



Characterization and genomic analysis of highly efficient thermotolerant oil-degrading bacterium *Gordonia* sp. 1D

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Abstract

A thermotolerant bacterial strain 1D isolated from refinery oil-contaminated soil was identified as *Gordonia* sp. based on the analysis of 16S rRNA and *gyrB* gene sequences. The strain was found to utilize crude oil, diesel fuel, and a wide spectrum of alkanes at temperatures up to 50 °C. Strain 1D is the first representative of *Gordonia amicalis* capable of utilizing alkanes of chain length up to C₃₆ at a temperature of 45–50 °C. The degree of crude oil degradation by *Gordonia* sp. 1D at 45 °C was 38% in liquid medium and 40% in soil (with regard to abiotic loss). There are no examples of so effective hydrocarbon-oxidizing thermotolerant *Gordonia* in the world literature. The 1D genome analysis revealed the presence of two alkane hydroxylase gene clusters, genes of dibenzothiophene cleavage, and the cleavage of salicylate and gentisate – naphthalene metabolism intermediates. The highly efficient thermotolerant strain *Gordonia* sp. 1D can be used in remediation of oil-contaminated soils in hot climates.

Introduction

Oil spills and refinery accidents are the main causes of hydrocarbon emission into the environment. Hydrocarbon pollution poses a serious man-made threat for the modern world. Microbial degradation is considered as one of the most effective and environmentally friendly approaches to removing hydrocarbons from contaminated ecosystems.

Gordoniae encompass a rich reservoir of metabolic diversity, which makes them attractive candidates for crude oil bioremediation (Arenskötter et al. 2004). The presence of the dibenzothiophene (DBT) desulfurization pathway in *Gordonia amicalis* DSM44461T is consistent

with their emerging status as a source of metabolic diversity rivaling that of rhodococci (Kim et al. 2000). *Gordonia* strains utilize aliphatic (Shen et al. 2010; Lo Piccolo et al. 2011) and aromatic (Lin et al. 2012) hydrocarbons and heterocyclic compounds (Santos et al. 2005).

However, the data about the ability of *Gordoniae* to degrade hydrocarbons at elevated temperatures are very limited (Young et al. 2005; Lin et al. 2008). Thus, it is of great importance to isolate and characterize novel effective *Gordonia* strains capable of oil degradation at high temperatures. Such strains can be effective agents of remediation of oil-contaminated soils at hot climates with temperatures up to 50 °C. In addition, of importance is further research into the mechanism of alkane degradation by *Gordonia* strains based on genomics because they are some of the most frequently reported bacterial strains for alkane degradation.

In this study, we describe the isolation and characterization of *Gordonia* sp. strain 1D capable of vigorous utilization of crude oil and a wide range of alkanes. The peculiar properties of the degradation of main crude oil fractions during the growth of strain 1D at different temperatures were also investigated. In addition, we made the whole genome sequencing and assembly to analyze clusters of genes responsible for alkane degradation.

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Materials and methods

Chemicals and media

Hydrocarbons (phenanthrene, anthracene, fluorene, octane, nonane, decane, hexadecane, heptamethyl nonane) of high-grade purity (> 98%) (Sigma–Aldrich, USA; Merck and Fluka, Germany) were used. Crude oil used as a carbon source was characterized by concentration of 0.868 g/mL and the following contents: water, 0.06%; salts, 45 mg/mL; mechanical impurities, 0.008%; and sulfur, 1.42%.

Lysogeny broth (LB) liquid medium consisted of (per liter of distilled water) 10 g of tryptone, 5.0 g of yeast extract, and 5.0 g of sodium chloride. Evans mineral medium contained the following components (per L): 8.71 g K₂HPO₄, 1 mL 5 mol solution of NH₄Cl, 1 mL 0.1 mol solution of Na₂SO₄, 1 mL 62 mmol solution of MgCl₂, 1 mL 1 mmol solution of CaCl₂, 1 mL 0.005 mmol solution of (NH₄)₆Mo₇O₂₄·4H₂O; trace elements, 1 mL; pH 7.0. The trace element solution consisted of the following components (in 1% HCl, g/L): ZnO, 0.41; FeCl₂·6H₂O, 5.4; MnCl₂·4H₂O, 2.00; CuCl₂·2H₂O, 0.17; CoCl₂·6H₂O, 0.48; H₃BO₃, 0.06.

