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(Original Article)

Isolation and Identification of Potassium-Solubilizing Bacteria from Wheat Rhizosphere (*Triticum aestivum* L.)

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Abstract

More than ninety percent of the potassium (K) in the soil is found in the form of silicate minerals that are insoluble and cannot be used by plants directly. K-solubilizing bacteria (KSB), as biofertilizers, are capable to dissolving silicate minerals and release insoluble K into soluble forms, hence increasing soil fertility and plant growth.

In this study, twenty bacterial isolates were obtained from wheat rhizosphere soils and grown on Aleksandrov medium containing orthoclase as a K-source. Eight isolates showed ability to solubilize orthoclase based on inducing clear zones around their colonies. The solubilization index of bacterial isolates KSB1, KSB2, KSB3, KSB4, KSB5, KSB6, KSB7and KSB8 were 3.60, 2.72, 3.69, 3.67, 3.16, 2.8, 2.73, and 2.78, respectively. The highest solubilization index was recorded by the strain KSB3 and the lowest by the strain KSB2. Determination of quantitative K solubilization was carried out in broth Aleksandrov medium. The isolated KSB3 and KSB4 showed the highest K^+ solubilizing abilities, whereas recorded (29.57, 69.36) and (30.64, 68.42) mg L^{-1} after 20 and 30 days from incubation, respectively, in the broth Aleksandrov medium.

The isolated KSB3 and KSB4 were identified by the cell morphological, physiological, and biochemical characteristics. Also, they were identified as *Bacillus subtilis* (accession no. OR856236) and *Bacillus velezensis* (accession no. OR856237), respectively; based on 16S rDNA sequences.

Keywords: *Bacteria، Orthoclase ، Phylogenetic، Release potassium، Rhizosphere.*

Introduction

As a dynamic natural system, soil contains several mineral elements. Potassium (K) is the third essential macronutrient found in soil and is most abundantly absorbed by plants (Hassan and Arshad, 2010). Multiple physiological and metabolic activities in plants, such as photosynthesis, plant growth, metabolism, rate of assimilation, sugar accumulation, and overall growth and development depend on this element (Sparks and Huang, 1985; White and Karley 2010; Almeida *et al*., 2015, and Hussain *et al.,* 2016). An adequate amount of K is required for plants (Bhattacharj *et al,* 2023, and White, 2003).

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The availability of many agricultural sites, despite their initial high levels of K, is severely constrained for crops due to issues such as insufficient solubilization, surface runoff, leaching, and soil erosion (Xiafang and Weiyi, 2002). More prevalent than phosphorus (P), About 2.6% of the Earth's crust is composed of K, the seventh most abundant element (Sangeeth *et al*., 2012). Of this, only one to two percent is accessible to plants. Feldspar and mica are the most common soil components carrying 90 to 98% potassium (McAfee, 2008).

One such soil characteristic that is crucial to soil weathering is microorganisms, which dissolve nutrients from insoluble minerals (Hu *et al.,* 2018). For example, a variety of microorganisms, including actinomycetes, bacteria, and fungi, can solubilize K minerals through the excretion of organic acids (Sarikhani *et al*., 2018). The type of microbe able to release potassium from feldspar and aluminosilicate minerals is called potassium-solubilizing bacteria, or KSB. In this situation Acidolysis, chelation, exchange processes, and complexation releases K. (Meena *et al.,* 2015; Etesami *et al*., 2017; Sattar *et al.,* 2019). The application of K-solubilizing bacteria plays a key role in releasing K from K-bearing minerals and is a promising approach for increasing K availability in soils (Barker *et al*., 1998 and Maurya *et al*., 2014). Potassium-solubilizing bacteria (KSB) increased K nutrition and yield in a number of crops, such as pepper and cucumber (Han and Lee, 2006), wheat (Kumar *et al*., 2014), and rice (Bakhshandeh *et al*., 2017). One of the most efficient ways of improving plant utilization of potassium in the soil is to use potassium solubilizing microbes, which can make potassium ions available from minerals of both igneous and sedimentary origins. The use of potassium solubilizing microbes as biofertilizers may be the awaited solution to increasing crop productivity, concerns linked to chemical fertilizer application, and earth resource diminution.

Therefore, the objective of this study was to isolate the bacterial strains capable of solubilizing k from k-mineral, screening, characterizing KSB, identifying the selected strains based on molecular identification, and evaluate their ability to release K from orthoclase under laboratory conditions.

Materials and Methods

1-Mineral samples

Potassium-feldspar ($KAlSi_3O_8$) powder (containing 8.25 % K_2O; obtained from the Department of Geology, Faculty of Sciences, Assiut University) was passed through a 0.5 mm mesh size. This size has been previously used by (Zhang *et al*.,2023) The sieved powder was submerged in sterile water for three days, to remove any soluble K.

