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EXPERIMENTAL ARTICLES

Bacterial Degraders of Polycyclic Aromatic Hydrocarbons Isolated from Salt-Contaminated Soils and Bottom Sediments in Salt Mining Areas

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Abstract—Fifteen bacterial strains capable of utilizing naphthalene, phenanthrene, and biphenyl as the sole sources of carbon and energy were isolated from soils and bottom sediments contaminated with waste products generated by chemical- and salt-producing plants. Based on cultural, morphological, and chemotaxonomic characteristics, ten of these strains were identified as belonging to the genera *Rhodococcus, Arthrobacter, Bacillus*, and *Pseudomonas*. All ten strains were found to be halotolerant bacteria capable of growing in nutrient-rich media at NaCl concentrations of 1–1.5 M. With naphthalene as the sole source of carbon and energy, the strains could grow in a mineral medium with 1 M NaCl. Apart from being able to grow on naphthalene, six of the ten strains were able to grow on phenanthrene; three strains, on biphenyl; three strains, on octane; and one strain, on phenol. All of the strains were plasmid-bearing. The plasmids of the *Pseudomonas* sp. strains SN11, SN101, and G51 are conjugative, contain genes responsible for the degradation of naphthalene and salicylate, and are characterized by the same restriction fragment maps. The transconjugants that gained the plasmid from strain SN11 acquired the ability to grow at elevated NaCl concentrations. Microbial associations isolated from the same samples were able to grow at a NaCl concentration of 2.5 M.

Key words: polycyclic aromatic carbohydrates, biodegradation, halotolerance, plasmids, bacteria.

Polycyclic aromatic hydrocarbons (PAHs), which are present in petroleum, petroleum products, and sewages of the coking and petrochemical industries, are abundant environmental pollutants. Many PAHs are carcinogenic and mutagenic, which makes their cleanup especially challenging. One of the approaches to the bioremediation of PAH-polluted areas is the use of microbial degraders. In connection with this, there is increasing research interest in the degraders of naphthalene [1] and higher molecular weight PAHs, such as phenanthrene and pyrene [2].

The introduction of microbial degraders to contaminated soils for the purpose of bioremediation [3] is sometimes ineffective. One of the reasons for this is a high concentration of salts in the soil, a fact that has stimulated interest in natural halotolerant microbial degraders [4, 5].

It is obvious that such degraders can be isolated from the areas that are heavily polluted with salts, such as the Berezniki area in Perm oblast, where several large chemical and salt producing plants are situated. These plants pollute the environment with both PAHs and salts, which poses the challenge to search for microbial communities and particular bacterial species that are capable of degrading various xenobiotics in the presence of high salt concentrations.

The aim of the present work was to isolate and study PAH degraders from salt-contaminated soils near a salt waste pile, from the bottom sediments of a slime-settling pit (the OAO Uralkalii salt plant, Berezniki), and in the neighboring Zyryanka River.

MATERIALS AND METHODS

Sampling, enrichments, and the isolation of PAH degraders. Soil samples were taken at distances of 1, 10, 50, 300, and 500 m from a rock salt waste pile at a depth of 5–10 cm from the soil surface at the OAO Uralkalii salt plant in Berezniki. The content of NaCl in the soil samples varied from 1 to 9 mg per g of dry soil. Sediment samples were taken from the surface layer of sludges in a slime-settling pit and in the nearby Zyryanka River. The content of NaCl in the bottom sediment samples varied from 7 to 31 mg per g of dry matter. Samples taken directly from the rock salt (halite) waste pile contained about 790 mg NaCl per g of sample. The pH of all the samples ranged from 6.1 to 7.1.

