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Isolation and identification of highly effective potassium solubilising bacterial strain from coastal soils

Simmanna Nakka, Uday Bhaskar Sajja

Department of Biotechnology, Dr.B.R. Ambedkar University, Etcherla, Andhra Pradesh, India.

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Abstract

Soil potassium supplementation relies heavily on the use of chemical fertilizer, which has a considerable negative impact on the environment. Potassium-solubilizing bacteria (KSB) could serve as inoculants. They convert insoluble potassium in the soil into a form that plants can access. This is a promising strat-egy for the improvement of plant absorption of potassium and so reducing the use of chemical fertilizer. In order to overcome poor potassium availability, potassium solubilising microorganisms can be used to ensure agriculture production. Hence, the study was aimed to isolate, identify, and characterise a potent bacterial species from coastal soils that can efficiently solubilise phosphate for plant absorption. From the five soil samples, a total of eight different discrete bacterial colonies were identified and they were named as MGK-01, MGK-02, MGK-03, MGK-04, MGK-05, MGK-06, MGK-07 and MGK-08. The screening of potassium solubilisation demonstrates that the greatest potassium solubilisation ability was found in the MGK-06 strain. Furthermore, the greatest potassium solubilisation under increasing NaCl concentrations were found in the MGK-06. Due to better potassium solubility and plant growth promoting activities, bacterial isolate MGK-06 was selected for molecular identification and bioinoculation studies. The molecular phylogeny revealed that the bacterial isolate MGK-06 is belongs to *Azotobacter chrococcum*species.

Keywords: Potassium-solubilizing bacteria, bioinoculation studies, *Azotobacter chrococcum*species.

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***Corresponding Author**

Simmanna Nakka

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Introduction

With increasing population food requirement also increases, thus putting pressure on more yields in agriculture. To meet these requirements, sustainable agricultural practices should be adopted. One of the significant challenges in agriculture is replacing the nutrients that are consumed by the crops. Plant growth will gradually decrease with the lack of essential nutrients. For improving the soil nutrients farmers have become dependent on chemical fertilizers [1]. Even though their usage improved the plant growth , mainly phosphorous, nitrog- enous, and potassic fertilizers have harmful effects on the environment (Adesemoye and Kloepper, 2009). As we all know potassium (k) is the key element in regulating plant growth. It promotes the activation of enzymes, the utilization of nitrogen (N), and the synthesis of protein and sugar. It also boosts plant photosynthesis [2]. Potassium deficiency causes incomplete development of the root system and effects its growth [3].

The chemical grade potassium effects the environment and its deficiency effects the plant growth, so the only solution to tackle the situation is exploiting the potassium reserves in soil properly. Soil has rich reserves of K, among which only 1–2% can be directly absorbed by plants [4]. About 90 - 98% of the soil potassium exists as silicate minerals [5]. Soil is both potassium rich and deficient, as potassium is present in the soil but is converted slowly into available forms. Studies have revealed that certain microbes are capable of producing organic acids which solubilize the rock and release potassium ions into soil [6, 7]. Some studies

have shown that the application of potassium-solubilizing bacteria (KSB) and K bearing minerals increases the amount of available K in the soil and promotes plant uptake of K [8, 9, 10,] Using the KSB to improve the available concentration of potassium in soil is more eco-friendly than using chemical fertilizers [13].

Materials and methods

Sample collection

Sample collection for isolation of potassium solubilising bacteria

Rhizospheric soil samples were collected from five cereal forming areas of Andhra Pradesh state that covers four districts such as East Godavari, West Godavari, Visakhapatnam, and Vizianagaram. Free rhizospheric soil samples collected from 6 to 10 cm soil layer, where roots were concentrated. From about 0 to 2.5 cm away from the root surface, a zone of soil is located that is significantly influenced by bacterial population and is referred as rhizosphere. Rhizosphere soil and roots were separated by hand. The samples were collected in order to identify the potassium solubilising bacteria and to screen their potassium solubilising capabilities as well as the plant growth promoting activity on rice plants.

All the soil samples were taken from the top 5 to 10 cm of soil profile, where maximum microbial activity occurs, and therefore where the majority of the bacterial population is concentrated. Approximately, 100 gm of soil sample was collected from each site by using clean, dry, and sterile plastic containers along with sterile spatula, marking pen, rubber band, and other accessories. The sampling site selection was done by considering as many different qualities as feasible in terms of organic matter, moisture content, particle size, temperature, humidity, and light in order to minimise contamination as much as possible. Samples were stored in ice boxes and transported to the laboratory, where they were kept in refrigerator at 4°C for further analysis.

