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Evaluation of the Biotechnological Potential of New Bacterial Strains Capable of Phenol Degradation

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Abstract—A number of bacteria, including rhizospheric bacteria, capable of transforming aliphatic and aromatic compounds to varying degrees were isolated from clean soils and soils contaminated with polycyclic aromatic hydrocarbons. Seven bacteria capable of decomposing phenol at a concentration of at least 500 mg/L were selected. Identification by the 16S rRNA gene showed that they belong to the genera *Rhodococcus*, *Pseudomonas*, *Stenotrophomonas*, *Lysinibacillus,* and *Isoptericola*. Determination of the activity of phenol destruction enzymes in these cultures showed the absence of a *meta*-pathway of catechol cleavage during phenol degradation. The activity of catechol 1,2-dioxygenase and protocatechuate 3,4-dioxygenase was detected in cell-free extracts. The activity of the latter enzyme was 15 times higher than the catechol 1,2-dioxygenase, that was described for the first time for members of the genus *Isoptericola*. Strains isolated from the rhizosphere of plants growing in contaminated soil were capable of destroying up to 15 individual pollutants, such as aliphatic hydrocarbons, chlorophenols, 2,4,5-trichlorophenoxyacetic acid, and caprolactam. The presence of enzymes, such as β-galactosidase and lysine-decarboxylase, was shown with the use of biochemical test systems for representatives of the genera *Stenotrophomonas* and *Isoptericola*. The isolated bacteria can be used both to create biopreparations for bioremediation and to create producer strains for the target enzymes.

Keywords: Rhodococcus, *Pseudomonas*, *Stenotrophomonas*, *Lysinibacillus*, *Isoptericola*, phenol, degradation, pollutants

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INTRODUCTION

Aromatic compounds and their derivatives are widespread in nature and lead to significant environmental pollution. Phenol is one of the widespread industrial pollutants. Its sources are petrochemical enterprises. Some phenol enters the environment as a result of the decomposition of plant and animal residues. Phenol and, especially, its derivatives used in various industries, are toxic to animals, humans, and microorganisms. Therefore, biological treatment is difficult for industrial wastewater with a high phenol content. Phenol and its derivatives, both natural and anthropogenic origin, are classified by the Agency for Toxic Substances and Disease Registry (ATSDR) as priority hazardous substances because of their potential toxic, mutagenic, carcinogenic, and teratogenic effects.

Biological purification methods, in particular, microbial biodegradation, have great potential and competitive advantages, which are primarily associated with their environmental safety and lower cost [1]. Many bacteria are capable of phenol destruction, among them gram-negative bacteria of the genera *Pseudomonas*, *Sphingomonas*, *Burkholderia*, *Alcaligenes*, *Acinetobacter*, and *Flavobacterium* and representatives of gram-positive bacteria of the genera *Arthrobacter*, *Nocardia*, *Rhodococcus,* and *Bacillus* [2–6]. Microorganisms decompose toxic aromatic compounds into substrates that are non-hazardous for living organisms and are easily recyclable; therefore, the isolation and characterization of new strains of destructors is a promising area of research to create effective biologics suitable for bioremediation.

The goal of the work was to characterize new soil microorganisms that can degrade aromatic compounds and were isolated isolated from various sources: contaminated and uncontaminated soils in Russia and Kazakhstan.

EXPERIMENTAL

Organisms and cultivation methods. Microorganisms were isolated from soil taken in the vicinity of

Pushchino, Moscow Region (Russia) and from the rhizospheric uncontaminated and contaminated soil of the city of Saratov (Saratov Oil Refinery, Russia) and Kazakhstan. Samples were taken from a depth of 5–10 cm. The soil was used to isolate microorganisms after mixing of the selected samples. The soil samples (5 g) were added to Erlenmeyer flasks with 100 mL of mineral medium of the following composition (g/L): Na_2HPO_4—0.7; KH_2PO_4—0.5; NH_4NO_3—0.75; $MgSO_4 \cdot 7H_2O$—0.2; $MnSO_4$—0.001; $FeSO_4$—0.02, $NaHCO_3$—0.25 containing 100 mg/L benzoate. The samples were cultivated on a shaker (180 rpm) at 28°C for 7 days. Cells were spreaded on Luria Bertani (LB) agarized medium after dilution to 10^{-6}–10^{-8}. Separate colonies differing in morphotype were subcultured on LB medium for further work.