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Table 1. Strains isolated from salt-contaminated soils and bottom sediments

Sample	Location of sampling site	Strain		
		naphthalene*	biphenyl*	phenanthrene*
Soil 1	1 m from a salt waste pile	SN11	SB13*	SF14**
		SN17		
Soil 10	10 m from a salt waste pile	SN101	SB102	
Soil 50	50 m from a salt waste pile	SN501	SB502	SF503**
Soil 300	300 m from a salt waste pile nearby a slime pit	SN31		SF32
Soil 500	500 m from a salt waste pile nearby an automobile bridge	SN21		SF27
Bottom sediment 1	Zyryanka River	DN13	DB11	DF14
Bottom sediment 2	Slime pit outlet	DN20	DB22**	DF23
		DN21**		

*Substrate used for enrichment culture.

**Strains representing mixed cultures.

To obtain enrichment cultures, the samples (1 g) were placed in flasks with 50 ml of cultivation medium K1 containing either naphthalene, phenanthrene, or biphenyl as the sole source of carbon and energy, and were incubated at 28°C for 1 to 4 months. In these experiments, three types of cultivation media were used: Raymond medium [6] containing 0.5 to 5 M NaCl; modified LB broth containing 5 g/l tryptone, 2.5 g/l yeast extract, and 150 g/l NaCl; and NaCl-free K1 mineral medium. Actively growing enrichments were plated on agar media to obtain PAH degraders in pure cultures. The purity of cultures was tested by plating them on nutrient agar.

Strains G10 and G51 were previously isolated from soils contaminated with halogenated aromatic compounds (the AO Halogen, Perm) using the enrichment medium K1 with naphthalene as the sole source of carbon and energy.

Growth characteristics were studied by growing PAH degraders at 28°C in liquid or on agar-solidified K1 mineral and Raymond media containing growth substrates at concentrations of 0.5 to 1 g/l. A modified Raymond medium containing 5 g/l of tryptone and 2.5 g/l of yeast extract was used as the nutrient-rich medium. During cultivation on agar media, naphthalene, biphenyl, phenol, and octane were placed on the lids of inverted petri dishes. Growth on phenanthrene was studied using only liquid media containing 0.5 g/l of this compound. The optical density (OD) of the cultures was measured at 540 nm using an FEK-56M photoelectrocolorimeter and 0.5-cm-pathlength cuvettes. The number of viable cells was determined by the serial dilution method using nutrient agar plates. The results were expressed as colony-forming units (CFU).

Identification of microorganisms. The life cycle and morphology of microorganisms were studied by growing them on the respective agar media and then examining them by phase-contrast light microscopy [7].

To study their chemotaxonomic characteristics, gram-positive bacteria were grown aerobically in a medium containing (g/l) glucose, 5; peptone, 5, yeast extract, 3; and K_2HPO_4, 0.2 (pH 7.2–7.4). The compositions of the cell wall amino acids and sugars, peptidoglycan amino acids, the isoprenoid quinones of the respiratory chain, and mycolic acids, were determined as described earlier [8]. Amino acids were quantified on a Hitachi amino acid analyzer (Japan). Menaquinones were analyzed using a model MAT 8430 mass spectrometer (Germany).

Bacteria were identified by conventional methods using the identification criteria of *Bergey's Manual* [9, 10].

Plasmid DNA was isolated by the method of Birnboim and Doly [11] with minor modifications. Plasmid size was determined using plasmids of known size as standards.

Restriction and electrophoresis were carried out using restriction endonucleases purchased from Amersham (United Kingdom), according to the instructions described in [12].

Conjugal transfer and plasmid mobilization to the recipient strain *Pseudomonas putida* BS394 (*nah*$^-$ *sal*$^-$*cys*$^-$) were performed after the method of Dunn and Gunsalus [13] with minor modifications.

Strain curing was carried out by eliminating plasmids with mitomycin C, as described by Rheinwald *et al.* [14].

