MICROBIOLOGY



# Degradation of diesel fuel by *Dietzia* sp. Ndt10 in saline conditions

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## Abstract

This study investigated the degradation of diesel fuel (DF) by an aerobic halotolerant strain, *Dietzia* sp. NDT10 (VKM Ac-2994D), under high salinity conditions. *Dietzia* sp. strain NDT10 has been isolated from diesel-contaminated rhizosphere soil of *Dactylis glomerata* L. on the territory of industrial production and processing of potassium salts (Solikamsk, Perm Krai, Russia). The 16S rRNA gene sequence analysis showed that the strain NDT10 is phylogenetically close (99.89 % similarity) to the type strains of two species, *Dietzia maris* DSM 43672$^T$ and *Dietzia kunjamensis* subsp. The ability of the strain NDT10 to degrade diesel fuel without salt and in the presence of up to 125 g NaCl/L was found. When adding 30, 50, and 70 g NaCl/L to the culture medium, the diesel fuel degradation ability of strain NDT10 was markedly increased, especially in the case of long-chain hydrocarbons ($C_{15}$–$C_{20}$) compared with short-chain hydrocarbons ($C_9$–$C_{14}$). An improvement in the degradative activity of *Dietzia* sp. NDT10 correlated with an increase in cell surface hydrophobicity in the presence of NaCl in the medium. Using the NDT10 strain as an example, a positive effect of diesel fuel components on the salt tolerance of bacteria was established. The results obtained can be used to develop biotechnological strategies for the clean-up of contaminated sites with DF and other petroleum products.

**Keywords:** diesel fuel, *Dietzia*, biodegradation, cell surface hydrophobicity, halotolerance.

## Introduction

At present, petroleum products are the most common and dangerous environmental pollutants. Using microorganisms to biodegrade organic pollutants is an effective biotechnological application for environmental bioremediation (Chandra, Sharma, Singh, and Sharma, 2013; Abatenh, Gizaw, Tsegaye, and Wassie, 2017; Xu et al., 2018; Tarfeen et al., 2022). The microbial degradation studies are essential to develop the remediation techniques of ecosystems contaminated with petroleum products. However, the effectiveness of this method is limited by extreme environmental conditions, particularly in cases of high salinity (Margesin and Schinner, 2001; Abed, Al-Kharusi, and Al-Hinai, 2015; Edbeib, Wahab, and Huyop, 2016; Khalid et al., 2021). Some members of the genus Dietzia are capable of degrading oil, petroleum products, aliphatic and aromatic hydrocarbons (Bødtker, Hvidsten, and Torsvik, 2009; Wang et al., 2011; Chen et al., 2017; Mikolasch et al., 2024), and also withstand a wide range of temperatures, pH and salinity, which allows them to be recommended for bioremediation of contaminated habitats (Gharibzahedi, Razavi and Mousavi, 2014; Nazina et al., 2015). Thousands of compounds, mainly saturated hydrocarbons, the number of carbon atoms per molecule ranges from 8–12 to 25–27, have been found in diesel fuel (DF). It is noted that petroleum products containing hydrocarbons with fewer carbon atoms, such as gasoline, can be degraded by microorganisms more eas-

**Citation:** Nazarov, A., Pyankova, A., Korsakova, E., and Plotnikova, E. 2024. Degradation of diesel fuel by *Dietzia* sp. Ndt10 in saline conditions. Bio. Comm. 69(2): 59–68. https://doi.org/10.21638/spbu03.2024.201

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**Manuscript Editor:** Kirill Antonets, Department of Cytology and Histology, Faculty of Biology, Saint Petersburg State University, Saint Petersburg, Russia

**Received:** September 5, 2023;

**Revised:** November 6, 2023;

**Accepted:** April 2, 2024.



**Funding:** The work was carried out within the state task (AAAA-A19-119112290009-1).

**Ethics statement:** This paper does not contain any studies involving human participants or animals performed by any of the authors.

**Competing interests:** The authors have declared that no competing interests exist.

ily and faster than heavy DF fractions (Marchal, Penet, Solano-Serena, and Vandecasteele, 2003; Auffret et al., 2009). However, the influence of high salinities on the degradation of DF by the members of the *Dietzia* genus has been insufficiently studied (Brzeszcz and Kaszycki, 2018), especially in the case of the degradation of individual hydrocarbon fractions of DF.

It should be noted that the initial stage of the process of hydrocarbon degradation is bacterial adhesion, which is determined by the properties of the cell surface, primarily the degree of its hydrophobicity, therefore the hydrophobicity of the cell surface determines the efficiency of biodegradation of hydrocarbons and petroleum products by bacteria (Bastiaens et al., 2000). It is known that the hydrophobicity of the cell surface of bacteria is actively influenced by physicochemical environmental factors (Bos, van der Mei, and Busscher, 1999). In the literature there are studies of the effect of salinity on the hydrophobicity of the cell surface of bacteria of the genera: *Erythrobacter* (Longang, Buck, and Kirkwood, 2016), *Exiguobacterium* (Cao et al., 2020), *Halomonas* (Hart and Vreeland, 1988), *Rhodococcus* (de Carvalho, 2012; Rubtsova, Kuyukina and Ivshina, 2012; Longang, Buck and Kirkwood, 2016). However, the specific effects of salinity on the cell surface hydrophobicity of bacteria of the genus *Dietzia* have not been studied.

