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Isolation, Biochemical Characterization and Potassium Solubilzation Efficiency of Different Microbial Isolates

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A B S T R A C T

Microbial isolates were collected from rhizospheric soil of Meerut-Muzaffarnagar district of western Uttar Pradesh to identify the potassium solubilizing efficiency of different soil microbes. A total of thirty-eight potassium solubilizing microbes were identified and isolated on Aleksandrow Agar media. It was found that thirty-five of them were bacterial isolates and three were fungal isolates. They were further characterized based on morphological and biochemical characteristics. Among them SS 7-6, P-4-1, S-6, SS 5-3, P-1-1, SS 9-2 and SS 7-1 were Gram negative, while SS-13, SS 7-7 and P-21, were Gram positive. The highest solubilization efficiency was observed in case of SS7-6 i.e. 11.50 mm which was followed by SS-13 i.e. 11.30 mm. All isolated KSB were also tested separately for their phosphorous solubilization efficiency. It was observed that the isolates namely SS 5-1, SS 5-2, SS 5-4, SS 7-1, SS7-4, S-6, SS-6, SS 9-1, SS 9-2, SS 9-3, P-1-1, PYS-1, PYS-5B, PYS-5A, P-17, P-21 and three fungal isolates efficiently solubilized phosphorous. Such 'dual-solubilizers' can convert fixed potassium and phosphorous source in soil to available form for plants.

Introduction

Potassium is one of the essential nutrient required for higher and sustainable productivity of crops. It is third important plant nutrient after Nitrogen (N) and Phosphorus (P). Potassium (K) is required for activation of enzymes which are fundamental to metabolic processes, especially the production of proteins and sugars (Johnston, 1986). It is also essential for opening and closure of stomatal guard cells or daily changes in the orientation of leaves (Shehata and El-Khawas, 2003). K is present in soil with a range between 0.04 - 3% and only 1 to 2% of this is available to plants.

While rest of the soil bound with other minerals and is unavailable to plants (Sparks and Huang, 1985). With the progressive intensification of agriculture due to small landholdings and debut of high-yielding crop

varieties and hybrids during green revolution, the soils are depleted in macronutrients including potassium at a speedy rate.

Moreover, available soil K levels have also dropped due to leaching, runoff, and erosion (Sheng and Huang, 2002a). As a after effect, potassium deficiency is becoming one of the major constraint in crop production, and therefore, many crops do respond to K fertilization in soils. Recently, K deficiency has also been recited in most of the crop plants (Meena, *et al.*, 2014; Xiao, *et al.*, 2017).

To overcome this quandary and to secure higher plant yields, farmers are reliant on chemical sources of fertilizers (Glick, 2012). While the chemical fertilizers have helped for achieving higher plant growth and yield but they have been detrimental to soil health (Adesemoye and Kloepper, 2009). Such chemical fertilizers are applied at higher than recommended doses to enhance crop yields, which have created environmental pollution problems (Brady 1990; Akande, *et al.*, 2008).

Therefore, direct application of rock phosphate and rock potassium materials in soils may be agronomically more useful and environmentally safer than application of soluble P and K applied as chemical fertilizers. However, application of complex minerals requires presence or incorporation of their solubilizers as well (Rajan, *et al.*, 1996).

Thus, Eco-friendly agricultural system has emerged as an important thrust area globally for long-term soil environmental sustainability and to minimize the environmental pollution associated with extensive use of chemical fertilizers. Therefore, plant growth-promoting rhizobacteria (PGPR) along with nitrogen-fixing, phosphate and potassium-solubilizing bacteria are being used as biofertilizers to conserve our existing resources and to minimize environmental pollution hazards (Vessey, 2003; Ekin, 2010; Bahadur, *et al.*, 2014; Sindhu, *et al.*, 2014c; Meena, *et al.*, 2014a).

Materials and Methods

Sample collection

The samples were collected from rhizospheric soils of different crops from 7-8 inches soil surface of Meerut-Muzaffarnagar District. The samples were collected in aseptic bags and transported to lab for further processing. Each sample consisted of 200 g soil. The details of the place and the crops of rhizospheric soil samples are furnished in Table 1.

Isolation of potassium solubilizing bacteria and fungi

One gram of rhizosphere soil was mixed thoroughly in 10 ml distilled water and was processed by serial dilution agar plate technique (Aneja, 2002). Suitable dilutions (10^{-5} and 10^{-6}) of rhizosphere solutions were plated on Aleksandrow medium (Hu, *et al.*, 2006): 0.5% glucose, 0.05% magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$), 0.0005% iron (III) chloride ($FeCl_3$), 0.01% calcium carbonate ($CaCO_3$), 0.2% calcium phosphate ($Ca_3\{PO_4\}_2$), and 0.2% potassium-aluminum-silicate ($KAlSi_3O_8$), pH 7.0–7.5.