Isolation and identification of crude oil-degrading microorganism

A crude oil-contaminated soil sample was obtained from the territory of a petroleum refinery plant in the Moscow Region (Russia). To enrich petroleum-degrading bacteria, the sample was cultivated in Erlenmeyer flasks with 100 mL of Evans medium with crude oil (2% v/v) for 3 weeks. The cultivation temperature was 30 °C. After that, the sample of enrichment culture was transferred to a flask with the same media and substrate and cultivated for 2 weeks at a temperature of 45 °C. That technique allowed us to obtain thermotolerant strains able to grow at medium as well as elevated temperatures.

To obtain single bacterial colonies, a series of standard tenfold dilutions of enrichment culture was carried out. Then, 100 µL from the last dilution was plated on a petri dish with agarized Evans medium supplemented with 2% of crude oil as a sole carbon and energy source. Single colonies were thrice re-plated by dashing, using inoculation loop, on the same medium to obtain pure cultures (Miller 1972).

Pure cultures were phylogenetically characterized using 16S rRNA and *gyrB* gene sequencing. Universal primers 27f/1492r (Eden et al. 1991) were used for PCR amplification of the 16S rRNA genes. The PCR thermal cycling consisted of an initial denaturation at 95 °C for 10 min, followed by 35 cycles at 94 °C for 45 s, 56 °C for 45 s, and 72 °C for 90 s, plus the final step at 72 °C for 10 min. Amplification was performed with a GeneAmp PCR System 2400. For PCR amplification of a *gyrB* gene fragment, we used the primer pair

UP1/UP2r (Yamamoto and Harayama 1995). The conditions of PCR cycling were as follows: a total of 30 cycles of amplification was performed with template DNA denaturation at 94 °C for 1 min, primer annealing at 60 °C for 1 min, and primer extension at 72 °C for 2 min.

All amplification products were checked by electrophoresis on 1% agarose gels stained with ethidium bromide. The products were sequenced with an Applied Biosystems 3130 × 1 sequencer (USA) and a BigDye v.3.1 sequencing kit. The obtained sequences were subjected to BLAST homology search (<http://www.ncbi.nlm.nih.gov/BLAST>). We used the Clustal algorithm for sequence alignment and MEGA7 software for phylogenetic tree building (Tamura et al. 2013). All the sequences of type strains to compare with the sequences of our strains were taken from the Genbank NCBI database.

Hydrocarbon utilization experiments

To examine the ability of strain *Gordonia* sp. 1D to degrade various hydrocarbons, it was cultivated in 10 mL of test tubes with 5 mL of liquid Evans medium and the substrates at an initial concentration of 2% (w/v or v/v) (100 µL of liquid hydrocarbons or 100 mg solid hydrocarbons per tube). As it is impossible to assess the microbial growth on hydrocarbons by measuring the increase of biomass by absorbance (A₆₀₀), we assessed the growth visually and classified the ability of the strain to grow on different substrates in three levels: vigorous, normal growth, and no growth.

Evaluation of the efficiency of crude oil utilization by strain 1D under various conditions

Thermotolerant strain *Gordonia* sp. 1D was cultivated in flasks with 50 mL of Evans medium and crude oil (2%) for 14 days at 24 and 45 °C. The total content of oil hydrocarbons was measured by IR spectrometry. After the 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). Sterile Evans medium with 2% oil was used as a control.

The crude oil concentration in liquid samples was calculated by the formula:

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

where *C* is the crude oil concentration in the eluate found by instrument's readings or the calibration dependence, mg/L; *V_I* is eluate volume, L; *V* is the water sample volume, L; and *η* is the degree of eluate dilution. In the case of no dilution, *η* = 1.

To study the process of crude oil destruction in soil (sand), we constructed a model system with the following contents:

sterile sand with sea salt (final concentration, 3%), crude oil (2%), sterile liquid mineral medium (10%), and strain *Gordonia* sp. 1D to a final concentration of 1×10^4 CFU/g. The sand was loosened every 3–4 days. The experiment was carried out both at 24 and at 45 °C. After the experiment, the residual concentration of hydrocarbons was measured by IR spectroscopy on an AN-2 oil analyzer (Russia).

The crude oil concentration in soil samples was calculated by the formula:

$$C_i = \frac{D \times (K \times C_{H_k} - C_{H_0}) \times V_0}{P}, \text{ [mg/kg of soil]},$$

where D is the proportionality coefficient obtained by processing the calibration dependence based on actual and measured oil concentrations in the soil (established for this type of soil); K is the coefficient of eluate dilution by CCl_4 , mL/mL; C_{H_k} is the crude oil concentration in the diluted eluate determined based on the calibration dependence, mg/L; C_{H_0} is the concentration of non-specific humus components and residual oil products that can occur in the soil taken as a control, mg/L; V_0 is the initial volume of CCl_4 taken for crude oil extraction from a soil sample, L; and P is the soil sample, kg.

