*Journal of Applied and Natural Science*
16(1), 196 - 201 (2024)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

*Research Article*

# Efficiency of *Bacillus mucilaginosus* isolated from the soil in dissolving potassium in its microenvironment

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***Article Info***
https://doi.org/10.31018/jans.v16i1.4728
Received: October 31, 2023
Revised: January 28, 2024
Accepted: February 7, 2024

***How to Cite***
Naji, N.S. *et al.* (2024). Efficiency of *Bacillus mucilaginosus* isolated from the soil in dissolving potassium in its microenvironment. *Journal of Applied and Natural Science*, 16(1), 196 - 201. https://doi.org/10.31018/jans.v16i1.4728

**Abstract**
Soil bacteria have an effective role in dissolving soil potassium. *Bacillus mucilaginosus* plays an effective role in dissolving potassium in the soil so that the plant may absorb it easily. The present study aimed to test the efficiency of bacteria in dissolving potassium present in the soil surrounding the roots of crops. *B. mucilaginosus* was isolated and diagnosed from the rhizosphere soil of Celery, Wheat, Basil and Alfalfa plants. The diagnosis included studying the isolates' culture, microscopic and biochemical characteristics. The laboratory study also included testing the efficiency of these bacterial isolates in dissolving potassium compounds in Modified Aleksandrov agar medium and estimating the dissolution coefficient. The results of isolation and identification of bacteria isolated from 19 out of 50 soil samples planted with different crops (Celery, Wheat, Basil and Alfalfa) showed that 8 isolates could dissolve potassium. The results of the microscopic examination of these eight isolates showed that they were sticky in shape, positive for Gram-staining, forming spores and the capsule, while the movement examination showed that they were positive for these tests (movement test). The biochemical tests and cultural characteristics showed that the eight isolates bear the characteristics of *B. mucilaginosus*. The results showed that the dissolution coefficient of potassium for the different isolates ranged between 2.28 and 1.14, while the type of sugar added to the culture medium increased the efficiency of bacterial isolates for potassium solubility. The study demonstrated the bacteria's efficiency in the rhizosphere region in dissolving potassium, which helps the plant use it easily.

**Keywords:** *Bacillus mucilaginosus*, Coefficient of potassium, Modified Aleksandrov agar, Rhizosphere

## INTRODUCTION

Microorganisms play an important role in nature and have a role in the potassium cycle, as some types of rhizosphere bacteria are capable of dissolving potassium compounds easily from the soil and bacteria silicates dissolve potassium, silicon and specifically aluminum is an insoluble potassium-bearing metal mica and illite by organic acids that dissolve rock potassium or chelated silicon ions to release potassium in the solution(Antoun and Prevost, 2015). Potassium-soluble bacteria *Bacillus mucilaginosus* produces many enzymes such as nuclease, endoglucanase, cellobiase, deoxyribonuclease, ribonuclease, protease and phosphomonoesterase which plays a role in liberating potassium (Tauson and Vinogrado, 2018).

The smelting of illite and feldspar by living microorganisms is due to the production of organic acids such as oxalic acid, and tartaric acid and the production of capsular polysaccharides that help dissolve minerals to release potassium. It also breaks down silicate minerals by *B. mucilaginosus* (Liu *et al.*, 2016).

Potassium is one of the elements that the plant needs as a nutritious mineral. Sometimes, the accumulation of potassium in large quantities at the roots of the plant may negatively affect the plant. Therefore, the bacteria in the decomposition of potassium have a very effective role in providing potassium to the plant on the one hand and reducing its harmful effects on the other hand (Mohi *et al.,* 2023). Potassium-degrading bacteria affect

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the movement of potassium, its spread in the soil, and its availability to plants. Potassium decomposes in several ways, including acid decomposition in highly acidic soils rich in organic compounds, and some potassium decomposes in water. These compounds are called unbreakable potassium. Microorganisms, including bacteria, can dissolve the other types of potassium to increase soil fertility and its readiness for plants (Uroz *et al* (2018). ,

Hence, the present study aimed to isolate and diagnose *B. mucilaginosus* from the soil rhizosphere of a group of crops in the Baghdad and Babylon governorates and test its efficiency in dissolving potassium and analyzing it so that it can be used in practical application to improve soil moisture.

## MATERIALS AND METHODS

### Samples collection

Fifty soil samples were collected from the rhizosphere area for a group of crops from the city of Hilla in Iraq during the period from October 2022 to March 2023 as mentioned in Table 1. The samples were placed in sterile nylon bags. Then they were transferred to the laboratory and placed in the refrigerator for the isolation and diagnosis process.

