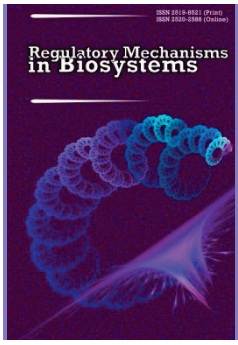

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Nitrogen-fixing, phosphate-potassium-mobilizing ability of *Rahnella* bacteria isolated from wheat roots

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As the number of people on earth increases, so does the need for food. Providing the population with environmentally friendly agricultural food is one of the urgent problems of our time. Currently, the main direction of modern organic farming is the use of biofertilizers. Bacterial preparations are capable of influencing the physiological processes of plants in small quantities, leading to increase in plant productivity. The objective of this work was to study rhizobacteria associated with wheat roots. For this purpose, we took more than 100 isolates of rhizobacteria from the rhizosphere and root surface of wheat plants grown in irrigated fields of Tashkent, Syrdarya, Andijan, Kashkadarya regions. Rhizobacteria were grown on nutrient media of Döbereiner, Ashby, Pikovsky, and Zack, and 25 isolates of associative rhizobacteria were selected based on the characteristics of absorption of molecular nitrogen, mobilization of phosphorus and potassium. They actively dissolved $Ca_3(PO_4)_2$ and $KAlSiO_4$ for 3 days. They were found to produce organic acids. In organic farming, nitrogen-fixing, phosphorus- and potassium-mobilizing rhizobacteria are of great practical importance, while our experiments on obtaining biological products are considered as an environmentally friendly and cost-effective way to increase crop yields. From the surface of wheat roots grown in different zones of Uzbekistan, when screening for nitrogen fixation, we selected 3 isolates with acetylene reductase activity of 79–91 nmol C_2H_4/flacon/24h. We determined that bacteria completely mobilized phosphate, forming 100% acid when grown in a medium containing $Ca_3(PO_4)_2$ for 5 days. The ability of the bacteria to mobilize potassium was studied on a nutrient $KAlSiO_4$-containing medium. The bacteria were observed to mobilize potassium, forming 90–100% acid within 15 days. Based on the study of the 16S rRNA gene of bacteria, we identified rhizobacteria UT3, UT4, and UT9 as *Rahnella aquatilis*.

Keywords: rhizobacteria; *Rahnella*; mobilization; 16S rDNA.

Introduction

Currently, there are environmentally friendly technologies of biological farming, one of which is the use of bacterial biofertilizers (Stamenković et al., 2018). According to modern views, associative diazotrophs are microorganisms that form associations on the roots of non-legume plants (Lira et al., 2020). Associative microorganisms are able to increase the solubility of soil phosphates, produce physiologically active substances that inhibit the development of pathogenic microflora through the release of antibiotics, stimulate germination seeds, etc. (Alori et al., 2017; Elhaissoufi et al., 2022). Although phosphorus is contained in the soil in inorganic and organic forms in sufficient amounts, it is a limiting factor in plant growth, since the bulk of phosphorus is in a form inaccessible to absorption by plant roots (Bechtaoui et al., 2021; Mardamootoo et al., 2021). It is found in parent rocks more often in the form of fluorapatite $Ca_5F(PO_4)_3$, $[Ca_5(PO_4)_3F]$ hydroxylapatite $Ca_5OH(PO_4)_3$, iron phosphate $Fe_2(OH)_3PO_4$ and dihydroxoaluminum phosphate $Al(OH)_2PO_4$. When these primary phosphorus-containing minerals are destroyed, secondary mineral phosphorus compounds salts of orthophosphoric acid are formed (Ricardo et al., 2000; Marius et al., 2022). In acidic soils (i.e., soddy podzolic and red soils), $AlPO_4$ phosphates and $FePO_4$ oxides are formed as well as basic iron and aluminum salts, which are characterized by very low solubility and availability for plants (Bache, 2006). Moreover, in soils saturated with bases, there develop calcium phosphates, such as octocalcium phosphate $Ca_4H(PO_4)_3$, which are usually poorly soluble in water. Gradually, they dissolve under the influence of the organic acids from microorganisms living in soils. As a result, phosphorus becomes more available to plants than apatite and phosphates. In all soils, a small amount of water-soluble one-substituted calcium and magnesium phosphates and one- and two-substituted potassium, sodium, and ammonium phosphates, are present in small amounts. Some of these phosphates are quickly used by plants, and the rest are converted into insoluble phosphates upon interaction with Ca, Mg, Al, and Fe (Devau et al., 2011). Organic phosphorus compounds that accumulate in soils are hydrolyzed by the activity of alkaline or acid phosphatase of microorganisms (Baneras et al., 2010, Gandhi et al., 2012; Obidi et al., 2018). Research on the rhizosphere have shown that numerous bacteria inhabit the rhizosphere of plants, which consists of various genera of bacteria, in particular the genera *Rahnella*, *Pseudomonas*, *Bacillus*, *Enterobacter*, *Pantoea*, *Serratia*, *Frateuria*, *Achromobacter*, *Acidothiobacillus*, *Paenibacillus*, *Rhizobium*, *Agrobacterium*, *Azospirillum*, *Azotobacter* and *Burkholderia*. These bacteria mobilize both phosphate and potassium and also fix molecular nitrogen. Currently, the rhizobacteria of the *Rahnella* genus are being intensively studied. *Rahnella aquatilis* was first studied and described in 1976 by the

