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EXPERIMENTAL ARTICLES

Occurrence of the SAL$^+$ Phenotype in Soil Pseudomonads

I. A. Kosheleva[a, b, 1], O. I. Sazonova[a], T. Yu. Izmalkova[a], and A. M. Boronin[a, b]

[a] *Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, pr. Nauki 5, Pushchino, Moscow oblast, 142290 Russia*

[b] *Pushchino State Institute of Natural Sciences, Pushchino, Russia*

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Abstract—Genetic systems of salicylate catabolism were studied in 75 strains of fluorescent pseudomonads and in 30 exogenously isolated SAL plasmids. All exogenously isolated SAL plasmids were found to contain the classical *nahG* gene in combination with the genes of the *meta*-pathway of catechol cleavage. In most studied strains, salicylate catabolism was controlled by the chromosomal genes, the *nahU* gene being the key gene of salicylate utilization and subsequent catechol cleavage occurring via the *ortho*-pathway. It is suggested that the *nahU*-like sequences play a key role in occurrence of the Sal$^+$ phenotype in strains degrading salicylate, but not naphthalene.

Keywords: fluorescent pseudomonads, biodegradation, salicylate, plasmids, salicylate hydroxylase genes

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Salicylic acid is a commonly occurring natural compound, and an important metabolic intermediate in plants, animals, and microorganisms. Presumably, most salicylate present in nature is of plant origin. Moreover, salicylate is a key intermediate produced in the course of microbial biodegradation of polycyclic aromatic hydrocarbons [1, 2]. Bacteria of the genus *Pseudomonas* can utilize salicylate in two ways: either by salicylate 1-monooxygenase (salicylate hydroxylase)-mediated transformation to catechol in the presence of oxygen and NAD(H) and subsequent catechol degradation via the *ortho*- or the *meta*-pathway [3], or by salicylate 5-hydroxylase-mediated oxidation to gentisic acid [4, 5].

Although salicylate is widespread in nature, until recently the genetic and biochemical aspects of its metabolism were studied in bacterial strains carrying NAH plasmids of naphthalene biodegradation, the heavier salicylate precursor [6, 7]. The available data suggest that the genetic means employed by currently known fluorescent pseudomonads for catabolizing both naphthalene and salicylate are fairly well conserved. The genetic control of naphthalene and salicylate catabolism has been studied in detail using NAH7, an archetypical conjugative plasmid that carries complete genetic information required for naphthalene conversion to pyruvate and acetaldehyde [6]. SAL plasmids have been studied less comprehensively, although our data suggest that more soil microorganisms may be capable of salicylate degradation than it was assumed previously [8]. The number of currently known salicylate catabolism genes includes *nahG*, which is considered the classical salicylate hydroxylase gene, as well as *nahW* and *nahU*, which encode the isofunctional enzyme salicylate 1-monooxygenase enzyme [6, 9, 10]. The *nahU* sequence differs both from the classical salicylate hydroxylase gene (approximately 65% homology) and from the *nahW* sequence of *P. stutzeri* strain AN10 (43% homology). Recently we identified a new salicylate hydroxylase gene *scpA* in salicylate/caprolactam biodegradation plasmids; it exhibits no more than 72–74% nucleotide sequence homology to the known genes and has approximately equal phylogenetic distances from its nearest homologues: *nahG* (NAH7 plasmid), *salA* (*Pseudomonas reinekei* MT1), and *nahU* (pND6-1). The enzyme exhibits a broad range of substrate specificity and is most active towards 4-methylsalicylate and unsubstituted salicylates [11]. It is also known that salicylate hydroxylase genes can be located not only in plasmids but also within the bacterial chromosome [12, 13].

The organization of salicylate catabolism genes and the pathways employed for salicylate utilization by bacterial strains that use it as an initial substrate, rather than as an intermediate in the degradation of heavier compounds, may be different from those in naphthalene-catabolizing strains.

The goal of the present study was to investigate the occurrence of salicylate-catabolizing microorganisms, as well as of salicylate biodegradation plasmids and of the genes encoding salicylate hydroxylases.

