Short Communication

Physiological and biochemical characterization and genome analysis of *Rhodococcus qingshengii* strain 7B capable of crude oil degradation and plant stimulation

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A R T I C L E   I N F O

A B S T R A C T

Rhodococci are typical soil inhabitants which take part in remediation of soil polluted with hydrocarbons. In this paper, we describe a new strain, *Rhodococcus qingshengii* 7B, which is capable of growth and hydrocarbon degradation at 45°C and in the presence of up to 10% NaCl in the medium. The genome of the 7B strain consists of a 6,278,280 bp chromosome and two plasmids. The circular plasmid is 103,992 bp in length. The linear plasmid is 416,450 bp in length. Genome analysis revealed the genes of degradation of various hydrocarbons, resistance to salt stress and plant growth promoting activity. This strain is promising for use in remediation of oil-contaminated soils, because it has a pronounced ability to utilize crude oil, oil sludge and individual hydro-carbons in a wide temperature range. Over 15 days of the experiment, the strain utilized 51% of crude oil at 28°C and 24% at 45 °C.

1. Introduction

Rhodococci are versatile degraders of various resistant pollutants [1]. Among hydrocarbon-oxidizing bacteria, representatives of the genus *Rhodococcus* are distinguished by a diversity of the genomic organization and functional diversity. Their biotechnological importance is associated with metabolic and genetic flexibility, as well as with the resistance of these strains to various types of stress [2]. Indeed, rhodococci are Gram-positive bacteria capable of utilizing a wide range of organic compounds, including toxic ones [3,4].

One of the priority pollutants is crude oil that consists of more than 300 individual compounds. Some of the components of crude oil are highly toxic, non-volatile, and resistant to degradation. Self-remediation of soil ecosystems from pollution caused by crude oil takes on average 50 years. Intensification of bioremediation processes may occur due to the introduction of nutrients (biostimulation) or microbial preparations

(augmentation) [5,6]. The most common approach to biostimulation is soil enrichment with compounds containing nitrogen and phosphorus [7,8]. Bioaugmentation is essentially the supplementation of contaminated sites with additional gene pools [9]. A high cleaning effect is achieved with the use of bacterial strains characterized by a high ability to decompose crude oil and its individual components [10]. Successful bioaugmentation requires the use of bacterial strains that are resistant to suboptimal environmental conditions [11,12]. The need to include such strains into the biological preparations is determined by the fact that oil production is carried out under climatic conditions which are stressful for many micro- and macroorganisms. These conditions include low or high temperatures, high salinity or acidity of soils, sharp fluctuations in temperature during the day. Such conditions hamper the natural processes of self-remediation of the environment. To a large extent, the success of the elimination of oil pollution depends on the presence of microbial strains that are adapted to extreme habitat conditions and

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Table 1

Information about primers used in RT-qPCR.

Gene name	Primer name	Primer sequence	Amplicon size
16S rRNA	16s_rt_for	ACATGCAAGTCGAGCGGTAA	141
	16s_rt_rev	CCACCATGCGATAGGAGGTC	
alkB1	alkb7499_rt_for	GCAACTTGGAGAGACCGGAA	67
	alkb7499_rt_rev	TGAAAGTTGCCGATGACGGA	
alkB2	alkb1948_rt_for	CAGTGGCGTGACAAGAAGAG	165
	alkb1948_rt_rev	CGGGAGCAGGATGTAGACGA	
alkB3	alkb0788_rt_for	TATTTGCACCTCGGCCTGTT	149
	alkb0788_rt_rev	CACCAGCGGTAGTAGCGGTC	
alkB4	alkb2279_rt_for	TGGGTTTGCTGGTTCCTCTC	146
	alkb2279_rt_rev	GCTGTTACCGTCCTCGCC	
alkB5	alkb0490_rt_for	TTCCACTCAGCCCGTTCATC	136
	alkb0490_rt_rev	GGACTTTCGCCGTCTTCACC	
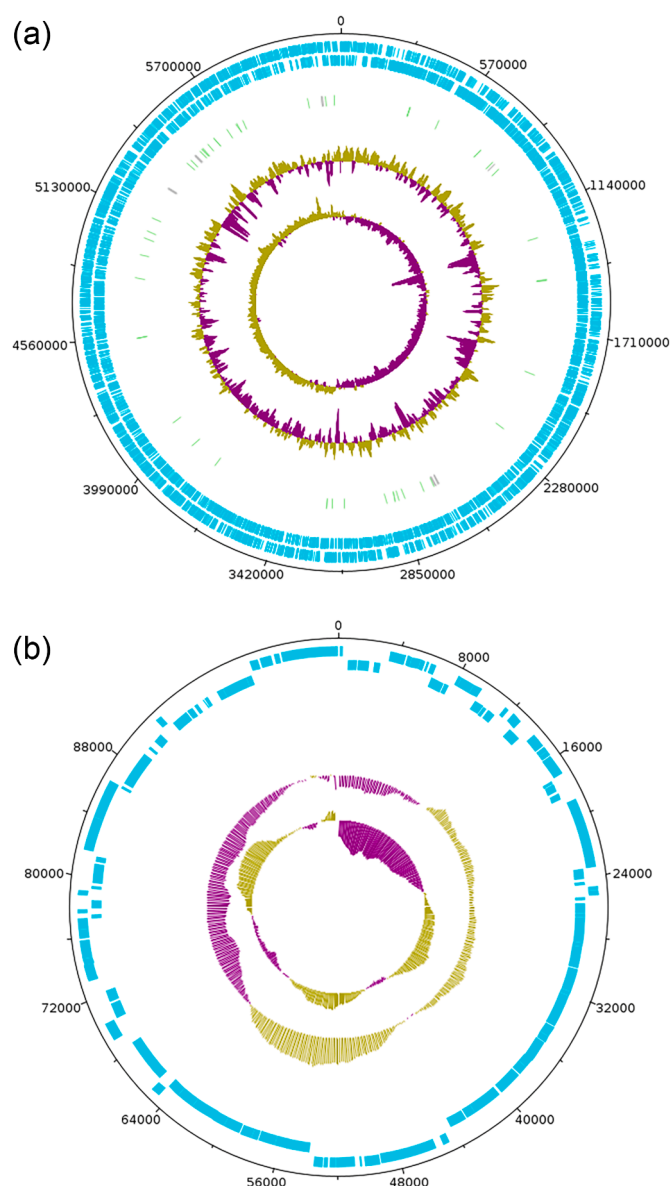
Fig. 1. Circular maps of R. qingshengii 7B chromosome (a) and plasmid pCP3 (b). The outer tracks represent the predicted CDSs of the forward and reverse strands. The green tracks represent RNAs. Two inner tracks display the GC plot and the GC skew.

capable of synthesizing surfactants, as well as possess a set of biodegradation genes [13–20].

Oil-oxidizing strain 7B has been isolated from the soil on the territory of the Saratov oil refinery. The strain has been tentatively identified as *Rhodococcus qingshengii* 7B based on sequence analysis of the 16S rRNA gene fragment. This strain has been found to be capable of utilizing caprolactam, phenol, benzoate, octane, nonane, decane, hexadecane, dodecane, undecane as growth substrates [21].

