**Development and Characterization of Efficient K-Solubilizing Rhizobacteria and Rhizobial Inoculants for Chickpea**

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**Running Title:**  Development and characterization of K-solubilizing bacteria for chickpea

**Abstract**

Use of mineral fertilizers have long been associated to improve growth of crop plants as well as increase their yield potential per unit area. However, incessant practice of imbalanced fertilizers application has mounted the economic and environmental costs for agricultural sector. The deficiency of potassium (K) has been identified as primary crop production challenge in certain semi-arid regions where soil-K reserves are increasingly being depleted. This study was aimed to isolate and characterize K-solubilizing bacterial and isolates from chickpea rhizosphere and root nodules. Initially, fifty isolates were isolated by serially-dilution method using Aleksandrov’s medium. Furthermore, 25 isolates were screened-out following a rigorous qualitative screening based on different physiological, morphological and biochemical tests. Among these, five isolates, each of rhizosphere and nodule origin were further screened and selected based on qualitative as well quantitative determination of various growth promoting traits. Besides efficient potassium and phosphate solubilization, selected isolates displayed better growth conditions as evident by glucose substrate use at 25°C and pH 7. In this study, we found that isolates, SKB3 (rhizosphere) and JKR7 (rhizobia), were recorded as the most efficient K-solubilizer. Additionally, they possessed diverse plant growth promoting traits like root colonization, synthesis of siderophores, exopolysaccharides, chitinase activity, indole-acetic acid production and 1-aminocyclopropane-1-carboxylic acid deaminase activity. Overall, our results suggested that application of bacterial K-solubilizer could be employed as a useful K-supplement in K-limited agroecosystems. Moreover, use of these K-inoculants may lead in alleviating the negative impact associated with chemical fertilizer use for environment.

**Key words:** Bacterial co-inoculants, K-solubilizing activity, screening, characterization, legumes

**Introduction**

Pakistan is the second largest chickpea producer in the world. In 2017-2018, chickpea production increased by 3% due to increase in cultivated area and favorable weather conditions prevalent at the time of sowing (GOP 2018). The high nutritional value of chickpea and its financial significance, the production of chickpea in Pakistan is very low, at 583 kilograms per hectare (Hassan and Khan 2007). Many factors are responsible for poor performance but among them, the use of traditional or low-yielding varieties and poor adaptation of management practices are of utmost importance (Ahmed *et al.* 2013).

Biological nitrogen fixation (BNF) has a number of benefits compared to N fertilizers such as; a) higher N use efficiency, b) minimize N leaching, and c) contamination in soil and water body (Graham and Vance 2000). While, the amount of N fixed by a legume varies depending on the crop type, soil and growth stage of the crop, as well as management practices (Chianu *et al.* 2011). Crop yield is limited due to availability of nutrients especially phosphorus (P). Therefore, the studies on the response of legumes and rhizobia to P fertilization have received substantial attention (Sheng *et al.* 2008). While to a lesser extent, research has focused on the response of legume-rhizobia to K, which is also scarce in many Pakistani soils.

Potassium is the fourth most abundant nutrient of the earth’s lithosphere and its concentration is varied in soil ranging 0.04 to 3.0 percent (Bhatti 2011). It plays a vital role in the production of amino acids and protein from NH4+ ions, which are absorbed by plant roots from the soil. In addition, K also participates to better root growth, and has been proved to increase the size and number of nodules in legumes. The use of K in soil can promote both numbers and size of nodules in legumes (Sheng *et al.* 2008). It means that higher K allocation in nodule is very essential to sustain BNF potential, as K deficiency can inhibit or reduce nitrogenase activity, thus disrupt the symbiotic potential of the plant (Hafsi *et al.* 2014). As a result, legumes that gain N through BNF, generally have a higher need for K than those that only release N and P from the soil. As the total quantity of N fixed by bacteria increases, so does their need for energy sources to reduce or change N in to NH4+ ions. The imperative role of K in photosynthetic activity that makes it an important benefactor to efficient N2 fixation by leguminous crop plants (Pettigrew 2008). The process of N fixation is influenced by K for very distinctive reasons and depends on it. Moreover, K is the predominant cation in the plant body as like to calcium in the soil (Pettigrew 2008). While in Pakistan, current evaluation of the level of soil K in Punjab have revealed an average decrease in soil K up to 3 mg kg-1 per year. This means a reduction of about 60 mg kg-1 K in Punjab soils over the past two decades, and as a result, soil K levels that were once considered adequate for plant growth are now approaching the deficiency threshold level (Sheng *et al.* 2008; Mian *et al.* 2010). Furthermore, the plant growth prompting rhizobacteria have been reported to be the key elements for better plant growth under nutrients deficient conditions. While, their application in the sector of agriculture can be in the favor of reduction of agro-chemicals use and give help to eco-friendly crop production (Arif *et al.* 2017). While, plant up takes K from soil and its availability from soil depends on the dynamics of the K, as well as the total K content in soil (Ahmed *et al.* 2013).

But the second form of non-exchangeable K about 10 percent of K in the soil is primarily the intermediate layer of K such as lattice and elite in K feldspar (Archana 2008). Moreover, the release of non-exchangeable K to the exchangeable form takes place when the concentration of exchangeable K and in solution reduced by plant uptake, erosion, leaching, and/ or runoff (Parmar and Sindhu 2013). Multifarious microbes in soil are capable of solubilizing unavailable forms of K containing minerals, like micas, illite and orthoclases by excreting organic acids that directly dissolve the K rock or bounded silicon ions to change the K in soil solution. While, silicate bacteria were also observed to dissolve Si, K, and Al from insolvable minerals (Aleksandrov *et al.* 1967). Most of the K in soil is believed to exist as silicate minerals. Potassium becomes available to crop plants when silicate minerals slowly disintegrated in smaller fragments or dissolved (Bertsch *et al.* 1985). It has been reported that a wide range of soil bacteria release K in to available form from minerals containing K in soils (Liu *et al.* 2019). Application of K solubilizing co-inoculants could be more efficient due to their manifold effects on plant health by many growth promoting mechanisms (Singh *et al.* 2010). In addition, the benefits of co-inoculation can be enhanced by retaining high population of effective K solubilizing bacteria in the rhizosphere soil. Application of root associated bacteria having K-solubilizing activity could be helpful to reduce the effects of biotic and abiotic stresses on plant health throughout its life cycle (Hafsi *et al.* 2014). Therefore, the integrated use of PGPR and rhizobia could be very effective for improving nodulation and yield of chickpea. It restores the prerequisite to focus on a balanced K fertilization strategy to promote the efficacy of legume symbiosis under nutrient deficient conditions. The successful identification of an elite microbial strain capable of solubilizing K that can conserve present resources and reduce the risks of environmental contamination caused by the intensive application chemical fertilizers.

Therefore, in the light of above discussion, this study was planned with the following objectives; a) Isolation, screening, and selection of the most efficient rhizobacteria and rhizobial strains having K solubilizing activity on qualitative as well as quantitative basis, b) Optimization of growth conditions for selected bacterial strains to solubilize K from WM, and c) Characterization of the most effective bacterial strains having K-solubilization activity

**Materials and Methods**

A series of laboratory studies were carried out for isolation, purification and characterization of K solubilizing rhizobacteria (KSR) and rhizobial isolates from rhizosphereic soil and root nodule samples of chickpea (*Cicer arietinum* L.), respectively in the Department of Soil and Environmental Sciences, CA, University of Sargodha, Sargodha, Pakistan, and SWSD, IFAS, University of Florida, USA.

**Collection of soil and plant samples**

Soil, and plant (chickpea) samples were collected from different sites (irrigated and rainfed) of Punjab [Sargodha (32.0740° N, 72.6861° E), Noorpur Thal (31.8449° N, 71.8571° E), Jhang (31.2781° N, 72.3317° E)]. The chickpea plants were uprooted along with adjacent soil adherent to the plant roots. The uprooted chickpea plants were packaged in polythene bags and brought to the laboratory for isolation, and purification of rhizobia and rhizobacterial isolates having trait of K solubilization.

**Isolation of potassium solubilizing rhizobacteria**

For this, K-solubilizing bacteria from soil samples were isolated using dilution plate technique on Aleksandrov’s medium (Hu *et al.* 2006). For this, 10 g of rhizosphere soil from every sample was weighed and mixed with 95 mL sterile solution of NaCl (0.85 percent) in a 250 mL conical flasks. Flasks were shaken energetically for 10 minutes to form uniform suspensions of soil. The soil suspensions were then incubated under agitation for 45 minutes followed by filtration through sterile filter paper. Using 1.0 mL of filtered supernatant of soil suspension, samples were consecutively diluted up to 10-6 and 0.1 mL of aliquot from each of the dilution was shifted, and equally straighten out on Aleksandrov’s agar medium (Aleksandrov 1967). After this, media plates were incubated for 72 hours at 28 ± 2ºC. Morphologically well grown and prominent colonies (color, shape, size, and growth rate) were selected and purified by additional streaking on freshly prepared Aleksandrov’s agar medium using a four way streak plate technique. The diameter of the halo zone was measured and articulated in millimeters, and for further studies the pure cultures on agar slants were stored in the refrigerator at -40 ºC.

