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EXPERIMENTAL ARTICLES

Molecular Biological Characterization of Biphenyl-Degrading Bacteria and Identification of the Biphenyl 2,3-Dioxygenase α-Subunit Genes

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Abstract—Bacterial isolates from soils contaminated with (chlorinated) aromatic compounds, which degraded biphenyl/chlorinated biphenyls (CB) and belonged to the genera *Rhodococcus* and *Pseudomonas*, were studied. Analysis of the 16S rRNA gene sequences was used to determine the phylogenetic position of the isolates. The *Rhodococcus* cells were found to contain plasmids of high molecular mass (220–680 kbp). PCR screening for the presence of the *bphA1* gene, a marker indicating the possibility for induction of 2,3-dioxygenase (biphenyl/toluene dioxygenase subfamily), revealed the presence of the *bphA1* genes with 99–100% similarity to the homologous genes of bacteria of the relevant species in all pseudomonad and most *Rhodococcus* isolates. A unique *bphA1* gene, which had not been previously reported for the genus, was identified in *Rhodococcus* sp. G10. The absence of specific amplification of the *bphA1* genes in some biphenyl-degrading bacteria (*Rhodococcus* sp. B7b, B106a, G12a, P2kr, P2(51), and P2m), as well as in an active biphenyl degrader *Rhodococcus ruber* P25, indicated the absence of the genes encoding the proteins of the biphenyl/toluene dioxygenase subfamily and participation of the enzymes other than this protein family in biphenyl/CB degradation.

Keywords: bacterial degraders, biphenyl, chlorinated biphenyls, plasmids, *bphA1, Rhodococcus, Pseudomonas*

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Anthropogenic contamination of the environment with stable toxic compounds is one of the most urgent ecological problems. Polychlorinated biphenyls (PCB), a type of highly toxic aromatic compound, which are extremely resistant to physical and chemical treatment, are among common pollutants. Despite the ban on industrial production and application of PCB since the 1970s, the problem of PCB utilization and remediation of contaminated soils and bottom sediments remains a pressing one. Bacteria are known to play an important role in processes of self-purification of natural environments contaminated with various aromatic compounds, including the polychlorinated biphenyls. Bacterial degraders of biphenyl and chlorinated biphenyls have been found in several phylogenetic groups. Among these, the biodegradable potential of gram-negative bacteria has been most fully studied, while gram-positive bacteria are less studied, although the latter group is of interest due to its ability to decompose a wide spectrum of stable xenobiotics and stability to unfavorable environmental factors [1–4].

Bacterial metabolism of unsubstituted biphenyl and its chlorinated derivatives to benzoic or chlorobenzoic acids follow the same biochemical route. That is why many biphenyl-utilizing bacteria are capable of complete or partial degradation of chlorinated biphenyls [1]. Biphenyl, due to its lower toxicity compared to its chlorinated derivatives, is used as a model compound in studies of enzymes and genetic systems involved in bacterial degradation of PCB. The first reaction of biphenyl/PCB oxidation, hydroxylation of the aromatic ring with formation of (chloro)biphenyl dihydrodiol, is performed by biphenyl 2,3-dioxygenase (BDO), a representative of a large family of hydroxylating dioxygenases containing the Rieske-type cluster and nonheme iron [5]. This is the key enzyme in bacterial biphenyl/PCB degradation, determining the spectrum of PCB congeners converted by bacteria. BDO consists of three large (α-) and three small (β-) subunits (BphA1 and BphA2, respectively), ferredoxin (BphA3), and ferredoxin reductase (BphA4). Since the α- subunit is responsible for substrate recognition and binding [1], the *bphA1* gene is an important genetic marker of aerobic bacterial degraders of biphenyl/PCB.

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Study of these genetic biomarkers may provide information on the biodegradation potential of bacteria, as well as the knowledge required to solve practical problems (development of strains and enzymes with preset characteristics for pollutant degradation; diagnostics of the biodegradation properties of bacterial populations, especially in connection with the important contribution of uncultured species of bacteria to the degradation of stable xenobiotics). Investigation of phylogenetic relationships between the key genes of aromatic compound degradation will provide insight on bacterial strategies used to adapt to the changing environment (for example, to the presence of PCB in their habitat), or in other words, to get closer to the solution of the fundamental problems concerning the evolution of enzymatic systems and interactions between organism and environment.

