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Thermotolerant Oil-Degrading Bacteria Isolated from Soil and Water of Geographically Distant Regions

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Abstract—Oil-degrading bacteria were isolated from soil and water samples taken in Russia, Kazakhstan, and the Antarctic; 13 of 86 strains proved to be thermotolerant. These bacteria utilized crude oil at 45–50°C; their growth optimum (35–37°C) and range (20–53°C) differ from those of mesophilic bacteria. Thermotolerant strains were identified as representatives of the genera *Rhodococcus* and *Gordonia*. It was shown that their ability to degrade petroleum products does not differ at 24 and 45°C. The strains *Rhodococcus* sp. Par7 and *Gordonia* sp. 1D utilized 14 and 20% of the oil, respectively, in 14 days at 45°C. All of the isolated thermotolerant bacteria grew in a medium containing 3% NaCl; the medium for the strains *Gordonia amicalis* 1B and *Gordonia* sp. 1D contained up to 10% NaCl. The bacteria *G. amicalis* and *Rhodococcus erythropolis* were able to utilize crude oil and individual hydrocarbons at higher (up to 50°C) temperatures.

Keywords: bioremediation, biodegradation, crude oil, oil-degrading bacteria, thermotolerant bacteria, *Gordonia*, *Rhodococcus*

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The efficiency of biopreparations for the elimination of oil pollution is determined by the metabolic potentials of the soil-degrading strains comprising these preparations and the indigenous microflora on the treated sites. It has been shown that representatives of many bacterial genera are able to degrade oil. Oil destruction by bacteria has been studied in all climatic zones, since environmental temperature is one of the major factors influencing their vital activity and population dynamics.

Oil-degrading bacteria are divided into the following physiological groups depending on habitat temperature: thermophilic, mesophilic, and psychrophilic. Psychrotolerant and thermotolerant microorganisms belong to intermediate groups located between psychrophils and mesophils and between mesophils and thermophils, respectively. However, this differentiation is rather nominal, because temperature is a permanently acting factor and it is impossible to draw a distinct line (with 1-degree accuracy) between the microorganism habitats. The effects of intermediate temperatures at the boundaries between the regions in the classification of physiological groups of microorganisms have not been studied in detail [1].

The group of thermotolerant microorganisms is not quite clear. In [2], a narrow temperature range for the survival of this group was specified (the maximum growth temperature was 45–48°C); in [3], it was shown that thermotolerant species grew within a range of 10 to 60°C. However, the maximum growth temperature mentioned above (55–60°C) is slightly overestimated, because these species are close to the mesophilic and differ from them mainly in their ability to grow at high temperatures [3].

Thermotolerant oil-oxidizing bacteria were usually isolated from oil-polluted sites in regions with a hot climate, though thermophilic and thermotolerant bacteria were also found in cold climate regions [4], as well as in intact areas not polluted with oil. Investigation of the group of thermotolerant bacteria with oil-destructor properties will make it possible to supplement and systematize the data on the bacterial degradation of hydrocarbons at higher (up to 50°C) temperatures.

The goal of this work was to analyze the oil oxidation properties of thermotolerant bacteria (oil destructors) isolated from soil and water samples in different geographically distant regions and to assess their diversity.

Table 1. Primers used in the work

Gene	Primer	Annealing T, °C	Product size, b.p.	Reference
16S rRNA	63f CAGGCCTAACACATGCAAGTC 1387r GGGCGGWGTGTACAAGGC	55	1300	[10]
*gyr*B	UP1 GAAGTCATCATGACCGTTCT GCAYGCNGGNGGNAARTTYGA UP2-r AGCAGGGTACGGATGTGCGA GCCRTCNACRTCNGCRTCNGTCAT	60	1100	[11]
*alk*B	alkBF ATCAAYRCVGCVCAYGAR YTVGGBCACAAG alkBR SGGRTTCGCRTGRTGRTCR CTGTGNSGYTG	66	558	[12]

MATERIALS AND METHODS

Reagents. Hydrocarbons (phenanthrene, anthracene, fluorene, octane, nonane, decane, hexadecane, heptamethyl nonane) of a high grade purity (>98%) (Sigma-Aldrich, United States; Merck and Fluka, Germany) and Taq polymerase (5 U/μL, Silex, Russia) were used in the work.