**Isolation of potassium solubilizing rhizobia**

Rhizobial isolates from nodules of chickpea were isolated using standard protocol. For this, healthy plant of chickpea (45-60 days old) were selected from field and then uprooted, and shifted to the lab using polythene bags. Bulk soil from roots of chickpea was detached by mild agitation and then the soil firmly adhering (rhizosphere soil) to the roots was removed. For isolation of rhizobia, the roots of chickpea plants were washed gently by tap water, and then nodules were detached from roots with the help of scissor. The collected nodules were surface disinfected for a moment (<10s) by immersing in 95 percent solution of ethanol, followed by immersing in 0.2 percent mercuric chloride solution for three minutes (Russell *et al.* 1982). Nodules were then washed several times with sterilized water to remove surface disinfectant. To obtain a milky suspension, the surface sterile/or disinfected nodules were crushed with a glass rod in sterile 5 mL water. A loop of milky suspension was striped on the Yeast Extract Mannitol (YEM) agar medium plates (Holt *et al.* 1994), and then incubated for 72 hours at 28 ± 2ºC. The isolated single colonies of rhizobial isolates were selected and picked with streaking needle and then restreaked on freshly prepared YEM agar medium plates. This process was repeated 3 to 4 times on freshly prepared YEM agar plates to obtain a pure cultures. In this way, 50 rhizobial isolates were isolated and designated with sampling sites. These isolates were stored in glycerol (20%) at -40 °C for further use.

**Screening of potassium solubilizing rhizobacteria and rhizobial isolates**

Potassium solubilizing ability of both bacterial isolates was tested on qualitative and quantitative basis from insoluble K bearing minerals.

**Qualitative assessment of potassium released from insoluble K bearing mineral**

For qualitative estimation of K, purified bacterial strains were initially selected for screening of K-solubilization on the basis of halo zone formation on agar medium having modified Aleksandrov’s medium via spot test method (Sindhu *et al.* 1999). Bacterial isolates showing the zone of solubilization in Aleksandrov’s agar medium were further tested to evaluate their potential to release K from the broth medium containing 1.0 percent mica mineral. For this purpose, 1.0 mL culture (overnight) of each bacterial isolates was inoculated to Aleksandrov’s broth having volume 25 mL (Aleksandrov 1967). All inoculated flasks were incubated for 3 weeks at 28±2°C. After this, the quantity of K was determined at 7, 14 and 21 days of incubation compared to control. Finally, the available K content was estimated by Flame Photometry method (Sugumaran and Janarthanam 2007).

**General characterization of bacterial isolates having trait potassium solubilization**

The bacterial isolates were tested for general characterization using the following procedures. These isolates were tested to determine the color, motility, shape, gram reaction, halo tolerance, as well as their ability to produce spores using the method described by Schaad *et al.* (2001). The selected isolates were tested for their ability to use divergent sources of carbon (C), namely sucrose, glycerol, maltose, and citrate. The sources of C were used at the rate of 2% in agar medium. After this, 24 hours old cultures were streaked on the medium and incubated for 24 hours at 28 ± 2°C. The degree of growth on media comprising various sources of C was generally observed and the growth was noted without growth (-) or growth (+). MR-VP Medium (Glucose phosphate broth) was used to perform Methyl Red test as described by Seeley and Vandemark (1981). While, the pre-sterilized tubes comprising broth test cultures MR-VP were inoculated for Voger-Proskauer test as studied by Seeley and Vandemark (1981). The method of Alariya *et al.* (2013) was used to detect Amylase activity of isolates. Freshly prepared cultures were streaked on Modified Czapek Mineral salt medium for the detection of the activity of cellulose enzyme (Aneja 2001). Catalase activity of isolates was tested by adding 3% H2O2 on developed growth colonies of selected isolates using method of Blazevic and Ederer (1975).

Tribibutyrene agar medium (TAM) was used for lipase activity. Lipolytic activity of selected isolates was verified by halo zone formation around the inoculation line and then inoculated plates were incubated for 72 hours at 25ºC (Anisha *et al.* 2012). Protease activity of isolates was checked using SMA (Skimmed Milk Agar) media (Smibert *et al.* 1994). The urease activity in selected isolates were determined as described by James and Sherman (1992). Qualitatively N fixing ability of bacterial cultures was determined using standard procedure as described by (Gothwal *et al.* 2008). Selected cultures were evaluated for P-solubilizing activity using protocol described by Pikovskaya (1948). Eckford (1927) method was used to test the potential of selected isolates to hydrolyse starch. In order to test for indole production assay, pre-sterilized sulfide-indole motility (SIM) agar tubes were inoculated with selected isolates. After this, SIM agar tubes were incubated for 48 hours at 28±2ºC. After the inoculation, Kovac’s reagent (10 drops) was added into each tube. Cherry Red production was considered positive for indole-production (Cheesborough 2006). While, the gelatin liquefaction of the bacterial cultures were determined as described by Blazevic and Ederer (1975). For hydrogen sulfide production assay was done using sterilized test tubes containing SIM (sulfide, indole, motility) agar medium (Cappuccino and Sherman 1992). Casein hydrolysis was determined by the method of Seeley and Vandemark (1981). The production of HCN (hydrogen cyanide) was estimated using modified King’s B agar medium (Baker *et al.* 1987)

**Quantitative characterization of plant growth promoting traits in selected isolates of bacteria**

For this, selected isolates were characterized for various plant growth promoting traits using the following protocols. Gordon and Weber (1951) method was used for estimation of indole-acetic acid (IAA) in selected isolates. The exopolysaccharides production was measured through inoculation of isolates on RCV (Rhodobacter-Capsulatus V) mineral medium after enrichment with Mannitol, glucose or sucrose, with and without NaCl (Ashraf *et al.* 2004). While, total carbohydrate content in the precipitated EPS was measured by standard procedure described by Dubois *et al.* (1956). Penrose and Glick (2003) protocol was used to determine the activity of ACC-deaminase activity in the cells of selected bacterial isolates in the form of α-ketobutyrate that resulting from the cleavage of ACC.

For the K-solubilization assay, 48 hours old bacterial cultures were used for inoculation of 25 mL

AMB spiked with WM in 50 mL capacity Erlenmeyer flask and then incubated in shaking incubator for six days at 28 ± 2ºC. The growth suspension of cultures was centrifuged at 7,000 × *g* for 10 minutes to separate the supernatant from cell growth, and the insoluble K and filtered. After this, filtered supernatant (1 mL) was taken in to a 50 mL flask and made the volume up to 50 mL using distilled water and then mixed well. Finally sample was analyzed for water soluble K content using flame photometer (Sugumaran and Janarthanam 2007).

For chitinase activity, the amount of N-Acetyl glucosamine (GlcNAc) produced from the colloidal chitin substrate was measured using procedure described by Reissig *et al.* (1955). The quantitative measurement of siderophores of K-solubilizing bacteria was done using CAS Shuttle Assay (Schwyn and Neilands 1987). For root colonization test, surface sterilized chickpea seeds inoculated for 48 hours old bacterial cultures and were sown in a glass jars filled with sterilized sand modified by Simon *et al.* (1996).

**Bacterial growth optimization for potassium solubilization**

Growth conditions (i.e. carbon sources, pH and temperature) were optimized for final selection of the most efficient K solubilizing isolates. Efficiency of bacterial strains for K solubilization from mica powder in Aleksandrov’s broth medium (ABM) was assessed using different combinations of above-mentioned growth conditions. The effect of glucose, galactose and cellulose as C sources at different temperatures (15, 25, 35 and 45 °C) and pH levels (6.5, 7.0, 7.5 and 8.5) was measured in terms of K solubilization potential of bacterial cultures. Each selected isolate was injected in 25 mL of amended ABM (Aleksandrov 1967), while, glucose was exchanged by either of two C sources (cellulose and galactose). After this, all flasks were incubated for 10 days at 28 ± 2ºC. The quantity of K released in to broth was measured compared to control though flame photometric method (Sugumaran and Janarthanam 2007).

**RSULTS**

**Isolation and purification of effective K-solubilizing rhizobacteria and rhizobial isolates**

For this, samples of rhizosphere soil and plant were collected from the chickpea cultivated areas of Punjab, Pakistan. Preliminary, 50 well grown and morphologically distinct colonies of each rhizobacteria and rhizobial isolates having trait K-solubilization were selected for purification. Selection of KSR isolates was based on growth of colonies on specific medium (i.e. Aleksandrov’s medium).

**Qualitative screening of selected K-solubilizing rhizobacterial isolates**

After purification, isolates were tested for their K-solubilization from WM on qualitative basis. For this, isolates were selected based on halo zone formation on Aleksandrov’s medium agar plates in 72

hours. Results of qualitative test showed that isolates with +, ++ and +++ signs produced halo zone of <2, >2 and >3mm, respectively (Table 1). While, isolates having negative (-ve) sign were found unable to form any halo zone on medium during incubation period. Out of 50 isolates, 25 gave maximum growth on medium spiked with WM as an insoluble K source. Twenty one isolates produced halo zone of >2mm while, five isolates produced halo zone of >3mm. These 25 isolates were selected for further quantitative screening which produced halo zones efficiently.