Genome sequencing, assembly, and annotation

High molecular mass genomic DNA was isolated from *Gordonia* sp. 1D using a DNA isolation kit (QIAGEN DNeasy Blood and Tissue Kit, Cat No/ID: 69506).

An Illumina Nextera XT reagent kit (FC-131-1024) was used to prepare a DNA library. Sequencing was carried out using the Illumina MiSeq platform with an MiSeq Reagent Kit v3 (600-cycle), MS-102-3003.

We obtained 1,143,191 paired-end reads of < 300 bp in size. Adapter and low-quality sequences ($Q < 20$) were deleted using Trimmomatic-0.36 software (Bolger et al. 2014). The remaining sequences were assembled to contigs using SPAdes-3.10.1 software (Bankevich et al. 2012). The number of contigs obtained was 191 with average k -mer coverage ($k = 127$) of 26.5659. The contigs were filtered using BLASTN and only 176 contigs were retained for further assembly.

Initial genome annotation of *Gordonia* sp. strain 1D was carried out using Prokka software (Seemann 2014). Additional gene prediction and functional annotation analyses were performed using RAST (Aziz et al. 2008) with k -mer size of 12 and Tigr Glimmer with minimum gene length of 300 (Delcher et al. 2007). Functions of some genes were checked manually by BLAST.

The circular map of the chromosome was made with DNAPlotter (Carver et al. 2009). Alkane hydroxylase cluster maps were performed using Artemis software (Carver et al. 2012).

Results and discussion

Isolation and identification of thermotolerant alkane-degrading strains

Several thermotolerant bacterial strains were isolated from refinery oil-contaminated soil (Moscow, Russia) after enrichment cultivation in Evans medium with crude oil for 3 weeks (Delegan et al. 2016). Gram-positive strain named 1D and capable of highly efficient degradation of crude oil at a temperature up to 50 °C was selected for further study. Colonies of the strain were orange-colored; cells were aerobic, non-motile, and featured short rods. The phylogenetic analysis of 16S rRNA and *gyrB* gene fragments showed the strain to be the closest to *G. amicalis* (99% similarity of the 16S rRNA gene, 94% similarity of the *gyrB* gene), but the similarity percentage of the marker genes, especially gyrase-encoding, was too low to unambiguously attribute the strain to *G. amicalis*. Based on the results of the morphological and genetic analysis, strain 1D was identified as *Gordonia* sp. (Fig. 1). The strain was deposited at the All-Russian Collection of Microorganisms as VKM Ac-2120 D.

Gordoniae are known for their ability to utilize crude oil and its individual components (alkanes, polycyclic aromatic compounds), including sulfur-containing components (DBT). However, there are little data about the metabolic peculiarities of *Gordonia* at elevated temperatures. Young et al. (2005) isolated strain *G. alkanivorans* CC-JG39 from oil-contaminated dryer sludge of a gas station in Taiwan. The strain grew on media with diesel fuel at temperatures up to 45 °C.

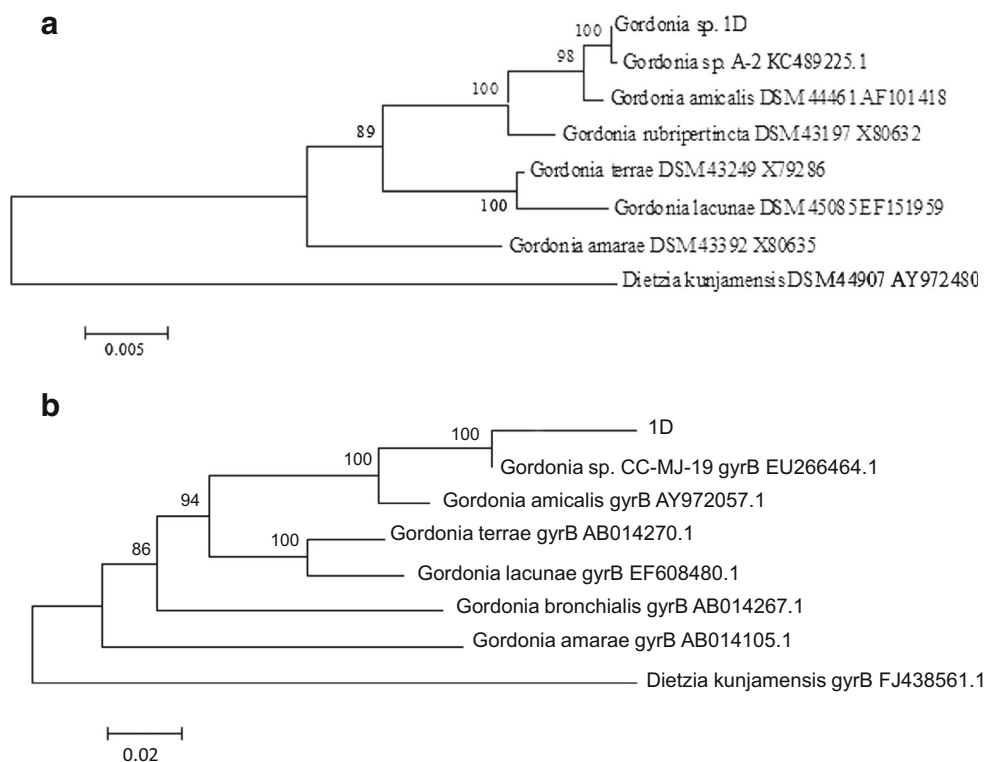
Analysis of the growth kinetics and temperature dependence of the specific growth rate of *Gordonia* sp. strain 1D within the range of 20–56 °C showed a maximum at 35–37 °C; at the same time, the maximum cell number was observed at the beginning of the stationary phase of culture growth. This temperature was optimal for strain growth. The maximal specific growth rate of strain 1D at 45 °C was 0.33 1/h (0.39 1/h at 35 °C) (Fig. 2).