Table: 1. sampling sites for isolation of Potassium solubilising bacteria

Sampling sites	Coordinates	Soil Characteristics			
		pH	Temp. (°C)	DO (mg/l)	Colour
Amalapuram	16°35' 2.19''N; 82°2' 23.33'' E	6.4	24.6	5.8	Brown
Bheemavaram	16°34' 26.47''N; 81°30' 25.49'' E	5.7	25.5	5.3	Brown
S. Kota	18°3' 5.89''N; 83o15' 21.78'' E	6.2	24.6	6.3	Brown
Simhachalam	17°46' 53.43''N; 83°14' 48.92'' E	6.4	22.7	5.9	Brown
Bobbili	18°34' 1.75''N; 83°22' 6.00'' E	5.8	21.4	6.4	Brown

Isolation of potassium solubilising bacteria (KSB)

The isolation of potassium solubilising bacteria was performed from the enriched soil bacterial culture by sub-cultured on the Aleksandrov agar medium according to the methodology of Meena et al., (2015a) with slight modifications. The Aleksandrov agar medium was prepared by dissolving 5 gm Glucose, 0.5 gm Magnesium sulphate ($MgSO_4.7H_2O$), 0.006 gm Iron (III) chloride ($FeCl_3.2H_2O$), 0.6 gm Calcium carbonate ($CaCO_3$), 2 gm Calcium phosphate ($CaPO_4$), 3 gm Mica, 30 gm agar in 1000 ml of distilled water and finally, phenol red indicator was added to the media. The pH of the medium was adjusted to 7.0 by using 0.1 M NaOH. Then, the prepared culture media was sterilised in an autoclave at 121°C for 15 minutes. After sterilisation of the medium, 1 ml of culture was spread on the Aleksandrov agar plates, and the plates were incubated at 27±2°C in an incubator for 7 days. After incubation, the phosphate solubilising bacterial colonies were identified on the basis of halo zones formed around the colonies. The morphological characteristics of phosphate solubilising colonies that grown on each plate were noted. The morphologically discrete bacterial strains were selected and purified on Aleksandrov agar medium by the recurrent streak plate technique. For the isolation of pure cultures, 7 days olds prominent colonies which exhibit clear zones around them were picked and subcultured repeatedly for 5 times on Aleksandrov agar medium. The dominant bacterial colonies with different colony characteristics were chosen to screen their potassium solubilising potentials and the best isolates were selected for further research.

Table: 2. Composition of Aleksandrov agar medium.

S. No	Material	Weight (gm/L)
1	$MgSO_4.7H_2O$	0.5
2	FeCl3.2H2O	0.006
3	$CaCO_3$	0.6
4	$CaPO_4$	2
5	Mica	3
6	Glucose	5
8	Agar	30

Phenotypic and Biochemical characterisation of the bacterial isolates

The presumptive identification of nitrogen fixing, phosphate and potassium solubilising, bacterial isolates were done by phenotypic examinations including cell and colony morphology, Gram's reactivity, sporulation, Motility. (Holt et al., 1994). Furthermore, the physiological and biochemical characterisation including sugar fermentation, IMViC (Indole, Methyl-red, Voges-Proskauer, and Citrate), catalase, nitrate reductase, and urease test were carried out for the bacterial isolates.

Phenotypic characterisation of bacterial isolates

The isolated bacterial strains were identified phenotypically based on the following methods.

1. **Colony morphology**-Colonial observations directly made from spread and streak plates that gave isolated colonies of the bacterial strains under the colony counter.
2. **Microscopic evaluation**-The microscopic evaluation was carried out to examine the bacterial strains' shape, size, organisation, gram's reaction properties and motility. Endospore staining is used to look for bacteria that might produce endospores. All of the staining procedures were performed according to the protocols illustrated in the Microbiology Laboratory Manual by Sherman and Cappuccino, (2008).