Pasteur Institute. *Rahnella* belongs to the Yersiniaceae family and is a Gram-negative, rod-shaped, fast-moving microaerophilic bacterium that is common in various environments (Adeolu, 1998), i.e. in soil, plant phyllosphere, water and food (Berge et al., 1991; Rhodes et al., 1998). There are currently 6 species of bacteria belonging to the *Rahnella* genus, namely *R. aquatilis*, *R. variigena*, *R. inusitata*, *R. bruchi*, *R. woolbedingensis* and *R. victoriana*. Over the recent years, studies have shown that bacteria of the *Rahnella* genus can be used to fight plant diseases or as a plant growth promoters. For example, *R. aquatilis* JZ-GX1 had released volatile organic compounds that inhibited the growth of the mycelium of *Colletotrichum gloeosporioides*, thus inhibiting the infection and spread of anthracnose in leaves (Kong et al., 2020). Thus, *R. strains* improve plant growth and development through various mechanisms such as nitrogen fixation, phytohormone production, phosphate solubilization, organic acid biosynthesis, and biocontrol. Studies by Li et al. (2019, 2021), showed that *R. strains* were widespread and adapted to diverse environments, which may be due to their resistance to acids, salts, antibiotics, and heavy metals. Thus, the introduction of effective microorganisms into the rhizosphere of plants can become one of the tools for increasing the availability of soil phosphorus, potassium, and nitrogen for plants. The use of these bacteria in agriculture leads to the restoration of soil fertility.

The objectives of this study were isolation of rhizospheric bacteria from the surface of wheat roots, their taxonomic classification and study of their activity in the assimilation of minerals such as nitrogen, phosphate and potassium.

Materials and methods

Isolation of local strains of rhizobacteria and screening of strains that actively fixed nitrogen and had phosphorus and potassium mobilization properties. We used roots of wheat seedlings cultivated in the fields of the Tashkent and Kashkadarya regions of the Republic of Uzbekistan. To isolate bacteria from the surface of the wheat roots, the roots were washed in tap water for 1 h, then washed in sterile distilled water and transplanted into 0.2% nitrogen-free Döbereiner nutrient medium. This media was incubated at 28 ºC for 10 days. Nitrogen-fixing rhizobacteria were isolated from the cultures grown in nitrogen-free nutrient medium. The medium contained (g/L): sodium malate – 0.50, KH_2PO_4 – 0.91, K_2HPO_4 – 1.74, NaCl – 0.50, $MgSO_4*7H_2O$ – 0.30, $CaCl_2*6H_2O$ – 0.10, $FeCl_3*6H_2O$ – 0.01, agar – 0.20, pH 7.0.

