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Isolation and identification of potassium-releasing bacteria in soil and assessment of their ability to release potassium for plants

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Summary

The application of potassium (K)-releasing microorganisms is a promising approach for increasing K availability in soil. The objectives of this study were to isolate and characterize the K-releasing bacteria (KRB) and to evaluate their contribution to the solubilization of K from muscovite and biotite, and to the assimilation of released K by tomato in a pot culture experiment. Soil samples were screened in both solid and liquid Aleksandrov media to isolate bacteria with the potential to release K from biotite and muscovite. Our results from *in-vitro* experiments revealed that more K was released in treatments with KRB than in the uninoculated media (control). Under the best conditions an increase of 188 and 127% was obtained for biotite and muscovite, respectively; among the isolates with the largest releasing ability it was 49 mg l^{-1}. The most efficient bacteria were identified as the *Pseudomonas* genus. Results of the pot culture experiment showed that the concentration and content of K in plant tissue were considerably more than those in any of the controls with no living organisms present. Results revealed that significantly more biomass was accumulated and K acquired in most pots treated with bacterial strains than in the control, especially for *Pseudomonas* sp. strain S10-3. This treatment increased K concentation and content by more than 50 and 70%, respectively, in tomato aerial tissue. Further research is necessary to examine the effects of these bacterial strains on the mobilization of K-bearing minerals under field conditions.

Highlights

- The aim was to determine if bacterial isolates can accelerate K release from micas for plants.
- We investigated the potential of some isolated bacteria to solubilize K from muscovite and biotite.
- Identification of efficient isolates showed that KRB belonged to the *Pseudomonas* genus.
- *Pseudomonas* sp. strain S10-3 increased K concentration in tomato aerial tissue by more than 50%.

Introduction

Potassium (K), like nitrogen (N) and phosporus (P), is a major macronutrient essential for plant growth (Zörb *et al.,* 2014). The amount of K required for plants is much greater than any other soil-supplied nutrient except nitrogen (Zörb *et al.,* 2014). Agricultural soil may contain from 4.1 to 62 Mg ha^{-1} of total K in the upper 15 cm. However, much of this is strongly bound within insoluble minerals and is unavailable, or only slowly available to the plants (Anon, 1998). Therefore, plants cannot use these forms of K directly unless it is released by weathering (Zörb *et al.,* 2014).

Available soil K levels have decreased because of crop removal, leaching, runoff and erosion. Many soils have been depleted of available and even slowly available K by decades of exhaustive farming, with crop removal exceeding nutrient inputs (Anon, 1998). To meet the K demand of the crop, replenishment of the K-depleted soil solution occurs through the release of exchangeable K from clay minerals and organic matter. Meanwhile, exchange sites need to be replenished continuously with K through the release of non-exchangeable K from K reserves (e.g. micas and feldspars) (Sparks & Huang, 1985) or the addition of K fertilizers.

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Many microorganisms in the soil can solubilize unavailable forms of K-bearing minerals, such as micas (including muscovite ($K[(Si_3Al)Al_2O_{10}(OH)_2]$) and biotite ($K[(Si_3Al)(Mg,Fe)_3O_{10}(OH)_2]$), illite (or hydrous and weathered mica) and orthoclase ($KAlSi_3O_8$), by excreting organic acids that either dissolve rock K directly or chelate silicon ions to leach the K into solution (Bennett *et al.,* 1998). Therefore, the application of K-solubilizing microorganisms (KSM) is a promising approach for increasing K availability in KSM-amended soil. Several studies have documented the release of K during the degradation of silicate minerals by bacteria (e.g. Barker *et al.,* 1998; Sheng, 2005). Some soil microorganisms (e.g. *Pseudomonas vancouverensis*, *P. aeruginosa*, *Burkholderia cepacia*, *Paenibacillus glucanolyticus*, *Acidothiobacillus ferrooxidans*, *Bacillus mucilaginosus*, *Bacillus edaphicus*, *Bacillus megaterium* and fungal species such as *Aspergillus niger* and *A. terreus*) can release K from K-bearing minerals. The main mechanism of K-solubilizing microorganisms is acidolysis, chelation, exchange reactions, complexolysis and production of organic acid (Sheng *et al.,* 2002; Meena *et al.,* 2014; Zörb *et al.,* 2014). The production of various types of organic acids plays the main role in potassium solubilization. These acids are accompanied by acidolysis and complexolysis exchange reactions, which are key processes attributed to the conversion of structural K compounds in soluble form. Acidolysis by organic acids can either directly dissolve the minerals containing K as a result of slow release of exchangeable K or readily available exchangeable K, or can chelate both Si and Al ions associated with K minerals (Meena *et al.,* 2014).