The petri-plates were incubated for 3 days at room temperature (30±1°C) and the colonies exhibiting clear zones were selected as potassium solubilizers from the 10^{-5} dilution containing plates. Cultures from these colonies were then streaked on Nutrient Agar plates for obtaining pure culture. These pure cultures were again transferred to fresh Aleksandrow medium to confirm their solubilization efficiency.

Estimation of potassium (K) solubilization potential of native isolates

Qualitative analysis of Potassium solubilizing activity of various isolated microbes on Aleksandrow Agar medium was calculated on the basis of solubilization zone ratio (zone diameter/colony diameter) or solubilization index (Hu, *et al.,* 2006). Solubilization efficiency and index was evaluated according to the ratio of the total diameter (halo zone – colony diameter) /2 as described by Edi-Premono, *et al.*, 1996.

Solubilization Efficiency (SE)
= (D-d)/2 (> 5mm)

Solubilization zone ratio / Solubilization Index (SI) = D/ d

Where,
d = Diameter of Mycelium/colony+growth zone.
D = Diameter of zone of clearance (Mycelium/colony + Clear zone)

Morphological and biochemical characteristics of KSB isolates

For morphological characterization, all the selected isolates were examined for the colony morphology, cell shape, Gram reaction and ability to form spores according to the standard procedures (Barthalomew and Mittewer, 1950). Colony characteristics such as size, shape, texture, colour, opacity and consistency were examined. The biochemical characterizations of the isolates were carried out as per the procedures mentioned in Bergey's Manual of Systematic Bacteriology 9th Edition (1993). Sugar utilization, Methyl red test (Seeley and Vandemark, 1970), Voges-Proskauer (VP) test (Seeley and Vandemark, 1970), Nitrate reduction test, Catalase test (Blazevic and Ederer, 1975), Citrate test, Motility test, Triple sugar iron test, oxidase test, lysine decarboxylase and ornithine decarboxylase test were performed.

Results and Discussion

Screening and isolation of potassium solubilizing microbial isolates

A total of eleven soil samples were collected from various places and analyzed for presence of K-solubilizing fungal and bacterial isolates. Out of eleven soil samples screened for mixed culture on Aleksandrow agar medium, the presence of 21 potassium solubilizing bacteria and 3 potassium solubilizing fungi were observed which were isolated in pure culture. Fourteen isolates were obtained from co-worker in lab working on P solubilizers (soil samples marked with */**). Hence, a total of thirty-eight microbes were tested for potassium solubilization on Aleksandrow agar medium as presented in Table 1.

Above isolates were tested for their potential to solubilize potassium and their ranking was done on basis of their solubilization efficiency (SE). Results showed that all thirty-five bacterial isolates and three fungal isolates have the potential to solubilize potassium although with differing solubilization efficiency (SE value). Isolates named SS 7-6, SS-13, P-21, SS 7-7, P-1-1, S-6, SS 5-3, P-4-1, SS 9-2 and SS 7-1 solubilized potassium with higher solubilization efficiency. The results observed are presented in Table 2 and Fig 1. The maximum clear zone measured on Aleksandrow agar medium was 29 mm for sample code SS 7-6 with solubilization efficiency (SE) of 11.50 mm followed by sample code SS-13 with clear zone of 28.67 mm and solubilization efficiency (SE) of 11.30 mm after 15 days of incubation at 28°C. Isolates with solubilization efficiency of 5.0 mm or more are generally considered as efficient solubilizers, which was demonstrated by isolates in following order namely SS 7-6

(11.50 mm) > SS 13 (11.33 mm) > P-21 (10.33 mm) > SS 7-7 (8.67 mm) >P-4-1 (8.33 mm) >S 6 (7.50 mm) > SS 5-3 (7.50 mm) > P-1-1 (7.33 mm) > SS 9-2 (7.17 mm) > SS 7-1 (7.00 mm) > SS-17 (5.42 mm) > SS 9-1 (5.17 mm) > SS 9-3 (5.00 mm). It was found that more number of solubilizers were isolated from rhizosperic soils of Meerut region compared to Muzaffarnagar region. Among crop rhizospheres the efficiency and presence of solubilizers were observed in following order namely potato > sugarcane > turmeric > mustard > mango > lemon plant field. In similar studies (Prajapati, *et al.,* 2012) had selected the colonies which were morphologically distinct and exhibiting zone of clearance indicating Potassium solubilization; a total of 14 bacterial isolates were isolated by them as potassium solubilizers and named as KSB1 to KSB14.