The genetic stability of PAH degraders under nonselective conditions was studied by subculturing their clones in LB broth. For this purpose, material taken from individual colonies was incubated in 5 ml of the broth up to the late logarithmic phase (24 h of growth). The culture obtained was then subcultured ten times by the successive transfer of 50 μl of culture broth into 5 ml of fresh medium every 24 h. The third, seventh, and tenth subcultures were analyzed by plating them on LB agar and then replicating 100 grown colo-

Table 2. Characterization of the PAH-degrading strains isolated from salt-contaminated soils and bottom sediments

Strain	Growth substrate									Plasmid size, kb
	naphthalene	biphenyl	phenanthrene	salicylate	*o*-phthalate	gentisate	octane	1-hydroxy-2-naphthoate	5-methylsalicylate	
Pseudomonas sp. SN11	+	–	±*	+	–	–	–	+	+	~85
Pseudomonas sp. SN21	+	–	±*	+	–	–	–	±	+	~85
Pseudomonas sp. SN101	+	–	±*	+	–	–	–	+	+	~85
Pseudomonas sp. G51	+	–	±*	+	–	–	–	±	+	~85
DN13 (unidentified)	+	–	±*	+	–	–	–	+	+	~85, +?
Rhodococcus sp. SN31	+	±	–	+	–	+	–	–	±	~100
Rhodococcus sp. DB11	+	+	–	+	+	+	+	–	±	ND
Rhodococcus sp. G10	+	+	–	+	+	+	–	–	–	~85, ~60, and 3 small
Arthrobacter sp. SN17	+	±	–	+	+	+	+	–	+	~85
Arthrobacter sp. DF14	+	–	+	+	+	+	–	+	–	~120
Arthrobacter sp. SF27	+	–	+	+	+	+	–	±	–	~120
Bacillus sp. SN501	+	–	–	+	+	–	+	–	–	ND

Note: "ND" stands for "not detected". "+", "±", and "–" stand for "good growth", "poor growth", and "no growth", respectively.
*Growth was evaluated after 2-week incubation with phenanthrene.

nies on the respective selective media containing succinate, salicylate, 1-hydroxy-2-naphthoate, or naphthalene as the sole source of carbon and energy. The genetic stability of the PAH degraders was defined as the percentage of clones retaining their ability to grow on particular growth substrates in the process of the aforesaid multiple reinoculations.

RESULTS

Isolation of PAH-degrading strains. Several enrichment cultures that we recovered from salt-contaminated soil and sediment samples differed in their sources of carbon and energy and in the concentration of NaCl in the media used for their recovery.

In the first set of these experiments, we attempted to recover PAH-degrading bacteria from samples incubated in Raymond medium containing NaCl at different concentrations (from 0.5 to 5 M) and either naphthalene or phenanthrene as the sole source of carbon and energy. Weak growth was observed in the enrichments that were obtained by incubating soil sample 300, bottom sediment 2 (Table 1), and a halite waste sample in the medium containing naphthalene and NaCl at concentrations of 1 and 2.5 M. However, we failed to obtain PAH degraders from these enrichments in pure cultures.

In the second set of experiments, we incubated bottom sediment and halite waste samples in modified LB broth (see the *Materials and Methods* section) and succeeded in the obtaining several pure bacterial cultures that grew in LB broth with 3 M NaCl. Some of these halophilic cultures grew on the aliphatic hydrocarbon octane; however, none of them could grow on naphthalene as the sole source of carbon and energy.

In the third set of experiments, we incubated soil and bottom sediment samples in K1 medium without NaCl (the enrichments surely contained some amounts of salt present in the samples) and obtained 5 mixed and 15 pure bacterial cultures that were capable of utilizing particular PAHs as the sole source of carbon and energy (Table 1). The greatest number of PAH-degrading isolates were obtained from the enrichments incubated with naphthalene. Ten of the isolates obtained in this set of experiments were chosen for further analysis. Among these, seven isolates (strains SN11, SN101, SN21, SN17, SN501, SN31, and DN13) were obtained from the enrichments incubated with naphthalene as the sole source of carbon and energy; two isolates (strains DF14 and SF27), from the enrichments incubated with phenanthrene; and one (strain DB11) from the enrichment incubated with biphenyl.

