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Original article

Characterization, enzymatic and biochemical properties of endophytic bacterial strains of the medicinal plant *Ajuga turkestanica* (Rgl.) Brig (*Lamiaceae*)

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ABSTRACT

This research work aims to isolate endophytic bacteria from the *Ajuga turkestanica* plant that have plant growth promoting properties. The microscopic, enzymatic and biochemical properties of the isolated endophytic bacteria are described. Twelve isolates of endophytic bacteria were isolated by standard microbiological methods. Isolated endophytic bacteria were identified by (MALDI-TOF MS). Endophytic bacteria isolated from the plant were detected in MALDI-TOF MS. Bacterial isolates were dominated by endophytic bacteria belonging to the genus *Bacillus*, *Pseudomonas*, *Siccibacter*. When the growth-enhancing properties of cultivated plants used in sustainable agriculture were studied, the isolated bacterial strains showed positive results in siderophore, ammonia, indole acetic acid production, and nitrogen fixation properties. Numerous endophytic bacteria have been found to have the enzymatic activity of oxidase, catalase, amylase, lecithinase and lipase. The results showed that endophytic bacteria isolated from the plant *A. turkestanica* are have plant stimulating properties which can be shown as a candidate for the production of bioinoculants individually or as part of a consortium.

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1. Introduction

In pharmaceuticals plant-derived substances are used in the treatment of cardiovascular diseases (myocardial infarction) (Egamberdieva and Jabborova, 2018; Egamberdieva and Jabborova, 2020). The plant extract has a wide range of antimicrobial and antifungal properties. The bioactive substances of the medicinal plant *Ajuga turkestanica* have antioxidant, anti-

leukemic, cardiotoxic and cytotoxic properties and are used in many clinics in Russia and Uzbekistan (Mamatkhanov et al., 1998; Zadeh and Kor, 2014).

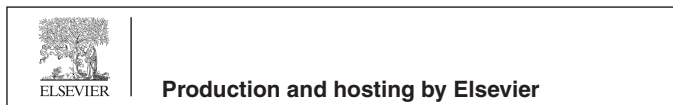
A. turkestanica (Regel) Brig. (*Lamiaceae*) is rich in biologically active substances the leaves and stems contain up to 3.76% flavonoids, iridoids, phytoecdysteroids and up to 0.1% of the plant leaves contain a compound called turkesterone (Baltaev 2000; Ramazanov 2005). The medicinal plant *A. turkestanica* contains biologically active substances with high pharmacological effects, such as ecdysterone, turkesterone, cyasterone, ajugasterone, ajugalactone, cyasterone-22 acetate and phytoecdysteroid used in the treatment of cancer, malaria, gastrointestinal, in cardiovascular problems (Mamatkhanov et al., 1998; Syrov, 2001; Abdudakirov et al., 2005; Shakhmurova et al., 2010).

In recent years, scientific hypotheses have been put forward that endophytic bacteria, which make up the plant microbiota, create optimal conditions for the normal growth and development of

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the host plant under adverse conditions (Singh and Padmavathy, 2014; Szilagyi-Zecchin et al., 2014, Selim et al., 2011; Kumar et al.; 2016, Bezerra et al., 2012). Bio stimulants and foliar application is another way to get optimal growth of host plant (Abbas et al., 2020; Ullah et al., 2020; Khan et al., 2020) Endophytic bacteria dissolve insoluble phosphate compounds in the soil by producing organic acids thereby stimulating plant growth (Sharma et al., 2013; Jabborova et al., 2015; Jabborova et al., 2020a). Soil properties changes with the land use (Marfo et al., 2019a; Marfo et al., 2019b). As the medicinal plant *A. turkestanica* contains pharmacologically important biologically active substances, it is important to analyze its endophytic microbiome. This study was performed to investigate characterization, enzymatic and biochemical properties of endophytic bacterial strains of the medicinal plant *A. turkestanica*.

2. Materials and methods

2.1. Sample collection

Ajuga turkestanica (Rgl.) Brig (*Lamiaceae*) grows on stony slopes of gypsum and red sandstone, soil and rock mixed areas at an altitude of 600–1000 m on the south-western slopes of the Pamir-Alay mountains of Boysun district of Surkhandarya region (Fig. 1).

2.2. Surface sterilization of plant samples

Surface sterilization was performed using Miral and Verma methods to isolate endophytic microorganisms from the medicinal plant *A. turkestanica* (Verma et al., 2014). Plant samples prepared for surface sterilization were cut into small pieces using sterile tweezers. Plant parts prepared for surface sterilization were washed in running water for fifteen minutes. The washed plant parts were then washed with copious amounts of distilled water. In the next step, plant samples were transferred to a laminar box (Thermo scientific Maxisafe 2020 Germany) provided with aseptic conditions. Plant parts (roots, stems, leaves) were cut into 3–5 cm pieces to facilitate surface sterilization and sterilized for one minute in 70% absolute ethyl alcohol. At the stage of surface steriliza-

tion of plant samples are stored in a solution of hypochlorite sodium (NaClO) five percent. Also, plant parts divided into small volumes are thoroughly washed in sterile deionized water. To determine how successful the sterilization process was, a pre-prepared Chapek doks agar medium was dripped from the last washed water using a sterile glass rod. If no microorganisms grow from the water droplet medium it confirms that the surface sterilization process was positive.