The aim of this work was to study the organization of the genome, as well as to assess the potential of using strain 7B in the field of environmental biotechnology.

2. Materials and methods

2.1. Bacterial strain and cultivation conditions

The strain *Rhodococcus qingshengii* 7B was deposited in the All-Russian Collection of Microorganisms (VKM) under the number Ac-2905D. As a nutritionally rich medium, we used the GRM broth [22], pH 6.5-7.5. As a minimal medium, we used the Evans mineral medium [23].

2.2. Determination of extent of crude oil degradation

As the only carbon source, we used crude oil at a concentration of 0.868 g/ml with the following admixtures: 0.06% water, 45 mg/ml salts, 0.008% mechanical impurities, and 1.42% sulfur. Oil sludge from Kuwait with total hydrocarbon content of 190 g/kg had the following composition: the sum of *n*-alkanes – 1.3%, medium-boiling fraction – 4.83%, high-boiling fraction – 5.67%, PAH – 0.0004%, 52.8% mechanical impurities.

The *R. qingshengii* strain 7B was cultivated in flasks containing 100 ml of the Evans medium and crude oil (20 g/L) for 28 days at 28°C. The residual content of oil hydrocarbons after cultivation of bacteria was measured by the infrared spectrometry in 7, 15 and 28 days of the experiment. Infrared spectrometry was employed to determine specific hydrocarbons by relative number of C–C and C–H bonds [24].

For experiments on the oil sludge degradation, the following model systems were used: 10 ml of cell suspension of strain 7B at a concentration of 5×10^8 CFU/ml were added to 1 kg of oil sludge (humidity 50-55%). The sludge was mixed daily. The experiment was carried out in 3 biological replicates.

After the cultivation, the remaining oil was extracted with carbon tetrachloride (1:1). The content of hydrocarbons in the extract was determined using an AN-2 petroleum product analyzer (Neftekhimavtomatika, Saint Petersburg, Russia). The sterile Evans medium supplemented with crude oil (20 g/L) was used as a control. The crude oil concentration in liquid samples was calculated using the formula:

$$X = \frac{C \times V_1 \times \eta}{V}, \ [mg/L],$$

where *C* is the concentration of crude oil in the eluate determined from the instrument's readings or the calibration dependence, mg/L; *V*i is the volume of the eluate, L; *Y* is the volume of the water sample, l; η is the degree of eluate dilution. In the absence of dilution, η = 1.

A degree of crude oil degradation was calculated in comparison with the control (blank experiment) using the formula:

$$D = \frac{X_k - X_i}{X_k} \times 100\%$$

where D is the degradation degree, %; X_k is the concentration of hydrocarbons in the control (liquid Evans medium with crude oil); X_i is the concentration of oil hydrocarbons in the samples with microorganisms
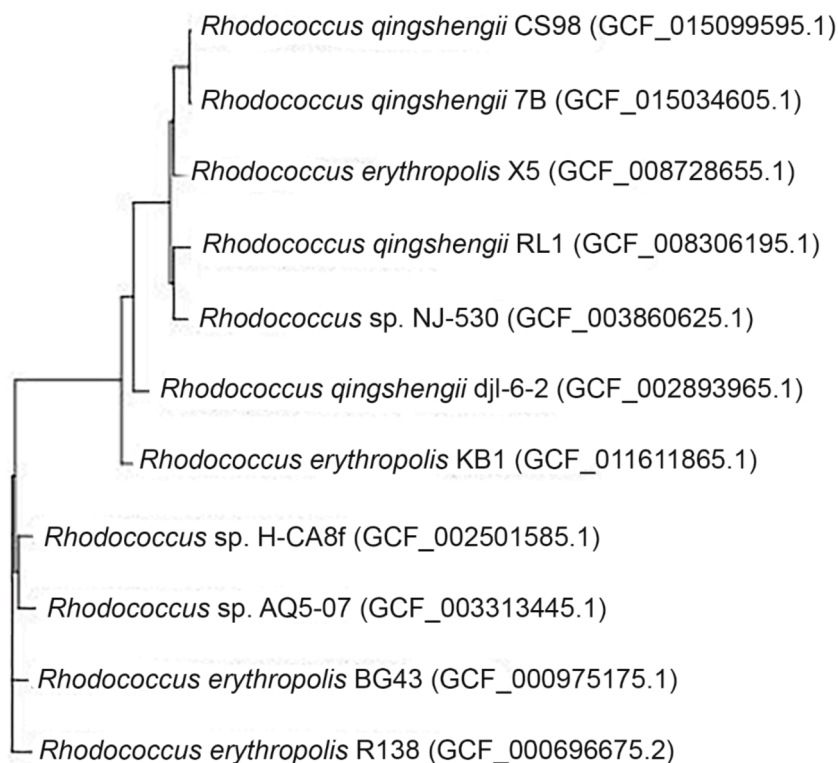
Fig. 2. Neighbor-joining phylogenetic tree of *Rhodococcus* based on whole-genome alignment

Table 2
The results of calculating the ANI value and DDH of strain 7B with type strains of *R. erythropolis* and *R. qingshengii*.

Strain	ANI value, %	DDH, %
R. erythropolis NBRC 15567T	95.41	82.50
R. qingshengii JCM 15477T	98.81	87.90

2.3. Chemical analysis of residual petroleum after degradation by the strain 7B

Chemical analysis of petroleum hydrocarbons in Evans liquid medium was carried out after extraction of oil from samples by the gravimetric method of column chromatography and fractionation using gas chromatography and high-performance liquid chromatography (HPLC).

The total concentration of substances extracted by chloroform was determined and a fractional analysis of hydrocarbons was performed after chloroform was evaporated and the samples were brought to a constant weight. Residual oil from the liquid phase of each sample was extracted with chloroform in separation funnels, rinsing the medium with chloroform three times after each separation of the oil layer.

Fractional analysis was carried out using Diapak C cartridges (Bio-ChemMak (Russia)). Based on the results of the analysis, the percentage of fractions was calculated: petroleum products (non-polar hydrocarbons); polyaromatic hydrocarbons and naphthenes (weakly polar hydrocarbons); resinous-asphaltene substances; oxidized substances (polar products of oxidation and destruction of hydrocarbons).