**Qualitative screening of selected K-solubilizing rhizobial isolates**

After purification, rhizobial isolates were tested for their K-solubilization from WM on qualitative basis. In this screening, isolates were selected based on halo zone formation on Aleksandrov’s medium agar plates in 72 hours. Results showed that isolates with +, ++ and +++ signs produced halo zone of <2, >2 and >3mm, respectively (Table 2). While, K-solubilizing isolates with - sign were unable to develop any halo zone on Aleksandrov’s medium during incubation period. Twenty five (25), out of 50 isolates showed maximum growth on medium spiked with WM as an insoluble K source. Twenty one isolates produced halo zone of >2mm size while, five isolates produced halo zone of >3mm. These 25 isolates were selected for further quantitative screening which produced halo zones efficiently.

**Quantitative screening of selected K-solubilizing rhizobacterial isolates**

For quantitative test, 25 isolates were selected for K-solubilization potential from WM in Aleksandrov’s broth within 72 hours of incubation (Table 3). Out of 25 isolates, five (5) isolates had shown maximum solubilization of K that was more than 26 mg L-1 from WM. Maximum K-solubilization was 31.6 mg L-1 due to inoculation with JKB20 isolate while, minimum was 10.7 mg L-1 compared to uninoculated control. Most of isolates had shown solubilization of K in the range of 16-22 mg L-1. The other most effective isolates (i.e. SKB3, SKB11, SKB13 and JKB10) had shown K-solubilization 29.3, 27.4, 26.8 and 26.5 mg L-1, respectively from WM in Aleksandrov’s broth after 48 hours of incubation.

**Quantitative screening of selected K-solubilizing rhizobial isolates**

For this, 25 isolates of rhizobia were selected to evaluate their K solubilization potential from WM in Aleksandrov’s broth within 72 hours (Table 3). Out of twenty five, 5 isolates had shown effective solubilization of K that was more than 15 mg L-1 from WM. While, maximum K-solubilization was recorded up to 18.6 mg L-1 due to inoculation with SKR10 isolate and minimum was found 6.1 mg L-1 over uninoculated control. Most of the isolates showed solubilization of K in the range of 9.0 to 17.0 mg L-1. The other most effective isolates (JKR7, SKR13, JKR16 and JKR4) had also shown K-solubilization 17.3,

17.1, 15.8 and 15.5 mg L-1, respectively from WM in Aleksandrov’s broth after 72 of incubation.

**General characterization of rhizobacteria and rhizobial isolates having trait K-solubilization**

For this, five most efficient isolates of each were subjected to general characterization (morphological and biochemical traits) and further experimentation (Table 4). Out of five rhizobacterial isolates, three (JKB10, SKB3, SKB11) were rod shaped and two (JKB20 and SKB13) were coccus shaped while, all isolates of rhizobia (JKR4, JKR7, JKR16 and SKR13 and SKR10) were rod shaped. Regarding color, all isolates of rhizobacteria were yellowish and three rhizobial isolates were creamy (JKR4, JKR7 and SKR10) and two (JKR16 and SKR13) were white in color. All bacterial isolates were positive in motility. Regarding sporulation and gram staining, three isolates (JKB10, SKB11 and SKB13) of rhizobacterial were positive while, two (JKB20 and SKB3) were negative. In case of rhizobia, all isolates were negative in sporulation and gram staining. For halo-tolerance, bacterial isolates (JKB10, SKB3, SKB11 and SKB13) were positive while, JKB20 was negative and three isolates of rhizobia (JKR4, JKR7 and JKR16) were positive while, SKR10 and SKR13 were found negative.

In case of biochemical characteristics, two isolates of rhizobacteria (JKB10 and SKB3) were positive for methyl red while, three isolates (JKB20, SKB11 and SKB13) were negative and all isolates of rhizobia were negative for methyl red. Regarding Voger-Proskaur test, four isolates rhizobacteria (JKB10, SKB3, SKB11 and SKB13) were positive and one (JKB20) was negative while, three isolates (JKR7, SKR10 and SKR13) were negative and two (JKR4 and JKR16) were positive in case of rhizobia. Indole production was observed in all isolates of rhizobacteria and rhizobia and it was found double in SKB3 isolate of rhizobacteria. However, production of H2S and HCN were negative in all bacterial isolates. Three rhizobacterial isolates (JKB10, SKB3 and SKB13) were found positive for amylase activity and two (JKB20 and SKB11) were negative while, three isolates of rhizobia (JKR4, JKR16 and SKR10) were negative for amylase activity and two isolates (JKR7 and SKR13) were positive. In case of cellulase activity, three isolates of rhizobacteria (JKB10, SKB3 and SKB13) were observed positive and absent in two isolates (JKB20 and SKB11) while this activity was absent in four isolates of rhizobia (JKR4, JKR16, SKR10 and SKR13) and present in only one isolate (JKR7). Catalase activity was observed in two isolates of rhizobacteria (JKB10 and SKB3) and absent in other three (JKB20, SKB11 and SKB13) while, this activity was found absent in four rhizobial isolates (JKR4, JKR16, SKR10 and SKR13) and present in JKR7 isolate. In case of lipase activity, three isolates of rhizobacteria (JKB10, SKB11 and SKB13) were negative and two (JKB20 and SKB3) were positive while, in case of rhizobia, it was present in two isolates (JKR7 and SKR13) and absent in three isolates (JKR4, JKR16 and SKR10). Urease activity was positive in all isolates of rhizobacteria and rhizobia except one isolate (SKR10). Similarly, in case of oxidase activity and starch hydrolysis, all isolates bacterial were found positive except isolate SKR13 in both cases. The rhizobacterial isolates (JKB20, SKB3 and SKB13) were negative and JKB10 and SKB3 were positive for casein hydrolysis and three isolates of rhizobia (JKR4, JKR7 and SKR13) were negative and two (JKR16 and SKR10) were positive. N2-fixing activity was observed in all bacterial isolates, and it was highly prominent in JKR7 isolate of rhizobia. Protease activity was observed positive in two isolates of rhizobacteria (JKB29 and SKB3) and absent in three isolates (JKB10, SKB11 and SKB13) and this activity was also absent in four isolates of rhizobia (JKR4, JKR16, SKR10 and SKR13) and it was present in JKR7 rhizobial isolate. It was also observed that gelatin liquefaction was present in all isolates while, it was highest in SKB3 rhizobacterial isolate.

**Utilization of different carbon sources by bacterial isolates having K-solubilization activity**

All bacterial strains had shown ability to utilize a variety of C sources is an excellent trait to perform in actual soil conditions. Growth and activity of microbial strains can be correlated with ease of utilization of C sources which are not easily degradable. Selected isolates of both rhizobacteria and rhizobia having trait K-solubilization were tested for their efficacy to utilize different C sources by growing on Aleksandrov’s media where glucose was replaced by arabinose, cellulose, citrate, galactose, sucrose and xylose (Table 5). All isolates were able to utilize all type of selected C sources efficiently except cellulose source which was utilized by only two isolates of rhizobacteria (JKB20 and SKB3). All rhizobial isolates have no potential to utilize the cellulose.

**Quantitative screening of effective combination of PGPR × Rhizobium**

For this, the most efficient twenty five (25) combinations were selected and evaluated for their K-solubilization potential from WM in Aleksandrov’s broth within 72 hours of incubation (Table 6). Out of twenty five (25), two (2) combinations (SKB3 × JKR7 and SKB11 × SKR13) had shown highest K solubilization that was 47.4 and 41.9 mg L-1, respectively from WM. Next effective combinations (i.e. JKB20 × SKR10, SKB3 × SKR10 and SKB13 × SKR10) had also exhibited K solubilization 29.3, 28.9 and 28.1 mg L-1, respectively from WM after 72 hours of incubation. While, minimum K was recorded up to 13.9 mg L-1 due to SKB13 × JKR16 combination. It was observed that most of the bacterial combinations had shown solubilization of K in the range of 15 to 26 mg L-1 in broth culture.

**Characterization of selected K-solubilizing bacterial isolates for plant growth promoting activities**

The selected bacterial isolates were tested for some plant growth promoting activities under lab conditions. Overall SKB3 and JKR7 performed better compared to the other remaining isolates. Results about plant growth promoting activities are mentioned in Table 7.

**ACC-deaminase activity**

Maximum ACC-deaminase activity (up to 304.4 α-KB μmol g-1 protein h-1) was recorded in JKB10

rhizobacterial isolate followed by SKB3, SKB13, JKB20 and SKB11 isolates, respectively that ranged from 177 to 284.7 α-KB μmol g-1 protein h-1 (Table 7). While in case of rhizobial isolates, highest ACC-deaminase activity (up to 30.4 α-KB μmol g-1 protein h-1) was recorded in JKR16 isolate of rhizobia. Rest of the isolates (JKR4, SKR13, JKR7 and SKR10) also showed ACC-deaminase activity in the range of 17.4 to 24.6 α-KB μmol g-1 protein h-1 more over control.

**Indole acetic acid production**

Production of IAA in the selected bacterial isolates was observed without and with L-TRP under lab conditions (Table 7). In case of rhizobacteria, maximum IAA production was recorded up to 34.3 and 73 mg L-1 without and with L-TRP, respectively over control. Rest of isolates had also shown the promising increase in IAA that was ranging from 19.4 to 30.6 mg L-1 (-L-TRP) and 34.3 to 65.8 mg L-1 (+L-TRP). While, in case of rhizobial isolates, maximum IAA production was observed up to 46.7 and 91.3 mg L-1 without and with L-TRP, respectively. Other isolates of rhizobia also exhibited a better production of IAA in the range of 16.9 to 32.6 mg L-1 (-L-TRP) and 37.9 to 64.2 mg L-1 (+L-TRP) compared to control.