Meena *et al.* (2015) reported the isolation and identification of 12 K-solubilizing rhizobacteria (KSR) from the rhizosphere of some crops, such as maize (*Zea mays* L.), banana (*Musa acuminata* colla), sugarcane (*Saccharum officinarum* L.), potato (*Solanum tuberosum* L.), pigeon pea (*Cajanus cajan* (L.) Millsp.) and tobacco (*Nicotiana tabacum* L.), based on their ability to solubilize mica minerals, including muscovite and biotite, in plate and liquid medium assays. Molecular identification showed that these bacteria belong to the genera *Agrobacterium*, *Rhizobium* and *Flavobacterium*, and the strains of *A. tumefaciens* OPVS 11 and *R. pusense* OPVS6 had the greatest K-solubilizing efficiency. They observed a significant decrease in pH and an increase in K release with the length of the incubation period. Zhang & Kong (2014) also reported the isolation and identification of some KRB from rhizosphere soil under tobacco and their effects on tobacco plant growth. In this study, 27 isolated KRB strains were identified by sequencing 16S ribosomal DNA. The genera *Klebsiella*, *Enterobacter*, *Pantoea*, *Agrobacterium*, *Microbacterium*, *Myroides* and *Burkholderia* were recognized as KSR. All isolated KRB strains could solubilize K-feldspar powder in solid and liquid media. Keshavarz Zarjani *et al.* (2013) identified some KSR belonging to *Bacillus* and *Arthrobacter* genera. They tested the ability of all isolates in three treatments, including acid-leached soil, biotite and muscovite, by analysing the soluble K content after 5 days of incubation at 28 ±2°C.

More attention has recently been given to the isolation and inoculation of K-solubilizing microorganisms. Beneficial effects of inoculation of soil with KRB on plant K uptake have been reported in cotton (*Gossypium herbaceum* L.), oilseed rape (*Brassica napus* L.), pepper (*Capsicum annuum* L.), cucumber (*Cucumis sativus* L.) and sudangrass (*Sorghum vulgare* var. *sudanense* (Piper) Hitchc.) (Sheng *et al.,* 2002; Han & Lee, 2005; Han *et al.,* 2006). Potassium-releasing bacteria can play an important role in reducing negative effects on the environment through their use as inoculants by replacing chemical fertilizers. They convert insoluble forms of soil K to a plant-available form. This could be a suitable strategy for the improvement of K uptake by plants and thereby reduce the use of chemical fertilizers.

Most Iranian soils are fairly rich in K resources as primary and secondary clay minerals (Keshavarz Zarjani *et al.,* 2013); therefore, increasing K availability to meet plant requirement is an alternative that needs to be considered in crop production. However, available K in some Iranian soil is not sufficient for crops that require a large uptake of K, such as potato, soya bean (*Glycine max* (L.) Merr.) and tomato (*Solanum lycopersicum* L.). Potassium supply to plants could potentially be improved by the application of KRB. This potential of KRB isolated from soils in Iran to improve the growth of tomato, which has a large demand for K, has not been determined. In addition, the effectiveness of KRB in solubilizing K and supplying K to tomato plants, especially in a sand medium containing mica minerals, has not been investigated. Therefore, the objective of this study was to assess the ability of isolated K-releasing bacteria to release K from mica and to evaluate their inoculation effects on the mobilization of K from muscovite mica to improve the growth of tomato plants.

Material and methods

Soil sampling

Twenty-one rhizosphere and non-rhizosphere soil samples were taken from 0 to 20-cm depth of arable soil cultivated with wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and maize, pasture and fallow in two provinces (Eastern Azerbayjan and Ardabil) of north-west Iran. The soil samples were collected in July 2011. Soil samples were brought to the laboratory and kept at 4°C for isolation of KRB. The remaining soil samples were air-dried, passed through a 2-mm sieve and analysed for particle-size distribution (Bouyoucos, 1962), pH, electrical conductivity (EC), organic carbon (OC) (Nelson & Sommers, 1982) and available K (Knudsen *et al.,* 1982). All experiments were performed in triplicate.

Minerals

Muscovite and biotite minerals were obtained from Zamanabad (Hamadan, Iran) and Qharabagh (Urmia, Iran) Mica Ores, respectively. Mica flakes were crushed and passed through a 0.5-mm sieve (Figure 1). Determination of the size distribution of micas was carried out after processing in a Wiley mill (Figure 1). Pretreatments to use micas in *in-vitro* assays were described by Sarikhani *et al.*

(2016). For the tomato pot culture, muscovite with a diameter of 2 mm and without any pretreatment was used.

Isolation of K-releasing bacteria and growth conditions

In this study, the Aleksandrov medium was used to screen KRB from soil samples. Samples were diluted (1/10 with distilled water) and spread on Aleksandrov medium at 26°C for 72 hours. Aleksandrov medium contained 0.5% glucose, 0.05% $MgSO_4.7H_2O$, 0.0005% $FeCl_3$, 0.01% $CaCl_2$, 0.2% potassium aluminosilicate (muscovite or biotite micas) and 0.2% $(Ca_3(PO_4)_2$ as a source of phosphorus (Hu *et al.,* 2006). The initial pH of the culture medium was adjusted to 7. The flasks were plugged with cotton and sterilized at 121°C and 0.1 MPa for 20 minutes in an autoclave. Ten grams of the soil sample were diluted sequentially to $10^{-6}-10^{-7}$ suspensions using 90 ml sterilized water. The soil suspension of 100 µl was then spread over a petri dish containing the culture medium for KRB. Each of the fast-growing isolates that showed solubilization zones on the agar plates were selected and a fresh culture of bacteria was prepared in a nutrient broth (NB) medium to be stored in 15% glycerol. The bacteria with more growth and larger diameter in the clear or halo zone were selected as candidate isolates for dot culture in the solid Aleksandrov medium for the primary screening. An inoculum of the grown cells of bacteria containing 10^8 cfu ml^{-1} in NB was used for the assessment of K-releasing ability. The plate assay was based on the diameter of clear zones produced by the bacterial colonies (Figure 2). The agar was inoculated with 10 µl of inoculum and the plates were sealed with parafilm. In the primary screening process to isolate KRB in the solid Aleksandrov medium, muscovite was used, but to quantify K release in the liquid Aleksandrov medium, both muscovite and biotite were used individually.

