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Isolation and Characterization of Potential Potassium Solubilizing Bacteria with Various Plant Growth Promoting Traits

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In soil, microorganisms participate in diverse processes such as C, N, P and S conversion, breakdown of xenobiotic organic compounds, soil structure development and plant nutrient uptake intensification. Plant growth promoting rhizobacteria (PGPR) serve as biofertilizer in both direct and indirect ways. In this study, two potassium solubilizing bacteria strains designated as AKY2 and HPY10 were isolated from rhizospheric soil. The bacterial isolate HPY10 was characterized as Serratia marcescens by using 16s rRNA sequencing. The potassium solubilisation index of strain HPY10 was 3.2. The potassium released by isolates AKY2 and HPY10 was 7.29 and 8.66 mg/L after 10 days of incubation respectively. Both isolates were showing different plant growth promoting activity. The present study, suggests use of isolates AKY2 and HPY10 as biofertilizers which is beneficial for crop cultivation by enhancing growth and yield due to the production of phosphate solubilization, IAA (indole-3-acetic acid) and also having antagonistic potential against Fusarium oxysporum.

Keywords: IAA; PGPR; Potassium solubilizing bacteria (KSB); Potassium solubilization; Serratia marcescens.

Potassium is required for metabolic and physiological activities of plants as well as to provide the resistance against biotic and abiotic stress to plants[1]. Potassium is also necessary for photosynthesis and acts as an activator for enzymes that break down carbohydrates to produce amino acids and proteins[2]. Potassium (90-98 %) is present in the soil as non-exchangeable mineral sources[3]. A wide range of rhizobacteria play an important role in the breakdown of mineral present and make accessible to plants by generating K from non-soluble materials[4,5]. Potassium as positive cation K is absorbed by roots and translocated within the plant[6]. To overcome the shortcomings of synthetic fertilizers, potassium solubilizing microorganisms are being commercialized as biofertilizer. Through solubilization, the KSB are effective at releasing K from inorganic and insoluble reservoirs of soil available K[7]. A wide range of rhizobacteria like *Bacillus circulans, Bacillus edaphicus, Bacillus mucilaginosus, Paenibacillus sp.,* and nitrogen fixing rhizobacteria (NFR) is responsible in solubilisation of potassium minerals. This is an environmentally friendly strategy to develop the

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sustainable food production systems in many nations across the world[8]. Plant growth promoting rhizobacteria (PGPR) has the ability to accelerate plant growth by producing plant growth hormones, siderophores, antibiotics and solubilizing ability of phosphate, zinc and potassium[9,10]. Because of its eco-friendliness and practicality in replacing the increasing use of synthetic insecticides, PGPR utilisation has the potential to increase in sustainable farming[10]. PGPRs are useful for the plants to provide protection against phytopathogen[11]. PGPR strains have the potential of biocontrol, increase crop yields, promoting legume nodulation and increase the incidence of seedlings. PGPR has the ability to accelerate plant development by creating plant growth hormones such as gibberellic acid, indole acetic acid, ethylene and cytokines, siderophores, antibiotics, and the ability of dissolving phosphate (P), zinc (Zn) and potassium (K). The ways in which PGPR taxa mitigate the negative effects of invading plant pathogens differ[12]. Diverse types of plant growth promoting rhizobacterial strains have the ability of biocontrol approaches, increase crop yields, boost susceptibility to microorganisms responsible for foliar spoilage, promoting legume nodulation and increase the incidence of seedlings[13]. Reported PGPRs include members of the genera *Aeromonas, Agrobacterium, Allorhizobium, Arthrobacter, Azorhizobium, Azospirillum, Azotobacter, Bacillus, Bradyrhizobium, Burkholderia, Chromobacterium, Delftia, Enterobacter, Flavobacterium, Gluconacetobacter, Klebsiella, Mesorhizobium, Micrococcus, Paenibacillus, Pantoea, Pseudomonas, Rhizobium, Serratia, Streptomyces, Thiobacillus,* etc.[14,15,16]. Present study aims to investigate the ability of potential bacterial isolates for Potassium Solubilizing Efficiency and their Plant Growth Promoting (PGP) Traits, where objectives include- isolation of bacterial isolates from rhizospheric soil and characterization of isolates for Potassium Solubilization Efficiency and PGP traits.