2.3. Isolation and identification of endophytic bacterial strains

In order to isolate endophytic bacteria from plant specimens the pieces which were initially cut into small pieces, were transferred to Petri dishes containing sterile tweezers Tryptic Soy Agar medium (NaCl 5.0 g; Dextrose 2.5 g; K_2HPO_4 2.5 g; Agar 15.0 g). Plant specimens were incubated at 28° C for ten to fifteen days after transfer to medium. After incubation, morphologically diverse bacterial colonies were selected and their morphological microscopic and biochemical properties were examined. The isolated bacterial isolates were grown overnight in the Tryptic Soy Broth medium. Cultured endophytic bacterial cultures are centrifuged at a rate of 15,000 revolutions per minute. The supernatant portion of the culture fluid was drained and a precipitate was removed for processing in subsequent stages. The separated precipitate was first washed using 250 μ l of sterile deionized water and then 800 μ l of ethyl alcohol. The resulting clutural fluid was again centrifuged at a rate of 15,000 beats per minute to remove the supernatant portion. The precipitate obtained was then dried in a laminar cabinet under aseptic conditions for 5–7 min. The precipitate was then mixed by adding 25 μ l of formic acid and acetonitrile. The resulting mixture was then centrifuged again and a total of 1 μ l of supernatant was placed on a 96-point tablet and dried. One microliter of MALDI MS solution was then poured into the samples and allowed to dry under aseptic conditions. MALDI TOF MS mass spectra were analyzed using bacterial identification system MALDI Biotyper®. Each sample was analyzed three times to confirm the reliability of the results. The control was carried out on the relevant spectra of existing strains in the collection of sanitary and epidemiological control of the Main Department of Medicine under the Presidential

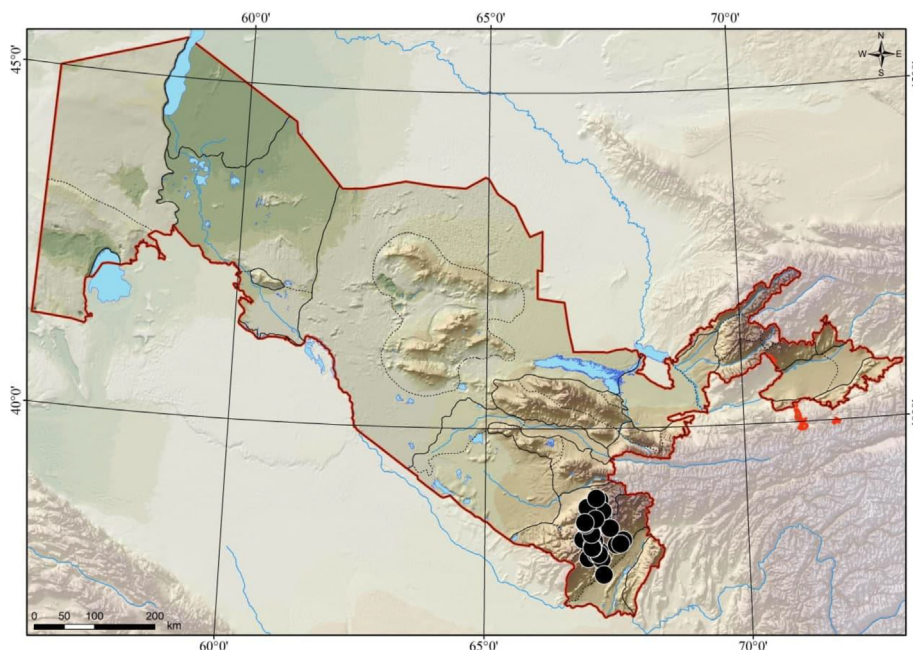


Fig. 1. Map of the area where the medicinal plant *A. turkestanica* is collected. Boysun district of Surkhandarya region Uzbekistan.

Administration of the Republic of Uzbekistan. According to the results obtained, the bacterial identification scores are as follows: ≥ 2000 species-level identification means 1700 1999 species-level identification. If the score is < 1700 , it means that the isolate is not identified.

2.4. Morphological characterization of endophytes

After incubation, the endophytic bacteria isolated from plant parts were purified according to their phenotypic properties. The morphology of endophytic bacterial isolates (color of bacterial colonies, gram staining, shape of colony margins, growth dynamics and nature of colonies) was studied according to Berge's (Bergey and Holt, 2000, Sayed et al., 2019). The gram method was used for morphological analysis of endophytic bacteria. Reagents used in staining of bacterial isolates by Gram reaction: (crystal violet (basic dye), iodine solution (purple color stabilizer in cell wall, color decolorization of samples (ethanol 70%) and distilled water).

2.5. Biochemical properties of endophytic bacterial isolates

Endophytic bacteria were grown in Simmons citrate agar medium and incubated for 24 h at 28 ± 2 °C. If the bacterial isolates have the property of assimilating citrate the medium color will change from green to blue. Such a change by endophytic bacteria is a positive reaction.

The carbohydrate fermentation property of the isolated bacterial isolates was determined by cultivation in Triple Sugar Iron agar medium (3.0 g beef extract; 20.0 g peptone; 3 g yeast extract; 10.0 g sugar; 10.0 g lactose; 1.0 g dextrose; 0.2 g ferrous sulfate; 5.0 g sodium chloride, 0.3 g sodium thiosulfate; 0.024 g phenol red and 12.0 g agar). Bacterial strains were incubated at 30 ± 2 °C for one day in TSI (Triple Sugar Iron agar) medium. The medium in which endophytic bacteria are grown exhibits an alkaline / acidic state. As a result, if the slope of the medium inside the test tube is red and the bottom is yellow, it means that the bacteria have only carried out dextrose fermentation. When endophytic bacteria grow in a medium acidic state, the slope and bottom of the medium turn yellow. This indicates that the bacteria carried out the fermentation of dextrose, lactose and sucrose.

