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EXPERIMENTAL ARTICLES

Diversity of Genetic Systems Responsible for Naphthalene Biodegradation in *Pseudomonas fluorescens* Strains

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Abstract—The genetic systems that are responsible for naphthalene catabolism were analyzed in 18 naphthalene-degrading *Pseudomonas fluorescens* strains isolated from oil-contaminated soils in different regions of Russia. It was found that 13 strains contain plasmids, from 20 to 120 kb in size, at least 5 of which are conjugative and bear the catabolic genes responsible for the complete utilization of naphthalene and salicylate. Five plasmids belong to the P-7 incompatibility group, and two plasmids belong to the P-9 incompatibility group. The naphthalene biodegradation genes of *P. fluorescens* are highly homologous to each other. The study revealed a new group of the *nahAc* genes and two new variants of the *nahG* gene. The suggestion is made that the key genes of naphthalene biodegradation, *nahAc* and *nahG*, evolve independently and occur in *P. fluorescens* strains in different combinations.

Key words: *Pseudomonas fluorescens*, diversity, naphthalene catabolism genes, plasmids, polymerase chain reaction.

Polycyclic aromatic hydrocarbons (PAHs) are severe environmental pollutants. In nature, PAHs are mainly degraded by microorganisms, the most efficient PAH degraders being bacteria of the genus *Pseudomonas*.

The genetic control of naphthalene biodegradation has been studied in detail with reference to the archetypal plasmid NAH7, which harbors all genetic information necessary to convert naphthalene to pyruvate and acetaldehyde [1]. The catabolic genes carried by plasmid NAH7 are organized in two operons, *nah1* (*nahAaAbAcAdBFCED*), which controls the conversion of naphthalene to salicylate, and *nah2* (*nahGTHINLOMKJ*), which controls the utilization of salicylate to the tricarboxylic acid cycle intermediates. Both operons are expressed under the positive control of the regulatory *nahR* gene [1]. Habe and Omori described several groups of plasmid and chromosomal *nah*-like genes (*nah, ndo, pah*, and *dox*) that are conservative and highly homologous to the *nah* genes of the archetypal plasmid NAH7. The transposon properties of catabolic operons imply the possibility of their independent evolution by means of transposition and recombination, which, together with horizontal gene transfer, are powerful factors responsible for the distribution of such operons within and between microbial populations and, in the final analysis, for the adaptation of natural microbial communities to the ever-increasing pollution of the environment. The study of the genetic systems that are responsible for PAH degradation can greatly contribute to our understanding of the evolution of various catabolic pathways.

The aim of this work was to analyze the genetic systems responsible for naphthalene catabolism in naphthalene-degrading *Pseudomonas fluorescens* strains isolated from oil-contaminated soils in different regions of Russia.

MATERIALS AND METHODS

The bacterial strains and plasmids used in this work are listed in Table 1.

The strains were grown overnight in LB broth [2] or mineral Evans medium [3] on a shaker, with 1 g/l naphthalene or salicylate as the carbon source. The *Pseudomonas fluorescens* strains were grown at 28°C, and the *Escherichia coli* strain JM109 was grown at 37°C. Upon cultivation on agar plates, naphthalene was placed on the inner side of the lids of inverted petri dishes.

Naphthalene-degrading strains were isolated through enrichment cultures incubated in Evans medium containing naphthalene as the sole source of carbon and energy. These strains were tested for the ability to grow on naphthalene derivatives (2-methyl-

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Table 1. Bacterial strains and plasmids used in this work

Strain	Phenotype	Plasmid	Plasmid size, kb	Gene type		Source
				nahAc	*nahG*	
Pseudomonas putida **PpG7**	Nah^+Sal^+	NAH7, IncP-9	83	C18	NAH7	I.C. Gunsalus, United States
P. fluorescens **NK21K**	Nah^+Sal^+	pNK21K-1	30	AN10	AN10	Nizhnekamskneftekhim sludge deposit
		pNK21K-2	20			
P. fluorescens **NK22**	Nah^+Sal^+	pNK22-1	30	AN10	AN10	Ditto
		pNK22-2	20			
P. fluorescens **NK32**	Nah^+Sal^+	pNK32-1	30	AN10	AN10	″
		pNK32-2	20			
P. fluorescens **NK33**	Nah^+Sal^+	pNK33 IncP-7	100	C18	pDTG1	″
P. fluorescens **NK43**	Nah^+Sal^+	pNK43 IncP-7	100	C18	pDTG1	″
P. fluorescens **NKNS3**	Nah^+Sal^+ $2MeN^+$	ND	–	AN10	NKNS	″
P. fluorescens **NKNS5**	Nah^+Sal^+ $2MeN^+$	pNKNS5 IncP-9	60	AN10	NKNS	″
P. fluorescens **NKNS7**	Nah^+Sal^+	ND	–	AN10	NKNS	″
P. fluorescens **NKNS15**	Nah^+Sal^+	ND	–	AN10	AN10	″
P. fluorescens **OS18P$^+$**	$Nah^+Sal^+Phn^+$ Tol^+ $mXyl^+oXyl^+Tra^+$	pOS18 IncP-7	100	C18	pDTG1	″
P. fluorescens **OS19P$^+$**	$Nah^+Sal^+Phn^+$	pOS19 IncP-7	100	C18	pDTG1	″
P. fluorescens **A24**	$Nah^+Sal^+Tra^+$	pA24 IncP-7	100	A88	NAH7	West Siberian soil contaminated with fuel oil
P. fluorescens **A88**	$Nah^+Sal^+Tra^+$	pA88 IncP-9	60	A88	A88	Ditto
P. fluorescens **A89**	$Nah^+Sal^+Phn^+$	ND	–	A88	A88	″
P. fluorescens **A96**	$Nah^+Sal^+Tra^+$	pA96	60	A88	A88	″
P. fluorescens **I-16**	Nah^+Sal^+	pI-161	120	A88	A88	″
		pI-162	100			
P. fluorescens **I-1**	$Nah^+Sal^+Tra^+$	pI-1	60	AN10	KF715	Voskresensk, Nizhni Novgorod oblast
P. fluorescens **II-9**	Nah^+Sal^+	ND	–	AN10	KF715	Ditto
P. putida **KT2442**	Nah^-Sal^- *gfp*Km^R	–	–	–	–	K. Smalla, Germany
Escherichia coli **JM109**	*rec*A1, *gyr*A96, *thi*, *hsd*R17, *sup*E44, *rel*A1, Δ(*lac-pro*AB), F'[*tra*D36, *pro*AB *lac*I^qZΔM15]	–	–	–	–	Promega, United States
	*Col*E1, *lacZ'*, Ap^R	pGEM-T Easy	3.015	–	–	Promega, United States