Identification of PAH degraders. Two of the ten isolates, SN31 (VKM Ac-2067) and DB11 (VKM Ac-2066), were classified as gram-positive *Rhodococcus* spp. They produced pinkish or pink–cream dull colonies; young cultures were dominated by irregular long rods or hyphae fragmented by senescence into short rod-shaped cells and cocci. The whole-cell hydrolysate contained *meso*-diaminopimelic acid, arabinose, and galactose; their mycolic acids were found to be typical of the genus *Rhodococcus*.

Three isolates (strains SF27, DF14, and SN17) were assigned to the genus *Arthrobacter* [10]. They produced colorless or yellowish concave colonies and had the life cycle (coccus–rod–coccus) and types of pepti-

Table 3. Growth of PAH-degrading strain at different NaCl concentrations in Raymond medium with tryptone plus yeast extract or naphthalene as the growth substrates

Strain	Growth on tryptone plus yeast extract at a NaCl concentration of		Growth on naphthalene at a NaCl concentration of		
	1.0 M	1.5 M	0.6 M	0.8 M	1.0 M
SN11	+	±	+	+	+
SN21	+	–	+	+	+
SN101	+	±	+	+	+
DN13	±	–	+	+	±
SN31	+	+	+	+	+
DB11	±	–	+	+	±
SN17	+	+	+	+	+
DF14	±	–	+	+	±
SF27	±	–	+	+	±
SN501	±	–	±	–	–
G51	+	–	+	+	+
G10	±	±	+	+	±

Note: "+", "±", and "–" stand for "good growth", "poor growth", and "no growth", respectively.

doglycan and menaquinones typical of arthrobacters. The amino acid composition of peptidoglycan (lysine, alanine, and glutamic acid at a ratio of 1 : 4 : 1) and the predominant menaquinone (MQ-9(H_2)) allowed strains SF27 (VKM Ac-2063) and DF14 (VKM Ac-2064) to be assigned to the *A. globiformis* group of species. Based on the amino acid composition of peptidoglycan (lysine, alanine, and glutamic acid at a ratio of 1 : 3 : 2) and the prevalence of MQ-8 among the respiratory chain menaquinones, strain SN17 (VKM Ac-2065) was assigned to the *A. nicotianae* group of species [15].

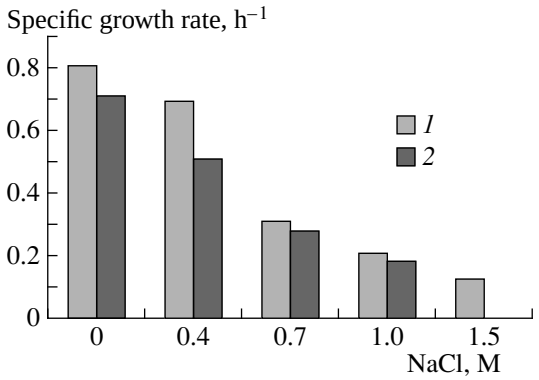

Fig. 1. Specific growth rates of *P. putida* strain SN11 during its batch cultivation at different NaCl concentrations in Raymond medium with (*1*) tryptone plus yeast extract or (*2*) naphthalene as the growth substrates.

Strain SN501, which represents a gram-positive rod-shaped endospore-forming bacterium, was identified as *Bacillus* sp. The cultural and morphological characteristics of the gram-negative strains SN11, SN21, and SN101, were found to be similar to two *Pseudomonas* species, *P. fluorescens* (strain SN21) and *P. putida* (strains SN11 and SN101). The gram-negative strain DN13 was not identified at the species level.

Metabolic characterization of PAH degraders. The ten isolates under study were tested for their ability to grow on naphthalene, phenanthrene, biphenyl, and their derivatives and metabolites, as well as on some aliphatic and monocyclic hydrocarbons (Table 2). All of these strains were found to be able to grow on naphthalene and salicylate, which is an intermediate of naphthalene catabolism. Five gram-positive strains, *Rhodococcus* sp. DB11, *Rhodococcus* sp. SN31, *Arthrobacter* sp. SN17, *Arthrobacter* sp. SF27, and *Arthrobacter* sp. DF14, grew on gentisate, which agrees with the data of Schmitz *et al.* [16] that bacteria of the genus *Rhodococcus* commonly degrade naphthalene through gentisate [16]. The ability of some strains to grow on 5-methylsalicylate suggests that they possess the functional metacleavage pathway of catechol metabolism [1].