MATERIALS AND METHODS

The study was performed with salicylate-catabolizing strains isolated from samples of petroleum-contaminated soil using enrichment cultures in Evans's

[1] Corresponding author; e-mail: kosheleva@ibpm.pushchino.ru

Table 1. Bacterial strains and plasmids used in the study

Strain	Plasmids	PCR results	Source
P. putida BS3701	pBS1141 IncP-9b, pBS1142	G G1 U A H	Laboratory collection
P. putida AK5	pAK5 IncP-7	*sgpG*	
P. putida g15 F, g20 F, g24 F	pG15, pG20, pG24	G1 U	Highway roadside, Kandalaksha, Murmansk oblast
P. putida NS11, NS12, NS20, NS22	pNS11-1, pNS11-2, pNS12-1, pNS12-2, pNS20-1, pNS20-2, pNS22-1, pNS22-2	G1 U A	Shchekinoazot chemical plant, Shchekino, Tula oblast
P. putida NS7, NS15, NS17, NS18, NS24	pNS7-1, pNS7-2, pNS15, pNS17, pNS18, pNS24	U A	
P. putida 4s1, 4s2, 4s4, 4s5,4s6, 4s7, 4s8, 4s9	ND**	U A	Car ramp of petrol filling station no. 1, Pushchino, Moscow oblast
P. putida 5s3	ND	A	Fuelling site of petrol filling station no. 3, Pushchino, Moscow oblast
P. putida 5s1, 5s2, 5s4, 5s5, 5s6, 5s7, 5s8, 5s9, 5s10	ND	U A	
P. fluorescens 6s1, 6s5	p6s1 IncP-9, p6s5 IncP-9	A	Thawed soil of petrol filling station no. 2, Pushchino, Moscow oblast
P. fluorescens 6s2	ND	U A	
P. fluorescens 6s7	ND	G1 A	
P. fluorescens 7s1, 7s2, 7s3, 7s4, 7s5, 7s6,	ND	A	Prioksko-Terrasnyi Biosphere Reserve
P. putida 8s1, 8s2, 8s3, 8s4, 8s5, 8s6, 8s7, 8s8, 8s9	ND	U A	Car ramp of petrol filling station no. 4, Pushchino, Moscow oblast
P. putida 9s5, 9s6, 9s13	ND	U A	Oka River bank, Pushchino, Moscow oblast
P. putida 9s1, 9s2, 9s3, 9s4, 9s8, 9s10, 9s11	ND	G1 U A	
P. fluorescens Vs6, Vs8	ND	U A H	Petrol filling station, Voskresensk, Nizhni Novgorod oblast
P. fluorescens Vs7	ND	A H	
P. fluorescens Nks1, Nks2, Nks4, Nks9	ND	U	Nizhnekamskneftechim sludge collector, Nizhnekamsk
P. fluorescens s1F, s2F, s4F, s6F, s9F, s10F, s17F3, s26F, s30F	ps6F, IncP-7	U A	Soil from highway roadside, Kandalaksha, Murmansk oblast

* Results of PCR tests for the principal salicylate catabolism genes: G1, *nahG1*; U, *nahU*; G, *nahG*; A, *catA*, H, *nahH*.

** ND, no plasmids detected.

mineral medium [14] containing salicylate as the sole source of carbon and energy, as described previously [15]. Bacterial strains and plasmids used in this study are listed in Table 1. The strains were grown overnight in LB medium [16] and in mineral medium at 28°C with shaking. The media were supplemented with 1 g/L salicylate or succinate as a carbon source.

Total bacterial DNA was isolated as described in [17]. Plasmid DNA was isolated by alkaline lysis as described in [16] with minor modifications. DNA concentration was determined on a TKO-100 fluorimeter (Hoefer Scientific Instruments, United States) with the Hoechst 33258 dye (Bio-Rad, United States) according to the manufacturer's instructions.

Total RNA was isolated using an UltraClean™ Microbial RNA Isolation Kit (MO BIO, United States) as recommended by the manufacturer.

Plasmid DNA was visualized using field inversion gel electrophoresis (FIGE) in agarose inserts prepared as proposed in [18]. FIGE was performed according to the protocol recommended by the manufacturer (Bio-Rad).