To these were added 1 mL/L each of a vitamins and a microelements mixture. The mixture of vitamins comprised (µ/mL: biotin 10, cyanocobalamin 2, riboflavin 200, and 100 of each thiamine, pyridoxine, pantothenate and nicotinic acid) (Shilyaeva, 1988). The mixture of microelements contained (g/L): H_3BO_3 – 0.50, $Na_2MoO_4*2H_2O$ – 0.50, $MnSO_4*H_2O$ – 0.30, KCl – 0.05, NaBr – 0.05, $ZnSO_4*7H_2O$ – 0.02, $Al_2(SO_4)_3*12H_2O$ – 0.03; the solutions were prepared in distilled water. Ashby's nitrogen-free nutrient growth medium (g/L): sucrose – 20.0, K_2HPO_4 – 0.2, $MgSO_4*7H_2O$ – 0.2, NaCl – 0.2, K_2SO_4 – 0.1, $CaCO_3$ – 5.0. To those media, we added 1 mL/L of the mixture of microelements (Smirnov, 1970).

Determination of nitrogen-fixing activity. Nitrogenase (nitrogen-fixing) activity was estimated in cultures grown in 10 mL flacon containing 4 mL of 0.2% agar free nitrogen Döbereiner medium and a gaseous phase of 2% oxygen using the acetylene-reductase activity (ARA) assay described by Hardy et al. (2022). After 3 days' growth at 28 ºC temperature, the vials were tightly sealed with rubber caps and acetylene was injected into the final concentration of 10% (v/v). After 1 day of incubation with acetylene, concentration of ethylene in the gaseous phase was measured on a gas chromatograph "Hewlett Packard-5890" (Minnesota, USA, 2005). The acetylene-reductase activity of the plants was expressed in nmoles C_2H_4/flacon/day.

Determining the mobilization of phosphorus and potassium in rhizobacteria. A preliminary determination of the mobilization of phosphorus and potassium by rhizobacteria was carried out on Muromsev media, which are composed as follows (g/L): glucose – 10, asparagine – 1, K_2SO_4 – 0.2, $MgSO_4$ – 0.2, corn extract – 0.02, agar – 20, pH 7.0 (Egorshina, 2011). The ability of rhizobacteria to mobilize phosphorus and potassium by forming acid was studied using the Pikovskaya and Zaka nutrient media with the addition of bromcresol violet indicator. Composition of the Pikovskaya nutrient medium (g/L) is as follows: $Ca_3(PO_4)_2$ – 5, glucose – 20, NaCl – 0.2, $MgSO_4$ – 0.1, $MnSO_4$ – 0.003, $FeSO_4$ – 0.004, bromcresol violet 0.04% – 0.1 mL, agar – 20, pH 6.8. The Zaka nutrient medium (g/L) is comprised of: sucrose – 20, $KAlSiO_4$ – 2, $CaCO_3$ – 2, $Ca_3(PO_4)_2$ – 1.5, $MgSO_4*7H_2O$ – 0.15, NaCl – 0.15, $MnSO_4$ – 0.003, $FeSO_4$ – 0.004, bromcresol violet 0.04% – 0.1 mL, agar – 20, pH 6.8 (Zvyagintsev, 1991).

Isolation of genomic DNA of rhizobacteria. Genomic DNA was isolated from rhizobacteria using a modified Marmur method (Serra, 2018). To accomplish this, the bacterial cultures grown at 37 ºC for 16 h in the Lauria-Bertani (LB, l) (1% Trypton, 0.5% yeast extract, and 1% NaCl) nutrient media were centrifuged at 13 000 × g for 10 min. This step was repeated, and the solution was vortexed by adding 800 µL of A1 solution (0.01 M EDTA, 0.15 M NaCl) buffer to the formed precipitate. Another 10 µL of E1 buffer (lysozyme solution) was added and mixed. Then, 175 µL of High-Salt RNAase were added with the following incubation at 37 ºC for 40 min. Then, they were incubated for 10 min and vortexed for 10 s at 65 ºC by adding 80 µL of A2 buffer (25% SDS). To this mixture, we added 250 µL of A3 buffer (5 M NaCl), which was then vortexed for 10 s. Then, 400 µL of chloroform/isoamyl alcohol was added. The solution was well mixed, centrifuged at 14 000 g for 15 min, and the supernatant portion was transferred to a new plastic tube. This chloroform–isoamyl phase was then repeated. Ninety µL of A4 buffer (pH 8.0) and 600 µL of isopropanol were added to the supernatant, stirred vigorously, and centrifuged at 14 000 g for 10 min. After removal of supernatant, the precipitate had been dried at the room temperature and then dissolved in 100 µL of TE buffer. The DNA samples were stored at +4 ºC for short-term use and at –20 ºC for long-term use. A 0.9% agarose gel was used to detect DNA samples.

