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

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Abstract: The use of mineral fertilizers has long been associated with the improved growth of crop plants as well as increased yield potential per unit area. However, the incessant practice of imbalanced fertilizers application has increased the economic and environmental costs for the agricultural sector. The deficiency of potassium (K) has been identified as a primary crop production challenge in certain semi-arid regions where soil-K reserves are increasingly being depleted. This study aimed to isolate and characterize K-solubilizing bacterial strains from the rhizosphere and root nodules of chickpea. Initially, 50 rhizobacterial strains and 50 rhizobial strains were isolated using Aleksandrov's medium. Each of these collections was narrowed down to 25 strains, following a rigorous qualitative screening based on different physiological, morphological and biochemical tests. From these, five strains each of rhizosphere and nodule origins were selected based on qualitative as well quantitative determination of various growth promoting traits. In addition to efficient potassium and phosphate solubilization, the selected strains displayed better growth conditions, as evident by glucose substrate use at 25 °C and pH 7. In this study, we found that strains SKB3 (rhizosphere) and JKR7 (rhizobia) were the most efficient K-solubilizers. Additionally, they possessed diverse plant growth promoting traits such as root colonization, the synthesis of siderophores, exopolysaccharides, chitinase activity, indole-acetic acid production and 1-aminocyclopropane-1-carboxylic acid deaminase activity. Overall, our results suggest that the application of bacterial K-solubilizers could be employed as a useful K-supplement in K-limited agroecosystems. Moreover, the use of these K-solubilizers may help lead in alleviating the negative environmental impacts associated with chemical fertilizer.

Keywords: bacterial co-inoculants; K-solubilizing activity; screening; characterization; legume

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1. 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 [1]. Moreover, chickpea has a higher nutritional value and financial significance but its production in Pakistan is very low, at 583 kg per hectare [2]. 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 [3].

Biological nitrogen fixation (BNF) has a number of benefits compared to N fertilizers such as (a) higher N use efficiency, (b) minimal N leaching and (c) minimal contamination of soil and water bodies [4]. The amount of N fixed by a legume varies depending on the crop type, soil and the growth stage of the crop, as well as management practices [5]. Crop yield is limited due to unavailability of nutrients especially phosphorus (P). Therefore, the studies on the response of legumes and rhizobia to P fertilization have received substantial attention [6]. 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 in the Earth's lithosphere, and its concentration is varied in soil, ranging from 0.04 to 3.0 percent [7]. It plays a vital role in the production of amino acids and protein from NH_4^+ ions, which are absorbed by plant roots from the soil. In addition, K also contributes to better root growth and has been proven to increase the size and the number of nodules in legumes. The use of K in soil can promote both the numbers and size of nodules in legumes [6]. This means that higher K allocation in nodules is very essential to sustain BNF potential, as K deficiency can inhibit or reduce the nitrogenase activity and thus disrupt the symbiotic potential of the plant [8]. 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_2 fixed by bacteria increases, so does their need for energy sources to reduce or change N into NH_4^+ ions. The imperative role of K in photosynthetic activity makes it an important benefactor to efficient N_2 fixation by leguminous crop plants [9]. The process of N_2 fixation is influenced by K for very distinctive reasons and is dependent on it. Moreover, K is the predominant cation in the plant body as calcium is in the soil [9]. In Pakistan, the current evaluation of the level of soil K in Punjab has revealed an average decrease in soil K up to 3 mg kg^{-1} per year. It means a reduction of about 60 mg K kg^{-1} in Punjab soils over the past two decades, and as a result, soil K level that was once considered adequate for plant growth, is now approaching the deficiency threshold level [6,10]. Moreover, the plant growth promoting rhizobacteria have been reported to be the key elements for better plant growth under nutrient-deficient conditions. Their application in the sector of agriculture can be in favor of the reduction of agro-chemicals use and give help to eco-friendly crop production [11]. Plant uptakes of K from the soil and its availability from soil depends on the dynamics of K, as well as the total K content in soil [3].

However, the second form of non-exchangeable K, about 10 percent of K in the soil, is primarily the intermediate layer of K such as in lattice and feldspar minerals [12]. Moreover, the release of non-exchangeable K to the exchangeable form takes place when the concentration of exchangeable K and K in solution reduced by plant uptake, erosion, leaching and/or runoff [13]. Multifarious microbes in the soil are capable of solubilizing unavailable forms of K containing minerals, such as 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. Moreover, silicate bacteria were also observed to dissolve Si, K and Al from insolvable minerals [14]. 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 [15]. It has been reported that a wide range of soil bacteria release K in available form from minerals containing K in soils [16]. The application of K-solubilizing co-inoculants could be more efficient due to their manifold effects on plant health by many growth-promoting mechanisms [17]. In addition, the benefits of co-inoculation can be enhanced by retaining a high population of effective K-solubilizing bacteria in the rhizosphere soil. The 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 lifecycle [8]. Therefore, the integrated use of plant growth promoting rhizobacteria (PGPR) and rhizobia could be very effective for improving the 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 soil conditions. The successful identification of an efficient 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 light of the above discussion, this study was planned with the following objectives: (a) the isolation, screening and selection of the most effective rhizobacteria and rhizobial strains having K-solubilizing activity on a qualitative as well as quantitative basis, (b) the optimization of growth conditions for selected bacterial strains to solubilize K from waste mica (WM) and (c) the characterization of the most effective bacterial strains having K-solubilization activity.

2. Materials and Methods

A series of laboratory studies were carried out for isolation, purification and characterization of K-solubilizing rhizobacteria (KSR) and rhizobial strains from rhizosphereic soil and root nodule samples of chickpea (*Cicer arietinum* L.), respectively, in the Department of Soil and Environmental Sciences, College of Agriculture, University of Sargodha, Sargodha, Pakistan, and Soil and Water Sciences Department, Institute of Food and Agricultural Sciences, University of Florida, USA.

2.1. 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 soil adherent to the plant roots. The uprooted chickpea plants were packaged in polythene bags and brought to the laboratory for isolation of rhizobia and rhizobacterial strains having trait of K solubilization. The physicochemical properties of soil sampling sites are mentioned in Table 1.

