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Research Article

ISOLATION AND CHARACTERIZATION OF POTASSIUM SOLUBILIZING BACTERIA FROM CERAMIC INDUSTRY SOIL

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ABSTRACT
Potassium is vital component of plant nutrition package limiting crop yield and Quality that performs a multitude of important biological functions to maintain plant growth, Isolation of potassium solubillizers was carried out using feldspar (insoluble potassium) from the soil samples of ceramic industries, on Aleksandrow's agar medium. From the 14 isolated bacterial isolates 5 bacterial strains were selected which exhibiting highest potassium solubilization on solid medium and characterized on the basis of cultural, morphological and biochemical characteristics. Solubilization of potassium from the potassium aluminium silicate minerals by the selected bacterial strains resulted to the action of different organic acids like Citric, Oxalic, Malic, succinic and Tartric acid.

Key Words: *Nutrition, Multitude, Solubilization, Feldspar,*

INTRODUCTION
Among the Nitrogen (N), Phosphorus (P) and Potassium (K), Potassium is the third important plant nutrient. Potassium is essential macronutrient for plant growth and plays significant roles in activation of several metabolic processes including Protein synthesis, Photosynthesis, Enzymes, as well as in Resistance to diseases and insects etc (Rehm and Schmitt, 2002). Potassium though present in as abundant element in soil or is applied to fields as natural or synthetic fertilizers, only one to two percent of this is available to plants, the rest being bound with other minerals and therefore unavailable to plants. The most common soil components of potassium, 90 to 98%, are feldspar and mica (McAfee, 2008). Soil microorganisms influence the availability of soil minerals, playing a central role in ion cycling and soil fertility (Bin Lian *et al*., 2010). Very little of this potassium source is available for plant use. Silicate bacteria were found to resolve potassium, silicon and aluminum from insoluble minerals (Alexander, 1985).Their uses as biofertilizers or biontrol agents for agriculture improvement and environmental protection have been a focus of recent research.
Certain bacteria are capable of decomposing alumino silicate minerals and releasing a portion of the potassium contained therein (Biswas and Basak, 2009). A detailed understanding of how bacteria affect mineral dissolution rates is essential to quantify mineral weathering on global element cycling (Xiufang, Hu Jishuang and Jiangfeng, 2006).

MATERIALS AND METHODS
Sample Collection
Majorly the ceramic industries are using insoluble source of potassium i.e. feldspar as their raw material so Samples were collected from the various areas of insoluble potassium mineral containing soils from the ceramic industries nearby Kadi, Kalol and Himmatnagar region.
Adaptation and Enrichment
Collected soil samples were mixed with insoluble potassium (Feldspar) and incubated for 1 week at room temperature. After adaptation 1 gm of soil was inoculated in 100 ml liquid medium containing 1% glucose, 0.05%yeast extract and 0.5% feldspar and incubated at 37^0 C on 120 rpm for 1 week.
Isolation and Screening of Potassium Solubilizing Microbes

Enriched samples were inoculated after serial dilution up to 10^{-6} on Aleksandrov agar medium constituted 1% glucose, 0.05% $MgSo_4.7H_2O$, 0.0005% $FeCl_3$, 0.01% $CaCO_3$, 0.2% $CaPo_4$ and 0.5% potassium aluminium silicate, agar 3 % pH-6.5(Sugumaran and Janartham, 2007) and incubated at 37 ^{0} C for 1 week and incubated at 37^0c for 1 week. Colonies exhibiting clear zone of potassium solubilization were selected as potassium solubilizers from the 10^{-4} , $10^{-5}$and 10^{-6} dilutions containing paltes. Secondary Screening was carried out on the basis of study of zone activity of the different isolates by using Khandeparkar's selection ratio.

Ratio = D/d = Diameter of zone of clearance / Diameter of growth

Characterization of Potassium Solubilizing Bacterial Isolates

Bacterial isolates were characterized using different cultural, microscopical and biochemical characteristics. In addition to other studies including various enzymes production, Antibiogram pattern, pH optima, temperature optima, growth at different NaCl concentration, different organic acids productions (Osman, 2009).