The strains were cultured on a mineral medium to obtain the biomass. The cultures were inoculated in 750-mL flasks containing 200 mL of mineral medium with 0.2 g/L phenol as the sole source of carbon and energy. The bacteria were grown on a shaker at 220 rpm at a temperature of 28°C to an optical density (OD) of 0.7–0.8 units, and 0.2 g/L of phenol was then re-added, since it was consumed by the culture. The culture growth was evaluated spectrophotometrically by the OD at a wavelength of 590 nm, and the presence of phenol was determined by the absorption spectrum in the region of 220–350 nm on a UV-1800 spectrophotometer (Shimadzu, Japan).

Identification of bacteria. The strains were determined based on an analysis of the 16S rRNA genes. The genomic DNA was isolated with the Zymo Reseacher Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Reseach, United States) according to the manufacturer's recommendation. The 16S rRNA gene was amplified by polymerase chain reaction (PCR) using universal primers for 16S rRNA prokaryotes: 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1525r (5'-AAGGAGGTGATCCAGCC-3') [7]. PCR was performed on a My-Cycler, Tetrad 2 instrument (Bio-Rad Laboratories, United States).

Phylogenetic analysis. Primary phylogenetic screening of the obtained sequences was performed with the BLAST program [http://www.ncbi.nlm.nih.gov/blast] and the EzBioCloud database (www.ezbiocloud.net). For phylogenetic analysis, we used 16S rRNA gene sequences taken from the GenBank database (www.ncbi.nlm.nih.gov). The obtained nucleotide sequences of the 16S rRNA gene were manually aligned with sequences of reference strains of the nearest microorganisms with the CLUSTAL W program [8].

Testing of the degradative activity of bacteria in relation to phenol. The strains were cultured in a mineral medium with phenol (initial concentration of 100 mg/L) as a source of carbon and energy. The culture growth was monitored with the measuring of OD at 590 nm. The presence of phenol was controlled by collecting spectral data in the range 220–350 nm. The pH of the medium was maintained in the range of 7.0–7.2 via the addition of NaOH. Cultures utilizing phenol at a concentration of 100 mg/L were reinoculated in fresh mineral medium, and the phenol concentration was increased to 300 mg/L. Cultures capable of growing on phenol at a concentration of 300 mg/L were reinoculated in fresh medium with phenol at a concentration of 500 mg/L.

Determination of the ability of bacteria to utilize various pollutants. Selected bacterial cultures were tested for their ability to utilize various aromatic, aliphatic, and chlorinated compounds, which were added to the mineral medium as the sole source of carbon and energy. The substrates were used in the following concentrations: phenanthrene, anthracene, fluorene, acenaphthene, phenol, caprolactam—0.2–1.0 g/L; salicylate, gentisate, protocatechuate, *o*-phthalate, 2-hydroxycinnamic acid, catechol, benzoate, chlorobenzoates (2-, 3-, 4-chlorobenzoate, 3,5-dichlorobenzoate), 2,4,5-trichlorophenoxyacetic acid –0.2 g/L; chlorophenols (2-, 3-chlorophenol, 2,3-, 2,4-, 2,5-, 2,6-, 3,4-dichlorophenol, 2,3,4-, 2,4,5-, 2, 4,6-trichlorophenol, pentachlorophenol)—0.1 g/L. When strains were grown on an agar medium, the volatile aromatic and aliphatic compounds naphthalene, benzene, toluene, ethylbenzene, hexane, octane, nonane, decane, undecane, dodecane, hexadecane, diesel fuel, camphor, and coumarin were applied to the cover of an inverted Petri dish.