Table 1. Physicochemical properties of soil sampling sites.

Sampling Sites	Soil Texture	Soil pH	EC (μS cm^{-1})	Available K (mg kg^{-1})
TSB-JNG	Sandy loam	7.84 ± 0.12	2134.39 ± 46.12	174.24 ± 6.32
TSB-JNG	Sandy loam	7.72 ± 0.06	1975.65 ± 39.78	165.03 ± 7.02
TSB-JNG	Sandy loam	7.90 ± 0.04	2034.52 ± 41.46	169.28 ± 8.11
GW-JNG	Sandy clay loam	8.02 ± 0.07	1864.90 ± 44.21	179.39 ± 6.87
GW-JNG	Sandy clay loam	8.11 ± 0.09	1643.78 ± 35.49	156.71 ± 4.53
SW-JNG	Sandy loam	7.94 ± 0.03	2314.39 ± 56.65	148.63 ± 6.34
RA-JNG	Sandy loam	7.56 ± 0.06	2056.14 ± 51.39	153.90 ± 5.19
RD-NPT	Sandy clay loam	7.80 ± 0.04	1758.64 ± 42.83	175.22 ± 7.42
RD-NPT	Sandy clay loam	8.13 ± 0.05	1759.27 ± 40.28	166.42 ± 8.03
SH-NPT	Sandy loam	7.82 ± 0.07	2431.08 ± 60.43	170.84 ± 7.94
SH-NPT	Loam	7.73 ± 0.03	1867.90 ± 44.90	175.44 ± 8.16
JK-NPT	Sandy loam	7.97 ± 0.09	2089.45 ± 48.66	157.24 ± 5.88
JK-NPT	Sandy loam	8.09 ± 0.08	2413.09 ± 62.39	139.54 ± 5.09
AK-NPT	Sandy clay loam	8.23 ± 0.09	2319.72 ± 54.28	146.78 ± 6.10
AK-NPT	Sandy clay loam	7.95 ± 0.07	2531.11 ± 67.13	168.34 ± 8.02
96NB-SGD	Sandy clay loam	7.98 ± 0.06	2131.62 ± 59.07	157.54 ± 6.48
96NB-SGD	Sandy clay loam	7.76 ± 0.03	1759.43 ± 46.55	169.38 ± 7.90
99NB-SGD	Sandy loam	7.82 ± 0.08	1866.70 ± 45.87	164.08 ± 6.17
99NB-SGD	Sandy loam	7.93 ± 0.04	2016.29 ± 53.64	170.90 ± 7.12
99NB-SGD	Sandy loam	7.90 ± 0.05	1509.48 ± 34.79	160.78 ± 5.54
MGR-SGD	Sandy clay loam	7.70 ± 0.07	1734.46 ± 40.29	152.90 ± 5.60
MGR-SGD	Sandy clay loam	7.81 ± 0.06	1873.41 ± 43.87	189.63 ± 9.03
SWA-SGD	Loamy sand	7.74 ± 0.04	1487.32 ± 32.94	178.16 ± 8.22
MHP-SGD	Sandy clay	7.49 ± 0.02	1564.12 ± 34.65	159.43 ± 5.37
MHP-SGD	Sandy clay	7.62 ± 0.05	1358.69 ± 31.92	164.23 ± 6.08

Values are means of three replicates. ±: Standard error of mean values; JNG: Jhang; SGD: Sargodha; NPT: Noorpur thal; TSB: Thall Sattan Bharai; GW: Gulab Wala; SW: Shero Wala; RA: Rehmatabad; RD: Rakh Dhamak; SH: Shah-Hussain; JK: Joura Kalan, AK: Adhi Kot; 96NB: Chak No. 96 NB; 99NB: 99 Chak No. 99; MGR: Mangoor; SWA: Shwala; MHP: Muhibpur.

2.2. Isolation of Potassium-Solubilizing Rhizobacteria

Isolation of K-solubilizing bacteria was completed using dilution plate technique on Aleksandrov's medium [18] from soil samples. First of all, bulk soil from the roots of chickpea was detached by mild agitation, and then the soil strongly adhering (rhizosphere soil) to the roots was also removed. After this, 10 g of rhizosphere soil from every sample was weighed and mixed with 95 mL sterile solution of NaCl (0.85 percent) in 250-mL conical flasks. Flasks were shaken energetically for 10 min to form uniform suspensions of soil. The soil suspensions were then incubated under agitation for 45 min 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 straightened out on Aleksandrov's agar medium [14]. After this, media plates were incubated for 72 h at 28 ± 2 °C. Morphologically well grown and prominent colonies (color, shape, size and growth rate) of 50 rhizobacterial strains 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 °C.

2.3. Isolation of Potassium-Solubilizing Mesorhizobial Strains

Mesorhizobial strains 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. For isolation of rhizobia, the roots of chickpea plants were washed gently with tap water, and then nodules were detached from roots with the help of scissors. The collected nodules were surface disinfected for a moment (<10 s) by immersion in 95 percent solution of ethanol, followed by immersion in 0.2 percent mercuric chloride solution for 3 min [19]. 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 5 mL sterile distilled water. A loop of milky suspension was striped on the Yeast Extract Mannitol (YEM) agar medium plates [20] and then incubated for 72 h at 28 ± 2 °C. The isolated single colonies of rhizobial strains 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 pure cultures. In this way, 50 rhizobial strains were isolated and designated with sampling sites. These strains were stored in glycerol (20%) at −40 °C for further use.

2.4. Screening of Potassium-Solubilizing Rhizobacteria and Mesorhizobial Strains

Potassium-solubilizing ability of both bacterial strains 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, bacterial strains were initially selected for screening of K-solubilization based on halo zone formation on agar medium having modified Aleksandrov's medium via spot test method [21]. Bacterial strains showing solubilization zone on Aleksandrov's agar medium were further tested to evaluate their potential to release K in broth medium containing 1.0 percent mica mineral. For this, 1.0 mL culture (overnight) of each bacterial strain was inoculated to Aleksandrov's broth, having a volume 25 mL [14]. 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 uninoculated control. Finally, the available K content was estimated by Flame Photometry method [22].