As per microscopic examination held among three isolated K-solubilizing fungi F1 and F2 were identified as *Aspergillus spp*. and F3 was identified as *Penicillium sp*. Similar study was conducted by Meena, *et al.,* 2016 and suggested that *Aspergillus spp*. and *Penicillium spp*. were more effective solubilizers than bacteria such as *Bacillus spp., Paenibacillus spp., Pseudomonas spp*., etc. Nineteen out of thirty-eight isolates namely SS 5-1, SS 5-2, SS 5-4, SS 7-1, SS7-4, S-6, SS-6, SS 9-1, SS 9-2, SS 9-3, P-1-1, PYS-1, PYS-5B, PYS-5A, PYS-7C1, P-21, F-1, F-2 and F-3 showed ability for solubilization of both potassium and phosphorous (Pikovskaya's agar medium, 7 days of incubation at 28°C). Such dual-solubilizers may significantly enhance the availability and uptake of P and K which may ultimately reflect in higher plant growth and yield.

Based on the efficiency of P and K solubilization these PGP bacterial isolates can be further investigated for their performance to enhance growth, nutrient uptake and yield of various crops. An increase in plant root length and plant growth promotion due to inoculation of *Bacillus edaphicus*, a potassium solubilizing bacteria (Sheng and He, 2006), maize (Wu, *et al.,* 2005), brinjal (Ramarethinam and Chandra, 2005), cotton and rape seed (Sheng, 2005) were previously reported. K-solubilizers, apart from solubilization of potassic minerals, are also known to possess other beneficial properties like solubilization of insoluble inorganic phosphates, polysaccharide production and production of plant growth promoting substances etc. Similar, observation for solubilization of insoluble phosphates by potassium solubilizing bacteria *Bacillus muciligenosus* was reported earlier by (Hu, *et al.,* 2006). Similarly, bacteria *Bacillus mucilaginosus* was isolated and characterized for their ability to solubilize two potassium bearing minerals like feldspar and illite. Their probable mechanism of action for solubilization of potassium bearing minerals was predicted and shown be to the action of organic acids like oxalic acid and capsular polysaccharides (Fang and Yan, 2006). The results in the present report are in agreement with the findings of (Norkina and Pumpynaskaya, 1956), they isolated two strains of Bacillus sp. and Pseudomonas from rhizosphere soil of various crop plants as mineral potassium solubilizers.

The variability among the bacterial solubilizing ability indicates that there is a need of time for exploration of different mineral potassium solubilizing bacteria and to understand their solubilizing mechanisms. Similar study was carried out by (Anjanadevi, *et al.,* 2015) with 36 different bacteria isolated from rocks of a major hill station at Ponmudi in Thiruvananthapuram, Kerala, India. A comprehensive characterization of K solubilization from feldspar was achieved with these isolates which indicated that the K

solubilizing efficiency increases with decrease in pH and increase in viscosity and viable cell count. When mica was replaced with KCl and K_2SO_4 it was found that maximum K solubilization was obtained with KCl as compared to mica which in term is another aspect to study the behavior of bacteria with different substrate (Liu, 2006).

Colony morphology and microscopic analysis of pure culture of different KSB isolates

After 24 hours of incubation of these isolates on nutrient agar media colony morphology and microscopic appearance were observed as shown in Table 3. The results showed that isolates consist of twenty-seven Gram positive rod, round shaped bacteria and eight Gram negative round shaped bacteria. Similarly, (Begum, *et al.,* 2017) collected soil samples from fish and vegetable waste dumping area of Dhaka city, Bangladesh, A total of 19 KSB single colonies were picked up and cultured. In order to characterize bacterial isolates, gram staining and biochemical tests were performed, it was found that 79% isolates were gram positive while 26% were gram negative. In microscopic analysis, some bacteria were of round shape and some were rod shape. Also (Prajapati, *et al.,* 2012) found that out of five selected KSB strains three were gram negative while two were gram positive bacteria. All were motile and four of them had capsule. The results are in agreement with the findings of (Norkina and Pumpynaskaya, 1956), they isolated two strains of *Bacillus sp.* and *Pseudomonas* from rhizosphere soil of various crop plants as mineral potassium solubilizers.

Biochemical analysis of different isolates

From the thirty-five bacterial isolates top ten efficient bacterial isolates were selected for studying their biochemical characteristics. Different biochemical tests (Triple sugar iron agar test, Citrate utilization test, Methyl Red Test, Voges-Proskauer test, Catalase test, Oxidase test, Motility tests, Lysine Decarboxylase Test, Ornithine decarboxylase test and Potassium Hydroxide test) were performed for these K solubilizing isolates. The results of different biochemical tests are summarized in Table 4.

All these ten isolates showed positive results for Citrate, Motility and Oxidase tests. For citrate utilization test appearance of dark blue color on Simmon's citrate slant from forest green color indicates positive result and confirms that citrate has been utilized. For motility test diffused growth along the stabbed line showed that bacteria were motile and indicated positive result.

All isolates showed diffused growth and hence are motile in nature. For oxidase test formation of blue color when culture was mixed with 1% solution of tetramethyl-p-phenylene-diaminedihydrochloride indicates positive result. All isolates showed positive result with appearance of dark blue color.