Microscopic research methods. Microscopic studies of the strains were carried out with Nikon Eclipse Ci microscopes (Nikon, Japan) with a ProgRes SpeedXT camera (Jenoptic, Germany).

Determination of the spectrum of utilized substrates by bacterial isolates. API 20 E and CH 50 tests (bioMerieux, France) were used according to the manufacturer's instructions to determine the spectrum of utilized substrates and the activity of the isolate enzymes.

Preparation of cell-free extracts. The cells were destroyed via extrusion disintegration on a Hughes-type press (IBPM-press, Russia) with a working pressure of 3200 kg/cm^2. After disintegration, the cell debris was removed via centrifugation at 10000 *g* (4°C, 30 min) in the presence of trace amounts of DNAase. The supernatant was used as a cell-free extract to determine the enzyme activity. The extract (5–50 μL) was added to 1.0 mL of the reaction mixture. The activity was determined at 25°C, and the reaction was started via the addition of cell-free extract; the OD was determined on a UV-1800 spectrophotometer (Shimadzu, Japan).

Determination of enzyme activity. The activity of catechol 2,3-dioxygenase was determined by the rate of formation of 2-hydroxymuconic semi-aldehyde in a reaction mixture containing 0.25 mM catechol, cell-free extract, and 50 mM Tris-HCl buffer (pH 7.5) (λ = 375 nm, ε = 33400 M^{-1} cm^{-1}) [9].

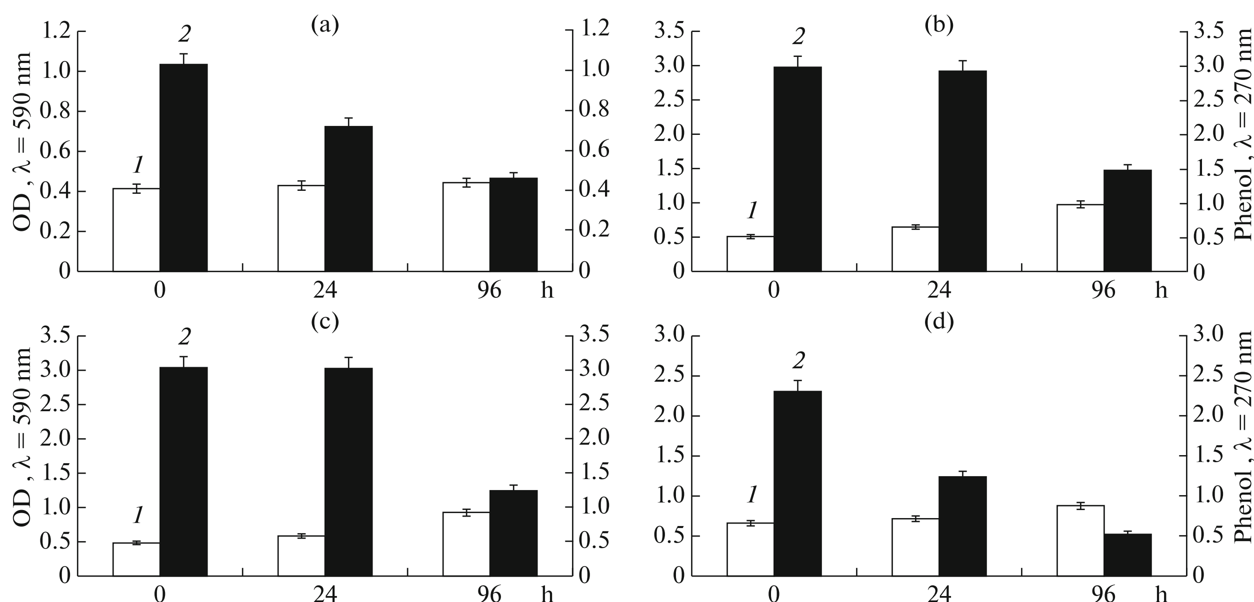

Fig. 1. Change in OD of the cells (*1*) and phenol concentration (*2*) during the growth of the strains *Stenotrophomonas* sp. Fch 8 (a), *Pseudomonas* sp. 13BN (b), *Isoptericola* sp. 8BN (c), *Rhodococcus* sp. 7B (d) in the mineral medium with phenol (phenol concentration 300 mg /L (a) and 500 mg /L (b–d)).