Determination of the hydrocarbon composition for the fractions of petroleum products was carried out on an Agilent 6890N gas chromatograph (Agilent Technologies) with a flame ionization detector, a DB5-ms 30m × 0.25mm × 0.25µm capillary column and a ChemStation data processing system. A quantitative mixture of *n*-alkanes
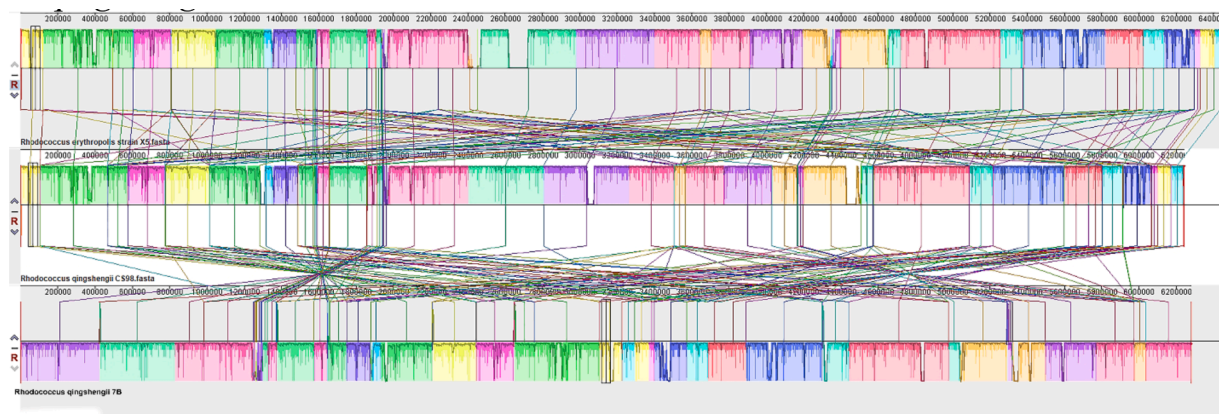

Fig. 3. Mauve visualization of locally collinear blocks identified between chromosomes of *R. qingshengii* 7B (bottom row), *R. erythropolis* X5 (top row)*,* and *R. qingshengii* CS98. Vertical bars mark interchromosomal boundaries

Table 3

Number and size of replicons of *R. qingshengii* strains from our institute collection.

Strain	Chromosome size, Mb	Number of plasmids	Name, size (kb) and configuration of plasmids
7B	6.28 (NZ_CP063234.1)	2	pCP3, 104, circular (NZ_CP063235.1) pLP4, 416, linear (NZ_CP063236.1)
X5 [44]	6.47 (NZ_CP044284.1)	1	pRhX5, 527, linear (NZ_CP044283.1)
VT6 [46]	6.45 (NZ_CP088906.1)	4	pCP1, 100, circular (NZ_CP088907.1) pCP2, 132, circular (NZ_CP088908.1) pLP1, 501, linear (NZ_CP088909.1) pLP2, 189, linear (NZ_CP088910.1)
F-2-2 [24]	6.31 (NZ_CP092101.1)	3	pLP156, 156, linear (NZ_CP092102.1) pCP209, 209, circular (NZ_CP092100.1) pLP337, 337, linear (NZ_CP092103.1)

(Connecticut *n*-Hydrocarbon Mix (Supelco)) was used as a standard.

2.4. Determination of halotolerance of strain 7B

To study NaCl resistance, the strain was cultivated at 28 and 45°C in the GRM broth, pH 6.5-7.5, containing 2, 5, 7.5 and 10% NaCl. The inoculum was added to reach the cell number equal to 10^6 CFU/ml. The growth was indicated by the turbidity of the medium in the tubes. The sterile GRM broth without microorganisms was used as a control.

2.5. Assessment of plant growth stimulation effect of *R. qingshengii* 7B

The cells were grown in 250 ml of the GRM broth in 750 ml flasks at 28°C for 48 h. The culture grown to OD 2.8-3 (wavelength: 525 nm) was diluted with saline solution to OD 0.3 and 2 ml of cell suspension were added to each Petri dish.

2.6. Microscopy

2.6.1. Light microscopy

To stabilize cell cultures during work, we used HEPES-KOH buffer (cat. # 11344041, ThermoFisher, USA) 50 mM + 330 mM Sorbitol, pH 7.2. To stain bacterial nucleic acids, we used SYTO 9 Green Fluorescent Nucleic Acid Stain (cat. # S34854, ThermoFisher, USA). For light microscopy, we used a Zeiss Axio Imager A1 microscope (Zeiss, Germany), an Axiocam 506C camera, and a Zeiss 56HE filter set (excitation: 470/27, emission: DBP 512/30 + 630/98).
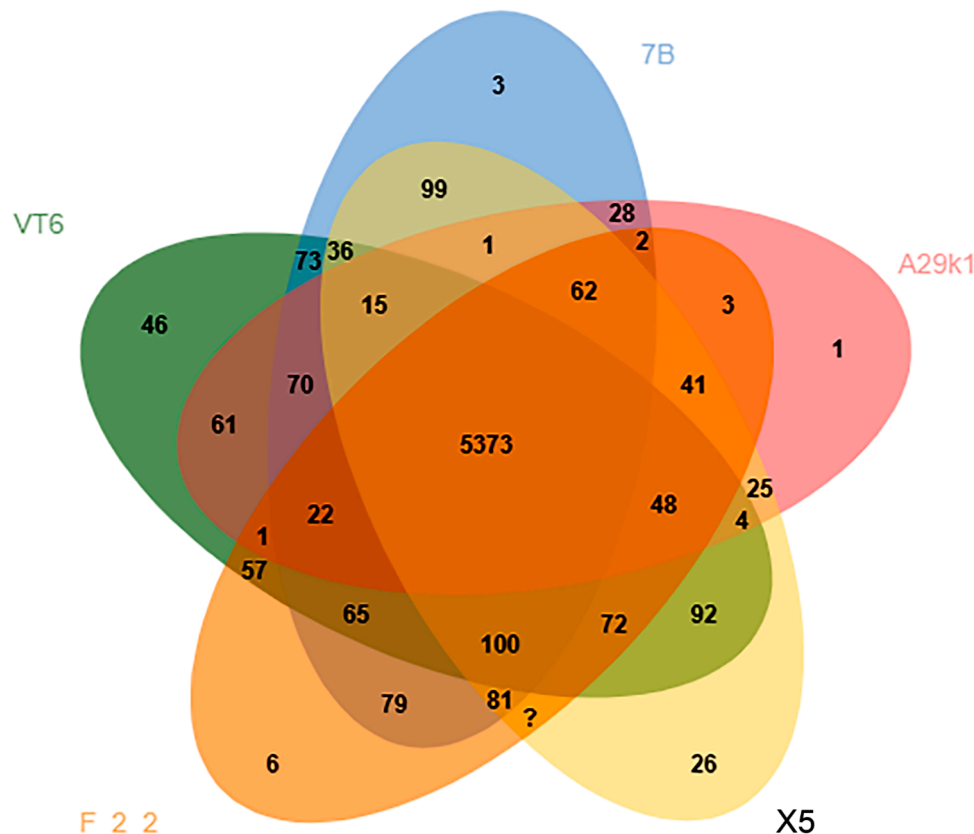
2.6.2. Scanning electron microscopy

Root samples were fixed in glutaraldehyde vapors at 4°C for 24 h, then fixed in osmium tetroxide vapors for 24 h at 20°C. After that, the samples were placed in a fume hood and subjected to long (7 days) drying in Petri dishes with a slightly open lid.

The surface of the dried samples was covered with gold in a JFC-1100

Table 4

The number of functionally annotated genes on replicons of the strain 7B.

Genome element	Total number of genes	Functionally annotated genes	Functionally annotated genes, %
chromosome	5820	2627	45.1
pCP3	107	19	17.8
pLP4	460	133	28.9

Fig. 4. Venn diagram depicting pangenome of *R. qingshengii* 7B, A29-k1, X5, F-2-2 and VT6.