When endophytic bacteria form a medium alkaline medium in which they grow, the slope and bottom of the medium inside the test tube remain unchanged. This result indicates that carbohydrate fermentation has not taken place. The blackening of the medium inside the solution indicates that hydrogen sulfide was produced by the bacterial isolates. The division of the medium and its rise in the solution indicates that the endophytic bacteria have formed a gas (Patel et al., 2016, Singh and Dubey, 2020).

Bacterial strains were incubated in Glucose Phosphate Broth (Buffered peptone 7.0 g; dextrose glucose 5.0 g; dipotassium phosphate 5.0 g; final pH 6.9 ± 0.2) for two days at 28 °C. At the end of the incubation period, 1 mL of bacterial fluid was transferred to a fresh sterile solution. Then 1–2 drops of methyl red solution were added to the solution. The rapid formation of a red color in the bacterial fluid when a methyl red solution was added was taken as a positive reaction.

2.6. Screening of plant growth promoting

Endophytic bacteria have been isolated from various parts of the *A. turkestanica* plant which is listed as an endemic species of flora of Uzbekistan. These endophytic bacteria have been found to have plant-enhancing properties used in sustainable agriculture.

The ability of endophytic bacterial strains to break down phosphate salts was studied in the Pikovskaya agar medium ($C_6H_{12}O_6$ 10.0 g; $Ca_3(PO_4)_2$ 5.0 g; yeast extract 0.5 g; $(NH_4)_2SO_4$ 0.5 g; KCl

0.2 g; NaCl 0.2 g; $MgSO_4$ 0.1 g; $MnSO_4 \cdot H_2O$, 0.002 g; $FeSO_4 \cdot 7H_2O$, 0.002 g agar 15 g; distilled water 1 L). Bacterial strains were stored for ten days at a temperature of 28 ± 2 °C after planting in agar medium. Bacterial colonies were monitored during incubation. Bacteria break down phosphate compounds by producing organic acids into the medium. The yellow color formed around the bacterial colony indicates that the phosphate compounds are broken down (Ogale et al., 2018; Jain et al., 2017).

Endophytic bacteria were grown in King's B medium (Proteose peptone 20.0 g; K_2HPO_4 1.5 g; $MgSO_4 \cdot 7H_2O$ 1.5 g; distilled water 1 L) in an incubator at a temperature of 28 °C in a shaker with a rotational speed of 170 times per minute for 72 h. The bacterial fluid was then taken in 1.5 mL small-volume solutions and centrifuged at 6000 rpm for ten minutes. Take 1 mL of the liquid from the supernatant portion and add 2% $FeCl_3$ from the aqueous solution. The appearance of orange and reddish-brown color in the solutions indicates that endophytic bacteria have synthesized siderophores.

Ammonia-synthesizing properties of bacterial strains were screened in a peptone medium. Bacterial cells were inoculated with peptone water without control. To assess the ammonia production of bacterial strains, 1 mL of Nessler reagent was added to a liquid medium with peptone. A change in color to light yellow indicates that minimal amounts of ammonia have been produced. A change from orange to brown indicates that the maximum amount of ammonia has been produced (ALKahtani et al., 2020; George et al., 2020).

The potential for IAA synthesis of bacterial strains was determined in the tryptophan Nutrient Broth medium. Bacterial strains were grown in a shaker incubator for 7 days at a temperature of 28 ± 2 °C after inoculation in the medium. At the end of the storage period, the bacterial fluid in which the strains were grown was centrifuged for ten to fifteen minutes at a speed of 6000 rpm. In the next step, the supernatant was isolated and 1 drop of Salkowski reagent (35 mL of 35% $HClO_4$ and 1 mL of 0.5 M $FeCl_3 \cdot 6H_2O$) was added to it. After the reagent was added to the supernatant, it was kept in a dark place for thirty minutes. The gradual development of cherry red in the supernatant indicated that bacterial strains synthesized indole acetic acid (Ogale et al., 2018).

Nitrogen fixation properties of endophytic bacteria were determined in Jensen's medium (Sucrose 20 g; K_2HPO_4 1.0 g; $MgSO_4 \cdot 7H_2O$ 0.5 g; NaCl 0.5 g; $FeSO_4 \cdot 7H_2O$ 0.1 g; Na_2MoO_4 0.005 g; $CaCO_3$ 2.0 g; Agar 15.0 g). Bacteria cultured in Jensen's medium were incubated for 7 days at 28 ± 2 °C. The appearance of endophytic bacterial colonies in Petri dishes during incubation indicates that bacterial cultures have the property of nitrogen fixation.

Endophyte bacterial strains were cultured in Nutrient Broth medium to test the activity of ACC deaminase. The cultured bacterial cultures were centrifuged at 8000 rpm for 8 min. Bacterial sediment was isolated and washed with sterilized 0.1 M Tris-HCl (pH 7.5). Bacterial cells were transplanted into a medium of minimal saline agar. A medium with the addition of 0.2 percent ammonium sulfate was used for positive control. Non-nitrogen source DF medium was used as a negative control. The cultures were incubated for 36 h at 28 ± 2 °C. The occurrence of endophytic bacterial colonies in DF minimal saline medium was evaluated as a positive control (Sánchez-Cruz et al., 2019; Ngoma et al., 2013; Mintoo et al., 2019).