Assessment of the solubilization ability of KRB in solid and liquid Aleksandrov media

After primary screening of KRB, the diameter of the halo zone in the dot culture was measured and mean values were reported for each isolate. Nutrient broth was used to prepare an overnight culture of bacteria to inoculate solid or liquid Aleksandrov media. The plates were then incubated at 26°C. After 7 days, the colonies with clear zones were considered to be KRB and selected for further study with the Aleksandrov broth medium. Quantitative determination of K release or solubilization was carried out in 100-ml Erlenmeyer flasks containing 30 ml of Aleksandrov medium. Each flask was inoculated with 0.5 ml of bacterial inoculum (approximately 10^8 cfu ml^{-1}) (Bakhshandeh *et al.,* 2017). Autoclaved, uninoculated medium served as the control to which 0.5 ml of sterile NB was added. The flasks were incubated at 26°C on a shaker-incubator at 120 revolutions per minute (rpm) for 7 days. At the end of the incubation time, cultures were centrifuged at 4100 ***g*** for 4 minutes. The supernatants were used to assay the solubilized K. The K concentration was determined by flame photometry (Corning model 410 flame photometer, Corning Inc., Corning, NY, USA).

Pot culture of tomato

To determine the ability of bacterial isolates to release K from muscovite and their effect on the K nutrition of tomato, a pot culture experiment was carried out in glasshouse conditions at the Agricultural Research Station of the University of Tabriz, Iran. The experiment was carried out under sterile conditions in a bed of sand and muscovite mixture. Briefly, the pots were filled with 1800 g of washed sand and 120 g of muscovite as a source of K, which were sterilized by autoclave (high pressure at 0.1 MPa, 121°C for 40 minutes) before use, then 10 disinfected seeds of tomato were sown. A carrier-based product for each isolate of K-releasing bacteria (0.5 ml inoculum with the optical density (OD) = 0.7 per seed) containing 1:1 bagasse and perlite was used for the inoculation of pot cultures. Muscovite of 2-mm diameter without any pretreatment was used for the pot experiment. Fifteen bacterial isolates (including S5-5, S5-9, S6-6, S10-3, S11-2, S12-3, S14-1, S14-3, S15-1, S16-3, S17-4, S19-1, S19-2, S20-7 and S21-1) were assessed for the growth and K nutrition of tomato. After germination of the seeds, seedlings were thinned and two healthy plants per pot were retained and grown for 90 days to ensure enough biomass was produced and enough nutrient removed from the bed of the culture. During the experiment, the pots were irrigated and supplied with other nutrients, excluding potassium, by Hoagland solution to maintain soil water content close to field capacity and to ensure that water and other nutrients were not the limiting factor. The growing period continued until the reproductive phase of the plants. Harvested plants were first rinsed with tap water and then with distilled water. Shoots and roots were blotted dry on filter paper and dried at 65°C for 2 days to determine plant dry weights. Some morphological and nutritional properties, including chlorophyll index, height, wet and dry weight of shoots and roots and K concentration in plant tissue, were measured. To determine element concentrations oven-dried samples were dry ashed in a muffle furnace at 550°C for 8 hours and then digested in diluted nitric acid (1:3, 14.4 M HNO_3:H_2O). The digested samples were dried on a heating plate and subsequently ashed at 550°C for another 3 hours. Samples were resuspended in 2 ml of 10% HCl and made up to volume by double distilled water (Anon, 1980). Concentration of P was determined spectrophotometrically by the ammoniumvanadate–molybdate method (Anon, 1980) and K was determined by flame photometry. Nutrient uptake was calculated for each pot as the sum of nutrient concentrations of shoots and roots for plants.

Molecular and biochemical identification of isolates

Molecular identification of the isolated bacteria was carried out without extraction of genomic DNA by application of the colony-polymerase chain reaction (PCR) method. Part of the bacterial single colony was used as a source of DNAg in the PCR reaction. Bacterial 16S rDNA was amplified with the universal bacterial 16S rDNA primers, 27F (5 AGA GTT TGA TCC TGG CTC AG 3) and 1492R (5 GGT TAC CTT GTT ACG ACT T 3). The PCR was performed with a 20-µl reaction mixture containing