The activity of catechol 1,2-dioxygenase was determined by the rate of *cis,cis*-muconate formation in a reaction mixture containing 5.0 mM Na EDTA, 0.25 mM catechol, cell-free extract, and 50 mM phosphate buffer (pH 7.0) (λ = 260 nm, ε = 16900 M^{-1} cm^{-1}) [9].

The activity of gentisate 1,2-dioxygenase was determined by the rate of the formation of maleyl pyruvate in a reaction mixture containing 0.1 mM gentisate, cell-free extract, and 100 mM of potassium phosphate buffer (pH 7.4) (λ = 330 nm, ε = 10800 M^{-1} cm^{-1}) [10].

The activity of protocatechuate 3,4-dioxygenase was determined via a reduction of protocatechuate extinction in a reaction mixture containing 0.25 mM protocatechuate and cell-free extract in Tris-acetate buffer (pH 7.5) (λ = 290 nm, ε = 2870 M^{-1} cm^{-1}) [11].

The specific activity of the enzymes was expressed in micromoles of the used substrate or the resulting product for 1 min per 1 mg of cellular protein. The protein concentration was determined via spectrophotometry with the modified Bradford method [12].

Statistical processing. The mean values and standard errors of the arithmetic mean were obtained from data from three independent experiments with Microsoft Excel 2007.

RESULTS AND DISCUSSION

Isolation of strains. The method of accumulative cultivation was used to create a collection of strains of destructors of aromatic compounds from samples of different soils. Strains capable of growing on benzoate were isolated from these soils, since this compound is less toxic than phenol and the complete destruction of benzoate and phenol involves the formation of catechol at the first stage, the further transformation of which is carried out by isofunctional enzymes [13]. In this way, ~80 strains were selected. The selected strains were subsequently examined for the ability to utilize phenol as the sole carbon source with its content in the medium from 100 to 500 mg/L. The strains isolated from various contaminated and uncontaminated soils were divided into three groups.

The first group consisted of eight strains isolated from the rhizosphere of plants grown in uncontaminated soil; these strains were designated Fch 1–8. They decomposed phenol at a concentration of 100 mg/L. All strains of this group were subcultured in a mineral medium with an increased phenol concentration of up to 300 mg/L. Strains (Nos. Fch 4–8) capable of degrading phenol at a concentration of 300 mg/L were subcultured into a mineral medium containing phenol at a concentration of 500 mg/L. Of these, three strains were the most active: Fch 5, 7, and 8.

Of the strains isolated from the rhizosphere of plants grown in contaminated soil, one Fg 1 strain capable of decomposing phenol at a concentration of 500 mg/L was selected with the same algorithm from six strains (strains numbers Fg 1, 3-7).

The third group of microorganisms included 17 strains isolated from contaminated soil (Saratov, Saratov Oil Refinery, Russia). From this group, three strains (8BN, 7B, 13BN) were grown in a liquid medium containing 500 mg/L of phenol.

Thus, of the 81 isolated strains, seven were able to decompose phenol at a concentration of 500 mg/L (Fig. 1).