To determine the amylolytic activity of endophytic bacteria, starch agar was incubated for 48 h at 28 ± 2 °C. At the end of the incubation period of bacteria, a solution of potassium iodide was poured into Petri dishes and soon drained. In the presence of amylase activity in endophytic bacterial strains, a colorless transparent zone is formed around the bacterial colonies (Ntabo et al., 2018; Malleswari and Bagyanarayana, 2013).

The protease activity of endophytic bacteria was determined in sterile Skim Milk Agar medium (Casein 5.0 g; glucose 1.0 g; yeast

extract 2.5 g; Agar 15.0 g; distilled water 1 L, added 7% skim milk). Bacterial strains were stored for two days at a temperature of $28 \pm 2 \text{ }^\circ\text{C}$ after inoculation on the medium. Degradation of proteolytic activity around bacterial colonies after incubation was detected. A transparent zone around the bacterial colonies indicated that the substrate was broken down by proteases (Ntabo et al., 2018).

Lipase and lecithinase activity of bacterial strains was determined by growing in Egg Yolk Agar. Bacterial strains were inoculated and stored at a temperature of $30 \pm 2 \text{ }^\circ\text{C}$ for two to three days. The formation of light zones around bacterial colonies after incubation indicates that lecithinase is synthesized by bacteria. The lipase activity of endophytic bacteria was determined as follows. A saturated solution of copper (II) sulfate was poured into the petri dishes and kept for 10–15 min and the excess reagent was discarded (Malleswari and Bagyanarayana, 2013). A greenish-blue color is formed around bacterial colonies that have lipase activity. This condition is considered a positive result.

A drop of endophytic bacterial culture grown in a nutrient broth medium was instilled into a glass plate and 3% hydrogen peroxide (H_2O_2) was added. Cultures with catalase activity form bubbles.

Whatman №1 filter paper was cut into 10 mm circles and immersed in a freshly prepared 1% solution of tetramethyl parphenyldiamine dihydroxychloride ($\text{C}_{10}\text{H}_{18}\text{Cl}_2\text{N}_2$) and dried. A small amount of endophytic bacterial colony was then instilled into the filter paper with a sterile Petri loop. The formation of a strong dark purple color in five to ten seconds was accepted as a positive reaction of the oxidase test. Whatman №1 filter paper color change was taken as a negative reaction.

Urea is a product of the decarboxylation of amino acids. Hydrolysis of urea results in the formation of ammonia and carbon dioxide. The formation of ammonia in the process alkalizes the medium and a change in pH (6.8–8.1) turns the light yellow to pink. When bacteria have urease activity, the medium turns light yellow to pink within 24 h. (Patel et al., 2016). Bacteria were cultured in Christensen medium to determine the urease enzyme activity of endophytic bacteria. Urea decomposes at high temperatures that are not resistant to heat. Therefore, a 40% solution of urea under aseptic conditions was prepared. 2 g urea was filtered in 5 mL of distilled water in a 0.22 nm millipore paper filter. After sterilization, the medium was cooled to $45\text{--}50^\circ$ and 5 mL sterile 40% urea solution was added under aseptic conditions and poured into Petri dishes. Bacterial strains were transplanted into medium and stored

in a thermostat at $28 \pm 2 \text{ }^\circ\text{C}$ for 24 h. When bacterial strains have urease activity the urea in the medium is broken down. The ammonia formed was determined by changing the color of the medium from light yellow to pinkish red.

The property of gelatin hydrolysis of bacterial isolates was determined in Nutrient gelatin (Nutrient agar 23 g/L, gelatin 8 g/L) agar medium. Bacterial strains were grown for three to seven days at a temperature of $30 \pm 2 \text{ }^\circ\text{C}$ in a gelatin agar medium. After incubation, bacterial isolates were poured into saturated Petri dishes from a saturated solution of ammonium sulfate $(\text{NH}_4)_2\text{SO}_4$ and kept for 8–10 min, then discarded. Ammonium sulfate precipitates gelatin that has not been hydrolyzed by bacterial isolates and zones are formed around the bacterial colonies (Gamalero et al., 2020).

3. Results

3.1. Morphological properties of bacterial strains

Twelve strains of bacteria were isolated from the medicinal plant *A. turkestanica* (root – 7, stem – 3, leaf – 3). Endophytic bacteria are white, yellow, whitish, yellowish in color and consist of flat and ascending colonies 1–2 mm in size. The morphological and microscopic appearance of endophytic bacteria is shown in Fig. 2, the morphological characteristics in Table 1.

3.2. Biochemical and plant growth promoting properties of endophytic bacteria

The ability to ferment carbohydrates and enzymatic activities (amylase, lipase, lecithinase, caseinase, protease, gelatinase, urease, catalase, oxidase) and plant growth promoting traits (phosphate solubility property, ACC production, siderophore production, indole acetic acid production) were studied. A citrate test is used to determine the ability of microorganisms to use citrate as a carbon source. *Pseudomonas kilonensis*, *Bacillus amyloliquefaciens* ssp. *bacterial strains of plantarum*, *Pseudomonas putida*, *Pseudomonas graminis*, *Siccibacter colletis*, *Pseudomonas chlororaphis* showed positive results in citrate use test (Table 2 and Fig. 3).