2.5. General Characterization of Bacterial Strains Having the Trait of Potassium Solubilization

The bacterial strains were tested for general characterization using the following procedures. These strains 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 [23]. The selected bacterial strains were also 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-h old cultures were streaked on the medium and incubated for 24 h 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 [24]. The pre-sterilized tubes comprising broth test cultures of MR-VP were inoculated for Voger–Proskauer test as studied by Seeley and Vandemark [24]. The method of Alariya et al. [25] was used to detect amylase activity of strains. Freshly prepared cultures were streaked on Modified Czapek Mineral salt medium for the detection of cellulose enzyme activity [26]. Catalase activity of strains was tested by adding 3% H_2O_2 on developed growth colonies of selected strains using method of Blazevic and Ederer [27].

Tribibutyrene agar medium (TAM) was used for lipase activity. Lipolytic activity of selected strains was verified by halo zone formation around the inoculation line, and then inoculated plates were incubated for 72 h at 25 °C [28]. Protease activity of strains was checked using SMA (Skimmed Milk Agar) media [29]. The urease activity in selected strains were determined as described by James and Sherman [30]. Qualitative N-fixing ability of bacterial cultures was determined using standard procedure as described by Gothwal et al. [31]. Selected strains were evaluated for P-solubilizing activity using protocol described by Pikovskaya [32]. Eckford's [33] method was used to test the potential of selected strains to hydrolyse starch. In order to test for indole production assay, pre-sterilized sulfide-indole motility (SIM) agar tubes were inoculated with selected strains. After this, SIM agar tubes were incubated for 48 h at 28 ± 2 °C. After inoculation, Kovac's reagent (10 drops) was added into each tube. Cherry Red production was considered positive for indole production [34]. The gelatin liquefaction of the bacterial strains were determined as described by Blazevic and Ederer [25]. The hydrogen sulfide production assay was performed using sterilized test tubes containing SIM (sulfide, indole, motility) agar medium [35]. Casein hydrolysis was determined as described by Seeley and Vandemark [24]. The production of HCN (hydrogen cyanide) was estimated using modified King's B agar medium [36].

2.6. Quantitative Characterization of Plant Growth Promoting Traits in Selected Strains of Bacteria

For this, selected strains were characterized for various plant growth promoting traits using the following protocols. Gordon and Weber [37] method was used for estimation of indole-acetic acid (IAA) in selected strains. The exopolysaccharides production was measured through inoculation of strains on RCV (Rhodobacter-Capsulatus V) mineral medium after enrichment with Mannitol, glucose or sucrose, with and without NaCl [38]. The total carbohydrate content in the precipitated EPS was measured by standard procedure described by Dubois et al. [39]. The Penrose and Glick [13] protocol was used to determine the activity of ACC-deaminase in the cells of selected bacterial strains in the form of α-ketobutyrate that resulted from the cleavage of ACC.

For K-solubilization assay, 48-h-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 6 days at 28 ± 2 °C. The growth suspension of cultures was centrifuged at 7000× *g* for 10 min to separate the supernatant from cell growth, and the insoluble K and then filtered. After this, filtered supernatant (1 mL) was taken in to a 50-mL flask and made 50 mL volume using distilled water and then mixed well. Finally, the sample was analyzed for water-soluble K content using flame photometer [22].

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. [40]. The quantitative measurement of siderophores of K-solubilizing bacteria was conducted using CAS Shuttle Assay [41]. For root colonization test, surface sterilized chickpea seeds

inoculated for 48-h-old bacterial cultures and were sown in a glass jars filled with sterilized sand modified by Simon et al. [42].

2.7. Optimization of Bacterial Growth Conditions for Potassium Solubilization

Growth conditions (i.e., carbon sources, pH and temperature) were optimized for final selection of the most efficient K solubilizing strains. Efficiency of bacterial strains for K solubilization from mica powder in Aleksandrov's broth medium (ABM) was assessed using different combinations of the 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 strains. Each selected bacterial strain was injected in 25 mL of amended ABM [14], while glucose was exchanged by either of the two C sources (cellulose and galactose). After this, all flasks were incubated for 10 days at 28 ± 2 °C. The quantity of K released into the broth was measured compared to uninoculated control though flame photometric method [22].

2.8. Statistical Analysis

By using ANOVA, following Statistix 10.0 statistical package, the data were analyzed [43] (Anonymous, 1986), and differences within KSB inoculation were tested by using Tukey's post-hoc test at 0.05 P [44] (Steel et al., 1997). All figures and tables represent means of four replicates followed by standard error of means. For preparation of the graphs, the Excel Graphics in computer package was used.

3. Results

3.1. Isolation and Purification of Effective K-Solubilizing Rhizobacteria and Mesorhizobial Strains

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

3.2. Qualitative Screening of Selected K-Solubilizing Rhizobacterial Strains

After purification, bacterial strains were tested for their K-solubilization on a qualitative basis using WM as the K source. For this, strains were selected based on halo zone formation on Aleksandrov's medium agar plates in 72 h. The results of this qualitative test showed that strains with +, ++ and +++ signs produced halo zones of <2, >2 and >3 mm, respectively (Table 2).

Table 2. Qualitative screening of potassium solubilizing rhizobacterial strains.

Bacterial Strain	Rating	Bacterial Strain	Rating
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	++

Table 2. *Cont.*

Bacterial Strain	Rating	Bacterial Strain	Rating
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 < 2 mm; ++: Halo size > 2 mm; +++: Halo size > 3 mm; –: No K-solubilizing activity.

Meanwhile, strains having negative signs were found unable to form any halo zone on medium during the incubation period. Out of 50 strains, 25 gave maximum growth on medium spiked with WM as an insoluble K source. Twenty strains produced a halo zone of >2 mm, while five strains produced halo zones of >3 mm. These 25 strains were selected for further quantitative screening, which produced halo zones efficiently.