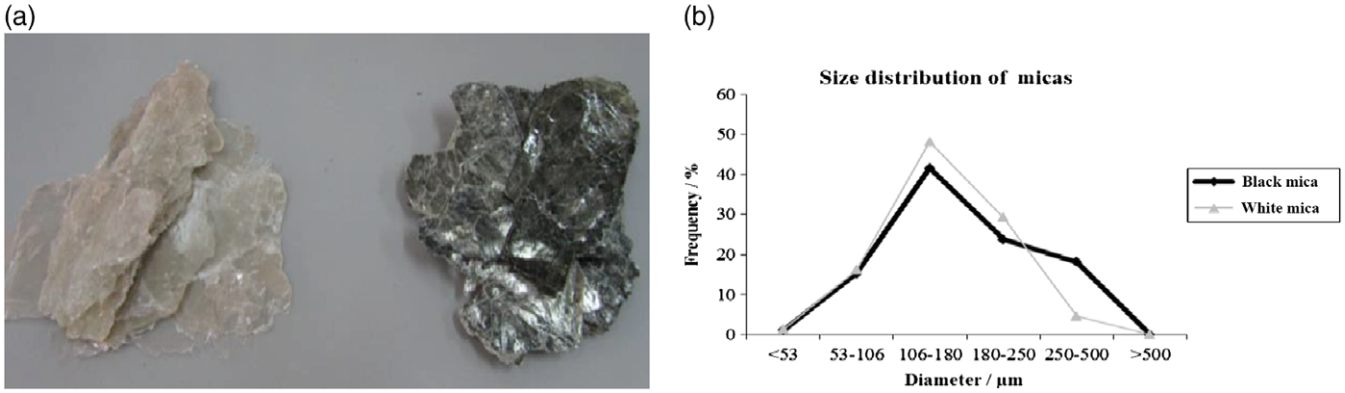

Figure 1 (a) Two types of mica mineral that were used in this experiment. Flakes of white (muscovite) and black (biotite) mica collected from mica mines at Zamanabad Hamedan and Gharabagh Urmia, Iran, respectively. (b) Determination of size distribution of micas after processing in a Wiley mill and sieved through a 0.5-mm sieve.

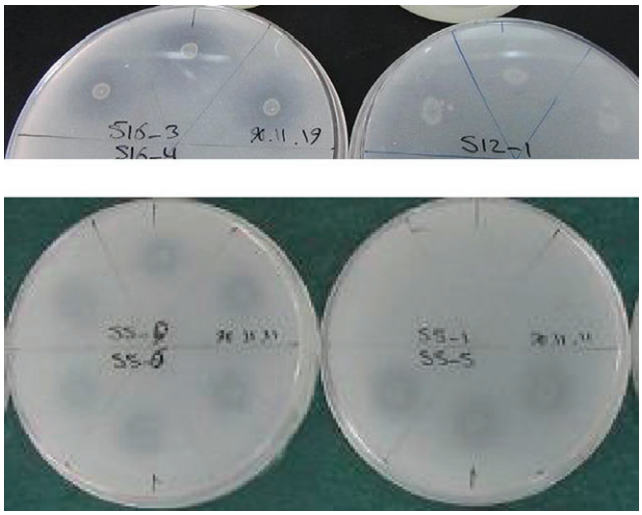

Figure 2 A sample of dot culture of some isolates on the solid Aleksandrov medium to check the growth and production of the clear (halo) zone, and to determine the halo diameter/colony diameter ratio after 7 days of incubation.

a bacterium colony as a template of DNA, 0.1 pmol of each primer, 2 mM $MgCl_2$ and dNTPs at a concentration of 0.2 mM, as well as 0.2 U of Taq DNA polymerase and buffer used as recommended by the manufacturer (Fermentas, Hanover, Germany). After the initial denaturation for 5 minutes at 94°C, there were 35 cycles consisting of denaturation at 94°C for 1 minute, annealing at 53°C for 1 minute, extension at 72°C for 1 minute and final extension at 72°C for 10 minutes, and PCR was carried out in a Flexigene thermocycler (Techne, Cambridge, UK). The PCR products were analysed by (1 g per 100 ml) agarose gel electrophoresis in 1X Tris/Borate/EDTA (TBE) buffer. A DNA fragment (approximately 1.5 kb) was eluted with the QIAgen Gel Extraction Kit (QIAgen, Hilden, Germany). The PCR products were sequenced and sequences were matched with previously published bacterial 16S rDNA sequences in the NCBI databases using BLASTn (Altschul *et al.,* 1997). Multiple sequence alignments of DNA were carried out using the ClustalW algorithm in the MEGA 6.0 software package (Tamura *et al.,* 2013). The phylogenetic tree for the identified nucleotide sequences was produced by the neighbour-joining method (Altschul *et al.,* 1997; Tamura *et al.,* 2013). To construct the phylogenetic tree, the *Bacillus coagulans* was used as an out-group strain.

Biochemical identification of the pure cultures was carried out using Bergey's Manual of Determinative Bacteriology. Catalase, nitrate reductase, oxidase, indole, hydrogen sulphide production and tryptophan deaminase tests were carried out according to Collins *et al.* (1995). Arginine hydrolysis, lysine decarboxylase and carbohydrate utilization tests, together with Gram staining and endospore staining, were also carried out following Collins *et al.* (1995).