- Protein families: genetic information process
- Carbohydrate metabolism
- Protein families: signaling and cellular processes
- Genetic Information Processing
- Environmental Information Processing
- Unclassified: metabolism
- Amino acid metabolism
- Unclassified
- Metabolism of cofactors and vitamins
- Nucleotide metabolism
- Unclassified: genetic information processing
- Cellular processes
- Lipid metabolism
- Protein families: metabolism
- Energy metabolism
- Unclassified: signaling and cellular processes
- Glycan biosynthesis and metabolism
- Metabolism of other amino acids
- Metabolism of terpenoids and polyketides
- Organismal systems
- Biodegradation of xenobiotics
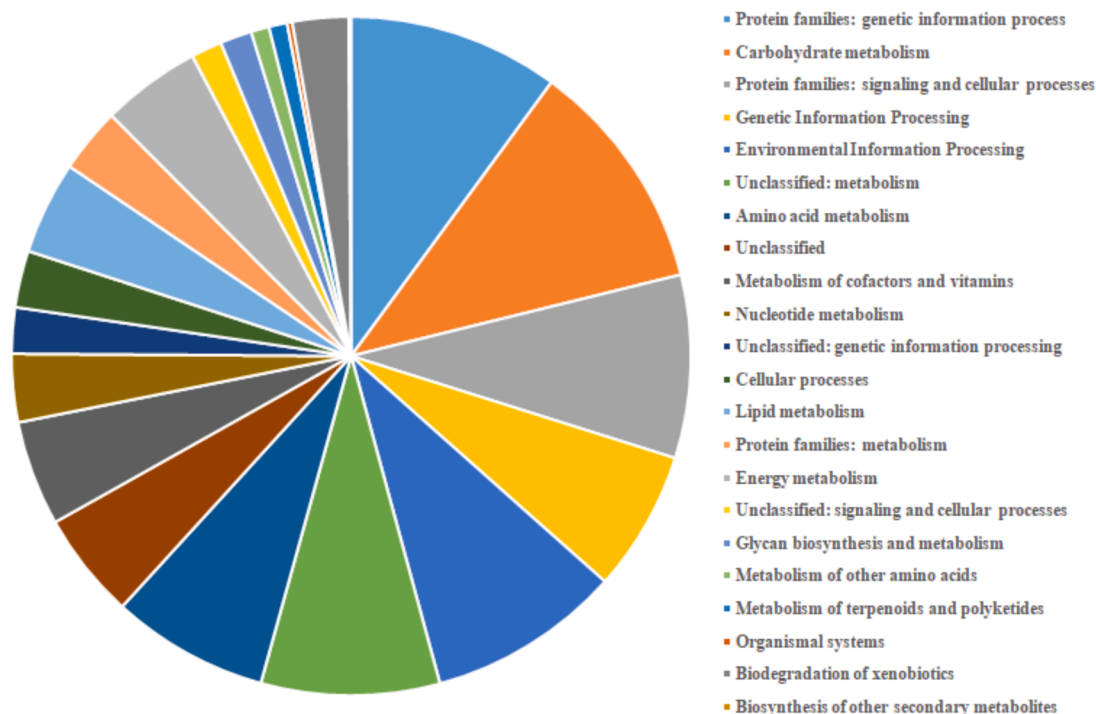- Biosynthesis of other secondary metabolites

Fig. 5. KEGG function classification of the *Rhodococcus qingshengii* strain 7B.

ion sputter (JEOL, Japan). Electron microscopy analysis was performed using a JSM-6510LV scanning electron microscope (JEOL, Japan).

2.7. Genome sequencing, assembly, and annotation

Genomic DNA was isolated from the biomass of a fresh culture (a colony) of *Rhodococcus* sp. strain 7B grown on the LB agar [25]. Commercial Kits, QIAamp DNA Mini Kit (cat. # 51304, Qiagen, Germany), Rapid Barcoding Kit (cat. # SQK-RBK00), MiSeq Reagent Kit v3 (cat. # MS-102-3003; 2 × 300 bp), NEBNext Ultra DNA Library Prep Kit (New England Biolabs, USA); Programs, Guppy 3.2.4, FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc), SPAdes 3.15.2 [26], the Flye assembler 2.6 [27], Bowtie2 2.3.5.1 [28], Pilon 1.23 [29], the Tablet [30] software; and Equipments, MinION sequencer with an R9.4.1 flow cell (Oxford Nanopore Technologies, UK), MiSeq System (Illumina, USA) were used as described earlier [31].

The base calling procedure yielded a total of 104 Mb distributed in 20,579 reads with a score Q>10. The N50 read length was 7442 bp. The data were submitted to the GenBank database under the accession numbers: BioProject – PRJNA669224, BioSample – SAMN16450133, GenBank – NZ_CP063234.1-NZ_CP063236.1.

The genome was annotated using Prokka [32] and RAST [33] software. The phylogenetic tree was constructed by the neighbor-joining method using the REALPHY service [34]. Genome sequences of *Rhodococcus* strains required for constructing the phylogenetic tree were taken from the WGS database (https://www.ncbi.nlm.nih.gov/Traces/wgs/?view=wgs). The circular map of the chromosome was made using DNAPlotter [35]. Genome alignment was carried out using the Mauve alignment algorithm [36].

The ANI value was calculated using the EzBioCloud service [37]. DDH was calculated using the Genome-to-Genome Distance Calculator 2.1 service [38]. To perform functional annotation using the blastp module, we used the Kyoto Encyclopedia of Genes and Genomes (KEGG) [39].

Comparative genomics methods were used for comparison the genome of strain 7B with the genomes of other *R. qingshengii*. The evolutionary relationships of *R. qingshengii* 7B with related strains were studied using collinearity and structural variations. Venn diagrams were applied to study the *R. qingshengii* pangenome and then visualize the results. The set of genes characteristic of all the studied strains of *R. qingshengii* is accepted as the core genome. The search and analysis of genes specific to each of the strains was also performed. Further, the possible correspondence of specific genes of strain 7B to its physiological properties was analyzed.

2.8. Analysis of differential expression of alkane hydroxylase genes of strain 7B

The strain was cultivated in Evans liquid medium with hexadecane (2%) at temperatures of 28°C (for 48 h) and 45°C (for 96 h). The different duration for cultivation is considered due to the fact that the temperature of 45°C is not optimal for the strain, and therefore the growth of the periodic culture is slower. Over the abovementioned time period, the culture at temperatures of 28 and 45°C undergoes the same phase that corresponds to late exponential growth.

Using the Primer-BLAST online tool, specific primers (Table 1) for the genes of interest were designed. The efficiency of amplification was determined using a series of tenfold dilutions of the DNA matrix, the specificity of the reaction was confirmed by electrophoresis in agarose gel.