Screening of potassium solubilising ability of active bacterial isolates

The potassium solubilising bacterial isolates were screened for their potassium solubilising ability by growing pure colonies in the Aleksandrov broth according to the methodology described by Hu et al., (2006) with minor modifications. Potassium aluminosilicate was added to the medium as a source of insoluble potassium. In this experiment, 1 ml of freshly subcultured 24-hour old isolated pure cultures of bacteria was allowed to grow in 40 ml of Aleksandrov broth under constant stirring at 150 rpm for 7 days at 30°C. Autoclave Aleksandrov broth without inoculation is used as a control. After incubation, the culture broth was centrifuged at 10,000 rpm for 10 minutes, and the supernatant was collected. The supernatant was used to estimate the potassium availability quantitatively by using atomic absorption spectrophotometry. Working parameters of the atomic adsorption spectrophotometer, such as wave length and band width for potassium estimation, were adjusted to 766.5 and 0.7 respectively. The calibration curve of the potassium was made with 0.1-1.0 μM/ml concentrations of potassium chloride.

Effect of salinity on potassium solubilisation by bacterial isolates

The potassium solubilising ability of isolated bacterial strains under salt stress was screened by growing bacterial strains in the Aleksandrov broth supplemented with potassium aluminosilicate. In this experiment the all the isolated colonies of bacteria were allowed grow in Aleksandrov broth which is supplemented with five different NaCl concentrations such as 1, 2, 3, 4, and 5%. These cultures were incubated at 30ºC for 7 days in an orbital shaking incubator at 150 rpm for adequate development. After incubation, the required quantity of broth was withdrawn from each conical flask and centrifuged at 10,000 rpm for 10 minutes. The supernatant was used to estimate the potassium availability quantitatively by using atomic absorption spectrophotometry. The calibration curve of the potassium was made with 0.1-1.0 μM/ml concentrations of potassium chloride.

Amplification and sequencing of 16S rRNA gene

PCR amplification and 16S rRNA gene sequencing were carried out as described Li *et al.,* (2010). The 16S rRNA genes from the bacterial isolates such as MGN-10, MGP-04, and MGK-06 were amplified by using PCR. Two universal primers 27F (5'AGAGTTTGATCMTGGCTCAG 3') and 907R (5'CCGTCAATTCMTTTRAGTTT3') were used to amplify 16S rRNA genes. PCR reaction mixture of 25 μl total volume, containing 1/10 volume 10× *Taq* buffer, 2 mm $MgCl_2$, 1-unit *Taq* DNA polymerase, 0.2 mM dNTP, 20 pmol forward primer, 20 pmol reverse primer and 100 ng DNA. DNA amplification was carried out in a Biorad Mini thermocycler with the following procedure: an initial denaturing step at 94°C for 5 min; 40 cycles for 1 min at 94°C (denature), 1 min at 48°C (annealing), 2

min at 72°C (extension) and a final elongation step at 72°C for 5 min and then cooled to 4˚C. Amplified PCR products were separated by electrophoresis on 1.5 % agarose gel containing 0.5 μg/ml ethidium bromide, and photographed. The standard DNA samples (100 bp DNA ladder marker) were used as molecular size marker. The purified PCR products was subjected to Sanger's di-deoxy sequencing, in both forward and reverse direction ns, using Big Dye terminator v3.1 cycle sequencing kit on ABI Prism3700 DNA Analyzer (Applied Biosystems Inc., USA).

Results and discussion

Morphological and phenotypic characterisation of K-solubilising bacterial isolates

The morphological and phenotypic characterisation of K-solubilising bacterial isolates indicated that among the 8 isolates, 5 isolates, such as MGK-01, MGK-02, MGK-05, MGK-07, and MGK-08 exhibited a circular configuration, whereas the remaining 3 isolates (MGK-03, MGK-04, and MGK-06) exhibited an uneven configuration. As well as, among the 8 bacterial isolates, 6 colonies, including MGK-01, MGK-02, MGK-03, MGK-06, MGK-07, and MGK-08 appear in white. While MGK-04 and MGK-05 colonies were cream in colour. The morphological characterisation of 8 isolates by Gram's staining revealed that 4 isolates, such as MGK-01, MGK-03, MGK-04, and MGK-07 exhibit rod-shaped morphology, and remaining 4 isolates (MGK-02, MGK-05, MGK-06, and MGK-08) were cocci. Furthermore, 7 of the 8 isolates (MGK-01, MGK-02, MGK-03, MGK-04, MGK-05, MGK-06, and MGK-08) exhibited positive reaction to Gram's staining, while the remaining 1 (MGK-07) was negative for Gram's staining. The endospore was observed in only 1 isolate (MGK-05) out of the 8 bacterial isolates. Motility was observed in 3 bacterial isolates (MGK-01, MGK-02, and MGK-06) and the remaining 5 (MGK-03, MGK-04, MGK-05, MGK-07, and MGK-08) were nonmotile. The results of morphological and phenotypic characterisation of K-solubilising bacterial isolates are shown in table 3.