The aim of this work was to evaluate the potential of the *Dietzia* sp. NDT10 to the biodegradation of diesel fuel under high salinity conditions. For this purpose, the effect of NaCl content in the medium on cell growth, cell surface hydrophobicity and the degree of degradation of diesel fuel, as well as individual hydrocarbon fractions of DF, were assessed.

## Materials and methods

**Bacterial strain**. The strain *Dietzia* sp. NDT10 was isolated from a sample of the rhizosphere of cocksfoot plants (*Dactylis glomerata* L.) growing on podzolic soil contaminated with diesel fuel (DF) with technogenic sodium-calcium chloride salinization. The soil sample was collected at 3 m from the halite waste dump (Solikamsk, Russia). The sampling site coordinates are latitude 59°38′00.1″, longitude 056°45′13.2″. The concentration of diesel fuel in the soil was 5.7 %, pH — 7.6, water-soluble salts — 1.3 %, $Ca^{2+}$ — 2824 mg/kg, $Na^{+}$ — 399 mg/kg, and $K^{+}$ — 85 mg/kg. The agrochemical analysis of soil was carried out using standard methods (Mineev, 2001), pH — by potentiometric method, the concentrations of $Ca^{2+}$, $Na^{+}$, $K^{+}$ were measured in water extracts from the soil using a device AA-6300 (Shimadzu, Japan).

**Media and cultivation conditions.** The bacteria were cultivated in a mineral Raymond medium (MRM) with the following composition (g/L): $NH_4NO_3$ — 2.0; $MgSO_4 \times 7H_2O$ — 0.2; $K_2HPO_4$ — 2.0; $Na_2HPO_4$ — 3.0; $MnSO_4 \times 2H_2O$ solution — 2 ml/L and 1 % $FeSO_4 \times 7H_2O$ solution — 1 ml/L of the medium (Raymond, 1961). To prepare a nutrient Raymond medium (NRM), 5 g/L tryptone (VWR Life Science Amresco, USA) and 2.5 g/L yeast extract (Biospringer, France) were added to an MRM as growth substrates. To obtain a solid cultivation medium agar (Helicon, Russia) was added to a final concentration of 15 g/L. Cultivation was carried out in a thermostat at 28 °C.

The growth characteristics of the strain were determined when grown in a liquid NRM without the addition of salt and with NaCl content in the cultivation medium: 30, 50, 70, 100 g/L. The growth of bacteria on DF was evaluated during cultivation in the liquid MRM without NaCl and with NaCl content of 30, 50, 70, 100, 125 g/L. DF was added to the medium to a final concentration of 1 g/L. The inoculum was a bacterial culture grown at 28 °C on NRM agar with 30 g/L NaCl within 5 days. The collected bacterial biomass was resuspended in the MRM containing 30 g/L NaCl. The resulting suspension of bacterial cells ($OD_{600}$ 1.0) was added to 100 ml of medium in an amount of 1 % v/v.

Cultivation was performed on a rotatory thermoshaker (Environmental Shaker-Incubator ES 20/60, Biosan, Latvia) at 28 °C and 140 rpm. The growth of the strain was assessed by determining the optical density of cultures at 600 nm ($OD_{600}$) on a UV-Visible BioSpecmini spectrophotometer (Shimadzu, Japan) in a cuvette with an optical path length of 1 cm. The specific growth rate ($\mu$, $h^{-1}$) was calculated according to a conventional method using the following formula:

$$\mu = (\mathrm{Ln}Cx - \mathrm{Ln}C_0) / (t_2 - t_1),$$

where $Cx$ — culture concentration at the highest growth point, $C_0$ — culture concentration at the initial point, and $t_1$ and $t_2$ — time at the beginning and the end of the exponential growth phase of the culture (Netrusov, 2005).

**Characteristics of the DF sample**. The diesel fuel used was EKTO Diesel Class 3, type II (DT-A-K5) (Lukoil-Permneftnorgsinez, Perm, Russia), with a density of 0.833 g/cm$^3$ at 15 °C, a kinematic viscosity of 1.46 mm$^2$/s at 40 °C, a cloud point 40 °C, cetane number 51 units, a mass fraction of polycyclic aromatic hydrocarbons < 1 %.

**Amplification of *alkB* genes**. Alk-BFB (5'–GGT ACG GSC AYT TCT ACR TCG A–3') and Alk-BRB (5'–CGG RTT CGC GTG RTG RT–3') primers were used to amplify the *alkB* gene encoding alkane-1-monooxygenase (Tourova et al., 2008). The DNA of *Rhodococcus wratislaviensis* strain KT112–7, the genome of which contains *alkB* (GenBank CP072193), was used as a positive control. Amplification was carried out on a C1000 Touch$^{TM}$ Thermal Cycler (Bio-Rad Laboratories, USA) according to the following conditions (Tourova et al., 2008).

