occur with incubation periods due to dissolution of TCP. The lowest pH at 21DAI was observed by PSB40 with the value 5.83 ± 0.12 (Table 6) followed by PSB56 with value 5.84 ± 0.06and PSB27 with value 5.9 ± 0.01 at 21DAI.Soluble salts (EC dSm^{-1}) production also influenced by incubation periods as well as particular isolates potentiality. The highest soluble salt content at 21 DAI was observed by PSB40 with the value 0.78 ± 0.03 followed by PSB30 and PSB56 with the value 0.63 ± 0.03 and 0.59 ± 0.03 at 21 DAI respectively (Table 6 and figure 8). Higher soluble salt content may be due to more dissolution of TCP by isolates. Similar findings were also notice by Ahmed and Jha (1968) [1] using the bacteria and fungi for the solubilization of tri-calcium phosphate. TCP was readily solubilized by the bacteria and fungi resulted in an increase in EC of culture media.P (µg mL^{-1}) released in TCP was significantly varied with isolates. Moreover the highest P (µg mL^{-1}) released by PSB40 with the value 7.17 ± 0.18, 10.31 ± 0.28, 13.70 ± 0.80 at 7DAI, 14DAI and 21DAI respectively (Table 6 and figure 8) followed by PSB27 13.40 µg mL^{-1} at 21DAI. However, The KSB isolates released P comparatively very low than PSB isolates it may be lack of acid and soluble salt production. The release of P from insoluble soil mineral (tri – calcium phosphate) was used to examine the isolates ability for its dissolution and the results indicated that the pH values decrease during the incubation period.The result is in agreement with Zhao *et al*. (2008) and Gundala *et al*. (2013) [12] who found that phosphate compounds are mobilized in the production of organic acids, accompanied by acidification of medium. The organic and inorganic acids convert tri calcium phosphate to di and mono phosphates (Sheng *et al*. 2002) [25-26]. Higher P release may be due the higher production of organic acids and lowering down the pH of the broth culture. The result of this study are strongly supported by the works of Parks *et al*. (2011) [21] he observed that P solubilization increase with increasing of organic acids and incubation periods. The results also indicated great variation between the P solubilizers the source of insoluble P. The difference in efficiency of bacteria to solubillization of insoluble or fixed form of P could be due to differences in their ability to release organic acids and inorganic acids.

Table 6: Effect of KSB and PSB on pH, EC and P-solubilize dynamics of TCP

Isolates	pH dynamics			EC dynamics(dS m^{-1})			P release (µg mL^{-1})		
	7DAI	14DAI	21DAI	7DAI	14DAI	21DAI	7DAI	14DAI	21DAI
Control	7.03 ± 0.03$_{a}$	6.87 ± 0.05$_{a}$	6.71 ± 0.07$_{a}$	0.39 ± 0.01$_{d}$	0.44 ± 0.01$_{g}$	0.49 ± 0.01$_{e}$	2.26 ± 0.13$_{h}$	2.43 ± 0.10$_{e}$	3.10 ± 0.12$_{e}$
KSB40	6.82 ± 0.04$_{bc}$	6.69 ± 0.04$_{b}$	6.65 ± 0.05$_{a}$	0.45 ± 0.02$_{c}$	0.51 ± 0.02$_{ef}$	0.56 ± 0.02$_{cd}$	3.26 ± 0.15$_{g}$	4.81 ± 0.15$_{d}$	6.26 ± 0.61$_{d}$
KSB17	6.70 ± 0.05$_{cd}$	6.59 ± 0.02$_{bc}$	6.45 ± 0.04$_{b}$	0.43 ± 0.01$_{c}$	0.53 ± 0.01$_{cde}$	0.56 ± 0.03$_{cd}$	4.40 ± 0.04$_{f}$	4.84 ± 0.15$_{d}$	6.47 ± 0.15$_{d}$
KSB31	6.68 ± 0.09$_{d}$	6.38 ± 0.03$_{d}$	6.32 ± 0.10$_{c}$	0.48 ± 0.00$_{b}$	0.54 ± 0.01$_{bcd}$	0.57 ± 0.01$_{bcd}$	5.09 ± 0.13$_{d}$	5.96 ± 0.13$_{c}$	6.19 ± 0.28$_{d}$
KSB94	6.81 ± 0.03$_{bc}$	6.73 ± 0.05$_{b}$	6.68 ± 0.03$_{a}$	0.49 ± 0.01$_{b}$	0.51 ± 0.01$_{def}$	0.52 ± 0.04$_{de}$	5.07 ± 0.08$_{d}$	5.48 ± 0.27$_{cd}$	7.03 ± 0.70$_{d}$
KSB105	6.85 ± 0.05$_{b}$	6.49 ± 0.07$_{cd}$	6.45 ± 0.05$_{b}$	0.42 ± 0.00$_{c}$	0.53 ± 0.01$_{cde}$	0.52 ± 0.03$_{de}$	4.73 ± 0.21$_{e}$	5.45 ± 0.62$_{cd}$	6.93 ± 0.15$_{d}$
PSB16	6.35 ± 0.05$_{f}$	6.19 ± 0.01$_{e}$	6.10 ± 0.01$_{d}$	0.54 ± 0.01$_{a}$	0.56 ± 0.01$_{b}$	0.57 ± 0.04$_{bcd}$	6.27 ± 0.16$_{b}$	9.77 ± 0.53$_{a}$	12.70 ± 0.40$_{bc}$
PSB27	6.00 ± 0.11$_{h}$	5.95 ± 0.06$_{f}$	5.90 ± 0.01$_{e}$	0.49 ± 0.01$_{b}$	0.49 ± 0.00$_{f}$	0.57 ± 0.00$_{bcd}$	7.04 ± 0.08$_{a}$	8.91 ± 0.57$_{b}$	13.40 ± 0.81$_{ab}$
PSB30	6.50 ± 0.07$_{e}$	6.45 ± 0.03$_{d}$	6.37 ± 0.02$_{bc}$	0.49 ± 0.00$_{b}$	0.50 ± 0.01$_{ef}$	0.63 ± 0.03$_{b}$	5.98 ± 0.11$_{c}$	8.73 ± 0.69$_{b}$	12.00 ± 0.36$_{c}$
PSB40	6.18 ± 0.07$_{g}$	6.17 ± 0.07$_{e}$	5.83 ± 0.12$_{d}$	0.56 ± 0.04$_{a}$	0.59 ± 0.02$_{a}$	0.78 ± 0.03$_{a}$	7.17 ± 0.18$_{a}$	10.31 ± 0.28$_{a}$	13.70 ± 0.80$_{a}$
PSB56	6.00 ± 0.11$_{h}$	5.90 ± 0.20$_{f}$	5.84 ± 0.06$_{e}$	0.55 ± 0.01$_{a}$	0.55 ± 0.00$_{bc}$	0.59 ± 0.03$_{bc}$	5.73 ± 0.33$_{c}$	8.71 ± 0.74$_{b}$	12.03 ± 0.49$_{c}$