Other biochemical tests showed mixed results for these 10 isolates. For VP test positive result was indicated by appearance of red color. Out of 10 isolates, sample code namely P-21, SS7-7, P-4-1, P-1-1, SS 7-1 and SS-13 showed positive result. Sample code SS 7-6, S6, SS 5-3, and SS 9-2 showed negative results. For catalase test formation of bubbles confirms that test was positive.

Out of 30 isolates, P-17 (PYS-7C1) and S-6 showed negative result while rest all 28 showed positive results. The Triple sugar iron agar (TSI) test was conducted on 28 isolates; it is named for its ability to test microorganism's ability to ferment sugars and to produce hydrogen sulphide.

Table.1 Details of Potassium solubilizing microbes isolated from different rhizosperic soil samples

S.No.	Soil Sample	Place	Date	Crop field	Culture Medium	Isolate code
1	S 1	Location 2 HRC, SVPUAT, Meerut	09/02/2019	Turmeric (*Curcuma longa*)	NA-KB-AM	No K- sol.
2	S 2	Phase 2 Pallavpuram, Meerut	12/02/2019	Onion (*Allium cepa)*	NA-KB-AM	No K- sol.
3	S 3	Sahaoli village, Muzaffarnagar, Uttar Pradesh	12/02/2019	Sugarcane (*Saccharumofficinarum*)	AM	No K- sol.
4	S 4	Makhyali village Muzaffarnagar, Uttar Pradesh	15/02/2019	Oat (*Avena sativa*)	AM	No K- sol.
5	S 5	Mataur Village, Daurala Block, Meerut District, Uttar Pradesh	18/02/2019	Yellow mustard (*B. hirta/ Sinapis alba*)	AM-NA	SS 5-1
						SS 5-2
						SS 5-3
						SS 5-4
6	S 6	ICAR-IIFSR Meerut (UP)	18/02/2019	Sugarcane(spring) +black gram+wheat	AM-NA/KB	SS 6
7	S 7	ICAR-IIFSR Meerut (UP)	18/02/2019	Potato (*Solanumtuberosum*)	AM-NA/KB	SS 7-1
						SS 7-2
						SS 7-3
						SS 7-4
						SS 7-5
						SS 7-6
						SS 7-7
						SS 7-8
					AM-PDA	SS F-1
					AM-PDA	SS F-3
8	S 8	Siwaya-jamalullapur Village, Daurala, Meerut	18/02/2019	Sugarcane (*Saccharumofficinarum*)	AM-NA	SS 8-1
						SS 8-2
9	S 9	Daurala Block , Meerut District, Uttar Pradesh	18/02/2019	Sugarcane (*Saccharumofficinarum*)	AM-NA/KB	SS 9-1 SS 9-2 SS 9-3
10	S 10	A2Z Colony Green Estate , Muzaffarnagar	18/02/2019	Garden soil	AM-PDA	SS F2
11	S 11	Bahrala Village , Daurala Block , Meerut District, U.P	18/02/2019	Sugarcane (*Saccharumofficinarum*)	AM-NA/KB	No K- sol.
12*	S 12	Sample 6 (PY) Vill. Shawali, MZN, UP	27/02/19	Fruit Field	KB-AM	SS-12

13*	S 13	Sample 3 Horticulture Research Center, SVPUAT, Meerut	27/02/19	Mango (*Mangifera indica* L.)	KB-AM	SS 13-1 SS 13-2
14*	S 14	Horticulture Research Center, SVPUAT, Meerut	27/02/19	Lemon (Citrus limon)	KB-AM	P-19
15*	S 15	Sample – 11 Horticulture Research Center, SVPUAT, Meerut	27/02/19	Turmeric (*Curcuma longa*)	NA/KB-AM	SS 15-1 P-3-2
16*	S 16	Sample – 11 Horticulture Research Center, SVPUAT, Meerut	27/02/19	Turmeric (*Curcuma longa*)	NA/KB-AM	P 4-1 SS 16-2
17*	S 17	Sample – 11 Horticulture Research Center, SVPUAT, Meerut	27/02/19	Turmeric (*Curcuma longa*)	NA/KB-AM	SS-17
18*	S 18	HRC	27/02/19	Uncultivated	NA/KB-AM	SS-18
19*	S 19	HRC	27/02/19	-	NA/KB-AM	SS-19
20*	S 20	HRC	27/02/19	Turmeric (*Curcuma longa*)	NA/KB-AM	SS-20
21**	S 21	HRC	27/02/19	-	KB-AM	P-1-1
22**	S 22	Sample 1 (PY) Horticulture Research Center, SVPUAT, Meerut	27/02/19	Turmeric (*Curcuma longa*)	KB-AM	PYS-1
23**	S 23	Sample 5 (PY) MZN, UP	27/02/19	Sugarcane (*Saccharum officinarum*)	KB-AM	PYS-5A PYS-5B
24**	S 24	Sample 7 (PY) Almaspur, Muzaffarnagar	27/02/19	Water chestnut *(Trapa natans)*	KB-AM	P-17
25**	S 25	Hill region, Uttrakhand.	27/02/19	Department of Soil Science, COA, SVPUAT, Meerut	KB-AM	P-21

\# AM - Aleksandrow Medium, PDA-Potato Dextrose Agar, KB-Kings B Medium. KS- potassium solubilizers.