Molecular analyses. The following universal oligonucleotide primers were used in PCR amplification: 27F (AGAGTTTGATCMTGGCTC AG) and 1492R (GGTTACCTTGTTACGACTT) (Reyes-Castillo, 2019). Samples of DNA isolated from the bacterial strains were PCR-amplified using a GenPak® PCR MasterMix kit (Isogene, Netherlands, 2021). The reaction was prepared in the total volume of 20 mL, consisting of 10 mL of the Dilution solution, containing Master Mix 8.2 mL of bidistilled water, 0.4 mL (27F and 1492R) primers, and 1 mL of the DNA samples. Then, PCR amplification optimization was conducted, with an initial denaturation at 94 °C for 3 min, followed by subsequent denaturation at 94 °C for 40 s, primer annealing at 55 °C for 40 s, elongation at 70 °C for 90 s, final elongation at 70 °C for 7 min, and the reaction was repeated in 35 cycles. Amplicones were detected by electrophoresis in a 2% agarose gel stained with etidium bromide.

Identification of rhizobacteria by 16S rRNA gene sequencing. Partial sequencing of the 16S rRNA gene was amplified by polymerase chain reaction primers using oligonucleotide 27F/1492R. Sequencing analysis was performed using an ABI 3100 capillary sequence analyzer (Applied Biosystems, Foster City, CA, USA). The FASTA sequence was analyzed and compared using BLASTN from a local tool from the National Center Biotechnology Information (NCBI) and RDP (database project on ribosomes).

The phylogenetic tree was created based on 16S rRNA gene of *R. aquatilis* bacterial strains. The optimal tree with the overall network length (equal to 0.19803035) is shown. In the bootstrap test (1000 replications) was shown next to the networks. In the phylogenetic tree, the length of the branches is given as a percentage, indicating the evolutionary distances of the genes. Evolutionary distances were calculated using the p-distance method. The analysis included 11 nucleotide sequences. Codon positions were 1 + 2 + 3 (unencoded). All positions containing gaps and missing information were removed. There were 733 positions in the final dataset. Evolutionary analyses were performed using MEGA 5 (Saitou & Nei, 1987; Tamura et al., 2011).

Results

Selection of nitrogen-fixing bacteria. From the surface of wheat roots grown in different zones of Uzbekistan, 25 isolates of associative rhizobacteria were isolated. To identify nitrogen-fixing bacteria among the isolates of rhizobacteria, they were cultivated on a nitrogen-free semi-liquid 0.2% Döbereiner agar medium. The bacteria were observed to grow from the surface of the nutrient medium towards a lower oxygen content due to aerotaxis. This feature of the isolates allowed us to consider tham microphilic nitrogen fixers (Fig. 1, Tables 1 and 2).

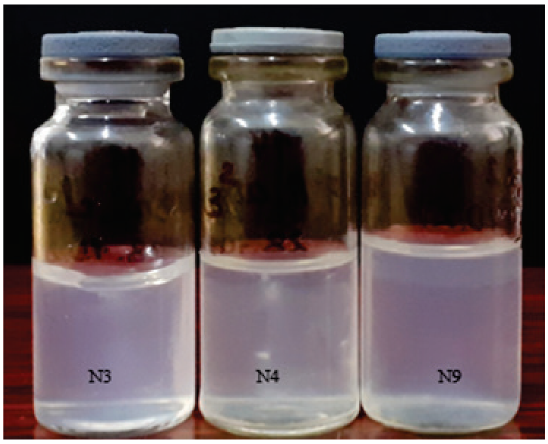

Fig. 1. Nitrogen-free semiliquid 0.2% agar: the growth of the N3, N4 and N9 isolates in the Döbereiner's culture medium according to the microphilic conditions

Table 1
Nitrogen-fixing activity of the isolates of rhizobacteria in nitrogen-free Döbereiner nutrient medium

Isolates of rhizobacteria	Nitrogen-fixing activity, nmol C_2H_4/flacon/24h
UT1	23
UT 2	29
UT 3	88
UT 4	91
UT 6	21
UT 8	42
UT 9	79
UT 10	21
UT 11	24
UT 14	31
UT 17	46
UT 18	39
UT 20	31
UT 21	25

Table 2
The formation of organic acids in the Pikovskaya nutrient medium of the UT3, UT4 and UT9 isolates (%)

Isolates of rhizobacteria	Days					
	1	3	5	7	9	15
UT3	80	97	100	100	100	100
UT4	90	97	100	100	100	100
UT9	90	100	100	100	100	100

Phosphorus and potassium mobilization by rhizobacteria. During the incubation of the UT3, UT4 and UT9 isolates for a day in wells on a Muromsev media containing $Ca_3(PO_4)_2$, phosphate was mobilized, forming a transparent 15–20 mm diameter circles (Fig. 2).