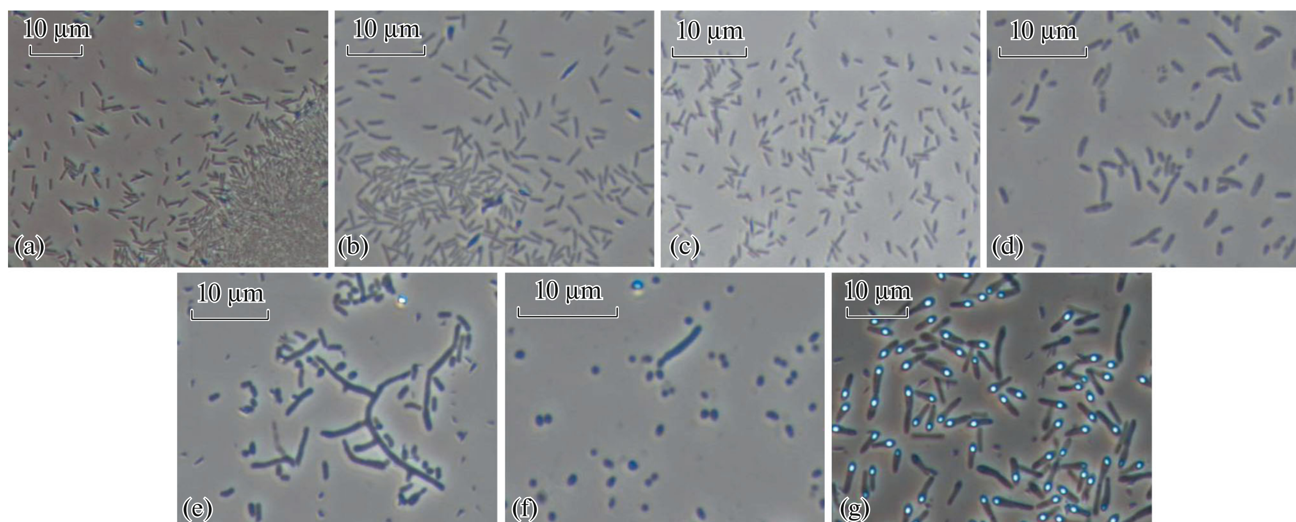

Fig. 2. Phase contrast microscopy of isolated strains: *Stenotrophomonas* sp. Fch 5 (a), *Stenotrophomonas* sp. Fch 7 (b), *Stenotrophomonas* sp. Fch 8 (c), *Pseudomonas* sp. 13BN (d), *Rhodococcus* sp. 7B (e) *Isoptericola* sp. 8BN (f), *Lysinibacillus* sp. Fg 1 (g).

Systematics. Analysis of the 16S rRNA gene showed that the selected cultures can be attributed to the following phylogenetic groups: strain 7B, genus *Rhodococcus*; strain 13BN, genus *Pseudomonas*; strains Fch 5, 7, and 8, genus *Stenotrophomonas*; strain Fg 1, genus *Lysinibacillus* sp.; and strain 8BN, genus *Isoptericola*.

Cell morphology. Microscopic studies have made it possible to determine the characteristic morphological features of strain cells (Fig. 2). The cells of culture 7B, which was isolated from contaminated soil, are represented by long, branching, motionless bacilli characteristic of *Rhodococcus*. The cells of strain 13BN are represented by medium rods 2–3 nm long; a distinctive feature of the culture growth is the formation of so-called “gas bubbles” on the colony growth surface, which we identified via phase-contrast microscopy, and requires further study. Strains Fch 5, 7 and 8 are thin rods 1–2 nm long, and strain 8BN is small cocci. In one of the phases of their development, these cocci are in the form of long rods (3–4 nm long) that can be crushed to form small cocci. The cells of the strain Fg 1 are of a bacillary form. They produce terminally located spores and form a “club-shaped” cell shape (Fig. 2).

Biochemical properties. API 32E and 50CH tests (bioMerieux, France) were used to determine the physiological and biochemical characteristics of the cultures. The studies of selected strains revealed the following features (Table 1).

Almost all of the studied strains utilized several organic compounds. The exception was strain *Isoptericola* sp. 8BN, which was able to utilize many of the studied substrates, including N-acetylglucosamine, the main component of the bacterial cell wall. The strain *Lysinibacillus* sp. Fg 1, which grew only on medium with N-acetylglucosamine, possessed the same property. This fact indicates the potential antimicrobial activity of these strains.