Table: 3. Morphological and phenotypic characterization of K-solubilising bacterial isolates

Characteristics	Bacterial isolates							
	MGK-01	**MGK-02**	**MGK-03**	**MGK-04**	**MGK-05**	**MGK-06**	**MGK-07**	**MGK-08**
Configuration	Circular	Circular	Uneven	Uneven	Circular	Uneven	Circular	Circular
Colony colour	White	White	White	Cream	Cream	White	White	White
Surface	Chalky	Chalky	Smooth	Chalky	Smooth	Smooth	Chalky	Smooth
Grams Reaction	Positive	Positive	Positive	Positive	Positive	Positive	Negative	Positive
Shape	Rods	Cocci	Rods	Rods	Cocci	Cocci	Rods	Cocci
Spore	-	-	-	-	+	-	-	-
Motility	+	+	-	-	-	+	-	-

Physiological and Biochemical characterisation of K-solubilising bacterial isolates

In the present study, among the eight isolates of K-solubilising bacteria, two bacterial isolates such as MGK-02 and MGP-03 were positive for the indole test. The remaining six isolates, MGK-01, MGK-04, MGK-05, MGK-06, MGK-07, and MGK-08 were indole negative. For MR test, three isolates such as MGK-03, MGK-07, and MGK-08 exhibit positive, whereas, the remaining 5 isolates were negative. The VP test, determines that among the eight isolates, four isolates such as MGK-03, MGK-04, MGK-05, and MGK-08 showed positive. In the current study, among the eight bacterial isolates, seven isolates such as MGK-02, MGK-03, MGK-04, MGK-05, MGK-06, MGK-07, and MGK-08 were tested as positive for citrate utilisation. The catalase assay demonstrates that the bacterial isolates such as MGK-01, MGK-02, MGK-03, MGK-05, MGK-06, MGK-07, and MGK-08 possess catalase among eight isolates.

The results of urease test demonstrated that four isolates such as MGK-02, MGK-05, MGK-06, and MGK-08 have urease enzyme. The oxidase test revealed that among the eight isolates, five isolates such as MGK-01, MGK-03, MGK-04, MGK-06, and MGK-07 contains oxidase enzyme. The nitrate reductase assay determines that, seven bacterial isolates among the eight isolates, such as MGK-01, MGK-02, MGK-03, MGK-04, MGK-05, MGK-06, and MGK-07 possess an enzyme nitrate reductase. Whereas, the starch hydrolysis assay determines that, four bacterial isolates among the eight isolates, such as MGK-02, MGK-04, MGK-06, and MGK-07 have amylase enzyme.For lipid hydrolysis test, among the eight isolates, five isolates such as MGK-02, MGK-04, MGK-05, MGK-06, and MGK-08 were tested as positive that indicates the presence of lipases. The tests of casein hydrolysis demonstrates that four isolates such as MGK-01, MGK-04, MGK-05, and MGK-06 were tested positive for the presence of protease. The tests of gelatinase hydrolysis demonstrates that five isolates such as

MGK-02, MGK-03, MGK-05, MGK-06, and MGK-07 were tested positive for the presence of gelatinase. The results of physiological and biochemicalcharacterisation of K-solubilising bacterial isolates are shown in table 4.

Table: 4. Physiological and biochemicalcharacterisation of K-solubilising bacterial isolates

Test Name	Bacterial isolate names							
	MGP-01	**MGP-02**	**MGP-03**	**MGP-04**	**MGP-05**	**MGP-06**	**MGP-07**	**MGP-08**
Indole	-	-	+	+	-	-	-	-
Methyl Red	-	-	+	-	-	-	+	+
Voges-Proskauer	-	-	+	+	+	-	-	+
Citrate	-	+	+	+	+	+	+	+
Catalase	+	+	+	-	+	+	+	+
Urease	-	+	-	-	+	+	-	+
Oxidase	+	-	+	+	-	+	+	-
Nitrate reductase	+	+	+	+	+	+	+	-
Starch hydrolysis	-	+	-	+	-	+	+	-
Lipid hydrolysis	-	+	-	+	+	+	-	+
Casein hydrolysis	+	-	-	+	+	+	-	-
Gelatin hydrolysis	-	+	+	-	+	+	+	-