The oil used as a carbon source was characterized by a density of 0.868 g/cm^3 and the following contents: water, 0.06%; salts, 45 mg/mL; mechanical impurities, 0.008%; and sulfur, 1.42%.

Strains and media. The mineral media were the Evans medium [5] and M9 medium [6]; Luria–Bertani medium (**LB**) was used as a rich medium [7]. Oil-degrading bacteria were isolated from natural objects by enrichment cultivation in a liquid Evans medium containing 2% oil for 21 days at 24°C. The enrichment culture was then plated onto agarized Evans medium with phenanthrene or diesel fuel (**DF**) and incubated at 24°C. Morphologically different colonies were hatched on agarized LB medium to verify culture purity. Thermotolerant strains were selected by the cultivation of isolated microorganisms in the liquid oil-containing Evans medium at 45°C.

Analysis of temperature dependence of the specific growth rate of thermotolerant strains. The thermotolerant strains were grown in liquid Evans medium containing 2% (vol/vol) hexadecane. The inoculum concentration was 5×10^5 CFU/mL. The cultivation was performed for 4 days at 20, 24, 28, 30, 35, 37, 40, 45, 50, 53, and 56°C.

The specific growth rate of the strains μ was calculated by the formula [8]:

$$\ln x = \ln x_0 + \mu t$$

where x is the concentration of microbial cells at a time t; x_0 is the initial cell concentration ($t = 0$); and μ is the specific growth rate.

The specific growth rate of the strains in a period of time from t_1 to t_2 was calculated as a slope of a straight line at the respective part t_1–t_2 of the plot of the growth dynamics of microorganisms.

Phylogenetic analysis of the 16S rRNA, gyrB, and alkB genes. Bacterial DNA was isolated as described [9]. PCR was performed with a GeneAmp PCR System 2400 amplifier (Perkin-Elmer, United States) according to the standard protocols, with the primers listed in Table 1. PCR products were purified according to the protocol [13]. The products were sequenced with an Applied Biosystems 3130 × 1 sequencer (United States) and the BigDye v.3.1 sequencing kit.

The BLAST program was used to search for nucleotide sequences homologous to the obtained gene fragments. MEGA 6 software was used for phylogenetic analysis and the construction of phylogenetic trees.

The GenBank provided accession numbers for the 16S rRNA gene nucleotide sequences (KR919788–KR919798) and the *alk*B genes of strains 1B, 1D, and 1G (KT862535, KT894216, and KT894217, respectively).

Determination of Physiological Characteristics of Thermotolerant Bacteria

Determination of the spectrum of substrates utilized by the strains under study. The microorganisms were cultivated in test tubes with the liquid Evans medium containing DF, polycyclic aromatic hydrocarbons (**PAH**), naphthalene, phenanthrene, anthracene, fluorine, and aliphatic hydrocarbons (hexane, octane, nonane, decane, hexadecane, and heptamethyl nonane). Solid hydrocarbons were added as a fine powder. The final substrate concentration was 2%. Culture growth was determined visually by the turbidity of the medium.

Investigation of the growth and DF degradation abilities of the strains in the presence of different sodium chloride concentrations. The strains were cultivated in test tubes with liquid Evans medium containing 3, 5, 7, and 10% NaCl and 2% (vol/vol) DF. The cultivation was performed for 7 days at 24 and 45°C. The growth

Table 2. Thermotolerant strains and places of their isolation

Sample	Strain	Source
From oil-polluted sites	1B, 1D, 1G	Soil from a sludge collector system (Moscow)
	Par5, Par7	Soil from a territory of oil spill (Kazakhstan)
	Par6, Par18	Oil sludge from a landfill (Kazakhstan)
From nonpolluted sites	4D, Par14	Soil from a lake coast (Baikal)
	5A, 6E	Water from Lake Baikal (the settlement of Bolshiye Koty)
	Par2, Par10	Lake water (Antarctic)

of microorganisms was also assessed visually by the degree of turbidity of the culture liquid.