2-Isolation of K-solubilizing bacteria from soil and growth conditions

Soil samples were collected from wheat rhizosphere growing at the Experimental Farm of the Faculty of Agriculture, Assiut University. The samples were mixed with a potassium-bearing mineral (Orthoclase) and incubated at room temperature for one week. A modified Alexandrov medium was used, which contained (20g Glucose, 0.5 g $MgSO_4$. $7H_2O$, 0.1g $CaCO_3$., 0.006 g $FeCL_3$., 2.0 g

$Ca_3.PO_4$, 3.0 g insoluble potassium source (Feldspar), and 20.0 g agar in 1 liter of deionized water. Alkali or diluted acid were used to adjust the medium's pH lower than 7. Serial dilution technique was employed, and the cultures were incubated at 30°C for 1 week (Sugumaran and Janardham, 2007). Several bacterial colonies on the dilution plates displayed a clear zone that suggested the capacity to solubilize potassium from insoluble sources were selected and grown on Alexandrov agar medium. The colonies were selected using the streak plate method.

According to Ramesh *et al.* (2014), secondary screening of the isolates by studying the capacity to the potassium solubilization Index (SI), where:

$$\mathbf{SI = D / d}$$

Where: (D) Diameter of colony + clear zone and (d) Diameter of colony

3-Evaluation of KSB capacity to dissolve k^+ in broth Aleksandrov medium.

Determination of Quantitative K release, or solubilization, was carried out in 250 ml Erlenmeyer flasks containing 100 ml of Aleksandrov medium. The bacterial cultures were withdrawn after 20 and 30 days (Bakhshandeh *et al.*, 2017). Autoclaved uninoculated medium served as the control to which 0.5 ml of sterile nutrient broth medium (NB) was added. After the flasks were incubated at 30°C for 20 and 30 days, the cultures were centrifuged for four minutes at 4,000 g supernatants were utilized to evaluate the solubilized K. Flame photometry was used to determine the K concentration. A pH meter was used to determine the pH of the media. Three replicates were arranged for each treatment. A control without inoculation was also included.

4-Screening and Characterization of KSB

Clear zone formation and an assessment of the solubilization capacity of KSB in broth Aleksandrov medium were used to qualitatively evaluate the bacterial isolates' K solubilization ability after incubation. The morphological and biochemical characteristics of the most effective isolate were studied.

The bacterial isolates were biochemically characterized by catalase test, Acid production, starch hydrolysis, fermentation gas production of different sugars patterns, as well as morphological traits such as colony color, shape, size, elevation, and density. Results were then compared to Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994). Bacterial culture was then stored in Aleksandrov agar medium at 4°C For future studies.

5-Scanning electron microscope Examination of tissue

Two days After fixation in 5^0C cold buffered gluteraldehyde, two or three 0.5 to 1 cm samples were removed from the bacterial culture. After that, the samples were post-fixed in 1% osmium tetroxide for two hours and cleaned three times, each for thirteen minutes, using a cacodylate buffer. After three thirteen-minute washes in cacodylate buffer, samples were dehydrated using an ascending sequence of ethanol (30, 50, 70, 90) for two hours, 100% for two days, and finally amyl acetate for two days. It is important to note that liquid carbon dioxide was used to apply drying to the samples.

By using gold sputter coating apparatus, samples were evenly gold coated in a thickness of 15nm. Samples were examined by uSIng JEOL JSM 5400 LV scanning electron microscope 15-25. kv and photographed. (Nafady *et al.,* 1988; Bozzola and Russell 1991).

6-Molecular identification of bacterial isolates

According to Zimbro *et al*. (2009), bacterial isolates were cultivated in sterile test tubes with 10 ml of nutritional broth medium before being sent to the Molecular Biology Research Unit at Assiut University for DNA extraction. Isolates were cultured for 48 hours at 28ºC. The Intron Biotechnology Company, Korea, supplied the patho-gene-spin DNA/RNA extraction kit, which was employed. Samples of extracted DNA were sent to SolGent Company in Daejeon, South Korea, for gene sequencing and polymerase chain reaction (PCR). Two universal primers were used for the PCR, which were 27F (5'-AGAGTTTGATCC TGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTA CGACTT-3').

Using a size nucleotide marker (100 base pairs), the purified PCR products (amplicons) were confirmed by electrophoreses on 1% agarose gel. Dideoxynucleotides (dd NTPs) were added to the reaction mixture and 27F and 1492R primers were used to sequence the purified amplicons in both the sense and antisense directions (White *et al*., 1990).

Sequences were further analyzed using Basic Local Alignment Search Tool (BLAST) from the National Center of Biotechnology Information (NCBI) website. Phylogenetic analysis of sequences was done using MegAlign (DNA Star) software version 5.05.

7-Statistical analysis

One-way ANOVA was applied to the data using the SPSS 16.0 software. The three replicates' means, and standard deviations were computed, and at the 5% probability level, Duncan's multiple range test was used to compare them.

Results and discussion

1-Isolation of K-solubilizing bacteria from soil

Twenty bacterial isolates were isolated from wheat rhizosphere soils on Aleksandrov medium containing orthoclase as a k-source. Eight isolates demonstrated the capacity to dissolve orthoclase based on inducing clear zones around their colonies. which indicates the ability to secrete acids. Data in Table.1 and Fig.1 show the label of the isolated bacterial strains and the solubilization index (SI). The solubilization index of bacterial isolates KSB1, KSB2, KSB3, KSB4, KSB5, KSB6, KSB7and KSB8 were 3.60, 2.72, 3.69, 3.67, 3.16, 2.8, 2.73and 2.78, respectively. Strain KSB3 had the highest solubilization index while strain KSB2 had the lowest.

