Microbiology, Vol. 72, No. 6, 2003, pp. 672–677. Translated from Mikrobiologiya, Vol. 72, No. 6, 2003, pp. 759–765.

EXPERIMENTAL ARTICLES

A New Aerobic Gram-Positive Bacterium with a Unique Ability to Degrade *ortho*- and *para*-chlorinated Biphenyls

D. O. Rybkina*, E. G. Plotnikova*, L. V. Dorofeeva, Yu. L. Mironenko***, and V. A. Demakov***

**Institute of Ecology and Genetics of Microorganisms, Ural Division, Russian Academy of Sciences, ul. Goleva 13, Perm, 614081 Russia*
***Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, pr. Nauki 5, Pushchino, Moscow oblast, 142290 Russia*
****Omutninsk Industrial Scientific Base, Vostochnyi settlement, Kirov oblast, Russia*

Received April 21, 2002; in final form, July 21, 2002

Abstract—Strain B51 capable of degrading polychlorinated biphenyls (PCB) was isolated from soil contaminated with wastes from the chemical industry. Based on its morphological and chemotaxonomic characteristics, the strain was identified as a *Microbacterium* sp. Experiments with washed cells showed that strain B51 is able to degrade *ortho*- and *para*-substituted mono-, di-, and trichlorinated biphenyls (MCB, DCB, and TCB, respectively). Unlike the known PCB degraders, *Microbacterium* sp. B51 is able to oxidize the *ortho*-chlorinated ring of 2,2'-DCB and 2,4'-DCB and the *para*-chlorinated ring of 4.4'-DCB. The degradation of 2,4'-DCB and 4,4'-DCB was associated with the accumulation of 4-chlorobenzoic acid (4-CBA) in the medium in amounts comprising 80–90% of the theoretical yield. The strain was able to utilize 2-MCB, 2,2'-DCB, and their intermediate 2-CBA and to oxidize the mono(*ortho*)-chlorinated ring of 2,4,2'-TCB and the di(*ortho-para*)-chlorinated ring of 2,4,4'-TCB. A mixed culture of *Microbacterium* sp. B51 and the 4-CBA-degrading bacterium *Arthrobacter* sp. H5 was found to grow well on 1 g/l 2,4'-DCB as the sole source of carbon and energy.

Key words: biodegradation, polychlorinated biphenyls, chlorobenzoic acids, *Microbacterium*, mixed culture.

Polychlorinated biphenyls (PCBs) are widely used in industry due to their valuable chemical and physical properties (such as good dielectric characteristics, inflammability, and resistance to elevated temperatures, acids, and alkali). On the other hand, PCBs are toxic compounds, which, because of their extensive use and persistence, accumulate in soil and bottom sediments and may come into contact with animals and even humans, causing some diseases [1]. For this reason, the problem of PCB biodegradation is of great social importance.

The PCB family amounts to 209 isomers, differing in the number and position of substituents in the biphenyl molecule. In nature, PCBs are mainly degraded through microbial attack [2, 3]. At the first step, polychlorinated biphenyls are reductively dehalogenated by anaerobic microorganisms with the production of PCB isomers with one to three substituents occurring mostly in the *ortho* or *para* position. The further degradation of PCB molecules is accomplished by aerobic microflora [3–5]. The key role in the primary attack at the biphenyl molecule is played by biphenyl dioxygenase (BphA), which, together with the subsequent enzymes of PCB metabolism, accounts for the substrate specificity of PCB-degrading microorganisms [6–10].

Most of the known microbial degraders of PCBs degrade them to the respective chlorobenzoic acids (CBAs) and pentadienoic acids (Fig. 1) [3, 4]. CBAs are also ecologically hazardous compounds [1]. There are only a few known natural bacterial strains capable of utilizing monochlorinated biphenyls in low amounts and one *Burkholderia* sp. strain SK-3 capable of degrading 2,4'-dichlorobiphenyl (2,4'-DCB) [3, 11]. The complete mineralization of PCBs in nature is accomplished by microbial communities containing both PCB and CBA degraders [2, 3]. Some mixed cultures that are able to utilize monochlorinated and dichlorinated biphenyls (MCB and DCB) have been described [5, 12]. The range of PCB isomers and the efficiency of their transformation by mixed microbial cultures depend on the activity of relevant enzymes in the degrading bacteria [7, 9, 10].