Investigation of the ability of oil destructors to grow on oil hydrocarbons and to degrade them at different pH values of the medium. The strains were cultivated in test tubes with liquid Evans medium, with pH value adjusted to 4, 6, 7, 8, and 10 by the addition of concentrated hydrochloric acid or EDTA-Na. DF (2% vol/vol) was used as a carbon and energy source. The cultivation was performed for 7 days at 24 and 45°C. Microbial growth was visually assessed by the degree of turbidity of the culture liquid.

Determination of the oil utilization ability of thermotolerant bacteria at different oil concentrations in the medium. The bacteria were cultivated in test tubes with liquid Evans medium containing 5, 10, 15, and 20% crude oil. The cultivation was performed for 14 days at 24 and 45°C. Microbial growth was visually assessed by the degree of turbidity of the culture liquid.

Determination of oil-oxidative activity of microorganisms. The strains of thermotolerant bacteria were cultivated in flasks with 50 mL of liquid Evans medium containing 2% oil for 14 days at 24 and 45°C. The total content of oil hydrocarbons was measured by IR spectrometry. After cultivation, the residual oil was extracted with carbon tetrachloride (1 : 1). The hydrocarbon content in the extract was determined with an AN-2 oil product analyzer (Russia) according to the protocol described in [14]. The sterile Evans medium with 2% oil was used as a control.

Statistical processing. The results were statistically processed with Microsoft Office software.

RESULTS AND DISCUSSION

Isolation of pure cultures of thermotolerant microorganisms and analysis of the temperature dependence of basic-strain growth parameters. The ability of many bacteria to utilize hydrocarbons in ecosystems, including those with low contents, is known [15]. Oil destructors (86 bacterial strains) were isolated from water and soil samples; 34 of them were taken in regions not polluted with hydrocarbons (lake water from the Antarctic, water and soil from the shore of Lake Baikal). The strains of thermotolerant bacteria used in the work are listed in Table 2.

The selection criterion for thermotolerant bacteria was their ability to grow on oil hydrocarbons for 4 days at 45°C. The culture growth was visually assessed on a three-point scale: good growth, weak growth, and no growth. It was shown that 13 strains were characterized by good growth at 45°C, while other strains grew weakly or did not grow at all under these conditions. Two thermotolerant strains were isolated from the Antarctic lake water (Table 2). The previously described thermotolerant microorganisms isolated in the Antarctic were found in the volcanoes and hot springs of the Antarctic Continent [16, 17].

Analysis of the temperature dependence of the specific growth rate of all 13 strains within a range of 20–56°C showed a maximum at 35–37°C; at the same time, the maximum cell number was observed at the beginning of the stationary phase of culture growth. Such a temperature was optimal for strain growth. However, their maximal specific growth rates at 45°C were as follows: 0.33 h^{-1} for 1D (0.39 h^{-1} at 35°C); 0.27 h^{-1} for Par7 (0.34 h^{-1} at 35°C); 0.22 h^{-1} for 4D (0.31 h^{-1} at 35°C) (Fig. 1).

Thus, the strains under study can be defined as thermotolerant, because the bacteria differed from mesophilic cultures in the optimal growth at 35–37°C.

Phylogenetic analysis and identification of thermotolerant bacteria. The nucleotide sequences of 16S rRNA gene fragments were analyzed for all 13 thermotolerant strains. The comparison and alignment of resultant sequences with the related sequences from the GenBank database showed the presence of members of the genera *Gordonia* and *Rhodococcus* (GenBank reference numbers KR919788–KR919798). So, the strains Par2, Par5, Par6, Par7, Par10, Par14, Par18, 5A, 4D, and 6E were assigned to the genus *Rhodococcus,* while the strains 1B, 1D, and 1G were assigned to the genus *Gordonia*.