The nucleotide sequence of the *alkB* gene was determined using the Big Dye Terminator Cycle Sequencing Kit v. 3.1 (Applied Biosystems, USA) on a Genetic Analyzer 3500XL (Applied Biosystems, USA) as recommended by the manufacturer. The nucleotide sequence data were analyzed using Sequence Scanner v2.0 and MEGA v11 software (http://www.megasoftware.net). The search of homologous sequences was done using the GenBank database (http://www.ncbi.nlm.nih.gov).

**Utilization of diesel fuel by the strain *Dietzia* sp. NDT10.** Cells of the strain were cultured in 150 ml conical flasks with ground glass stoppers in 10 ml of MCP with DF (1 g/l). The ratio of the volume of the cultivation medium and the air gas phase was 1 : 15, which excluded the limitation of the process of hydrocarbon oxidation by oxygen (Ivanova et al., 2014).

Determination of the content of DF hydrocarbons in the medium was carried out after 14 days of cultivation of bacteria, in chloroform extracts on an Agilent 7890B gas chromatograph (Agilent, USA) with an Agilent 7010B triple quadrupole mass spectrometer (Agilent, USA) and with a RESTEK RTx-5MS quartz column (Restek, USA). Helium was used as the carrier gas, the flow rate was 1 ml/min, evaporator temperature was 260 °C. The initial oven temperature was 130 °C for 3 min, and the final temperature was 280 °C, with an increasing rate of 10 °C/min. Chromatograms were analyzed using the MassHunter v10 program (Agilent, USA). Calculation of hydrocarbon concentrations was carried out based on the peak areas in comparison with the peak areas of control samples. To identify and calculate the concentrations of hydrocarbon fractions, chloroform solutions of *n*-alkanes ($C_9$–$C_{20}$: nonane, decane, undecane, dodecane, tridecane, tetradecane, pentadecane, hexadecane, heptadecane, octadecane, nonadecane and eicosane) were used. The calculation of the DF hydrocarbon content after 14 days of cultivation of the *Dietzia* sp. NDT10 in MRM was carried out relative to sterile controls incubated for 14 days (hereinafter referred to as "sterile control samples") according to the method (Ivanova et al., 2014).

**Cell surface hydrophobicity** was measured using the MATH test (Rosenberg, 1984; Maneerat and Dikit, 2007). After cultivation for 14 days, bacterial cells were separated from the culture medium by centrifugation at 12000 g for 30 min at 4 °C and were washed twice with a solution containing $K_2HPO_4$ at a concentration of 8.7 g/L and NaCl — 0, 30, 50, 70, 100, 125 g/L (pH 7.0). Then the cells were resuspended in this solution to the $OD_{600}$ of 0.5. The content of NaCl in the solution used for washing and resuspension of the cells corresponded to the concentration of sodium chloride in the medium where the bacteria were grown. Then, 0.5 ml of hexane was added to 3 ml of the cell suspension, and the resulting mixture was vortexed for 3 min. After 10 min, the aqueous phase was taken and its $OD_{600}$ was measured on a UV-Visible BioSpec-mini spectrophotometer (Shimadzu, Japan). The hydrophobicity of the cell surface (H, %) was calculated as follows:

$$H, \% = (A_0 - A) / A_0 \times 100\,\%,$$

where $A_0$ is the $OD_{600}$ of the cell suspension before adding hexane equal to 0.5 and $A$ is the $OD_{600}$ of the cell suspension after vortexing and settling for 3 min with hexane.

**Statistical analysis**. Statistical data processing was performed using the Statistica 6.0 (http://statsoft.ru). Standard methods of parametric statistics were used to describe the results of the study: the mean and standard deviation were calculated. To assess the significance of differences, Student's *t*-test was used with the calculation of a 95 % confidence interval.

## Results and discussion

The strain NDT10 (VKM Ac-2994D) on an agarized NRM forms round shiny smooth opaque convex orange colonies 2–3 mm in diameter, with regular borders, uniform structure and soft consistency. Comparison of the 16S rRNA gene fragment nucleotide sequence (915 bp) of the NDT10 and the type strains of the genus *Dietzia* showed that the NDT10 strain is phylogenetically close (99.89 % similarity) to the type strains of two species, *Dietzia maris* DSM 43672$^T$ and *Dietzia kunjamensis* subsp. *kunjamensis* DSM 44907$^T$. The 16S rRNA gene nucleotide sequence of the NDT10 strain was deposited in the GenBank database at number ON527781.

It was shown that the strain NDT10 is capable of growing in liquid MSR on diesel fuel as the sole source of carbon and energy. The amplification of alkane-1-monooxygenase gene (*alkB*), a key enzyme for the aerobic degradation of *n*-alkanes (the main components of DF), using primers Alk-BFB and Alk-BRB (Tourova et al., 2008) led to the production of a PCR product of the expected size (about 500 bp). However, sequencing and analysis of the *alkB* gene revealed matrix heterogeneity, which is characterized by the content of several homologous DNA nucleotide sequences in the sample. It may indicate the presence of more than one copy of the *alkB* gene in the genome of the strain NDT10. The genomes of some strains of the genus *Dietzia* have several copies of the *alkB* gene (https://img.jgi.doe.gov/).