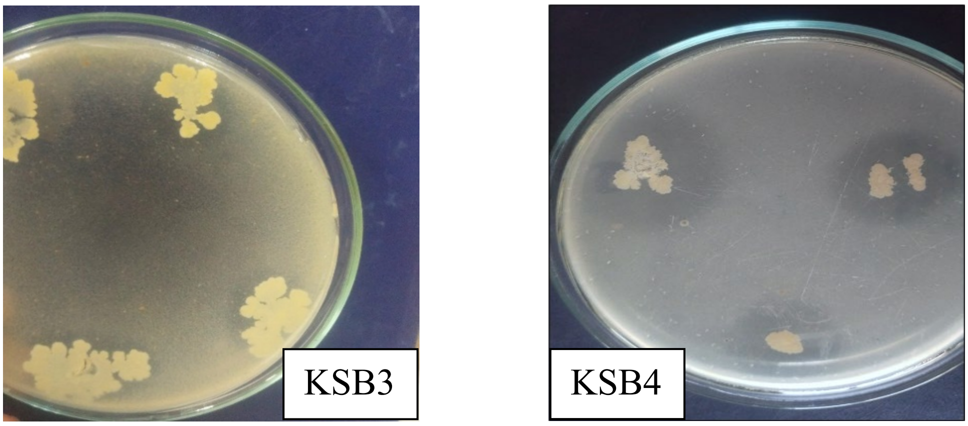

Figure 1. Clear zone around colonies of isolated bacterial strains KSB3 and KSB4 grown on Aleksandrov agar media for 7 days.

Table 1. The solubilization index (SI)* of isolated bacterial strains grown for 7- days on Aleksandrov medium.

Isolates	D (cm)	d (cm)	SI
KSB1	2.77	0.77	3.60
KSB2	1.36	0.5	2.72
KSB3	1.92	0.52	3.69
KSB4	2.2	0.6	3.67
KSB5	1.36	0.43	3.16
KSB6	1.4	0.5	2.80
KSB7	2.46	0.9	2.73
KSB8	2.5	0.9	2.78

*SI: is the ratio of total diameter (colony + halo zone) and the colony diameter

According to Setiawati and Mutmainnah's (2016), the KSB was able to create solubilization zones on the solid medium and solubilize K from sources of insoluble K-containing minerals, such as feldspar. Based on the dissolving index (DI), microbes involved in K solubilization can be classified as low (SI<2), moderate (SI 2<4), or high (SI >4) (Marra *et al*., 2011). Additionally, media acidification or the chelation of cations that typically bind to K are implicated in the mechanism of K solubilization (Etesami *et al*., 2017). Concerning the above-mentioned reports of several authors, our results indicated that the highest solubilization index was recorded by the strain KSB3 and KSB4.

These strains were isolated from Egypt, which located in arid and semi-arid regions. Therefore, in such conditions, strains with a high ability to dissolve potassium can be isolated.

2-The KSB solubilizing ability in broth Aleksandrov medium

All the eight isolates proved their ability to dissolve potassium from the K mineral in the Alexandrov broth medium as shown in Table 2 and Fig. 2.

Table 2. Determination of solubilizing capacity of K-feldspar on broth Aleksandrov medium (30ºC, pH 7).

Treatments	(Potassium concentration		PH-value (ppm)	
	20 days	30 days	20 days	30 days
CK*	14.53±0.34^{i}	14.53±0.05^{i}	7.06±0.66^{a}	7.06±0.11^{a}
KSB1	27.15±1.2^{f}	66.60±0.92^{e}	6.32±0.33^{c}	5.27±0.04^{h}
KSB2	19.39±1.3^{h}	59.76±0.66^{h}	6.63±0.11^{b}	6.61±0.11^{d}
KSB3	29.56±0.18^{b}	69.30±1.4^{a}	5.38±0.29^{i}	5.37±0.04^{g}
KSB4	30.64±0.72^{a}	68.60±0.73^{b}	5.53±0.11^{h}	4.70±0.03^{i}
KSB5	27.23±0.29^{e}	66.00±0.75^{f}	6.03±0.05^{e}	7.02±0.04^{b}
KSB6	29.03±0.08^{c}	67.00±0.51^{d}	6.00±0.11^{f}	6.98±0.24^{c}
KSB7	23.73±0.78^{g}	67.40±1.2^{c}	5.92±0.07^{g}	5.73±0.05^{e}
KSB8	28.71±0.58^{d}	61.53±1.9^{g}	6.21±0.11^{d}	5.43±0.07^{f}

*CK= uninoculated Aleksandrov medium as control.