The *alk*B gene, which encodes alkane monooxygenase (EC 1.14.15.3), was used as a phylogenetic marker to determine the species status of strains of the genus *Gordonia*. It was shown previously [12] that the variability of nucleotide sequences of the *alk*B gene, together with other phylogenetic markers (the 16S rRNA, *gyr*B and *cat*A genes), can be used in species differentiation of strains from the genus *Gordonia*. A phylogenetic tree was constructed by the Neighbor

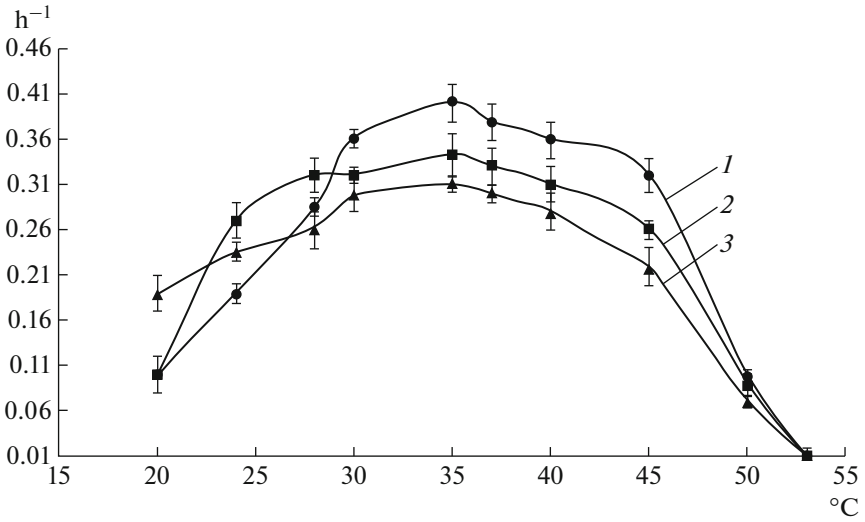

Fig. 1. Temperature dependence of specific growth rate (μ, h^{-1}) of thermotolerant strains 1D (*1*), Par7 (*2*), and 4D (*3*).