Earlier, we isolated bacterial biphenyl degraders of the genera *Rhodococcus* and *Pseudomonas* from the technologically contaminated soils; their ability to decompose various PCB congeners was studied [6–8]. The goal of the present work was further investigation of the biodegradation potential of the isolated bacteria, in particular, detection of non-chromosomal genetic structures (plasmids) and exploration of the key genes of biphenyl degradation (*bphA1*).

MATERIALS AND METHODS

Bacterial strains. Bacteria used in the present work have been isolated from soil contaminated with (halo)aromatic compounds (JSC Galogen, Perm, Russia): *Rhodococcus* sp. P1, P2, P2m, P2kr, P2(51), P12, P13, P20 [6], *Rhodococcus ruber* P25 (=IEGM896) [8], and *Rhodococcus* sp. G10 [7]; and from soil contaminated with PCB (Serpukhov, Moscow oblast, Russia): *Pseudomonas* sp. S13, S210, S211, and S212 [6]. Strains of bacteria degrading aromatic compounds (from the working collection of the Laboratory of Microbiology and Biotechnology, Institute of Ecology and Genetics of Microorganisms, Russian Academy of Sciences), which were also used in the present work, were designated as P23a and G12a (soil, JSC Galogen, Perm, Russia), and B7b, B106a (soil, JSC Uralkalii, Berezniki, Perm krai, Russia). Bacteria were isolated from enrichment cultures in K1 mineral medium [9] with biphenyl (1 g/L) inoculated with soil samples.

Phenotypic characteristics of the isolated strains were studied by conventional techniques [10].

Ability of the bacteria to grow on aromatic compounds was determined by cultivation on agarized K1 mineral medium and in liquid K1 medium at 28°C with aeration on a temperature-controlled shaker (100 rpm). One of the following compounds was added to the medium as the only source of carbon and energy: benzene, toluene (0.5 g/L), biphenyl, or naphthalene (1 g/L). In the case of bacteria cultivated on solid medium, the substrates were applied to the cap of the overturned petri dish. Bacterial growth was evaluated as previously described [7]. Optical density of the cultures was determined on a TCC-240A (Shimadzu, Japan) spectrophotometer at 600 nm in a quartz cuvette with optical path of 1 cm.

Plasmid DNA was isolated by pulse-field electrophoresis using the CHEF DR II (Bio-Rad Laboratories, United States) as previously described [11].

Isolation of total DNA was performed according to the conventional procedure [12].

Amplification of the 16S rRNA genes using the isolated total DNA as a template was performed with the standard primers f27 and r1493 under conditions described by Tiirola et al [13]. PCR was performed on a MyCycler (Bio-Rad Laboratories).

Amplification of the *bphA1* genes coding for the α-subunit of enzymes of the biphenyl/toluene dioxygenase (B/T DO) subfamily [5] was performed using the degenerate primers bphAf668-3 and bphAr1153-2 under reaction conditions proposed by Witzig et al. [14]. The primers were chosen on the basis of alignment of known nucleotide sequences of bacterial genes coding for α-subunits of terminal oxygenase of the B/T DO subfamily (*bphA1*). The fragment of the *bphA1* gene flanked by the primers codes for the active center of the enzyme [14].

Reaction products were separated by electrophoresis in agarose gel (1%) at 10 V/cm, stained with ethidium bromide (5 μg/mL), and visualized under UV light using the Gel Doc™ XR (Bio-Rad Laboratories) documenting system.

DNA typing of the isolates was performed by the BOX-PCR method according to the standard protocol [15].