This work was undertaken to characterize the aerobic strain *Microbacterium* sp. B51 with a unique ability to degrade *ortho*- and *para*-chlorinated biphenyls and to study the possibility of using this strain in mixed cultures for the degradation of a wide range of PCBs.

0026-2617/03/7206-0672$25.00

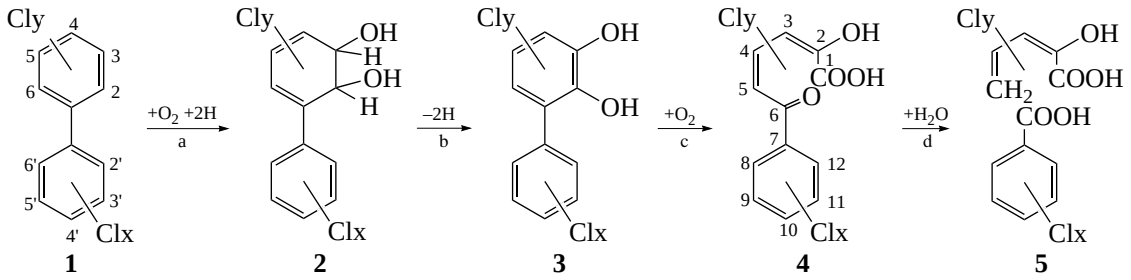

Fig. 1. Putative scheme of the microbial degradation of polychlorobiphenyls. Compounds: (**1**) polychlorobiphenyl, (**2**) chlorobiphenyldihydrodiol, (**3**) chlorohydroxybiphenyl, (**4**) chloro-2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoic acid (HOPDA), (**5**) chlorobenzoic acid. The enzymes involved: (a) biphenyl dioxygenase (BphA), (b) biphenyldihydrodiol dehydrogenase (BphB), (c) hydroxybiphenyl 2,3-dioxygenase (BphC), (d) HOPDA hydrolase (BphD).

MATERIALS AND METHODS

The PCB-degrading strain *Microbacterium* sp. B51 was isolated from an enrichment culture using a mineral K1 agar [13] with biphenyl as the sole source of carbon and energy. To obtain the enrichment culture, a soil sample (1 g) contaminated with wastes from the chemical plants of Berezniki (Perm oblast) was placed in a flask with 50 ml of the liquid K1 medium with biphenyl. The flask was incubated at 28°C for 3 months. The morphological, physiological, and biochemical characteristics of the isolate were studied by conventional methods [14] and its chemotaxonomic characteristics were investigated as described earlier [15]. Experiments with mixed cultures were carried out using the *Arthrobacter* sp. strain H5 capable of degrading 4-CBA [16].

To study the products of PCB degradation, *Microbacterium* sp. B51 was grown at 28°C in the K1 medium with biphenyl (1 g/l) to a cell density corresponding to OD_{615} = 1.0. Cells were harvested, washed twice with the fresh K1 medium, and suspended in the same medium to a density OD_{615} = 2.0. The cell suspension was dispensed (in 1-ml aliquots) into vials with Teflon caps. The vials were supplemented with MCB (94.25 mg/l), or DCB (22.3 mg/l), or TCB (12.8 mg/l) and incubated at 28°C on a shaker. Each of the experimental variants was performed in triplicate.

Chlorobenzoic acids in the incubation medium were analyzed using an HPLC chromatograph from LKB Bromma (Sweden) equipped with a (250 × 4.6 mm ID) reversed-phase column RP-18 (Alltech, United States) [4]. CBAs were identified by their retention times using the authentic samples of 2-CBA, 4-CBA, and 2,4-dichrolobenzoic acid (2,4-DCBA). The other products of PCB transformation, namely, 2-hydroxo-6-oxo-(chlorophenyl)-hexa-2,4-dienoic acids, were determined by measuring their absorbance at 390 to 430 nm using a Perkin-Elmer 402 spectrophotometer [4]. The dehalogenation of chlorinated substrates by bacterial cells was studied by analyzing the reaction product of silver nitrate with the chlorine liberated from the chlorinated substrates into the culture liquid [13]. The absorbance of the reaction product (silver chloride) was measured at 460 nm.