Note: Nah^+, $2MeN^+$, Sal^+, Phn^+, Tol^+, $mXyl^+$, and $oXyl^+$ indicate the ability to grow on naphthalene, methylnaphthalene, salicylate, phenanthrene, toluene, *m*-xylene, and *o*-xylene, respectively. Ap^R, Km^R, and Tra^+ stand for ampicillin resistance, kanamycin resistance, and capability for conjugal transfer, respectively. *Gfp* is green fluorescent protein. ND stands for "not detected."

Table 2. The PCR primers used in this work

Gene	Primer	Nucleotide sequence	PCR product size, kb	Ref.
	BOXA1R	5'-CTA CGG CAA GGC GAC GCT GAC G-3'	–	[5]
16S rRNA	8f	5'-AGA GTT TGA TCM TGG CTC AG-3'	1484	[6]
	1492r	5'-TAC GGH TAC CTT GTT ACG ACT T-3'		
IncP-9	repF	5'-CCAGCGCGGTACWTGGG-3'	554	[7]
repAB	repR	5'-GTCGGCAICTGCTTGAGCTT-3'		
IncP-7	Upper	5'-CCCTATCTCACGATGCTGTA-3'	524	United Kingdom
rep region	Lower	5'-GCACAAACGGTCGTCAG-3'		
nahAc	Ac149f	5'-CCC YGG CGA CTA TGT-3'	865	[8]
	Ac1014r	5'-CTC RGG CAT GTC TTT TTC-3'		
	Ac596r	5'-CRG GTG YCT TCC AGT TG-3'		[9]
nahG	shc1_up	5'-CGG CKT THG GTG ARG TCG GTG C-3'	893	[10]
	shc1_lo	5'-GGC GAG GAA RTA GGC GTC CTC AAG-3'		
nahR	nahR_1f	5'-ATG GAA CTG CGT GAC CTG G-3'	585	[10]
	nahR_585r	5'-GCC GTA GGA ACA GAA GCG-3'		
xylE	23OF	5'-ATG GAT DTD ATG GGD TTC AAG GT-3'	721	[11]
	23OR	5'-ACD GTC ADG AAD CGD TCG TTG AG-3'		
	pBlue_23	5'-ACG TTG TAA AAC GAC GGC CAG TG-3'		M.G. Shlyapnikov, IBPM
	M13r+26	5'-GTG AGC GGA TAA CAA TTT CAC ACA GG-3'		

Note: IBPM is the Skryabin Institute of Biochemistry and Physiology of Microorganisms.

naphthalene, salicylate, and gentisate), toluene, xylene, and other PAHs (phenanthrene, anthracene, dibenzothiophene, biphenyl, and pyrene).

Total bacterial DNA was isolated as described in the handbook [4]. Plasmid DNA was isolated by the alkaline lysis method [2] with minor modifications. DNA was quantified by using a TKO-100 fluorimeter (Hoefer Scientific Instruments, United States) and the dye Hoechst 33258 (Bio-Rad, United States) according to the manufacturer's instructions.

The conjugal transfer of plasmids to the recipient strain *P. putida* KT2442 was carried out overnight on agar media with Millipore membrane filters. Transconjugants were tested for the presence of plasmids and the *gfp* gene.

The polymerase chain reaction (PCR) was performed under standard conditions in a GeneAmp 2400 thermal cycler (Perkin-Elmer, United States). The reaction mixture contained 200 μM of each deoxyribonucleotide triphosphate; 1.5 mM $MgCl_2$; and, in some experiments, 5% dimethylsulfoxide (Sigma, United States). The oligonucleotide primers used in the work are listed in Table 2.