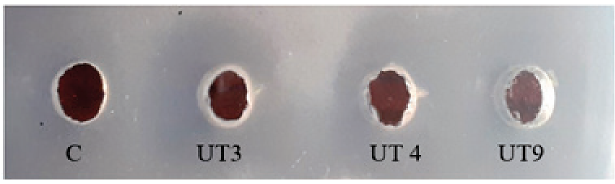

Fig. 2. $Ca_3(PO_4)_2$ in the nutrient medium was dissolved under the action of organic acids of the UT3, UT4, and UT9 isolates and transparent 15–20 mm in diameter circles formed around the cultures

The total organic acid formation of rhizobacteria in the Pikovskaya $Ca_3(PO_4)_2$-containing nutrient medium, was 85–90% on the 1st day of the study and 100% on the 5th day (Table 3, Fig. 3).

Table 3
The formation of organic acids by the UT3, UT4, UT9 isolates in the Pikovskaya nutrient medium

Isolates of rhizobacteria	Days, %					
	1	3	5	7	9	15
UT3	80	97	100	100	100	100
UT4	90	97	100	100	100	100
UT9	90	100	100	100	100	100

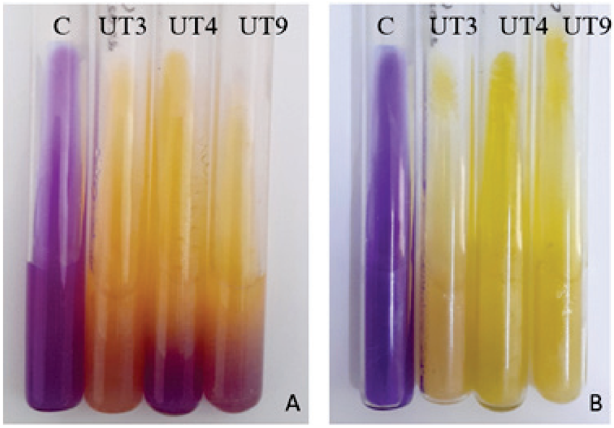

Fig. 3. Synthesis of organic acids of UT3, UT4 and UT9 isolates in the Pikovskaya nutrient media depending on time: *A* – 1-day culture, *B* – 5-day culture

It was found that cultures that mobilized phosphate were also able to mobilize potassium by forming acids in the Zaka nutrient medium, which contains $KAlSiO_4$. The yield of organic acids was 50–70% on the 1st day of the cultivation and 90–100% on the 15th day (Table 4, Fig. 4). It should be noted that during the mobilization of phosphorus and potassium, the pH of the medium was observed to decrease to 4.1–5.4, and the main change occurred during the second day of bacterial growth.

Table 4
Formation of organic acids by the UT3, UT4 and UT9 isolates of rhizobactera in the Zaka nutrient medium (%)

Isolates of rhizobacteria	Days					
	1	3	5	7	9	15
UT3	70	80	85	90	90	100
UT4	50	60	75	80	85	90
UT9	60	60	70	80	80	90

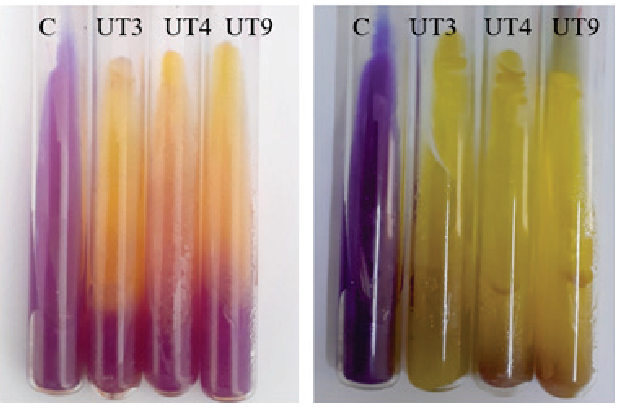

Fig. 4. Synthesis of organic acids of the UT3, UT4 and UT9 isolates in the Zaka nutrient medium depending on time: *a* – 1-day culture, *b* – 15-day culture