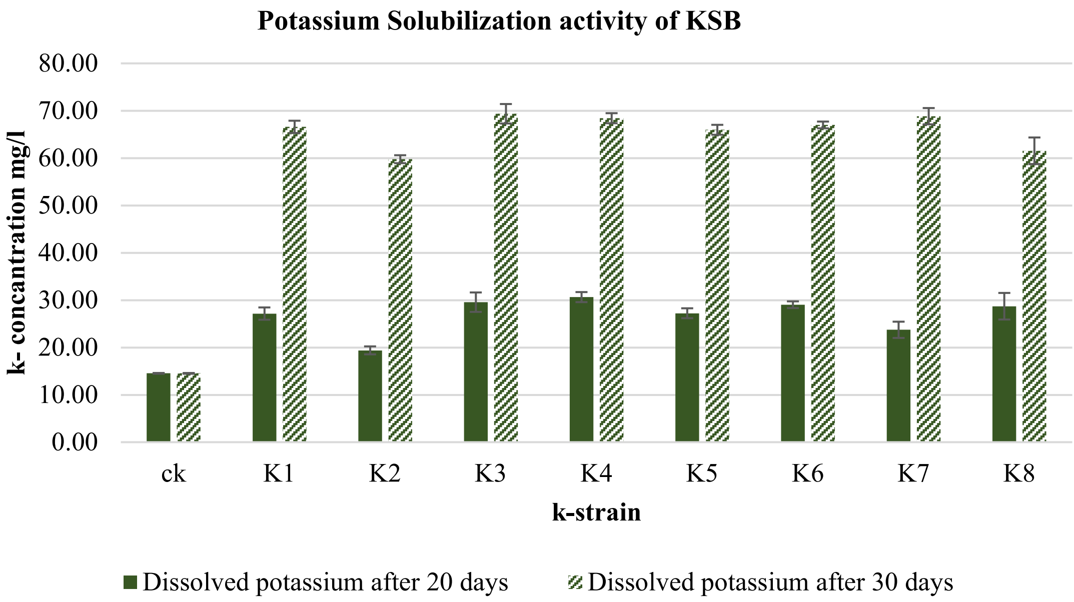

Figure 2. Dissolved potassium after 20 days and Dissolved potassium after 30 days of inoculation with the eight isolates in the liquid medium. Uninoculated media were used as a control (CK).

Potassium solubilizing strains (KSB3 and KSB4) showed enhanced K+ concentration of soluble K in the medium apparently by 29.57and 69.36 after 20 days and 30.64 and 68.42 mg/ L after 30 days by exhibiting K+ releasing ability. Means with different letters are statistically different significantly.

On the other hand, the uninoculated medium contained 14.38 and 60.48 mg/L after 20 and 30 days respectively. Moreover, the pH of inoculated medium showed clear decline after incubation (Fig.3).

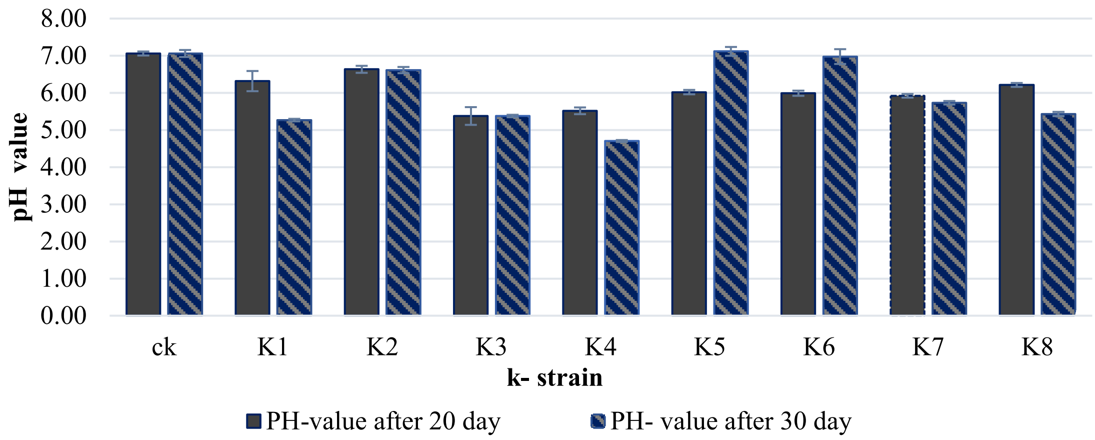

Figure 3. The media pH after 20 and 30 days after inoculation with various strains. The media initial pH was 7.

The results showed that solubilization increased with the number of incubation days. The capacity of KSB to dissolve insoluble potassium in liquid Aleksandrov broth medium has been explained by several research studies (Maurya *et al*., 2014). These results are supported by (Liu *et al*., 2006), who believed metabolic activities may be the cause of the highly acidic environments.

Recent investigations have shown that organic exudates of some bacteria played a declared role in the release of K from K-bearing minerals such as Basak and Biswas (2009), Prajapati *et al.* (2013), Zorba *et al*. (2013), and Zhang *et al.* (2014). Results proved that the KSB isolates produced various types of organic acids during their metabolism which affected dissolution by decreasing the pH of the environment.

So, our results indicated that the isolate KSB3and KSB4 showed the highest K^+ solubilizing abilities.

3- Characterization and identification of potassium-solubilizing strains

The isolates KSB3 and KSB4 showed stronger K^+ releasing abilities, and identified by the cell morphological, physiological, and biochemical characteristics. Results of the tested morphological, cultural, and physiological characteristics of the isolates are shown in (Table 3). The cell shape of the isolate is rod, ranging from 0.5 to 0.6 µm in diameter and from 2 to 3 µm in length. On nutrient agar medium, the isolate produced smooth, circular, convex colonies 0.5-0.7 cm in diameter. According to the Gram stain, the two were gram-positive. All isolates were unable to produce acid, also all isolates were unable to ferment glucose, sucrose, lactose, fructose, maltose, and arabinose.

Each isolate exhibited the ability to hydrolyze casein and starch. Based on Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994), considering all the identifying characteristics selected isolates were identified as *Bacillus* sp.