Since the strain NDT10 was isolated from soil with high salinity, we investigated its capability of the growth in a medium with high mineralization. *Dietzia* sp. strain NDT10 grew in NRM both without NaCl and at salt concentrations up to 70 g/L (Fig. 1). At the same time, the duration of the lag phase and the maximal optical density of the culture were similar when growing on a medium without the addition of NaCl and with a con-

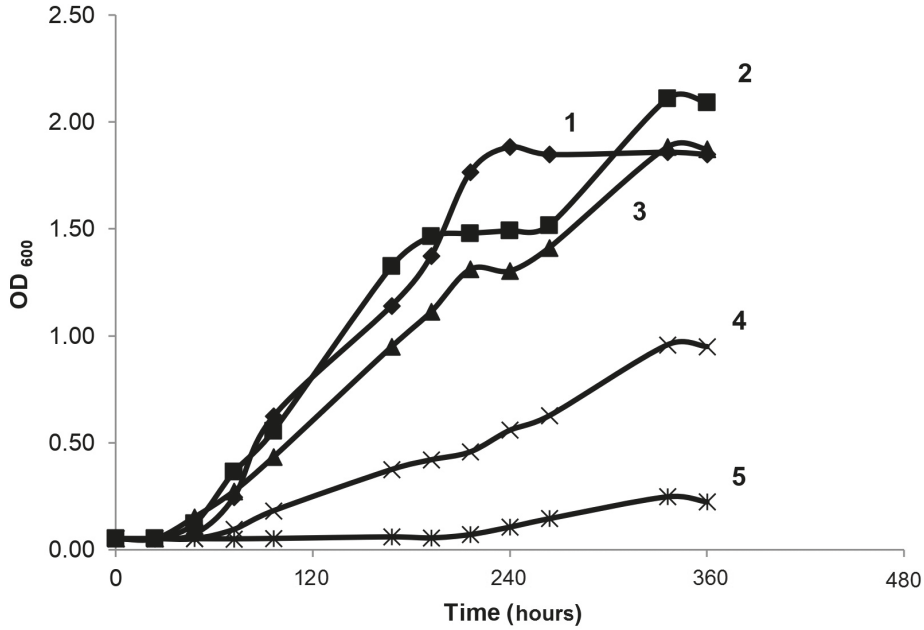


**Fig. 1.** The growth of *Dietzia* sp. strain NDT10 in NRM at different NaCl concentrations (g/L): 1 — without NaCl; 2 — 30; 3 — 50; 4 — 70; 5 — 100. The experiment was performed in triplicate.

tent of 30 g NaCl/L and 50 g NaCl/L. Based on the data obtained, it can be argued that the strain NDT10 is a halotolerant organism (Kushner, 1978). An increase in the salt concentration in the medium to 70 g NaCl/L led to an increase in the duration of the lag phase (up to 100 h) and a decrease in the maximal $OD_{600}$ to 0.96 (Table 1). With an increase of the salt content in the NRM to 100 g NaCl/L, the growth of the strain NDT10 was not detected.

The strain NDT10 was able to grow in the MRM with DF at NaCl concentrations up to 125 g/L (Fig. 2). The most efficient growth of the strain NDT10 was observed in diesel fuel without sodium chloride and in the presence of 30, 50 and 70 g NaCl/L. The lag phase was 70–83 h, followed by a significant increase in biomass. The maximal $OD_{600}$ value (equal to 0.93) was observed after 7 days and 14 days of cultivation with 30 g NaCl/L and without the addition of sodium chloride, respectively (Fig. 2, Table 1).

With an increase of sodium chloride concentration in the medium at the initial stages of cultivation, slow growth of the culture was observed ($OD_{600}$ value did not exceed 0.2). An active increase in biomass was noted only after 7 days and 18 days when the content in the medium was 100 and 125 g NaCl/L, respectively. The maximal $OD_{600}$, equal to 0.7, was observed during cultivation in a medium containing 100 g/L sodium chloride. At a content of 125 g NaCl/L in the medium, the maximum $OD_{600}$ was 0.45 (Fig. 2, Table 1).

Interestingly, the strain *Dietzia* sp. NDT10 in a nutrient medium (NRM) was able to grow at sodium chloride concentrations not higher than 70 g/L (Fig. 1, Table 1). While the strain NDT10 can effectively grow at higher concentrations of NaCl (up to 125 g/L) in a mineral medium (MCP) with DF as a substrate (Fig. 2, Table 1). The growth characteristics of the strain did not decrease to the concentration of NaCl up to 50 g/L in NRM, when cultivation on MSR with DF the growth did not decrease up to 70 g NaCl/L (Table 1).

It is known that microorganisms are able to grow at higher concentrations of NaCl in the presence of substances with an osmoprotective effect in the cultivation medium — betaine, ectoine, glutamate, etc. (Sleator and Hill 2001; Plakunov, Zhurina and Belyaev, 2008; Hu et al., 2020). In this work, it was noted for the first time that aliphatic hydrocarbons can have a positive effect on the growth of bacteria under high salinity conditions.