DNA samples were electrophoresed in 0.8% agarose gel in 0.5× Tris–borate buffer [2]. DNA bands were visualized by staining the developed agarose gels with ethidium bromide. DNA fragments were extracted from the agarose gel with the aid of a Qiaex II Agarose Gel Extraction System (Qiagen, Germany) as recommended by the manufacturer.

Escherichia coli cells were transformed with plasmid DNA as described in the handbook [4].

The nucleotide sequence of DNA was determined by using the CEQ™ 2000XL DNA Analysis System and DTCS reagents (Beckman, United States) according to the manufacturer's instructions. The nucleotide sequence of cloned *nahAc* gene fragments was determined with the pBlue_23, M13r+26, and Ac596R primers (Table 2).

RESULTS

The isolation and characterization of naphthalene-degrading strains. As can be seen from Table 1, all the naphthalene-degrading strains isolated from oil-contaminated soils of different regions of Russia were able to grow on naphthalene and salicylate. In addition, two strains could grow on 2-methylnaphthalene (the $2MeN^+$ phenotype) and three strains could grow on phenanthrene. Strain OS18 could grow on naphthalene and salicylate at low temperatures (down to 4°C) and was able to utilize phenanthrene, toluene, and xylene.

The screening of 100 naphthalene-degrading strains on King B medium allowed 60 strains of fluorescent bacteria to be selected. Based on their morphological and physiological properties and using the identifica-

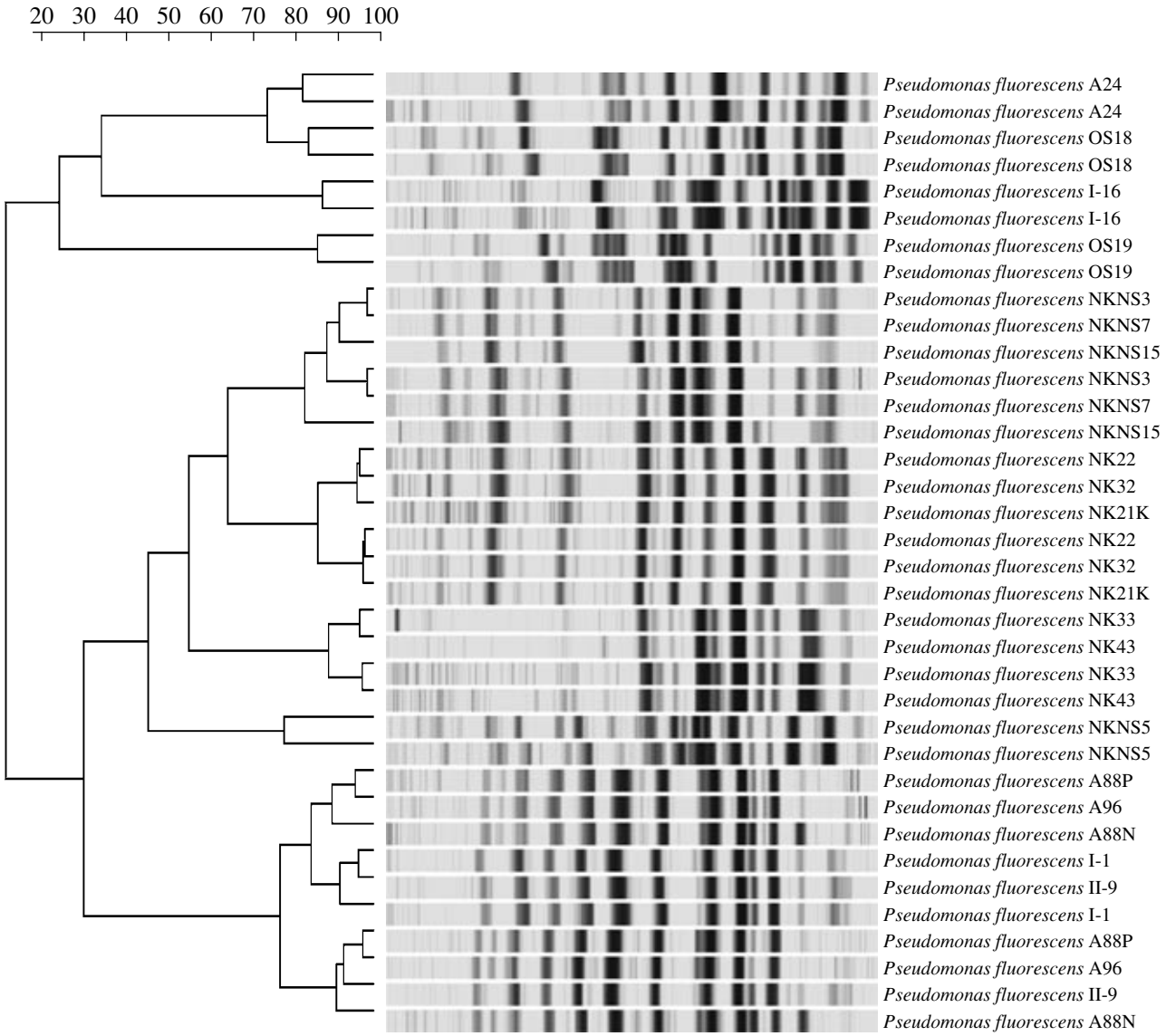

Fig. 1. The cluster analysis of the REP-PCR products of DNA samples. The tree was constructed with the aid of GelCompar 4.0 (UPGMA algorithm).