### Treatment of the collected samples

One hundred grams of soil samples were collected from the previously mentioned regions and kept in polyethylene bags 20x 40 cm. Soil samples were pretreated with CaCO3 at a ratio of 10:1 soil: CaCO3 and kept at an ambient temperature 37ºC to reduce the contamination with molds and yeast, as described by (Aziz, 2022).

### Isolation and purification of *Bacillus* from soil samples

After homogenizing each sample was sieved through the 2mm pore to remove gravel, large stones and debris. Then, 1 g of each soil sample was added to 9 ml of distilled water, and successive dilutions were made up to $10^{-1}$ - $10^{-5}$ . And placed in a water bath at 80°C for 30 minutes to eliminate vegetative cells. Each of the serial dilution was spread and placed on the surface Nutrient agar and incubated at a temperature of 28 °C for 7 days. The isolated colonies were then selected, and a full tube was transferred to Modified Aleksandrov broth and incubated for seven days at a temperature of 28°C. For isolating bacteria that dissolve potassium compounds. It was observed that turbidity and color change in the manure indicate the decomposition of insoluble potassium (Prajapati and Modi, 2022).

After obtaining pure colonies, the bacterial isolates were kept in the freezer until used. When used, it was incubated in tubes at 28 C˚ for 3 days, and after it grew, 0.1 ml of it was taken and it was grown on Modified Aleksandrov agar (Al-Zari medium)

**Table 1.** Sites and number of soil samples in the study area

| Rizosphere region type | Sample No. |
|---|---|
| Celery | 13,17,16,18,22,29,30,27,34,37,46,49 |
| Wheat | 1,2,7,8,9,6,10,14,25,31,33,38,42,43,44 |
| Basil | 32,35,26,28,11,12,24,35,41,45,50 |
| Alfalfa | 3,6,4,5,15,19,20,21,23,36,39,40,47,48 |

### Identification of *B. mucilaginosus* Culture characteristics

To diagnose bacterial isolates, phenotypic characteristics of the developing colonies such as their shapes , colors , surface , edges of the colonies, and the presence of odors distinctive, transparent and textured on the surface of Nutrient agar were observed .

### Microscopically tests

### Gram stain test

A dried bacterial swab was stained thermally with a violet crystal dye on a glass slide for 20 seconds and then washed with distilled water for 2 seconds. Iodine dye was added for 60 seconds, the stain was removed with ethanol for 10-20 seconds, and the slide was washed off with distilled water for 2 seconds. Dye was added Sufranin for 20 seconds and washed off with water distilled for 2 hrs, then examined again and air-dried slide with a light microscope to see if it preserved Bacteria stain (Violet Crystal). The cell shapes, their arrangement, and their interaction with the dye were examined under 100 x magnification in an oil-powered microscope (MacFaddin, 2000).

### Motility test

The agar medium test tubes were inoculated with semi-solid feed with bacteria using stapling and incubating at a temperature of 28 ° C for 24 hours. The spread of growth outside the stabbing area indicates the ability of isolation on movement (Shanware *et al*.,2014).

### Endospore staining

This test was conducted as reported by Drits (2020).

### Capsule staining

Capsular staining was made, as reported by Morello *et al.* (2016).

### Biochemical tests

The most important biochemical tests viz., Nitrate reduction test, Red methyl (MR) test, Focus Proscauer (VP) test, Catalase enzyme production, Oxidase enzyme production test, Urease enzyme production, Hydrolysis gelatin and Phenylalanine deaminase test were adopted in the diagnosis of bacteria following

methodology cited in Dahal (2023) .

**Genetic identification**

The G-spinTM Genomic DNA Extraction Kit was used for bacteria to purify DNA from small amounts of starting materials. The sample was not overloaded to obtain optimum DNA yield and quality. The 1-2 ml volume was suitable for gDNA prep. (Ideal OD600 value is 0.8-1.1).

**Protocol**

From 1-2 ml of cells (OD 600:0.8–1.0) were harvested by centrifugation at 13,000 rpm for 1 min. Remove supernatant. After centrifugation, vortexing or tapping removed the supernatant and completely resuspended it. Fifty µl of pre-buffer and 3 µl of lysozyme solution were added and mixed well. Incubated at 37°C for at least 15 minutes. To assist the cells, the mixture in the tube was stirred every 5 min during incubation. An amount of 250 microliters of G-Buffer solution was added, and the mixture was stirred well. It was incubated at 65°C for 15 minutes.