When cultivating *Dietzia* sp. NDT10 on the medium with the addition of 30, 50, and 70 g NaCl/L, there is an increase in the DF degradation, in comparison with the variant of the experiment without the addition of NaCl to the medium, by 1.2, 1.3, and 1.4 times, respectively (Fig. 3). A decrease in the degradation of diesel fuel by 4.7 times in comparison with the variant of the experiment without the introduction of NaCl was noted at the content of NaCl in the amount of 125 g/L.

In experimental variants with a positive effect of increased salinity on the DF degradation the decomposition of long-chain hydrocarbons ($C_{15}$–$C_{20}$) by the strain NDT10 increased greater than that of short-chain hydrocarbons ($C_9$–$C_{14}$) (Table 2). For example, in the experiment with the addition of 70 g NaCl/L, in which the greatest decrease in the concentration of hydrocarbons was noted, the content of short-chain hydrocarbons

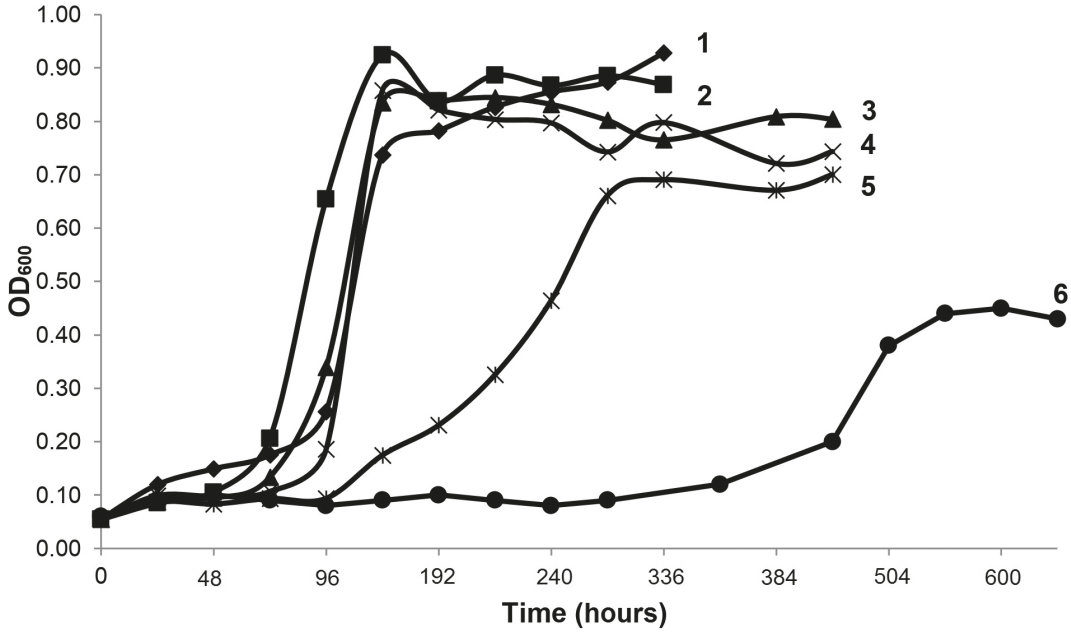


**Fig. 2.** The growth of *Dietzia* sp. strain NDT10 in MRM enriched with diesel fuel (1 g/L) at different NaCl concentrations (g/L): 1 — without NaCl; 2 — 30; 3 — 50; 4 — 70; 5 — 100; 6 — 125. The experiment was performed in triplicate.

**Table 1. The growth of *Dietzia* sp. strain NDT10 in NRM and MRM with DF at different concentrations of NaCl**

| Growth parameters | NaCl concentrations in the medium, g/L | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 30 | 50 | 70 | 100 | 125 |
| The growth of the strain in NRM | | | | | | |
| Specific growth rate, $h^{-1}$ | 0.010 ± 0.002 | 0.010 ± 0.003 | 0.007 ± 0.002 | 0.004 ± 0.001 | — | n. d. |
| Maximum $OD_{600}$ | 1.88 | 2.11 | 1.88 | 0.96 | — | n. d. |
| Lag phase, h | 72 | 72 | 72 | 100 | — | n. d. |
| The growth of the strain in MRM with DF | | | | | | |
| Specific growth rate, $h^{-1}$ | 0.007 ± 0.002 | 0.008 ± 0.001 | 0.008 ± 0.003 | 0.010 ± 0.002 | 0.005 ± 0.002 | 0.003 ± 0.001 |
| Maximum $OD_{600}$ | 0.93 | 0.93 | 0.84 | 0.86 | 0.70 | 0.45 |
| Lag phase, h | 83 | 70 | 70 | 96 | 168 | 432 |

— the growth was not observed; n. d. — no data.

($C_9$–$C_{14}$) decreased by 1.2–1.4 times, and that of long-chain hydrocarbons ($C_{15}$–$C_{20}$) — by 3.9–10.9 times in comparison with the variant of the experiment without adding NaCl.