Apart from being able to grow on naphthalene and salicylate, strains *Rhodococcus* sp. DB11, *Arthrobacter* sp. SN17, and *Arthrobacter* sp. SN31 grew on biphenyl as the sole source of carbon and energy. One of these strains, DB11, which was isolated from the enrichment that was incubated in the presence of biphenyl, was also able to grow on gentisate, *o*-phthalate, and octane (Table 2). Two other biphenyl degraders, SN17 and SN31, which were isolated from the enrichment incubated in the presence of naphthalene, grew on biphenyl more poorly than strain DB11.

Among the ten strains under study, three strains (*Rhodococcus* sp. DB11, *Bacillus* sp. SN501, and *Arthrobacter* sp. SN17) grew on octane, and six strains grew on phenanthrene (Table 2). The gram-negative strains *Pseudomonas* sp. SN11, *Pseudomonas* sp. SN101, *Pseudomonas* sp. SN21, and strain DN13, acquired the ability to grow on phenanthrene (as well as on its intermediate, 1-hydroxy-2-naphthoate) only after long-term cultivation on this PAH.

Two other phenanthrene degraders, *Arthrobacter* sp. DF14 and *Arthrobacter* sp. SF27, which were isolated from the enrichment that was incubated in the presence of phenanthrene (Table 1), grew on this PAH more efficiently than the other pseudomonads. These strains were also able to utilize 1-hydroxy-2-naphthoate, salicylate, *o*-phthalate, and gentisate (Table 2).

Growth of PAH degraders at high salt concentrations. The effect of NaCl on PAH degraders was studied upon their growth either in nutrient-rich medium or in a mineral medium with naphthalene as the sole source of carbon and energy. All of the strains studied were able to grow without NaCl in the growth medium