Sequencing and analysis of the data. Nucleotide sequences were determined using the Big Dye Terminator Ready Reaction Kit v. 3.1 (Applied Biosystems, United States) on a Genetic Analyser 3500XL (Applied Biosystems) automated sequencer according to the manufacturer's recommendations. Search for homologous sequences was performed in GenBank (http://www.ncbi.nlm.nih.gov) and EzTaxon (http://www.eztaxon.org) databases. Percent similarity of the 16S rRNA genes with homologous genes from the type strains was calculated using online resources of the EzTaxon server. Search for the *bphA1* homologues and preliminary analysis were performed with the BLAST (http://www.ncbi.nlm.nih.gov) software package.

Deposition of nucleotide sequences in GenBank. Data on nucleotide sequences of the *bphA1* gene of the strains *Pseudomonas* sp. S13, S210, S211, S212 and *Rhodococcus* sp. P1, P12, P13, P20, G10 were deposited in the GenBank international database under accession numbers FJ752168, FJ752169, KC832468, FJ752170, FJ752167, FJ765412, FJ752171, KC832467, and KC832469, respectively.

Table 1. Comparison of nucleotide sequences of 16S rRNA in degrader strains with the homologous sequences of the type strains

Strain	Type strain (GenBank accession no.)	Homology, %
B7b	*Rhodococcus erythropolis* DSM 43066^T (X79289)	99.1
B106a	*Rhodococcus erythropolis* DSM 43066^T (X79289)	100.0
G10	*Rhodococcus wratislaviensis* NCIMB 13082^T (Z37138)	99.0
G12a	*Rhodococcus erythropolis* DSM 43066^T (X79289)	98.0
P1	*Rhodococcus wratislaviensis* NCIMB 13082^T (Z37138)	100.0
P2kr	*Rhodococcus erythropolis* DSM 43066^T (X79289)	100.0
P2m	*Rhodococcus erythropolis* DSM 43066^T (X79289)	99.9
P2(51)	*Rhodococcus baikonurensis* GTC 1041^T (AB071951)	100.0
P12	*Rhodococcus wratislaviensis* NCIMB 13082^T (Z37138)	99.8
P13	*Rhodococcus wratislaviensis* NCIMB 13082^T (Z37138)	99.8
P20	*Rhodococcus wratislaviensis* NCIMB 13082^T (Z37138)	100.0
P23a	*Rhodococcus erythropolis* DSM 43066^T (X79289)	100.0
S13	*Pseudomonas xanthomarina* KMM 1447^T (AB176954)	99.5
S210	*Pseudomonas xanthomarina* KMM 1447^T (AB176954)	98.9
S211	*Pseudomonas xanthomarina* KMM 1447^T (AB176954)	98.9
S212	*Pseudomonas xanthomarina* KMM 1447^T (AB176954)	99.0

RESULTS

Identification of bacteria. Strains P23a, G12a, B7b, and B106a were capable of growth on biphenyl. Optical density of these cultures in the liquid K1 medium with biphenyl as the only source of carbon and energy reached 0.3–0.42 OU after 48 h. When grown on solid medium, the strains formed round convex colonies with a smooth surface and even edge, which were shiny, oily, and of creamy rose shade. The bacteria under study were capable of growth in a wide temperature range: from 8 to 42°C, with the optimum at 25–30°C. Active growth was observed at pH 6–9.

Analysis of nucleotide sequences of the 16S rRNA genes in strains P23a, G12a, B7b, and B106a revealed that they were phylogenetically most closely related to the *R. erythropolis* type strain (Table 1). The results of BOX-PCR demonstrated that these strains differed at molecular genetical level from each other and from the previously studied *Rhodococcus* strains (Figs. 1a and 1b).

BOX-PCR profiles of the *Pseudomonas* sp. strains S211 and S212 were similar to each other and different from those of the strains *Pseudomonas* sp. S13 and S210 (Fig. 1c).

Table 1 presents the experimental results that specify the taxonomic position of the bacteria previously identified at the phenotypic level as the representatives of the genera *Rhodococcus* and *Pseudomonas*.

Plasmid DNA of degrader bacteria. Pulse-field electrophoresis of native DNA revealed plasmids in most of the *Rhodoccocus* strains studied. Three plasmids of 30, 220, and 550 kbp were observed in *Rhodococcus* sp. G10 (Fig. 2a).