Currently, there are very few data on the features of degradation of DF by bacteria of the genus *Dietzia* at high mineralization of the medium, while a positive effect of NaCl on DF degradation by representatives of this genus has not been previously revealed. The ability of the strain *Dietzia maris* NWWC4 to degrade diesel fuel hydrocarbons ($C_{10}$–$C_{21}$) under conditions of low temperature (10 °C) and high salinity (5 % NaCl) was found. At the same time, the loss of DF hydrocarbons with salinity after 18 days was 21 %, without salinity — 37 % (Chang, Akbari, David, and Ghoshal, 2018). The bacterial association consisting of strains of *Dietzia*, *Cellulomonas*, *Bacillus* and *Halomonas* species carried out DF degradation at the environment high salinity. The biodegradation of diesel fuel after 53 days was 64 % without salt in the cultivation medium; when the medium contained high salt concentrations (10 % and 17.5 % NaCl), there was no significant decrease in the diesel fuel degradation (62 % and 58 %, respectively) (Riis, Kleinsteuber, and Babel, 2003). For comparison, in our experiments, the DF hydrocarbons degradation by the strain NDT10 after 14 days in a medium without NaCl was 52 %, at a concentration of 30 g NaCl/L — 63 %, 50 g/L — 67 %, 70 g/L — 72 %, 100 g/L — 56 %, 125 g/L — 11 % (Fig. 3).

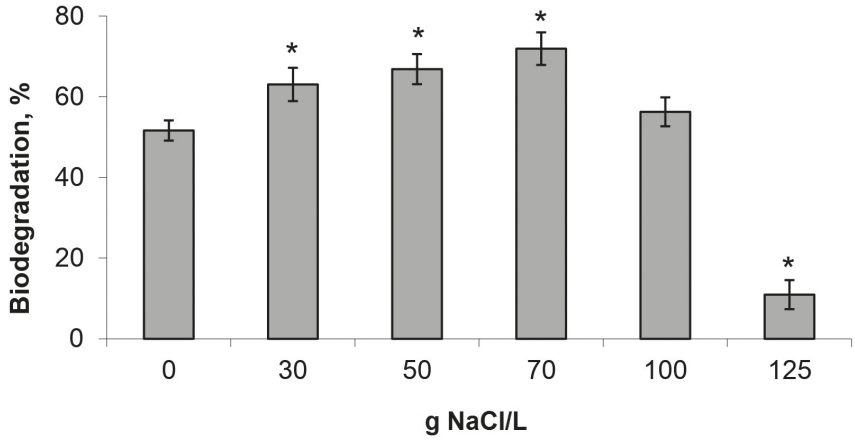


**Fig. 3.** The degradation of diesel fuel by *Dietzia* sp. strain NDT10 after 14 days of cultivation with various concentrations of NaCl. Error bars correspond to standard deviations. (*) Indicates that the difference between the experimental variants with and without the addition of NaCl is significant at the 5 % confidence level.

**Table 2. Content (% of concentration in sterile control samples) of DF hydrocarbons after 14 days of cultivation of the strain *Dietzia* sp. NDT10 in MRM**

| NaCl concentrations in the medium, g/L | The number of carbon atoms in hydrocarbons | | | | | |
|---|---|---|---|---|---|---|
| | 9 | 10 | 11 | 12 | 13 | 14 |
| 0 | 12.2 ± 0.9 | 14.7 ± 3.4 | 20.5 ± 1.2 | 38.9 ± 2.8 | 66.1 ± 4.3 | 64.9 ± 4.7 |
| 30 | 4.4 ± 1.1 | 12.8 ± 3.0 | 19.2 ± 1.6 | 36.3 ± 3.8 | 65.3 ± 5.9 | 63.0 ± 5.4 |
| 50 | 7.7 ± 1.5 | 5.9 ± 3.1 | 19.7 ± 2.1 | 23.1 ± 3.1 | 63.3 ± 5.7 | 63.8 ± 5.6 |
| 70 | 9.1 ± 1.8 | 12.6 ± 2.3 | 17.8 ± 1.9 | 27.9 ± 2.3 | 49.3 ± 2.9 | 47.3 ± 2.1 |
| 100 | 15.8 ± 6.4 | 26.3 ± 4.6 | 26.8 ± 5.9 | 43.4 ± 9.9 | 51.0 ± 9.6 | 53.8 ± 7.1 |
| 125 | 51.2 ± 5.8 | 48.0 ± 4.0 | 69.7 ± 5.4 | 99.8 ± 8.3 | 105.9 ± 12.3 | 100.4 ± 15.2 |
| NaCl concentrations in the medium, g/L | The number of carbon atoms in hydrocarbons | | | | | |
| | 15 | 16 | 17 | 18 | 19 | 20 |
| 0 | 73.2 ± 8.4 | 70.9 ± 2.1 | 75.2 ± 6.8 | 66.6 ± 7.8 | 70.6 ± 5.1 | 103.7 ± 15.4 |
| 30 | 14.1 ± 4.7 | 9.8 ± 2.3 | 8.8 ± 3.0 | 25.5 ± 5.5 | 46.1 ± 5.9 | 38.6 ± 4.0 |
| 50 | 8.0 ± 1.6 | 8.6 ± 1.7 | 11.9 ± 1.2 | 32.3 ± 1.2 | 41.7 ± 6.9 | 21.5 ± 2.6 |
| 70 | 6.7 ± 1.8 | 8.1 ± 2.6 | 8.4 ± 3.2 | 17.1 ± 3.4 | 13.4 ± 3.1 | 15.2 ± 4.1 |
| 100 | 54.3 ± 4.6 | 51.9 ± 4.8 | 54.7 ± 12.7 | 62.6 ± 3.2 | 64.1 ± 4.6 | 82.4 ± 7.3 |
| 125 | 101.0 ± 12.1 | 100.5 ± 11.4 | 100.8 ± 10.2 | 101.9 ± 10.2 | 101.2 ± 8.2 | 106.6 ± 10.3 |