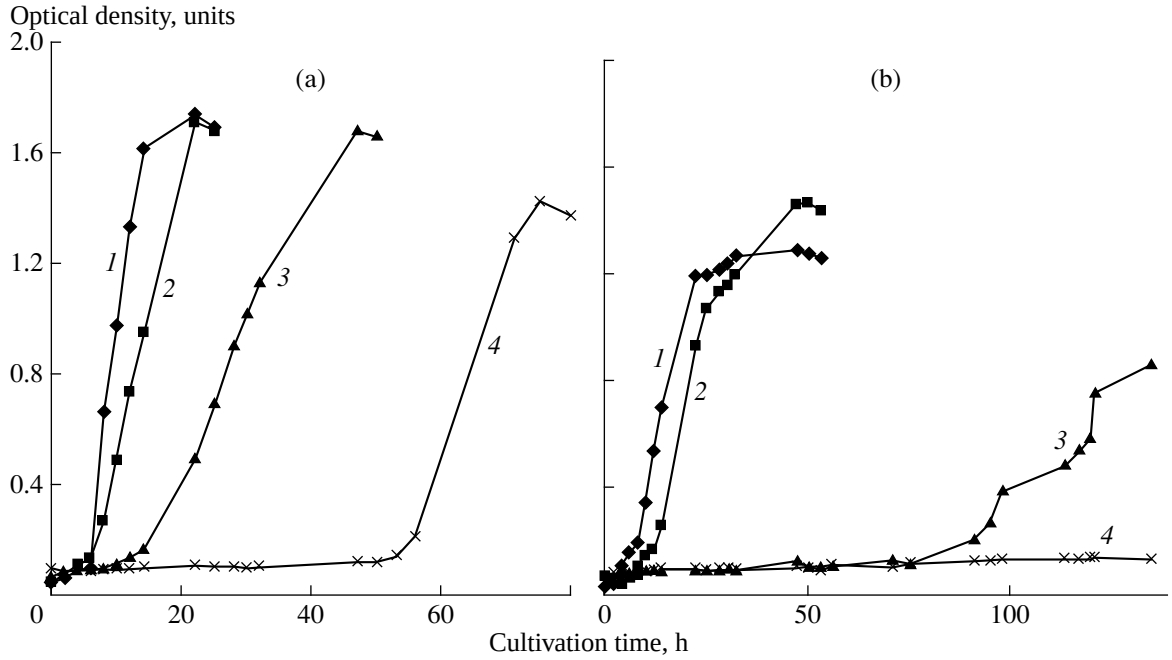

Fig. 2. Growth of *P. putida* strain SN11 on (a) tryptone plus yeast extract or (b) naphthalene at different NaCl concentrations (M): *1*, 0; *2*, 0.4; *3*, 0.7; and *4*, 1.5.

and at high salt concentrations (Table 3). In nutrient-rich medium, PAH degraders could grow at a NaCl concentration of 1 M (all strains) and 1.5 M (strains SN11, SN17, SN31, and SN101). Therefore, they can be considered to be moderately halotolerant bacteria [4, 17]. All but one of the strains (*Bacillus* sp. SN501) grew on naphthalene as the sole source of carbon and energy in the presence of 1 M NaCl.

The effect of NaCl on bacterial growth was studied in more detail using the naphthalene degrader *P. putida* SN11, which was cultivated in nutrient-rich medium (Raymond medium supplemented with 5 g/l tryptone and 2.5 g/l yeast extract) and Raymond medium with naphthalene as the growth substrate. The specific growth rate of strain SN11 in both media did not essentially depend on the NaCl concentration when it was below 0.4 M, but noticeably decreased at a NaCl concentration of 0.7 M or higher (Fig. 1) with a concurrent lengthening of the lag phase (Figs. 2a and 2b). The lag phase of strain SN11 cultivated in the nutrient-rich medium at high salt concentrations lengthened insignificantly (Figs. 2a and 2b). These data are consistent with those of Ventose *et al.* [4], who showed the beneficial effect of yeast extract in the medium on bacterial growth at high NaCl concentrations.

The role of plasmids in PAH degradation and strain halotolerance was studied using the ten strains described above and two strains (*Rhodococcus* sp. G10 and *Pseudomonas* sp. G51) previously isolated from soils contaminated with halogenated organic compounds (the AO Halogen, Perm).

Ten of the twelve strains studied were found to contain large plasmids (Table 2 and Fig. 3). All *Pseudomonas* strains and strains *Arthrobacter* sp. SN17 and *Rhodococcus* sp. G10 bear plasmids about 85 kb in size; *Rhodococcus* sp. SN31 bears plasmid about 100 kb in size; and *Arthrobacter* sp. strains DF14 and SF27 bear plasmids about 120 kb in size.

To discern the location of genes involved in naphthalene degradation, we attempted to cure *Pseudomonas* sp. strain SN11 of its plasmid, but failed to obtain plasmid-free derivatives of this strain. The conjugal transfer of plasmid pSN11 to the recipient strain *P. putida* BS394 (*cys*$^-$*nah*$^-$*sal*$^-$) gave rise to transconjugants with the phenotype Cys$^-$Nah$^+$Sal$^+$Phn$^+$. Three of the transconjugants obtained were tested for the presence of plasmids. All three were found to contain plasmid pSN11 and its deletion derivative, which was about 60 kb in size. These data suggest that pSN11 is a conjugative plasmid bearing genes that are responsible for the degradation of naphthalene, salicylate, and phenanthrene.