3.3. Qualitative Screening of K-Solubilizing Mesorhizobial Strains

After purification, rhizobial strains were tested for their K-solubilization from WM on qualitative basis. In this screening, the strains were selected based on halo zone formation on Aleksandrov's medium agar plates in 72 h. The results showed that strains with +, ++ and +++ signs produced halo zones of <2, >2 and >3 mm, respectively (Table 3).

Table 3. Qualitative screening of potassium solubilizing Mesorhizobial strains.

Rhizobial Strains	Rating	Mesorhizobial Strains	Rating
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 < 2 mm; ++: Halo size > 2 mm; +++: Halo size > 3 mm; –: No K-solubilizing activity.

Meanwhile, K-solubilizing strains with negative signs were unable to develop any halo zone on Aleksandrov's medium during the incubation period. Twenty-five out of fifty strains showed maximum growth on the medium spiked with WM as an insoluble K source. Twenty-one strains produced halo zones of >2 mm size, while five strains produced halo zones of >3 mm. These 25 strains were selected for further quantitative screening, which produced halo zones efficiently.

3.4. Quantitative Screening of Selected K-Solubilizing Rhizobacterial Strains

For the quantitative test, 25 strains were selected for K-solubilization potential from WM in Aleksandrov's broth within 72 h of incubation (Table 4).

Table 4. Quantitative screening of K-solubilizing bacterial strains.

Rhizobacterial Strains	Soluble K (mg L^{-1})	Mesorhizobial Strains	Soluble K (mg L^{-1})
JKB1	214 ± 11.3 ^{e}	JKR1	104 ± 2.96 ^{fg}
JKB4	197 ± 10.9 ^{f}	JKR2	117 ± 3.32 ^{ef}
JKB10	265 ± 13.6 ^{c}	JKR4	155 ± 4.85 ^{c}
JKB11	188 ± 10.6 ^{fg}	JKR6	98 ± 2.79 ^{gh}
JKB14	227 ± 12.4 ^{d}	JKR7	173 ± 5.82 ^{b}
JKB19	174 ± 9.8 ^{g}	JKR8	83 ± 2.60 ^{i}
JKB20	316 ± 19.6 ^{a}	JKR9	117 ± 3.24 ^{ef}
NKB2	198 ± 10.3 ^{f}	JKR10	138 ± 3.89 ^{d}
NKB4	203 ± 11.3 ^{ef}	JKR12	123 ± 3.79 ^{e}
NKB6	174 ± 9.2 ^{g}	JKR13	74 ± 2.37 ^{j}
NKB7	169 ± 8.9 ^{h}	JKR14	109 ± 3.19 ^{f}
NKB8	225 ± 12.7 ^{d}	JKR16	158 ± 5.06 ^{c}
NKB9	203 ± 10.3 ^{ef}	JKR19	63 ± 1.72 ^{k}
NKB11	179 ± 09.1 ^{g}	JKR20	129 ± 3.40 ^{de}
NKB12	194 ± 10.1 ^{f}	JKR22	94 ± 2.73 ^{h}
SKB1	132 ± 8.3 ^{i}	NKR1	102 ± 3.04 ^{g}
SKB2	171 ± 9.5 ^{gh}	NKR4	61 ± 1.54 ^{k}
SKB3	293 ± 18.2 ^{b}	NKR7	123 ± 3.69 ^{e}
SKB4	217 ± 12.0 ^{de}	NKR13	97 ± 2.70 ^{g}
SKB6	168 ± 8.6 ^{h}	SKR1	118 ± 3.26 ^{e}
SKB7	107 ± 6.7 ^{k}	SKR4	91 ± 2.60 ^{h}
SKB8	206 ± 11.5 ^{e}	SKR10	186 ± 6.23 ^{a}
SKB11	274 ± 18.7 ^{c}	SKR12	105 ± 3.11 ^{g}
SKB13	268 ± 17.9 ^{c}	SKR13	171 ± 5.60 ^{b}
SKB14	199 ± 11.2 ^{f}	SKR14	89 ± 2.75 ^{h}

Values are means of four replicates. For each parameter, under each column, values sharing different letters differ significantly from each other at $p < 0.05$. K-solubilization in Aleksandrov's broth from waste mica in 48 h = mg L^{-1} = (ppm).

Out of 25 strains, 5 strains had shown a maximum solubilization of K that was more than 26 mg L^{-1} from WM. The maximum K-solubilization was 31.6 mg L^{-1} due to inoculation with the JKB20 isolate while the minimum concentration was 10.7 mg L^{-1} compared to the uninoculated control. Most of the strains had shown a solubilization of K in the range of 16–22 mg L^{-1}, while the other most effective strains (i.e., SKB3, SKB11, SKB13 and JKB10) had shown K-solubilization of 29.3, 27.4, 26.8 and 26.5 mg L^{-1}, respectively, from WM in Aleksandrov's broth after 48 h of incubation.

3.5. Quantitative Screening of Selected K-Solubilizing Mesorhizobial Strains

For this, 25 strains of rhizobia were selected to evaluate their K-solubilization potential from WM in Aleksandrov's broth within 72 h (Table 4). Out of 25 strains, 5 had shown an effective solubilization of K that was more than 15 mg L^{-1} from WM, while the maximum K-solubilization concentration was recorded up to 18.6 mg L^{-1} due to inoculation with the SKR10 strain, and the minimum concentration was found to be 6.1 mg L^{-1} over the

uninoculated control. Most of the strains showed solubilization of K in the range of 9.0 to 17.0 mg L^{-1}. The other most effective strains of rhizobia (JKR7, SKR13, JKR16 and JKR4) had also shown K-solubilization of 17.3, 17.1, 15.8 and 15.5 mg L^{-1}, respectively, from WM in Aleksandrov's broth after 72 of incubation.

3.6. General Characterization of Rhizobacteria and Mesorhizobial Strains Having the Trait of K-Solubilization

For this, the five most efficient strains of each test were subjected to general characterization (morphological and biochemical traits) and further experimentation (Table 5).