An increase in the petroleum products degradation under the influence of NaCl by the bacteria *Dietzia maris* INMI 101 was noted (Zvyagintseva et al., 2001). The biodegradation of turbine oil by this strain was observed at NaCl concentrations from 0.5 to 7 %, reaching maximum values (the amount of emitted $CO_2$) at a salinity of 5 %.

A study of saturated hydrocarbons ($C_{12}$–$C_{16}$) degradation by *Rhodococcus erythropolis* showed a positive effect of NaCl at concentrations up to 5 % on the biodegradation of long-chain alkanes ($C_{12}$–$C_{16}$), while these NaCl concentrations did not affect the degradation of short-chain alkanes ($C_6$–$C_{11}$) (de Carvalho, 2012). Presumably, this positive effect of NaCl on alkanes degradation was associated with an increase in the cell surface hydrophobicity (de Carvalho, 2012), since an increase in medium salinity increased the concentration of lipids with long-chain fatty acids in bacteria, which cause high cell hydrophobicity (de Carvalho, Fatal, Alves, and da Fonseca, 2007). A similar effect was also observed in the study of the strain NDT10, but at higher concentrations (up to 70 g NaCl/L).

It was found that with an increase in the concentration of NaCl, both in the medium with trypton and yeast

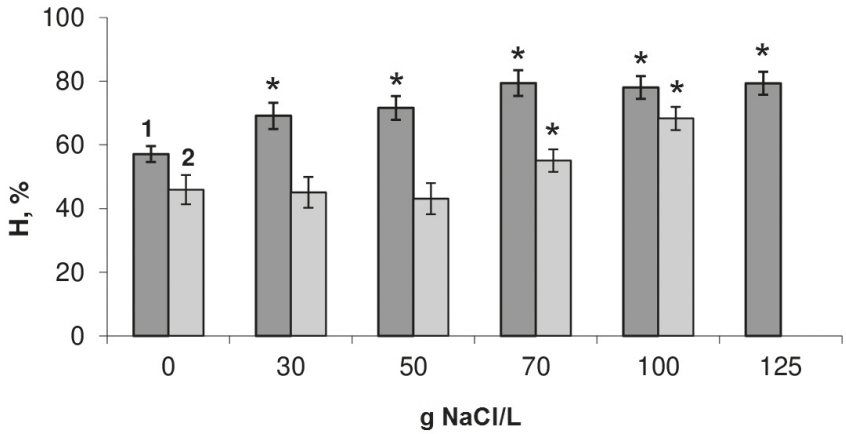


**Fig. 4.** Effect of NaCl concentrations on cell surface hydrophobicity (H, %) of *Dietzia* sp. strain NDT10 after 14 days of cultivation in MRM enriched with diesel fuel (1), in NRM (2). Error bars correspond to standard deviations. (*) Indicates that the difference between the experimental variants with and without the addition of NaCl is significant at the 5 % confidence level.

extract (NRM) and in the MRM with DF, the cell surface hydrophobicity of *Dietzia* sp. NDT10 increased (Fig. 4). Thus, the cell surface hydrophobicity of the strain during cultivation in the medium with DF was by 1.1–1.7 times higher than during cultivation in the NRM. At the same time, the highest values of the cell surface hydrophobicity of the strain were found when cultivated on a medium with the addition of diesel fuel and at concentrations of 70, 100, and 125 g NaCl/L.

Hypersaline ecosystems are widely distributed around the world and include both natural and man-made habitats, such as soda and salt lakes, limans, saline soils (Oren, 2002). To be able to live at high salt concentrations, halophilic and halotolerant bacteria use diverse strategies of adaptation. To maintain turgor and preserve cell viability, most of the salt-tolerant and moderately halophilic bacteria accumulate osmoprotectants in the cytoplasm. These low-molecular-weight substances are amino acids, carbohydrates or their derivatives, sugars, and polyols, that have osmotic activity and are non-toxic to the cells in high concentrations (Shivan and Mugeraya, 2011; Oren, 2013; Banciu and Sorokin, 2013).