Table 2. PCR primers used in the study

Gene	Primers	Sequences	PCR product size, bp	Reference
	BOXA1R	5'-CTA CGG CAA GGC GAC GCT GAC G-3'	–	19
16S rRNA	8f 1492r	5'-AGA GTT TGA TCM TGG CTC AG-3' 5'-TAC GGH TAC CTT GTT ACG ACT T-3'	1484	20
nahG	shc1_up shc1_lo	5'-CGG CKT THG GTG ARG TCG GTG C-3' 5'-GGC GAG GAA RTA GGC GTC CTC AAG 3'	893	21
nahG1	KT_136f KT_1129r	5'-ATT CAT ATC GGC CCT AAC G-3' 5'-CAA GCTGCT GCC CAT AGA G-3'	994	8
nahU	nahGU_244f nahU_898r	5'-GACATCTGGTTCGAATGGCG-3' 5'-CAAGATCATGCAGCGCCC-3'	654	8
nagG	458f1 224r	5'-CCT GAC CAA GCT SAA GGT-3' 5'-CGT YTC GGT SAC CAT GTG-3'	766	5
nahH	23OF 23OR	5'-ATG GAT DTD ATG GGD TTC AAG GT-3' 5'-ACD GTC ADG AAD CGD TCG TTG AG-3'	721	22
catA	C12O_UP C12O_LOW2	5'-GCG HAC VAT CGA AGG NCC RYT GTA-3' 5'-TCR CGS GTN GCA WAN GCA AAG TC-3'	462	5
IncP-9 *repAB*	repF repR	5'-CCAGCGCGGTACWTGGG-3' 5'-GTCGGCAICTGCTTGAGCTT-3'	554	23
IncP-7 *rep* region	Upper Lower	5'-CCCTATCTCACGATGCTGTA-3' 5'-GCACAAACGGTCGTCAG-3'	524	5

Plasmids were transferred by conjugation into the recipient *P. putida* strain KT2442 as described in [5].

Plasmids were exogenously isolated as described in [11]. The recipient strain was *P. putida* KT2442. The transformants were tested for the presence of plasmids and *gfp*.

Enzymes and buffer solutions used in this work were purchased from Fermentas (Lithuania). All procedures were performed according to the manufacturer's protocols.

Polymerase chain reaction (PCR) was carried out in a Mastercycler Gradient thermal cycler (Eppendorf, Germany) following a standard protocol; the reaction mixture contained 200 μM dNTPs, 1.5 or 2 mM $MgCl_2$ (for *nahAc* amplification), and, in some cases, 5% DMSO (Sigma, United States). The oligonucleotide primers used are described in Table 2.

The first cDNA strand was synthesized using RevertAid™ M-MuLV Reverse Transcriptase (Fermentas) according to the manufacturer's protocol.

DNA was analyzed by conventional electrophoresis in 0.8% agarose gels in 0.5× Tris-borate buffer and visualized using ethidium bromide staining [16].

RESULTS

Isolation and characterization of salicylate-catabolizing strains. This study was performed with salicylate-catabolizing strains isolated from oil-polluted soil samples from Moscow, Murmansk, Nizhni Novgorod, and Tula oblasts, as well as from Tatarstan, using enrichment cultures in a mineral medium containing salicylate as the sole source of carbon and energy. Altogether, over 200 strains were isolated, and 126 of them were identified as fluorescent pseudomonads after testing on King's medium B. Based on phenotypic traits and the results of a REP–PCR test with the BOXA1R primers [19, 24], 75 salicylate-catabolizing strains were selected for further analysis (Table 1). The species affiliation of the 75 isolates was determined by amplified ribosomal DNA restriction analysis (ARDRA) of the 16S rRNA gene amplicons. The strains *P. putida* BS203, *P. fluorescens* 2.79, *P. chlororaphis* 17411, *P. aeruginosa* PAK NP1, and *P. aureofaciens* 30.84 were used as the controls (data not shown). It was found that 49 strains (i.e., approximately two thirds of the total number) belonged to the species *P. putida*, and 26 were *P. fluorescens* strains.