Isolating in a solid medium is usually the first step in screening, followed by culture in a liquid medium. Osman (2009) isolated KSB using only the solid

Aleksandrov medium, although Zhang and Kong (2014) and Meena *et al*. (2015) followed primary KSB isolation in solid media with liquid investigations in the Aleksandrov broth medium.

Table 3. Morphology, physiology, and cultural characteristics of KSB3 and KSB4 isolates.

Tests	Isolates	
	KSB3	KSB4
Colony morphology		
Colony Size (cm)	0.5	0.7
Colony shape	Circular	Circular
Colony edge	Wavy	Entire
Colony type	Smooth	Smooth
Cell shape	Rod	Rod
Cell size (μm)	0.5	0.6
Biochemical properties		
Starch hydrolysis	+	+
Casein hydrolysis	+	+
Catalase test	+	+
Methyl red test	+	+
Acid production	+	+
Gas production	-	-
Carbohydrate fermentation		
Glucose	-	-
Sucrose	-	-
Lactose	-	-
Fructose	-	-
Maltose	-	-
Arabinose	-	-

Based on their morphological, physiological, and biochemical properties, these KSB isolates were classified up to the genus level. According to Gram's method, colonies of the strains KSB3 and KSB4 were found on broth media plates. The stains' cell morphologies were also examined under a microscope. (Figure 4 and 5). According to Bergey's Manual of Determinative Bacteriology (Holt *et al.,* 1994), keeping in mind all the identifying characteristics the isolates were identified as *Bacillus* sp.

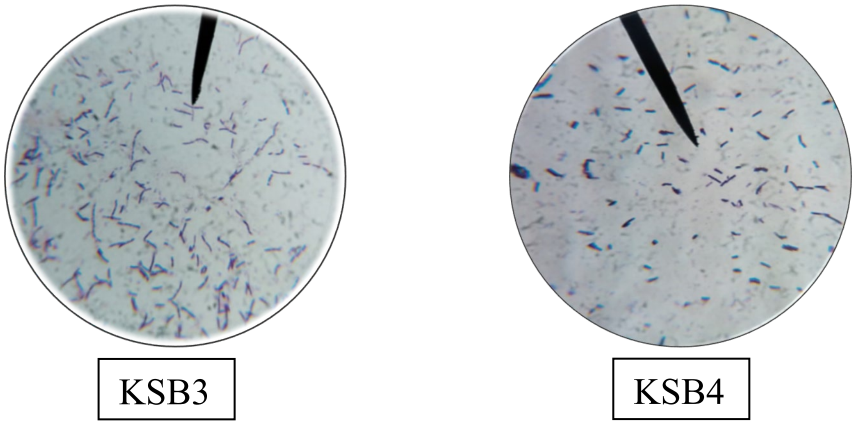

Fig. 4. Vegetative cells of potassium solubilizing bacteria (KSB3 and KSB4) from 48 hrs old cultures on nutrient agar medium. (X 1000)

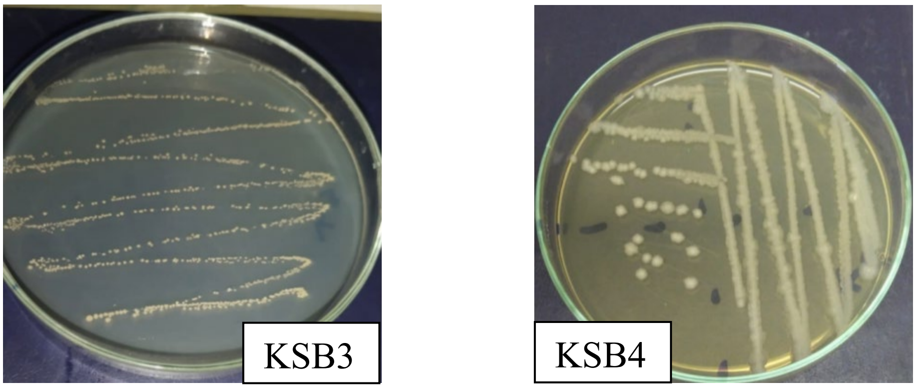

Fig. 5. Colonies of KSB3 and KSB4 potassium solubilizing bacteria strains on nutrient agar medium after 3 days of incubation.

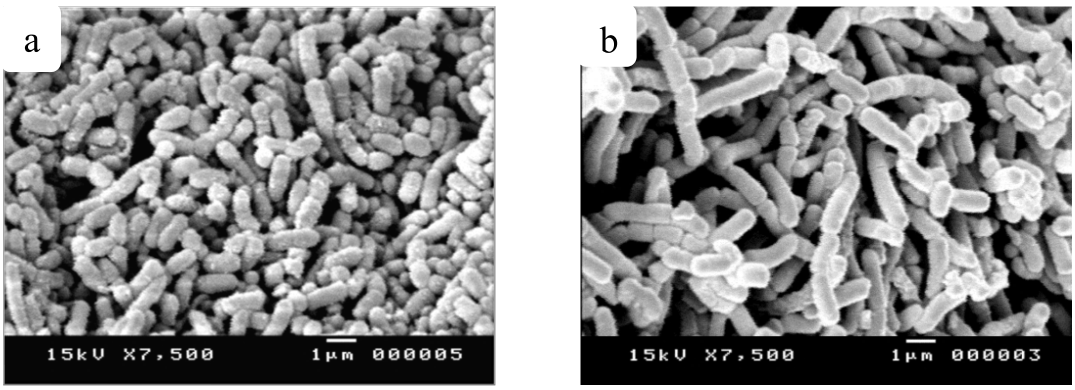

Fig 6. The scanning electron micrograph of *Bacillus subtilis* AUMC B-529 (a) and *Bacillus velezensis* AUMC B-530 (b) respectively