Taxonomy of the rhizobacteria based on morphology and the 16S rRNA gene sequence. The cells of the selected rhizobacteria were rod-shaped, 0.3–0.4×0.6–1.5 µm in size, mobile, Gram-negative. The strain colonies were circular, smooth, light-yellow, translucent and convex with an entire edge, 2.0–3.0 mm (Fig. 5).

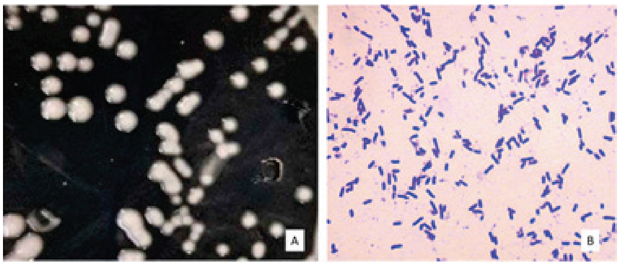

Fig. 5. Bacterial colonies (*A*) and rod-shaped cells of *R. aquatilis* UT3 (*B*)

Using the modified Marmur method, genomic DNA was isolated from the UT3, UT4, and UT9 bacteria. The concentration and purity of genomic DNA were determined. At a later stage, 0.9% agarose gel was used to check samples of 16S rRNA gene PCR products of bacterial genome DNA (Fig. 6).

Partial sequence of 16S rRNA gene isolated from agarose gel was determined using an ABI 3100 capillary sequencer model (Applied Biosystems, Foster City, CA, USA). Using DNA sequencing-large dye, terminator cycle sequencing-ready ABI was developed using universal primer pairs. The nucleotide sequences in the FASTA file were analyzed and compared using the NCBI (National Biotechnology Information Center) Local program BLASTN. The study of the 16S rRNA gene nucleotide sequence of the bacterial genes showed that the bacteria under study belonged to the Gammaproteobacteria class, the Enterobacterales order, the Yersiniaceae family, the *Rahnella* genus, and the *R. aquatilis* species. As a result of the BLAST analysis of the sequence of 16S rRNA nucleotides of the bacterial genes, the UT3, UT4, and UT9 strains were identified as *R. aquatilis* (Table 5).

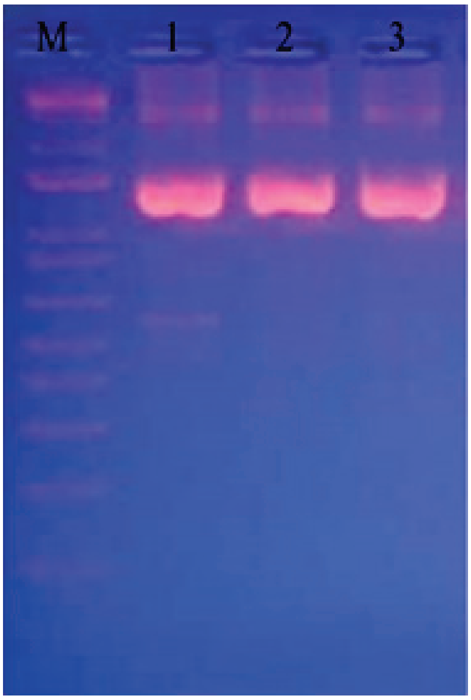

Fig. 6. Electrophoresis of 16S rRNA gene in PCR product samples of bacterial genome DNA in 0.9% agarose gel: *M* – marker, *1* – UT3, *2* – UT4, *3* – UT9; PCR products = 1400 bp

Table 5
Comparative BLAST analysis of homology of the nucleotide sequence of 16S rRNA genes of the local rhizobacteria with certain bacteria in the data bank of the National Biotechnological Information Center, GenBank (USA)