Strains *Rhodococcus* sp. P1, P12, and P13 were characterized by similar plasmid profiles (Fig. 2a). The DNA pool between the marker fragments of 450 and 565 kbp probably contains at least two plasmids of similar sizes. In *Rhodococcus* sp. P20 one plasmid was distinctly visualized at the level of 450 kbp; another one, at approximately 680 kpb (Fig. 2a). In all these strains, a plasmid of approximately 365 kbp was detected.

Another group with similar plasmid profiles was represented by *Rhodococcus* sp. P2kr, P2m, B7b, and G12a: only one plasmid with electrophoretic mobility close to the fragment of 565-kbp marker DNA was revealed (Fig. 2b).

In strain *Pseudomonas* sp. S211, two plasmids of approximately 100–110 kbp were detected, and in strain *Pseudomonas* sp. S212, a small plasmid of about 23 kbp was found (data not shown).

Bacterial growth on mono(poly)aromatic hydrocarbons. Bacteria under study were screened for similarities/differences in the key enzymes of biphenyl/PCB degradation by means of evaluation of their ability to utilize aromatic compounds that are the substrates of the B/T DO subfamily enzymes (biphenyl, benzene,

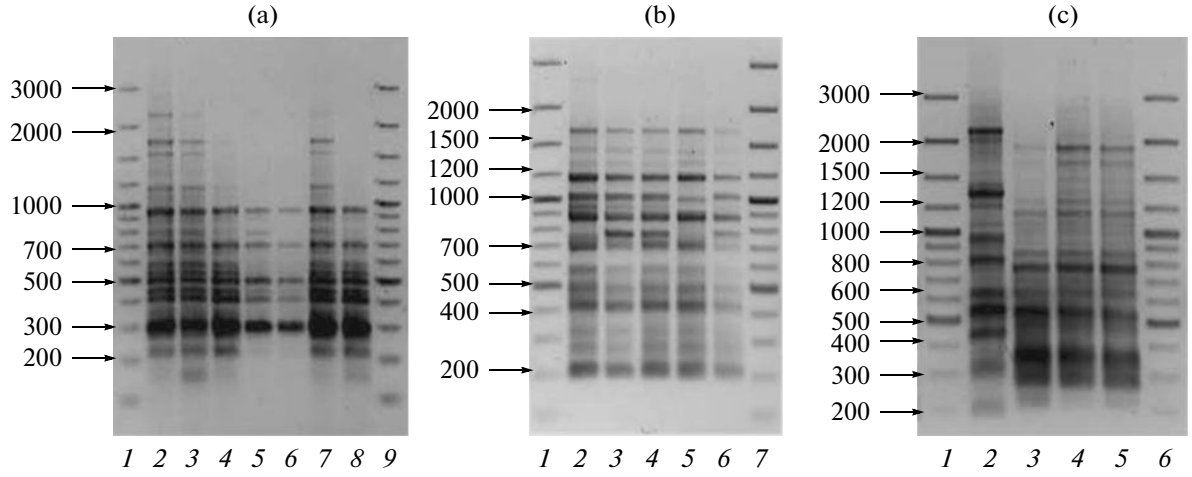

Fig. 1. BOX-PCR analysis of biphenyl-degrading strains (a, b, strains of the genus *Rhodococcus*; c, strains of the genus *Pseudomonas*). (a) *1, 9*, O'GeneRuler^{TM} 100 bp Plus DNA Ladder (Fermentas, Lithuania); *2–8*, strains of the *Rhodococcus* genus: *2*, P2m; *3*, Pkr; *4*, P2(51); *5*, P23a; *6*, G12a; *7*, B7b; *8*, B106a; (b) *1, 7,* O'GeneRuler^{TM} 100 bp Plus DNA Ladder (Fermentas, Lithuania); *2–6*, strains of the genus *Rhodococcus*: *2*, P1; *3*, P12; *4*, P13, *5*, P20; *6*, G10; (c) *1, 6,* O'GeneRuler^{TM} 100 bp Plus DNA Ladder (Fermentas, Lithuania); *2–5*, strains of the genus *Pseudomonas*: *2*, S13; *3*, S210; *4*, S211; and *5*, S212. Arrows indicate size of fragments of marker DNA, bp.

and toluene) as the only source of carbon and energy. Since the spectra of oxidized substrates for biphenyl and naphthalene dioxygenases (NDO) overlap [16], we also studied the ability of bacteria to grow on naphthalene.