The results imply that the thermotolerant representatives of the genus *Gordonia* may have a great scientific significance due to physiological and possible genetic differences from other *Gordonia* species that are mainly mesophilic.

Substrate spectrum of *Gordonia* sp. strain 1D

The degradation of crude oil, diesel fuel, alkanes of different chain length, and polycyclic aromatic hydrocarbons (PAHs) with two or three rings was measured. We analyzed the growth of bacteria at temperatures of 24 and 45 °C and found no differences between their hydrocarbon-oxidizing capacities at different temperatures in a good agreement with our preliminary results (Delegan et al. 2016) (Table 1).

Fig. 1 Phylogenetic tree derived from **a** 16S rRNA and **b** *gyrB* gene sequences and the sequences of the closest neighbor type strains. The distances were calculated using the neighbor-joining method. Numbers at branch points are bootstrap values based on 1000 samplings. *Dietzia kunjamensis* type strain (FJ438561.1) was used as the outgroup



We established that *Gordonia* sp. 1D could not degrade any of the tested PAHs. Amplification with specific primers also showed that there were no genes of PAH catabolism in the strain. Meanwhile, the strain was a vigorous alkane degrader; it utilized alkanes with a wide range of chain length.

The results of the fractional analysis showed that strain 1D was capable of utilizing a wide spectrum of odd and even alkanes from C₈ to C₃₆, including branched ones (pristane, phytane). There are no examples of such effective hydrocarbon-oxidizing thermotolerant *Gordonia* species in the world literature. The strain utilized petroleum alkanes at temperatures up to 50 °C.

Efficiency of crude oil utilization by *Gordonia* sp. 1D under different conditions

The results of our investigations have shown that *Gordonia* representatives can actively degrade crude oil and a wide spectrum of hydrocarbons not only under moderate conditions. To evaluate the efficiency of crude oil degradation by strain 1D, it was grown both in Evans medium with crude oil and introduced into oil-contaminated soil at a temperature 45–50 °C. After the cultivation, the residual hydrocarbon content was measured and the degree of crude oil degradation was evaluated (Fig. 3).

This paper is the first report of the peculiarities of crude oil degradation by a strain close to *G. amicalis* at

Fig. 2 Temperature dependence of specific growth rate (μ , 1/h) of thermotolerant strain *Gordonia* sp. 1D

