JOURNAL OF PURE AND APPLIED MICROBIOLOGY, December 2013. Vol. 7(4), p. 3051-3057

Isolation and Performance Evaluation of Indigenous Soil Bacteria for Biodegradation of Polychlorinated Biphenyl

Liu Feng*, Jing Wang and Shun-yan Shi

Department of Environmental Science and Engineering,
Beijing University of Chemical Technology, Beijing 100029 P. R. China

(Received: 23 September 2013; accepted: 06 November 2013)

PCBs polluted sites have been widely concerned due to their potential risks of carcinogenicity, teratogenicity, and mutagenicity, the governments and environmental researchers in the world were working on the economic and effective remediation technologies to solve this problem. Among of them, there would be no choices better than the isolation and acclimation of high-performance indigenous PCBs degradation bacterium from polluted sites. For this purpose, a new polychlorinated biphenyl (PCB)-degrading bacterium strain, PS-11, was isolated from PCB-contaminated soils using an enrichment culture, and identified as Stenotrophomonas maltophilia based on the 16S rDNA sequence analysis results. The strain PS-11 presented fair degrading capability to PCBs. At 30 °C, pH 7.0, and 2 mg/l substrate concentration, the PCB52 removal efficiency using the PS-11 strain was 31.1% and 52.9%, after 4-day and 7-day of culture, respectively, whereas the removal efficiency was 10.9% for refractory PCB153 after 7-day of culture. With the strangest growth capability obtained at sucrose source and the shortest lag time obtained at dextrose source, it grew well at various carbon sources, and presented an improved degrading capability to PCB52 when sucrose, glucose and Tween-80 were acted as carbon sources. Additionally, with good resistance to high concentrations of several heavy metal ions, the strain exhibited a very good environmental tolerability. The research hereof could provide a new choice for the remediation of PCBs polluted sites.

Key words: Polychlorinated biphenyl, Microbial degradation, Isolation of degrading strain, Soil.

Polychlorinated biphenyl (PCB) is one of the 12 persistent organic pollutants that have received attention globally[1]. PCBs, once released into the environment, can bioaccumulate throughout the food chain with carcinogenicity, teratogenicity, and mutagenicity. PCBs have been detected in the human adipose tissue, milk and serum[2,3].

The microbial degradation method is the most potentially beneficial technique because it provides several advantages over traditional methods, such as lower cost, absence of secondary pollution, and bioaugmentation in situ[4]. Microbial degradation of PCBs is usually realized through two types of microorganisms, namely anaerobic and aerobic bacteria[5]. In general, highly chlorinated PCBs (≥ 5 chlorines) are degraded by anaerobic microbes through dechlorination[6, 7], whereas lowly chlorinated PCBs are degraded by aerobic microbes through the destruction of benzene ring structure[8].

The reported microorganisms to date that can degrade PCBs are mainly aerobic bacteria. Among them, only a small number of degrading bacteria, such as *Alcaligenes eutrophus* H850[9], *Burkholderia xenovorans* LB400[10] (*Pseudomonas* sp. LB400)[11]and *Rhodococcus* sp. RHA1[12,13], can degrade PCBs with less than 6 chlorine atoms. Most of the strains are limited in the degradation of less than 4 chlorine-substituted PCBs[14]. Also thermophilic and psychrophilic bacteria *Bacillus* sp. JF8, isolated from compost could degrade several PCB congeners, including tetra- and pentachlorobiphenyls[15]. Antarctic marine bacteria

* To whom all correspondence should be addressed.
Tel.: +86-10-64427356;
E-mail: fengliu-buct@vip.sina.com(L.Feng)

Pseudoalteromonas, Psychrobacter, and Arthrobacter could degrade PCB as the sole carbon and energy source at both 4°C and 15°C[16].

In addition to bacteria, some fungi could also degrade PCBs[17]. *Pleurotus ostreatus* isolated from contaminated soil removed approximately 40% of Delor 103 in 2 months[18]. Degradation of [U-^{14}C]-Aroclor 1254 by *Phanerochaete chrysosporium* resulted in the release of $^{14}CO_2$ and water-soluble degradation products [19].

Those strains mentioned above provided available resources for the bioremediation of different PCB-polluted areas. As a contribution to the family of PCB degradation microorganisms, and also in accord with the different site characteristics, we first isolated and characterized a soil bacterium capable of utilizing PCBs as the sole carbon source. We then investigated the effect of certain abiotic factors and additives on PCB degradation and bacterial growth in liquid cultures to provide practical information for the design of an effective strategy suitable for the remediation of PCB contaminated sites.