The ability of *Microbacterium* sp. B51 and *Arthrobacter* sp. H5 to utilize 2,4'-DCB was studied either with washed cells of these bacteria and a concentration of 2.4'-DCB equal to 0.1 g/l or with a mixed culture of these bacteria and a concentration of 2,4'-DCB equal to 1 g/l. In the latter case, the mixed culture was incubated at 28°C on a shaker (100 rpm) in 250-ml flasks containing 50 ml of the K1 medium with 2,4'-DCB as the sole source of carbon and energy.

Colony-forming units (CFU) were determined by plating serial dilutions onto LB agar.

RESULTS

Strain B51 grown on LB agar produced convex, pasty, yellow colonies. In young cultures, cells were thin, irregular rods, sometimes occurring in V-shaped pairs. In old cultures cells were short rods. The strain was gram-positive, acid-sensitive, chemoorganotrophic, catalase-positive, oxidase-positive, did not produce spores, and showed good growth under aerobic conditions and poor growth under microaerobic conditions. The peptidoglycan was found to contain ornithine, alanine, glycine, homoserine, glutamic acid, and hydroxyglutamic acids in a proportion of 1.0 : 1.1 : 3.0 : 0.7 : 0.3 : 0.7. Such an amino acid composition is typical of B2β peptidoglycan and has thus far been reported only for bacteria of the genus *Microbacterium* [17]. The major isoprenoid quinones of the respiratory chain were found to be menaquinones with a nonhydrated 11-unit isoprenoid chain (MQ-11). Based on these data, the isolate B51 was identified as a *Microbacterium* sp. [17].

Experiments with washed cells showed that *Microbacterium* sp. B51 was able to degrade 2-MCB, 4-MCB, 2,2'-DCB, 2,4'-DCB, 4,4'-DCB, 2,4,2'-TCB, and 2,4,4'-TCB (table). The incubation of B51 cells with monochlorinated biphenyls led to the accumulation of the respective chlorobenzoic acids in the medium. The oxidation of the unsubstituted ring of 2-MCB was accompanied by the formation of 2-CBA

The degradation of various chlorobiphenyls by *Microbacterium* sp. B51

PCB	Initial concentration, mg/l	Incubation time, h	Concentration of chlorine ions produced, mg/l	Chlorobenzoic acid produced			HOPDA	
				Position of chlorine atom	Concentration		λ_{max}, nm	Absorbance
					mg/l	%*		
2-MCB	94.25	0	ND	2	–	–	395	–
		5	ND		56.6 ± 0.2	72.3		–
		24	ND		11.82 ± 0.01	15.1		>0.1
4-MCB	94.25	0	ND	4	–	–	434	–
		5	ND		31.11 ± 0.08	39.7		>0.1
		24	ND		28.31 ± 0.05	36.0		>0.1
2,2'-DCB	22.3	0	–	2	–	–	–	–
		5	3.8 ± 0.2		14.13 ± 0.03	90.3		–
		24	6.3 ± 0.1		1.65 ± 0.02	10.6		–
2,4'-DCB	22.3	0	1.7 ± 0.5	4	0.96 ± 0.01	6.1	–	–
		5	2.7 ± 0.3		13.66 ± 0.01	87.3		–
		24	2.7 ± 0.2		14.46 ± 0.01	92.4		–
4,4'-DCB	22.3	0	–	4	–	–	432	–
		5	–		3.59 ± 0.01	23.0		0.36 ± 0.03
		24	–		12.69 ± 0.06	81.1		0.75 ± 0.02
2.4,2'-TCB	12.8	0	–	2.4	–	–	–	–
		5	–		–	–		–
		24	–		1.41 ± 0.02	14.8		–
2.4,4'-TCB	12.8	0	–	4	–	–	–	–
		5	–		–	–		–
		24	–		0.70 ± 0.08	9.0		–

Note: ND stands for “not determined”. The symbol “–” stands for “not detected”.
* Percent of the theoretical yield.

in an amount comprising 72.3% of the theoretical yield (in the course of 5-h incubation). After 24 h of incubation, the amount of 2-CBA in the medium decreased by 4.8 times (table). During the degradation of 4-MCB, the amount of 2-CBA accumulated in the medium reached 36–39% of the theoretical yield.