Cultural, Morphological and Biochemical Characteristics of Bacterial Strains

The isolates were cultured on Aleksandrov'S agar medium. Colony Characteristics such as size, shape, texture, consistency and transparency were examined. Gram staining,endospore staining and capsule staining were carried out for the isolates. Voges-proskauer, Catalase test,Ornithine utilization, Lysine utilization, H_2S production ,Nitrate reduction, Phenylalanine deamination test were performed (Cappuccino, 1998).Production of acids from various carbohydrates and enzymatic activities were determined as per procedure outlined by cappuccino and Sherman. Various carbohydrate utilization were also studied (John, Noel, Peter, James and Stanley, 1997).

Antibiotic sensitivity test.

Antibiotic sensitivity tests were performed according to the Kirby – Bauer method (Prescott, Harley and Kelin, 2002).

Effect of pH on Growth of K solubilizers

The effects of pH, was studied by using Nutrient broth medium and pH adjusted to the required level by 0.1 M HCl and NaOH. Medium was inoculated with 1 loopful of previously activated bacterial culture in 10 ml N.broth medium and incubated at 37^0 C for 48 hours. Then the growth was checked visually.

Effect of Temperatures on Growth of K solubilizers

The effects of temperatures was studied by using Nutrient broth medium. Medium was inoculated with 1 loopful of previously activated bacterial culture in 10 ml N.broth medium and incubated at different Temperatures for 48 hours. Then the growth was checked visually.

Effect of NaCl concentration on Growth of K solubilizers

The effects of NaCl concentration was studied by using N.broth medium with different NaCl concentration. Medium was inoculated with 1 loopful of previously activated bacterial culture in 10 ml N.broth medium and incubated at 37^0 C for 48 hours. Then the growth was checked visually.

Detection of Organic acids produced by bacterial isolates

Organic acids identification was performed by using solvent system n- butanol: Acetic acid: Water (4:1:5) and Developing Reagent 0.04% Bromocresol Green in Alcohol from the 1/10 th volume concentrated supernatant of Aleksandrov broth culture incubated at 30 ^{0} C for 10 days (Isherwood and Hanes, 1953).

Potassium solubilisation and identification of best solubilizer

The all five isolates were grown on K enriched 100 ml broth containing 1% Glucose, 0.05% yeast extract, 0.5% feldspar, pH-6.5 and incubated on shaking condition at 30^0 C on 120 rpm for 7 days by using two insoluble source of potassium feldspar. After each 24 hours the potassium released were determined by using Sodium cobaltinitrite and Folin-Ciocalteu Phenol reagent (Abul Fadl, 1948)**.** The higher potassium solubilizing strain was identified using standard cultural, morphological and biochemical methodology, but its identity was re-evaluated by 16S rRNA gene sequence analysis.

RESULTS AND DISCUSSION:

Isolation and screening

Colonies exhibiting zone of clearance indicating Potassium solubilization were selected.
The colonies were selected which were morphologically distinct. Total 14 bacterial isolates were isolated as potassium solubilizers and named as KSB1 to KSB14.(Table-1).

Table-1: Potassium solubilization values of bacterial isolates by Khandeparkar's selection ratio.

Isolates	Diameter of zone of clearance (D) mm	Diameter of growth (d) mm	D/d (ratio)
KSB1	7	6	1.66
KSB2	8.5	7	1.21
KSB3	10	7.5	1.33
KSB4	7.5	6	1.25
KSB5	6	5	1.20
KSB6	12	10	1.20
KSB7	13	9	1.44
KSB8	11	7	1.57
KSB9	8.5	7	1.21
KSB10	6.5	5	1.3
KSB11	9	7	1.28
KSB12	10.5	9	1.16
KSB13	7.2	6.9	1.04
KSB14	6.5	6.1	1.06

From that isolates five bacterial KSB 1, KSB 3 KSB 7 , KSB 8, and KSB 11 isolates exhibiting higher ratio of clear zone of potassium solubilization by Khandeparkar's selection ratio were selected.