MATERIALS AND METHODS

Chemicals

2,2',5,5'-Tetrachlorobiphenyl (PCB52) and 2,2',4,4',5,5'-Hexachlorobiphenyl (PCB153) were purchased from Cerilliant Corporation (Round Rock, Texas USA). Normal hexane and acetone (chromatographic pure) were used. All other chemicals were of highest purity and are commercially available.

Culture medium

Both the soil enrichment medium and the mineral culture medium were prepared with 1L sterilized water at pH 7.0. Among them, the soil enrichment was composed of 10g peptone, 5g yeast extract, 5g NaCl and 0.5g biphenyl; the mineral culture medium contained 0.5g K_2HPO_4, 0.5g KH_2PO_4, 1.0g $(NH4)_2SO_4$, 0.2g $MgSO_4$, 0.1g $CaCl_2$, 0.2g NaCl and 2g biphenyl.

All culture media (excluding biphenyl) were sterilized for 20min at 121°C and 0.1MPa before use. Biphenyl (with acetone as solvent) was added to the sterilized culture media after filtration sterilization.

Enrichment and Isolation of bacteria

Soil from both sites near a Chemical Plant and a Coking Plant were collected for the enrichment culture of PCB-degrading bacteria. A 2-month preliminary acclimation was conducted in an organic glass device (37cm×37cm×32cm). Nutrient solution (containing 5g/l glucose, 2g/l urea, 1g/l KH_2PO_4 and 10g/l biphenyl) was added periodically to maintain adequate soil moisture and oxygen, and pH was kept between 6.5 and 7.5.

Enrichment was performed by successive subculturing of samples. Soil sample (1g) obtained from the preliminary domestication soil was used as the inoculum for the 100 mL soil enrichment medium supplemented with biphenyl (2 g/l final concentration). These cultures were incubated at 30°C with agitation (150rpm) for 1 week and then transferred into 100 mL inorganic salt culture medium supplemented with 2 g/l biphenyl as the sole carbon source. After sub-culturing in inorganic salt culture medium for six generations, the isolates were obtained using the dilution-plate method and separately re-inoculated in inorganic salt culture medium. The isolates that grew fastest and had the highest removal efficiency were selected for further investigation and kept in glycerol (30% final concentration) at -80 °C.

Determination of bacterial growth curve

The growth curve was detected by the absorbency value (OD_{600nm}) at different time points. Strains with quicker and better growth were selected for the determination of the degradation efficiency. The selected strains used benzene-containing organic carbon source for growth.

Biodegradation test of bacteria dormancy system

The inocula for the PCB degradation experiments were prepared during the late phase of the exponential growth by centrifugation (6,000rpm) for 5 min. The cells were washed twice with sodium phosphate buffer (pH 7.0) and resuspended in sodium phosphate buffer as suspensions (OD_{600nm}=1.0) for use in the succeeding experiments. The bacteria exhibiting the highest destruction efficiency were selected through the biodegradation test of bacterial dormancy system to 2 mg/l PCB52 and PCB153.

Extraction of PCBs in the bacteria dormancy system

Each sample with 5 ml n-hexane and 0.7 g ammonium sulfate as demulsifier was shaken for 10 min in a fast vortex mixer and extracted for 5 min with ultrasound. After a standing-and-layering

process, 1 ml of the upper organic phase that was dehydrated by anhydrous sodium sulfate was analyzed using a gas chromatography equipped with an ECD.

Identification of a PCB-degrading bacterium

The bacterial colony was identified according to the results of the morphological, physiologic, and phylogenetic analysis of the 16S rDNA sequence. The DNA of a single colony was extracted and used as a template to amplify the 16S rDNA by PCR using the universal primers 16F (AGAGTTTGATCCTGGCTCAG) and 16R (GGTTACCTTGTTACGACTT). The isolate was identified by Beijing Sun Base Biotech Co., Ltd. (Beijing, China). The sequencing result was submitted to the GenBank database (http:// www.ncbi.nlm.nih. gov/), and a similarity search of the nucleotides was conducted using the Basic Local Alignment Search Tool.