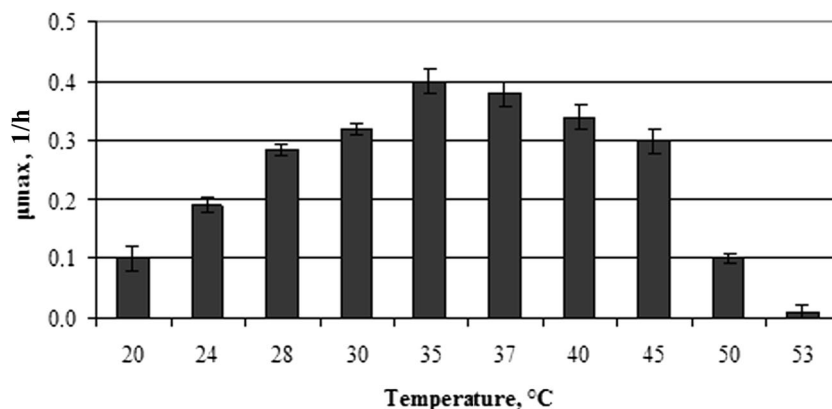


Table 1 Hydrocarbon utilization profile of *Gordonia* sp. strain 1D

Substrates	Utilization		Substrates	Utilization	
	24 °C	45 °C		24 °C	45 °C
Naphthalene	–	–	Decane	++	++
Phenanthrene	–	–	Hexadecane	++	++
Anthracene	–	–	Heptamethyl nonane	+	+
Fluorene	–	–	Diesel fuel	++	++
Octane	++	++	Crude oil	++	+
Nonane	++	++			

(++) Vigorous growth; (+) growth; (–) no growth. The concentration of each substrate was 2% (v/v or w/v)

different temperatures. The full spectrum of alkanes utilized by the thermotolerant *Gordonia* strain was also investigated for the first time.

Overview of *Gordonia* sp. 1D genome

The draft genome of *Gordonia* sp. 1D has a single chromosome and no plasmids (Fig. 4). The size of the genome is 5,151,623 bp with a G + C content of 67.3%. Average gene size is 965 bp, giving a coding density of 89.4%. The genome contains 4772 predicted protein coding sequences (CDS), 47 tRNA genes, and 3 rRNA operons. Average nucleotide identity (ANI) parameter analysis revealed that the closest phylogenetic relatives of strain 1D are *Gordonia* sp. KTR9 (NC_018581.1) and *Gordonia terrae* 3612 (CP016594.1) both having the OrthoANIu value of 80.9%. There are no fully assembled genomes of *G. amicalis* strains in the Genbank NCBI database, so we could compare the genome of 1D

only with suitable contigs of *G. amicalis* strains CCMA-559 and NBRC 100051.

Of the 4772 CDS found, 4091 could be assigned to 23 different categories of clusters of orthologous groups (COGs). These results suggest that the thermotolerant strain 1D possesses efficient lipid, carbohydrate, amino acid transport, and metabolism (Fig. 5).

In the present paper, the thermotolerant strain *Gordonia* sp. 1D was found to be able to utilize alkanes of different chain length. The analysis of the assembled sequence of the strain confirmed that *Gordonia* sp. 1D possesses different alkane hydroxylase systems. Moreover, the strain bears the enzymes of haloalkanes and alkanal degradation.

The alkane hydroxylase systems in *Gordonia* sp. strain 1D

The first group of alkane catabolic genes in strain 1D was represented by a cluster of four genes consisting of an alkane 1-monoxygenase *alkB*, two rubredoxin units and rubredoxin reductase (Fig. 6). It differed from the same cluster in *Gordonia* sp. KTR9 by the absence of transposase gene directly after the gene *alkB*, as shown in strain KTR9.

The RubR amino acid sequence has a 67% similarity with that of *Gordonia* sp. KTR9, and the sequence of the gene *alkB* has an 89% similarity.

The search for genes coding for the enzyme system CYP153 in the genome of strain 1D revealed the presence of ten copies of cytochrome P450 monoxygenase, only one of which was included in a full cluster with 2Fe-2S ferredoxin and ferredoxin reductase. The enzymes encoded by the genes of this cluster probably gave strain 1D the ability to oxidize short (from C₈) alkanes. This process was also active at high

Fig. 3 The degree of crude oil degradation by *Gordonia* sp. 1D in liquid Evans medium and soil in liquid Evans medium and soil at 24 and 45 °C (with regard to abiotic loss)