**Chitinase activity assay**

All bacterial isolates showed chitinase activity under lab conditions. Highest chitinase activity was observed in SKB3 isolate of rhizobacteria (37.8 µmol of Glc NAc min-1 mg-1 protein) and lowest chitinase activity was recorded in JKB20 isolate. While in rhizobial isolates, this activity was poor as compared to rhizobacterial isolates. But maximum chitinase activity in rhizobial isolate was recorded in JKR7 isolate of rhizobia that was up to 3.6 µmol of Glc NAc min-1 mg-1 protein and lowest chitinase activity was recorded in SKR3 (2.1 µmol of Glc NAc min-1 mg-1 protein)

**Exopolysaccharides production**

All selected isolates showed production of exopolysaccharides (EPSs). Maximum EPSs production was observed up to 78.5 µg mL-1 due to inoculation of SKB3 isolate of rhizobacteria and minimum EPSs was 42.8 µg mL-1 in case of SKB11 isolate. While, in rhizobial isolates, highest EPSs (321.2 µg mL-1) was noted in JKR7 and lowest EPSs was produced by SKR13 isolate that was 122.8 µg mL-1.

**Potassium solubilization activity**

All isolates had shown K-solubilizing activity in Aleksandrov’s medium spiked with 0.5% WM. Maximum K-solubilization was observed in case of SKB3 isolate that was up to 113.7 mg L-1 followed by JKB10 isolate (74.4 mg L-1). But in rhizobial isolates, maximum K-solubilization was observed in JKR16 that was up to 45.3 mg L-1 followedby JKR7 isolate (up to 41.6 mg L-1).

**Phosphate solubilization activity**

Both rhizobacteria and rhizobial isolates had shown potential to solubilize phosphate in liquid medium. Maximum P-solubilization activity was found to be associated with SKB3 isolate of rhizobacteria (72.5 mg L-1) followed by JKB10 isolate of rhizobacteria. While in rhizobial isolates, highest P-solubilization activity was observed in JKR7 isolates (17.3 mg L-1) and minimum in isolate JKR16.

**Siderophores production**

Maximum siderophores production was recorded (up to 43.7%) because of SKB3 isolate of rhizobacteria followed by JKB10 isolate. Rest of the isolates had also shown siderophores production that ranged from 18.9 to 24.8% compared to control. While, rhizobial isolate JKR7 showed maximum production of siderophores that was up to 36.8% followed by isolate JKR16. Other remaining isolates also exhibited a promising activity of siderophores that was ranging from 17.9 to 26.9% over control.

**Root colonization**

Highest root colonization was observed by SKB13 isolate of rhizobacteria that was 4.78 x 107 CFU g-1 FRB) and minimum was by JKB20 isolate (7.68 x 105 CFU g-1 FRB). While in case of rhizobial isolates, maximum root colonization was found to be associated with isolate SKR7 (3.94 x 107 CFU g-1 FRB) and minimum was by rhizobial isolate SKR13 (6.34 x 105 CFU g-1 FRB).

**Optimization of growth conditions for K-solubilizing bacterial isolates from mica in broth medium**

Results about K-solubilization showed that bacterial isolates SKB3 and JKR7 performed efficiently at all C sources (i.e. glucose, galactose and cellulose). Maximum and minimum K-solubilization was observed in a media having glucose and cellulose as a C source, respectively. This trend was found consistent with all temperature and pH values. It was observed that the increase in temperature negatively affected the K-solubilizing activity at any pH values and C source. Maximum K solubilization was observed at 25-30°C with all C sources and pH values, and subsequently decreased in K-solubilization was recorded with increase in temperature. Similarly, maximum amount of K-solubilization was recorded at pH 7.0 at all temperature with all C sources. Optimum conditions of growth medium for maximum K-solubilization and K-solubilizing activity were found as; glucose was the best source of C at 25-30 °C and pH of 6.8-7.2. Graphical description of results had shown in the Figures S1, S2, S3, S4, S5, S6, S7, S8 and S9.

**DISCUSSION**

The current study was executed to carry out the isolation, screening and characterization of K-solubilizing

bacteria based on qualitative as well as quantitative traits of plant growth promoting under axenic conditions.

**K-solubilizing rhizobacteria and rhizobial isolates**

Plant-microbe beneficial interactions in rhizosphere are very important component of eco-friendly agricultural system. In this study, rhizosphere as well as nodule bacteria were preliminary isolated from chickpea dominated areas. Although 25 isolates showed prolific growth in the presence of WM spiked Aleksandrov media, only five isolates each from rhizosphere and nodule of chickpea were able to form halo zone of > 3mm (Table 1, 2). The apparent affinity of bacteria to solubilize K is often reflected by halo zone formation (Kumar *et al.* 2015). All KSB isolates were re-evaluated for another test of K solubility by screening on the basis of amount of K solubilized *in-vitro* after 72 h incubation. Our results confirmed the efficacy of KSB isolation approach as both isolates with highest halo zone formation were able to solubilize highest amount of K among rest of the isolates (Table 3). Numerous reports have shown the tendency of some microbes to solubilize the insoluble K caused by bacterial metabolic activity resulting in the dissolution of K (Sarikhani *et al.* 2018; Liu *et al.* 2019).

The KSB isolates displayed variety of morphological characteristics such as mortility sporulation, gram staining and halotolerance (Table 4). The results had shown that SKB3 isolate was able to utilize all carbon substrates, while none of the rhizobial isolate was able to use cellulose (Table 5). Different bioactive compounds and growth metabolites produced by plant beneficial bacteria could greatly be influenced both by the availability of different C substrates and the extent of their utilization (Kaur *et al.* 2019).

In this study, quantitative screening for effective co-inoculants was carried out based on their cumulative K solubilization activity in K-enriched medium. The combined K releasing ability of co-inoculants ranged 13.9 to 47.4 mg L-1 (Table 6). The largest amount of soluble K was recovered with co-inoculation of SKB3×JKR7 followed by SKB11×SKR13, whereas lowest release of soluble K was obtained with SKB13×JKR16 (Table 6). Low grade K originating from feldspars and mica minerals can be mobilized and/or solubilized by some bacteria, and for this very reason their application in K-deficient soil could increase K availability for crop plants (Sattar *et al.* 2019).

The ACC-deaminase activity is one of the main growth promoting attribute which plays a vital role in plant growth regulation both in normal as well as stressed conditions (Shahzad *et al.* 2017). Ethylene, a phytohormones often produced in abundance under stress, could negatively affect plant growth and development. However, plant inoculation with bacteria having ACC-deaminase can regulate the level of ethylene by converting ACC (ethylene precursor) into ammonia and α-ketobutyrate (Penrose and Glick 2003). In present research, rhizobacteria have relatively higher ACC-deaminase activity than rhizobial isolates. Some plant beneficial bacteria can modify level of indigenous phytohormone production in plant, such as IAA, which promotes root elongation, lateral root development and root hair formation. The improvement in the root system often lead to higher water and nutrient uptake efficiency of plant. Coupling of root as well as bacterial release IAA can facilitate as a potential energy resource for the introduced bacterium for higher growth, survival and root colonization (Shahzad *et al.* 2017). In this study, rhizobial isolates showed more IAA production than rhizobacterial isolates both with and without L-tryptophan (Table 7). In some previous studies, strong IAA activity was reported in several rhizobial isolates (Ghosh *et al.* 2013). An elevated chitinase activity was displayed by rhizobacteria ranging 12.5 to 37.8 µmol Glc NAc min-1 mg-1 protein, whereas rhizobial isolates had relatively lower chitinase activity between 2.1 to 3.6 µmol Glc NAc min-1 mg-1 protein (Table 7). Chitinase activity usually reflects the availability as well accessibility of substrate because of its key role in organic matter degradation (Shahzad *et al.* 2017). These findings elicit that rhizobacteria are more inclined towards the available C substrate resource than rhizobia. In addition, various rhizobacteria have been reported to establish plant growth by releasing fungal cell wall degrading chitinase enzyme to safeguard plant against pathogen (Liu *et al.* 2019).

In present study, isolates of rhizobia participate more in secreting biopolymer compounds such as exopolysaccharide (EPS), which was in higher amount between 122.8 to 321.2 µg mL-1 than rhizobacteria (Table 7). A number of recent reports described that rhizobial EPS production is generally linked to the formation of an adaptive mechanism at cell surface scale under stressful conditions (Zhao *et al.* 2019; Liu *et al.* 2019). The EPS would thus be released by the bacteria to shield the plants against exposed stressor, indirectly benefiting from its growth and development under stress (Skorupska *et al.* 2006).