Effects of different culture conditions on the biodegradation of PCB

Three different incubation temperatures (20°C, 30°C, and 40°C) were applied in the temperature-effect experiment to evaluate the effects of temperature on the microbial growth in culture medium at pH of 7.0.

The pH values of the culture medium were adjusted to 6.0, 7.0, 8.0, 9.0, 10.0, and 11.0 to evaluate the effect of pH on PCB biodegradation at 30°C. The PCB removal efficiencies, with the substrate concentration ranging from 2 mg/l to 50 mg/l, were tested at pH 7.0 and 30°C. The inorganic salt culture media were enriched by adding dextrose, sucrose, sodium dodecyl benzene sulfonate (SDBS), sodium acetate, β-cyclodextrins, and Tween-80 to evaluate the effect of extra carbon source on microbial growth at 30°C. Then, Cu^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+} were added into the inorganic salt culture media with different concentrations to evaluate the effect of metal ion on microbial growth at 30°C.

Under these conditions, the flasks were incubated in a rotary shaker at 150 rpm in the dark. Samples were removed at designated intervals for the analysis of residual PCB, and microbial growth was determined by phototurbidometry.

All experiments were performed in triplicate. The uninoculated controls were maintained in all experiments. The results are reported as the average of three replicates.

Analytical method

PCB was measured by GC 2010 (Shimadzu, Japan) equipped with an electron capture detector and a DB-5 capillary column (30m×0.32mm×0.25μm). GC parameters were as follows: high pure nitrogen carrier gas, 8.8 ml/min; injection port temperature, 270°C; ECD temperature, 300°C; and oven program: 150°C for 1 min, increased to 200°C at 10°C/min, increased to 265°C at 35°C/min; held at 265°C for 5 min, increased to 300°C at 10°C/min; and held at 300°C for 1 min.

RESULTS AND DISCUSSION

Screening of degrading bacteria

Six strains of bacteria, labeled from A to F, were obtained according to the morphology of colonies on the solid plate after preliminary domestication, enrichment culture, and purification. The growth of E was the fastest in the biphenyl-containing medium followed by that of A, in contrast to the slow growth of B and C, meanwhile D as well as F did not grow at all (Fig. 1). Therefore, strains A, B, C, and E were selected for further screening.

PCB biodegradation by strains A, B, C and E

The PCB52 biodegradation efficiencies determined through A, B, C, and E strains were respectively 31.1%, 15.7%, 2.5%, and 12.6% after 4d, and 52.9%, 18.1%, 10.1%, and 15.4% after 7d culture, respectively, at the concentration of 2 mg/ l (Fig. 2a). Strain A clearly exhibited the highest removal efficiency of PCB. Meanwhile, this strain also showed the highest removal efficiency for 2 mg/l of refractory PCB153 in 7 d (Fig. 2b). Strain A was consequently named *PS-11* and was identified. All PCB153 degradation efficiencies were lower than those of PCB52 (Fig. 2) because of the difficulty in chlorine removal as the number of chlorine increased, resulting in greater toxicity to microorganisms. This result is consistent with the reported literature[21]. PCBs could be degraded after domestication for a period, although they exhibit certain toxicity to microorganisms. The toxicity of PCBs varies just like their diversity. Therefore, the *PS-11* strain laid a foundation for the study of the degradation of other highly toxic PCBs

Identification of PCB-degrading bacterium

A soil bacterial strain capable of utilizing PCBs as its sole carbon and energy source was

isolated via the enrichment technique. The isolate was identified as a *Gram-negative* and aerobic bacterium. *PS-11* showed the greatest similarity (99%) with the *Stenotrophomonas maltophilia* type sequence (GenBank No. HQ166115.1) based on the 16s rDNA sequences analysis.

The PCB biodegradation by *Pseudomonas* has been widely reported[20,21,22], unlike that by *S. maltophilia*. *S. maltophilia* is abundant in the natural world and plays an important role in the degradation and transformation of pollutants. A previous study has reported that *S. maltophilia* accounts for 3.8% of *Gram-negative* bacteria in the effluent of municipal water treatment [23]. Another previous study on the bacterial diversity analysis of large yellow croaker from several farms in East China Sea have found that *S. maltophilia* was the second beneficial bacterium group in fish, accounting for 21.0% [24].