The indole test determines an organism's ability to breakdown the amino acid tryptophan. The existence of indole when bacteria is cultured in a tryptophan-rich medium shows that the organism can break down tryptophan. It is employed as a part of the IMViC assays, which are a series of tests intended to discriminate between members of the Enterobacteriaceae family. The detection of indole in the media is based on the chemical reaction that occurs in acidic conditions involving indole and p-dimethylaminobenzaldehyde (DMAB) to form the red dye rosindole (Winn et al., 2006). Methyl Red (MR) is a pH indicator that gives a red colour when pH is less than 4.4. The methyl red test is used to determine an organism's capacity to make and retain as a final product of glucose fermentation. Several bacterial species can synthesise acids in higher amounts from glucose by fermentation, and these acids surpass the bacterial system's buffering effect. Generally, glucose can synthesise numerous organic acids such as lactic acid, acetic acid, succinic acid, and formic acid in mixed acid fermentation. As a result of high acid accumulation during mixed acid fermentation, the pH of the phosphate buffer in the media will fall to less than 4.4. When the pH is less than 4.4, the culture tubes containing the pH indicator methyl red turn red colour. If the methyl red becomes yellow, the pH is greater than 6.0 and it indicates mixed acid fermentation is not employed. The Voges-Proskauer test is used to determine the presence of acetoin, a precursor of 2,3 butanediol, hence it is used to identify the organism that synthesis acetoin via the butylene glycol pathway. The acetoin is oxidised to diacetyl upon the addition of potassium hydroxide (KOH), and VP reagents to the MR-VP broth that has been inoculated with bacterial culture that employs butylene glycol pathway. Diacetyl then combines with oxygen to generate red colour. If the culture contains acetoin, it will change colour from reddish-brown to pink. If the culture is acetoin-free, it will change colour from brownish-green to yellow. The citrate test is used to identify if a microbe could use citrate as its only source of carbon. The synthesis of enzyme citrase by bacteria involved in the breakdown of citrate into oxaloacetate and acetate resulted in a blue colour in the culture tubes. Oxaloacetate is broken further to produce pyruvate and carbon dioxide. The alkaline pH of the media is attributed to the production of sodium bicarbonate (NaHCO3) and ammonia (NH3) from sodium citrate and ammonium salts. Aerobic bacteria use citrate because they have a complete tricarboxylic acid cycle and just need a citrate adsorption mechanism to use it. The catabolism of citrate by anaerobic bacteria such as *Klebsiella pneumoniae*, *Escherichia coli* (Bott, 1997) and *Lacticacid bacillus* (Drider et al., 2004) uses an enzyme, citrate lyase that cleaves citrate into oxaloacetate and acetate (Schneider et al., 2000).

Screening of K-solubilising ability of KSB isolates

In the present study, all the isolated bacterial strains showed significant potassium solubilisation with increased incubation times. The results of potassium solubilising abilities for eight isolated bacterial strains are shown in table 5. From these results, the greatest potassium solubilisation ability was found in the MGK-06 strains, whereas MGK-08 had

the lowest potassium solubilisation ability. The isolates, MGK-04, and MGK-07 have moderate potassium solubilising ability.On the third day of incubation, the potassium solubilisation of eight bacterial isolates such as MGK-01, MGK-02, MGK-03, MGK-04, MGK-05, MGK-06, MGK-07, and MGK-08 was observed to be 8.87, 14.8, 10.6, 22.5, 12.7, 28.9, 19.5, and 7.4 µg/ml respectively. On the sixth day of incubation, the potassium solubilisation of eight bacterial isolates such as MGK-01, MGK-02, MGK-03, MGK-04, MGK-05, MGK-06, MGK-07, and MGK-08 was observed to be 15.8, 19.4, 13.5, 28.4, 16.2, 33.7, 23.7, and 14.7 µg/ml respectively. On the ninth day of incubation, the potassium solubilisation of eight bacterial isolates such as MGK-01, MGK-02, MGK-03, MGK-04, MGK-05, MGK-06, MGK-07, and MGK-08 was observed to be 21.8, 28.1, 18.4, 29.7, 19.5, 48.5, 29, and 18.6 µg/ml respectively. On the 12th day of incubation, the potassium solubilisation of eight bacterial isolates such as MGK-01, MGK-02, MGK-03, MGK-04, MGK-05, MGK-06, MGK-07, and MGK-08 was observed to be 22.9, 33.7, 19.1, 33.2, 22.6, 56.2, 33.2, and 22.4 µg/ml respectively. On the 15th day of incubation, the potassium solubilisation of eight bacterial isolates such as MGK-01, MGK-02, MGK-03, MGK-04, MGK-05, MGK-06, MGK-07, and MGK-08 was observed to be 22.9, 34.1, 19.7, 33.9, 22.8, 65.3, 33.7, and 22.9 µg/ml respectively.