The following results were obtained when studying the carbohydrate fermentation properties of endophytic bacteria. Bacterial endophytes *P. kilonensis*, *B. subtilis* ssp., *B. amyloliquefaciens* ssp. *plantarum* performed only glucose fermentation. *Bacillus amyloli-*

Code	Identity according to MALDI-TOF MS	Typical images of the colonies	Microscopic appearance	Code	Identity according to MALDI-TOF MS	Typical images of the colonies	Microscopic appearance	Code	Identity according to MALDI-TOF MS	Typical images of the colonies	Microscopic appearance
M1	<i>Pseudomonas kilonensis</i>			M2	<i>Bacillus amyloliquefaciens</i> ssp. <i>plantarum</i>			M3	<i>Pseudomonas putida</i>		
M4	<i>Bacillus subtilis</i> ssp.			M5	<i>Pseudomonas graminis</i>			M6	<i>Bacillus amyloliquefaciens</i> ssp. <i>Plantarium</i>		
M7	<i>Siccibacter colletis</i>			M9	<i>Bacillus amyloliquefaciens</i>			M10	<i>Bacillus subtilis</i> ssp.		
M11	<i>Bacillus mojavensis</i>			M12	<i>Pseudomonas chlororaphis</i>			M13	<i>Bacillus amyloliquefaciens</i> ssp. <i>plantarium</i>		

Fig. 2. Macroscopic and microscopic view of endophytic bacterial strains.

Table 1
Description of morphological characteristics of bacterial isolate colonies isolated from the medicinal plant *A.turkestanica* (Rgl.) Brig (*Lamiaceae*).

Isolates	Identity according to MALDI-TOF MS	Colour	Texture	Margins of colony	Degree of growth	Nature	Gram staining	Size
M1	<i>Pseudomonas kilonensis</i>	yellowish	raised	undulate	profuse	creamy	-ve	bacilli
M2	<i>Bacillus amyloliquefaciens</i> ssp. plantarum	yellow	flat	undulate	profuse	creamy	-ve	bacilli
M3	<i>Pseudomonas putida</i>	yellowish	flat	undulate	profuse	creamy	-ve	bacilli
M4	<i>Bacillus subtilis</i> ssp.	white	flat	entire	profuse	discrete	-ve	bacilli
M5	<i>Pseudomonas graminis</i>	yellowish	flat	entire	profuse	creamy	-ve	bacilli
M6	<i>Bacillus amyloliquefaciens</i> ssp. plantarum	whitish	flat	entire	profuse	discrete	-ve	bacilli
M7	<i>Siccibacter colletis</i>	yellowish	raised	entire	profuse	creamy	+ve	bacilli
M9	<i>Bacillus amyloliquefaciens</i>	white	flat	entire	scanty	discrete	-ve	bacilli
M10	<i>Bacillus subtilis</i> ssp.	whitish	raised	entire	scanty	discrete	-ve	bacilli
M11	<i>Bacillus mojavenis</i>	yellowish	flat	entire	profuse	creamy	-ve	bacilli
M12	<i>Pseudomonas chlororaphis</i>	whitish	flat	undulate	profuse	creamy	-ve	bacilli
M13	<i>Bacillus amyloliquefaciens</i> ssp. plantarum	yellowish	flat	entire	scanty	discrete	-ve	bacilli

+ Positive; -negative.

Table 2
Features of carbohydrate fermentation of bacterial strains isolated from the plant *A. turkestanica*.

Isolates	Identity according to MALDI-TOF MS	Plant parts	Results (slant/butt)	Interpretation
M1	<i>Pseudomonas kilonensis</i>	root	red and yellow	Glucose fermentation and peptone catabolism
M2	<i>Bacillus amyloliquefaciens</i> ssp. plantarum	root	yellow and yellow	
M3	<i>Pseudomonas putida</i>	stem	yellow and yellow	
				Fermentation of glucose, lactose and sucrose
M4	<i>Bacillus subtilis</i> ssp.	leaf	red and yellow	Glucose fermentation and peptone catabolism
M5	<i>Pseudomonas graminis</i>	root	yellow and yellow	
M6	<i>Bacillus amyloliquefaciens</i> ssp. plantarum	stem	red and yellow	Glucose fermentation and peptone catabolism
M7	<i>Siccibacter colletis</i>	root	yellow and yellow	
M9	<i>Bacillus amyloliquefaciens</i>	leaf	yellow and yellow	Fermentation of glucose, lactose and sucrose
M10	<i>Bacillus subtilis</i> ssp.	stem	red and yellow	Glucose fermentation and peptone catabolism
M11	<i>Bacillus mojavenis</i>	leaf	yellow and yellow	
M12	<i>Pseudomonas chlororaphis</i>	root	yellow and yellow	Fermentation of glucose, lactose and sucrose
M13	<i>Bacillus amyloliquefaciens</i> ssp. plantarum	root	yellow and yellow	