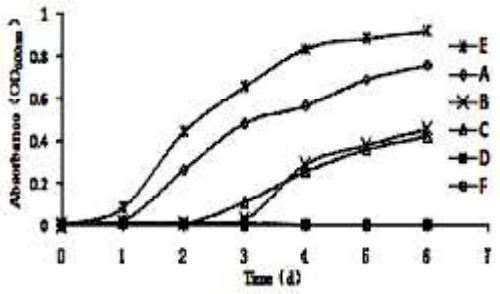

Fig. 1. Growth curves of strains A to F in inorganic salt culture medium with 2 g /l biphenyl as the sole carbon source

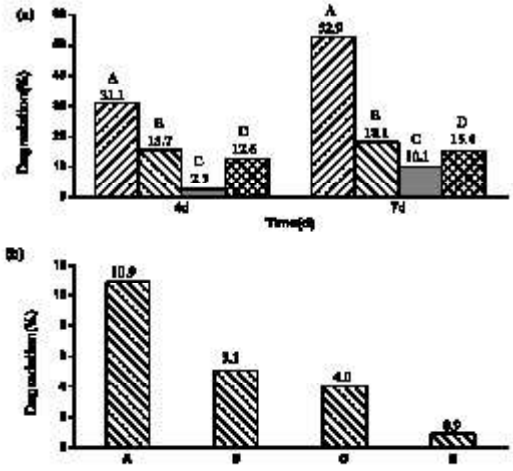

Fig. 2 a) PCB52 degradation rates by A, B, C, and E bacterium resting cells; **b**) PCB153 degradation rates by A, B, C, and E bacterium resting cells on 7 days

Effect of pH on PCB degradation and PS-11 growth

PS-11 growth and PCB52 degradation were observed over a wide pH range from 6 to 11 (Fig 3). The optimal pH for *PS-11* growth was found to be alkaline (pH 7.0 to pH 9.0). A pH lower than 6.0 or higher than 10.0 was shown to be less suitable for PS-11 growth (Fig 3a). However, the highest degradation efficiency was at pH 7.0 (Fig. 3c). Hence, the optimal pH for *PS-11* growth and PCB degradation was 7.0.

The pH value decreased in the culture system of well-grown *PS-11* (Fig 3b). The fastest pH decline was in the culture system with initial pH level of 9.0, in which *PS-11* growth was the fastest. Therefore, these results could explain that the acid was generated during the *PS-11* growth. The growth of *PS-11* was inhibited when the pH of the culture system was below 7.0. Thus, *PS-11*

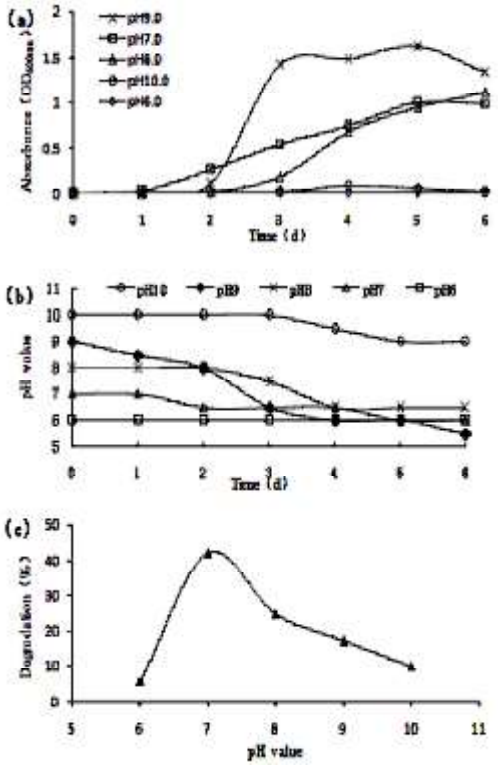

Fig. 3(a). Growth curve of PS-11 cells with different pH levels; (b) Time course of culture pH during PS-11 cell growth; (c) Effect of different pH levels on PCB52 percent degradation on day 6

screened in this study could be used for the degradation of PCB-contaminated alkaline soil restoration.

Effect of temperature on PS-11 growth

The impact of temperature on *PS-11* growth is shown in Fig 4. The PS-11 growth curves were measured at 20°C, 30°C and 40°C. The greatest growth of PS-11 was observed at 30°C, whereas 20°C and 40°C were unfavourable for *PS-11* growth.