Strain B51 efficiently degraded DCB molecules containing one substituent in the *ortho* or *para* position (table). In the incubation medium with 2,2'-DCB, the product of the *meta* cleavage of PCB (2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoic acid, HOPDA) was not detected, although 2-CBA and free chlorine ions were found to be accumulating. After 5 h of incubation, the amount of 2-CBA in the medium reached 90% of the theoretical yield. After 24 h of incubation, the amount of 2-CBA decreased ninefold. Strain B51 degraded 2,4'-DCB to 4-CBA. In this case, no HOPDA was detected in the incubation medium. After 24 h of incubation, the amount of 4-CBA in the medium reached 90% of the theoretical yield. The amount of free chlorine ions accumulated in the medium by the end of the incubation period corresponded to the complete dehalogenation of one ring of the 2,4-DCB molecule. At the same time, the degradation of 4,4'-DCB was accompanied by the accumulation of 3,10-chlorinated HOPDA with λ_{max} = 432 nm, which gave yellow color to the medium [10]. By the end of the incubation period, the amount of 4-CBA in the medium comprised 81% of the theoretical yield. Free chlorine ions were not detected in the medium. Thus, the experiments showed that strain B51 can efficiently dioxygenize the *ortho*-chlorinated ring of the 2,2'-DCB and 2,4'-DCB molecules and the *para*-chlorinated ring of the 4,4'-DCB molecule.

The analysis of the degradation products of 2,4,2'-TCB and 2,4,4'-TCB revealed the presence of 2,4-DCBA in the medium (indicating that strain B51 accomplished the oxidation of the mono(*ortho*)-chlorinated ring of 2,4,2'-TCB), as well as of 4-CBA (indicating the oxidation of the di(*ortho-para*)-chlorinated ring of 2,4,4'-TCB).

During the degradation of *ortho*-chlorinated biphenyls (2-MCB and 2,2'-DCB) by strain B51, the product

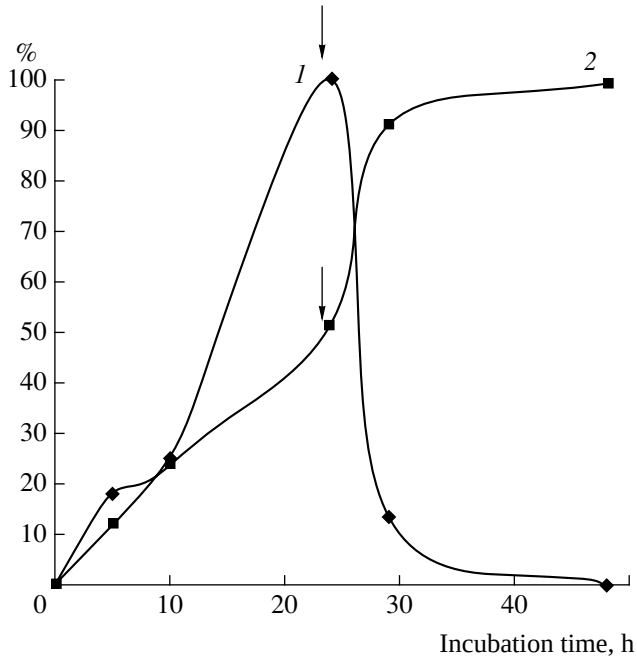

Fig. 2. The degradation of 2,4'-dichlorobiphenyl by *Microbacterium* sp. B51 and *Arthrobacter* sp. H5 cells: (*1*) 4-CBA and (*2*) chlorine ions in the medium. The arrows show the instant of the addition of *Arthrobacter* sp. H5 cells.