MATERIALS AND METHODS

Isolation, screening and identification of potassium solubilizing bacteria

The spread plate method was used for the isolation of bacteria from rhizospheric soil of Solan, Himachal Pradesh by using NA plates. The plates were incubated at 37°C for two days. Different colonies were selected, purified and stored in 10 % glycerol vials at -20°C for further study. 16S rRNA sequencing of best potassium solubilizing bacteria was done.

The isolated bacterial cultures were spot inoculated on Aleksandrow Agar medium (2.0 g potassium alumina silicate, 5.0 g dextrose (glucose), 0.1 g $CaCO_3$, 0.005 g $FeCl_3$, 0.5 g $MgSO_4.7H_2O$, 2.0 g Ca_3PO_4 and 20.0 g agar) followed by incubation at 28 °C for 3 to 7 days to observe the zone formation[17]. After 10 days growth in Aleksandrow liquid medium, quantitative analysis of potassium solubilization was checked and media was analysed by using Flame photometer after purification using centrifugation. The potassium solubilization efficiency (KE) on agar medium was calculated by given formula.

KE = (Diameter of potassium solubilization zone + Diameter of colony) / (Diameter of colony)

The effect of pH on K solubilisation was also analysed by using Aleksandrow agar plates with changed pH of medium (i.e. acidic and basic).

Modified plate assay for K solubilisation

Modified Aleksandrow agar plates were prepared by adding Bromothymolblue (BTB) dye[18]. After spot inoculation, the ability of bacterial isolates to dissolve potassium was analysed based on the appearance of clear zones and a transformation in colour from greenish blue to yellow.

Plant Growth Promoting Activity

Ammonia production, HCN production and Phosphate solubilization

The fresh inoculum of isolates was transferred in to 4% peptone water followed by incubation at 37 °C for 2-3 days. Nessler's reagent was added after incubation, the colour changed from brown to yellow, signifying the release of ammonia[19]. For detection of synthesis of hydrogen cyanide by the isolates modified agar plates with 0.44% glycine were prepared and isolates were streaked on to the plates. Filter paper was soaked in 2% sodium carbonate and 0.5% picric acid solution (yellow solution) and was placed on the top lid of the petri plate and incubation was done at 28± 2°C for 72 to 96 hours. Formation of orange to red colour indicated HCN production[19]. Isolates

were test for phosphate solubilizing activity by spot inoculations of the isolates on Pikovskaya plates. The plates were then kept at 28°C for 5–7 days. The formation of solubilization zone by different isolates was measured[20]. Phosphate solubilisation index (PSI) was calculated as by using below formula.

PSI = (Colony diameter + zone of solubilization diameter) / (Colony diameter)

Zinc solubilisation, IAA production, Antagonistic activity and salt tolerance

To check the zinc solubilization on modified agar plates containing 0.1 % zinc carbonate, isolates were spot inoculated and incubated for 7 days at 28 °C[21]. To check the production of IAA, isolates were transferred into nutrient broth containing 0.1% tryptophan and incubated at 28 °C for 48 hr. After centrifugation, culture supernatant was mixed with equal amount of Salawaski's reagent and kept it for 30 min to observe the red colour formation. The quantitative production of IAA was measured by taking optical density at 530 nm[22]. To check the antagonistic activity inoculum of *Fusarium oxysporum* was spreaded over PDA plates and 5 mm diameter disc punched on the centre of plate. Isolated bacteria were inoculated into the wells of PDA (Potato Dextrose Agar) plates and incubated for 28 °C for 6 days. The antagonistic activity was determined by measuring the inhibition of mycelial growth of *Fusarium oxysporum*. The salt tolerance of isolates was checked at different concentration of NaCl (2%, 4%, 6%, and 8%) in nutrient broth. After inoculation, broth was incubated at 37 °C for 48