Effect of PCB concentration

The PCB52 degradation efficiencies by *PS-11*, with different PCB52 concentrations ranging from 2 mg/l to 50 mg/l, were tested in inorganic salt culture medium at pH7.0 and 30 ºC. The results showed that effective degradation efficiencies were hampered as the initial PCB52 concentration increased (Fig. 5). For example, 42.1% of PCB52 was depleted within 6d when the initial PCB52 concentration was 2 mg/l, whereas the PCB52 degradation efficiencies were 29.7% and 27.0% at 5 mg/l and 10 mg/l, respectively. A slight degradation of PCB52 efficiency was observed at a concentration from 20 mg/l to 50 mg/l for a period of 6 d.

Effect of extra carbon source

Given that biphenyl itself is a pollutant[25], the selection of new carbon sources was considered by examining the growth of the *PS-11* strain in inorganic salt culture with different carbon sources (2 g/l) at 30°C. The effect of extra carbon source detected by absorbency value (OD_{600nm}) was: sucrose > dextrose > Tween-80 > sodium

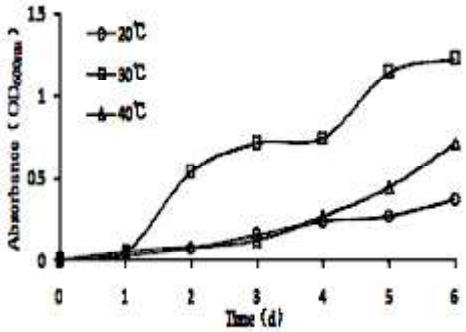

Fig. 4. Growth curve of PS-11 cells at different temperatures

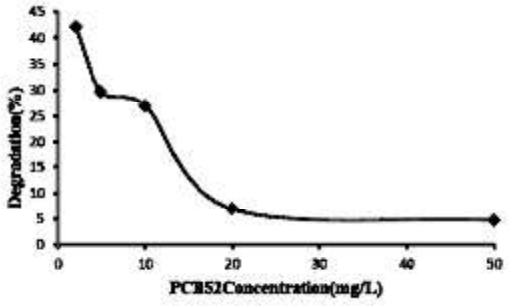

Fig. 5. Effects of different PCB52 initial concentrations on the PCB52 percent degradation by PS-11 on day 6

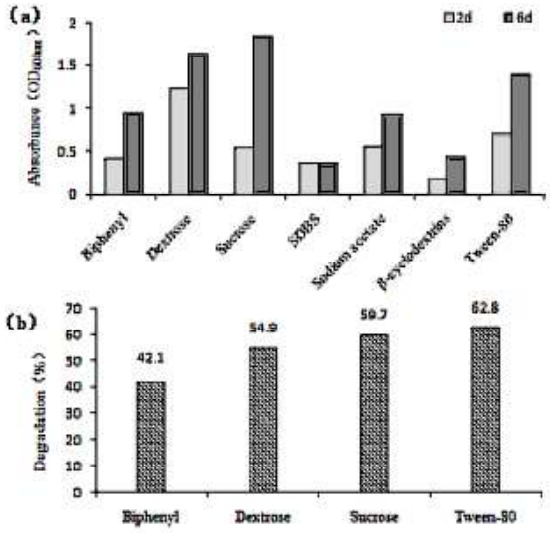

Fig.6(a). Effect of different carbon sources on *PS-11* growth; **(b)** PCB52 percent degradation on day 6

acetate > biphenyl > β-cyclodextrins > SDBS (Fig 6a). The strain had the strangest growth capability when sucrose was used as a carbon source and the shortest period of adjustment when glucose was used as a carbon source. *PS-11* growth was almost inhibited in the inorganic salt culture with β-cyclodextrins and SDBS. In addition, sucrose, dextrose, Tween-80 and biphenyl increased the PCB52 degradation efficiencies by *PS-11* by 59.7%, 54.9%, 62.8%, and 42.1%, respectively (Fig 6b).

The *PS-11* strain was resistant to high concentrations of some heavy metal ions (Table 1). The order of tolerance was: Pb^{2+}>Cd^{2+}> Zn^{2+}> Cu^{2+}. *PS-11* growth was satisfactory when the Cu^{2+} concentration was less than 0.05 mg/l, but was almost inhibited when the Cu^{2+} concentration was higher than 0.05 mg/l. *PS-11* can grow well at 0.5 mg/l of Zn^{2+} concentration, but not at over 50 mg/l concentrations. *PS-11* growth was remarkable at 10 mg/l of Cd^{2+} concentration, and was adversely affected at a concentration higher than 10 mg/L. *PS-11* exhibited highest tolerance to Pb^{2+} such that *PS-11* still grew well within the 50 mg/l to 200 mg/l range. Thus, *PS-11* was adequate for Pb, Cd, and Zn-contaminated soil, but not Cu.