Cultural, Microscopical and Biochemical Characteristics

The morphological and the Biochemical characteristics of the 5 good Potassium solubilizers are presented in Tables 2. Most of these isolates are aerobic, capsulated, motile and 2 of them are Gram positive while 3 are gram negative. The biochemical characters are studied for the five isolates of potassium solubilizing bacteria presented in (Table-2).

Table-2: Colonical, Morphological And biochemical characteristics of best potassium solubilizing bacterial isolates.

Isolates	Colony characters	Morphological characters								
		Gram reaction & cell shape	Spore Formation	Capsule Formation	Motility	Biochemical tests				
						1	2	3	4	5
KSB 1	Small Creamy, transparent, smooth, Raised,	-ve, Small rod	Non sporulating	Non capsulated	Motile	-	+	-	+	+
KSB 3	Large,White,opaque, smooth circular, Slimy,	+ve, Thick rod	Sporulating	Capsulated	Motile	+	-	-	-	-
KSB 7	Large,White,opaque,smooth circular, Slimy.	+ve, Thick rod	Sporulating	Capsulated	Motile	-	-	-	-	-
KSB 8	Medium,creamy,opaque, smooth, spreading, gummy.	-ve, Small rod	Non sporulating	Capsulated	Motile	+	+	-	+	+
KSB11	Medium,creamy,opaque, smooth, spreading, gummy	-ve, Small rod	Non sporulating	Capsulated	Motile	+	+	-	+	+

1-V-P test, 2- Ornithine utilization, 3- H_2S Production, 4- Lysine utilization,5- Citrate utilization.

Out of five selected bacterial strains three are gram negative while two are gram positive bacteria. All are motile and four of them are capsule forming organisms.

KSB 8 and KSB 11 shows V-P, ornithine utilization,lysine utilization and Citrate utilization tests positive while KSB 7 gives all the five test negative. KSB 1 utilize ornithine, lysine and Citrate. All the five bacterial strains do not produce H_2S.

Total nine different enzymes activity and eleven various carbohydrate sources were checked for all the five bacterial strains. Among them KSB 3 and KSB 7 shows a wide range of carbohydrate utilization while KSB 1 only able to utilize Glucose as a carbon source.

Table-3: Enzymatic Activity and various carbohydrates utilization by potassium solubilizers.

Isolates	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
KSB 1	-	+	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
KSB 3	+	+	-	-	-	+	+	-	-	+	-	+	+	-	+	+	+	+	+	+
KSB 7	+	+	-	-	-	+	+	-	-	+	-	+	+	-	+	+	+	+	+	-
KSB 8	-	-	-	+	+	-	+	-	+	+	-	-	+	+	+	+	+	-	-	-
KSB11	-	-	-	+	+	-	+	-	+	+	-	-	+	+	+	+	+	-	-	-

1-Amylase, 2- Protease, 3- Lipase, 4- Urease, 5- Catalase, 6- Alkaline phosphatise, 7- β –galactosidase, 8- Phenylalanine deaminase, 9- Nitrate reducase, 10- Glucose, 11- Adonitol, 12- Lactose, 13- Arabinose, 14- Sorbitol, 15- Sucrose, 16- Mannitol, 17- Trehalose, 18- Maltose,19- Raffinose.

Table-4: Antibiogram pattern of potassium solubilizing bacteria.