Microbial mediated nutrient solubilization is another crucial trait that can improve their availability for plant uptake in limited nutrient environment. In current study, we identified that both rhizobia and rhizobacteria were able to solubilize K and P in the culture medium (Table 7). Selected isolates were more efficient K solubilizers whereas rhizobacteria had shown stronger capacity to solubilize K and P than rhizobial isolates (Table 7). These findings further corroborate the previous reports where organic acids produced as a result of bacterial metabolic activities contributed to increase solubility of nutrients such P (Arif *et al.* 2017) and K (Zhao *et al.* 2019). Besides nutrients solubility, selected isolates had varied in siderophores production (Table 7). Microbial siderophores are low molecular weight iron-scavenging ligands produced mainly under iron deficient conditions (Arif *et al.* 2017). Establishment of successful root colonization by microbial inoculant is an essential criterion to confer associated plant growth and development benefits (Sattar *et al.* 2019). Results of this study showed that rhizosphere as well as rhizobial isolates were efficient root colonizer (Table 7). Failure of microbial inoculant to colonize plant roots often caused the diffusion of their metabolic substances into the root-zone and eventually consumed by variety of root inhabiting microbes. In the absence of root persistence, introduced bacteria can evade away from the rhizosphere thus making root interface more vulnerable to deleterious root microflora (Mia *et al.* 2010).

**Optimization of growth conditions for K-solubilizing rhizobacteria and rhizobia**

Optimization of conditions for maximum growth ofSKB3 and JKR7 isolates and their K-solubilization activity, resulted in glucose being the best source of C when maintained at 25°C by regulating pH 7.0 of growth medium. Parmar and Sindhu (2013) conducted a study to investigate the effect of various growth conditions on the K-solubilization potential of K-solubilizing bacteria, resulting in a neutral pH range at 25°C, while using glucose as a source of C. The findings of this study is similar to Sheng *et al.* (2008) that some strains of K-solubilizing bacteria also documented with remarkable K solubilization activity at relatively higher temperatures, up to 42°C, but maximum activity was detected in the range of 25-30°C. All researchers also agreed that glucose is the best source of C for the maximal activity of almost all K solubilizing bacterial strains studied in many experiments (Kumar *et al.* 2015; Liu *et al.* 2019). The bacterial strains SKB3 and JKR7 could have a low K solubility potential at a temperature of 25 °C and a pH of 7.0 using cellulose as a C source. However, the minute use of cellulose as a C source could be an outstanding feature of any strain of PGPR to improve better performance under natural soil environments.

**Conclusions**

It is concluded that the application of rhizobacteria and rhizobial K-solubilizer could be employed as a useful K-supplement in K-limited agroecosystems. Furthermore, use of these K-inoculants may lead in alleviating the negative impact of associated with chemical fertilizer use for environment. This technique can also be used alone as well as nutritional partner of K-fertilizers for different crops depending upon the extent of crop K-requirements.

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**Table 1:** Qualitative screening of potassium solubilizing rhizobacterial isolates

|  |  |  |  |
| --- | --- | --- | --- |
| Bacterial isolate | Score | Bacterial isolate | Score |
| JKB1 | ++ | NKB6 | ++ |
| JKB2 | + | NKB7 | ++ |
| JKB3 | + | NKB8 | ++ |
| JKB4 | ++ | NKB9 | ++ |
| JKB5 | – | NKB10 | – |
| JKB6 | + | NKB11 | + |
| JKB7 | – | NKB12 | – |
| JKB8 | + | SKB1 | ++ |
| JKB9 | + | SKB2 | ++ |
| JKB10 | +++ | SKB3 | +++ |
| JKB11 | ++ | SKB4 | ++ |
| JKB12 | + | SKB5 | + |
| JKB13 | + | SKB6 | ++ |
| JKB14 | ++ | SKB7 | ++ |
| JKB15 | – | SKB8 | ++ |
| JKB16 | + | SKB9 | + |
| JKB17 | – | SKB10 | – |
| JKB18 | + | SKB11 | +++ |
| JKB19 | ++ | SKB12 | + |
| JKB20 | +++ | SKB13 | +++ |
| NKB1 | + | SKB14 | ++ |
| NKB2 | ++ | SKB15 | ++ |
| NKB3 | – | SKB16 | + |
| NKB4 | ++ | SKB17 | ++ |
| NKB5 | – | SKB18 | – |

+: Halo size < 2mm; ++: Halo size > 2mm; +++: Halo size > 3mm

**Table 2:** Qualitative screening of potassium solubilizing rhizobial isolates

|  |  |  |  |
| --- | --- | --- | --- |
| Rhizobial isolate | Score | Rhizobial isolate | Score |
| JKR1 | ++ | NKR4 | ++ |
| JKR2 | ++ | NKR5 | + |
| JKR3 | – | NKR6 | + |
| JKR4 | +++ | NKR7 | ++ |
| JKR5 | – | NKR8 | – |
| JKR6 | ++ | NKR9 | + |
| JKR7 | +++ | NKR10 | – |
| JKR8 | ++ | NKR11 | + |
| JKR9 | ++ | NKR12 | + |
| JKR10 | ++ | NKR13 | ++ |
| JKR11 | + | SKR1 | ++ |
| JKR12 | ++ | SKR2 | + |
| JKR13 | ++ | SKR3 | + |
| JKR14 | ++ | SKR4 | ++ |
| JKR15 | + | SKR5 | – |
| JKR16 | +++ | SKR6 | + |
| JKR17 | – | SKR7 | – |
| JKR18 | + | SKR8 | + |
| JKR19 | ++ | SKR9 | + |
| JKR20 | ++ | SKR10 | +++ |
| JKR21 | + | SKR11 | + |
| JKR22 | ++ | SKR12 | ++ |
| NKR1 | ++ | SKR13 | +++ |
| NKR2 | + | SKR14 | ++ |
| NKR3 | – | SKR15 | – |

+: Halo size < 2mm; ++: Halo size > 2mm; +++: Halo size > 3mm

**Table 3:** Quantitative screening of K–solubilizing rhizobacteria and rhizobial isolates

|  |  |  |  |
| --- | --- | --- | --- |
| Rhizobacterial isolate | Soluble K (mg L–1) | Rhizobial isolate | Soluble K (mg L–1) |
| JKB1 | 214 ± 113 | JKR1 | 104 ± 082 |
| JKB4 | 197 ± 109 | JKR2 | 117 ± 089 |
| JKB10 | 265 ± 136 | JKR4 | 155 ± 098 |
| JKB11 | 188 ± 106 | JKR6 | 98 ± 076 |
| JKB14 | 227 ± 124 | JKR7 | 173 ± 108 |
| JKB19 | 174 ± 098 | JKR8 | 83 ± 064 |
| JKB20 | 316 ± 196 | JKR9 | 117 ± 080 |
| NKB2 | 198 ± 103 | JKR10 | 138 ± 110 |
| NKB4 | 203 ± 113 | JKR12 | 123 ± 104 |
| NKB6 | 174 ± 092 | JKR13 | 74 ± 043 |
| NKB7 | 169 ± 089 | JKR14 | 109 ± 081 |
| NKB8 | 225 ± 127 | JKR16 | 158 ± 102 |
| NKB9 | 203 ± 103 | JKR19 | 63 ± 035 |
| NKB11 | 179 ± 091 | JKR20 | 129 ± 101 |
| NKB12 | 194 ± 101 | JKR22 | 94 ± 082 |
| SKB1 | 132 ± 083 | NKR1 | 102 ± 088 |
| SKB2 | 171 ± 095 | NKR4 | 61 ± 039 |
| SKB3 | 293 ± 189 | NKR7 | 123 ± 097 |
| SKB4 | 217 ± 120 | NKR13 | 97 ± 0982 |
| SKB6 | 168 ± 086 | SKR1 | 118 ± 090 |
| SKB7 | 107 ± 067 | SKR4 | 91 ± 086 |
| SKB8 | 206 ± 115 | SKR10 | 186 ± 114 |
| SKB11 | 274 ± 187 | SKR12 | 105 ± 090 |
| SKB13 | 268 ± 179 | SKR13 | 171 ± 104 |
| SKB14 | 199 ± 112 | SKR14 | 89 ± 059 |

Values are mean of three replicates followed by (±) standard error of mean K–solubilization in Aleksandrov’s broth from waste mica in 48 hours = mg L–1 = (ppm)

**Table 4:** General characterization of rhizobacteria and rhizobial isolates having trait K-solubilization

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Characters | Rhizobacterial isolates | | | | | Rhizobial isolates | | | | |
| JKB10 | JKB20 | SKB3 | SKB11 | SKB13 | JKR4 | JKR7 | JRK16 | SKR10 | SKR13 |
| Morphological traits |  | | | | | | | | | |
| Shape | Rod | Coccus | Rod | Rod | Coccus | Rod | Rod | Rod | Rod | Rod |
| Color | Y | CW | Y | MW | Y | CW | C | W | CW | MW |
| Motility | + | + | + | + | + | + | + | + | + | + |
| Sporulation | + | – | – | + | + | – | – | – | – | – |
| Gram staining | + | – | – | + | + | – | – | – | – | – |
| Halotolerance | + | – | + | + | + | + | + | + | – | – |
| Biochemical traits | | | | | | | | | | |
| Methyl red | + | – | + | – | – | – | – | – | – | – |
| Voger–Proskauer test | + | – | + | + | + | + | – | + | – | – |
| Indole production | + | + | ++ | + | + | + | + | + | + | + |
| H2S production | – | – | – | – | – | – | – | – | – | – |
| HCN production | – | – | – | – | – | – | – | – | – | – |
| Amylase activity | ++ | – | + | – | + | – | + | – | – | + |
| Cellulase activity | + | – | ++ | – | + | – | + | – | – | – |
| Catalase activity | + | – | + | – | – | – | + | – | – | – |
| Lipase activity | – | + | ++ | – | – | – | + | – | – | + |
| Urease activity | + | + | + | + | + | + | + | + | – | + |
| Oxidase activity | + | + | + | + | + | + | + | + | + | – |
| Starch hydrolysis | + | + | + | + | + | + | + | + | + | – |
| Casein hydrolysis | + | – | + | – | – | – | – | + | + | – |
| N2–fixing activity | + | + | + | + | + | + | ++ | + | + | + |
| Protease activity | – | + | + | – | – | – | + | – | – | – |
| Gelatin liquefaction | + | + | ++ | + | + | + | + | + | + | + |