CONCLUSION

A new PCB-degrading bacterium strain, *PS-11*, was isolated from contaminated soils and identified as *S. maltophilia* based on the 16S rDNA sequence analysis results. As indicated above, the strain *PS-11* could grow well at such carbon sources as sucrose, glucose, and Tween-80, and presented fair degrading capability to PCBs, inclusive of the refractory ones such as PCB153. Furthermore, being resistant to high concentrations of several heavy metal ions, such as Pb^{2+}, Cd^{2+}, $Zn^{2+}$and Cu^{2+}, it also showed good environment tolerability. To sum up, the *PS-11* should be a potential strain that could be applied in the bioremediation of PCB-contaminated soil.

Table 1. Effect of heavy metal ions on the PS-11 growth

Metal ion	c/mg·L^{-1}	Biomass(OD_{600nm})
-	0	1.106
Cu^{2+}	0.005	1.177
	0.05	0.782
	≥0.5	0
Zn^{2+}	5	1.022
	10	0.632
	≥50	<0.1
Pb^{2+}	50	1.231
	200	0.576
	500	0.038
Cd^{2+}	10	1.25
	25	0.383
	≥50	<0.2

ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial support of this work by both the Ministry of Land and Resources of P. R. China (Grant No. 201211039-03-03) and the National Natural Science Foundation of P. R. China (Grant No.20777007).

REFERENCES

1. Batterman S., Chernyak S., Gouden Y., Hayes J., Robins T, Chetty S, PCBs in air, soil and milk in industrialized and urban areas of KwaZulu-Natal, South Africa. *Environment Pollution*. 2009; **157**: 654-663.
2. Harrad S.J., Sewart A.P., Alcock R., Boumphrey R., Burnett V., Duarte-Davidson R., Gaisall C., Sanders G., Waterhouse K., Wild S.R., Jones K.C., Polychlorinated biphenyls(PCBs) in the British environment: Sinks, sources and temporal trends. *Environment Pollution*. 1994; **85**(2): 131-146.
3. Turrio-Baldassarri L., Alivernini S., Carasi S., Casella M., Fuselli A., Iacovella N., Iamiceli A.L., Rocca C.L., Scarcella C., Battistelli C.L., PCB, PCDD and PCDF contamination of food of animal origin as the effect of soil pollution and the cause of human exposure in Brescia. *Chemosphere.* 2009; **76**(2): 278-285.
4. Furukawa K., Fujihara H., Microbial degradation of polychlorinated biphenyls: Biochemical and molecular features. *Journal of Bioscience and Bioengineering*. 2008; **105**(5): 433-449.
5. Field J.A., Sierra-Alvarez R., Microbial transformation and degradation of polychlorinated biphenyls. *Environment Pollution.* 2008; **155**(1): 1-12.
6. Bertin L., Capodicasa S., Fedi S., Zannoni D., Marchetti L., Fava F., Biotransformation of a highly chlorinated PCB mixture in an activated sludge collected from a Membrane Biological Reactor (MBR) subjected to anaerobic digestion. *Journal of Hazardous Materials*. 2011; **186**(2-