Statistical analysis

The experiment was based on a completely randomized design with three replicates each for *in vitro* and plant culture assessment. Analysis of variance (ANOVA) and mean comparison by LSD (least significant difference) test were carried out using MSTATC software and differences were considered to be significant at the $P = 0.05$ level. In carrying out an ANOVA, two main assumptions were checked (i.e. that the residuals from ANOVA were normally distributed (skewness and kurtosis) and the variances were homogeneous (Levene's test)). The association between the traits studied was determined by Pearson correlation coefficient analysis using SPSS 16 software.

Results

Soil samples

Some properties measured on the soils studied are given in Table 1. Available K content ranged from 121 to 628 mg kg^{-1}, organic carbon content varied between 0.39 and 5.07% and calcium carbonate equivalent was 6.6 to 39.7%. The electrical conductivity of all samples was less than 1 dS m^{-1} (EC < 1 dS m^{-1}) and soil pH was higher

than 7.15 and lower than 8.10. Soil textures such as clay, loam, clay loam, sandy loam and sandy clay loam were prominent in soil samples.

Screening, isolation and determination of K-releasing activity of bacteria

Isolation and screening of the bacteria was carried out in four steps. In total, 138 isolates were selected among all the bacteria grown in the Aleksandrov medium. Some of the isolates were disregarded because of phenotype similarity; this reduced the number of selected bacteria for simultaneous dot culture in the solid medium to 77 isolates. A total of 77 phenotypically different colonies were selected by primary screening on the Aleksandrov medium; these colonies grew better on this medium. The number of bacteria decreased to 44 with the dot culture of selected isolates on the solid Aleksandrov medium (Figure 2). An acid-washed muscovite powder agar plate was used in the semi-quantitative measurement of K-solubilization ability of the KRB. Fifteen bacterial isolates were found to be capable of solubilizing K and the solubilization index (HD/CD) ranged from 1.4 to 3.4 (Table 2). Furthermore, for the liquid assay, biotite or muscovite mica powders were added to the liquid Aleksandrov medium as the sole source of K to test the K-solubilization ability of the isolates. The amount of K released in the presence of biotite in the 7-day incubation period was two to four orders of magnitude greater than that in the presence of muscovite (Table 2). Isolate S5-5 showed the most pronounced ability to solubilize K from biotite (49 mg l^{-1}). It was followed by S19-2 (46 mg l^{-1}), S19-1 (41 mg l^{-1}) and S10-3 (38.5 mg l^{-1}). The isolate S16-3 solubilized the least K (20.5 mg l^{-1}), whereas the uninoculated treatment contained 17 mg l^{-1} soluble K (Table 2). The K-releasing ability of 15 selected isolates ranged from 12 to 20.5 mg l^{-1} for the isolates S17-4 and S11-2, respectively, whereas the uninoculated treatment showed 9 mg l^{-1} released K from muscovite in the Aleksandrov medium. Measurements over a period of 7 days showed that, irrespective of mineral type, the concentrations of K released in the culture were considerably larger than those in any of the control experiments where there were no living organisms. Finally, according to the K released in the *in vitro* assay, 15 isolates were used in the pot experiment (Table 2).

Molecular and biochemical identification of efficient KRB

The isolated bacteria were Gram− or Gram+ in rod, cocci, cocobacill or streptobacill shapes. Colony morphology revealed slime, with white, creamy and whitish texture and with smooth and rough surfaces on the Aleksandrov medium. Colonies formed by most of the isolates appeared to be translucent, but some were opaque. Based on the phenotypic characteristics of bacteria and their similarity, the number of unique bacteria was decreased to 15. The data related to these bacteria are given in Table 2. Finally, regarding the main properties of 15 isolates, especially considering pot culture results (Table 3) and K solubility of isolates in *in vitro* assays (Table 2), five isolates, namely S6-6, S10-3, S14-3, S19-1 and S21-1, were chosen for further molecular and biochemical identification. Our molecular identification revealed that all these isolates belonged to the genus *Pseudomonas*, with GenBank accession numbers KU179048.1, MG687290, MG687371, MG687374 and MG687382, respectively (Figure 3). Some biochemical tests to identify these chosen isolates are listed in Table 4.

Plant growth and K uptake by tomato in the presence of KRB

Height of plant, chlorophyll index, total dry matter and K content in shoots and roots were significantly ($P < 0.05$) affected by bacterial isolates. Mean values of these properties are given in Table 3. At the end of experiment, the highest plant grew with the S21-1 isolate (48.1 cm) and the 16.6% increase was largest after the inoculation of tomato by KRB. The chlorophyll index varied between 9.2 (S5-9) and 13 (S14-3). Largest total dry matter was achieved by S19-1 (6.3 g), and inoculation of pots with isolate S19-1 increased biomass yield by 12.5% over the control. Potassium uptake in shoots was affected by bacterial inoculation, especially with isolate S10-3, which resulted in a 70.1% increase of K content in plants, whereas increases of 41.5 and 40.2% over the control were observed with the isolates S20-7 and S21-1, respectively. Addition of muscovite into pots and inoculation with isolate S21-1 increased K uptake in roots by 124% over the control and the isolates S6-6 (4.9 mg pot^{-1}) and S19-1 (4.5 mg pot^{-1}) were the next in order (Table 3).