Table 5. General characterization of rhizobacteria and Mesorhizobial strains having the trait of K-solubilization.

Parameters	Rhizobacterial Strains					Mesorhizobial Strains				
	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	+	+	++	+	+	+	+	+	+	+
H_2S production	–	–	–	–	–	–	–	–	–	–
HCN production	–	–	–	–	–	–	–	–	–	–
Amylase activity	++	–	+	–	+	–	+	–	–	+
Cellulase activity	+	–	++	–	+	–	+	–	–	–
Catalase activity	+	–	+	–	–	–	+	–	–	–
Lipase activity	–	+	++	–	–	–	+	–	–	+
Urease activity	+	+	+	+	+	+	+	+	–	+
Oxidase activity	+	+	+	+	+	+	+	+	+	–
Starch hydrolysis	+	+	+	+	+	+	+	+	+	–
Casein hydrolysis	+	–	+	–	–	–	–	+	+	–
N_2-fixing activity	+	+	+	+	+	+	++	+	+	+
Protease activity	–	+	+	–	–	–	+	–	–	–
Gelatin liquefaction	+	+	++	+	+	+	+	+	+	+

Single positive sign means halo size <2 mm, while double positive means halo size >2 mm; Y: Yellowish; CW: Cream white; MW: Milky white; C: Creamy; –: Character is not present.

Out of five rhizobacterial strains, three (JKB10, SKB3, SKB11) were rod-shaped and two (JKB20 and SKB13) were coccus shaped, while all strains of rhizobia (JKR4, JKR7, JKR16 and SKR13 and SKR10) were rod-shaped. Regarding color, all strains of rhizobacteria were yellowish and three rhizobial strains were creamy (JKR4, JKR7 and SKR10) and two (JKR16 and SKR13) were white in color. All bacterial strains were positive in motility. Regarding, sporulation and gram staining, all strains of rhizobacteria and rhizobia were found to be negative in sporulation and gram staining. For halo-tolerance, bacterial strains (JKB10, SKB3, SKB11 and SKB13) were positive, while JKB20 was negative, and three strains of rhizobia (JKR4, JKR7 and JKR16) were positive, while SKR10 and SKR13 were found negative.

In case of biochemical characteristics, two strains of rhizobacteria (JKB10 and SKB3) were positive for methyl red, while three strains (JKB20, SKB11 and SKB13) were negative, and all strains of rhizobia were negative for methyl red. Regarding the Voger–Proskaur test, four strains rhizobacteria (JKB10, SKB3, SKB11 and SKB13) were positive and one (JKB20) was negative while, three strains (JKR7, SKR10 and SKR13) were negative and two (JKR4 and JKR16) were positive in the case of rhizobia. Indole production was observed in

all strains of rhizobacteria and rhizobia, and it was found to be double in the SKB3 isolate of rhizobacteria. However, the production of H_2S and HCN were negative in all bacterial strains. Three rhizobacterial strains (JKB10, SKB3 and SKB13) were found positive for amylase activity and two (JKB20 and SKB11) were negative, while three strains of rhizobia (JKR4, JKR16 and SKR10) were negative for amylase activity and two strains (JKR7 and SKR13) were positive. In the case of cellulase activity, three strains of rhizobacteria (JKB10, SKB3 and SKB13) were observed positive and it was absent in two strains (JKB20 and SKB11), while this activity was absent in four strains of rhizobia (JKR4, JKR16, SKR10 and SKR13) and present in only one isolate (JKR7). Catalase activity was observed in two strains of rhizobacteria (JKB10 and SKB3) and absent in the other three (JKB20, SKB11 and SKB13), while this activity was found absent in four rhizobial strains (JKR4, JKR16, SKR10 and SKR13) and present in the JKR7 isolate. In the case of lipase activity, three strains of rhizobacteria (JKB10, SKB11 and SKB13) were negative and two (JKB20 and SKB3) were positive, while, in the case of rhizobia, it was present in two strains (JKR7 and SKR13) and absent in three strains (JKR4, JKR16 and SKR10). Urease activity was positive in all strains of rhizobacteria and rhizobia except for one isolate (SKR10). Similarly, in the case of oxidase activity and starch hydrolysis, all bacterial strains were found positive, except isolate SKR13 in both cases. The rhizobacterial strains (JKB20, SKB3 and SKB13) were negative and JKB10 and SKB3 were positive for casein hydrolysis, and three strains of rhizobia (JKR4, JKR7 and SKR13) were negative and two (JKR16 and SKR10) were positive. N_2-fixing activity was observed in all bacterial strains, and it was highly prominent in the JKR7 isolate of rhizobia. Protease activity was observed positive in two strains of rhizobacteria (JKB29 and SKB3) and absent in three strains (JKB10, SKB11 and SKB13). This activity was also absent in four strains of rhizobia (JKR4, JKR16, SKR10 and SKR13), and it was present in the JKR7 rhizobial isolate. It was also observed that gelatin liquefaction was present in all strains, while it was highest in the SKB3 rhizobacterial isolate.

3.7. Utilization of Different Carbon Sources by Bacterial Strains Having K-Solubilization Activity

All bacterial strains had shown the ability to utilize a variety of C sources, which is an excellent trait to perform in actual soil conditions. The growth and activity of microbial strains can be correlated with the ease of utilization of C sources, which are not easily degradable. Selected strains of both rhizobacteria and rhizobia having the trait of K-solubilization were tested for their efficacy in utilizing different C sources by growing on Aleksandrov's media where glucose was replaced by arabinose, cellulose, citrate, galactose, sucrose and xylose (Table 6).

Table 6. Utilization of different carbon sources by rhizobacteria and Mesorhizobial strains having K-solubilization activity.