Isolates from S.no 12 to S. no 25 were taken from co-worker in lab.

* Isolates above were not solubilizing phosphorus but solubilized potassium.

** Isolates above were solubilizing phosphorus and potassium both.

Table.2 Potassium solubilizing efficiency of different microbial isolates

S.	Isolates Codes	Colony Size (d) (mm)		Solubilization Zone (D) (mm)		Solubilization Efficiency (mm)	Rank	Phosphorus solubilizing Ability
		d	SD	D	SD	(D-d)/2		
1	SS 7-6	6.00	±0.00	29.00	±0.00	11.50	1	+
2	SS 13	6.00	±0.00	28.67	±0.58	11.33	2	-
3	P-21	9.00	±1.00	29.67	±0.58	10.33	3	+
4	SS 7-7	6.67	±2.08	24.00	±1.00	8.67	4	-
5	P-4-1	9.00	±0.00	25.67	±0.58	8.33	5	-
6	S 6	6.00	±0.00	21.00	±1.00	7.50	6	+
7	SS 5-3	6.00	±0.00	21.00	±1.00	7.50	7	-
8	P-1-1	8.33	±0.58	23.00	±1.00	7.33	8	+
9	SS 9-2	5.33	±0.58	19.67	±2.08	7.17	9	+
10	SS 7-1	6.00	±0.00	20.00	±0.00	7.00	10	+
11	SS-17	7.33	±0.58	18.17	±0.29	5.42	11	-
12	SS 9-1	6.33	±1.15	16.67	±1.53	5.17	12	+
13	SS 9-3	5.67	±0.58	15.67	±4.04	5.00	13	+
14	PYS-1	7.33	±1.15	16.33	±0.58	4.50	14	+
15	SS 7-5	7.00	±2.00	16.00	±0.00	4.50	15	-
16	SS 7 -3	7.00	±1.73	16.00	±0.00	4.50	16	-
17	SS 5-1	7.67	±0.58	15.67	±0.58	4.00	17	+
18	SS 7-4	6.33	±1.15	13.67	±2.31	3.67	18	+
19	P-19	6.67	±0.58	13.67	±2.31	3.50	19	-
20	P-17	4.33	±0.58	11.00	±1.00	3.33	20	+
21	SS 5-2	8.00	±0.00	14.67	±0.58	3.33	21	+
22	SS 7-8	8.00	±0.00	14.33	±1.15	3.17	22	-
23	SS 15-1	8.67	±0.58	15.00	±0.00	3.17	23	-
24	SS-18	10.33	±0.58	16.00	±1.00	2.83	24	-
25	SS-6	7.33	±3.21	12.33	±2.31	2.50	25	-
26	SS-19	4.33	±0.58	8.33	±0.58	2.00	26	-
27	SS 5-4	8.67	±0.58	11.33	±0.58	1.33	27	+
28	SS 16-2	10.00	±1.00	12.00	±0.00	1.00	28	-
29	P 3-2	8.00	±0.00	10.00	±0.00	1.00	29	-
30	SS 7-2	5.67	±0.76	7.67	±0.58	1.00	30	-
31	PYS-5A	8.33	±0.58	10.17	±0.76	0.92	31	+
32	SS 8-1	6.33	±0.29	8.17	±0.29	0.92	32	-

* d- Colony diameter, D- diameter of potassium solubilization zone. Readings in three replica.
+: Phosphorus solubilizing Ability Present
-: Phosphorus solubilizing Ability Absent

Table.3 Colony morphology and microscopic characteristics of top ten KSB isolates

S.	Isolate code	Colony Morphology						Remarks
		Form	Elevation	Size	Opacity	Color	Surface	
1	SS 7-6	Irregular	Flat	Small	Translucent	White	Glistering	G-ve cocci
2	SS 13	Regular	Flat	Small	Opaque	White	Dull	G+ve cocci
3	P-21	Regular	Flat	Medium	Opaque	White	Dull	G+ve small rods
4	SS 7-7	Regular	Flat	Small	Translucent	White	Glistering	G+ve cocci
5	P-4-1	Filamentous	Raised	Medium	Translucent	White	Glistering	G-ve cocci
6	S-6	Regular	Flat	Small	Transparent	White	Glistering	G-ve small rods
7	SS 5-3	Irregular	Flat	Medium	Translucent	White	Smooth	G-ve small rods
8	P-1-1	Regular	Flat	Small	Transparent	White	Rough	G-ve cocci
9	SS 9-2	Irregular	Flat	Medium	Translucent	White	Smooth	G-ve small rods
10	SS 7-1	Regular	Flat	Small	Translucent	White	Smooth	G-ve small rods