Antibiotics	**Concentration µg/ml**	**Strain KSB1**	**Strain KSB3**	**Strain KSB7**	**Strain KSB8**	**Strain KSB11**
Ampicillin	10	18 (R)	-	-	-	-
Ciprofloxacin	10	26 (S)	18 (S)	22 (S)	24 (S)	24 (S)
Colistin	10	16 (I)	16 (I)	12 (R)	10 (R)	10 (R)
Co-Trimoxazol	25	-	16 (S)	16 (S)	22 (S)	22 (S)
Gentamicin	10	24 (S)	18 (S)	22 (S)	18 (S)	16 (S)
Nitrofurantoin	300	-	17 (S)	24 (S)	-	-
Streptomycin	10	14 (I)	-	-	16 (I)	16 (I)
Tetracyclin	30	12 (R)	28 (S)	24 (S)	-	-
Amikacin	10	-	18 (S)	17 (S)	20 (S)	14 (R)
Amoxycillin	10	-	20 (S)	20 (S)	-	12 (R)
Bacitracin	10 U	-	16 (I)	-	-	-
Cephalothin	30	-	14 (R)	16 (I)	-	-
Erythromycin	15	-	24 (S)	20 (S)	-	-
Novoblocin	30	-	24 (S)	22 (S)	8 (R)	-
Oxytetracyclin	30	18 (I)	24 (S)	-	-	-
Vancomycin	30	-	20 (S)	22 (S)	-	-

(S) Susceptible, (R) Resistant, (I) Intermediate

Various 16 antibiotics effect were checked for all the bacterial strain all the isolates having various effects against the different antibiotics which shown in (Table-4).

Table-5: Growth of isolates on different PH levels.

Sr. No.	pH	Strain KSB1	Strain KSB3	Strain KSB7	Strain KSB8	Strain KSB11
1	2	-	-	-	-	-
2	4	-	-	-	-	-
3	5	+	++	++	++	++
4	6	+	++	++	++	++
5	7	++	++	++	+++	+++
6	8	+	++	-	++	++
7	10	-	+	-	-	-
8	12	-	-	-	-	-

- *No Growth, + Slight growth, ++ Moderate growth, +++ Vigorous growth*

Table- 6: Growth of l isolates on different temperatures.

Sr No.	Temp	Strain KSB1	Strain KSB3	Strain KSB7	Strain KSB8	Strain KSB11
1	5^0C	-	-	-	-	-
2	10^0C	-	+	-	-	-
3	25^0C	+++	++	++	+++	+++
4	30^0C	+++	+++	+++	+++	+++
5	35^0C	+++	++	++	+++	+++
6	40^0C	+	+	+	-	-

- *No Growth, + Slight growth, ++ Moderate growth, +++ Vigorous growth*

Table-7: Growth of isolates on different NaCl concentrations.

Sr. No.	NaCl Concentration	Strain KSB1	Strain KSB3	Strain KSB7	Strain KSB8	Strain KSB11
1	1 %	+++	+++	+++	+++	+++
2	2 %	++	++	+	++	++
3	4 %	+	+	+	+	+
4	5 %	+	+	+	+	+
5	7 %	-	+	-	-	-
6	10 %	-	-	-	-	-

-*No Growth, + Slight growth, ++ Moderate growth, +++ Vigorous growth*

All the five selected isolates were checked at various pH, temperatures and NaCl concentrations, among them KSB 3 having a wide range of pH and NaCl concentrations as compare to other isolates. While KSB 1, KSB 3 and KSB 7 able to grow up to 40^0 C and KSB 3 can grow at $10\ ^0$ C also. KSB 3 can grow at wide range of temperature, pH as well as in higher saline environment as compare to the other isolates (Table-5, 6, 7).

Organic acids productions

All the five bacterial isolates were solubilize the potassium from the insoluble source of potassium i.e. feldspar through the acid production and oxalic and citric acid is likely involved in potassium solubilization (Table-8).

Table-8: Differnt organic acids produced by isolates.