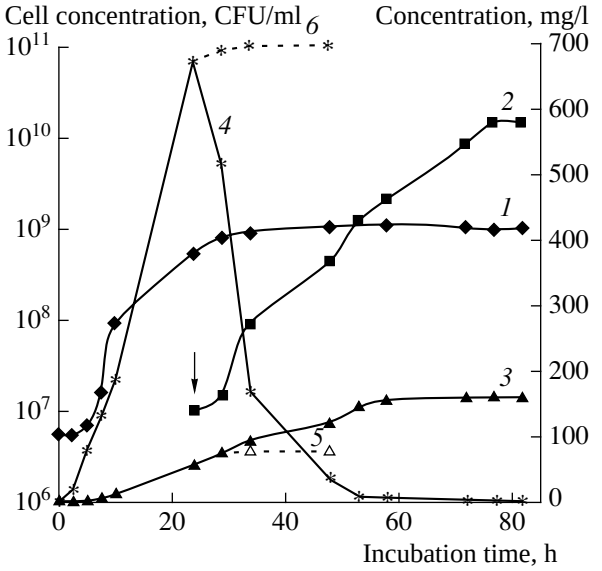

Fig. 3. The degradation of 2,4'-dichlorobiphenyl by a mixed culture of *Microbacterium* sp. B51 and *Arthrobacter* sp. H5: (*1*) growth of *Microbacterium* sp. B51; (*2*) growth of *Arthrobacter* sp. H5; (*3*) chlorine ions; (*4*) 4-CBA; (*5*) concentration of chlorine ions in the absence of *Arthrobacter* sp. H5; (*6*) concentration of 4-CBA in the absence of *Arthrobacter* sp. H5. The arrow shows the instant of the addition of *Arthrobacter* sp. H5 cells.

2-CBA was found to be utilized (table). Further studies showed that the strain is able to grow on 2-CBA as the sole source of carbon and energy. When cultivated in medium with 1 g/l 2-CBA for 5 days, the strain degraded half this amount of 2-CBA with the production of free chlorine ions in a stoichiometric amount (data not presented). At the same time, strain B51 was unable to utilize 4-CBA.

Thus, *Microbacterium* sp. B51 is able to transform *para*-substituted chlorobiphenyls into 4-CBA but is unable to utilize this product. It was of interest to study the degradation of chlorinated biphenyls by strain B51 in the presence of the *Arthrobacter* sp. strain H5, which is able to efficiently utilize 4-CBA [16]. For this study, we chose 2,4'-DCB, which is chlorinated at the *para* and *ortho* positions in the different rings of the biphenyl molecule.

Experiments with washed *Microbacterium* sp. B51 cells showed that they could completely transform 0.1 g/l 2,4'-DCB to 4-CBA in 24 h (Fig. 2) with the accumulation of free chlorine ions in the medium in an amount comprising about 50% of the theoretical yield. This finding provided further evidence that the 2,3-dioxygenation of the *ortho*-chlorinated ring of the 2,4'-DCB molecule is accompanied by the cleavage of chlorine ion in the *ortho* position. The subsequent addition of *Arthrobacter* sp. H5 cells at the 24th hour of incubation of *Microbacterium* sp. B51 cells (this instant is shown by the arrows in Fig. 2) led to a rapid decrease in the amount of 4-CBA and an increase in the concentration of chlorine ions. As a results, in the course of the next 24 h of incubation, 4-CBA was entirely absent from the medium, whereas the concentration of chlorine ions reached the maximum possible level (100% of the theoretical yield). Thus, the mixture of washed *Microbacterium* sp. B51 and *Arthrobacter* sp. H5 cells completely utilized 0.1 g/l 2,4'-DCB in 24 h.

In the next experiment performed under growth conditions, an *Arthrobacter* sp. H5 culture was added to the *Microbacterium* sp. B51 culture after the latter had been incubated in the medium with 1 g/l 2,4'-DCB for 24 h (Fig. 3). As can be seen from this figure, the transformation of 2,4'-DCB by the mixed culture composed of strain B51 and *Arthrobacter* sp. H5 was inhibited neither by the high concentration of 2,4'-DCB nor by the accumulation of transformation products. By the end of incubation (82 h), the culture liquid contained only traces of 4-CBA (less than 0.02%), and the amount of chlorine ions in the medium reached 100% of the theoretical yield. The specific growth rates of *Microbacterium* sp. B51 and *Arthrobacter* sp. H5 in the mixed culture were 0.19 and 0.12 h^{-1}, respectively, being comparable with the specific growth rates of these strains in monocultures on the respective substrates. Namely, the specific growth rate of strain B51 on 1 g/l biphenyl was found to be 0.21 h^{-1}, and that of *Arthrobacter* sp. H5 grown on 1 g/l 4-CBA was 0.08 h^{-1}. Thus, the bacteria *Microbacterium* sp. B51 and *Arthrobacter* sp. H5 do not exert any detrimental effect on each other when incubated in mixed cultures in the presence of chlorinated biphenyls.