Data bank	Similar to database NCBI, %	Similar to database NCBI, %	Similar to database NCBI, %	Similar to database NCBI, %	Similar to database NCBI, %
NCBI GenBank	MN826572.1 *R. aquatilis* cqsV2	CP036490.1 *R. aquatilis* MEM40	MN192429.1 *R. aquatilis* SM015A	CP034483.1 *R. aquatilis* KM05	MF943238.1 *R. aquatilis* TES09B
UT3 similar %	98.89	98.89	98.89	98.89	98.89
NCBI GenBank	MN826597.1 *R. aquatilis* cqsR2-2	MN826570.1 *R. aquatilis* qsR5	MT256284.1 *R. aquatilis* KCOM 1451	CP036490.1 *R. aquatilis* MEM40	CP034483.1 *R. aquatilis* KM05
UT4, similar, %	99.03	99.03	99.03	99.03	99.03
NCBI GenBank	CP034482.1 *R. aquatilis* KM12	CP034481.1 *R. aquatilis* KM25	CP032296.1 *R. aquatilis* ZF7	MG966290.1 *R. aquatilis* PRE12	MF943242.1 *R. aquatilis* TES13A
UT9 similar %	98.90	98.90	98.90	98.90	98.90

In particular, the nucleotide sequence of the 16S rRNA gene of the *R. aquatilis* UT3 strain had 98.9% matching with analogous genes of *R. aquatilis* MN826572.1, CP036490.1, MN192429.1, CP034483.1, and MF943238.1 strains. The genes of the *R. aquatilis* UT4 strain were 99% similar to the nucleotide sequences of *R. aquatilis* MN826597.1, MN826570.1, MT256284.1, CP036490.1, CP034483.1 strains and the genes of *R. aquatilis* UT9 strain were 98% identical to the genes of *R. aquatilis* CP034482.1, CP034481.1, CP032296.1, MG966290.1, and MF943242.1 strains. The phylogenetic tree of the local *R. aquatilis* strains was created based on the nucleotide sequence of the studied *R. aquatilis* 16S rRNK gene. The local *R. aquatilis* strains formed a single cluster on the phylogenetic tree (Fig. 7), that is, the local *R. aquatilis* UT3, UT4, and UT9 bacteria belong to this cluster.

Discussion

The study of the acetylene reductase activity of isolates grown on a nitrogen-free Döbereiner medium for 3 days revealed that 14 isolates, which we named as UT1-UT14 (UT – Uzbekistan, Tashkent), had the property of nitrogen fixation. Of them, isolates UT3, UT4 and UT9 showed high acetylene-reducing activity (79–91 nmol C_2H_4/flacon/24h). Berge et al. (1991) reported the *R. aquatilis* CF1, CF3, CF4, TR01, TR02, and TR03 rhizospheric strains exerted acetylene reductase activity measuring 60–306 nmol C_2H_4/tube (Berge et al., 1991). It should be noted that those strains of rhizobacteria also showed growth on the Ashby's nitrogen-free nutrient medium, which means that their nitrogen-fixing properties have been confirmed once again. Based on the results, UT3, UT4 and UT9 isolates were selected for further studies.

The mechanism of dissolution of mineral phosphates and potassium by rhizospheric bacteria has been studied by several researchers. Kim et al. (1997) found that the mechanism of dissolution of inorganic phosphorus is the formation of organic acids and decrease in soil pH. In previous works, it was noted that the mobilization of phosphate and potassium occurs mainly due to the production of gluconic acid by bacteria. Aliyat et al. (2022). Tactek et al. (2017) observed that rhizobacteria exopolysaccharides mobilized P from igneous phosphate rocks, forming biofilms from living cells and organic acids (Tactek et al., 2017).

In our studies, during the incubation of the UT3, UT4 and UT9 isolates for a day in wells on Muromsev media containing $Ca_3(PO_4)_2$, phosphate was mobilized, forming a transparent circle, measuring 15–20 mm in diameter. The ability of rhizobacteria to mobilize phosphate ($Ca_3(PO_4)_2$) and potassium ($KAlSiO_4$) by the formation of organic acids was studied dynamically in the Pikovskaya and Zaka nutrient media. The rhizobacteria were observed to mobilize phosphorus and potassium by the formation of acid for 15 days.

Kong et al. (2022) reported that the genome identity of the *Rahnella* sp. JZ-GX1 with *R. victoriana* strains was near 98%; and below 90% when comparing the strain with other species of *Rahnella*. Those data confirm that the *Rahnella* sp. JZ-GX1 belonged to *R. victoriana* (Kong et al., 2022).