All isolated salicylate-catabolizing strains were tested for their ability to grow on naphthalene and gentisate. Only four strains isolated with salicylate as the sole carbon and energy source could also grow on naphthalene, a higher-molecular-weight compound: *P. putida* 5s7 and *P. fluorescens* Vs6, Vs7, and Vs8. Thirteen salicylate-catabolizing strains were also capable of growth on gentisate: *P. putida* g15F, NS11, NS15, NS20, 5s7, 8s3, 8s8, 9s1, 9s2, 9s3, 9s10, 9s13, and *P. fluorescens* s30F.

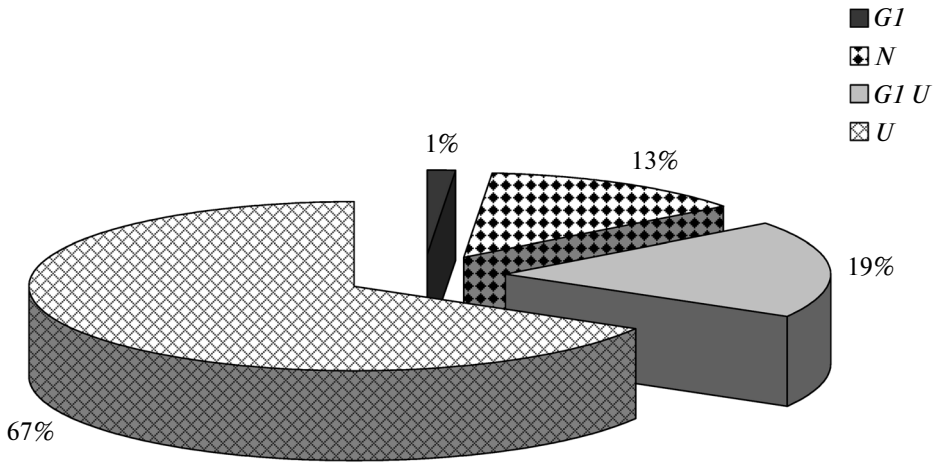

Fig. 1. Occurrence of different salicylate hydroxylase genes in the *Pseudomonas* strains studied. *G1, nahG1*; *U*, *nahU* gene; *N*, salicylate hydroxylase-encoding sequence not identified.

It is known that in fluorescent pseudomonads salicylate catabolism genes can be located both on the chromosome and on large conjugative plasmids, usually of the P-7 and P-9 incompatibility groups [5, 7, 12, 13]. Using FIGE and PCR with IncP-7/IncP-9-specific primers (Table 2), 15 salicylate-catabolizing strains were found to carry plasmids (Table 1). Experiments on conjugation transfer into the recipient *P. putida* strain KT2442 on a salicylate-containing selective medium determined that the *P. fluorescens* strain s6F carried an IncP-7 salicylate catabolism plasmid. The role of other detected plasmids in the genetic control of salicylate catabolism could not be established.

The presence of salicylate hydroxylase genes in salicylate-catabolizing strains. To identify the principal salicylate catabolism genes in the new strains of fluorescent pseudomonads, PCR amplification of the following target sequences was carried out: *nahG*, the classical salicylate hydroxylase gene; *nahG1*, a chromosomal pseudogene from *P. putida* KT2440; and *nahU*, a salicylate hydroxylase gene from the pND6 plasmid not included in the *nah2* operon. In addition, the strains were tested for the presence of the *nagG* (*sgpG*) gene of the large subunit of salicylate 5-hydroxylase involved in the alternative salicylate catabolism pathway via gentisate [5]. While *nahG* is usually located on plasmids, *nahG1* (which we designated as the KT-type salicylate 1-hydroxylase gene) is a nonfunctional gene located on the *P. putida* KT2442 chromosome. In our previous study, we detected *nagG* (first described in *Ralstonia* sp.) in *P. putida* strain AK5 [5]. The laboratory *P. putida* strains AK5 and BS3701, which carries all three variants of the salicylate hydroxylase gene, were used as the controls. Oligonucleotide primers used for PCR are listed in Table 2; the results are shown in Table 1 and Fig. 1.