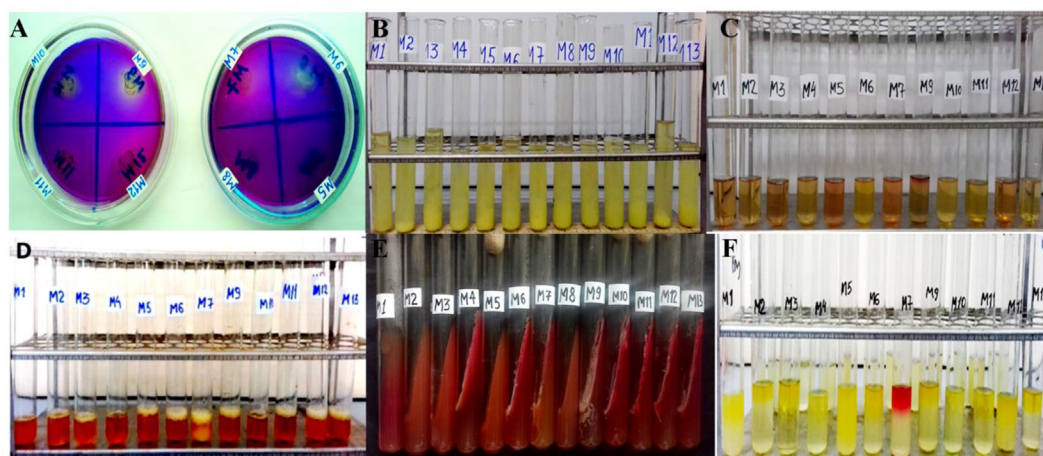


Fig. 3. Plant Growth promoting properties of the bacterial strains. A – Detection of phosphate solubilisation. B – Detection of ammonia production. C – Detection of IAA production. D – Detection of siderophore production. E – Detection of carbohydrate fermentation. F Detection of methyl red test.

uefaciens ssp. plantarum, *P. putida*, *P. graminis*, *S. colletis*, *B. amyloliquefaciens*, *B.subtilis* ssp., *B. mojavenis*, *P. chlororaphis*, *B.amyloliquefaciens* ssp. plantarum endophytic bacterial isolates have been found to carry out the fermentation of glucose, lactose and sucrose (Table 2).

The acid production potential of microorganisms during glucose fermentation is determined by methyl red test screening. Some endophytic bacteria synthesize the final products of glucose when

they use glucose. The results showed a positive result of *S. colletis* bacterial endophyte. Other bacterial isolates presented a negative result. The following results were obtained when the amyolytic activity of endophytic bacteria was examined. Bacterial endophytes *P.putida*, *B. subtilis* ssp., *P. graminis*, *B. amyloliquefaciens* ssp. plantarum, *S. colletis*, *B. amyloliquefaciens*, *B. subtilis* ssp., *B. mojavenis* and *P. chlororaphis* were found to have high amyolytic activity. The results obtained showed high protease (caseinase)

Table 3
Screening of enzymatic and biochemical properties of endophytic bacterial strains.

Biochemical tests	Bacterial endophytes											
	<i>Pseudomonas kilonensis</i>	<i>Bacillus amyloliquefaciens</i> ssp. plantarum	<i>Pseudomonas putida</i>	<i>Bacillus subtilis</i> ssp.	<i>Pseudomonas graminis</i>	<i>Bacillus amyloliquefaciens</i> ssp. plantarum	<i>Siccobacter colletis</i>	<i>Bacillus amyloliquefaciens</i> ssp. plantarum	<i>Bacillus subtilis</i> ssp.	<i>Bacillus mojavensis</i>	<i>Pseudomonas chlororaphis</i>	<i>Bacillus amyloliquefaciens</i> ssp. plantarum
<i>Test for enteric bacteria</i>												
Methyl red test	+	+	+	+	+	+	+	+	+	+	+	+
Citrate utilization	+	+	+	+	+	+	+++	+	+	+	+	+
<i>Enzyme assay</i>												
Oxidase	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+	+
Amylase	+	+	+	+	+	+	+	+	+	+	+	+
Lecithinase	+	+	+	+	+	+	+	+	+	+	+	+
Lipase	+	+	+	+	+	+	+	+	+	+	+	+
Protease	+	+	+	+	+	+	+	+	+	+	+	+
caseinase	+	+	+	+	+	+	+	+	+	+	+	+
urease	+	+	+	+	+	+	+	+	+	+	+	+
<i>PCPB properties</i>												
Phosphate solubilization	+	+	+	+	+	+	+	+	+	+	+	+
Siderophore production	+	+	+	+	+	+	+	+	+	+	+	+
ACC deaminase production	+	+	+	+	+	+	+	+	+	+	+	+
Ammonia production	+	+	+	+	+	+	+	+	+	+	+	+
IAA production	+	+	+	+	+	+	+	+	+	+	+	+
Nitrogen fixation	+	+	+	+	+	+	+	+	+	+	+	+
Gelatin hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+

activity of *B. subtilis* ssp., *B. amyloliquefaciens*, *B. subtilis* ssp. and *B. mojavensis* bacterial endophytes. Enzymatic screening revealed *B. subtilis*, *B. amyloliquefaciens*, *B. mojavensis* strains with lipase and lecithinase activity. (Table 3 and Fig. 4).

All bacterial endophytes of the medicinal plant *A. turkestanica* showed a positive oxidase test result. The results of the scientific research showed that the bacterial endophytes *P. kilonensis*, *B. amyloliquefaciens* ssp. plantarum, *B. mojavensis* and *P. chlororaphis* exhibit urease activity. Gelatin hydrolysis is a two-step process. The enzyme gelatinase hydrolyzes gelatin and results in the formation of polypeptides. Polypeptides are converted into amino acids and used by the cell during metabolism. When the properties of gelatin hydrolysis of endophytic bacteria were studied *B. amyloliquefaciens* ssp. plantarum, *B. amyloliquefaciens*, *B. subtilis* ssp., *B. mojavensis* and *B. amyloliquefaciens* ssp. plantarum gave a positive result on gelatin hydrolysis (Table 3).