Total RNA was isolated from the fresh biomass using Aurum total RNA mini kit (Bio-Rad, USA), the RNA concentration was determined using Nanodrop equipment (Thermo Fisher Scientific), and the quality of the preparations was assessed by agarose gel electrophoresis. For the synthesis of the first cDNA strand, the RevertAid RT Reverse Transcription Kit (Thermo Fisher Scientific, USA) was used. All the experiments were performed in 5 biological replicates; the 16S rRNA gene was used as reference. Quantitative reverse transcription PCR was performed with qPCRmix-HS SYBR (Evrogen). qRT-PCR data were normalized against 16S rRNA gene. PCR reactions were carried out using CFX Real-Time PCR System (Bio-Rad, USA). Statistical data processing was performed in the RStudio program, the difference between the mean values of the samples was determined using Student's T-test for two independent samples.
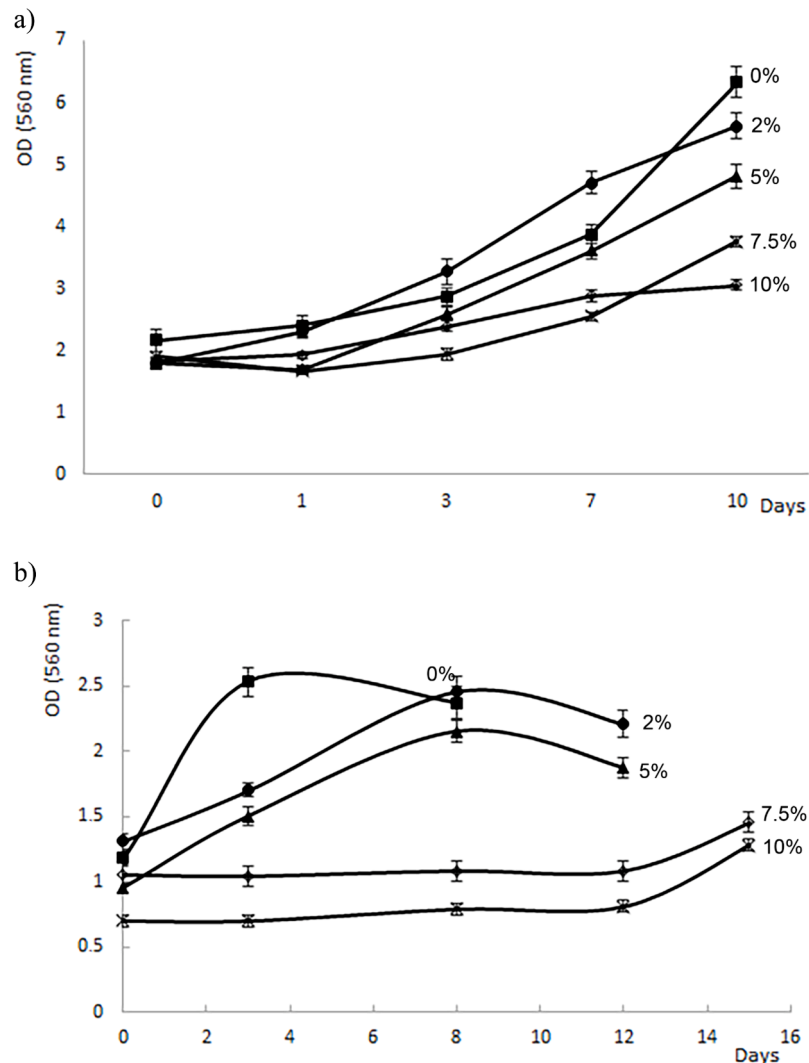
Fig. 6. Growth curves of *R. qingshengii* 7B in GRM broth at a) 28 °C, b) 45 °C.

Table 5
Degree of degradation of crude oil by the *Rhodococcus qingshengii* strain 7B (initial concentration of crude oil – 20 g/L).

	28°C			45°C	
	After 7 days	After 15 days	After 28 days	After 15 days	After 28 days
Percentage of crude oil degradation, %	41±5	51±6	62±6	24±3	44±7

3. Results and discussion

3.1. Sequencing and genome analysis of R. qingshengii strain 7B

The *Rhodococcus qingshengii* 7B genome consists of a 6,278,280 bp circular chromosome with a GC content of 62.49% (Fig. 1a) and two plasmids. The first plasmid, pCP3, is circular; it is 103,992 bp in length and has a GC content of 62.40% (Fig. 1b). The second plasmid, pLP4, is linear; it is 416,450 bp in length and has a GC content of 62.29%. The circularization of the chromosome and plasmids was ensured by overlapping of the ends. The chromosome was found to contain 5894 genes, the pCP3 plasmid – 107 genes, and the pLP4 plasmid – 460 genes.

3.2. Strain identification

The closest relatives of the strain 7B are: *R. qingshengii* CS98 (ANI value: 97.76%) AP023172.1, *R. erythropolis* X5 (98.05%) CP044284.1, *R. qingshengii* RL1 (98.94%) CP042917.1 (Fig. 2).

R. erythropolis and *R. qingshengii* — are very closely related species, and modern taxonomy is not always able to draw a clear line between them. The *R. erythropolis* species group includes the type strains *R. erythropolis, R. enclensis* [40], *R. globerulus* [41] and *R. qingshengii* [42, 43]. The results of the calculation of the ANI value and DDH allow us to define the strain 7B as *Rhodococcus qingshengii* (Table 2).

3.3. Comparative analysis of the genomes of the strain 7B and the closest strains

A Mauve-based comparison was performed between the chromosomes of the strain 7B and the closest strains (Fig. 3). The diagram shows the homology regions of the three genomes. Note that the main blocks (marked with one color) retain a similar arrangement on the chromosomes and, in general, the structures of the genomes are similar. However, there are numerous small rearrangements that may be the result of the activity of a large number of transposases in the strains.

For this work, several strains of *R. qingshengii* were sequenced and assembled (Table 3). Previously, strain X5 [44] was identified as
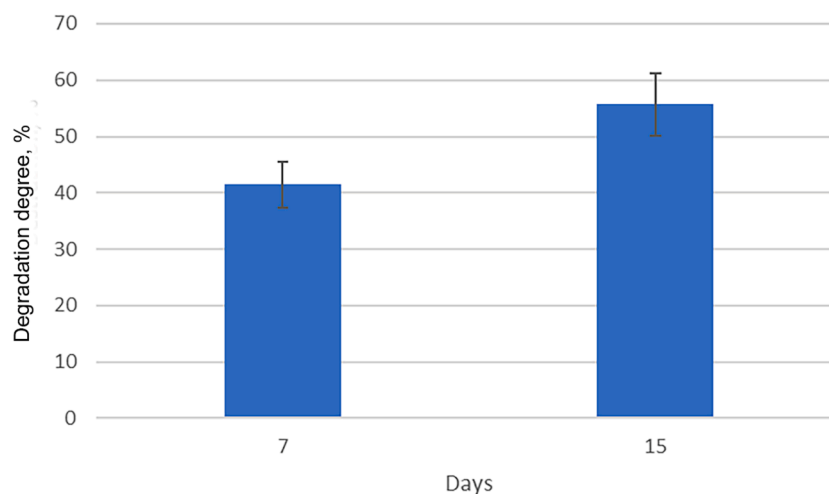
Fig. 7. Degree, %, of Kuwait oil sludge degradation by *R. qingshengii* 7B after 7 and 15 days of cultivation. Initial concentration of hydrocarbons in oil sludge – 190 g/kg.

R. erythropolis. However, in accordance with the classification proposed in the article [45], later we redefined it as *R. qingshengii.* All the strains have great metabolic potential: they utilize complex hydrocarbon mixtures, oil sludge and alkanes from C_8 to C_{36} in length at both low (F-2-2, X5, A29-k1 [45]) and elevated (7B) temperatures. Interestingly, among all analyzed *R. qingshengii*, only strain 7B is able to grow and utilize hydrocarbons at temperatures above 40°C, the remaining strains do not grow at such temperatures.