Among the bacteria under study, several groups differing in their substrate specificity were revealed (Table 2): (1) *Rhodococcus* sp. B7b, B106a, G12a, P2kr, P2m, and P23a grew well on biphenyl but showed poor or no growth on benzene, toluene, and naphthalene; (2) *Rhodococcus* sp. P1, P12, P13, and P20 were distinguished by their ability to grow actively on practically all substrates; (3) *Rhodococcus* sp. G10 grew well on benzene, toluene, and naphthalene, and grew poorly on biphenyl, producing yellow coloration of the medium, which indicated biphenyl transformation and extracellular accumulation of the *meta*-cleavage product [9]; (4) strain *R. ruber* P25 grew actively on biphenyl and naphthalene and poorly on benzene and toluene; and (5) bacteria of the genus *Pseudomonas* grew only on biphenyl.

Screening of the degrader bacteria for the presence of genes coding for the α-subunit of biphenyl dioxygenases. Seventeen strains (Table 3) were screened for the presence of nucleotide sequences coding for the α-subunit of enzymes of the B/T DO subfamily [14] in their genome. PCR product of expected size of 500 bp was obtained using DNA template of 9 strains (*Rhodococcus* sp. G10, P1, P12, P13, and P20 and *Pseudomonas* sp. S13, S210, S211, and S212). At the same time, no specific amplification was observed from DNA of a number of active biphenyl degraders, in particular, *Rhodococcus* sp. B7b, B106a, G12a, P2kr, P2m, P2(51), and P23a and the biphenyl degrader *R. ruber* P25.

Analysis of nucleotide sequences of the *bphA1* genes. Amplified fragments of the functional genes from *Rhodococcus* sp. P1, P12, P13, and P20 were similar to those of the genes of the α-subunit in the enzymes of the B/T DO subfamily in the known *Rhodococcus* degraders of aromatic compounds (Table 3). The genes of the strains under study were most similar to the *bphA1* gene coding for the α-subunit of BDO of a biphenyl/PCB-degrading *R. opacus* BIE-20 [17] with the homology level of 98.7–99.4%.

Fragment of the *bphA1* gene amplified on the DNA template from *Rhodococcus* sp. G10 was by 99.8% similar to the gene of the α-subunit hydroxylating DO of a toluene-degrading *Arthrobacter* sp. 3YC3 [14] and differed considerably from the homologous genes of rhodococci.

Fragments of the genes amplified on a DNA template from biphenyl-degrading *Pseudomonas* sp. S210, S211, and S212 possessed 100% homology to the *bphA1* genes of the known gram-negative biphenyl-degrading strains, *Achromobacter* sp. BP2 and *Pseudomonas* sp. B2a (Table 4). For the amplicon obtained on a DNA template from *Pseudomonas* sp. S13, maximum homology of 99.6% was observed with *bphA1* of the *Pseudomonas* sp. B2a strain [17].

DISCUSSION

Genome analysis is presently often applied for the study of the processes of degradation of persistent aromatic compounds by microorganisms prospective for biotechnology applications. In the present work, we