The classical salicylate hydroxylase gene *nahG* was not amplified from the DNA material of the isolated strains. Although 13 of these strains could grow on a gentisate-containing medium, there was no amplification of the *nagG* (*sgpG*) gene encoding the large subunit of salicylate 5-hydroxylase.

The *nahU* sequence was detected in 64 of the 75 strains tested; that is, it was the most common salicylate catabolism gene in the newly isolated salicylate-degrading bacteria (Fig. 1). Amplification of the *nahG1* sequences produced 994 bp PCR products in 15 strains; other strains did not carry this gene. Moreover, no known salicylate hydroxylase gene sequences were detected in ten of the Sal^+ strains studied.

Identification of the sequences encoding functional salicylate hydroxylases. The laboratory *P. putida* strain BS3701 carries three salicylate hydroxylase-encoding gene sequences, *nahG1*, *nahG*, and *nahU*, which is the reason it was selected as the control strain in the experiments on determining the expression levels of salicylate hydroxylase genes. In our previous study, we showed that *nahG*, which encodes a classic salicylate hydroxylase and is located within the *nah2* operon on the pBS1141 conjugative plasmid, is a silent gene [25]. To find out whether the other two genes, *nahU* and *nahG1*, were functional, we tested the cells grown on a salicylate medium using RT-PCR (Fig. 2). It was found that *nahU* was a functional gene, while *nahG1* was silent, as it was the case in *P. putida* KT2440. The *nahG1* sequences present in the other strains also were probably nonfunctional, whereas the Sal^+ phenotype was determined by *nahU*.

Analysis of the genes encoding catechol 2,3-dioxygenase (*nahH*) and catechol 1,2-dioxygenase (*catA*). To find out whether salicylate-catabolizing strains utilized the *meta*- or the *ortho*-pathway of catechol cleavage, we performed PCR amplification of the *nahH* and *catA* sequences encoding catechol 2,3-dioxygenase

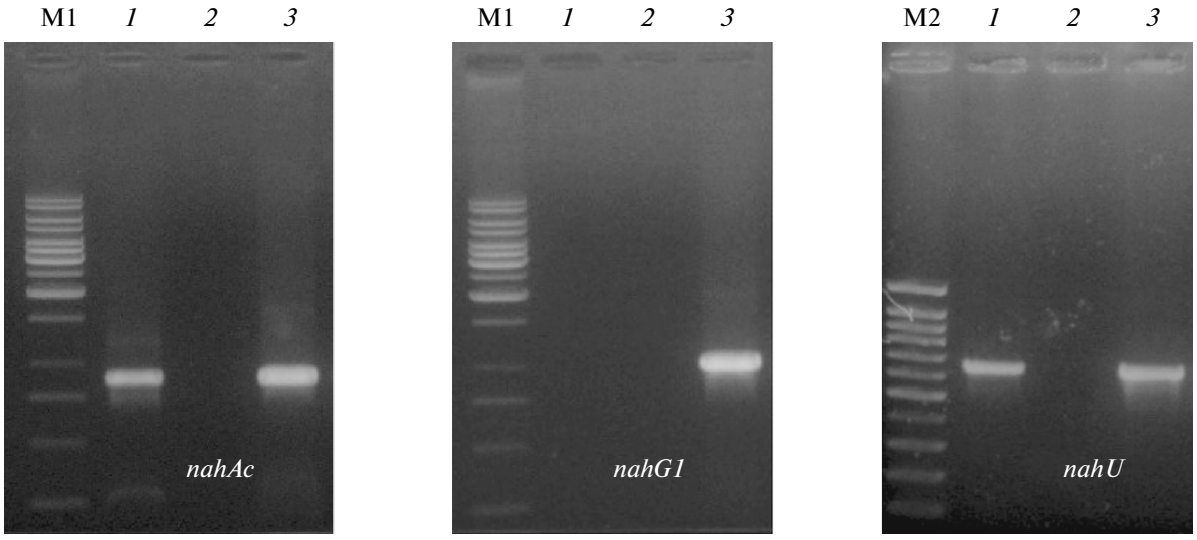

Fig. 2. Products of RT-PCR of *nahG1* and *nahU* sequences from *P. putida* strain BS3701 templates. M1, 1 kb DNA ladder (Fermentas); M2, DNA marker M6 (Ecobiotechnologiya, Russia); (*1*), cDNA; (*2*), total RNA without reverse transcriptase treatment; (*3*), total DNA of *P. putida* BS3701. Naphthalene 1,2-dioxygenase gene *nahAc* served as a positive control.