Table.4 Biochemical test of top ten KSB isolates

S.	Biochemical Characteristics	KSB isolates									
		SS 7-6	SS-13	P-21	SS 7-7	P-4-1	S-6	SS 5-3	P-1-1	SS 9-2	SS 7-1
1.	Citrate utilization	+	+	+	+	+	+	+	+	+	+
2.	Vogues Proskauer (VP)	-	+	+	+	+	-	-	+	-	+
3.	Motility	+	+	+	+	+	+	+	+	+	+
4.	Catalase	+	+	+	+	+	-	+	+	+	+
5.	Oxidase	+	+	+	+	+	+	+	+	+	+
6.	TSI	+	+	+	+	+	-	-	-	-	+
7.	Methyl Red (MR)	+	-	-	-	-	-	+	-	-	+
8.	Lysine Decarboxylase	+	-	-	-	-	+	+	-	+	-
9.	Ornithine Decarboxylase	+	-	-	-	-	+	+	-	+	-
10.	Nitrate Reduction	-	-	-	-	+	+	-	-	+	-
11.	KOH Test	+	-	-	+	+	+	+	+	+	+

+ Positive test, - Negative test

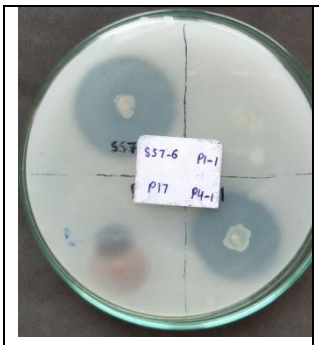
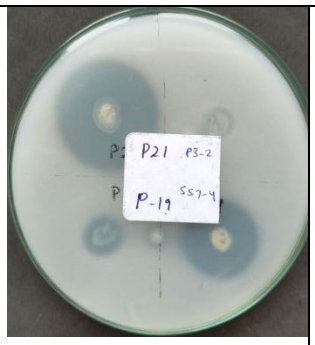
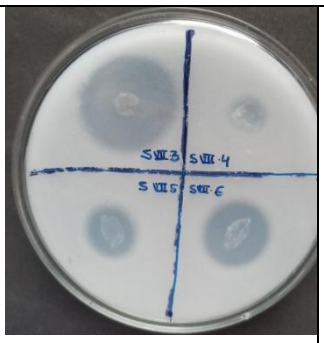
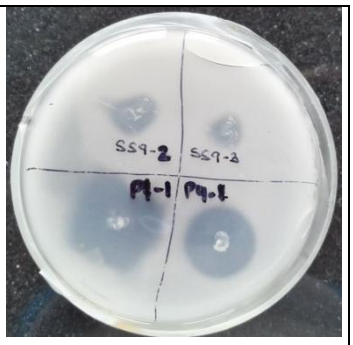
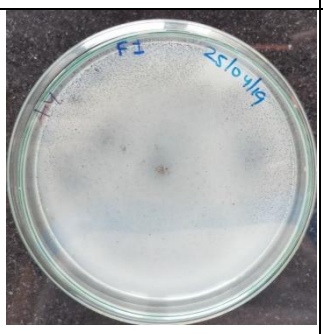
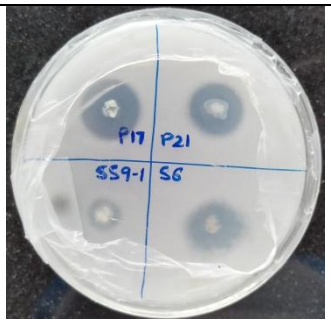
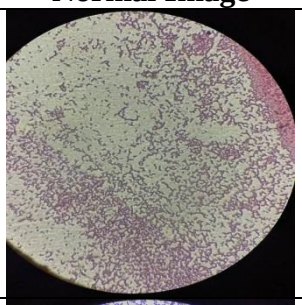
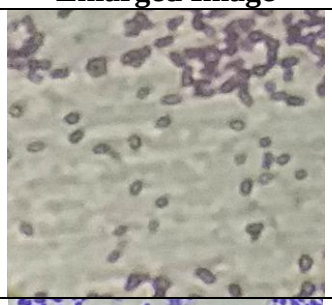
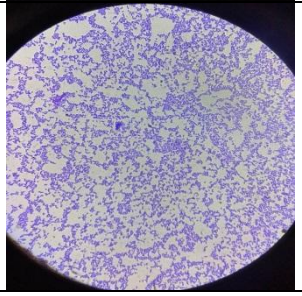
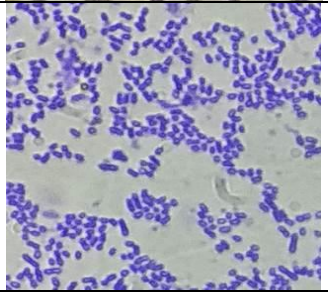
			