DISCUSSION

Microbacterium sp. B51 has a wide substrate specificity for different *ortho*- and *para*-chlorinated biphenyls and is able to completely utilize 2-MCB, 2,2'-DCB, 2,4'-DCB, 4,4'-DCB, and 2-CBA. The data presented in the previous section suggest that strain B51 attacks the *ortho*-chlorinated ring of the 2,2'-DCB and 2,4'-DCB molecules, dioxygenizing it at positions 2 and 3 (Fig. 1). This process results in the dechlorination of dichlorobiphenyls (table, Fig. 2). The accumulation of 2-CBA in the medium in great amounts and the absence of the products of the *meta* cleavage of dichlorobiphenyls suggest that strain B51 has a high activity of HOPDA hydrolase (BphD). Earlier, this activity was reported for *Burkholderia* sp. LB400, one of the best studied degraders of PCBs [3, 7]. It was shown that the BphA of strain LB400 exhibits dioxygenase activity towards the 2 and 3 carbon atoms of the *ortho*-chlorinated ring, cleaving a chlorine atom, whereas the BphD of this strain exhibits high hydrolase activity towards 8-chlorinated and 10-chlorinated HOPDA [7, 10].

The analysis of the degradation products of 2,2'-DCB and 2,4'-DCB in strain B51 showed that the PCB-degrading activity of this strain is comparable with that of *Burkholderia* sp. LB400. It should be emphasized that strain B51 surpasses *Burkholderia* sp. LB400 in the ability to utilize and grow on 2-CBA (the degradation product of 2-MCB and 2,2'-DCB) as the sole source of carbon and energy, whereas strain LB400 is unable to utilize 2-CBA and grows poorly on 2-MCB and 2,2'-DCB, utilizing a fragment of these compounds containing five carbon atoms [12]. To the best of our knowledge, there are only a few genetically modified strains that are able to completely metabolize small amounts of 2-MCB [18, 19].

Furthermore, strain B51 has a unique ability to oxidize both the *para*- and *ortho*-chlorinated rings of PCB molecules, including 4,4'-DCB. An analysis of the data available in the literature concerning the substrate specificity of PCB degraders shows that these degraders can be divided into two groups: the group of *Burkholderia* sp. LB400 and the group of *Pseudomonas pseudoalcaligenes* KF707. The members of the first group have a broad substrate specificity toward *ortho*- and *para*-chlorinated biphenyls, are able to degrade 2,5,2',5'-tetraCB, but fail to degrade 4,4'-DCB. The members of the second group have a narrow substrate specificity, are unable to degrade 2,5,2',5'-tetraCB, but are able to degrade 4,4'-DCB [3, 7]. The data obtained in this study suggest that the BphA of strain B51 catalyzes the 2,3-dioxygenation of one of the mono(*para*)-chlorinated rings of particular chlorobiphenyls with the production of 3,10-chlorinated HOPDA. The transformation of 4,4'-DCB via this pathway was described for *P. pseudoalcaligenes* KF707 and some other strains capable of degrading di(*para*)-chlorinated biphenyls [3]. It should also be noted that the HOPDA hydrolase of strain B51 is highly active toward 3,10-chlorinated HOPDA, producing 4-CBA in amounts reaching 81% of the theoretical yield. In contrast, the BphD of strain LB400 is unable to hydrolyze 3,10-chlorinated HOPDA with the production of 4-CBA [10].

Microbacterium sp. B51 is able to oxidize the mono(*ortho*)-chlorinated ring of 2,4,2'-TCB and the di(*ortho-para*)-chlorinated ring of 2,4,4'-TCB with the production of chlorobenzoates in small amounts (table), whereas the products of the *meta* cleavage of chlorobiphenyls were not detected. These data suggest that strain B51 degrades 2,4,2'-TCB and 2,4,4'-TCB in the same way as does strain LB400, but the primary enzymatic activities of strain B51 are less specific for TCBs than are those of strain LB400 [4]. In general, *Microbacterium* sp. B51 is a more efficient degrader of PCBs than is *Burkholderia* sp. LB400, because the former bacterium is able to partially degrade 4,4'-DCB and to completely degrade 2-MCB and 2,2'-DCB.