tion criteria of *Bergey's Manual* [12], the fluorescent strains were attributed to the genus *Pseudomonas.* The species affiliation of the fluorescent pseudomonads was determined by the amplified ribosomal DNA restriction analysis (ARDRA) of the 16S rRNA gene. The *Rsa*I, *Msp*II, and *Hae*III restriction fragments of the 16S rDNA were separated by electrophoresis in 2% agarose gel with *P. putida* BS203, *P. fluorescens* 2.79, *P. chlororaphis* 17411, *P. aeruginosa* PAK NP1, and *P. aureofaciens* 30.84 as the reference strains. As a result of this analysis, 26 naphthalene degraders (18 of which were studied in this work) were assigned to the species *P. fluorescens.*

The genotypic analysis of the naphthalene-degrading *P. fluorescens* strains by the genome fingerprint method (REP-PCR) with the BOXA1R primer [5] showed that these strains can be divided into six groups (Fig. 1). Some strains were found to be allied in spite of the fact that they were isolated from geographically distant regions. Group 1 comprised strains I-1 and II-9, which were isolated from soils of Nizhni Novgorod oblast, and strains A88, A89, and A96, which were isolated from West Siberian soils (Table 1). The largest group, 2, comprised eight NK strains, which were recovered from Nizhnekamsk soils. Two strains, A24 (from West Siberian soil) and OS18 (from Nizhnekamsk soil), constituted group 3. Groups 4, 5, and 6 each included single strains (I-16, OS19, and NKNS5, respectively).

Thirteen strains (*P. fluorescens* NK21K, NK22, NK32, NK33, NK43, NKNS5, OS18, OS19, A24, A88, A96, I-16, and I-1) were found to contain plasmids, from 20 to 120 kb in size (Table 1). The PCR analysis

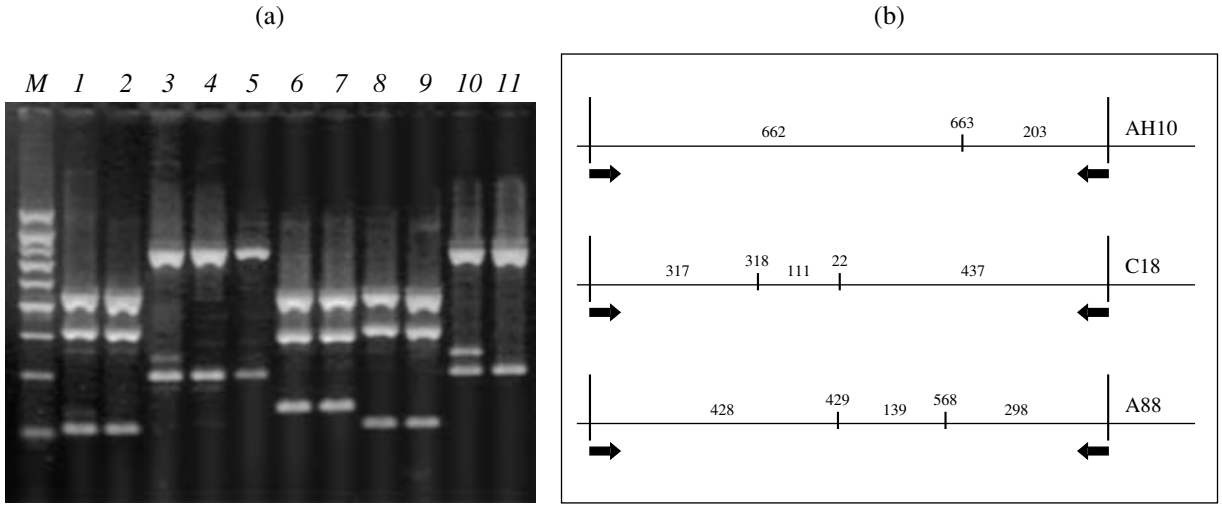

Fig. 2. (a) Electrophoresis of the 865-kb PCR products of the *nahAc* genes of various strains digested with *Hae*III restriction endonuclease [8]. Lanes: (*M*) Hyper Ladder 100 bp (Bioline); (*1*) NAH7; (*2*) NK33; (*3*) NK21K; (*4*) NK22; (*5*) NK32; (*6*) A24; (*7*) A88; (*8*) OS18; (*9*) OS19; (*10*) I-1; (*11*) the purified PCR product of strain I-1. (b) The *Hae*III restriction maps of the 865-kb PCR products of the *nahAc* genes of various type strains.

of these plasmids with the primers specific to the incompatibility groups P-7 and P-9 showed that five plasmids (pNK33, pNK43, pOS18, pOS19, and pA24) belong to the P-7 incompatibility group and two (pA88 and pNKNS5) belong to the P-9 incompatibility group. The affiliation of the other plasmids to incompatibility groups remains unknown. Experiments on the conjugal transfer of plasmids to the recipient strain *P. putida* KT2442 showed that plasmids pA88, pA96, pI-1, pOS18, and pA24 (the first three about 60 and the last two about 100 kb in size) possess the whole set of genes necessary for naphthalene and salicylate conversion. The conjugal transfer frequency of plasmid pOS18 was 10^{-4}, and that of plasmids pA24, pA88, pA96, and pI-1 was 10^{-5}. The study of the role of the other plasmids in PAH catabolism is in progress in our laboratory.