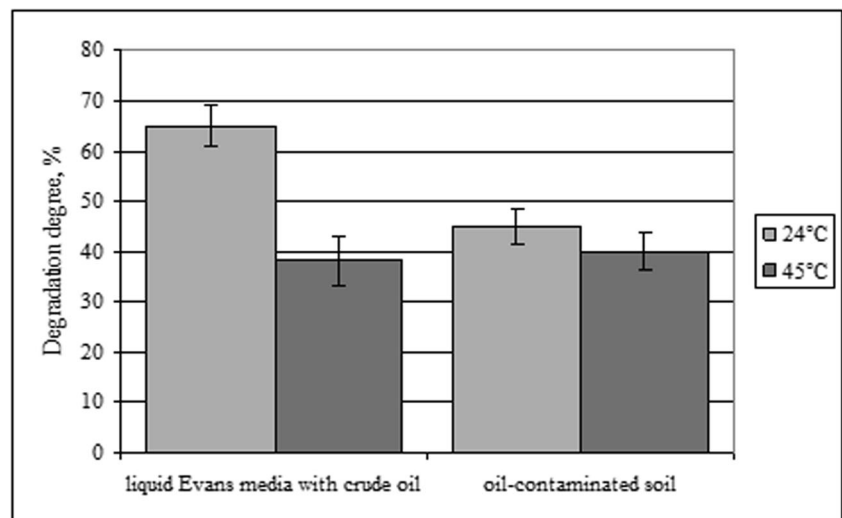


Fig. 4 Circular map of *Gordonia* sp. strain 1D. Outer tracks represent predicted CDS of the forward (cyan) and reverse (purple) strands. Red tracks represent tRNA; violet strings, rRNA clusters. Two inner tracks display the GC plot and the GC skew

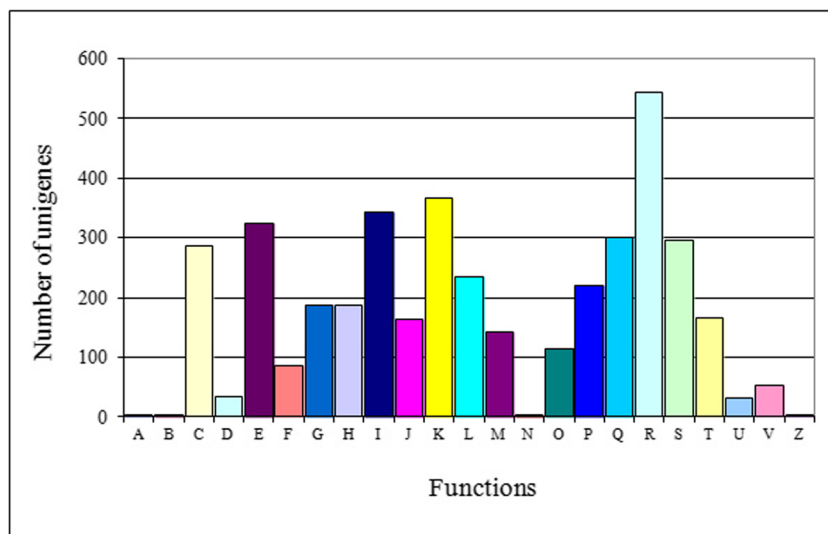
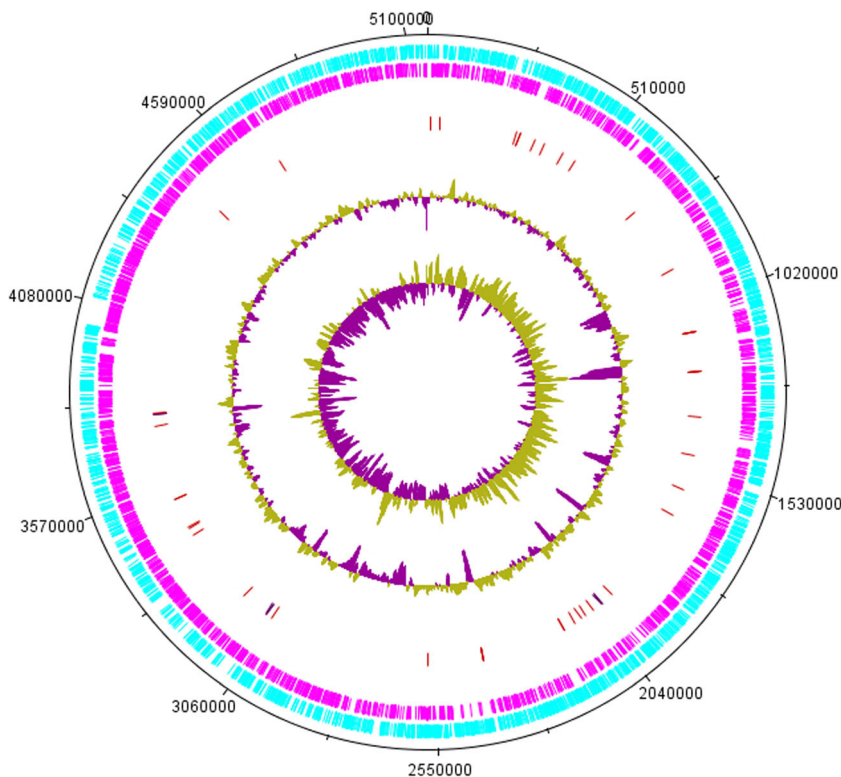