Discussion

Isolation and identification of KRB and determination of K-releasing activity of bacteria

Table 1 shows that the soil properties EC and pH were constant and varied little, but calcium carbonate equivalent (CCE), OC and available K were variable. The primary screening process in this research resulted in 138 isolates in the solid Aleksandrov media, which decreased to 15 isolates with secondary screening by dot culture and assessment of potassium release in the liquid Aleksandrov medium. Screening usually starts with isolating in the solid medium followed by culture in the liquid one. For example, Osman (2009) used only the solid Aleksandrov medium for isolation of KRB, whereas Meena *et al.* (2015) and Zhang & Kong (2014) followed primary isolation of KRB in solid media with liquid assays in the Aleksandrov broth medium. In some studies, efficiency of K-releasing bacteria has been assessed not only in *in vitro* conditions (Liu *et al.,* 2006; Meena *et al.,* 2015) but this potential has also been checked after inoculation of target plants with isolated KRB (Basak & Biswas, 2010; Zhang & Kong, 2014; Bakhshandeh *et al.,* 2017). Twelve KRB were isolated from the rhizosphere of common Kharif crops based on their ability to solubilize waste mica (muscovite and biotite) in a plate assay. All these KRB were capable of K-solubilization from waste mica in both solid and liquid media *in vitro* (Meena *et al.,* 2015).

In our study, primary screening was carried out based on the larger rate of growth of bacteria in the Aleksandrov solid medium, and the final indicator for selection of KRB isolates for the

Table 1 Physicochemical properties of the soils of study sites

Sampling site	Soil sample	K available/ mg kg^{-1}	O.C/ %	Soil texture	$CaCO_3$/ %	ECe/ dS m^{-1}	pH
1	1	121	2.92	Clay loam	28.30	0.65	7.85
2	2	273	1.17	Sandy clay loam	14.80	0.52	8.10
3	3	265	1.95	Sandy loam	9.90	0.63	7.95
4	4	565	3.12	Loam	20.60	0.59	7.82
5	5	402	2.34	Clay	32.00	0.74	7.95
6	6	335	4.87	Clay loam	15.20	0.74	7.59
7	7	371	2.53	Clay	16.00	0.45	7.60
8	8	548	4.29	Clay loam	12.50	0.54	7.48
9	9	317	1.56	Clay	14.30	0.54	7.75
9	10	311	2.34	Clay	6.60	0.55	7.15
9	11	253	4.10	Clay	18.60	0.72	7.65
9	12	382	2.14	Clay	25.40	0.46	7.85
10	13	375	3.51	Loam	17.80	0.76	7.71
10	14	388	3.31	Clay loam	16.30	0.66	7.65
11	15	262	3.51	Clay loam	13.80	0.56	7.64
11	16	572	4.48	Clay loam	15.00	0.66	7.60
12	17	628	1.56	Clay	22.70	0.55	7.87
13	18	453	5.07	Sandy loam	16.00	0.66	7.82
14	19	507	0.39	Clay loam	6.80	0.67	7.73
15	20	428	2.34	Clay	19.30	0.59	7.70
15	21	369	1.95	Clay loam	39.70	0.50	7.88

Table 2 A comparison of the means of potassium-releasing ability, solubilization index (halo zone diameter/colony diameter), P solubility, EC tolerance and gram staining reaction of 15 selected isolates for pot culture

Isolates	Gram and shape	HD/CD	pH	P solubility / mg l^{-1}	K-muscovite/ mg l^{-1}	K- biotite/ mg l^{-1}	Tolerance of EC 5%
S5-5	G−, rod	3.1	3.8	340	14.0	49.0	Sensitive
S5-9	G−, rod	1.9	4.3	275	13.0	37.5	Resistant
S6-6	G−, rod	1.8	4.6	220	13.0	36.0	Resistant
S10-3	G−, rod	1.8	4.4	270	13.5	38.5	Partly sensitive
S11-2	G−, cocobacill	1.5	4.6	280	20.5	28.0	Resistant
S12-3	G+, streptobacill	1.4	3.4	90	16.5	36.0	Sensitive
S14-1	G−, rod	3.0	3.8	200	15.5	37.0	Resistant
S14-3	G−, rod	2.3	4.1	240	13.0	36.5	Resistant
S15-1	G−, cocci	1.5	4.5	120	14.5	22.0	Sensitive
S16-3	G−, rod	3.4	3.5	600	13.0	20.5	Resistant
S17-4	G+, cocci	1.5	7.2	100	12.0	24.5	Partly resistant
S19-1	G−, rod	2.7	4.2	300	17.5	41.0	Partly sensitive
S19-2	G−, rod	2.0	4.3	340	14.5	46.0	Resistant
S20-7	G−, rod	2.0	4.3	280	19.0	36.0	Resistant
S21-1	G−, rod	1.7	4.6	250	18.0	34.5	Sensitive
Control		–	7.4	90	9.0	17.0	
SE		0.07	0.08	6.55	2.59	4.15	
LSD		0.20	0.24	19.64	7.81	12.51	

LSD, least significant difference, at $P = 0.05$; SE, standard error of the mean determined from ANOVA; HD/CD, halo diameter/colony diameter ratio; EC, electrical conductivity.

pot culture experiment was their potential to solubilize K from biotite and muscovite in the liquid medium. The K-releasing ability of 15 selected isolates from biotite, ranged from 20.5 to 49 mg l^{-1} for S16-3 and S5-5, respectively, whereas uninoculated treatment released only 17 mg l^{-1} K. These data varied between 9 and 20.5 mg l^{-1} when muscovite was used as the source of K-containing mineral in the Aleksandrov medium. The largest amount of K released was obtained with inoculation of isolate S11-2 into this medium, and the smallest release of K in the presence of muscovite was measured with isolate S17-4 (Table 2).