Single positive sign means halo size <2mm while double positive means halo size >2mm; Y: Yellowish; CW: Cream white; MW: Milky white; C: Creamy

**Table 5:** Utilization of different carbon sources by rhizobacteria and rhizobial isolates having K-solubilization activity

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Isolates | Different carbon sources | | | | | |
| Arabinose | Cellulose | Citrate | Galactose | Sucrose | Xylose |
| Rhizobacterial isolates | | | | | | |
| JKB10 | + | – | + | + | + | + |
| JKB20 | – | + | + | + | + | + |
| SKB3 | + | + | + | + | + | + |
| SKB11 | – | – | + | – | + | + |
| SKB13 | – | – | + | – | + | + |
| Rhizobial isolates | | | | | | |
| JKR4 | + | – | + | + | + | – |
| JKR7 | + | – | + | + | + | + |
| JKR16 | – | – | + | + | + | + |
| SKR10 | – | – | + | – | + | + |
| SKR13 | – | – | + | – | + | – |

**Table 6:** Quantitative screening of effective combination of PGPR x *Rhizobium*

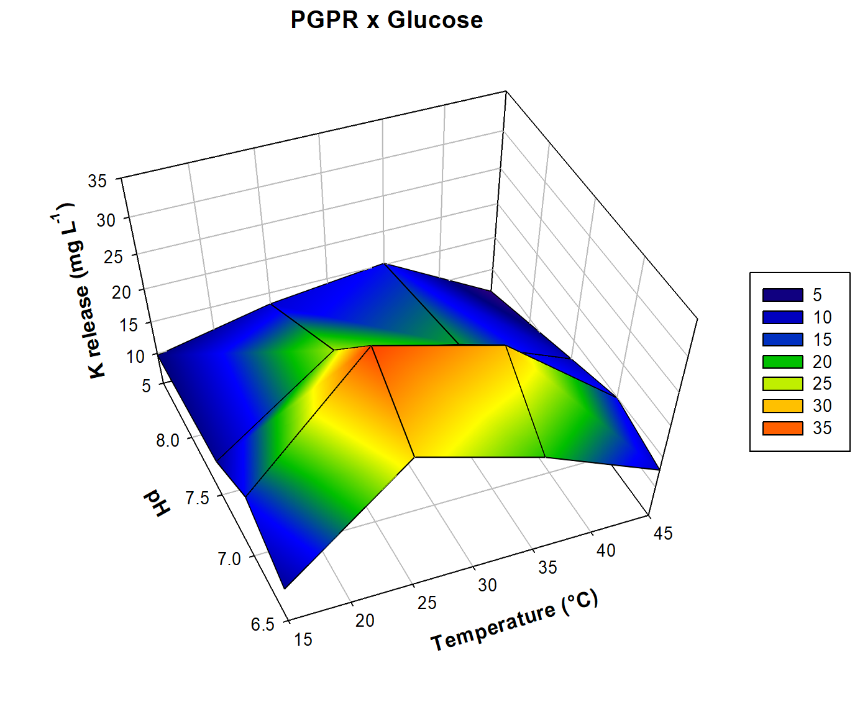
|  |  |  |  |
| --- | --- | --- | --- |
| PGPR x *Rhizobium* | Soluble K (mg L–1) | PGPR x *Rhizobium* | Soluble K (mg L–1) |
| JKB10 x JKR4 | 227 ± 134 | SKB3 x SKR10 | 289 ± 214 |
| JKB10 x JKR7 | 243 ± 172 | SKB3 x SKR13 | 265 ± 184 |
| JKB10 x JKR16 | 172 ± 108 | SKB11 x JKR4 | 217 ± 147 |
| JKB10 x SKR10 | 262 ± 190 | SKB11 x JKR7 | 265 ± 188 |
| JKB10 x SKR13 | 258 ± 173 | SKB11 x JKR16 | 154 ± 102 |
| JKB20 x JKR4 | 274 ± 204 | SKB11 x SKR10 | 277 ± 207 |
| JKB20 x JKR7 | 257 ± 170 | SKB11 x SKR13 | 419 ± 239 |
| JKB20 x JKR16 | 282 ± 210 | SKB13 x JKR4 | 194 ± 087 |
| JKB20 x SKR10 | 293 ± 213 | SKB13 x JKR7 | 236 ± 169 |
| JKB20 x SKR13 | 262 ± 192 | SKB13 xJKR16 | 139 ± 094 |
| SKB3 x JKR4 | 254 ± 177 | SKB13 x SKR10 | 281 ± 211 |
| SKB3 x JKR7 | 474 ± 278 | SKB13 x SKR13 | 259 ± 179 |
| SKB3 x JKR16 | 239 ± 167 |  |  |

Values are mean of three replicates followed by (±) standard error of mean K–Solubilization in Aleksandrov’s broth from waste mica in 48 hours = mg L–1 = (ppm)

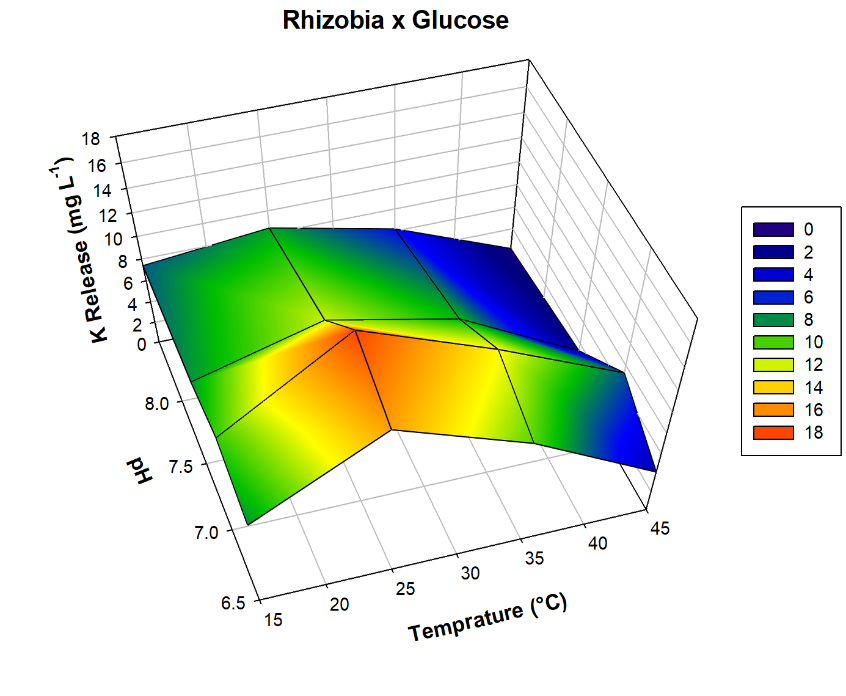
**Table 7:** Characterization of selected K–solubilizing rhizobacteria and rhizobial isolates for plant growth promoting activities

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Bacterial isolates | Plant growth promoting (PGP) activities (Quantitative) | | | | | | | | |
| ACC-deaminase (α-KB μmol g-1 protein h-1) | Indole-acetic acid production (mg L-1) | | Chitinase (µmol of Glc NAc min-1 mg-1 protein) | EPSs production (µg mL-1) | K-solubilization (mg L-1) [05% WMEM] | Phosphate solubilization [05% RP-EM] | Siderophores production (SU %) | Root colonization  (CFU g-1 FRB) |
| -L-TRP | +L-TRP |
| Rhizobacterial isolates | | | | | | | | | |
| JKB10 | 3044 ± 232 | 278 ± 319 | 445 ± 43 | 226 ± 282 | 636 ± 47 | 744 ± 432 | 672 ± 96 | 314 ± 27 | 373 x 107 ± 487 x 106 |
| JKB20 | 1925 ± 223 | 306 ± 267 | 658 ± 53 | 178 ± 194 | 513 ± 36 | 518 ± 415 | 473 ± 8 9 | 248 ± 39 | 289 x 106 ± 523 x 105 |
| SKB3 | 2847 ± 194 | 343 ± 379 | 736 ± 74 | 378 ± 226 | 785 ± 56 | 1137 ± 631 | 725 ± 55 | 437 ± 49 | 478 x 107 ± 467 x 106 |
| SKB11 | 1778 ± 279 | 264 ± 287 | 627 ± 64 | 125 ± 278 | 428 ± 32 | 609 ± 439 | 373 ± 693 | 189 ± 36 | 546 x 106 ± 513 x 105 |
| SKB13 | 2327 ± 172 | 194 ± 103 | 343 ± 53 | 215 ± 354 | 528 ± 48 | 566 ± 469 | 523 ± 743 | 219 ± 18 | 768 x 105 ± 359 x 104 |
| Rhizobial isolates | | | | | | | | | |
| JKR4 | 246 ± 33 | 326 ± 234 | 642 ± 433 | 24 ± 082 | 2436 ± 346 | 384 ± 356 | 128 ± 106 | 243 ± 244 | 484 x 106 ± 560 x 105 |
| JKR7 | 218 ± 26 | 467 ± 310 | 913 ± 653 | 36 ± 094 | 3212 ± 316 | 416 ± 389 | 173 ± 129 | 368 ± 323 | 394 x 107 ± 623 x 106 |
| JKR16 | 304 ± 46 | 298 ± 210 | 572 ± 524 | 23 ± 062 | 1542 ± 315 | 453 ± 431 | 75 ± 135 | 316 ± 452 | 512 x 106 ± 443 x 105 |
| SKR10 | 174 ± 29 | 201 ± 178 | 424 ± 732 | 25 ± 078 | 1628 ± 237 | 361 ± 418 | 113 ± 193 | 269 ± 288 | 359 x 106 ± 534 x 105 |
| SKR13 | 227 ± 39 | 169 ± 139 | 379 ± 547 | 21 ± 054 | 1228 ± 133 | 307 ± 334 | 93 ± 043 | 179 ± 318 | 634 x 105 ± 568 x 104 |