Analysis with the use of restriction endonuclease *Eco*RI showed that the plasmids of strains SN11 and G51 are probably identical. The conjugal transfer of plasmids from the *Pseudomonas* sp. strains SN101 and G51 to the recipient strain *P. putida* BS394 showed that these plasmids are also conjugative and bear the naphthalene degradation genes. The conjugal transfer of these plasmids was accompanied by the deletion of a DNA fragment. The *Eco*RI-based analysis of the deletion-containing plasmids isolated from transconjugants

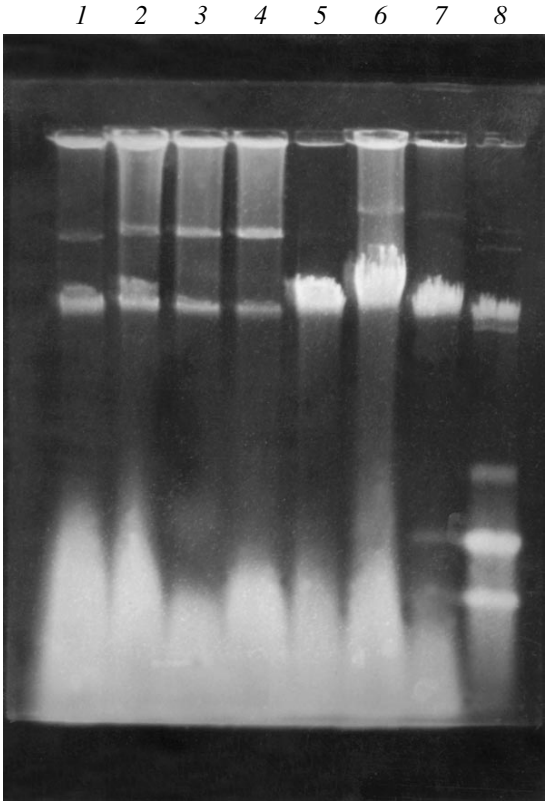

Fig. 3. Electrophoresis of plasmid DNA isolated from various strains. Lanes: *1*, PpG7(NAH)7; *2*, SN11; *3*, SN13; *4*, G51; *5*, SN17; *6*, SF23; *7*, DF14; and *8*, G10.

SN11-TC1 and G51-TC1 revealed the identity of these plasmids.

The plasmid location of the PAH degradation genes suggests that PAH degraders can easily lose their degrading ability during cultivation under nonselective conditions. However, the subculturing of two naphthalene-degrading strains, *Pseudomonas* sp. SN11 and *Rhodococcus* sp. DB11 in LB broth (i.e., under nonselective conditions) showed that they retain their ability to grow on naphthalene and salicylate even after 80 subcultures.

To study the dependence of the strain halotolerance on plasmids, transconjugants SN11-TC1 and G51-TC1 were grown at different NaCl concentrations in Raymond medium containing either tryptone and yeast extract or naphthalene as the growth substrates. Unlike the recipient strain *P. putida* BS394, which cannot grow on tryptone and yeast extract at a salinity of 0.7 M NaCl and higher or on naphthalene at a salinity of 0.4 M NaCl and higher (Table 4), the transconjugants grew well on tryptone and yeast extract at a salinity of 1 M NaCl and on naphthalene at a salinity of 0.7 M NaCl. As for *Pseudomonas* sp. SN11, this strain turned out to be more halotolerant than the transconjugants (Table 4).

DISCUSSION

The environment is commonly polluted by several contaminants at the same time. For instance, the contamination of soils by petroleum and polycyclic aromatic hydrocarbons is often accompanied by soil salinization. This leads to selective pressure on indigenous microflora to degrade the PAHs and to increase the halotolerance of microorganisms. As a result, the microbial diversity of polluted areas rises.

In the present work, the recovery of microorganisms from salt-contaminated samples using salt-free K1 medium led to the isolation of halotolerant naphthalene, phenanthrene, and biphenyl degraders that belonged to different gram-positive and gram-negative bacterial genera. Some of the microbial associations obtained using K1 medium with 1 or 2.5 M NaCl were also able to utilize naphthalene. However, we failed to obtain halotolerant naphthalene degraders in pure cultures from these isolates. This can be explained by the fact that the isolates contained halotolerant microorganisms capable of secreting osmoprotectants into the medium, which allowed nonhalotolerant degradative bacteria to utilize naphthalene in the presence of high concentrations of NaCl in the medium. We believe that such microbial associations containing halotolerant and degradative bacteria can be useful for the bioremediation of saline soils.