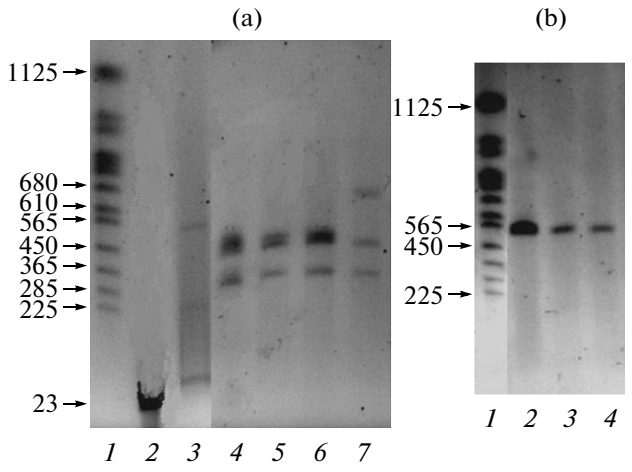

Fig. 2. Pulse-field electrophoresis of the native DNA of biphenyl-degrading bacteria of the genus *Rhodococcus*. (a) Yeast chromosomal (Bio-Rad Laboratories, United States) molecular weight marker (*1*); *Hind*III Ladder (Fermentas, Lithuania) (*2*); strains: G10 (*3*); P1 (*4*), P12 (*5*), P13 (*6*), and P20 (*7*). (b) Yeast chromosomal (Bio-Rad Laboratories, United States) molecular weight marker (*1*); strains: G12a (*2*); B7b (*3*); and P2kr (*4*). Arrows indicate the size of the fragments of marker DNA, kbp.

studied in detail the key genes of biphenyl/PCB degradation (*bphA1*) and determined the extrachromosomal genetic structures (plasmids) in bacterial degraders of the genera *Rhodococcus* and *Pseudomonas*.

Among the rhodococci isolated from technogenic soils, strains with broad substrate specificity and strains catabolizing biphenyl, but inactive toward other substrates, were revealed. The ability of bacteria to transform several substrates (benzene, toluene, and biphenyl) may be explained by the presence either of a biphenyl dioxygenase (BDO) with broad substrate specificity or of several dioxygenases hydroxylating the aromatic ring of each of the substrates. Since the structure of the BDO active center, and thus the spectrum of oxidized substrates and other properties of the enzyme, depends on the amino acid composition of the BphA1 α-subunit, the *bphA1* gene is an important target for investigation of the biodegradation potential of bacteria [18]. To reveal homology/differences (at molecular genetic level) among biphenyl dioxygenases of the strains belonging to different groups according to their substrate specificity, we performed screening of the *bphA1* genes coding for the α-subunit of BDO.

It is known that most biphenyl dioxygenases of rhodococci resemble benzene/toluene dioxygenases (subfamily of B/T DO) and may oxidize compounds with similar chemical structure. Different enzymes of the subfamily of B/T DO of gram-positive bacteria (biphenyl, toluene, benzene, and isopropylbenzene dioxygenases) are more similar between each other than with the BDO of gram-negative bacteria [5]. This may explain active growth of rhodoccoci belonging to group 2 on all substrates, while pseudomonads (group 5) grew only on biphenyl (Table 2). In bacteria of group 2 (*Rhodococcus* sp. P1, P12, P13, and P20), which were phylogenetically close to *R. wratislaviensis* (Table 1) by their 16S rRNA gene sequence, the *bphA1* genes similar to the genes of the α-subunits of the B/T DO subfamily of the known *Rhodococcus* strains degrading aromatic compounds were revealed (Table 3). High level of similarity of the revealed gene fragment to the genes of toluene, benzene, and isopropylbenzene DO confirms the hypothesis that when the strains are cultured on biphenyl, benzene, and toluene, the same enzyme—biphenyl dioxygenase with broad substrate specificity—is induced.

Despite the enzyme specialization toward specific substrates developed in the course of evolution, overlapping of the spectra of oxidized compounds is observed even for oxygenases of different subfamilies. Some of the described naphthalene dioxygenases (NDO) of the naphthalene dioxygenases subfamily [16] are known to be able to oxidize chlorinated biphenyls. For example, NDO of the *P. putida* G7 strain efficiently transformed PCB [16]. *Sphingomonas aromaticivorans* F199, *Bacillus* sp. JF8, *Rhodococcus* sp. R04, and *Rhodococcus* sp. K37 were also shown to degrade biphenyl using dioxygenases homologous to the phenanthrene and naphthalene dioxygenases [1, 4, 19–21]. One may not exclude the presence of similar enzymes in the biphenyl degraders under study, in which no genes of the biphenyl/toluene dioxygenases subfamily were detected (strains *Rhodococcus* sp. B7b, B106a, G12a, P2kr, P2m, P2(51), P23a, and *R. ruber* P25). This hypothesis may be supported by the fact that in *R. ruber* P25, an active degrader of aromatic compounds/PCB, the genes coding for the α and β-subunits of naphthalene dioxygenases (data not shown) and exhibiting 99% similarity to NDO genes of rhodococci—*Rhodococcus* sp. DB11, *Rhodococcus* sp. P400, etc.—were revealed [22, 23]. Therefore, one may assume that biphenyl decomposition by the bacteria for which no specific amplification of the *bphA1* gene was observed is performed by the enzymes different from those of the B/T DO subfamily of dioxygenases.