and catechol 1,2-dioxygenase, respectively. It was found that *nahH* sequences were present only in Vs strains isolated from a soil sample collected at a petrol filling station in the Nizhni Novgorod oblast (Table 1). It should be noted that the strains carrying the *nahH* gene sequence were able to grow on naphthalene.

The results of PCR tests with *catA*-specific primers showed that a large majority of Sal+ strains carried *catA* sequences and therefore exhibited catechol 1,2-dioxygenase activity. This observation agrees with the data from other publications, suggesting that pseudomonads usually possess chromosome-encoded enzymes of the *ortho*-pathway of catechol degradation [26]. For this reason, the fact that *catA* sequences were not amplified in strains Nks1, Nks2, Nks4, and Nks9 isolated from the Nizhnekamskneftekhim sludge collector attracts special attention.

Exogenous isolation and characterization of salicylate catabolism plasmids from polluted soils. Since analysis of the salicylate-catabolizing isolates identified only one plasmid responsible for the degradation of this compound, ps6F, we also applied exogenous isolation to retrieve SAL plasmids. This technique is an efficient approach used to investigate the diversity and ecology of naturally occurring plasmids. The well-characterized *P. putida* strain KT2442 was used as recipient. Exogenous plasmid isolation was performed with samples of oil-polluted soil collected in Nizhni Novgorod oblast (no. 8) and Pushchino (NP10 and NP11); we also used the soil samples collected in oil fields of the Yamal Peninsula: Y1, Y2, Y3, Y4, and Y5. In these experiments, samples NP11, Y1, Y2, and Y5 gave rise to *P. putida* KT2442 transconjugants capable of growing on salicylate.

Plasmid DNA was obtained from exogenous isolates, and the size of plasmids was determined by electrophoresis (Fig. 3). Analysis of 30 isolates revealed at least two types of salicylate biodegradation plasmids of 50 and 75 kb in size (Fig. 3). The *Eco*RI restriction mapping of the isolated plasmids showed that even a single soil sample contained plasmids differing significantly from each other. In particular, the plasmids pY1-2 and pY1N obtained from a soil sample from the Yamal oil field are worth special discussion (Fig. 4). Their restriction profiles exhibited a considerable similarity; however, pY1N controlled both naphthalene and salicylate degradation, whereas pY1-2 was

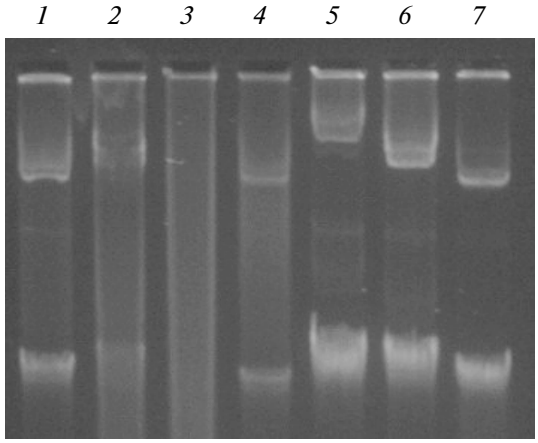

Fig. 3. Plasmid DNA of exogenous isolates obtained from a soil samples collected in Pushchino. (*1*), EI no. 1, (*2*), EI no. 2; (*3*), EI no. 5; (*4*), EI no. 6; (*5*), NPL-1; (*6*), pNF142; (*7*), 8909-2. Plasmids NPL-1 (100 kb), pNF142 (83 kb), and 8909-2 (50 kb) from the laboratory collection were used as controls.