As mentioned above, the isolated halotolerant PAH degraders were both gram-positive and gram-negative bacteria. From the enrichments incubated in the presence of phenanthrene, we obtained two strains belonging to the genus *Arthrobacter* that were capable of utilizing phenanthrene, naphthalene, and their intermediates. These strains, *Arthrobacter* sp. SF27 and DF14, were deposited in the All-Russia Collection of Microorganisms as VKM Ac-2063 and VKM Ac-2064, respectively. It should be noted that the majority of the known PAH degraders belong to the genus *Burkholderia* [18], and only a few of them belong to the genus *Arthrobacter* [16].

Other isolates from these enrichments (the *Pseudomonas* strains SN11, SN21, SN101, and G51 and nonidentified strain DN13) acquired the ability to grow on phenanthrene and its intermediate, 1-hydroxy-2-naphthoate, only after long-term cultivation on this PAH. Earlier, we described the mutants of naphthalene-degrading fluorescent pseudomonads that were able to grow on phenanthrene [19] by degradation through 1-hydroxy-2-naphthoate, salicylate, and catechol. The halotolerant pseudomonads obtained in the present study catabolize phenanthrene in the same way (data not presented). As for the gram-positive PAH degraders of the genus *Arthrobacter* (DF14 and SF27), we suggest that they catabolize phenanthrene through 1-hydroxy-2-naphthoate, which then cleaves by the reactions involving either salicylate or *o*-phthalate and protocatechuate [20].

Table 4. Growth of donor, recipient, and transconjugant strains at different NaCl concentrations in Raymond medium with tryptone plus yeast extract or naphthalene as the growth substrates

Strain	Growth on tryptone plus yeast extract at a NaCl concentration of			Growth on naphthalene at a NaCl concentration of		
	0.7 M	1.0 M	1.5 M	0.4 M	0.7 M	1.0 M
Pseudomonas sp. SN11*	++	++	++	++	++	+
P. putida BS394**	+	–	–	–	–	–
P. putida 394-(SN11-TC1)	++	++	–	++	+	–
P. putida 394-(SN11-TC2)	++	++	–	++	+	–

Note: "+", "++", and "–" stand for "good growth", "poor growth", and "no growth", respectively. * and ** mark the donor and recipient strains, respectively.

The PAH degraders that were isolated from salt-contaminated soil and sediment samples are moderately halotolerant bacteria. This is not surprising if one takes into account that, unlike saline aqueous habitats, saline soils are characterized by seasonal variations in the salt content [4]. In the nutrient-rich medium, *P. putida* SN11 is able to grow at a higher salt concentration than in the mineral medium with naphthalene (Fig. 2). This agrees with the data available in the literature indicating that yeast extract beneficially influences bacterial growth at high salt concentrations [4]. The data presented in Fig. 2b suggest that the metabolic pathways involved in naphthalene degradation are sensitive to high salt concentrations, which is evident from the fact that NaCl slows bacterial growth and increases the lag phase.

Naphthalene degradation is often controlled by plasmid genes [1]. The *Pseudomonas* strains isolated in the present work most likely contain identical plasmids involved in naphthalene biodegradation. The identity is confirmed by the deletion of the same DNA fragment during the conjugal transfer of these plasmids. Earlier, similar deletions associated with conjugal transfer were described for other naphthalene degradation plasmids [19].

The halotolerance of bacteria may also be associated with plasmids [4]. For instance, the curing of *Halomonas elongata* by eliminating plasmids led to the inability of this bacterium to grow at high salt concentrations [21]. The data presented in Table 4 suggest that the naphthalene degradation plasmid pSN11 of *P. putida* SN11 plays an important role in the halotolerance of this strain. On the other hand, the halotolerance of strain SN11 is higher than that of the transconjugants of this strain (Table 4) and, hence, halotolerance can be determined by both plasmid and chromosomal determinants.

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