Among these bacterial endophytes yellow zones were formed in medium *B. amyloliquefaciens* ssp. plantarum, *S. colletis*, *B. amyloliquefaciens*, *B. subtilis* ssp., and *B. mojavensis*. This result confirmed that the above five bacterial endophytes have phosphate dissolving properties. Bacterial strains in the DF saline medium were found to synthesize the enzyme ACC deaminase. The results showed that the bacterial isolates *P. kilonensis*, *P. putida*, *S. colletis* and *P. chlororaphis* have significantly higher property of forming the enzyme ACC deaminase (Table 3 and Fig. 4).

The synthesis of IAA in endophytic bacterial strains was studied. *P. kilonensis*, *B. amyloliquefaciens* ssp. plantarum, *P. putida*, *P. graminis*, *S. colletis*, *B. amyloliquefaciens* and *P. chlororaphis* showed a positive result. When 2–3 drops of Salkovsky reagent were added to the culture fluid in which endophytic bacteria were grown, a cherry red color was formed in the culture fluid. The bacterial strains identified during our scientific study were found to have the property of assimilating free nitrogen in the air (Table 3).

4. Discussion

Endophytic bacteria and plant growth promoting bacteria play an important role in increasing the plant growth (Jaborova et al., 2020b; Jaborova et al., 2021; Egamberdieva et al., 2013; Jaborova et al., 2013; Egamberdieva and Jaborova, 2013), plant nutrition (Egamberdieva et al., 2017; Egamberdieva et al., 2018; Jaborova et al., 2020), and nodule formation of different plants (Jaborova et al., 2013; Jaborova and Davranov, 2015; Jaborova et al., 2018).

Scientific studies on isolated endophytic bacteria have yielded the following results. Three of the endophytic bacterial strains were found to synthesize indole three acetic acid. (Table 3). Regulation of hormonal processes in root, stem and leaf development is closely related to indole acetic acid (IAA). Indole acetic acid (IAA), synthesized by endophytic microorganisms, is actively involved in all stages of the plant development process. This bioactive compound regulates biological processes such as tissue and cell formation in mechanisms that take place at the cellular level. These physiological processes play an important role in the formation of xylem and root tissue of plants.

The indole three acetic acid synthesized by the studied bacterial strains has a positive effect on the root system of plants and ensures that the root penetrates deep into the soil layers. Indole triacetic acid (IAA) acts in two directions on plants. The first is involved in the rapid division and proliferation of cells. The second effect plays a smoother role in the specialization of cells. Bacteria in the rhizosphere have the property of synthesizing 80% indole acetic acid, and the use of such microorganisms in agricultural plants increases the chances of high yields in plants. IAA synthe-

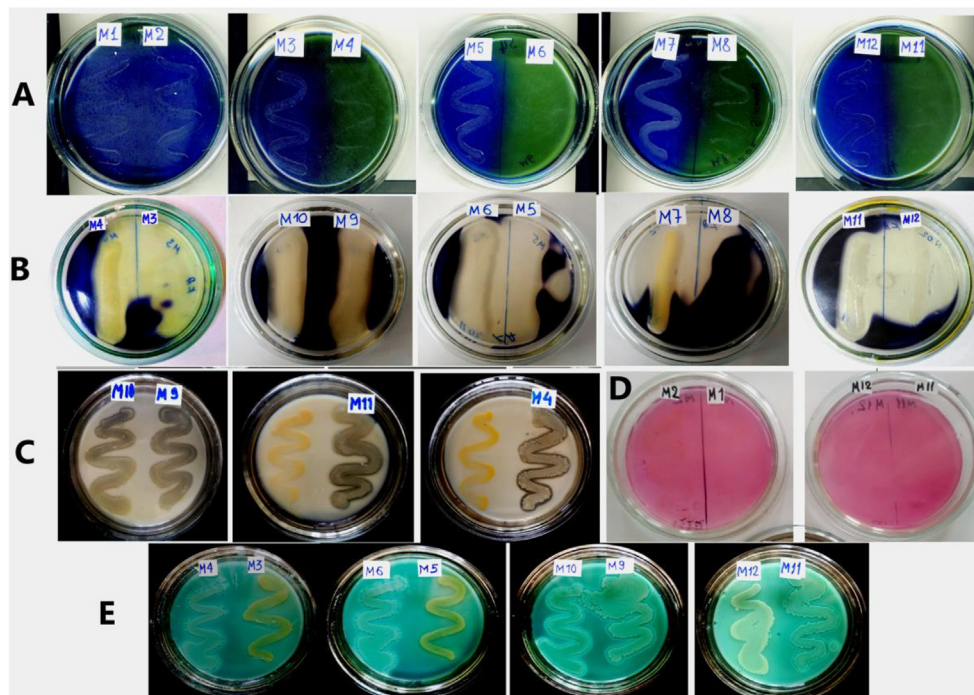


Fig. 4. Enzymatic activity of the bacterial strains. A – Detection of citrate utilization. B – Detection of amylase activity. C – Detection of proteolytic activity. D – Detection of urease activity. E – Detection of lecithinase and lipase activities.

sized by rhizobacteria affects the root system of plants. Plants increase weight, the number of branches on the branches, and the surface area of the root in contact with the soil. In rhizosphere bacteria, IAA metabolism is mainly dependent on L-tryptophan-dependent and independent pathways. Most rhizospheric bacteria have chosen the L-tryptophan pathway as the basis for IAA synthesis. Most root rhizosphere bacteria use L-tryptophan, which is secreted from root exudate, a precursor to IAA production (Ogale et al., 2018; Jabbarova et al., 2020c).