Sequence Obtained

GTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGAT
AACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGAC
CTTTCGGGCCTCTTGCCATCAGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTA
ATGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGG
AACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAAT
GGGCGCAAGCCTGATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAA
GCACTTTCAGCGAGGAGGAAGGTGGTGAGCTTAATACGTTCATCAATTGACGTTACT
CGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGC
AAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGA
TGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTTGAAACTGGCAAGCTAGAGTCT
CGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGA
ATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCG
TGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAAAGAAGTTACCTC
CGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATCGACCGCCTG
GGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCG
GTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCC
AGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCTGAGACAGGTGCTGCAT
GGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACC
CTTATCCTTTGTTGCCAGCGGTTCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAA
ACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGGCTACA
CACGTGCTACAATGGCATATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCT
CATAAAGTATGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAA
TCGCTAGTAATCGTAGATCAGAATGCTACGGTGAATACGTTCCCGGGCCTTGTACAC
ACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCGG
GAGGGCGCTTACCACTTTGTGATTCATGACTGGGGGTGAAGTCGTAACAAGGGTAAC
CGTAGGGG

Fig. 1. 16S rRNA sequence obtained of HPY10

hr. The growth of isolates was measured at 560nm using spectrophotometer.

RESULTS AND DISCUSSION

Isolation and screening of K solubilizing bacteria

In our study 30 morphologically different bacterial strain were isolated from the rhizospheric soil. Out of 30, only two bacterial isolates AKY2 and HPY10 were showing potassium solubilisation zone 10 and 22 mm respectively on Aleksandrow agar plates after 5 days of incubation as shown in Figure 2a. In Previous study the potassium solubilization bacteria were isolated from rhizospheric soil, it was observed that it depending on the isolate, the solubilizing zones on silicate culture media ranged from 0.65 cm to 1.50 cm[23].

The potassium solubilisation efficiency (KE) of AKY2 and HPY10 was measured as 2.6 and 23.2 respectively. The potassium solubilisation by bacterial isolate AKY2 and HPY10 on Aleksandrow agar amended with bromothymol blue is shown in Figure 2b. The quantitative estimation of potassium released by isolates AKY2 and HPY10 was