Strains Fch 5, 7, and 8 of the genus *Stenotrophomonas* and *Isoptericola* sp. strain 8BN synthesized β-galactosidase; two of them, Fch 5 and 8, also showed lysine-decarboxylase activity, and the *Pseudomonas* sp. strain 13BN showed arginine-hydrolase activity.

Degrading potential of selected cultures. The study of the characteristics of selected strains showed that they utilized to a greater extent such substrates as benzoate, phenol, naphthalene, and *n*-alkanes (the number of carbon atoms C6–C16) (Table 2). More than half of them grew on these compounds.

A much smaller number of strains could utilize benzene and its derivatives, toluene and ethylbenzene, as well as chlorine-containing phenols and benzoates. Most strains did not use these substrates. The strains, designated Fch 8 and Fg 1, grew on pentachlorophenol, a compound that the Stockholm Convention included in the list of persistent organic pollutants (POPs). Conversely, strains Fch 5 and Fg1 isolated from the rhizosphere of plants grown in clean and polluted soil, respectively, showed the ability to degrade more than 15 compounds, including those related to POPs (strain Fig. 1).

Activity of phenol-degradation enzymes. In the cells of the four cultures that grew most actively on phenol (Fch 8, 13BN, 8BN and 7B), the activity of the enzymes involved in the decomposition of phenol was determined: catechol 1,2-dioxygenase (Cat 1,2-DO), catechol 2,3-dioxygenase (Cat 2,3-DO), protocatechuate 3,4-dioxygenase (PCA 3,4-DO), muconate cycloisomerase (MCI) and gentisate 1,2-dioxygenase (GDO) (Table 3).

It was found that all of the studied strains showed Cat 1,2-DO activity, amounting to 0.08–0.16 units/mg

Table 1. Biochemical properties of studied strains

Indicator*	Strains						
	7B	8BN	13BN	Fch 7	Fch 8	Fg 1	Fch 5
Characteristic*							
β-galactosidase	–	+	–	+	+	–	+
Arginine dydrolase	–	–	+	–	–	–	–
Lysine decarboxylase	–	–	–	–	+	–	+
Urease	+	–	+	+	+	–	+
Citrate utilization	+	–	–	–	–	–	+
Liquefaction of gelatin	–	–	–	–	+	–	+
Reduction of N_2	–	–	–	+	+	+	+
Substrate utilization **							
L-arabinose	–	+	+	–	–	–	–
D-xylose	–	+	+	–	–	–	–
D-galactose	–	+	+	–	–	–	–
D-glucose	–	+	–	–	–	–	–
D-fructose	+	+	–	–	–	–	–
D-mannose	–	+	+	–	+	–	–
Inositol	+	–	–	–	–	–	–
D-mannitol	+	–	–	–	–	–	–
D-sorbitol	+	–	–	–	–	–	–
Eskulin	+	+	–	+	+	–	+
N-acetylglucosamine	–	+	–	–	–	+	–
Amygdalin	–	+	–	–	–	–	–
Arubutin	–	+	–	–	–	–	–
Salicin	–	+	–	–	–	–	–
D-cellobiose	–	+	–	–	–	–	–
D-maltose	–	+	–	+	+	–	+
D-lactose	–	+	–	–	–	–	–
D-melibiosis	–	+	–	–	–	–	–
D-sucrose	–	+	–	–	–	–	–
D-trehalose	–	+	+	–	–	–	–
D-melecitosis	–	+	–	–	–	–	–
D-raffinose	–	+	–	–	–	–	–
Amidone (starch)	–	+	–	–	–	–	–
Glycogen	–	–	–	–	–	–	–
Gentiobiose	–	+	–	–	–	–	–
D-turanose	–	+	–	–	–	–	–
D-tagatose	–	–	+	–	–	–	–
D-Arabite	+	–	–	–	–	–	–

*—negative reaction; +—positive reaction.
** All studied strains showed a negative reaction to the formation of H_2S and indole, the Foges-Proskauer reaction and utilization of glycerol, erythritol, D-arabinoses, D-ribose, D-xylose, D-adonitol, methyl-β D-xylopyranoside, L-sorbose, L-ramnose, dulcitol, methyl-α D-mannopyranoside, methyl-α D-glucopyranoside, inulin, xylitol, D-lyxose, D-fucose, L-arabitol, 2-ketogluconate, 5-ketogluconate.