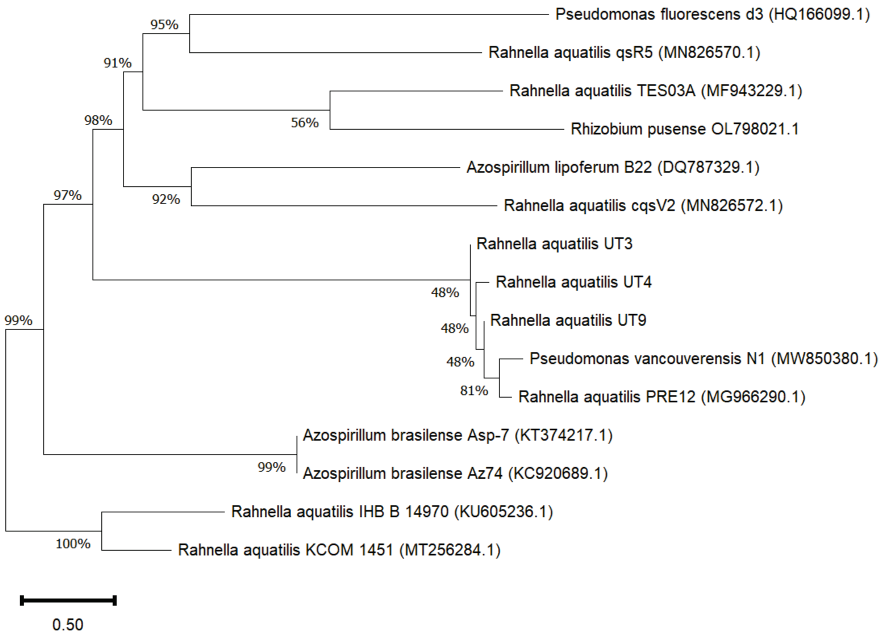

Fig. 7. Phylogenetic tree based on the 16S rRNA gene of the *R. aquatilis* UT3, *R. aquatilis* UT4 and *R. aquatilis* UT9 strains, *R. aquatilis* UT4 and *R. aquatilis* UT9 strains: the networked sample was obtained by the neighbor-joining method; GenBank sample numbers for applicable sequences are shown in parentheses

The networked sample was obtained by the neighbor-joining method. GenBank sample numbers for applicable sequences are shown in parentheses. The phylogenetic tree suggests that despite the fact that these bacteria were isolated from different soils and climatic conditions, the local *R. aquatilis* strains are closely related to each other in terms of evolutionary origin. It also shows that local *R. aquatilis* strains have a certain degree of similarity to the evolution of *Pseudomonas vancouverensis* N1 (MW850380.1) and *R. aquatilis* PRE12 (MG966290.1) strains.

In particular, the nucleotide sequence of the 16S rRNA gene of the *R. aquatilis* UT3 strain matched 98.89% with the analogous genes of *R. aquatilis* MN826572.1, CP036490.1, MN192429.1, CP034483.1, and MF943238.1 strains. The genes of the *R. aquatilis* UT4 strain were 99.0% identical to the nucleotide sequences of *R. aquatilis* MN826597.1, MN826570.1, MT256284.1, CP036490.1, CP034483.1 strains and the genes of *R. aquatilis* UT9 strain were 98.9% identical to the genes of *R. aquatilis* strains CP034482.1, CP034481.1, CP032296.1, MG966290.1, and MF943242.1. Therefore, all the data is included in the NCBI database (National Center for Biotechnology Information, 2021, www.ncbi.nlm.nih.gov).

Conclusions

Based on the fixation of molecular nitrogen, phosphorus and potassium mobilization, three active rhizobacteria of the wheat roots were selected. As a result of the implementation of the molecular-genetic taxonomy of the bacteria, those bacteria strains were identified to the species *R. aquatilis*. The results showed that those bacteria were genetically very close to other well known *R. aquatilis* strains and had similar abilities. The fixation of molecular nitrogen, with the ability to mobilize phosphate and potassium, demonstrated that the application of those bacteria as bio fertilizers has a great future potential. Practical application of *R. aquatilis* bacteria can promote increase in soil fertility, and the production of environmentally friendly yield of agricultural crops.

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