Bacterial Strains	Different Carbon Sources					
	Arabinose	**Cellulose**	**Citrate**	**Galactose**	**Sucrose**	**Xylose**
	Rhizobacterialstrains					
JKB10	+	−	+	+	+	+
JKB20	−	+	+	+	+	+
SKB3	+	+	+	+	+	+
SKB11	−	−	+	−	+	+
SKB13	−	−	+	−	+	+
	Mesorhizobialstrains					
JKR4	+	−	+	+	+	−
JKR7	+	−	+	+	+	+
JKR16	−	−	+	+	+	+
SKR10	−	−	+	−	+	+
SKR13	−	−	+	−	+	−

+: has the ability to utilize respective carbon source; −: does not have ability to utilize respective carbon source.

All strains were able to utilize all types of the selected C sources efficiently except the cellulose source, which was utilized by only two strains of rhizobacteria (JKB20 and SKB3). All rhizobial strains have no potential to utilize the cellulose.

3.8. Quantitative Screening of Effective Combination of Rhizobacteria × Mesorhizobium

For this, we evaluated all 25 combinations of the 5 most efficient rhizobacteria with the 5 most efficient rhizobia and evaluated the most effective combination based on the K-solubilization potential from WM in Aleksandrov's broth within 72 h of incubation (Table 7).

Table 7. Quantitative screening of effective combination of rhizobacteria and Mesorhizobial strains.

Rhizobacteria × *Mesorhizobium*	**Soluble K (mg L^{-1})**	**Rhizobacteria × *Mesorhizobium***	**Soluble K (mg L^{-1})**
JKB10 × JKR4	227 ± 13.4 ^{g}	SKB3 × SKR10	289 ± 21.4 ^{c}
JKB10 × JKR7	243 ± 17.2 ^{efg}	SKB3 × SKR13	265 ± 18.4 ^{de}
JKB10 × JKR16	172 ± 10.8 ^{i}	SKB11 × JKR4	217 ± 14.7 ^{g}
JKB10 × SKR10	262 ± 19.0 ^{de}	SKB11 × JKR7	265 ± 18.8 ^{de}
JKB10 × SKR13	258 ± 173 ^{e}	SKB11 × JKR16	154 ± 10.2 ^{j}
JKB20 × JKR4	274 ± 20.4 ^{d}	SKB11 × SKR10	277 ± 20.7 ^{d}
JKB20 × JKR7	257 ± 17.0 ^{e}	SKB11 × SKR13	419 ± 23.9 ^{b}
JKB20 × JKR16	282 ± 21.0 ^{cd}	SKB13 × JKR4	194 ± 8.7 ^{h}
JKB20 × SKR10	293 ± 21.3 ^{c}	SKB13 × JKR7	236 ± 16.9 ^{fg}
JKB20 × SKR13	262 ± 19.2 ^{de}	SKB13 ×JKR16	139 ± 9.4 ^{k}
SKB3 × JKR4	254 ± 17.7 ^{e}	SKB13 × SKR10	281 ± 21.1 ^{cd}
SKB3 × JKR7	474 ± 27.8 ^{a}	SKB13 × SKR13	259 ± 17.9 ^{e}
SKB3 × JKR16	239 ± 16.7 ^{fg}		

Values are means of four replicates. For each parameter, under each column, values sharing different letters differ significantly from each other at $p < 0.05$. K-solubilization in Aleksandrov's broth from waste mica in 48 h = mg L^{-1} = (ppm).

Out of 25, only 2 combinations (SKB3 × JKR7 and SKB11 × SKR13) had shown the highest K-solubilization, which were 47.4 and 41.9 mg L^{-1}, respectively, from WM. The next most effective combinations (i.e., JKB20 × SKR10, SKB3 × SKR10 and SKB13 × SKR10) had also exhibited K-solubilization of 29.3, 28.9 and 28.1 mg L^{-1}, respectively, from WM after 72 h of incubation. The minimum K was recorded up to 13.9 mg L^{-1} from the 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.

3.9. Characterization of Selected K-Solubilizing Bacterial Strains for Plant-Growth-Promoting Activities

The selected bacterial strains were tested for some plant-growth-promoting activities under lab conditions. Overall, SKB3 and JKR7 performed better compared to the other remaining strains. Results concerning the plant-growth-promoting activities are mentioned in Table 8.

Table 8. Characterization of selected K-solubilizing rhizobacteria and Mesorhizobial strains for plant-growth-promoting activities.