4-Sequences and phylogenetic analysis of potassium solubilizing bacteria strains KSB3 and KSB4

Sample K3: *Bacillus subtilis* strain AUMC B-529 (1422 letters) GenBank accession no. OR856236

TGCAAGTCGAGCGGACAGATGGGAGCTTGCTCCCTGATGTTAGCGGCG GACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGG GAAACCGGGGCTAATACCGGATGCTTGTTTGAACCGCATGGTTCAAACATAA AAGGTGGCTTCGGCTACCACTTACAGATGGACCCGCGGCGCATTAGCTAGTT GGTGAGGTAATGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGT GATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGC AGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTG AGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTA CCGTTCGAATAGGGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTA ACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAAT TATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCC CCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAG AGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGA ACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTGAGGAGCGA AAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAAC GATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGC ATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAA TTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAAC GCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAATCCTAGAGATAGGAC GTCCCCTTCGGGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTG TCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTT GCCAGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGG AAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACAC GTGCTACAATGGACAGAACAAAGGGCAGCGAAACCGCGAGGTTAAGCCAAT CCCACAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTGAA GCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCG GGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTC GGTGAGGTAACCTTTTAGGAGCAGCCGCCGAA

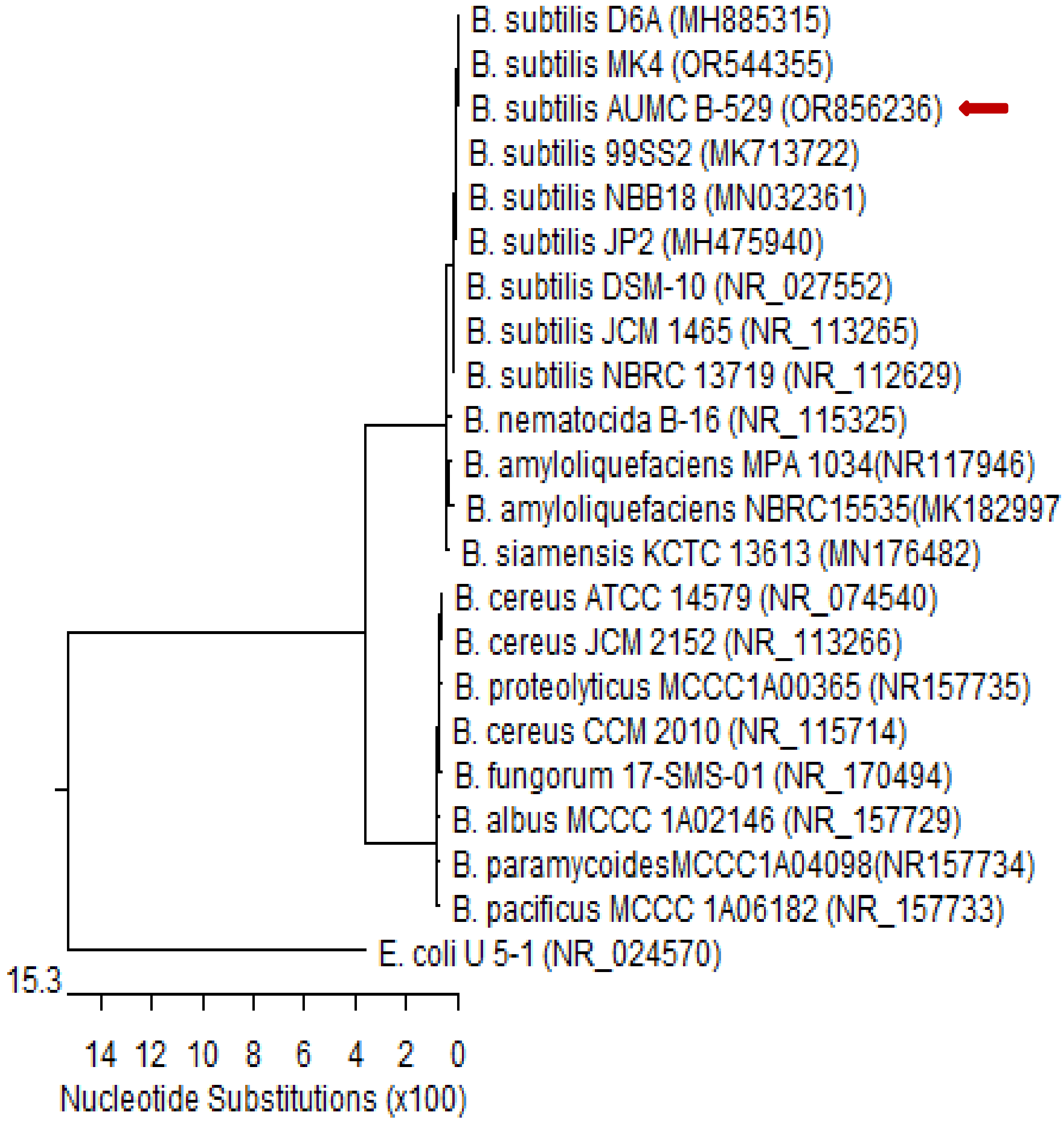

Fig. 7. Phylogenetic tree based on 16S rDNA sequences of the bacterial strain isolated in the present study (*Bacillus subtilis* AUMC B-529 with GenBank accession OR856236, arrowed) aligned with closely related strains accessed from the GenBank. This strain showed 100% identity and 100 % coverage with several strains of the same species including the type material *B. subtilis* NBRC13719 with GenBank accession no. NR_112629. *Escherichia coli* is included in the tree as outgroup strain, *B.*= *Bacillus, E.*= Escherichia