Add 250 µL of binding buffer, and mixed thoroughly by pipetting (at least 10 times) or gently vortexing. This step results in the efficient passage of cell lysates through the column and increased gDNA binding to the column resins and is important for efficient protein removal. The cell was loaded onto the column and centrifuged at 13,000 rpm for 1 minute. Note: Maximum volume of column reservoirs was 800 µL. For sample volumes greater than 800 µl, the sample was loaded and then spun again. 500 µL of wash solution A was added to the column and centrifuged for 1 minute at 13,000 rpm .The solution is removed and centrifuged for 1 minute at 13,000 rpm .The G-spin TM column was placed in a clean 1.5 ml microcentrifuge tube (not provided), and 50-200 µl of elution buffer was added directly to the membrane.Then, it was incubated at room temperature for 1 min and centrifuged for 1 min at 13,000 rpm .The concentration and purity of DNA were estimated using nanodrop methods (Antoun and Prevost, 2015).

**Experiment to test the efficiency of bacterial isolates in dissolving potassium in the medium solid implant**

**Determination of solubilization index (SI)**

The dissolution factor was estimated to Mica as a source of potassium for seven Isolated potassium solvents and Dietary Aleksandrov medium agar for this purpose. The medium was prepared and sterilized in an autoclave at room temperature and a pressure of 15 pound 121$^{o}$c for 20 minutes and then, poured the medium into Petri dishes was left to solidify and then transferred to 1.0 ml from isolates and spread on Surface of the medium with an L-shape diffuser using three replicates. The dishes were incubated at 28°C and after passing 3 Days after incubation, measured the diameter of the colony .The transparent areola was measured using a ruler and the following equation for estimating the solubility coefficient (Li *et al.,* 2017).

SI= D/C Eq.1

SI= solubilization index, D= The total diameter of the colony +The transparent halo, C = colony diameter only.

**Experiment to test the efficiency of the bacteria dissolving potassium in the liquid medium influencing the type of sugar**

A bacterial colony was taken and incubated in Alexandrov broth at 28°C for 10 days. Tubes with bacterial growth are centrifuged at 7000 rpm for 10 minutes to separate the suspension from growing cells and undissolved potassium. 1 ml of the suspension was placed in a 50 ml volumetric flask. The volume was supplemented to 50 ml with distilled water, mixed well, and measured with a spectrometer to estimate the potassium content (Lian, et al, 2018). Then different potassium concentrations were prepared starting from…,5.5,5,4.5, 5.4,3.5,3.0,2.5,2.0,1.5,0.5 10 mg/L.

## RESULTS AND DISCUSSION

The results of bacteria isolated from 19 ( 6 wheat, 5celery, 5basil and 3alfalfa) soil samples out of 50 soil samples of the rhizosphere region of different locations in Hilla city planted with different crops are shown in Fig. 1 and Fig.2 .

The rhizosphere is rich in organic materials and nutrients leaking from plant residues or root secretions. The presence of these high levels of nutrients stimulates the growth of large numbers of microorganisms, especially bacteria, which are characterized by the ability to adapt to various environmental conditions and their short life cycle, making them grow very active in the area around plant roots (Parmar and Sindhu. 2023).

**Isolation and identification of *B. mucilaginosus***

Among a series of dilutions of different soil samples sequenced from $10^{-1}$- $10^{-4}$, the concentration $10^{-4}$ was the most concentrated, giving bacterial growth in isolated and clear colonies. Isolated colonies were purified more than once by subculturing.

The microscopic and culture characteristics of the bacterial isolates are shown in Table 2.

Table 3 shows the results of the biochemical tests for the eight *B. mucilaginosus* isolates which were positive for catalase and urease test, while these isolates were negative for Voges Proskauer test, $H_2S$ production, the metabolism of orenes reductase and reduced nitrate. The isolates bearing the numbers 2, 3 and 6 were able to produce oxidase, while the rest of the isolates showed a negative result for this test. The overall re-