Fig. 5 COG function classification of thermotolerant strain *Gordonia* sp. 1D. (A) RNA processing and modification. (B) Chromatin structure and dynamics. (C) Energy production and conversion. (D) Cell cycle control, cell division, chromosome partitioning. (E) Amino acid transport and metabolism. (F) Nucleotide transport and metabolism. (G) Carbohydrate transport and metabolism. (H) Coenzyme transport and metabolism. (I) Lipid transport and metabolism. (J) Translation, ribosomal structure, and biogenesis. (K)

Transcription. (L) Replication, recombination, and repair, (M) Cell wall/membrane/envelope biogenesis. (N) Cell motility. (O) Posttranslational modification, protein turnover, chaperones. (P) Inorganic ion transport and metabolism. (Q) Secondary metabolites biosynthesis, transport, and catabolism. (R) General function prediction only. (S) Function unknown. (T) Signal transduction mechanisms. (U) Intracellular trafficking, secretion, and vesicular transport. (V) Defense mechanisms. (Z) Cytoskeleton

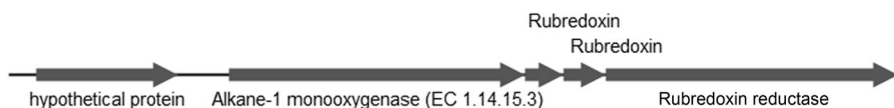


Fig. 6 Organization of the genes coding for the enzymes of AlkB hydroxylase cluster: *alkB*, alkane 1-monooxygenase, EC 1.14.15.3; *rubA*, *rubA2*, rubredoxins; *rubR*, rubredoxin reductase. The closest neighbors of the cluster are (left) hypothetical protein of 199 aa and (right) transcriptional regulator, TetR family, of 200 aa

temperatures. Strain *Gordonia* sp. KTR9, the closest phylogenetic relative of strain 1D (ANI value 80.9%), does not possess cytochrome P450 monooxygenases. There are also no data about the presence of these genes in other close relatives of strain 1D—*G. amicalis* CCMA-559 and NBRC 100051. Cloning and expression of these putative alkane hydroxylases would be helpful for the better understanding the mechanism of alkane degradation by *Gordonia* strains, including that process at high temperatures.

Besides the genes of alkane degradation, the genome of *Gordonia* sp. 1D contained genes of DBT cleavage, as well as the cleavage of salicylate and gentisate–naphthalene metabolism intermediates. The naphthalene cleavage pathway via salicylate and gentisate is present in most actinobacteria especially in *Rhodococci* (Di Gennaro et al. 2001). However, naphthalene degradation genes were not found in the genome of strain 1D. In this study, we observed no naphthalene degradation, and it was not surprising that no naphthalene catabolic gene cluster was identified by the genomic analysis of strain 1D. Meanwhile, the metabolic capabilities of *Gordonia* sp. 1D as a promising hydrocarbon degrader could be enhanced by introducing genetic constructions in the strain. Construction of genetically engineered strains based on 1D can overcome the incomplete degradation of naphthalene during the recultivation process. Further research is planned to test this possibility.

Conclusions

Thermotolerant strain 1D capable of vigorously degrading crude oil, diesel fuel, and alkanes of different chain lengths was isolated from refinery oil-contaminated soil (Moscow, Russia). 16S rRNA and *gyrB* gene sequencing and analysis have revealed that the strain belongs to the genus *Gordonia*. The results showed that hydrocarbon degradation occurred actively both at moderate and high (up to 50 °C) temperatures in liquid media and soil. Strain 1D is the first representative of *G. amicalis* capable of utilizing alkanes of chain length up to C₃₆ at a temperature of 45–50 °C. All the strains of *G. amicalis* known to date are mesophilic. There are no examples of such effective hydrocarbon-oxidizing thermotolerant *Gordonia* in the world literature. Information about the genome sequence of *Gordonia* sp. 1D offered an opportunity to expand the data about the metabolic potential of *Gordoniae*. Further experiments will be helpful to verify the functions of alkane degradation annotated genes.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest. The work was financially supported by RFBR (project №18-34-00329_mol_a).