Table: 5.Screening of Potassium solubilisation abilities from the eight KSB isolates with increasing incubation times.

S. No	Isolate	Potassium solubility (µg/ml)				
		3rd day	6th day	9th day	12th day	15th day
1	MGK-01	8.87	15.8	21.8	22.9	22.9
2	MGK-02	14.8	19.4	28.1	33.7	34.1
3	MGK-03	10.6	13.5	18.4	19.1	19.7
4	MGK-04	22.5	28.4	29.7	33.2	33.9
5	MGK-05	12.7	16.2	19.5	22.6	22.8
6	MGK-06	28.9	33.7	48.5	56.2	65.3
7	MGK-07	19.5	23.7	29.0	33.2	33.7
8	MGK-08	7.4	14.7	18.6	22.4	22.9

Effect of salinity on K-solubilisation by KSB isolates

The results of potassium solubilisation abilities under increasing salt concentrations for eight isolated bacterial strains are shown in table 6. From these results, it was observed that all the isolated bacterial strains exhibit significant decrease in potassium solubilisation with increasing NaCl concentrations.The greatestpotassium solubilisation under all NaCl concentrations were found in the MGK-06 isolate, whereas MGP-03 had the lowest potassium solubilisation ability under all NaCl concentrations. In this study, all the eight bacterial isolates exhibit potassium solubilisation up to 5% NaCl concentration.

At 1% NaCl concentration, the potassium solubilisation ability of eight bacterial isolates such as MGK-01, MGK-02, MGK-03, MGK-04, MGK-05, MGK-06, MGK-07, and MGK-08 was found to be 22.5, 34, 19.5, 33, 22.6, 65.1, 33.5, and 22.1 µg/ml respectively. At 2% NaCl concentration, the potassium solubilisation ability of eight bacterial isolates such as MGK-01, MGK-02, MGK-03, MGK-04, MGK-05, MGK-06, MGK-07, and MGK-08 was found to be 19.4, 29.1, 18.3, 28.7, 20.1, 64.3, 31.9, and 19 µg/ml respectively. At 3% NaCl concentration, the potassium solubilisation ability of eight bacterial isolates such as MGK-01, MGK-02, MGK-03, MGK-04, MGK-05, MGK-06, MGK-07, and MGK-08 was found to be 17.2, 27, 17.4, 26.2, 17.4, 61.5, 29.5, and 17.4 µg/ml respectively. At 4% NaCl concentration, the potassium solubilisation ability of eight bacterial isolates such as MGK-01, MGK-02, MGK-03, MGK-04, MGK-05, MGK-06, MGK-07, and MGK-08 was found to be 14, 21.7, 13, 23, 13.9, 57.2, 24.9, and 14.9 µg/ml respectively. At 5% NaCl concentration, the potassium solubilisation ability of eight bacterial isolates such as MGK-01, MGK-02, MGK-03, MGK-04, MGK-05, MGK-06, MGK-07, and MGK-08 was found to be 13.2, 19.3, 12.8, 21.9, 10.7, 55.1, 21.0, and 12.1 µg/ml respectively.

Table: 6. Effect of salinity on the K-solubilisation abilities of the KSB isolates with increasing NaCl concentrations.

S. No	Isolate	Potassium solubilization at 15 days of incubation (μg/ml)				
		1% NaCl	2% NaCl	3% NaCl	4% NaCl	5% NaCl
1	MGK-01	22.5	19.4	17.2	14.0	13.2
2	MGK-02	34	29.1	27.0	21.7	19.3
3	MGK-03	19.5	18.3	17.4	13.0	12.8
4	MGK-04	33	28.7	26.2	23.0	21.9
5	MGK-05	22.6	20.1	17.4	13.9	10.7
6	MGK-06	65.1	64.3	61.5	57.2	55.1
7	MGK-07	33.5	31.9	29.5	24.9	21.0
8	MGK-08	22.1	19.0	17.4	14.9	12.1

Sequence analysis

A sequence characteristic of 16S rRNA gene was calculated by seqstate v.1.21 (Muller, 2005). The PCR amplified 16S rRNA gene from bacterial isolates MGN-10, MGP-04, and MGK-06 have 1430, 1428, and 1266 nucleotides respectively.