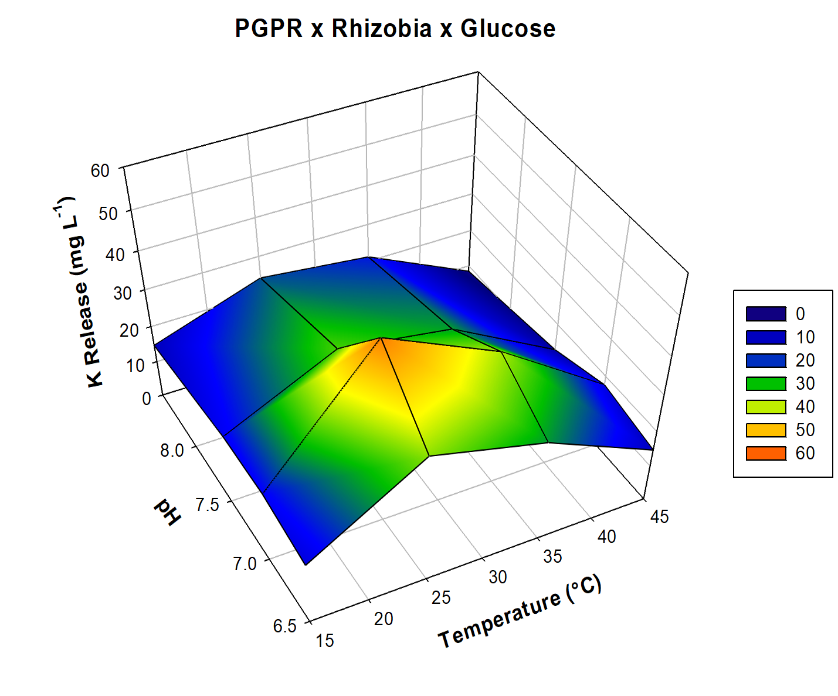
ACC: 1-aminocyclopropane-1-carboxylate; α-KB: α-ketobutyrate; L-TRP: L-tryptophan; Glc NAc: N-acetyl D-glucosamine; EPSs: Exopolysaccharides; WM-EM: Waste mica enriched medium; RP-EM: Rock phosphate enriched medium



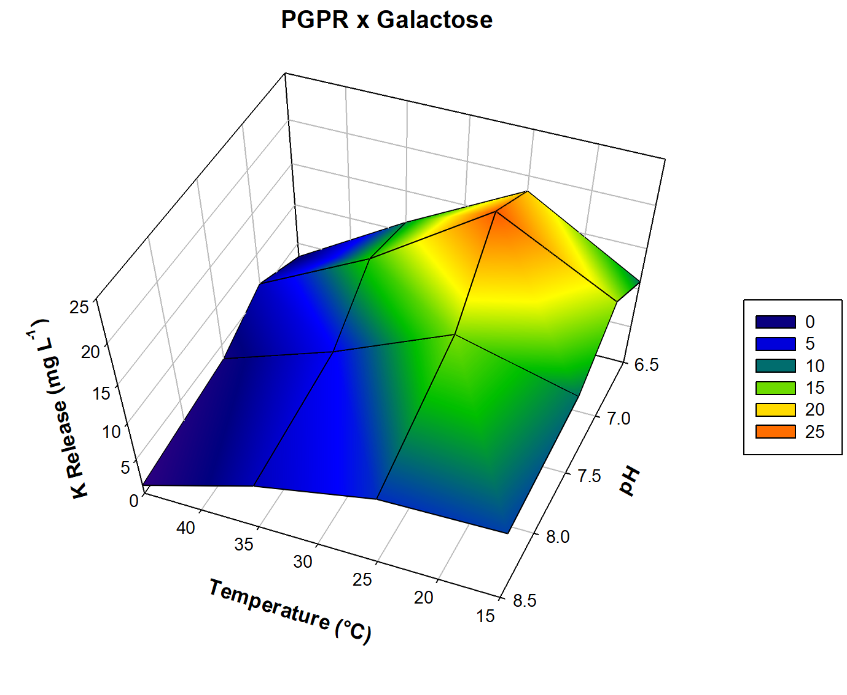
**Fig. S1:** 3D relationship between glucose (C source), temperature (°C), pH and K release from waste mica (WM) powder 3D Stoichiometric relationships = 3D stoichiometric relationships = Glucose and pH: R2 = 7730, p<0001, n = 0032; Glucose and temperature (°C): R2 = 7730, p<0001, n= 0002, pH and temperature (°C): R2 = 7730, p<0001, n = 0341



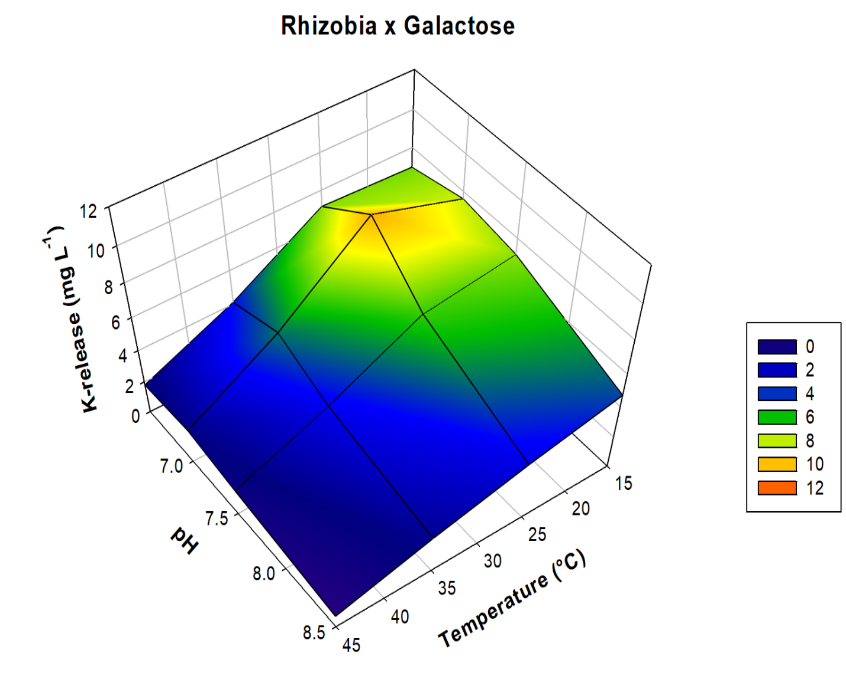
**Fig. S2:** 3D relationship between glucose (C source), temperature (°C), pH and K release from waste mica (WM) powder 3D Stoichiometric relationships = 3D stoichiometric relationships = Glucose and pH: R2 = 7730, p<0001, n = 0032; Glucose and temperature (°C): R2 = 7730, p<0001, n= 0002, pH and temperature (°C): R2 = 7730, p<0001, n = 0341



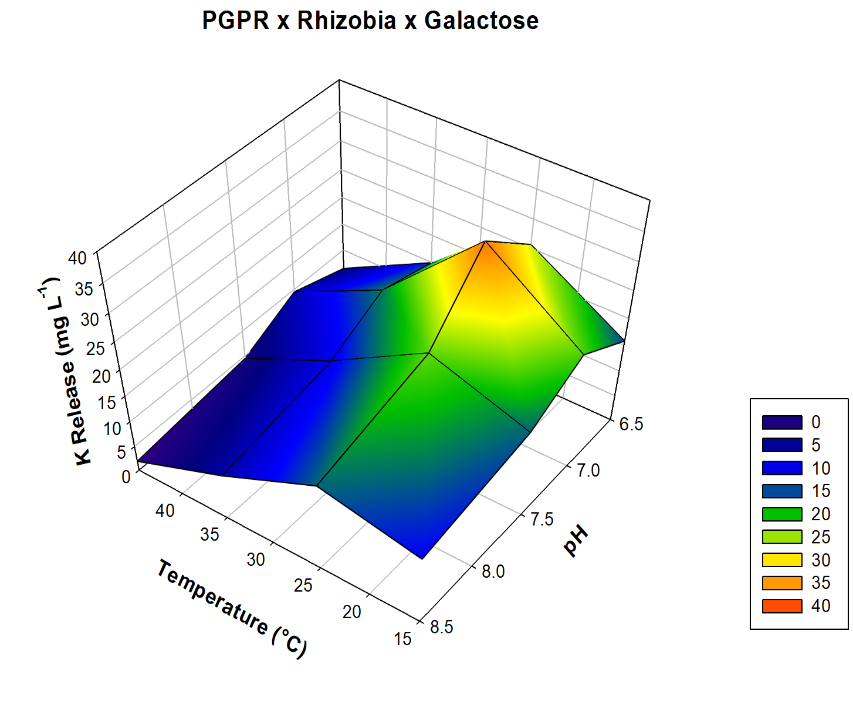
**Fig. S3:** 3D relationship between glucose (C source), temperature (°C), pH and K release from waste mica (WM) powder 3D Stoichiometric relationships = 3D stoichiometric relationships = Glucose and pH: R2 = 7730, p<0001, n = 0032; Glucose and temperature (°C): R2 = 7730, p<0001, n= 0002, pH and temperature (°C): R2 = 7730, p<0001, n = 0341