The amplification of the naphthalene degradation genes. The *nahAc, nahR, nahG*, and *nahH* genes, which code for the large subunit of naphthalene 1,2-dioxygenase, a regulatory protein, salicylate 1-hydroxylase, and catechol-2,3-dioxygenase, respectively, were subjected to PCR amplification with specific primers. As a result, four PCR products with sizes of 865, 893, 585, and 721 kb, respectively, were obtained. This implies that the four aforementioned naphthalene degradation genes were amplified in all the samples tested.

Analysis of the *nahAc* gene of various *P. fluorescens* strains. To study the diversity of the *nahAc* gene, which codes for the large subunit of naphthalene-1,2-dioxygenase, the 865-kb PCR products were digested with *Hae*III restriction endonuclease [8]. The restriction analysis of the replicons showed that nine *P. fluorescens* strains harbor the *nahAc* gene. The hydrolysis pattern of this gene with *Hae*III was similar to those of the *nahAc* gene of *P. stutzeri* AN10 and the *pah3A* gene of *P. aeruginosa* PaK1. Consequently, these genes were designated as type AN10 genes (Table 1). The nucleotide sequences of the *nahAc* genes of four other strains were found to be similar to those of the *doxB* gene of *Pseudomonas* sp. C18 and the *nahAc* gene of *P. putida* G7. Consequently, these genes were designated as type C18 genes (Table 1). The hydrolysis patterns of the *nahAc* genes of five other strains (A24, A88, A89, A96, and I-16) were identical and unique (Figs. 2a, 2b).

The amplification products of the *nahAc* genes of strains A88, A24, and I-16 were cloned on the pGEM-T Easy plasmid vector, and their nucleotide sequences were determined. The sequences of the *nahAc* amplicons of strains A88, A24, and I-16 were 99% identical. Accordingly, they were designated as the *nahAc* genes of type A88. The substitution of adenine for cytosine at position 318 removes the *Hae*III hydrolysis site, as is evident from a comparison of the *nahAc* genes of types A88 and C18 (Fig. 2b). The *Hae*III hydrolysis site at position 429 of the cloned fragments corresponds to the *Hae*III hydrolysis site of the *nahAc* genes of type C18. An extra *Hae*III site occurs at position 568 (Fig. 2b). These variations in the nucleotide sequences of the *nahAc* genes do not affect the amino acid sequences of the encoded proteins. Indeed, BLAST- and CLUSTAL-aided comparisons of the nucleotide sequence of the *nahAc* gene of strain A88 and its deduced amino acid sequence with those available in a database [13] showed that the nucleotide sequences of the *nahAc* genes of strains A88, A24, and I-16 are 87–88% similar to those of the *nahAc* gene of *P. stutzeri* AN10 and the *doxB* gene of *Pseudomonas* sp. C18. Cluster analysis showed that the *nahAc* gene of A88 comprises a separate group, which is intermediate between groups AN10 and C18 (Fig. 3). The amino acid sequence of the

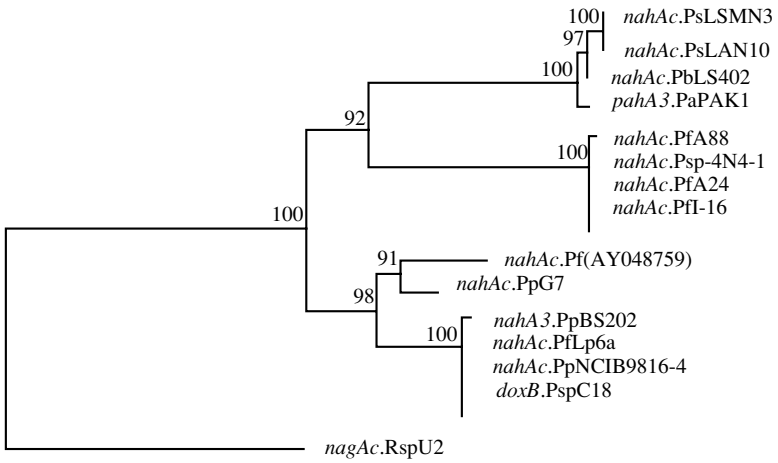

Fig. 3. A tree illustrating the evolutionary relationship between the internal regions of the *nahAc* genes of various bacterial strains. Relative distances are determined and the tree is constructed with the aid of the Treecon program [14]. The nucleotide sequences used in this work have the following accession numbers in GenBank: *Pseudomonas stutzeri* LSMN3 (AF306427), *P. stutzeri* AN10 (AF039533), *P. belearica* LS402 (AF306429), *P. aeruginosa* PAK1 (D84146), *P. fluorescens* A88 (AY433939), *Pseudomonas* sp. 4N4-1 (AJ496394), *P. fluorescens* A24 (AY433941), *P. fluorescens* I-16 (AY433941), *P. fluorescens* (AY048759), *P. putida* G7 (M83949), *P. putida* BS202 (AF010471), *P. fluorescens* Lp6a (AY125981), *P. putida* NCIB9816-4 (AF491307), and *Ralstonia* sp. U2 (AF036940).