Bacterial Strains	Quantitative Estimation of Plant-Growth-Promoting Activities								
	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}) [0.5% WM-EM]	Phosphate Solubilization [0.5% RP-EM]	Siderophores Production (SU %)	Root Colonization (CFU g^{-1} FRB)
		-L-TRP	+L-TRP						
Rhizobacterial strains									
JKB10	304.4 ± 23.2^{a}	27.8 ± 3.19^{ef}	44.5 ± 4.3^{c}	22.6 ± 2.82^{b}	63.6 ± 4.7^{b}	74.4 ± 4.32^{b}	67.2 ± 9.6^{ab}	31.4 ± 2.7^{b}	$3.73 \times 10^{7} \pm 4.87 \times 10^{6\,b}$
JKB20	192.5 ± 22.3^{d}	30.6 ± 2.67^{e}	65.8 ± 5.3^{b}	17.8 ± 1.94^{c}	51.3 ± 3.6^{c}	51.8 ± 4.15^{d}	47.3 ± 8.9^{c}	24.8 ± 3.9^{c}	$2.89 \times 10^{6} \pm 5.23 \times 10^{5\,d}$
SKB3	284.7 ± 19.4^{ab}	34.3 ± 3.79^{d}	73.6 ± 7.4^{a}	37.8 ± 2.26^{a}	78.5 ± 5.6^{a}	113.7 ± 6.31^{a}	72.5 ± 5.5^{a}	43.7 ± 4.9^{a}	$4.78 \times 10^{7} \pm 4.67 \times 10^{6\,a}$
SKB11	177.8 ± 27.9^{e}	26.4 ± 2.87^{f}	62.7 ± 6.4^{b}	12.5 ± 2.78^{d}	42.8 ± 3.2^{d}	60.9 ± 4.39^{c}	37.3 ± 6.93^{d}	18.9 ± 3.6^{e}	$5.46 \times 10^{6} \pm 5.13 \times 10^{5\,c}$
SKB13	232.7 ± 17.2^{c}	19.4 ± 1.03^{g}	34.3 ± 5.3^{d}	21.5 ± 3.54^{b}	52.8 ± 4.8^{c}	56.6 ± 4.69^{cd}	52.3 ± 7.43^{c}	21.9 ± 1.8^{de}	$7.68 \times 10^{5} \pm 3.59 \times 10^{4\,e}$
Mesorhizobial strains									
JKR4	24.6 ± 3.3^{b}	32.6 ± 2.34^{f}	64.2 ± 4.33^{b}	2.4 ± 0.82^{b}	243.6 ± 34.6^{b}	38.4 ± 3.56^{bc}	12.8 ± 1.06^{b}	24.3 ± 2.44^{cd}	$4.84 \times 10^{6} \pm 5.60 \times 10^{5\,b}$
JKR7	21.8 ± 2.6^{c}	46.7 ± 3.10^{d}	91.3 ± 6.53^{a}	3.6 ± 0.94^{a}	321.2 ± 31.6^{a}	41.6 ± 3.89^{ab}	17.3 ± 1.29^{a}	36.8 ± 3.23^{a}	$3.94 \times 10^{7} \pm 6.23 \times 10^{6\,a}$
JKR16	30.4 ± 4.6^{a}	29.8 ± 2.10^{g}	57.2 ± 5.24^{c}	2.3 ± 0.62^{b}	154.2 ± 31.5^{c}	45.3 ± 4.31^{a}	7.5 ± 1.35^{d}	31.6 ± 4.52^{ab}	$5.12 \times 10^{6} \pm 4.43 \times 10^{5\,b}$
SKR10	17.4 ± 2.9^{d}	20.1 ± 1.78^{h}	42.4 ± 7.32^{d}	2.5 ± 0.78^{b}	162.8 ± 23.7^{c}	36.1 ± 4.18^{c}	11.3 ± 1.93^{b}	26.9 ± 2.88^{c}	$3.59 \times 10^{6} \pm 5.34 \times 10^{5\,bc}$
SKR13	22.7 ± 3.9^{bc}	16.9 ± 1.39^{i}	37.9 ± 5.47^{de}	2.1 ± 0.54^{b}	122.8 ± 13.3^{d}	30.7 ± 3.34^{d}	9.3 ± 0.43^{c}	17.9 ± 3.18^{e}	$6.34 \times 10^{5} \pm 5.68 \times 10^{4\,d}$

Values are means of four replicates. For each parameter, under each column, values sharing different letters differ significantly from each other at $p < 0.05$. 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.

3.9.1. ACC-Deaminase Activity

The maximum ACC-deaminase activity (up to 304.4 α-KB μmol g^{-1} protein h^{-1}) was recorded in the JKB10 rhizobacterial isolate followed by the SKB3, SKB13, JKB20 and SKB11 strains that ranged from 177 to 284.7 α-KB μmol g^{-1} protein h^{-1} (Table 8). In the case of rhizobial strains, the highest ACC-deaminase activity (up to 30.4 α-KB μmol g^{-1} protein h^{-1}) was recorded in the JKR16 isolate of rhizobia. The rest of the strains (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 the control.

3.9.2. Indole Acetic Acid Production

The production of IAA in the selected bacterial strains was observed without and with L-TRP under lab conditions (Table 8). In the case of rhizobacteria, the maximum IAA production was recorded up to 34.3 and 73 mg L^{-1} without and with L-TRP, respectively, over the control. The rest of the strains had also shown a promising increase in IAA 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 the case of the rhizobial strains, the maximum IAA production was observed to be up to 46.7 and 91.3 mg L^{-1} without and with L-TRP, respectively, the other strains 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 the control.

3.9.3. Chitinase Activity Assay

All bacterial strains 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 strains, this activity was poor as compared to rhizobacterial strains. 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).

3.9.4. Exopolysaccharides Production

All selected strains 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 strains, 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}.

3.9.5. Potassium Solubilization Activity

All strains had shown K-solubilizing activity in Aleksandrov's medium spiked with 0.5% WM. The maximum K-solubilization was observed in the SKB3 isolate, which was up to 113.7 mg L^{-1}, followed by the JKB10 isolate (74.4 mg L^{-1}). However, in the rhizobial strains, the maximum K-solubilization was observed in JKR16, which was up to 45.3 mg L^{-1}, followed by the JKR7 isolate (up to 41.6 mg L^{-1}).

3.9.6. Phosphate-Solubilization Activity

Both the rhizobacteria and rhizobial strains had shown potential to solubilize phosphate in liquid medium. The maximum P-solubilization activity was found to be associated with the SKB3 isolate of rhizobacteria (72.5 mg L^{-1}), followed by the JKB10 isolate of rhizobacteria. In the rhizobial strains, the highest P-solubilization activity was observed in the JKR7 strains (17.3 mg L^{-1}) and the minimum in the JKR16 isolate.

3.9.7. Siderophores Production

The maximum siderophores production was recorded (up to 43.7%) because of SKB3 isolate of rhizobacteria followed by JKB10 isolate. The rest of the strains had also shown siderophores production that ranged from 18.9 to 24.8% compared to control. While the

rhizobial isolate JKR7 showed the maximum production of siderophores of up to 36.8%, followed by the isolate JKR16, the other remaining strains also exhibited a promising activity of siderophores that ranged from 17.9 to 26.9% over the control.

3.9.8. Root Colonization

The highest root colonization was observed by the SKB13 isolate of rhizobacteria, which was 4.78×10^7 CFU g^{-1} FRB), and the minimum was by the JKB20 isolate (7.68×10^5 CFU g^{-1} FRB). In the case of rhizobial strains, the maximum root colonization was found to be associated with isolate SKR7 (3.94×10^7 CFU g^{-1} FRB) and the minimum was by rhizobial isolate SKR13 (6.34×10^5 CFU g^{-1} FRB).