Isolate K4: Bacillus velezensis AUMC B-530 (1396 letters) GenBank accession no. OR856237.

GCAGTCGAGCGGACAGATGGGAGCTTGCTCCCTGATGTTAGCGGCGGACGG
GTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAAC
CGGGGCTAATACCGGATGGTTGTTTGAACCGCATGGTTCAGACATAAAAGGT
GGCTTCGGCTACCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGA
GGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCG
GCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAG
GGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGA
TGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTGCCGTT
CAAATAGGGCGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTAC
GTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTG
GGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGC
TCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGGA
GAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACAC
CAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTGAGGAGCGAAAGC
GTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGA
GTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAG
CACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACG
GGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAG
AACCTTACCAGGTCTTGACATCCTCTGACAATCCTAGAGATAGGACGTCCCCT
TCGGGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGA
GATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGC
ATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTG
GGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTAC
AATGGGCAGAACAAAGGGCAGCGAAACCGCGAGGTTAAGCCAATCCCACAA
ATCTGTTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGCTGGAA
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TA

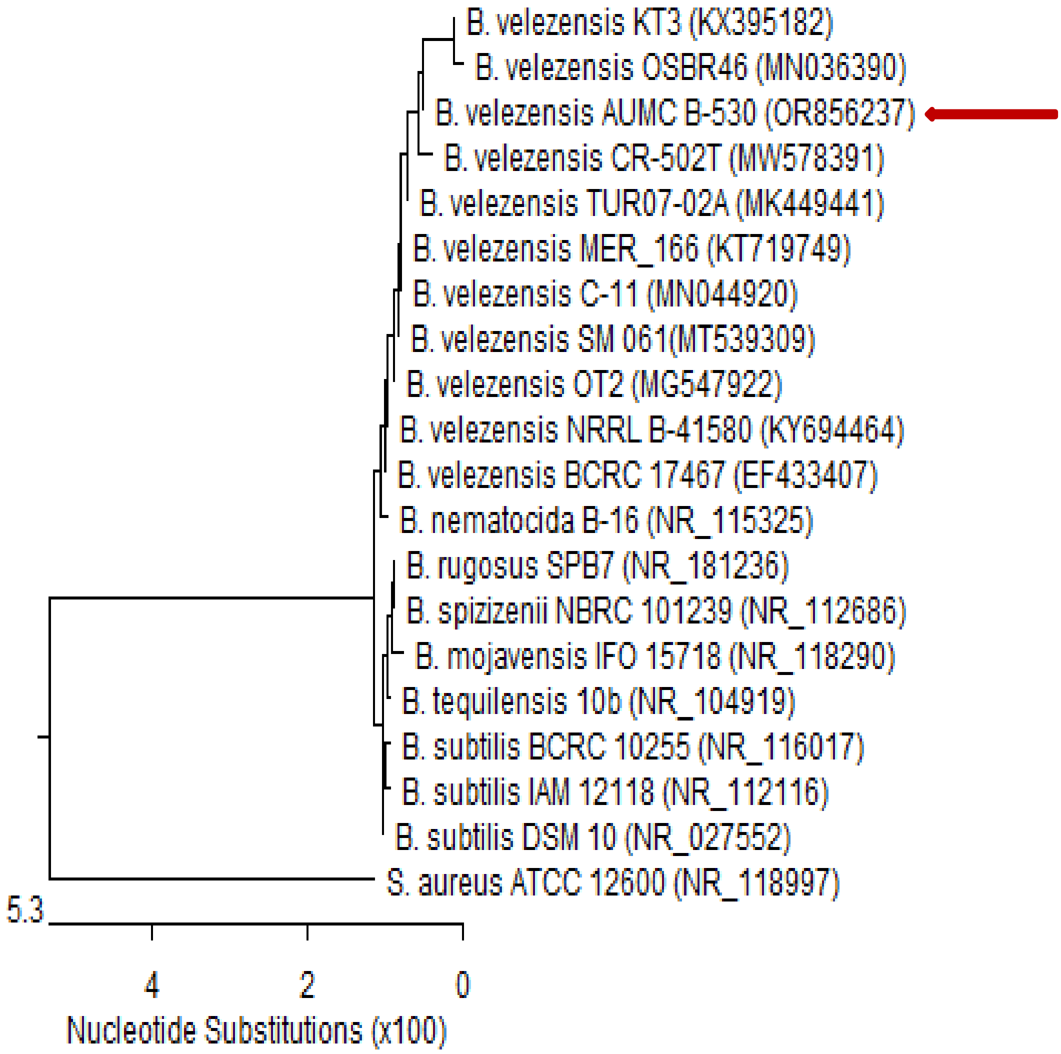

Figure 8. Phylogenetic tree based on 16S rDNA sequences of the bacterial strain isolated in the present study (*Bacillus velezensis* AUMC B-530 with GenBank accession no. OR856237, arrowed) aligned with closely related strains accessed from the GenBank. This strain showed 99.57% - 100% identity and 99% - 100 % coverage with several strains of the same species including the type material *B. velezensis* CR-502 with GenBank accession no. MW578391. *Staphylococcus aureus* is included in the tree as outgroup strain, *B.*= *Bacillus, S*= *Staphylococcus*