Cell surface hydrophobicity is known to affect bacterial membrane permeability. The hydrophilic cell surface promotes the entry of water-soluble substances and prevents the penetration of hydrophobic (water-insoluble) compounds into the cells, whereas the hydrophobic cell surface has the opposite properties (de Carvalho, 2012; de Carvalho, Marques, Hachicho, and Heipieper, 2014). Therefore, the decrease or increase in cell surface hydrophobicity can change the uptake of substances into bacterial cells. This mechanism is likely to be an adaptation to various environmental factors by bacteria, in particular, to salt conditions.

For example, increasing salinity levels in the growth medium has caused a reduction in the cell surface hydrophobicity of *Halomonas elongata* (Hart and Vreeland, 1988), *Erythrobacter* sp. (Longang, Buck, and Kirkwood, 2016), *Exiguobacterium* sp. (Cao et al., 2020). It has been suggested that the hydrophilic cell surface facilitates the entry of water into bacterial cells under saline conditions (Hart and Vreeland 1988; Cao et al., 2020). An increase in the cell surface hydrophobicity during salinization has been found in bacteria from the genus *Rhodococcus* (de Carvalho, 2012; Rubtsova, Kuyukina, and Ivshina, 2012; Longang, Buck, and Kirkwood, 2016) and in soil actinomycetes (Zahir et al., 2016). The cell surface hydrophobicity of halotolerant strains of the genus *Rhodococcus* has been increased with increasing concentration of NaCl in the culture medium, while in non-resistant strains it decreased or did not change depending on the conditions (Rubtsova, Kuyukina, and Ivshina, 2012). In many halotolerant fungi, salt tolerance could be mainly associated with hydrophobization of the cell wall. This reduces the loss of osmoprotectants as a result of diffusion into the medium and increases their concentration in cells (Gori, Mortensen, Arneborg, and Jespersen, 2005; Kogej et al., 2007). Therefore, the mechanisms that underlie the halotolerance of bacteria could be similarly related to an increase in the cell surface hydrophobicity under saline conditions. Likewise, an increase in the cell surface hydrophobicity can enhance microbial salt tolerance by reducing the influx of sodium ions into the cell (Banciu and Sorokin, 2013).

The degree of cell surface hydrophobicity is known to play an important role in adhesion and microbial uptake on hydrocarbons, so many actinobacteria have increased their cell surface hydrophobicity in a similar manner (Bredholt, Bruheim, Potocky, and Eimhjellen, 2002; de Carvalho et al., 2007; Hvidsten, Mjøs, Bødtker, and Barth, 2015). *Dietzia* sp. strain DQ12–45–1b grown on hexatriacontane ($C_{36}$) had a greater cell surface hydrophobicity rate compared to a medium with the addition of hexadecane ($C_{16}$) or tetracosane ($C_{24}$) (Wang

et al., 2013). Changing the bacterial cell hydrophobicity may lead to alterations in biodegradation of hydrocarbons under saline conditions. The positive impact of the salinity in concentrations of NaCl up to 5 % in the growth media in the degradation of alkanes ($C_{12}$–$C_{16}$) by *Rhodococcus erythropolis* has been revealed, presumably by increasing the hydrophobic surface of bacterial cells (de Carvalho, 2012). Herewith, the same level of NaCl had no effect on the degradation of short-chain alkanes ($C_6$-$C_{11}$) with greater solubility in water compared to long–chain alkanes ($C_{12}$–$C_{16}$). On the contrary, under high salinity, *Exiguobacterium* sp. N4–1P has decreased the hydrophobic cell surface, that led to a reduction in microbial degradation of long-chain alkanes and an enhancement of naphthalene degradation (Cao et al., 2020). Therefore, the hydrophobicity of the cell surface can affect both the halotolerance of bacteria and their ability to degrade hydrocarbons.

The highest hydrophobicity of the cell surface of *Dietzia* sp. NDT10 observed during bacterial growth in the MRM containing both DF and NaCl (Fig. 4). As a result, the ability to grow bacteria in the presence of DF was observed at higher concentrations of NaCl (125 g/L) compared to the Raymond's medium with the addition of tryptone and yeast extract (at 70 g NaCl/L) (Figs 1, 2). At 30, 50, and 70 g NaCl/L in the medium microbial degradation of DF was also increased (Fig. 3).

## Conclusion

The strain *Dietzia* sp. NDT10 was capable of growth in a medium with DF up to 125 NaCl g/L with biodegradation of $C_9$–$C_{20}$ hydrocarbon fractions at a salinity range of 0–100 g NaCl/L and the destruction of $C_9$-$C_{11}$ hydrocarbons fractions at a concentration of 125 g NaCl/L. We observed an increase in cell surface hydrophobicity of the studied bacteria with an increase of the concentration of NaCl in the medium, that resulted in better DF degradation at 30, 50 and 70 g NaCl/L. In addition, the strain NDT10 showed a high activity against long-chain hydrocarbons ($C_{15}$–$C_{20}$) in contrast to short-chain ones ($C_9$–$C_{14}$) in the presence of NaCl in the medium. Our results and further studies of hydrocarbon biodegradation by halotolerant bacteria can be implemented to develop the approaches to control biotransformation through an increase of cell surface hydrophobicity after the exposure to NaCl, and bioremediation of contaminated environment under saline conditions.

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