Table 2. Growth of isolated strains on single substrates

Strain	Isolation source	Growth substrate
Stenotrophomonas sp. Fch 5	Rhizosphere of plants grown in uncontaminated soil	Caprolactam, phenol, benzoate, salicylate, gentisic acid, octane, nonane, decane, hexadecane, dodecane, undecane, 2-chlorophenol, 2,4-dichlorophenol, 2,6-dichlorophenol, 2,4,6-trichlorophenol
Stenotrophomonas sp. Fch 7	Rhizosphere of plants grown in uncontaminated soil	Phenol, 2-chlorophenol
Stenotrophomonas sp. Fch 8	Rhizosphere of plants grown in uncontaminated soil	Phenol, pentachlorophenol, 2,5-dichlorophenol
Lysinibacillus sp. Fg 1	Rhizosphere of plants grown in contaminated soil	Caprolactam, phenol, benzoate, octane, nonane, decane, hexadecane, dodecane, undecane, 2-chlorophenol, 3-chlorophenol, pentachlorophenol, 2,5-dichlorophenol, 2,6-dichlorophenol, 2,4,5-trichlorophenoxyacetic acid, 2, 4,6-trichlorophenol, protocatechuic acid
Pseudomonas sp. 13BN	Contaminated soil (Saratov Oil Refinery, Russia)	Phenol, decane, undecane
Isoptericola sp. 8BN	Contaminated soil (Saratov Oil Refinery, Russia)	Phenol
Rhodococcus sp. 7B	Contaminated soil (Saratov Oil Refinery, Russia)	Caprolactam, phenol, benzoate, octane, nonane, decane, hexadecane, dodecane, undecane

Table 3. Activity (units/mg protein) of aromatic degradation enzymes

Strain	Cat 1,2-DO	Cat 2,3-DO	PCA 3,4-DO	MCI	GDO
Stenotrophomonas sp. Fch 8	0.086 ± 0.009	0.001 ± 0.001	<0.001	0.006 ± 0.002	≤0.001
Pseudomonas sp. 13BN	0.089 ± 0.011	0.002 ± 0.001	1.355 ± 0.121	0.079 ± 0.016	≤0.001
Isoptericola sp. 8BN	0.159 ± 0.028	0.009 ± 0.002	1.098 ± 0.025	0.097 ± 0.049	≤0.001
Rhodococcus sp. 7B	0.122 ± 0.029	<0.001	2.014 ± 0.185	0.046 ± 0.02	≤0.001

of protein, while the activity of PCA 3,4-DO in cell-free extracts of strains 13BN, 8BN, and 7B was almost 15 times higher in comparison with the activity of Cat 1,2-DO. In strain Fch 8, no PCA 3,4-DO activity was detected. MCI activity was also found only in cell-free extracts of strains 13BN, 8BN, and 7B and averaged 0.07 units/mg protein. The activities of the Cat 2,3-DO and GDO enzymes were not found in cell-free extracts of any of the studied strains. No activity of ornithine decarboxylase and tryptophan deaminase were also found in cellular homogenates.

From the collection of benzoate destructor strains, we selected seven strains capable of utilizing phenol at a concentration of up to 500 mg/L. Phenol was chosen to create a collection of destructors, since it is a widespread toxic compound, the content of which in drinking water and air should be strictly controlled [14, 15]. The isolated bacterial strains, which were capable of phenol degradation at concentrations above 500 mg/L, are of significant biotechnological interest.