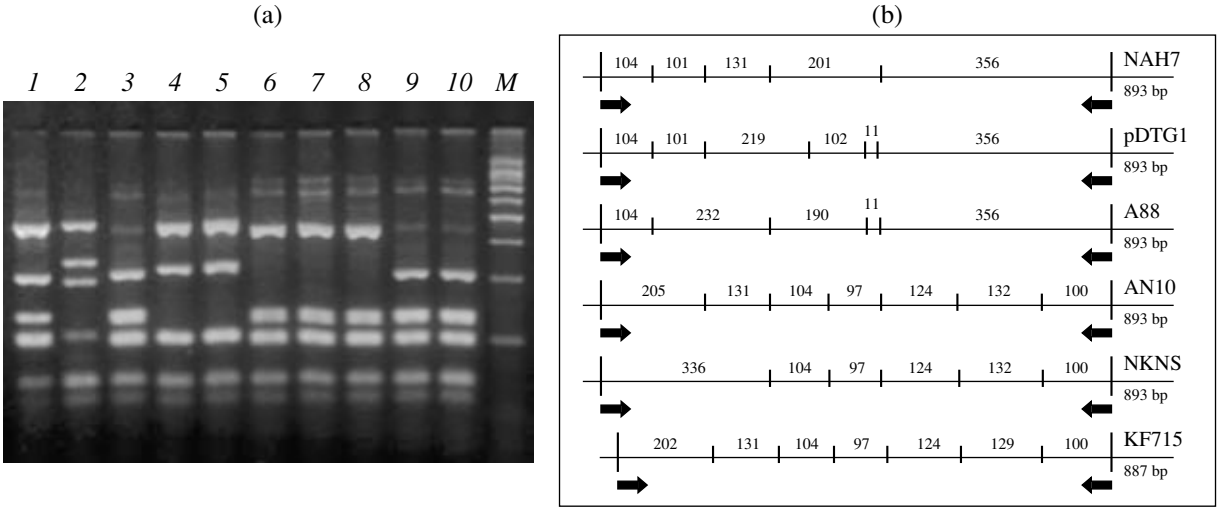

Fig. 4. (a) Electrophoresis of the 893-bp PCR products of the *nahG* genes of various strains digested with *Rsa*I restriction endonuclease. Lanes: (*M*) Hyper Ladder 100 bp; (*1*) NAH7; (*2*) A88; (*3*) NK22; (*4*) OS18; (*5*) OS19; (*6*) NKNS3; (*7*) NKNS5; (*8*) NKNS7; (*9*) NKNS15; (*10*) II-9. (b) The *Psa*I restriction maps of the PCR products of the *nahG* genes of *P. putida* G7 (type NAH7), *P. putida* NCIB9816 (type pDTG1), *P. fluorescens* A88, *P. stutzeri* AN10 (type AN10), *P. fluorescens* NKNS3, and *P. putida* KF715.

NahAc protein of strain A88 is 94–95% similar to that of the NahAc protein of strain C18 and 92% similar to that of the NahAc protein of strain AN10.

Analysis of the *nahG* genes of various *P. fluorescens* strains. The diversity of the *nahG* gene in various *P. fluorescens* strains was studied in much the same way as in the case of the *nahAc* gene. The 893-bp amplification products were treated with the *Rsa*I and *Msp*I restriction endonucleases (Figs. 4, 5). The fragments obtained were separated by horizontal electrophoresis in 3% agarose gel, and the results obtained were compared with the restriction maps of the known *nahG* sequences. The salicylate hydroxylase genes (*nahG*) of four strains were found to be analogous to the *nahG* gene of plasmid pDTG1 (type pDTG1), the *nahG* genes of four other strains were identical to the chromosomal *nahG* gene of *P. stutzeri* AN10 (type AN10), the *nahG* genes of two strains were identical to that of *P. putida* KF715 (type KF715), and the *nahG* gene of one strain (A24) was identical to that of plasmid NAH7 (type NAH7) (Figs. 4, 5, Table 1). The hydrolysis patterns of the *nahG* amplicons of three strains (NKNS3, NKNS5, and NKNS7) were identical to that of the *nahG* ampli-