Let us compare the 2,4'-DCB–degrading capacity of the mixed culture of *Microbacterium* sp. B51 and *Arthrobacter* sp. H5 (Fig. 3) with that of the other mixed cultures described in the literature. The mixed culture ECO3 made up of *Pseudomonas* sp CPE1, which is capable of utilizing biphenyl and 4-MCB, *Pseudomonas* sp. CPE2, which can grow on 2-CBA, and *Alcaligenes* sp. CPE3, which can grow on 4-CBA, was found to be able to utilize about 50% of the 2,4'-DCB initially present in the medium (the final concentration of this compound was 0.08 g/l) with the accumulation of the *meta* cleavage product of 2,4'-DCB in the medium [5]. For comparison, the mixed culture of *Microbacterium* sp. B51 and *Arthrobacter* sp. H5 almost completely degraded 2,4'-DCB in 24 h without accumulating HOPDA in the medium, irrespective of whether the initial concentration of 2,4'-DCB in the medium was 0.1 or 1 g/l (Figs. 2, 3). In the course of cultivation on 2,4'-DCB, the other known PCB-degrading mixed culture (*Burkholderia* sp. LB400 and the genetically modified strain *Pseudomonas putida* mt-2a) utilized 2,4'-DCB by about 30% in 24 h and almost completely in 3 days [12]. In this case, the number of viable cells of both *Burkholderia* sp. LB400 and *P. putida* mt-2a increased by an order [12], whereas the number of viable cells of *Microbacterium* sp. B51 and *Arthrobacter* sp. H5 increased by two and three orders, respectively (Fig. 3). All this shows that *Microbacterium* sp. B51 is a promising PCB degrader and can be used for compiling mixed cultures important for bioremediation of PCB-contaminated soils.

To conclude, the isolated strain *Microbacterium* sp. B51 is an active PCB degrader, which possesses unique enzymatic activities providing for the complete utilization of 2-MCB and 2,2'-DCB. Investigations into the biochemical and genetic systems controlling the degradation of PCB by this organism are in progress in our laboratory.

ACKNOWLEDGMENTS

We are grateful to O.V. Mal'tseva (Western Michigan University, Kalamazoo, United States) and L.I. Evtushenko (Skryabin Institute of Biochemistry and Physiology of Microorganisms) for their advice and discussion of the experimental data.

This work was supported by grant no. 02-04-96404 from the Russian Foundation for Basic Research.