Data are presented as mean± standard error (n=3), Mean followed by similar letter within a column for a particular parameters are not significantly different (*P*<0.05) level of significance according to DMRT.

3.5 Effect of KSB and PSB on pH, EC and P-solubilize dynamics of Rock Phosphate

Initial pH of the un-inoculated pikovskaya broth with rock phosphate also showed negligible change in pH. This may be low dissolution of rock phosphate due to lack of appropriate solubilizers. The lowest pH was observed by PSB27 (5.75±0.07), PSB56 (5.77±0.11), PSB40 (6.03±0.11) at 21DAI (Table 7). The isolates produce organic acids that influence rock phosphate dissolution by decreasing pH. Stella and Halimi. (2014) Gluconic acid production by PSB to liberate phosphorus from insoluble phosphate complexes. All the isolates were able to produce gluconic acid which could have contributed to mineral phosphate solubilization. Yadav *et al*. (2014) Poor soluble rock phosphate can be solubilize with PSB by gluconic acid production. Among the isolates PSB40 showed maximum EC (0.78±0.02 dSm^{-1}), which has significantly superior than other isolates. The EC values of the cultures in the experiments during the incubation period increase significantly with the incubation period. Highest P released was observed by PSB56 (5.09±0.29) and PSB40 (4.97±0.12) at 21DAI (Table 7). Although it was low comparative to tri-calcium phosphate as rock phosphate is more insoluble in nature.

Table 7: Effect of KSB and PSB on pH, EC and P-solubilize dynamics of Rock Phosphate.