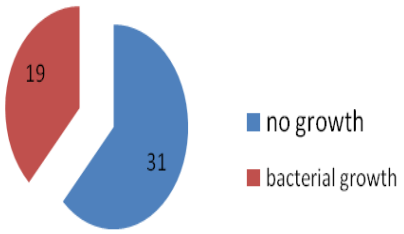



**Fig. 1.** *Number of soils that gave bacterial growth*

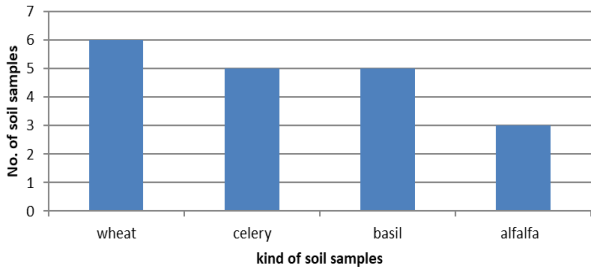



**Fig. 2.** *Distribution of soils that gave bacterial growth.*

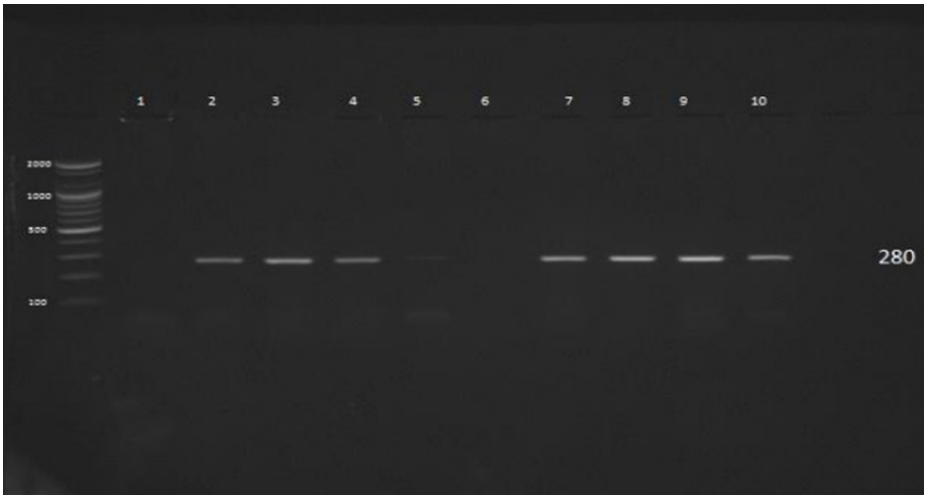



**Fig. 3.** *Agarose-gel-electrophoresis images showing the PCR product. investigation of 16S rRNA gene in B. mucilaginosus isolates. Lane (M): marker ladder (100- 2000bp), lane (1-10): 16S rRNA gene positive at 191bp PCR product size.*

sults of the microscopic, cultural, and biochemical tests, depending on the sources of relevant scientific studies (Hazen *et al*., 2019), showed that the eight isolates had the characteristics of *B. mucilaginosus* AB045091 and these results are consistent with what has been indicated in earlier studies (Kloepper *et al*., 2018 ; Vessy, and Kevin 2023; Sheng, and Huang, 2022; Styriakova *et al*., 2023; &Sheng and He, 2016) who isolated the *B. mucilaginous* from the soil of a wheat and barley field in China, they proved its ability to dissolve potassium.

**Molecular results**

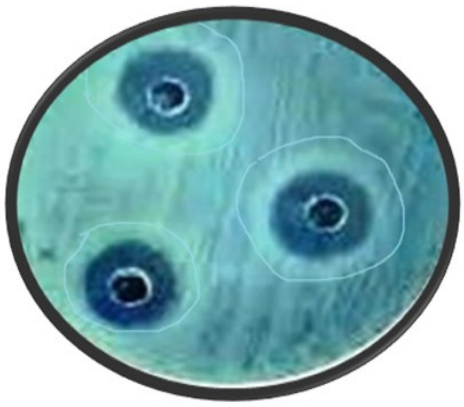
Molecular detection of 16S rRNA of *B. mucilaginosus* as 8 (100%), is shown in Fig. 3.