Analysis of the pangenome of *R. qingshengii* strains (Fig. 4) showed that the core (genes that all our strains have) makes up 86% of the whole pangenome. For comparison, the core of *E. coli* strains is 98% of the pangenome.

Of all our *R. qingshengii* strains, F-2-2 and X5 are the most related to strain 7B (7B/F-2-2 pair has 99 identical genes and 7B/X5 pair has 79 identical genes, which are unique to these pairs of strains but not found in other strains). Among the genes unique to the 7B/F-2-2 pair, it is interesting to note the genes belonging to the categories GO:0001659 "temperature homeostasis" and GO:0009651 "response to salt stress".

For the 7B/X5 pair, genes of terpene and steroids metabolism are identified among the unique genes (for example, GO:0016098 "monoterpenoid metabolic process"; GO:0008300 "isoprenoid catabolic process", GO:0006694 "steroid biosynthetic process"; GO:0008203 "cholesterol metabolic process").

3.4. Analysis of clusters of orthologous genes (COGs) in strain 7B

The chromosome and 2 plasmids of the strain contain 6387 genes in total. Out of the 6387 CDSs found, 2779 (43.5%) were functionally annotated (Table 4, Fig. 5). These results suggest that the *R. qingshengii* strain 7B has efficient lipid, carbohydrate and amino acid transport as well as metabolism.

Both plasmids, especially pCP3, contain a large number of genes encoding hypothetical proteins, as well as IS elements and genes the products of which are necessary for the maintenance of the plasmids themselves.

On plasmids of *R. qingshengii* 7B and its related strains, genes involved in catabolism of various compounds and responsible for the resistance to adverse environmental factors were found. For example, the plasmids pLP4 (7B) and pRhX5 (X5) bear genes for resistance to heavy metals (arsenic, mercury, cadmium, zinc, copper). Short alkane degradation genes (CYP153) were detected on plasmids in all strains.

The genes for the catabolism of hydrocarbons and their derivatives in the strain 7B are located mainly on the chromosome. We identified 5 copies of genes for alkane monooxygenases of the *alk*B family; out of

them, 3 are single (*alk*B3-*alk*B5), one (*alk*B1) is part of a cluster (with 2 copies of genes for rubredoxin and rubredoxin reductase), and one (*alk*B2) is accompanied by only two rubredoxin genes without reductase. Also, the chromosome contains genes involved in the degradation of biphenyl and dibenzothiophene.

The *alk*B gene designations are given here in accordance with the previously proposed classification [47,48]. It was shown that the change in the expression of *alk*B1, *alk*B3 and *alk*B5 genes due to temperature alterations is not statistically significant, while the expression of *alk*B2 and *alk*B4 genes when growing on hexadecane depends on temperature. Repression of these two genes occurred at 45°C (expression of *alk*B2 and *alk*B4 was 10.1- and 5.4-time weaker, respectively) compared to that at 28°C. This is generally consistent with the observation that the temperature of 45°C is not optimal for the strain. However, it is worthy to note that the process of alkane catabolism (in this case, hexadecane) still proceeds, albeit with less efficiency.

3.5. Resistance of strain 7B to NaCl content in the medium

It has been previously shown [21] that *R. qingshengii* 7B is a mesophilic strain with a temperature optimum of 28−32 °C. However, further research has shown that its survival limits are rather wide: the strain was capable of growth in a rich medium in the temperature range from 8°C to 55°C.

Oil production is often accompanied by the salinization of the soil. For this reason, the bacteria that are planned to be used in the course of remediation have to be halotolerant. The resistance of microorganisms to a salinity of the environment above 3% expands the opportunities of their use in bioremediation of oil-contaminated ground and water ecosystems. We analyzed the ability of the strain 7B to grow at an elevated salt content in a medium at an optimal (28°C) and at a stressful for this strain temperature (45°C) (Fig. 6a, b).

At a temperature of 45°C in a medium without the addition of NaCl, the phase of growth slowdown began as early as in 3 days, then converting into the phase of wasting away (Fig. 6b). An increase in the salt concentration in the medium to 5% shifted the onset of the stationary phase by about 5 days. A decrease in the amount of biomass was also observed. We assume that this is due to the synthesis of osmoprotectants by the strain 7B.

Moderate halotolerance is common in actinobacteria. To stabilize cells under conditions of salt stress, bacteria of this phylum use ectoine [43]. In the genome of the strain 7B, we identified a 10 kb cluster of genes involved in the biosynthesis of ectoine, including L-ectoine synthase, diaminobutyrate-2-oxoglutarate transaminase,
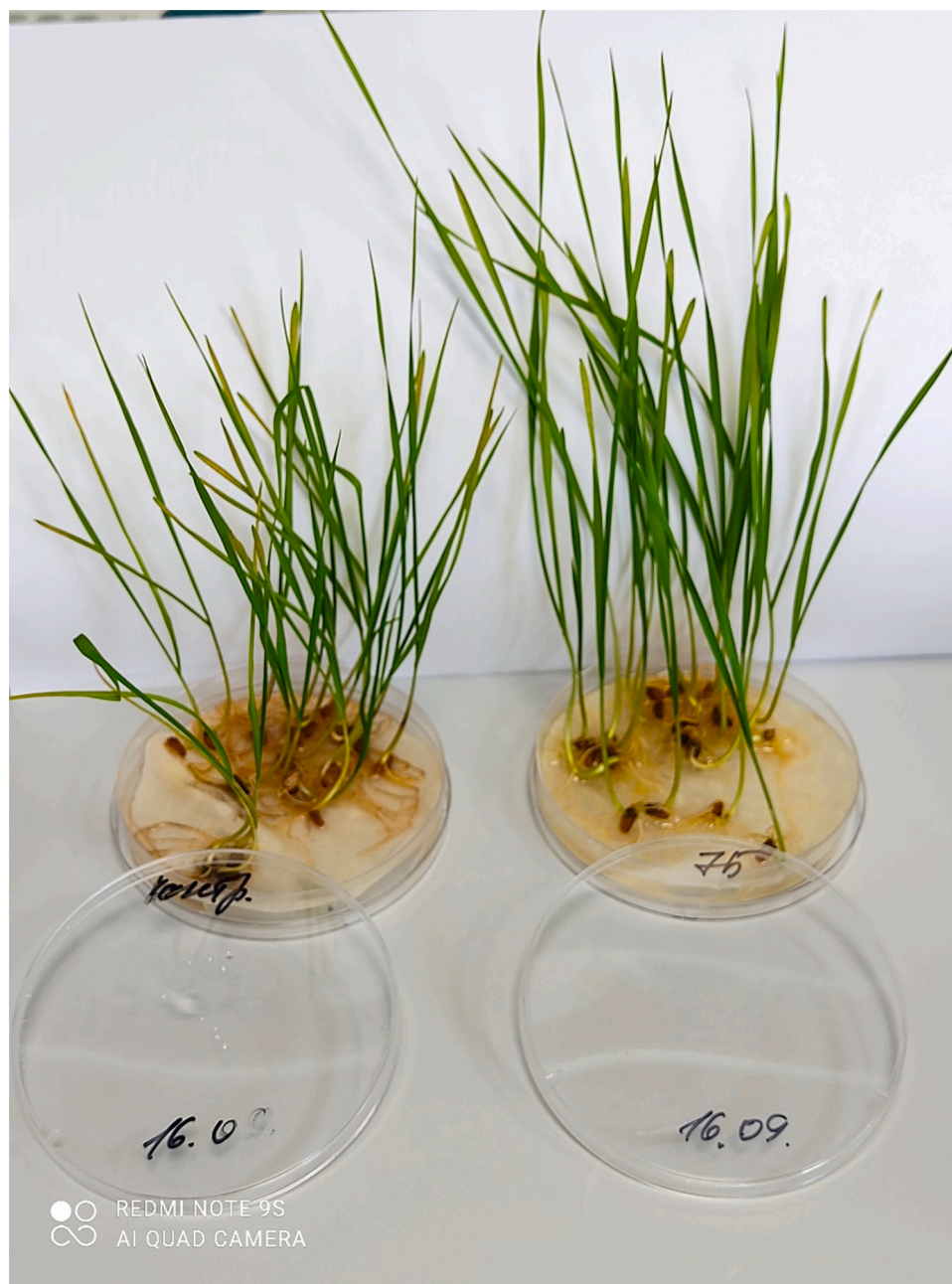
Fig. 8. Germination of corn kernels in the presence of cells of *R. qingshengii* 7B.