Both in pseudomonads and rhodococci, plasmids of various molecular masses were revealed. The presence of large plasmids in the cells is typical for the bacteria of the genus *Rhodococcus* [2]. The genes determining the ability of bacteria to metabolize aromatic compounds are known to be localized in plasmids [24]. The plasmids revealed in rhodococci under study (Fig. 2) were comparable in size to the plasmid DNA of known degraders of aromatic compounds. For example, three linear plasmids of 330, 450, and 1100 kbp, where biphenyl/PCB degradation genes are localized, were detected in *R. jostii* RHA1 [25]. In the cells of a (chloro)aromatic compound degrader *R. opacus* 1CP, a linear plasmid of 740 kbp responsible

Table 2. Bacterial growth on aromatic compounds*

Group no.	Strain	Biphenyl**	Benzene	Toluene	Naphthalene
1	*Rhodococcus* sp. B7b	++++	–	–	+
	Rhodococcus sp. B106a	++++	–	–	+
	Rhodococcus sp. G12a	++++	–	–	+
	Rhodococcus sp. P2m	++++	+	+	–
	Rhodococcus sp. P2kr	++++	+	+	–
	Rhodococcus sp. P23a	++++	–	–	–
	Rhodococcus sp. P2(51)	+++	+	–	–
2	*Rhodococcus* sp. P1	++++	++++	++	++++
	Rhodococcus sp. P12	++++	+++	+++	++++
	Rhodococcus sp. P13	++++	+++	+++	++++
	Rhodococcus sp. P20	++++	+++	+++	++++
3	*Rhodococcus* sp. G10***	++	++++	++++	++++
4	*R. ruber* P25	++++	+	+	+++
5	*Pseudomonas* sp. S13	+++	–	–	–
	Pseudomonas sp. S210	+++	–	–	–
	Pseudomonas sp. S211	+++	–	–	–
	Pseudomonas sp. S212	+++	–	–	–

* Growth was assessed as the size of colonies on day 7: "–", no growth, "+" to "++++" denote activity of growth (colonies of 0.1 cm were considered "+"; 0.1–0.2 cm, "++"; 0.2–0.3 cm, "+++"; over 0.3 cm, "++++").

** Growth on biphenyl was determined on agar and in liquid K1 medium. When grown in liquid medium, OD_{600} of 0.2–0.3, 0.3–0.4, 0.4–0.6, and >0.6, were designated as "+", "++", "+++", and "++++", respectively.

*** When the strain was cultured with biphenyl, the medium was colored yellow.

for catabolism of chlorocatechols [24] is located, and in the cells of *R. aetherivorans* I24, a plasmid of 340 kbp where the *tid* genes coding for toluene dioxygenase [26] are present. Therefore, one may not exclude that in the strains under study the genes controlling decomposition of aromatic compounds are located in the plasmids.

There are interesting data evidencing a high level of similarity (99.8%) of the *bphA1* gene fragment amplified on the DNA template from *Rhodococcus* sp. G10 to the α-subunit of hydroxylating DO of a toluene degrader belonging to another genus, *Arthrobacter* sp. 3YC3. Moreover, strain G10 contains three plasmids (Fig. 2a). These data may indicate the possibility of horizontal transfer of the gene under study between the representatives of the genera *Arthrobacter* and *Rhodococcus*.