Based on Molecular identification, the selected isolates were identified as *Bacillus subtilis* AUMC B-529 and *Bacillus velezensis* AUMC B-530 (Figures 7 and 8).

The function of solubilizing K by *B. velezensis* and *B. subtilis* were reported before (Moussa and Daoud, 2023; Nowocień and Sokołowska, 2022; Das and Pradhan, 2016)

Sivasakthi *et al*., (2014) reported that *B. subtilis* has been identified from the rhizosphere of distinct plants. (Pramanik *et al*., 2020; Saha *et al*., 2016) showed that Various *Bacillus* species have exhibited K solubilization abilities, e.g., *B.*

horikoshii, *B. circulans*, *B. subtilis*, *B. velezensis*, and *B. cereus*. Potassium-solubilizing *Bacillus* spp. were also found to tolerate environmental stresses (Saha *et al*., 2016).

Manzum and Al Mamun (2018) declared that *B. subtills* were isolated from the soil and identified. On the other hand, two *B. velezensis* strains were identified and showed that the two strains can promote plant growth by dissolving and improving nutrient uptake (Shi *et al*., 2022).

It has also been observed that *Bacillus* spp. increases the soil K availability, crop K levels, and yield (Pramanik *et al*., 2019; Shakeel *et al*., 2015). It is well known that several K-solubilizing bacteria (KSB) such as *Bacillus velezensis B. mucilaginosus,* and *B. subtilis* promote the solubilization of soil K-bearing minerals through various direct and indirect mechanisms (Fatharani and Rahayu 2018). Moussa and Daoud (2023) proved that the *B. subtilis* and *B. velezensis* produced several types of organic acids during their metabolism including acetic, lactic, citric, malic, and oxalic acids .These organic acids produced by the KSB might enhance the dissolution of K-bearing minerals by supplying protons, destabilizing the surface of K-bearing and complexing Ca^{+2}, Fe^{+2}, and Al^{+3} ions (Etesami *et al*., 2017 and Badr, 2006).

Finally, the two selected strains in this study had a great ability to release potassium from k- minerals. They will be a promising source of biofertilizers that improve food security and the quality of crops, which achieves sustainable development goals.

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عزل وتعريف البكتيريا المحررة للبوتاسيوم من ريزوسفير القمح (*Triticum aestivum L.*)

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الملخص

يوجد أكثر من 90% من البوتاسيوم (K) الموجود في التربة في أشكال غير قابلة للذوبان كمعادن السيليكات ولا يمكن للنباتات الاستفادة منه مباشرة. ولكن يمكن للبكتيريا المذيبة للبوتاسيوم (KSB) تحسين خصوبة التربة ونمو النباتات عند استخدامها كأسمدة حيوية عن طريق تحلل معادن السيليكات وإطلاق البوتاسيوم غير القابل للذوبان في أشكال قابلة للذوبان.

تم في هذه الدراسة عزل عشرين عزلة بكتيرية من التربة في منطقة انتشار الجذور لنبات القمح على وسط الكسندروف المحتوي على الاورثوكلاز كمصدر للبوتاسيوم. أثبتت ثماني عزلات قدرة على إذابة الأورثوكلاز اعتمادا على إحداث هاله شفافة حول مستعمراتها. بلغ مؤشر ذوبان البوتاسيوم العزلات البكتيريةKSB1 ، KSB2، KSB3، KSB4، KSB5، KSB6، KSB7، KSB8 النسب الأتية:- 2.78، 3.60, 2.72، 3.69، 3.67, 3.16، 2.73، 2.8على التوالي. تم تسجيل أعلى مؤشر للإذابة بواسطة السلالة KSB3 والأدنى بواسطة السلالةKSB2 . تم تحديد ذوبان البوتاسيوم الكمي في وسط مرق الكسندروف. أظهرت العزلتان KSB3 و KSB4 أعلى قدرة على إذابةK^+ ، حيث سجلت (29.57، 69.36)، (30.64، 68.42) ملجم/لتر$^{-1}$ بعد 20، 30 يوماً من التحضين، على التوالي في وسط المرق. تم التعرف على العزلات KSB3، KSB4 من خلال الخصائص المورفولوجية والفسيولوجية والكيميائية الحيوية للخلية. أيضًا، تم التعرف عليهم على أنهم *Bacillus subtilis* ، *Bacillus velezensis*، على التوالي؛ على أساس تسلسل الحمض النووي الريبوزي 16S rDNA.

الكلمات المفتاحية: *إطلاق البوتاسيوم، الأورثوكلاز، البكتيريا، التطور، منطقة الجذور*