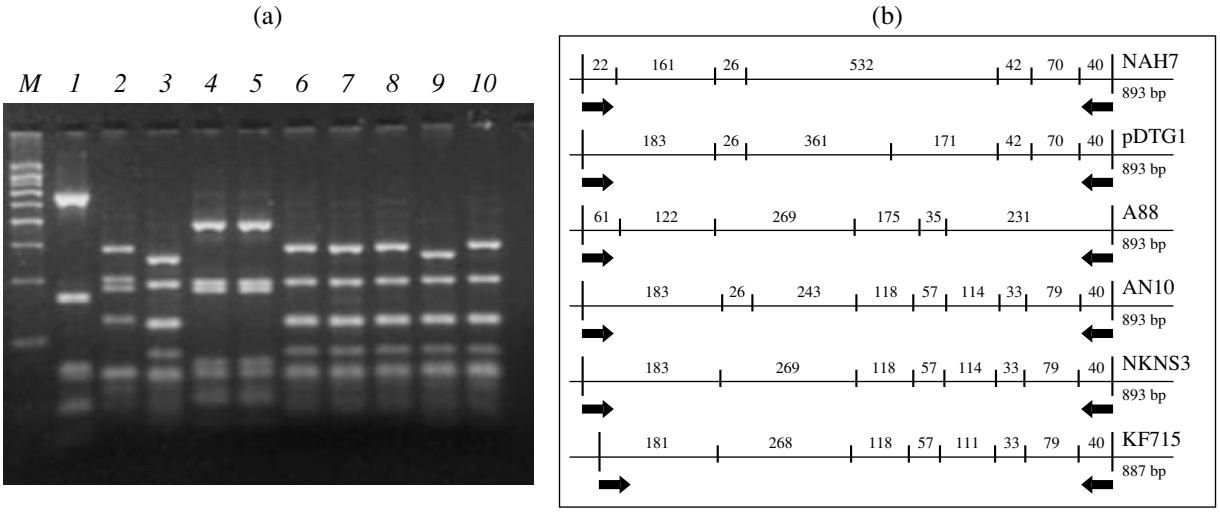

Fig. 5. (a) Electrophoresis of the 893-bp PCR products of the *nahG* genes of various strains digested with *Msp*I restriction endonuclease. Lanes: (*M*) Hyper Ladder 100 bp; (*1*) NAH7; (*2*) A88; (*3*) NK22; (*4*) OS18; (*5*) OS19; (*6*) NKNS3; (*7*) NKNS5; (*8*) NKNS7; (*9*) NKNS15; (*10*) II-9. (b) The *Msp*I restriction maps of the PCR products of the *nahG* genes of *P. putida* G7 (type NAH7), *P. putida* NCIB9816 (type pDTG1), *P. fluorescens* A88, *P. stutzeri* AN10 (type AN10), *P. fluorescens* NKNS3, and *P. putida* KF715.

con of *P. putida* KF715 when digested with the *Msp*I restriction endonuclease but not when digested with the *Rsa*I restriction endonuclease (Figs. 4a, 4b). A comparative analysis of the nucleotide sequences of the *nahG* genes of strains NKNS3 and KF715 showed that the fusion of 205- and 131-bp fragments gave rise to a new fragment 336 bp in size (Figs. 4a, 4b). Site-directed substitution mutagenesis eliminated the *Rsa*I restriction site at position 205 and led to a substitution of alanine (GCA) for valine (GTA). The amino acid sequence of the product of the *nahG* gene of strain NKNS3 showed 96% homology to those of strains KF715 and AN10 and 87% homology to those of the other known salicylate hydroxylases (Fig. 6).

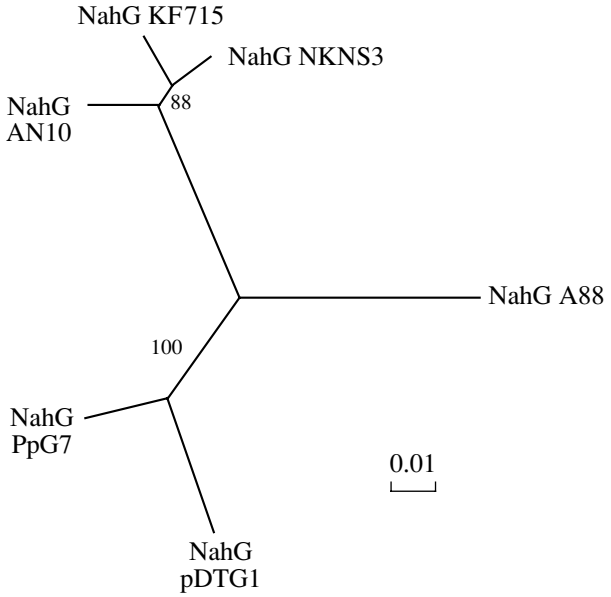

Fig. 6. A tree illustrating the evolutionary relationship between the internal regions of the *nahG* genes of various bacterial strains. Relative distances were determined and the tree constructed with the aid of the Treecon program [14]. The nucleotide sequences used in this work have the following accession numbers in GenBank: *P. stutzeri* AN10 (AF039534), *P. putida* KF715 (S80995), *P. fluorescens* NKNS3 (AY429511), *P. fluorescens* A88 (AY433938), *P. putida* G7 (M60055), and *P. putida* NCIB9816-4 (AF491307).

The *Rsa*I and *Msp*I restriction fragments of the amplicons of strains A88, A89, A96, and I-16 were identical to each other and differed from those of the other known *nahG* genes (Figs. 4, 5). The nucleotide sequence of the *nahG* amplicon of *P. fluorescens* A88 showed 81–82% homology to those of the *nahG* genes of the archetypal plasmids NAH7 and pDTG1 and 84–86% homology to those of the *nahG* gene of strain NKNS3 and the chromosomal *nahG* genes of *P. stutzeri* AN10 and *P. putida* KF715. The amino acid sequence of the *nahG* amplicon of strain A88 showed 86–88% homology to those of the aforementioned salicylate hydroxylases. Cluster analysis showed that the NahG of strain NKNS3 comprises a group with the salicylate hydroxylases of strains KF715 and AN10, whereas the NahG of strain A88 forms a separate cluster (Fig. 6).