Isolates	pH dynamics			EC dynamics(dS m^{-1})			P release (µg mL^{-1})		
	7DAI	14DAI	21DAI	7DAI	14DAI	21DAI	7DAI	14DAI	21DAI
Control	7.02 ± 0.02$_{a}$	6.81 ± 0.08$_{a}$	6.71 ± 0.07$_{a}$	0.39± 0.01$_{e}$	0.45 ± 0.02$_{g}$	0.52 ± 0.03$_{d}$	0.10 ± 0.01$_{g}$	0.21 ± 0.02$_{f}$	0.31 ± 0.01$_{h}$
KSB40	6.72 ± 0.04$_{b}$	6.59 ± 0.04$_{b}$	6.53 ± 0.02$_{b}$	0.45 ± 0.01$_{c}$	0.51 ± 0.03$_{ef}$	0.57 ± 0.01$_{bcd}$	1.27 ± 0.22$_{de}$	1.56 ± 0.04$_{d}$	2.45 ± 0.11$_{e}$
KSB17	6.63 ± 0.04$_{b}$	6.59 ± 0.02$_{b}$	6.47 ± 0.07$_{bc}$	0.43 ± 0.01$_{cd}$	0.54 ± 0.00$_{bcd}$	0.57 ± 0.01$_{bcd}$	1.42 ± 0.12$_{d}$	1.43 ± 0.10$_{d}$	1.68 ± 0.04$_{f}$
KSB31	6.61 ± 0.05$_{b}$	6.41 ± 0.06$_{c}$	6.41± 0.02$_{c}$	0.44 ± 0.00$_{c}$	0.54 ± 0.01$_{bcde}$	0.58 ± 0.01$_{bcd}$	0.91 ± 0.02$_{f}$	0.96 ± 0.01$_{e}$	1.02 ± 0.06$_{g}$
KSB94	6.71 ± 0.09$_{b}$	6.66 ± 0.03$_{b}$	6.41 ± 0.03$_{bc}$	0.40 ± 0.02$_{de}$	0.51 ± 0.01$_{def}$	0.54 ± 0.03$_{cd}$	0.93 ± 0.03$_{f}$	0.98 ± 0.01$_{e}$	1.08 ± 0.08$_{g}$
KSB105	6.49 ± 0.03$_{c}$	6.45 ± 0.07$_{c}$	6.41 ± 0.02$_{c}$	0.42 ± 0.00$_{cde}$	0.53 ± 0.01$_{cdef}$	0.54 ± 0.03$_{bcd}$	1.17 ± 0.05$_{e}$	1.54 ± 0.12$_{d}$	1.77 ± 0.08$_{f}$
PSB16	6.49 ± 0.06$_{c}$	6.39 ± 0.01$_{c}$	6.11 ± 0.02$_{d}$	0.54 ± 0.00$_{a}$	0.56 ± 0.01$_{b}$	0.57 ± 0.04$_{bcd}$	1.96 ± 0.12$_{c}$	2.69 ± 0.16$_{c}$	3.84 ± 0.28$_{d}$
PSB27	6.01 ± 0.12$_{e}$	5.84 ± 0.04$_{e}$	5.75 ± 0.07$_{e}$	0.49 ± 0.01$_{b}$	0.50 ± 0.02$_{f}$	0.58 ± 0.02$_{bc}$	1.48 ± 0.21$_{d}$	3.63 ± 0.23$_{b}$	4.55 ± 0.21$_{b}$
PSB30	6.47 ± 0.04$_{c}$	6.42 ± 0.03$_{c}$	6.37 ± 0.02$_{c}$	0.50 ± 0.02$_{b}$	0.50 ± 0.01$_{f}$	0.59 ± 0.05$_{b}$	2.13 ± 0.16$_{bc}$	3.41 ± 0.27$_{b}$	4.21 ± 0.10$_{c}$
PSB40	6.18 ± 0.07$_{d}$	6.15 ± 0.08$_{d}$	6.03 ± 0.11$_{d}$	0.56 ± 0.04$_{a}$	0.59 ± 0.02$_{a}$	0.78 ± 0.02$_{a}$	2.32 ± 0.13$_{ab}$	4.29 ± 0.29$_{a}$	4.97 ± 0.12$_{a}$
PSB56	6.11 ± 0.09$_{de}$	5.86 ± 0.19$_{e}$	5.77 ± 0.11$_{e}$	0.54 ± 0.01$_{a}$	0.55 ± 0.00$_{bc}$	0.59 ± 0.03$_{b}$	2.43 ± 0.11$_{a}$	3.97 ± 0.35$_{a}$	5.09 ± 0.29$_{a}$

Data are presented as mean± standard error (n=3), Mean followed by similar letter within a column for a particular parameters are not significantly different (*P*<0.05) level of significance according to DMRT.

Conclusions

Application of KSB and PSB on soil-plant system can be a valuable tool for increased crop productivity. Therefore in this study five KSB isolates and five PSB isolates were examined. Among all the isolates PSB40 was showing the overall satisfactory result. It was also apparent from the experimental data that PSBs cause more acidity to broth as compared to KSBs isolates. It was observed the isolates of K-solubilizers solubilize P from the insoluble sources of P. Isolates of PSB solubilize K from the insoluble sources of K as biotite and muscovite. Solubilization of P by KSBs were not in that extent as isolates of PSBs. Isolates of PSB solubilized more K than KSB isolates. Experimental findings indicated that efficient isolates of PSBs and KSBs may be helpful in solubilization of P as well as K from their insoluble minerals. Detail investigation is required to characterize the P and K isolates to understand the mechanism of K and P-solubilization from different nature of insoluble K and P-minerals. The KSBs and PSBs which can enhance the P and K vice versa availability in agricultural soils with combined use of waste mica/TCP/RP may reduce the negative impact on Indian economy while integrating with slogan of the sustainable development. Further studies on the mechanism by which KSBs and PSBs solubilized K and P and the effectiveness of their use in the field are needed to promote evergreen agriculture.

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