**Dissolving coefficient determination test**

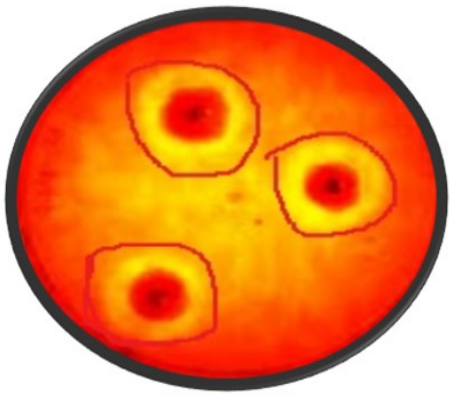
The results of this test showed heterogeneity of *B. mucilaginosus* AB045091 isolates. In its ability to dissolve potassium on the Agar culture medium when growing on Alexandrov medium at a temperature of 28 °C for seven days (Fig. 4). It was noted that these isolates varied in their ability to dissolve potassium compounds, as there were only 8 isolates which were able to dissolve potassium in Alexandrov broth medium while 12 isolate could not dissolve compounds potassium as evident in Table 4. It was noticed that Isolate 3 had the lowest dissolving factor Its amount was 1.18 and isolates 1,2.7 had a solubility coefficient of 1.20-1.25, followed by isolate 4, where its solubility factor was 1.44,

**Table 2.** Microscopic and culture characteristics of *Bacillus mucilaginosus* AB045091

| No. | Position | Appear. the colony | Cell form | Texture and transparency | Gram stain | Spore formation | formation capsule | Movement |
|---|---|---|---|---|---|---|---|---|
| 1 | Wheat | white circular | bacillus short | Sticky/semi transparent | + | + | + | + |
| 2 | Wheat | white circular | bacillus short | Sticky/transparent | + | + | + | + |
| 3 | Wheat | white circular | bacillus | Sticky/transparent | + | + | + | + |
| 4 | Celery | white circular | bacillus short | Sticky/ semi transparent | + | + | + | + |
| 5 | Basil | white circular | bacillus | Sticky/transparent | + | + | + | + |
| 6 | Basil | white circular | Bacillus | Sticky/transparent | + | + | + | + |
| 7 | Alfalfa | white circular | bacillus | Sticky/ semi transparent | + | + | + | + |
| 8 | Alfalfa | white circular | bacillus short | Sticky/transparent | + | + | + | + |



***A.*** *Showing Bacillus mucilaginosus AB045091 dissolving potassium on Alexandrov medium without phenyl red stain*



***B.*** *Showing Bacillus . mucilaginosus AB045091 dissolving potassium on Alexandrov medium with phenyl red stain*

**Fig. 4. A. and B.** *Showing the transparent halo round bacterial colonies that dissolved potassium*

**Table 3.** Biochemical test to identify the *B. mucilaginosus*AB045091

| No. isolates | Catalase | Urease | Oxidase | Voges proskauer | $H_2S$ | Orenes Reductase | Lysine utilization | Gelatin G | phenylalanine deaminase | reduces nitrate |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | + | + | - | - | - | - | + | + | + | - |
| 2 | + | + | + | - | - | - | - | + | - | - |
| 3 | + | + | + | - | - | - | - | - | - | - |
| 4 | + | + | - | - | - | - | - | - | - | - |
| 5 | + | + | - | - | - | - | - | - | - | - |
| 6 | + | + | + | - | - | - | - | - | - | - |
| 7 | + | + | - | - | - | - | - | - | - | - |
| 8 | + | + | - | - | - | - | - | - | - | - |

**Table 4.** Dissolving coefficient values of potassium compounds for *B. mucilaginosus* AB045091

| No. of isolate | Total diameter of the colony + Transparent halo (D) mm | Colonial diameter only (C) mm | Solubility coefficient (D/C) |
|---|---|---|---|
| 1 | 11 | 9 | 1.22 |
| 2 | 12 | 10 | 1.20 |
| 3 | 13 | 11 | 1.18 |
| 4 | 13 | 9 | 1.44 |
| 5 | 17 | 8 | 2.12 |
| 6 | 16 | 7 | 2.28 |
| 7 | 10 | 8 | 1.25 |
| 8 | 15 | 6 | 2.50 |

D= diameter of the colony and the transparent halo; C = Colonial diameter only

and the highest solubility factor was for Isolate 8 by 2.50.

These results are consistent with Sugumaran, and Janarthanam (2017) who isolated *B . mucilaginosus* from soil planted with Basil. They demonstrated its ability to dissolve potassium using Alexandrov medium with a phenyl red stain. The cause of melting may be due to the high efficiency of bacteria *B. mucilaginosus* in producing organic acids that have the primary role of dissolution. Warr (2021) reported that bacteria growing in insoluble silicates produce carbon dioxide, organic acids, and exopolysaccharides. *B. mucilaginosus* capsule, which contains exopolysaccharides (Yang *et al*., 2016) and converts organic acids into oxalic acid, oxalate (citrate acid), citric acid, tartaric acid (Zhao, *et al*., 2019; Sheng and He, 2016), and malic acid. and formic acid (formic acid Shanuer *et al*., 2014). These organic acids accelerate the weathering process of potassium-bearing minerals, leading to their liberation (Willey et al., 2019). This explains the ability of *B. mucilaginosus* to dissolve potassium present in soil with crops.