DISCUSSION

Knowledge of the genetic systems responsible for the degrading capacity of microbial strains is necessary for understanding the evolutionary processes that underlie the diversity of these strains and their adaptation to variable environmental conditions. This work deals with the study of 18 strains of *P. fluorescens* that were isolated from oil-contaminated soils and were found to be able to utilize naphthalene and salicylate as

sole sources of carbon and energy. In general, PAH-degrading *P. fluorescens* strains are as yet more poorly studied than PAH-degrading *P. putida* strains. In spite of the fact that the PAH-degrading *P. fluorescens* strains under study were isolated from distant regions of Russia, they showed a high degree of phylogenetic relatedness.

The adaptation of microorganisms to environmental conditions is largely determined by the allocation of genetic material within and between natural microbial populations. Horizontal gene transfer is one of the main mechanisms by which microorganisms acquire new metabolic properties and genetic combinations. Microorganisms often acquire biodegradation capacity by means of plasmids. Thirteen of the eighteen naphthalene-degrading *P. fluorescens* strains tested were found to contain plasmids, from 20 to 120 kb in size. The failure to detect such plasmids in the other naphthalene-degrading *P. fluorescens* strains does not imply that they are absent in the strains since large (with sizes larger than 100 kb) low-copy-number plasmids are difficult to isolate and, hence, to detect. Five plasmids (pOS18, pA24, pA88, pA96, and pI-1) contain all necessary genetic information for the utilization of naphthalene and salicylate and are capable of conjugal transfer to *P. putida* strains at a relatively high frequency (10^{-4}–10^{-5}). It is known that PAH biodegradation plasmids belong to the P-2, P-7, and P-9 incompatibility groups [15], the species *P. putida* being the major host of the IncP-9 plasmids [10] and the species *P. fluorescens* being the major potential host of the IncP-7 plasmids. Five plasmids (pNK33, pNK43, pOS18, pOS19, and pA24) are known to belong to the P-7 incompatibility group, and two plasmids (pA88 and pNKNS5) are known to belong to the P-9 incompatibility group.

Naphthalene dioxygenase and salicylate hydroxylase, which are synthesized under the positive control of the NahR protein, are the key enzymes of the microbial degradation of naphthalene, phenanthrene, and salicylate. Naphthalene dioxygenase can also be synthesized constitutively and independently of the presence of salicylate [16]. Catechol-2,3-dioxygenase is responsible for the *meta* cleavage of catechol (an intermediate product of the degradation of polycyclic and monocyclic compounds) and makes the host strain capable of growing on methylated naphthalene and salicylate. The presence of the *nahH* gene, which codes for catechol-2,3-dioxygenase, can serve as indirect evidence for the plasmid localization of biodegradation operons [17]. The *nahAc* genes of pseudomonads fall into two groups (types) [8]. The first group, AN10, comprises the *nahAc* genes that are digested by the *Hae*III restriction endonuclease like the *nahAc* gene of *P. stutzeri* AN10 and the *pah3A* gene of *P. aeruginosa* PaK1. The second group, C18, comprises the *nahAc* genes that are analogous to the *doxB* gene of *Pseudomonas* sp. C18 and the *nahAc* gene of *P. putida* G7. The new group of the *nahAc* genes (type A88) disclosed in this work is intermediate between types AN10 and C18 (Fig. 3).

The study of the diversity of the *nahG* genes of the naphthalene-degrading *P. fluorescens* strains showed that they fall into six types (NAH7, pDTG1, AN10, KF715, A88, and NKNS). The two last types of the *nahG* genes have not yet been described in the literature. The conservative organization of the naphthalene biodegradation genes in the *P. fluorescens* strains under study implies the presence of the regulatory *nahR* gene and the catechol-2,3-dioxygenase gene in all these strains.

Identical alleles of the *nahAc* and *nahG* genes were also found in phylogenetically distant *P. fluorescens* strains, such as OS18, NK33, A88, A96, and I-16 (Fig. 1; Table 1). The *nahAc* and *nahG* genes of two allied strains (A88 and A96) belong to one type (A88). However, in two other allied strains (I-1 and II-9), these genes belong to different types (AN10 and KF715, respectively). The nucleotide sequences of the *nahAc* genes of strains A24, A88, A88, A96, and I-16, which were isolated from West Siberian soils, are highly homologous (99%) to the nucleotide sequences of the *nahAc* genes of naphthalene-degrading *Pseudomonas* sp. strains isolated from Antarctic shelf bottom sediments (AJ496394), indicating that such nucleotide sequences are widespread in nature. A comparison of the *nahAc* and *nahG* gene types in one strain suggests that the key enzymes of naphthalene biodegradation, *nahAc* and *nahG*, evolve independently as separate blocks, forming various combinations in different strains. Due to recombination under the selective pressure of the environment, catabolic genes and operons produce new genetic combinations and give rise to degradative strains that are highly adapted to life in a given ecological niche.

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