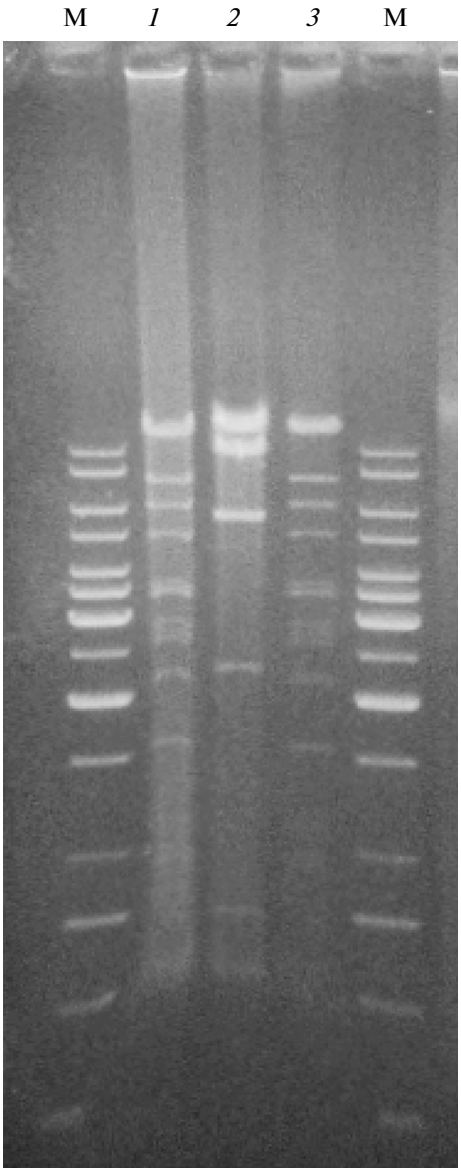

Fig. 4. Plasmid DNA of exogenous isolates obtained from soil samples of the Yamal Peninsula, *Eco*RI restriction endonuclease treatment. M, 1 kb DNA ladder (Fermentas);. (*1*), pY1-2; (*2*), pY5-1; (*3*), pY1N.

responsible only for salicylate metabolism. It is possible that pY1-2 was initially capable of controlling naphthalene biodegradation, but lost this capacity due to mutations. On the whole, the restriction profiles of both plasmids were similar to the profile of the archetypical pDTG1 plasmid of the P-9 group commonly occurring in polluted soils.

Using PCR with the *rep* region-specific primers (Table 2), we determined that all exogenously isolated plasmids belonged to the P-7 incompatibility group, which confirmed our earlier observation concerning the predominance of this plasmid group in highly polluted soils [27]. The pAK5 plasmid previously classified into the P-7 group served as a control [5]. It should be noted that all SAL plasmids described previously belonged to the IncP-9 group [28].

Analysis of the key genes of salicylate degradation in exogenously isolated plasmids. Exogenously isolated plasmids were tested for the presence of known salicylate hydroxylase-encoding sequences by PCR. All plasmids studied were found to carry the classic salicylate hydroxylase gene *nahG*, which was apparently responsible for the Sal^+ phenotype of the host strains. Thus, *nahG* was the most common gene found in the plasmids controlling salicylate or salicylate and naphthalene biodegradation. The catechol 2,3-dioxygenase gene, *nahH*, encodes the key enzyme of the *meta*-pathway of catechol degradation. Its presence indirectly suggests that the strain carries biodegradation plasmids containing the catabolic operons [29]. Exogenous isolate colonies growing on E-agar with salicylate or succinate as a sole carbon and energy source were sprayed with 100 mM catechol solution in water; this test revealed catechol 2,3-dioxygenase activity in all strains except Y1-7 (after treatment, the colonies turned yellow due to accumulation of 2-hydroxymuconic semialdehyde, a catechol oxidation intermediate). In all strains except Y1-Nah, this activity was salicylate-induced and was not observed in the isolates growing on succinate (i.e., the colonies did not turn yellow after spray treatment).These data suggest that, in addition to the classical *nahG* gene, the exogenously isolated plasmids apparently contained the *nah2* operon, and the plasmid-carrying strains express the *meta*-pathway of catechol degradation.