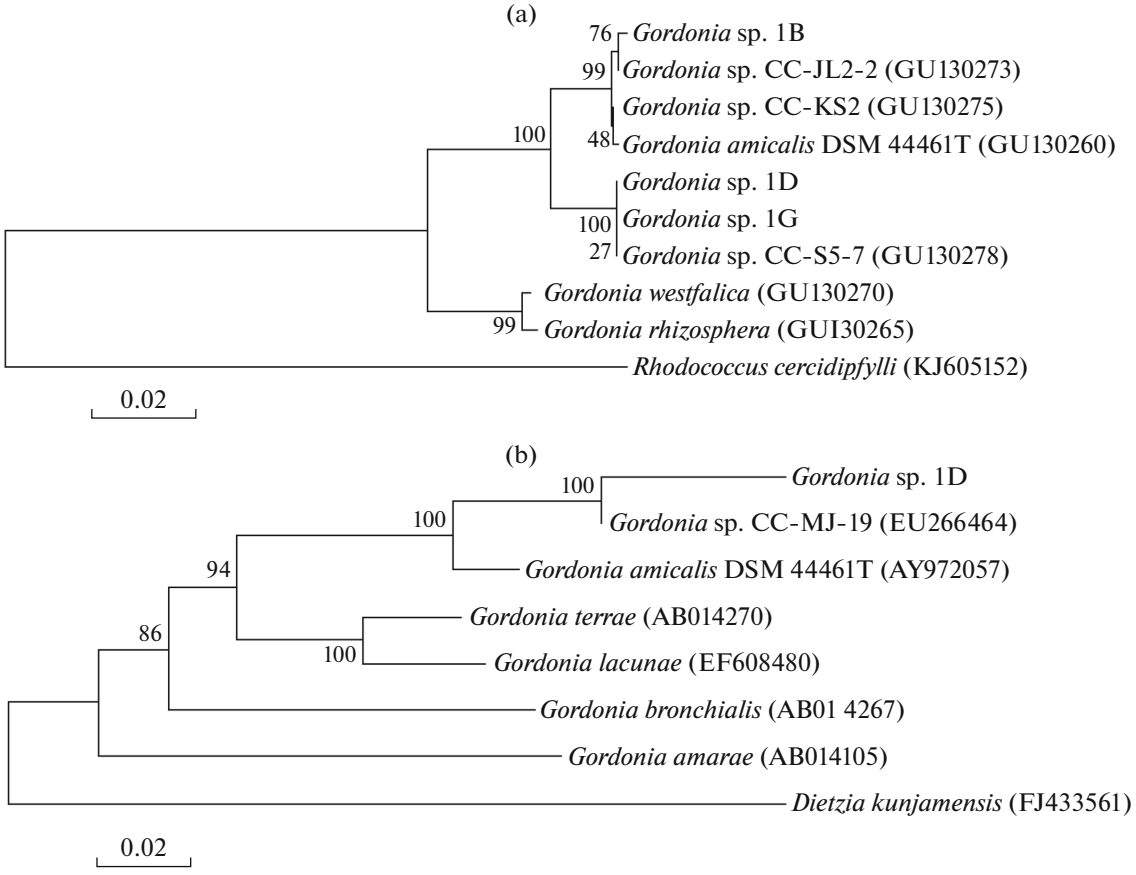

Fig. 2. Phylogenetic tree constructed by the NJ method based on the nucleotide sequence analysis of the *alk*B genes (a) and the *gyr*B gene (b) of strains of the genus *Gordonia*.

Joining (**NJ**) method based on the comparison of the *alk*B gene sequences of strains 1B, 1D, and 1G with the sequences of these genes in the type strains of the genus *Gordonia*. Its stability was also confirmed by the method of Maximum Parsimony (**MP**). On the phylogenetic tree, strain 1B was clustered together with the type strain of *G. amicalis* (Fig. 2a). The *alk*B sequences of strain 1B and the type strain of *G. amicalis* (Gen-

Table 3. Substrates utilized by oil-oxidizing bacteria at 24 and 45°C

Strain	Phenanthrene		Anthracene		Fluorene		Octane		Nonane		Decane		Hexadecane		Heptamethyl nonane		Diesel fuel	
	24°C	45°C	24°C	45°C	24°C	45°C	24°C	45°C	24°C	45°C	24°C	45°C	24°C	45°C	24°C	45°C	24°C	45°C
G. amicalis 1B	–	–	–	–	–	–	++	–	+	+	++	+	++	++	–	–	++	+
Gordonia sp. 1D	–	–	–	–	–	–	++	–	++	+	++	+	++	++	–	–	++	+
Gordonia sp. 1G	–	–	–	–	–	–	++	–	++	+	++	+	+	+	–	–	++	+
Rhodococcus sp. 4D	–	–	–	–	–	–	–	–	–	–	+	+	+	+	–		+	+
Rhodococcus sp. 5A	–	–	–	–	–	–	–	–	–	–	+		+	+	–	–	+	+
Rhodococcus sp. 6E	+	+	–	–	–	–	–	–	–	–	+	+	+	+	+		+	+
R. erythropolis Par2	–	–	+	+	–	–	–	–	+	+	+	+	+	+	–	–	++	+
Rhodococcus sp. Par5	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	–	++	+
R. erythropolis Par6	+	+			+	+	–	–	+	+	+	+	+	+	+	–	++	+
Rhodococcus sp. Par7	–	–	–	–	–	–	–	–	+	+	+	+	+	+	–	–	+	+
R. erythropolis Par10	–	–	–	–	–	–	–	–	+	+	+	+	+	+	–	–	++	+
Rhodococcus sp. Par14	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	+	+
Rhodococcus sp. Par18	+	–	–	–	–	–	–	–	–	–	+	+	+	+	–	–	+	+

"+", good growth; "++", very good growth; "–", no growth.