3.10. Optimization of Growth Conditions for K-Solubilizing Bacterial Strains from Mica in Broth Medium

Results concerning K-solubilization showed that the bacterial strains SKB3 and JKR7 efficiently performed in all C sources (i.e., glucose, galactose and cellulose). The maximum and minimum K-solubilization was observed in 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. The maximum K-solubilization was observed at 25–30 °C with all C sources and pH values, and subsequent decreases in K-solubilization were recorded with an increase in temperature. Similarly, the maximum amount of K-solubilization was recorded at pH 7.0 at all temperatures and with all C sources. Optimum conditions of growth medium for the maximum K-solubilization and K-solubilizing activity were found as follows: glucose was the best source of C at 25–30 °C and pH of 6.8–7.2. Graphical descriptions of the results are shown in Figures S1–S9.

4. 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 promotion under axenic conditions.

4.1. K-Solubilizing Rhizobacteria and Rhizobial Strains

Beneficial plant–microbe interactions in the rhizosphere are a very important component of eco-friendly agricultural systems. In this study, the rhizosphere as well as nodule bacteria were preliminary isolated from chickpea-dominated areas. Although 25 strains showed prolific growth in the presence of WM-spiked Aleksandrov's media, only 5 strains each from the rhizosphere and nodules of chickpea were able to form halo zones of >3 mm (Tables 2 and 3). The apparent affinity of bacteria to solubilize K is often reflected by halo zone formation [45]. All KSB strains were re-evaluated for another test of K solubility by screening based on amount of K solubilized in vitro after 72 h of incubation. Our results confirmed the efficacy of the KSB isolation approach as both strains with highest halo zone formation were able to solubilize the highest amount of K among rest of the strains (Table 4). 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 [16,46].

The KSB strains displayed a variety of morphological characteristics such as motility sporulation, gram staining and halotolerance (Table 5). The results had shown that the SKB3 isolate was able to utilize all carbon substrates, while none of the rhizobial isolate was able to use cellulose (Table 6). 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 [47].

In this study, quantitative screening for effective co-inoculants was carried out based on their cumulative K-solubilization activity in a K-enriched medium. The combined K-releasing ability of the co-inoculants ranged from 13.9 to 47.4 mg L^{-1} (Table 7). The largest amount of soluble K was recovered with co-inoculation of SKB3 × JKR7 followed by SKB11 × SKR13, whereas the lowest release of soluble K was obtained with SKB13 × JKR16

(Table 7). 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 [48].

The ACC-deaminase activity is one of the main growth-promoting attributes and plays a vital role in plant growth regulation, both in normal as well as in stressed conditions [49]. Ethylene, a phytohormone 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 [13]. In the present research, rhizobacteria have relatively higher ACC-deaminase activity than rhizobial strains. Some plant-beneficial bacteria can modify the level of indigenous phytohormone production in plants, such as IAA, which promotes root elongation, lateral root development and root hair formation. The improvement in the root system often leads to a higher water and nutrient uptake efficiency of the plant. The coupling of roots as well as the bacterial release IAA can facilitate a potential energy resource for the introduced bacterium for higher growth, survival and root colonization [49]. In this study, rhizobial strains showed more IAA production than rhizobacterial strains both with and without L-tryptophan (Table 8). In some previous studies, strong IAA activity was reported in several rhizobial strains [50]. An elevated chitinase activity was displayed by rhizobacteria ranging 12.5 to 37.8 µmol Glc NAc min^{-1} mg^{-1} protein, whereas rhizobial strains had relatively lower chitinase activity between 2.1 to 3.6 µmol Glc NAc min^{-1} mg^{-1} protein (Table 8). Chitinase activity usually reflects the availability as well as the accessibility of substrate because of its key role in the degradation of organic matter [49]. 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 plants against pathogens [16].

In the present study, strains of rhizobia participate more in secreting biopolymer compounds such as exopolysaccharide (EPS), which was in higher amounts between 122.8 to 321.2 µg mL^{-1} than rhizobacteria (Table 8). A number of recent reports described that rhizobial EPS production is generally linked to the formation of an adaptive mechanism at the cell surface scale under stressful conditions [16,51]. The EPS would thus be released by the bacteria to shield the plants against exposed stressors, indirectly benefiting from its growth and development under stress [52].

Microbially mediated nutrient solubilization is another crucial trait that can improve nutrient availability for plant uptake in limited nutrient environments. In the current study, we identified that both rhizobia and rhizobacteria were able to solubilize K and P in the culture medium (Table 8). Selected strains were more efficient K solubilizers whereas rhizobacteria had shown stronger capacity to solubilize K and P than rhizobial strains (Table 8). These findings further corroborate the previous reports that organic acids produced as a result of bacterial metabolic activities contribute to the increased solubility of nutrients such as P [11] and K [51]. In addition to nutrient solubility, selected strains had varied in siderophores production (Table 8). Microbial siderophores are low molecular weight, iron-scavenging ligands produced mainly under iron-deficient conditions [11,53,54]. The establishment of successful root colonization by microbial inoculants is an essential criterion to confer associated plant growth and development benefits [48]. The results of this study showed that the rhizosphere as well as rhizobial strains were efficient root colonizers (Table 8). The failure of microbial inoculants to colonize plant roots often caused the diffusion of their metabolic substances into the root zone and were eventually consumed by variety of root inhabiting microbes. In the absence of root persistence, introduced bacteria can evade away from the rhizosphere, thus making the root interface more vulnerable to deleterious root microflora [10].

4.2. Optimization of Growth Conditions for K-Solubilizing Rhizobacteria and Mesorhizobial Strains

The optimization of conditions for the maximum growth of SKB3 and JKR7 strains 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 [53] 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 are similar to Sheng et al. [6] in that some strains of K-solubilizing bacteria were also documented with remarkable K-solubilization activity at relatively higher temperatures, up to 42 °C, but the maximum activity was detected in the range of 25 to 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 [16,45]. 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.

5. Conclusions

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

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/su131810240/s1, Figures S1–S9: Results of Optimization of Growth Conditions for K-Solubilizing Bacterial Strains from Mica in Broth Medium.

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