REFERENCES

1. Fedorov, L.A., *Dioksiny kak ecologicheskaya opasnost': retrospectiva i perspektivy* (Dioxins as an Ecological Hazard: Retrospection and Prospects), Moscow: Nauka, 1993.
2. Abramowicz, D.A. and Daniel, A., Aerobic and Anaerobic PCB Biodegradation in the Environment, *Environ. Health Perspect.*, 1995, vol. 103, pp. 97–104.
3. Unterman, R., A History of PCB Biodegradation, *Bioremediation: Principles and Applications*, Crawford, R.L. and Crawford, D.L., Eds., 1996, pp. 209–253.
4. Maltseva, O.V., Tsoi, T.V., Quensen III, J.F., Fucuda, M., and Tiedje, J.M., Degradation of Anaerobic Reductive Dechlorination Products of Aroclor 1242 by Four Aerobic Bacteria, *Biodegradation*, 1999, vol. 10, pp. 363–371.
5. Fava, F., Di Gioia, D., Cinti, S., Marchetti, L., and Quattroni, G., Degradation and Dechlorination of Low-chlorinated Biphenyls by a Three-membered Bacterial Coculture, *Appl. Microbiol. Biotechnol.*, 1994, vol. 41, pp. 117–123.
6. Erickson, B.D. and Mondello, F.J., Enhanced Biodegradation of Polychlorinated Biphenyl after Site-directed Mutagenesis of a Biphenyl Dioxygenase Gene, *Appl. Environ. Microbiol.*, 1993, vol. 59, pp. 3858–3862.
7. Seeger, M., Zielinski, M., Timmis, K.N., and Hofer, B., Regiospecificity of Dioxygenation of Di- to Pentachlorobiphenyls and Their Degradation to Chlorobenzoates by the *bph*-encoded Catabolic Pathway of *Burkholderia* sp. Strain LB400, *Appl. Environ. Microbiol.*, 1999, vol. 65, pp. 3614–3621.
8. Kimura, N., Nishi, A., Goto, M., and Furukawa, K., Functional Analyses of a Variety of Chimeric Dioxygenases Constructed from Two Biphenyl Dioxygenases That Are Similar Structurally but Different Functionally, *J. Bacteriol.*, 1997, vol. 179, pp. 3936–3943.
9. Vaillancourt, F.H., Labbe, G., Droouin, N.M., Fortin, P.D., and Eltis, L.D., The Mechanism-based Inactivation of 2,3-Dihydroxybiphenyl 1,2-Dioxygenase by Catecholic Substrates, *J. Biol. Chem.*, 2002, vol. 277, pp. 2019–2027.
10. Seah, S.Y.K., Labbe, G., Kaschebek, S.R., Reifenrath, F., Reineke, W., and Eltis, L.D., Comparative specificities of Two Evolutionarily Divergent Hydrolases Involved in Microbial Degradation of Polychlorinated Biphenyls, *J. Bacteriol.*, 2001, vol. 183, pp. 1511–1516.
11. Kim, S. and Picardal, F.W., Microbial Growth on Dichlorobiphenyls Chlorinated on Both Rings As a Sole Carbon and Energy Source, *Appl. Environ. Microbiol.*, 2001, vol. 64, pp. 1953–1955.
12. Potrawfke, T., Lohnert, T.-H., Timmis, K.N., and Wittich, R.-M., Mineralization of Low-chlorinated Biphenyls by *Burkholderia* sp. Strain LB400 and by a Two-membered Consortium upon Directed Interspecies Transfer of Chlorocatechol Pathway Genes, *Appl. Microbiol. Biotechnol.*, 1998, vol. 50, pp. 440–446.
13. Zaitsev, G.M. and Karasevich, Yu.N., Utilization of 4-Chlorobenzoic Acid by an *Arthrobacter globiformis* Strain, *Mikrobiologiya*, 1981, vol. 50, pp. 35–40.
14. *Manual of Methods for General Bacteriology*, Gerhardt, P. *et al.*, Eds., Washington: Am. Soc. Microbiol., 1981. Translated under the title *Metody obshchei bakteriologii*, Moscow: Mir, 1983–1984, vol. 1–3.
15. Evtushenko, L.I., Dorofeeva, L.V., Subbotin, S.A., Cole, J.R., and Tiedje, J.M., *Leifsonia poae* gen. nov., sp. nov., Isolated from Nematode Galls on *Poa annua* and Reclassification of *"Corynebacterium aquaticum"* Leifson 1962 as *Leifsonia aquatica* (ex Leifson 1962) gen. nov., nom. rev., comb. nov. and *Clavibacter xyli* Davis *et al.* 1984 with Two Subspecies as *Leifsonia xyli* (Davis *et al.* 1984) gen. nov., comb. nov., *Int. J. Syst. Evol. Microbiol.*, 2000, vol. 50, pp. 371–380.
16. Rybkina, D.O., Plotnikova, E.G., and Demakov, V.A., Degradation of Chlorobenzoates by Soil Bacteria, *Ob. Nauchn. Zh.*, 2003, no. 1, pp. 73–82.
17. Takeuchi, M. and Hatano, K., Union of the Genera *Microbacterium* Orla-Jensen and *Aureobacterium* Collins *et al.* in a Redefined Genus *Microbacterium*, *Int. J. Syst. Bacteriol.*, 1998, vol. 48, pp. 739–747.
18. Havel, J. and Reineke, W., Total Degradation of Various Chlorobiphenyls by Cocultures and *In Vivo* Constructed Hybrid Pseudomonads, *FEMS Microbiol. Lett.*, 1991, vol. 78, pp. 163–170.
19. Hrywna, Y., Tsoi, T.V., Maltseva, O.V., and Tiedje, J.M., Construction and Characterization of Recombinant Bacteria That Grow on *ortho*- and *para*-substituted Chlorobiphenyls, *Appl. Environ. Microbiol.*, 1999, vol. 65, pp. 2163–2169.