3): 2060-2067.
7. Fagervold S.K., Watts J.E.M., May H.D., Sowers K.R., Effects of bioaugmentation on indigenous PCB dechlorinating activity in sediment microcosms. *Water Research.* 2011; **45**(13): 3899-3907.
8. Tsuneta T., Loch-Caruso R., Quensen III J.F., Boyd S.A., Hanna M., Grindatti C., Stimulatory effects of a microbially dechlorinated polychlorinated biphenyl (PCB) mixture on rat uterine contraction in vitro. *Environmental Research*. 2008; **107**(2): 185-193.
9. Bedar D.L., Wagner R.E., Brennan M.J., Haberl M.L., Brown J.F., Extensive Degradation of Aroclors and Evironmentally Transformed Polychlorinated Biphenyls by *Alcaligenes eutrophus* H850. *Applied and Environmental Microbiology.* 1987; 53(5): 1094-1102.
10. Ponce B.L., Latorre V.K., González M., Seeger M., Antioxidant compounds improved PCB-degradation by *Burkholderia xenovorans* strain LB400. *Enzyme and Microbial Technology.* 2011; **49**(6-7): 509-516.
11. Bopp L.H., Degradation of highly chlorinated PCBs by *Pseudomonas* strain LB400. *Journal of Industrial Microbiology & Biotechnology*. 1986; **1**: 23-29.
12. Mukerjee-Dhar G., Shimura M., Kimbara K., Degradation of polychlorinated biphenyl by cells of Rhodococcus opacus strain TSP203 immobilized in alginate and in solution. Enzyme *and Microbial Technology*. 1998; **23**(1-2): 34-41.
13. Seto M., Kimbara K., Shimura M., Hatta T., Fukuda M., Yano K., Novel transformation of polychlorinated biphenyls by *Rhodococcus* sp. strain RHA1. *Applied and Environmental Microbiology*. 1995; **61**(9): 3353-3358.
14. Tu C., Teng Y., Luo Y.M., Li X.H., Sun X.H., Li Z.G., Liu W.X., Christie P., Potential for biodegradation of polychlorinated biphenyls (PCBs) by *Sinorhizobium meliloti*. *Journal of Hazardous Materials*. 2011; **186**(2-3): 1438-1444.
15. Shimura M., Mukerjee-Dhar G., Kimbara K., Nagato H., Kiyohara H., Hatta T., Isolation and characterization of a *thermophilic Bacillus* sp. JF8 capable of degrading polychlorinated biphenyls and naphthalene. *FEMS Microbiology Letter*. 1999; **178**: 87-93.
16. Michaud L., Di Marco G., Bruni V., Giudice L.A., Biodegradative potential and characterization of psychrotolerant polychlorinated biphenyl-degrading marine bacteria isolated from a coastal station in the Terra Nova Bay (Ross Sea, Antarctica). *Marine Pollution Bulletin.* 2007; **54**: 1754-1761.
17. Ruiz-Aguilar G.M.L., Fernández-Sánchez J.M., Rodríguez-Vázquez R., Poggi-Varaldo H., Degradation by white-rot fungi of high concentrations of PCB extracted from a contaminated soil. *Advances in Environmental Research.* 2002; **6**(4): 559-568.
18. Kubátová A., Erbanová P., Eichlerová I., Homolka L., Nerud F., Sasek V., PCB congener selective biodegradation by the white rot fungus *Pleurotus ostreatus* in contaminated soil. *Chemosphere.* 2001; **43**(2): 207-215.
19. Eaton D.C., Mineralization of polychlorinated biphenyls by *Phanerochaete chrysosporium*: A ligninolytic fungus. Enzyme and Microbial Technology. 1985; 7(5): 194-196.
20. Komancová M., Jurcová I., Kochánková L., Burkhard J., Metabolic pathways of polychlorinated biphenyls degradation by *Pseudomonas* sp.2. *Chemosphere.* 2003; **50**(4): 537-543.
21. Hatamian-Zarmi A., Shojaosadati S.A., Vasheghani-Farahani E., Hosseinkhani S., Emamzadeh A., Extensive biodegradation of highly chlorinated biphenyl and Aroclor 1242 by *Pseudomonas aeruginosa* TMU56 isolated from contaminated soils. International Biodeterioration & Biodegradation. 2009; **63**(6): 788-794.
22. Nováková H., Vosahíková M., Pazlarová J., Macková M., Burkhardb J., Demnerová K., PCB metabolism by *Pseudomonas* sp.P2. International Biodeterioration & Biodegradation. 2002; **50**(1): 47-54.
23. Romano G., Stampi S., Zanetti F., De Luca G., Tonelli E., Occurrence of gram-negative bacteria in drinking water undergoing softening treatment. Zentralbl Hyg Umweltmed. 1997; **200**(2-3): 152-162.
24. Guo Q.Y., Xu Z., Yang X.S., Bacterial diversity in cultured Pseudosciaena crocea from the East China Sea. *Marine Fisheries*, 2005; **27**(3): 241-245.
25. Luo W., D'Angelo E.M., Coyne M.S., Organic carbon effects on aerobic polychlorinated biphenyl removal and bacterial community composition in soils and sediments. *Chemosphere*. 2008; **70**(3): 364-373.