Rhizosphere bacteria have the ability to dissolve complex phosphate compounds and mineralize organic phosphate in the soil. For example, these bacteria convert organic phosphate into an inorganic form that plants can assimilate. Endophyte bacterial strains break down phosphate compounds by forming organic acids. The phosphate compounds present in the soil are not dispersed in a form suitable for plant nutrition. The endophytic bacterial species that colonize the plant body play an important role in this process. Endophytic bacterial strains break down phosphate salts in the soil through the production of organic acids to form nutrients for the plant. That is, in the form of insoluble, sedimentary, plant roots cannot directly absorb this mineral compound. Endophytic microorganisms that colonize the plant body break down these insoluble phosphate compounds by producing organic acids into a form that the plant can assimilate. These endophytic microorganisms, which make up the plant microbiome, exhibit beneficial properties for their host plant. Among phosphorus-soluble microorganisms in the soil, the proportion of endophytic bacteria or rhizobacteria (PSB and PSR) and the phosphorus dissolution potential is 1–50%, while in phosphorus-soluble fungi it is 0.1–0.5%. Effective phosphate-soluble bacteria for plants include *Pseudomonas*, *Bacillus*. Phosphate-soluble bacteria dissolve inorganic forms of phosphate through organic acids, phosphate-bound cations or by lowering the pH of the rhizosphere (proton formation – with bicarbonate release). In our study six bacterial endophytes (*B. amyloliquefaciens* ssp. *plantarum*, *S. colletis*, *B. amyloliquefaciens*, *B. subtilis* ssp., *B. mojavensis*) were found to have the property of

dissolving phosphorus compounds in the soil (Table 3). Therefore, the solubility and mineralization of phosphate in the soil may be present in the same bacterial strains (Ogale et al., 2018). Similar investigations indicated that Li et al. (2018) reported endophytic bacteria with the same phosphate solubilization trait.

Endophytic bacteria prevent the growth of various pathogenic fungi in the rhizosphere by producing siderophores that bind to Fe^{3+} ions around the plant roots where they are located. It has been observed that endophytic bacteria belonging to the genus *Pseudomonas*, which form siderophores, destroy various pathogens found in the soil. Experiments have shown that wild-type siderophore-producing strains are more effective in preventing diseases caused by various pathogenic fungi in the rhizosphere environment than non-siderophore-producing mutant strains.

The word siderophore means iron carrier. This compound is produced by endophytic bacteria that colonize the plant body in the rhizosphere (Cornelis, 2010; Ogale et al., 2018; Firdous et al., 2019). Endophyte bacteria increase the movement of micronutrients from the soil to the root, which plants assimilate through the roots through siderophores synthesized by them. There are species such as *Bacillus*, *Rhizobium*, *Pseudomonas*, *Agrobacterium*, *E. coli*, endophytic bacteria that have the ability to synthesize such siderophores. All of the endophytic bacteria studied in our scientific study were found to synthesize siderophores. *Bacillus* sp., which has a similar property in scientific studies and *Pseudomonas* sp endophytic bacteria were detected.

The presence of atmospheric nitrogen uptake by bacteria is one of the important factors in the process of plant nutrition. Nitrogen assimilated by bacterial strains plays an important role in plant life activities and nutrition processes. Three main routes of nitrogen-fixing endophytic bacteria entering the root are described: 1) through root holes (caused by mechanical damage) and randomly emerging roots; 2) through the root hairs; 3) through undamaged epidermal cells [Cocking, 2003]. We know that there is enough nitrogen in the air, but this nitrogen cannot be absorbed by plants. Plants assimilate nitrogen (NH_4^{+}) and NO_3^{-} . The absorption of

NO_3^- occurs with the flow of protons, the absorption of NH_4^+ occurs with the release of protons. According to this process, it leads to saturation of the rhizosphere with hydroxides and acids and affects rhizosphere processes (Dekas et al., 2009). When living organisms die, organic matter is consumed by soil heterotrophs and the decomposition process takes place. In this process, nitrogen in the dead body is converted by microorganisms into a form that is important for plants. The process of denitrification of nitrite and nitrate nitrogen compounds is carried out by endophytic bacteria belonging to the genus *Pseudomonas*, *Bacillus*. Twelve endophytic bacterial strains isolated from the medicinal plant *A. turkestanica* were found to have ammonia-synthesizing properties (Li et al., 2018).

5. Conclusion

The results of our scientific research show that the medicinal plant *Ajuga turkestanica* which lives in a high mountainous region where naturally rocky and constant winds blow, is a microbiome inhabited by endophytic bacteria of different generations. Bacterial strains isolated from the medicinal plant *A. turkestanica*, which is the object of our study, were found to be dominated by *Bacillus*, *Pseudomonas*, *Siccibacter* genera. It has been found that these bacterial generations have many properties that enhance the growth of cultivated plants used in the development of sustainable agriculture. Compounds that stimulate and enhance plant growth synthesized by these endophytic bacteria, whose properties have been studied, increase soil fertility and create normal conditions for the host plant. Due to the fact that endophytic bacteria isolated from the plant *A. turkestanica* have the property of enhancing plant growth, they can be used to produce bioinoculants individually or in consortium. However, since studies on endophytic bacteria have been conducted in vitro, it is important to apply them to field conditions to prove their beneficial properties.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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