References

- Arenskötter M, Bröker D, Steinbuechel A (2004) Biology of the metabolically diverse genus *Gordonia*. Appl Environ Microbiol 70:3195–3204. <https://doi.org/10.1128/AEM.70.6.3195-3204.2004>
- Aziz RK, Bartels D, Best AA, DeJongh M, Disz T et al (2008) The RAST server: rapid annotations using subsystems technology. BMC Genomics 8(9):75. <https://doi.org/10.1186/1471-2164-9-75>
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M et al (2012) SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19(5):455–477. <https://doi.org/10.1089/cmb.2012.0021>
- Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>
- Carver T, Thomson N, Bleasby A, Berriman M, Parkhill J (2009) DNAPlotter: circular and linear interactive genome visualization. Bioinformatics 25(1):119–120. <https://doi.org/10.1093/bioinformatics/btm578>
- Carver T, Harris SR, Berriman M, Parkhill J, McQuillan JA (2012) Artemis: an integrated platform for visualization and analysis of high-throughput sequence-based experimental data. Bioinformatics 28:464–469. <https://doi.org/10.1093/bioinformatics/btr703>
- Delcher AL, Bratke KA, Powers EC, Salzberg SL (2007) Identifying bacterial genes and endosymbiont DNA with glimmer. Bioinformatics 23(6):673–679. <https://doi.org/10.1093/bioinformatics/btm009>
- Delegan Y, Vetrova A, Akimov V, Titok M, Filonov A, Boronin M (2016) Thermotolerant oil-degrading bacteria isolated from soil and water of geographically distant regions. Appl Biochem Microbiol 52(4):389–396. <https://doi.org/10.1134/S0003683816040025>
- Di Gennaro P, Rescalli E, Galli E, Sello G, Bestetti G (2001) Characterization of *Rhodococcus opacus* R7, a strain able to degrade naphthalene and o-xylene isolated from a polycyclic aromatic hydrocarbon-contaminated soil. Res Microbiol 152(7):641–651. [https://doi.org/10.1016/S0923-2508\(01\)01243-8](https://doi.org/10.1016/S0923-2508(01)01243-8)
- Eden PA, Schmidt TM, Blakemore RP, Pace NR (1991) Phylogenetic analysis of *Aquaspirillum magnetotacticum* using polymerase chain reaction-amplified 16S rRNA-specific DNA. Int J Syst Bacteriol 41(2):324–325. <https://doi.org/10.1099/00207713-41-2-324>
- Kim SB, Brown R, Oldfield C, Gilbert SC, Iliarionov S, Goodfellow M (2000) *Gordonia amicalis* sp. nov., a novel dibenzothiophenedesulfurizing actinomycete. Int J Syst Evol Microbiol 50:2031–2036. <https://doi.org/10.1099/00207713-50-6-2031>
- Lin CL, Shen FT, Tan CC, Huang CC, Chen BY, Arun AB, Young CC (2012) Characterization of *Gordonia* sp. strain CC-NAPH129-6 capable of naphthalene degradation. Microbiol Res 167:395–404. <https://doi.org/10.1016/j.micres.2011.12.002>
- Lin TC, Chang JS, Young CC (2008) Exopolysaccharides produced by *Gordonia alkanivorans* enhance bacterial degradation activity for diesel. Biotechnol Lett 30:1201–1206. <https://doi.org/10.1007/s10529-008-9667-8>
- Lo Piccolo L, De Pasquale C, Fodale R, Puglia AM, Quatrini P (2011) Involvement of an alkane hydroxylase system of *Gordonia* sp. strain SoCg in degradation of solid n-alkanes. Appl Environ Microbiol 77:1204–1213. <https://doi.org/10.1128/AEM.02180-10>

- Miller JH (1972) Experiments in molecular genetics. Cold Spring Harbor Laboratory. 466 pages
- Santos SCC, Alviano DS, Alviano CS, Pádula M, Leitão AC et al (2005) Characterization of *Gordonia* sp. strain F.5.25.8 capable of dibenzothiophene desulfurization and carbazole utilization. *Appl Microbiol Biotechnol* 71:355–362. <https://doi.org/10.1007/s00253-005-0154-z>
- Seemann T (2014) Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30(14):2068–2069. <https://doi.org/10.1093/bioinformatics/btu153>
- Shen FT, Young LS, Hsieh MF, Lin SY, Young CC (2010) Molecular detection and phylogenetic analysis of the alkane 1-monoxygenase gene from *Gordonia* spp. *Syst Appl Microbiol* 33:53–55. <https://doi.org/10.1016/j.syapm.2009.11.003>
- Tamura K, Stecher G, Peterson D, Filipiński A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 30(12):2725–2729. <https://doi.org/10.1093/molbev/mst197>
- Yamamoto S, Harayama S (1995) PCR amplification and direct sequencing of *gyrB* genes with universal primers and their application to the detection and taxonomic analysis of *Pseudomonas putida* strains. *Appl Environ Microbiol* 61:1104–1109
- Young CC, Lin TC, Yeh MS, Shen FT, Chang JS (2005) Identification and kinetic characteristics of an indigenous diesel-degrading *Gordonia alkanivorans* strain. *World J Microbiol Biotechnol* 21: 1409–1414. <https://doi.org/10.1007/s11274-005-5742-7>