Bank reference code GU130260.1) had a high degree of homology (99.6% of identical b.p.), which made it possible to identify strain 1B as *G. amicalis*.

However, *Gordonia* sp. strains 1D and 1G were not clustered on the phylogenetic tree (Fig. 2a) with any type strain of the genus *Gordonia*. It may be assumed that these strains are a novel species within this genus.

The separate species status of *Gordonia* sp. strain 1D was confirmed by the sequence analysis of the *gyr*B genes. So, the *gyr*B gene sequences of *Gordonia* sp. strain 1D and the type strain of *G. amicalis* (GenBank reference number AY972057.1) contained 92.2% of identical b.p., which prevented strain 1D from being unambiguously assigned to *G. amicalis*, despite their close positions on the phylogenetic tree (Fig. 2b).

The nucleotide sequence analysis of the *gyr*B genes of thermotolerant rhodococci demonstrates that the sequences of these genes are homologous (from 94.0 to 99.7% of identical b.p.) to *gyr*B sequences of the *R. erythropolis* type strain. It was shown previously [18] that the sequence similarity of the *gyr*B genes in *R. erythropolis* and the closely related species such as *R. jialingiae* and *R. qingshengii* is 95%. For unambiguous identification of strains as representatives of the species *R. erythropolis*, *R. jialingiae* or *R. qingshengii*, the homology between the *gyr*B genes and gyrase genes of the type strains of these species must be more than 95%. So, the strains Par2 (99.2%), Par6 (99.5%), and Par10 (99.7% of identical b.p.) were assigned to *R. erythropolis*, while the other seven strains could be representatives of a novel species of the genus *Rhodococcus*.

Thus, all of the isolated thermotolerant oil-oxidizing bacteria belonged to the genera *Gordonia* and *Rhodococcus*. The members of both genera are mesophilic oil destructors; however, their ability to utilize hydrocarbons at the temperatures above 40°C has not been previously reported. The identification of some of the studied thermotolerant bacteria as representatives of the species *G. amicalis* and *R. erythropolis* will provide new data on the known physiological characteristics of bacteria from these genera.

The spectrum of substrates utilized by thermotolerant strains. The bacterial strains used in the work were able to utilize crude oil and DF, as well as different hydrocarbons (Table 3). Bacterial destruction of hydrocarbons of different chemical structures occurred both at 24 and 45°C. Analysis of the results showed that the most easily utilized substrates were DF, decane, and hexadecane (Table 3). Rhodococci showed insignifi-

cant PAH degradation. Of the ten *Rhodococcus* representatives under study, only strains 6E, Par6, and Par18 utilized phenanthrene and Par2 utilized anthracene; Par6 grew also on fluorene.

The strains 5A and Par14 utilized some alkanes; Par14 utilized hexadecane only, while 5A utilized decane and hexadecane. The strains Par2, Par6, Par7, and Par10 oxidized nonane, decane and hexadecane (Table 3). The results demonstrated that the hydrocarbon degradation ability of rhodococci did not depend on temperature.

Only strains 6E, Par5, and Par6 were able to utilize branched-chain alkanes (heptamethyl nonane); however, they lost this ability once the temperature was increased. The destruction of branched alkanes was observed previously in the strain *Rhodococcus* oxidizing pristine at 20°C; at 30°C, no destruction of this compound was observed [19].

Gordonia strains are often known as alkane destructors [20–22]. Analysis of the spectrum of substrates utilized by the strains *G. amicalis* 1B, *Gordonia* sp. 1D and 1G showed that they, both at 24°C and at a higher (45°C) temperature, actively destroyed normal alkanes and DF (Table 3). However, once the temperature was increased, the strains of the genus *Gordonia* lost their ability to utilize octane. Thus, the studied *Gordonia* strains including *G. amicalis*, could grow on and utilize hydrocarbon substrates at the temperatures above 40°C.

The results confirm the information that strains of the genus *Rhodococcus* can also grow at high temperatures [23, 24]. It should be noted that, in contrast to the known thermotolerant rhodococci isolated from the soils of Kuwait, the rhodococci studied in this work have been isolated from soil and water not only in high-temperature regions (Kazakhstan) but also in low-temperature regions (Baikal and Antarctic).