The sequence of 16S rRNA gene from bacterial isolate MGN-10 have 355 bp of Adenine(A), 293 bp of Thymine (T), 457 bp of Guanine (G) and 325 bp of Cytosine (C). The % of GC was calculated as 53. The nucleotide composition of MGN-10 16S rRNA gene results has been showed. The sequence of 16S rRNA gene from bacterial isolate MGP-04 have 368 bp of Adenine(A), 301 bp of Thymine (T), 434 bp of Guanine (G) and 325 bp of Cytosine (C). The % of GC was calculated as 53.2. The nucleotide composition of MGP-04 16S rRNA gene results has been showed. The sequence of 16S rRNA gene from bacterial isolate MGK-06 have 313 bp of Adenine(A), 243 bp of Thymine (T), 412 bp of Guanine (G) and 293 bp of Cytosine (C). The % of GC was calculated as 55. The nucleotide composition of MGK-06 16S rRNA gene results has been showed.

Phylogenetic tree analysis

The selected homology sequences for the target 16S rRNA genes of MGN-10, MGP-04, and MGK-06 were extracted from the NCBI database for constructing a phylogenetic tree. The differences among all the selected sequences respectively for MGN-10, MGP-04, and MGK-06 were determined by calculating the distance matrix. Depending on the expressed differences in the distance matrix, the maximum-parsimony phylogenetic tree was built using MEGAX and the evaluation of phylogenetic tree topologies were done by the bootstrap method with 1000 replicates for all nodes (Felsenstein, 1985b). The evolutionary divergence of bacterial isolates such as MGN-10, MGP-04, and MGK-06with their relative members were determined. Satisfactory results were established by the use of 16S rRNA gene as a marker to evaluate the phylogenetic relationship.

In the phylogenetic tree of MGN-10 and their relative species, consists two main clades. The first main clade consists of three subclades and the first subclade of main clade 1 was composed of MGN-10, *Azotobacter chrococcum* strain SKU DBTABF111 (MN340247.1), KT374218.1 *Azotobacter chrococcum* strain Azt-7 (KT374218.1), and MK537389.1 *Azotobacter chrococcum* strain AZE-8 (MK537389.1). The second subclade of the main clade 1 was composed with the *Azotobacter chrococcum* strain PP30 (MW085001.1), *Azotobacter chrococcum* strain W5 (MK734329.1), and *Azotobacter chrococcum* strain M3 (OL348492.1). The third subclade of the main clade 1 was composed with the *Azotobacter chrococcum* strain ISSDS-397 (EF620440.1), *Azotobacter beijerinckii* (AB429527.1), and *Azotobacter chrococcum* strain CHB874 (KX710041). The second main clade consists of two subclades. The first subclade of main clade 2 was composed of *Azotobacter* sp. strain L27 (MN853548.1), *Azotobacter chrococcum* strain BPR IST062 (JF700513.1), and Azotobacter chrococcum strain Avi2 (KP099933.2). The second subclade of main clade 2 was composed of *Azotobacter* sp. strain P19 (MN853545.1), *Pseudomonas* sp. NBRC12994 (AB680361.1), *Azotobacter chrococcum* strain W5 (MT299751.1), Uncultured *Pseudomonas* bacterium clone B03-11B (FJ542980.1), and Uncultured *Pseudomonas* bacterium clone B10-09C (FJ543080.1). The phylogenetic tree indicated that the N2 fixing bacterial isolate MGN-10 has a close branch with *Azotobacter chrococcum* strain SKU DBTABF111 (GenBank Accession No. MN340247.1). On the basis of morphological studies, the molecular phylogenetics, it is revealed that the bacterial isolate MGN-10 is belongs to *Azotobacter chrococcum*strain SKU DBTABF111. The maximum-parsimony phylogenetic tree of MGN-10 was showed in figure 1.