**Measuring the efficiency of *B. mucilaginosus* AB045091 in dissolving potassium in broth containing various sugars**

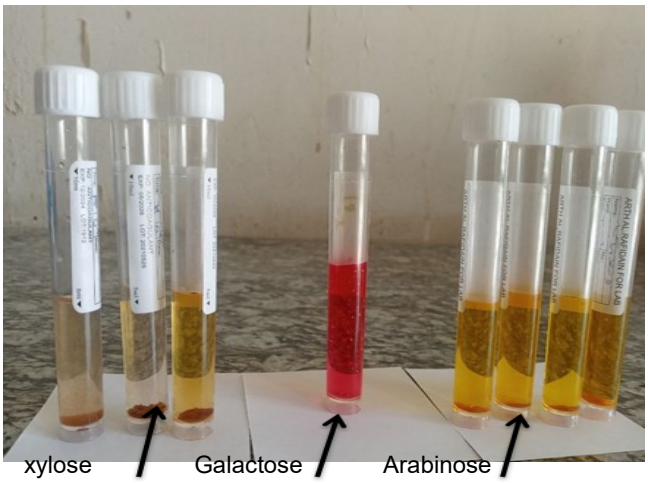
To know the effect of the type of sugar as a carbon source on the ability of bacterial isolates to dissolve potassium in broth, xylose, galactose, glucose, and arabinose were used (Fig. 5). The findings demonstrated that the ability of these *B. mucilaginosus* AB045091 to dissolve potassium was diminished when other oligosaccharides were substituted for glucose. Table 5 shows the lowest amount of dissolved potassium (39.6 mg \ L)when xylose was used for Isolation 1. The amount of dissolved potassium varied between isolates when using the four sugars previously mentioned; isolate number (7) had the highest amount of dissolved potassium at 89.7 mg \ L.

These results are consistent with Anton and Prevost (2015), Benizri *et al.* (2021) and Blomberg *et al.* (2021), who discovered that *B. mucilaginosus* is a bacteria present in the soil of the roots of some crops such as wheat, barley, and celery. They also proved that the presence of various sugars such as glucose, xylose, and galactose increases the efficiency of bacteria in

**Table 5.** Dissolution of potassium compounds by *Bacillus mucilaginosus* AB045091 isolates using different sugars

| Isolates No. | Glucose (mg\L ) | Xylose (mg\L ) | Galactose(mg\L ) | Arabinose (mg\L) |
|---|---|---|---|---|
| 1 | 54.9 | 39.6 | - | 45.5 |
| 2 | 67.6 | 54.6 | - | - |
| 3 | 77.8 | 57.8 | - | - |
| 4 | 43.5 | - | - | 78.2 |
| 5 | 56.8 | - | - | - |
| 6 | 53.6 | - | - | - |
| 7 | 78.9 | - | 89.7 | 65.8 |
| 8 | 65.4 | - | - | - |

**A**. *Alexandrov broth medium with glucose showing the ability B. mucilaginosus AB045091 to dissolve potassium in the presence of glucose as an energy source, and the change in color of the medium indicates fermentation of the sugar*




**B**. *Alexandrov broth medium with different sugar. It shows the ability of B. mucilaginosus AB045091 to dissolve potassium in the presence of xylose, galactose and arabinose as an energy source, and the change in color of the medium indicates fermentation of the sugar*

**Fig. 5. A. and B.** *Dissolution of potassium compounds by 7 and 1 Isolate using different sugars*

dissolving and decomposing potassium so that it becomes easier for plants (Antoun and Prevost, 2015).

## Conclusion

The present study concluded that the presence of *B. mucilaginosus* AB045091 in the root zone of herbal plants (Celery, Wheat, Basil and Alfalfa), from different areas in the city of Al-Hilla, Babil Governorate, in Iraq. Thus, *B. mucilaginosus* has the ability to dissolve potassium with high efficiency, and the solubility coefficient of potassium can be affected differently depending on the kind of carbohydrates (xylose, galactose, glucose, and arabinose) present in the soil.

## Conflict of interest

The author declare that they have no conflict of interest.

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