Resistance of the strains to NaCl content in the medium. The high daily mean and annual temperatures are distinctive features of arid regions. Such regions are usually characterized by low atmospheric precipitation and, as a consequence, soil and water salinization. The resistance of thermotolerant microorganisms to a more than 3% salt concentration in the medium expands the possibilities of their application in the bioremediation of oil-polluted ecosystems in arid regions. However, the intent of this work was not to search only for halophiles, because the introduction of an additional selective factor would restrict the potential application areas of the preparation based on thermotolerant strains.

All of the investigated strains could utilize hydrocarbons in the presence of 3% NaCl in the medium. Growth of the strains *Rhodococcus* sp. 4D, 5A, 6E, Par2, Par5, and Par7 was observed in the presence of 5% salt in the medium; growth of the strains *R. erythropolis* Par6 and Par10 was observed with up to 7% salt. A further increase in the salt concentration reduced the growth ability of the bacteria. The growth of *Gordonia* strains 1D and 1G on DF was observed in the presence of 10% NaCl.

The halotolerance of representatives of the genus *Gordonia* was previously reported in [25, 26]. The strain isolated from the *Chenopodium murale* rhizosphere could grow in the medium with a salt concentration up to 6% [25]. The novel species *Gordonia jinhuaensis* was also shown to grow in the medium with up to 9% salt [26].

The tolerance of some bacteria isolated in this work in the cultivation medium to enhanced salt concentrations suggests that they will utilize hydrocarbons in salinized (up to 7–10%) soil and water ecosystems, contributing to their restoration.

Hydrocarbon utilization by thermotolerant strains at different pH values of the medium. Bacterial isolation via enrichment cultivation was not aimed at obtaining strains that can grow in extremely acidified or alkaline media; the cultivation was performed at pH 7.2 (the pH value of Evans medium). All of the studied strains could grow on DF and utilize it in a medium with a pH in the range of 6–8, at both normal and high temperatures. However, the strain *G. amicalis* 1B was shown to be capable of growing in an alkaline medium (weak culture growth was observed at pH 10).

Crude oil utilization by thermotolerant strains at different concentrations in the medium. It is known that many microorganisms can utilize crude oil at low concentrations (1–5%); however, their metabolism is inhibited at higher oil concentrations [27]. All of the strains studied in this work grew in a medium with 10% oil. It has also been shown (Table 4) that oil destruction by *Gordonia* sp. strains 1D and 1G, as well as by *R. erythropolis* Par6 and *Rhodococcus* sp. Par7, could occur at an oil content of 15%. In the presence of 20% crude oil in the medium at 24°C, weak growth was observed only for *Gordonia* sp. 1D and 1G; the latter strain grew also at up to 45°C.

Different mechanical and physicochemical methods have to be combined for remediating soils polluted with high oil concentrations, followed by bio- and phytoremediation. The application of bacteria that can grow in the presence of 15–20% oil will expand the possibilities of bioremediation of soils with significant hydrocarbon contamination.

Efficiency of crude oil degradation by thermotolerant strains in a liquid mineral medium. The oil-degrading ability has been studied in five cultures: *R. erythropolis* Par2, *R. erythropolis* Par6, *Rhodococcus* sp. Par7, *Gordonia* sp. 1G and 1D. The results of the determination of the degree of oil destruction by these cultures are shown in Fig. 3.