Sr. No.	Isolates	Oxalic Acid	Citric Acid	Malic Acid	Succinic Acid	Tarteric Acid
1	KSB 1	+	+	-	+	-
2	KSB 3	+	+	+	-	-
3	KSB 7	+	+	-	+	-
4	KSB 8	+	+	-	-	-
5	KSB 11	+	-	-	-	-

Potassium solubilization and identification of best solubilizer

Among the all bacterial strains KSB 8 shows highest potassium solubilization when tested on liquid media and release the higher amount of potassium from the insoluble source of feldspar when determined by colorimetric method. From the cultural morphological and finally by 16S rRNA gene sequencing the KSB 8 is identified as *Enterobacter hormaechei.*

Conclusion

After secondary screening five bacterial isolates were selecte out of 14 isolates. Among them KSB1, KSB8 and KSB11 are gram negative, short rods while KSB3 and KSB7 are gram positive bacillus central spore formers. Except KSB 1 all the bacterial isolates are higher exopolysaccharide producers Among 5 bacterial strains KSB3 and KSB7 which are Gram Positive bacilli are utilizing wide range of the carbohydrate sources while KSB1 only capable of utilizing Glucose as a sole source of carbon. All the bacterial strains having various enzymatic activity as well as different Biochemical characterization.KSB3 having a high capacity to grow in saline condition and pH range. Oxalic acid and citric acids were likely to be involved in the solubilization of feldspar among the all 5 acids. So these all characteristics make these bacterial strains to solubilize the potassium. The best potassium solubilizing bacterial strain is identified as *Enterobacter hormaechei* from cultural, morphological and 16S rRNA gene sequencing.

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REFERENCES

Abul Fadl MAM (1948). Colorimetric Determination of Potassium by Folin-Ciocalteu phenol reagent. Postgraduate medical school London. *Biochemistry Journal* **44**(3) 282-285.

Alexander M (1985). Introduction to Soil Microbiology. John Wiley and Sons Inc., NewYork, USA 382-385.

Bin Lian, Bin Wang, Mu Pan, Congqiang Liu and H. Henry Teng (2010). Microbial release of potassium from K-bearing mineralsby thermophilic fungus Aspergillus fumigatus. *Geochimica et Cosmochimica Acta* **72** 87–98.

Biswas DR and Basak BB (2009). Influence of potassium solubilizing microorganism (Bacillus mucilaginosus) and waste mica on potassium uptake dynamics by sudan grass (Sorghum vulgare) grown under two Alfisols. *Plant Soil Environment Journal* **317** 235-255.

Cappuccino JG (1998). *Microbiology – A Laboratory Manual* 7 th Edition Pearson Education. Published by Dorling Kindersley (India) Pvt. Ltd.

Isherwood FA and Hanes CS (1953). Separation and estimation of organic acids on paper chromatograms. *Biochemistry Journal* **55**(5) 824-830.

John GH, Noel RK, Peter HS, James TS and Stanley TW (1997). Bergey´s Manual of Determinative Bacteriology (In Russian) 9th Edition. Moscow, Mir Publishers **2**.

McAfee J (2008). Potassium, A Key Nutrient for Plant Growth. Department of Soil and CropSciences http://jimmcafee.tamu.edu/files/potassium.

Osman AG (2009). Study of Some Characteristics of Silicate Bacteria. *Journal of Science and. Technology* 27-35.

Prescott LM, Harley JP and Kelin DA (2002). *In Antimicrobial Chemotherapy*: Kelvin K, Editor Microbiology, 5th edition England. Wm. C Brown Publisher 805-813.

Rehm G and Schmitt M (2002). *Potassium for crop production*. Retrieved February 2, 2011, from Regents of the University of Minnesota website: http://www.extension.umn.edu/distribution/cropsystems/dc6794.html.

Sugumaran P and Janartham B (2007). Solubilization of potassium minerals by bacteria and their effect on plant growth. *World Journal of Agricultural Sciences* **3**(3) 350-355.

Xiufang Hu, Jishuang C and Jiangfeng G (2006). Two phosphate- and potassium-solubilizing bacteria isolated from Tianmu Mountain Zhejiang, China. *World Journal of Microbiology & Biotechnology* **22** 983-990.