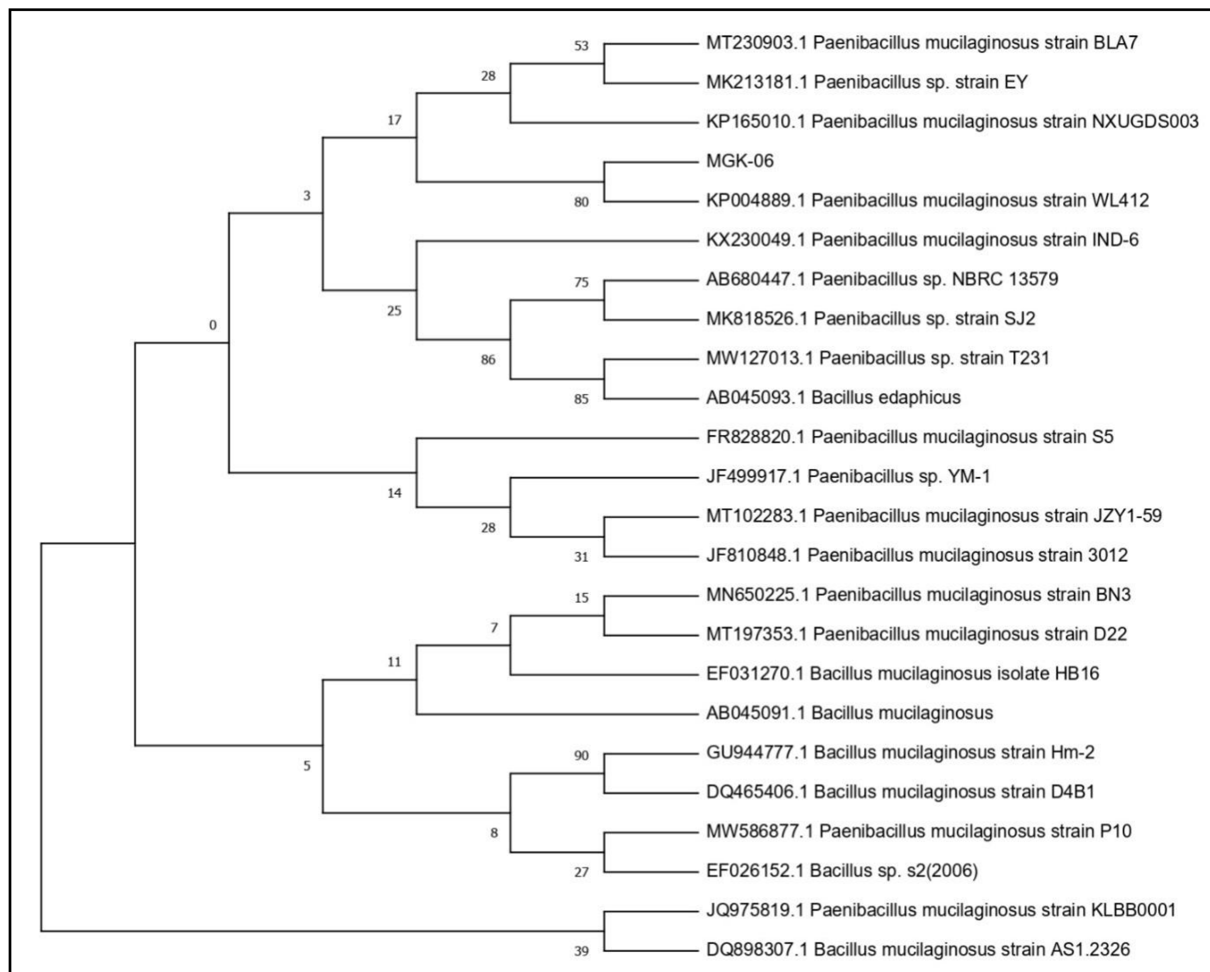

Figure: 1. Maximum Parsimony tree of potassium solubilising bacterial isolate MGK-06 and other relative species based on the 16S rRNA gene.

Conclusion

In conclusion, this study has demonstrated the potential of using potassium-solubilizing bacteria as inoculants to improve plant absorption of potassium, thus reducing the reliance on chemical fertilizer in agriculture. From the soil samples collected, a potent bacterial strain, identified as Azotobacter chrococcum strain MGK-06, was isolated, characterized and found to have the greatest potassium solubilization ability and plant growth promoting activities under increasing NaCl concentrations. The molecular identification of this strain confirms its potential as a bioinoculant for sustainable agriculture practices. The findings of this study provide valuable insights into the use of microbial technologies for reducing the negative impact of chemical fertilizers on the environment while ensuring agriculture production. Further research is needed to explore the practical application of this bacterial strain in field trials to assess its effectiveness in improving crop yields and reducing fertilizer usage.

Conflict of Interest

Authors are declared No Conflict of Interest

Acknowledgement

Not Applicable

Author Contribution

All Authors Contributed equally

Ethical Considerations

Not Applicable

Inform Consent

Not Applicable

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