The abiotic loss of crude oil at 24 and 45°C was 4 and 11%, respectively. Data analysis showed that the most efficient oil degradation at 45°C was observed for *Rhodococcus* sp. Par7 and *Gordonia* sp. 1D (Fig. 3). These strains utilized 14 and 20% of the oil, respec-

Table 4. The growth of bacteria at different oil concentrations (5–20%) in the cultivation medium

Strain	Oil concentration, %							
	5		10		15		20	
	24°C	45°C	24°C	45°C	24°C	45°C	24°C	45°C
G. amicalis 1B	+	+	+	+	±	–	–	–
Gordonia sp. 1D	+	+	+	+	+	±	±	–
Gordonia sp. 1G	+	+	+	+	+	±	±	±
Rhodococcus sp. 4D	+	+	+	±	–	–	–	–
Rhodococcus sp. 5A	+	+	+	±	–	–	–	–
Rhodococcus sp. 6E	+	+	+	±	–	–	–	–
R. erythropolis Par2	+	+	+	±	–	–	–	–
Rhodococcus sp. Par5	+	+	+	±	–	–	–	–
R. erythropolis Par6	+	+	+	+	+	±	–	–
Rhodococcus sp. Par7	+	+	+	+	+	±	–	–
R. erythropolis Par10	+	+	+	±	–	–	–	–
Rhodococcus sp. Par14	+	+	+	±	–	–	–	–
Rhodococcus sp. Par18	+	+	+	±	–	–	–	–

"+", good growth; "±", weak growth; "–", no growth.

tively. At 24°C, the maximum degree of oil destruction (59%) was observed for *Gordonia* sp. strain 1D.

Thus, efficient crude oil degradation by thermotolerant strains was observed in a broad temperature range. As seen from the results presented in Table 3, most of the strains utilizing individual hydrocarbons at 24°C are able to utilize them also at 45°C.

In bacterial preparations to remove oil pollutions, preference is given to the organisms that most completely utilize the multicomponent mixture of oil hydrocarbons within the shortest periods of time, irrespective of environmental factors. It is obvious that an effective bacterial preparation can be based on a consortium of organisms. It may be supposed that thermotolerant oil-oxidizing bacteria are promising for the development of biopreparations for soil and water remediation in hot climates.

It was demonstrated previously [3] that the optimal growth temperatures for mesophilic and thermotolerant bacteria are at the same level. Nevertheless, the bacteria under study had different temperature optimum (35–37°C) when compared to mesophilic microorganisms (25–30°C), and they had a growth range of 20–53°C. Hence, thermotolerant bacteria can be classified into a separate group of oil-oxidizing microorganisms.

Thus, it has been shown that the bacteria *G. amicalis* and *R. erythropolis* can effectively utilize crude oil

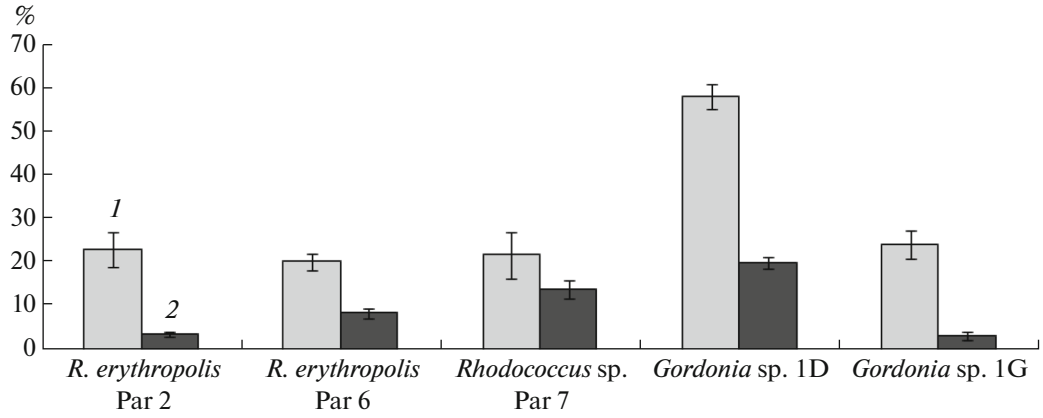

Fig. 3. Degree of crude oil destruction (%) by thermotolerant strains in liquid medium at 24 (*1*) and 45°C (*2*) after 14-day growth (with regard to abiotic loss).

and individual hydrocarbons at temperatures above 40°C, while thermotolerant bacteria can be assigned to the species and genera previously classified as mesophilic.

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