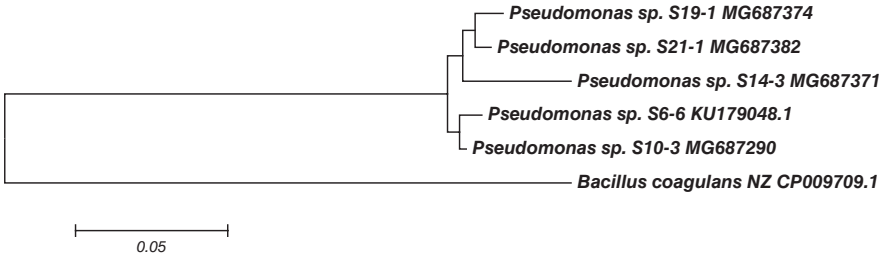

Figure 3 Phylogenetic tree of isolates identified. In this dendrogram, the *Bacillus* strain was used as an out-group strain. The tree was constructed by the MEGA 6.0 program.

Table 3 Means of growth properties of tomato plants after inoculation with selected KRB isolates

Isolates	Total dry matter/g	Height/ cm	Chlorophyll index	Potassium uptake in shoot / mg pot^{-1}	Potassium uptake in root/ mg pot^{-1}
S5-5	5.2	41.2	10.4	57.9	3.6
S5-9	4.8	44.3	9.2	62.8	1.5
S6-6	6.1	41.8	10.7	56.5	4.9
S10-3	6.0	46.2	10.6	78.6	3.0
S11-2	5.2	43.5	12.2	61.6	3.3
S12-3	6.2	41.6	10.4	55.9	4.6
S14-1	5.5	41.2	11.1	62.1	3.1
S14-3	6.0	40.6	13.0	55.2	4.3
S15-1	5.0	42.0	9.6	46.9	3.5
S16-3	5.5	39.1	12.1	56.1	3.1
S17-4	5.4	41.7	10.9	48.8	3.9
S19-1	6.3	44.6	9.4	45.2	4.5
S19-2	5.0	41.5	9.8	50.0	3.9
S20-7	5.4	42.6	12.5	65.4	3.6
S21-1	6.2	48.1	10.6	64.8	5.6
Control	5.6	41.2	11.4	46.2	2.5
SE	0.39	1.54	0.97	5.66	0.78
LSD	1.11	4.41	2.80	16.30	2.24

LSD, least significant difference, at $P = 0.05$; SE, standard error of the mean determined from ANOVA.

A comparison of K-containing minerals (biotite and muscovite) indicated that bacterial inoculation of the Aleksandrov media containing biotite further enhanced K solubilization by a factor of about 2.5. The correlation between the K released and changes in pH in the media was negative ($r = -0.54$, $P < 0.05$). The K in trioctahedral micas such as biotite and phlogopite is reported to be more readily released by weathering, and for this reason its application to K-deficient soil might enhance the plant-available K content of soil (Öborn *et al.,* 2005). Glowa *et al.* (2003) compared the ability of the fungus *Piloderma* to extract K from biotite, microcline and chlorite and found that this species could acquire K from all three minerals, although biotite was the more biodegradable.

Potassium is present in soil mainly in unavailable form not directly available to plants. Fortunately some soil microorganisms are efficient in the bioactivation of soil K reserves or unavailable forms of K-bearing minerals, such as micas, illite and orthoclases. This solubilization could be attributed to the excretion of organic acids, which either dissolve rock K directly or chelate silicon ions to bring K into solution (Ullman *et al.,* 1996; Bennett *et al.,* 1998). Certain soil microorganisms (e.g. *Pseudomonas* spp., *Burkholderia* spp., *Bacillus* spp.) can release K from K-containing minerals by different mechanisms (Sheng *et al.,* 2002). Lian *et al.* (2002) further suggested that, for phyllosilicates such as illite, smaller organic ligands might be forced into the interlayer spacing to drive out the K.