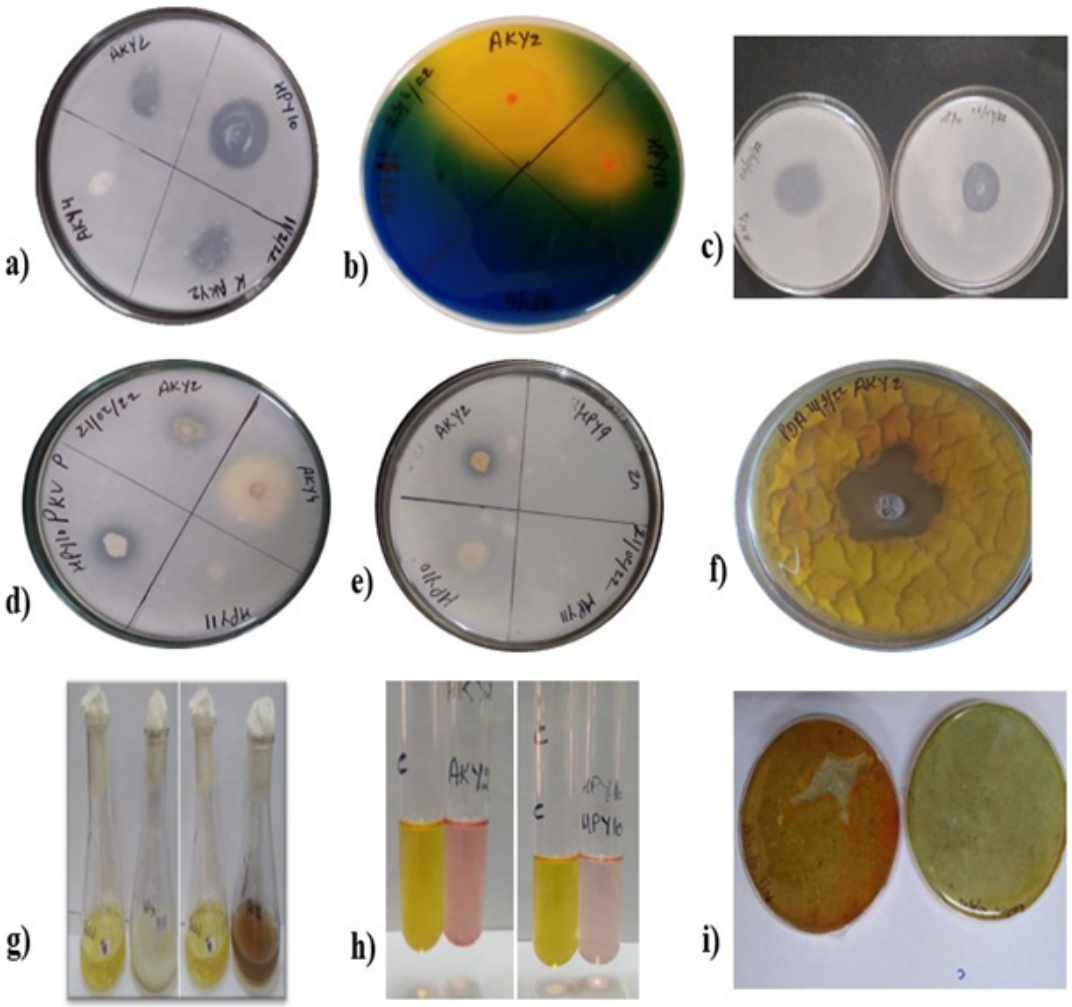

Fig. 2. PGPR activity of AKY2 and HPY10 (a) Potassium solubilisation (b) Potassium solubilisation on modified Aleksandrow agar (c) Potassium solubilisation at 7 pH (d) Phosphate solubilization (e) Zinc solubilization (f) Antagonistic activity of isolate AKY2 *(*g) Ammonium production by HPY10 and AKY2 (h) IAA production by AKY2 and HPY10 (i) HCN Production by AKY2 and HPY10

analysed by Flame photometer in ppm. The amount of potassium released was interpolated as 7.29 and 8.66 mg/L after 10 days of incubation respectively. A study has shown that bacterial isolates could show quantitative estimation of potassium by solubilizing the 13.71 to 23.88 mg L^{-1} of potassium mineral in liquid Aleksandrov broth medium[24]. The 16S rRNA sequencing of bacterial isolate HPY10 was carried out from National Collection of Industrial Microorganisms, CSIR-National Chemical Laboratory, Pune, India. The 16S rRNA sequence was compared with other sequences by using BLAST (Basic Local Alignment Search Tool) programme at NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi)[25,26]. The culture was identified as *Serratia marcescens*. The 16S rRNA sequence obtained as shown in figure 1.

Plant growth promoting activity

Bacterial strain AKY2 showed ammonia production, antagonistic activity against *Fusarium oxysporum*, zinc solubilisation, HCN production, IAA production and phosphate solubilisation index 3.4 and can grow at 2 and 4% NaCl concentration. Both isolates AKY2 and HPY10 were exhibiting very good potassium solubilization at 7 pH as shown in Figure 2c. In previous study Shree *et al.,* (2015) isolated the potassium solubilizing bacteria and found that all isolates grew in acidic condition (pH-5)[27]. Hydrogen Cyanide (HCN) synthesis by bacteria has been linked to the destruction and inhibition of other living things grow[28]. The isolated bacterial strain HPY10 exhibited good PGPR activity; zinc solubilisation, HCN and IAA (6.14 g/ml) production and phosphate solubilisation index (2.6) and displayed positive growth at 2 % NaCl concentration.

CONCLUSION

The results of present study suggested that *Serratia marcescens* HPY10 and AKY2 were Potassium solubilizing bacteria as well as demonstrated multiple plant growth promoting traits like Ammonia production, HCN production and Phosphate solubilization, Zinc solubilisation, IAA production, Antagonistic activity and salt tolerance. Further research and field trials are needed in order to acquire information on their ability to be a PGPR and hence their ability to improve crop yield.

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Conflict of Interest

I hereby declare that all the authors and corresponds author do not have any conflict of interest.

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