We also determined the ability of the selected strains to utilize a number of toxic organic compounds and showed that two rhizospheric strains, Fch 5 and Fg 1, are able to grow on more than 15 substrates, while one of the strains was isolated from uncontaminated soil (Fch 5). The studied strains belong to different phylogenetic groups: gram-negative—*Stenotrophomonas* sp. Fch 5, Fch 7, and Fch 8 and *Pseudomonas* 13BN; gram-positive—*Rhodococcus* sp. 7B, *Lysinibacillus* sp. Fg 1, and *Isoptericola* sp. 8BN. There are few descriptions in the literature of representatives of the last two genera as destructors of toxic aromatic compounds. Thus, the ability to grow on crude oil as the sole source of carbon and energy was found in 2018 for the bacteria *Isoptericola chiayiensis* [16]. Comparison of the substrate profile of the previously described strains and the strain isolated by us showed their similarity, but, unlike the previously isolated strains, 8BN demonstrated β-galactosidase activity and the ability to utilize N-acetylglucosamine [17–19].

Determination of the activity of the main enzymes involved in the degradation of aromatic compounds showed that the studied strains demonstrated Cat 1,2-DO activity, while the activity of PCA 3,4-DO was determined in strains 13BN, 8BN, and 7B, which was 15 times higher than the activity of Cat 1,2-DO. The simultaneous presence of Cat 1,2-DO and PCA 3,4-DO is found in bacteria of various genera, e.g., bacterial cells *Pseudomonas putida* KT2440 grown on benzoate, which were found to have high activity of Cat 1,2-DO and traces of PCA 3,4-DO [20], *Acinetobacter baumanii* DU202 cells grown on *p*-hydroxybenzoic acid [21], and some representatives *Amycolatopsis* and *Streptomyces* upon the disposal of benzoate [22]. In the *Rhodococcus* sp. RHA1 strain activities of the Cat 1,2-DO and PCA 3,4-DO were detected simultaneously with growth on benzoate and phthalate [23], but it was described for the first time for representatives of the genus *Isoptericola*. The high activity of the PCA 3,4-DO enzyme may indicate that the phenol-degradation process proceeds mainly via the formation of a protocatechuate, which is not characteristic of bacteria. Another possible explanation is the multiplicity of pathways for the degradation of aromatic compounds in bacteria that have not been adapted to grow on target substrates for a long time.

Biochemical test systems for the genera *Stenotrophomonas* and *Isoptericola* qualitatively showed the presence of activity of enzymes such as β-galactosidase and lysine decarboxylase. These enzymes are obtained in industry, including with the help of microorganisms; therefore, the studied strains can be used not only for bioremediation but also as a source of these enzymes.

The *Lysinibacillus* sp. strain Fg 1 is capable of utilizing *N*-acetylglucosamine, the main component of the bacterial cell wall. Analysis of the literature data on substrate profiles of strains of the genus *Lysinibacillus* showed that this feature was not previously shown and is a distinctive characteristic of the isolated strain [24, 25]. The literature describes examples of the antimicrobial and antifungal activity of strains of this genus, including activity against parasitic plant fungi [26]; thus, further study will reveal its biotechnological potential. It should be emphasized that, as a spore-forming bacterium, the Fg 1 strain is capable of surviving in adverse conditions; remaining in the form of spores, it can be transported in a dry form. Therefore, it is promising for the creation of biological products for cleaning areas remote from its production sites.

The problem of the disposal of compounds related to persistent organic pollutants is relevant. New compounds are gradually added to the primary list defined by the Stockholm Convention in 2001. The strains Fch 8 and Fg 1 are capable of utilizing pentachlorophenol, a compound related to POPs, and are potentially significant for the creation of biological products for the cleaning of territories exposed to POPs.

CONCLUSIONS

In general, we can say that the isolated strains are valuable for use in various branches of biotechnology. Their further study is necessary for a deeper characterization of the properties already studied and the identification of new features.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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