diaminobutyrate-2-oxoglutarate transaminase, L-2,4-diaminobutyric acid acetyltransferase. In addition, the genome contains a system of Na^+/H^+ antiporters that is primarily responsible for maintaining the homeostasis of pH and sodium [49].

An increase in the salt content to 10% led to a long lag phase associated with the adaptation of the culture to salt and the synthesis of osmoprotectants. A noticeable growth in the presence of 7.5 and 10% NaCl occurred only after 12 days of cultivation. Further increase in salt concentration led to growth inhibition. In the absence of visible growth, cell survival was confirmed by the formation of colonies when bacteria were cultured on a solid medium. Thus, the strain 7B can be characterized as a halotolerant organism that is promising for use in the remediation of saline soils.

Determination of the ability of the strain 7B to degrade petroleum products was carried out using crude oil (Table 5) and oil sludge. The strain was found to be capable of decompose up to 55% of hydrocarbons

of oil sludge over 15 days (Fig. 7). According to chromatographic analysis, the strain actively utilized even and odd alkanes from C_{14} to C_{32} in length, their content decreased approximately equally, degradation was ~65−70% at 28°C and 42−48% at 45°C. The strain poorly utilized terpenoid alkanes (pristane, phytane), their decrease was no more than 10% relative to the initial content, although the strain 7B bore the corresponding genes for this process.

The ability to efficiently metabolize and degrade hydrocarbons at elevated temperatures is rarely found in representatives of *R. erythropolis* and related species, including *R. qingshengii*. The *Rhodococcus qingshengii* strain TA13008 isolated from the Arabian Gulf sediments catalyzed 100% and 95% decomposition of naphthalene at 37°C and 25°C, respectively [50]. By cultivation at elevated temperatures, Delegan et al. [51] have isolated the strain Par7 identified as *R. erythropolis*. The strain Par7 utilized 15% of the crude oil over 14 days at a temperature of 45°C and an initial oil concentration of 2% (w/v). Sharma and Pant [52]
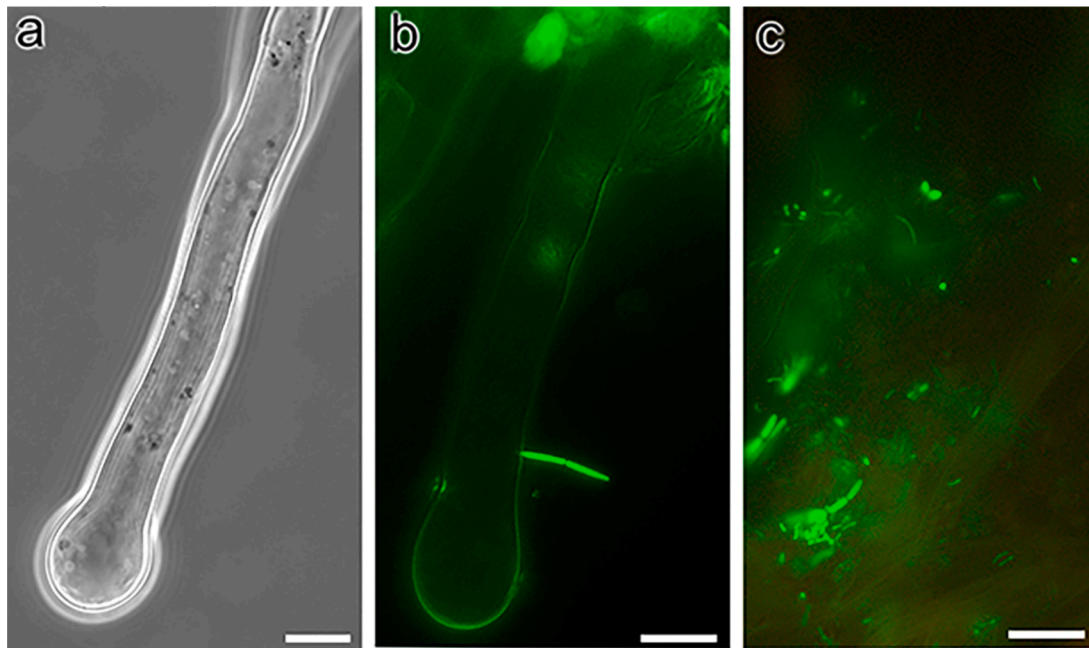
Fig. 9. Light (phase contrast) (a) and fluorescence (b) microscopy of wheat root hairs; (c) rhizoderm of the root colonized by the *R. qingshengii* strain 7B. Scale bar length – 10 μm.

reported that the *Rhodococcus* strain grew in the medium with crude oil at a temperature of 37°C, but at higher temperature cells could not grow. Rhodococci have extreme metabolic flexibility and are able to utilize pollutants of different origin, including hydrocarbons and their derivatives. However, in terms of growth temperatures, representatives of this genus are typical mesophilic strains, and therefore their use in biotechnology is possible only in temperate zones. This is the first time report about a strain of *R. qingshengii* that degrades oil and oil sludge at temperatures above 40°C.

The ability of the strain to utilize oil products in oil sludge samples at a temperature of 45°C was also assessed. With the initial content of oil products in the oil sludge of 190 g/kg, the degree of degradation of oil products was 38% and 47%, respectively (excluding abiotic loss).

The ability of the strain 7B to utilize crude oil and oil sludge is interesting from the point of view of using this strain in the reclamation of oil-contaminated soils. In addition, this strain was shown to be capable of growing on diesel fuel and engine oil. Since the strain 7B is able to grow at elevated temperatures, it can be considered as a promising bacterial strain for creating highly effective biological preparations for cleaning soils from crude oil.

3.6. Interaction of cells of R. qingshengii 7B and plants

Cell cultures of the *R. qingshengii* strain 7B had a pronounced stimulating effect on the growth and development of such plants as corn and wheat (Fig. 8).