DISCUSSION

Accumulation of salicylates and their substituted derivatives in the environment usually suppresses microbial growth; however, certain bacteria can metabolize these compounds and utilize them as sole sources of carbon and energy.

As a rule, the biochemical and genetic aspects of salicylate biodegradation have been considered together with the same issues concerning its higher-molecular-weight precursors, such as naphthalene and phenanthrene. In pseudomonads, naphthalene and phenanthrene degradation is most commonly controlled by plasmid genes [6, 7]. Salicylate is produced as an intermediate in the course of their oxidation and is further cleaved by salicylate 1-monooxygenase (salicylate hydroxylase). Conventionally, the classical salicylate hydroxylase gene is *nahG* from naphthalene-catabolizing *P. putida* strains G7 and NCIB 9816-4, where it is located on the NAH7 and pDTG1 plasmids, respectively [6, 7]. Other strains carrying several nonhomologous salicylate hydroxylase-encoding genes have been described: *P. putida* ND6 [10] and *P. stutzeri* AN10 [9]. Both NahG and NahW salicylate hydroxylases from *P. stutzeri* AN10 have broad substrate specificity, although the relative degradation rates vary considerably among the substituted salicy-

late derivatives. For instance, chlorosalicylates are more efficiently metabolized by NahW, whereas NahG is more active towards methylated salicylate derivatives [9]. NahG and NahU salicylate hydroxylases from *P. putida* ND6 also exhibit broad substrate specificity [10], although NahU has a higher catalytic activity and a higher affinity to salicylate and cofactors than NahG. The homology between the *nahU* and *nahG* nucleotide sequences is only 65%.

The exogenously isolated plasmids of salicylate biodegradation were also found to carry the *nahG* gene and to direct catechol degradation along the *meta*-pathway. The SAL plasmids exhibit considerable similarity to naphthalene biodegradation plasmids. For example, comparison of SAL (68 kb), SAL1 (85 kb), and NAH7 (83 kb) structures showed that the first two plasmids were NAH7 derivatives produced by a series of insertions and deletions that disrupted the expression of the *nah1* operon [30].

The ps6F plasmid from *P. fluorescens* strain s6F was the only salicylate degradation plasmid isolated from the 75 Sal+ strains studied; in contrast to the exogenously isolated SAL plasmids, it had a different restriction profile (data not shown), but also carried the *nahU* salicylate hydroxylase gene and not the classical *nahG*. In the pND6-1 plasmid (GenBank acc. no. AY208917), the *nahU* sequence lies separately and is not included in any catabolic operon; however, the adjacent *catA* gene encodes catechol 1,2-dioxygenase, the first enzyme of the *ortho*-pathway of catechol degradation. In pseudomonads, the genes of the *ortho*-pathway are usually located on the chromosome. The *ortho*-pathway is usually utilized for benzoate utilization, which may be carried out by many pseudomonad [26]. To obtain the ability to utilize salicylate, it would suffice to acquire a salicylate hydroxylase gene.

In the analyzed salicylate-catabolizing strains that lacked salicylate biodegradation plasmids, the key gene of salicylate utilization was *nahU,* and subsequent catechol degradation occurred via the *ortho*-pathway. It is possible that, similarly to the pND6-1 plasmid, *nahU* is located next to *catA* and further catechol oxidation genes.

Although in an overwhelming majority of known strains *nahU* is located on the chromosome, the fact that it was found in the pND6 and ps6F conjugative plasmids suggests that this sequence is capable of transposing between the chromosome and plasmid DNA, which serves for the wide occurrence of the Sal+ phenotype in microbial populations. Such genes that do not belong to any catabolic operons probably play a key role in the spreading of the Sal+ phenotype among salicylate-catabolizing bacteria.

The wide occurrence of *nahU* observed in our study in salicylate-catabolizing strains with chromosomal control of salicylate degradation indicates that this gene might be as classical in pseudomonads as *nahG* is considered in NAH and SAL plasmids.

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