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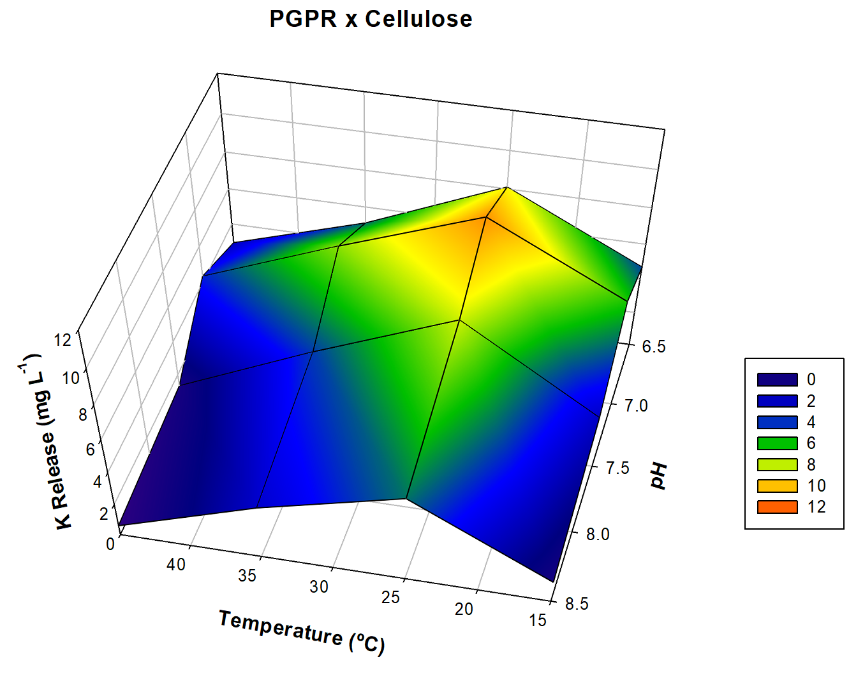
**Fig. S4:** 3D relationship between glucose (C source), temperature (°C), pH and K release from waste mica (WM) powder 3D Stoichiometric relationships = 3D stoichiometric relationships = Glucose and pH: R2 = 7730, p<0001, n = 0032; Glucose and temperature (°C): R2 = 7730, p<0001, n= 0002, pH and temperature (°C): R2 = 7730, p<0001, n = 0341



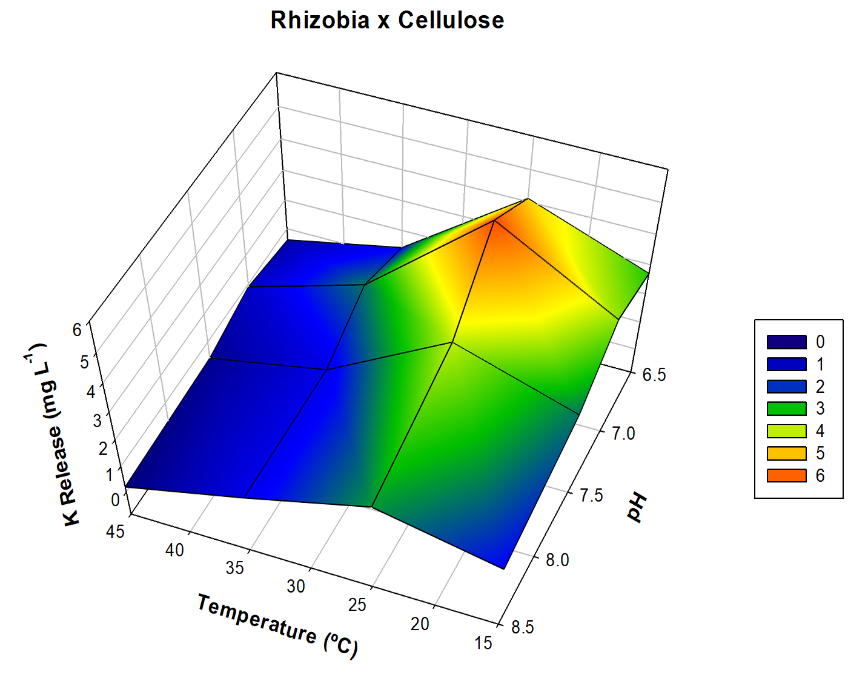
**Fig. S5:** 3D relationship between glucose (C source), temperature (°C), pH and K release from waste mica (WM) powder 3D Stoichiometric relationships = 3D stoichiometric relationships = Glucose and pH: R2 = 7730, p<0001, n = 0032; Glucose and temperature (°C): R2 = 7730, p<0001, n= 0002, pH and temperature (°C): R2 = 7730, p<0001, n = 0341



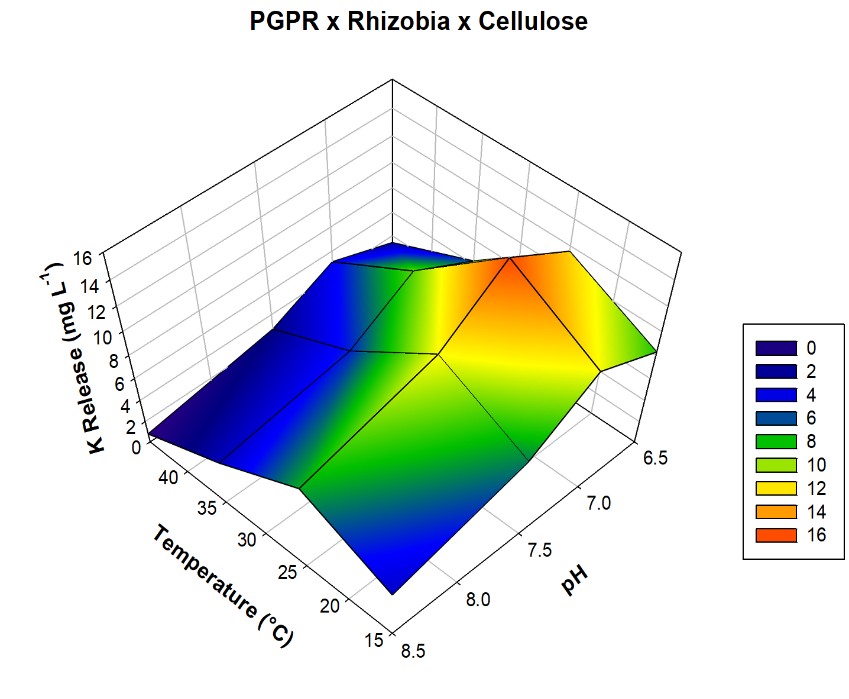
**Fig. S6:** 3D relationship between glucose (C source), temperature (°C), pH and K release from waste mica (WM) powder 3D Stoichiometric relationships = 3D stoichiometric relationships = Glucose and pH: R2 = 7730, p<0001, n = 0032; Glucose and temperature (°C): R2 = 7730, p<0001, n= 0002, pH and temperature (°C): R2 = 7730, p<0001, n = 0341



**Fig. S7:** 3D relationship between glucose (C source), temperature (°C), pH and K release from waste mica (WM) powder 3D Stoichiometric relationships = 3D stoichiometric relationships = Glucose and pH: R2 = 7730, p<0001, n = 0032; Glucose and temperature (°C): R2 = 7730, p<0001, n= 0002, pH and temperature (°C): R2 = 7730, p<0001, n = 0341



**Fig. S8:** 3D relationship between glucose (C source), temperature (°C), pH and K release from waste mica (WM) powder 3D Stoichiometric relationships = 3D stoichiometric relationships = Glucose and pH: R2 = 7730, p<0001, n = 0032; Glucose and temperature (°C): R2 = 7730, p<0001, n= 0002, pH and temperature (°C): R2 = 7730, p<0001, n = 0341



**Fig. S9:** 3D relationship between glucose (C source), temperature (°C), pH and K release from waste mica (WM) powder 3D Stoichiometric relationships = 3D Stoichiometric relationships = Glucose and pH: R2 = 7730, p<0001, n = 0032; Glucose and temperature (°C): R2 = 7730, p<0001, n= 0002, pH and temperature (°C): R2 = 7730, p<0001, n = 0341