The data of light and luminescence microscopy showed that rhodococci do not colonize the thin cellulose wall of the root hairs of the rhizoderm (epiblema). Using luminescence microscopy, we found that individual cells cling to the denser tops of the root hairs, but this is rather a random phenomenon.

Scanning microscopy showed that during cultivation rhodococci colonize the main root and bases at the root hairs (Fig. 10 a, b). Colonization occurs with the formation of biofilm. This is partially visible in photographs taken using fluorescence microscopy (Fig. 9 b, c).

Genome of the strain 7B contains a wide variety of genes, the products of which can positively affect the vital activity of plants. These genes are common among representatives of *R. qingshengii* and can be grouped into the following major metabolic pathways: indole-3-acetic

acid, cytokinin, siderophore, gibberellin production. In the genome of strain 7B, there are gene clusters of heterobactin and erythrochelin production, as well as individual elements related to the gene cluster of atratumycin production. Atratumycin is a cyclodepsipeptide with activity against *Mycobacteria tuberculosis* isolated from deep-sea derived *Streptomyces atratus* [53]. In turn, erythrochelin is a hydroxamate-type siderophore, the production of which is characteristic of *Saccharopolyspora erythraea* [54]. The production of heterobactin was previously observed in *R. erythropolis* [55] and *R. qingshengii* [56]. Analysis of the genomes of all known *R. erythropolis* and *R. qingshengii* strains revealed that the genes of siderophore biosynthesis are characteristic of all representatives of this group of strains. However, further experiments with the strains of our institute collection to study PGPR properties showed that not all *R. erythropolis* and *R. qingshengii*, even with such genes, have a stimulating effect on plants (data are not shown).

PGPR-bacteria increase the bioavailability of phosphorus due to its solubilization and nitrogen due to its fixation from the air, suppressing ethylene production via 1-aminocyclopropane-1-carboxylate deaminase activity [57–59]. The stimulatory effect of *Rhodococcus* cells on plants during co-cultivation has recently been shown for the *Rhodococcus qingshengii* strain RL1 [43]. Among the genes involved in the interaction of microbes with plants, the RL1 genome has been shown to contain *ami*E (amidase), *iaa*M (amine oxidase), as well as the genes involved in tryptophan metabolism, siderophore production, phosphate solubilization, and biofilm formation. Germination of wheat seeds in the presence of *R. qingshengii* 7B cells led to the formation of biofilms also at the level of the basal mature part of the root. The absence of the endophytic type of growth of *R. qingshengii* in the root zone of plants allows to attribute them to non-phytopathogens, in contrast to representatives of *R. fascians* and *R. corynebacterioides* known as endophytes which cause diseases of a number of plants, for example, pistachio, tobacco and *Arabidopsis thaliana* [60,61].

4. Conclusion

In this work, we report the complete assembly of the genome of the *Rhodococcus qingshengii* strain that can utilize crude oil and oil sludge at temperatures up to 45°C and salinity up to 7.5%. These findings suggest that the strain will be effective when used as part of microbial
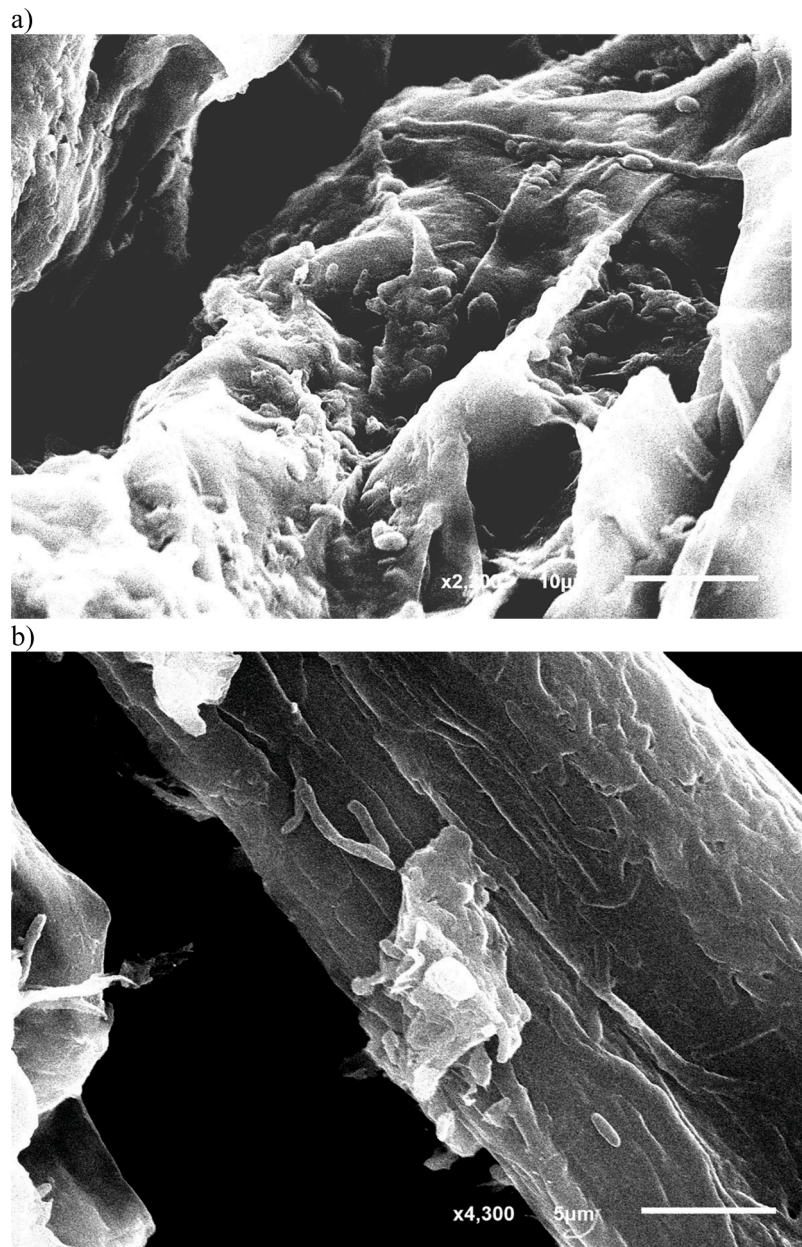
Fig. 10. Microphotographs (scanning microscopy) of the root and base of wheat root hairs colonized by *Rhodococcus* cells. Scale bar length – 10 μm (a) and 5 μm (b).

preparations for remediation of ecosystems under conditions of high temperatures and in saline soils.

The genome of strain 7B is represented by a chromosome and 2 plasmids (pCP3, pLP4). Genes for hydrocarbon catabolism are located on the chromosome and linear plasmid pLP4, the function of pCP3 is not yet clear.

An interesting feature of this strain is the fact that instead of root hairs, it colonizes the main root with the formation of a biofilm in the mucous sheath of calyptrogen and on the surface of the rhizoderm at the base of the root hairs. This can be an additional factor that protects the root from negative factors in the long term, since the lifespan of root hairs is short.

Funding

Declaration of Competing Interest

Let me inform you that all authors (Leila Iminova, Yanina Delegan, Ekaterina Frantsuzova, Alexander Bogun, Anton Zvonarev, Nataliya Suzina, Sadasivam Anbumani, Inna Solyanikova) declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

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