In clockwise direction isolates are SS7-6, P1-1, P4-1, P17	In clockwise direction isolates are P21, P3-2, SS 7-4, P-19	In clockwise direction isolates are SS 7-3, SS 7-4, SS7-6, SS 7-5	In clockwise direction isolates are SS9-2, SS9-3, SS16-1, P-1-1
			
Isolate F-3 (*Penicillium sp*)	Isolate F-2 (*Aspergillus sp)*	Isolate F-1 (*Aspergillus sp)*	In clockwise direction isolates are P-17, P-21, S6, SS9-1

Figure.1 Potassium solubilization by different isolates on Aleksandrow agar medium

S.no.	Isolates Code	Normal Image	Enlarged Image
1.	**SS 7-6**		
2.	**SS-13**		

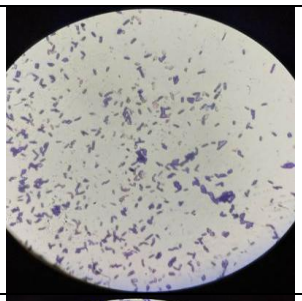
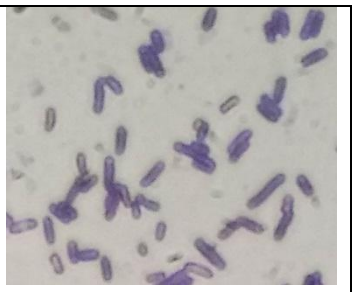
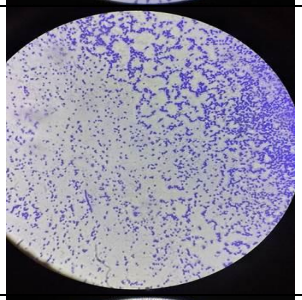
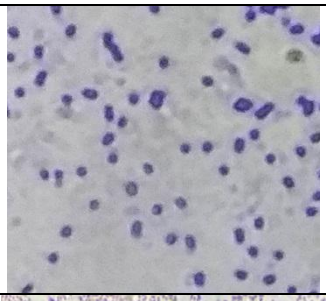
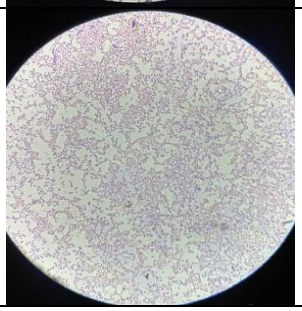
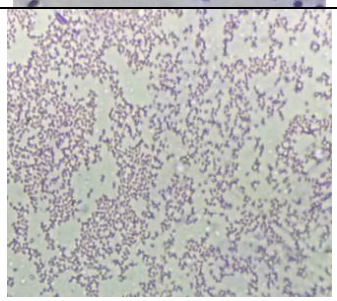
3.	**P-21**		
4.	**SS7-7**		
5.	**P 1-1**		

Figure.2 Microscopic characteristics and gram staining of different K solubilizing isolates

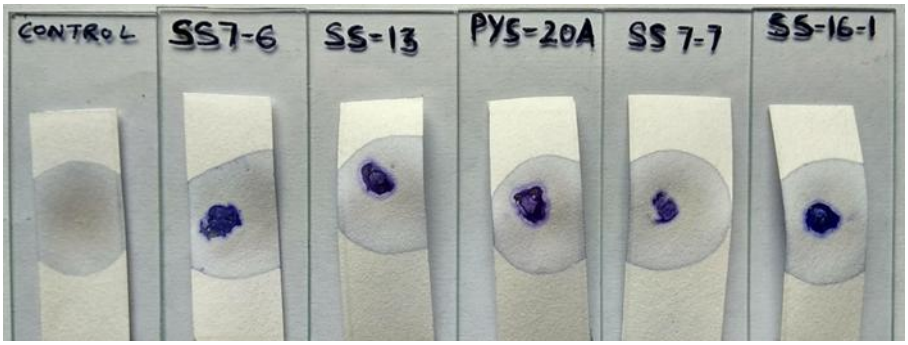
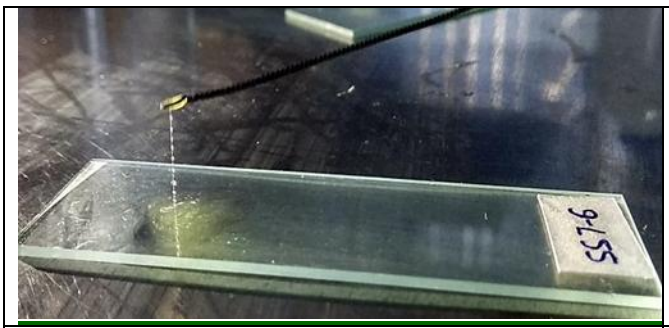
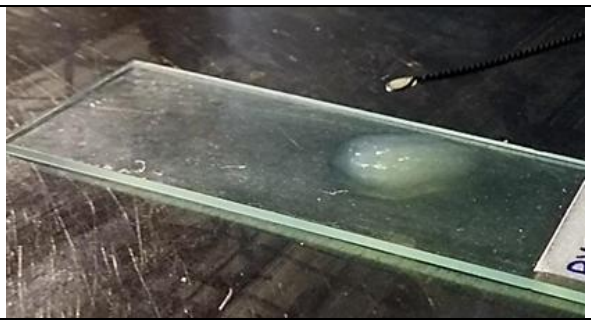
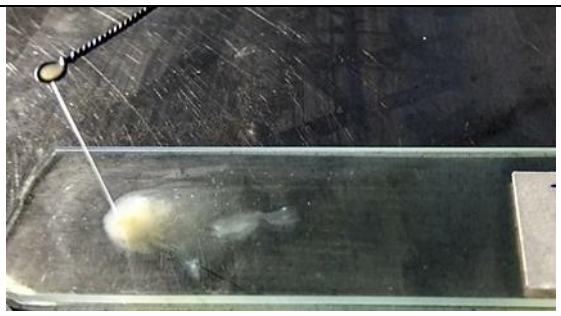