The results of our studies show that a number of biphenyl/chlorobiphenyls-degrading bacteria of the genus *Rhodococcus*, as well as of the genus *Pseudomonas*, possess the genes coding for biphenyl 2,3-dioxygenases of the B/T DO subfamily specific for each of the two taxonomic groups. However, in other degraders of the genus *Rhodococcus*, similar genes were not detected, and molecular genetic mechanisms providing for efficient degradation of biphenyl/chlorinated biphenyls in these bacteria require further investigation.

ACKNOWLEDGMENTS

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Table 3. Analysis of nucleotide sequences of the *bphA1* genes in bacterial degraders under study

Strain	Degrader strains, homologous genes (GenBank accession no.)	Homology, %	Reference
	Strains of the genus *Rhodococcus*		
P1	*R. opacus* BIE-20, *bphA1* (AJ544524)	99.4	[17]
	R. aetherivrans I24, gene of the α-subunit of the toluene-induced DO (AF452376)	99.4	[26]
	R. opacus B4, *bnzA1* (AP011117)	99.4	[27]
	R. erythropolis BD2, *ibpA1* (U24277)	98.7	[28]
	Rhodococcus jostii RHA1, *bphA1* (D32142)	96.5	[29]
P12	*R. opacus* BIE-20, *bphA1* (AJ544524)	99.2	[17]
	R. aetherivrans I24, gene of the α-subunit of the toluene-induced DO (AF452376)	98.2	[26]
	R. opacus B4, *bnzA1* (AP011117)	98.2	[27]
	R. erythropolis BD2, *ibpA1* (U24277)	97.6	[28]
	Rhodococcus jostii RHA1, *bphA1* (D32142)	95.5	[29]
P13	*R. opacus* BIE-20, *bphA1* (AJ544524)	98.7	[17]
	R. aetherivrans I24, gene of the α-subunit of the toluene-induced DO (AF452376)	98.7	[26]
	R. opacus B4, *bnzA1* (AP011117)	98.7	[27]
	R. erythropolis BD2, *ibpA1* (U24277)	98.1	[28]
	Rhodococcus jostii RHA1, *bphA1* (D32142)	95.7	[29]
P20	*R. opacus* BIE-20, *bphA1* (AJ544524)	99.1	[17]
	R. aetherivrans I24, gene of the α-subunit of the toluene-induced DO (AF452376)	99.1	[26]
	R. opacus B4, *bnzA1* (AP011117)	99.1	[27]
	R. erythropolis BD2, *ibpA1* (U24277)	98.4	[28]
	Rhodococcus jostii RHA1, *bphA1* (D32142)	96.0	[29]
G10	*Arthriacter* sp. 3YC3, putative Rieske non-heme iron-sulfur oxygenase α-subunit gene (DQ336942)	99.8	[14]
	Strains of the genus *Pseudomonas*		
S13	*Pseudomonas* sp. B2a, *bphA1* (AJ544518)	99.6	[17]
	Pseudomonas sp. B4, *bphA1* (U95054)	99.1	–
	B. xenovorans LB400, *bphA1* (CP000272)	97.6	[30]
S210	*Achromobacter* sp. BP3, *bphA1* (EU812171)	100	–
	Pseudomonas sp. B2a, *bphA1* (AJ544518)	100	[17]
	Pseudomonas sp. B4, *bphA1* (U95054)	99.6	–
	B. xenovorans LB400, *bphA1* (CP000272)	97.7	[30]
S211	*Achromobacter* sp. BP3, *bphA1* (EU812171)	100	–
	Pseudomonas sp. B2a, *bphA1* (AJ544518)	100	[17]
	Pseudomonas sp. B4, *bphA1* (U95054)	99.6	–
	B. xenovorans LB400, *bphA1* (CP000272)	97.8	[30]
S212	*Achromobacter* sp. BP3, *bphA1* (EU812171)	100	–
	Pseudomonas sp. B2a, *bphA1* (AJ544518)	100	[17]
	Pseudomonas sp. B4, *bphA1* (U95054)	99.8	–
	B. xenovorans LB400, *bphA1* (CP000272)	97.9	[30]

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