Plant growth and K uptake by tomato in the presence of KRB

Potassium is one of the major plant nutrients influencing plant growth, development and grain quality. It plays a key role in the synthesis of cells, enzymes, proteins, starch, cellulose and vitamins. Moreover, K not only participates in nutrient transport and uptake, but also confers resistance to abiotic and biotic stresses, leading to increased production and enhanced quality of crops, and provides resistance to plant diseases (Zörb *et al.,* 2014). Tomato is also known as a K-exhausting crop because it requires a substantial amount of K for its growth. Our results showed that significantly more biomass accumulated and nutrient was acquired in some pots treated with mica and bacterial isolates than the control. The largest dry biomass of tomato (6.3 g pot^{-1}) was obtained with S19-1, followed by S21-1, S12-3, S6-6, S10-3 and S14-3, which recorded 12.5 to 7.1% extra biomass production over the control. A more pronounced effect was observed for the treatment inoculated with the isolate S10-3, for which K uptake was 70.1% greater than for the control (Table 3). In the experiment with the mixture of sand and muscovite minerals, the differences among the treatments can be explained by the plant growth-promoting properties of bacteria (characteristics such as the production of hormones like indole-3-acetic acid or the K-releasing ability of bacteria). The pot culture substrate was an inert medium (sand) containing muscovite minerals. Therefore, increased plant biomass and K uptake related only to the K-releasing ability of bacteria because the washed sand was deficient in any nutrients and the main source of K was the muscovite. Apart from the inoculation effect of bacteria in increasing K uptake in plants, which has been reported in other studies (Singh *et al.,* 2010; Bakhshandeh *et al.,* 2017), root exudates and organic acids released into the rhizosphere could also be a mechanism for releasing K from minerals (Calvaruso *et al.,* 2006; Singh *et al.,* 2010). The appreciable amount of K present in shoots and roots, even in the control treatment, could be explained by this mechanism.

Basak & Biswas (2010) used a glasshouse trial to study the effect of co-inoculation of K-solubilizing (*B. mucilaginosus*) and

Table 4 Results of some biochemical tests to identify efficient isolates

Bacteria isolates	Catalase	Oxidase	Proline	Lysine	Arginine	Indole	Tryptophane	Fluorescent	Manitol	Sorbitol	Sucrose	Lactose	Glycerol	Glucose	Gelatin	Starch	Urea	Citrate	Nitrate	Sulphide
S6-6	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	−
S10-3	+	−	−	−	−	−	+	+	−	−	−	−	−	+	−	−	−	+	+	−
S14-3	+	+	−	−	−	−	+	−	−	−	−	−	−	+	+	−	−	−	−	−
S19-1	+	−	−	−	−	−	+	+	−	−	−	−	−	+	−	−	−	+	−	−
S21-1	+	+	−	−	−	−	+	+	−	−	−	−	−	+	+	−	−	+	−	−

+ and − show positive and negative response of each biochemical test, respectively.

nitrogen-fixing (*A. chroococcum* A-41) bacteria on the solubilization of waste mica and their effects on growth promotion and nutrient uptake by sudangrass. Their results showed that significantly more biomass accumulated and nutrients were acquired in all the pots treated with mica or a bacterial strain, or both, than the control. The *B. mucilaginosus* strain was a more effective and potent K solubilizer than *A. chroococcum* A-41. Growth promotion and increased K uptake by cotton and rapeseed by a K-releasing strain of *Bacillus edaphicus* was reported by Sheng (2005). A K-releasing bacterial strain, *B. edaphicus* NBT, was examined for plant growth-promoting effects and nutrient uptake on cotton and oilseed rape in K-deficient soil in pot experiments. Inoculation with the bacterial strain *B. edaphicus* NBT increased root and shoot growth of cotton and rapeseed. Strain NBT was able to mobilize K efficiently in both plants when illite was added to the soil. For cotton and rapeseed growing in soil treated with insoluble K and inoculated with the strain NBT, K content increased by 30 and 26%, respectively. Bacterial inoculation also resulted in larger N and P contents of above-ground plant components.

Soil reserves of K are generally large, but most are not plant available. Therefore, crops need to be supplied with soluble K fertilizers, the demand for which is expected to increase considerably, particularly in developing regions of the world. Recent investigations have shown that organic exudates of some bacteria and plant roots play a key role in releasing otherwise unavailable K from K-bearing minerals (Zörb *et al.,* 2014). Plant species that are effective in K uptake and K-solubilizing microbial populations might be two further key factors that control the release of K from soil minerals. Therefore, inoculation of K-solubilizing microorganisms in conjunction with the application of rock-K to soil has recently gained attention. Beneficial effects of inoculated-mica application to soil on plant K uptake have been reported in cotton, oilseed rape, pepper, cucumber and sudangrass (Sheng *et al.,* 2002; Han & Lee, 2005; Han *et al.,* 2006). This indicates that exudates of these microorganisms can effectively increase the release of K from clay minerals. Similarly, several incubation trials have shown that application of inoculated feldspars into soil increases K solubility and plant K uptake by about 40–60% (Han *et al.,* 2006; Basak & Biswas, 2009).

Conclusions

Application of K-solubilizing microorganisms is a promising approach for increasing K bioavailability in soil. Our *in-vitro* results showed that bacteria were able to release more potassium from biotite than muscovite. In inoculated treatments, KRB resulted in an increase in the release of K of almost 2.5–3.5-fold compared with the control in the presence of muscovite and biotite, respectively. The screening process in this study showed that bacteria belonging to *Pseudomonas* genus were efficient in releasing potassium. *Pseudomonas* sp. strain S10-3 increased K concentration in tomato shoots by more than 50%. We suggest that isolates S10-3, S6-6, S14-3 and S21-1 identified here should be used for further research. Further research is necessary to assess the effects of these bacterial strains on the mobilization of K-bearing minerals under field conditions.

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