Figure.3 Oxidase test for different KSB Isolates

	
Isolate SS7-6	Isolate SS-13



Isolate P-21



Isolate P-1-1

Figure.4 Potassium Hydroxide Test for KSB Isolates

It was observed that out of 28 isolates, Sample code namely SS 7-6, SS-13, PYS 20A, SS 7-7, P-4-1, SS 7-1, SS-17, SS 9-1, PYS-1, SS 7-5, SS 7-8, SS 5-1, SS 7-4, PYS-7C1, SS 7-8, SS 15-1, SS-18, SS 16-2, SS 15-2 showed positive results. Sample code namely S-6, SS 5-3, P-1-1, SS 9-2, SS 9-3, PYS-1, SS-14, SS 5-2, SS-6, SS-19 and SS 5-4 were not able to ferment any sugar. It was observed that none of the isolates produced hydrogen sulphide or any of the gas (CO_2/H_2). Sample code namely SS-13, P-21, SS 7-7, P-4-1, SS -17, SS 9-1, SS 5-1, SS 7-4, PYS-7C1, SS 15-1, SS-18, SS 16-2 and SS 15-2 were able to ferment only glucose because the color of the butt was observed to be yellow while slant remained red.

For methyl red test positive result was indicated by appearance of stable red color in the medium after the addition of methyl red indicator. Out of 10 isolates, sample code namely SS 7-6, SS 7-1 and SS 5-3 showed positive result. Sample code namely SS-13, P-21, SS 7-7, P-4-1, SS 9-2, P-1-1 and S-6 showed negative results. For lysine decarboxylase test if the inoculated medium is yellow, or if there was no color change, the organism was decarboxylase-negative for the amino acid. If the medium turns purple, the organism was decarboxylase-positive for amino acid. Out of 10 isolates namely SS 7-6, S6, SS 5-3, SS 9-2 showed positive result and SS-13, P-21, SS 7-7, P-4-1, P-1-1, SS 7-1 showed negative result.

For ornithine decarboxylase test if the inoculated medium was yellow, or if there was no color change, the organism was decarboxylase-negative for the amino acid. If the medium turns purple, the organism was decarboxylase-positive for amino acid. Out of 10 isolates namely SS 7-6, S6, SS 5-3, SS 9-2 showed positive result and SS-13, P-21, SS 7-7, P-4-1, P-1-1, SS 7-1 showed negative result. For nitrate reduction test appearance of red color indicated positive result. Out of 10 isolates, sample code namely P-4-1, S-6, and SS9-2 showed light red color and sample code SS 7-6, SS-13, P-21, SS 7-7, SS 5-3, P-1-1 and SS 7-1 showed negative result.

For Potassium hydroxide test if smear of organism become thick, stringy and form long strands within the first 30sec, it was seen as positive result for Gram negative bacteria. If organism leaves the suspension unaltered or absence of stringing, this was seen as indication for Gram positive bacteria. Eight out of 10 isolates showed positive result with long strand formation indicating gram negative bacteria and 2 isolates namely P-21 and SS 13 showed no strand formation; hence they were gram positive bacteria as shown in fig. 4.

The potassium fertilizer currently used in agriculture requires a greater input that makes them unaffordable by the farmers of developing nations. Since most soils are deficient in plant-available potassium and

chemical fertilizers are not cost-effective, scientists thus have a responsibility for society to find ways and means of making natural potassium resources available to crops, as an economically efficient substitute for expensive chemical fertilizers. Keeping the above in view it will be of crucial importance to identify other efficient rhizosphere competent bacteria (RCB) or soil microorganisms responsible for higher potassium-solubilizing ability. In current studies K solubilizing isolates namely SS 7-6, SS-13, P-21, SS 7-7 and P-4-1 could be further tested for their use as bio inoculants for various field crops.

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