World J Microbiol Biotechnol (2009) 25:165–170
DOI 10.1007/s11274-008-9875-3

SHORT COMMUNICATION

Degradation of polychlorinated biphenyl (PCB) by a consortium obtained from a contaminated soil composed of *Brevibacterium*, *Pandoraea* and *Ochrobactrum*

Juan Antonio Zermeño-Eguia Liz · Janet Jan-Roblero · Javier Zavala-Díaz de la Serna · Arturo Vera-Ponce de León · César Hernández-Rodríguez

Received: 4 April 2008 / Accepted: 27 September 2008 / Published online: 14 October 2008

Abstract An indigenous polychlorinated biphenyl (PCB)-degrading bacterial consortium was obtained from soils contaminated by transformer oil with a high content of PCBs. The PCB degrader strains were isolated and identified as *Brevibacterium antarcticum, Pandoraea pnomenusa,* and *Ochrobactrum intermedium* by 16S rRNA gene sequence phylogenetic analysis. The PCB-degrading ability of the consortium and of individual strains was determined by using GC/MS. The PCB-degrading capacities of the consortium were evaluated for three concentrations of transfomer oil ranging from 55 to 152 μM supplemented with 0.001% biphenyl and 0.1% of Tween 80 surfactant. PCB biodegradation by the consortium was favored in the presence of both additives and the greatest extent of biodegradation (67.5%) was obtained at a PCB concentration of 55 μM. Each bacterial species exhibited a particular pattern of degradation relating to specific PCB congeners. Isolated strains showed a moderate degradation capability towards tetra-, hepta-, and octachlorobiphenyls; although no effect on penta-, hexa-, and nona-chlorobiphenyls was observed. Recently, PCB degradation capacity was recognized in a *Pandorea* member; however, this is the first study that describes the ability of *Brevibacterium* and *Ochrobactrum* species to degrade PCBs.

Keywords Polychlorinated biphenyls · *Brevibacterium antarcticum* · *Pandoraea pnomenusa* · *Ochrobactrum intermedium*

J. Jan-Roblero · J. Z.-D. de la Serna · A. V.-P. de León · C. Hernández-Rodríguez (✉)
Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas, IPN. Prol. de Carpio y Plan de Ayala Col. Sto. Tomás, 11340 Mexico, D.F. Mexico
e-mail: chdez38@hotmail.com

J. Jan-Roblero
e-mail: jjan_r@yahoo.com.mx

J. A. Z.-E. Liz
Dirección de seguridad y medio ambiente, Instituto Mexicano del Petróleo. Eje Central Lázaro Cárdenas, 152, Col. San Bartolo Atepehuacán, 07730 Mexico, D.F. Mexico

Introduction

Polychlorinated biphenyls (PCBs) are ubiquitous contaminants of public concern because of their persistence and bioaccumulation in the environment, as well as their potential toxicity to humans and ecotoxicology (Borlakoglu and Haegeles 1991). The conventional methods for remediation of PCB-contaminated soil, that include incineration or relocation to specialized landfills, are often prohibitively expensive. The alternative biodegradation strategy for in situ PCB removal uses microorganisms capable of metabolizing PCBs; several bacteria capable of utilizing PCB as carbon source have been reported, including aerobic bacteria, such as *Arthrobacter, Comamonas*, *Moraxella*, *Alcaligenes*, *Pseudomonas*, *Burkholderia, Sphingomonas, Rhodococcus* and methanotrophic species (Pieper 2005; Lindner et al. 2003), as well as some fungi, such as *Phanerochaete chrysosporium* (Beaudette et al. 1998). Anaerobic bacteria, such as the *Clostridium* members, which remove chlorine from PCB, have also been studied (Hou and Dutta 2000). PCB-degrading bacteria usually have the ability to degrade various PCB congeners, and it is generally assumed that biphenyl-oxidizing enzymes with a wide substrate range are responsible for this action (Kohler et al. 1988). Frequently, PCBs found in an oxidative environment are

highly chlorinated compounds, indicating that the less chlorinated biphenyls were more rapidly degraded than the highly chlorinated and recalcitrant congeners (Furukawa et al. 1978). Additionally, anaerobic PCB dechlorination demonstrates that higher chlorinated PCBs are more susceptible to the reductive dechlorination in meta- and para-positions of the PCBs.

Usually, the degradation pathway of PCBs by aerobic bacteria is similar to other aromatic compounds, such as toluene and naphthalene (Arensdorf and Focht 1994; Finette et al. 1984). In brief, PCBs can be oxidatively degraded under aerobic conditions, but the process under anaerobic conditions can lead only to dechlorination of PCB's (Wiegel and Wu 2000); their mineralization is an exclusively aerobic process (Robinson and Lenn 1994).

In this work, an indigenous polychlorinated biphenyl (PCB)-degrading bacterial consortium was obtained from soils highly contaminated with PCBs. Basically, the consortium and individual strains degraded tetra-, hepta-, and octa-chlorobiphenyls, but no effect on penta-, hexa-, and nona-chlorobiphenyls was observed.

Materials and methods

Soil sampling and obtaining of a PCB-degrading consortium

Five different soil samples, contaminated with leaking transformer and heat interchanger oils, were collected from a petroleum industry storage site located in the state of Veracruz, in the Mexican Southeast. The contaminant transformer oil contained 80% w/v of highly chlorinated PCBs (Aroclor 1260) according to the USEPA 680 method (Alford-Stevens et al. 1985). A total of 10 g of representative soil, obtained by mixing uniform weight subsamples from each of the five soils, were suspended in 100 ml saline solution, and filtered through Whatman No. 4 paper to separate the soil. Later, bacterial cells were recovered by centrifugation (1500*g*, 15 min). The resulting pellet was suspended in 10 ml saline solution followed by blending and centrifugation. This step was performed twice. The cellular pellet was inoculated into flasks containing 100 ml isolation medium (IM), consisted of a mineral salts medium containing 0.001% biphenyl, and 0.02% transformer oil (Aroclor 1260, a PCB mixture containing 60% chlorine) as the sole carbon sources. This medium was prepared, according to Bedard et al. (1986) and cultured in an orbital shaker adjusted to 100 rev/min at 30°C for 7 days as an initial enrichment step. The culture was monitored for the presence of microorganisms until turbidity of the medium was observed by measuring at 600 nm. The biomass obtained was recovered by centrifugation and used in seven successive inoculations to flasks containing 100 ml IM supplemented with 0.02 ml transformer oil incubating at the same conditions described previously.

Isolation of strains with the ability to degrade PCB

Bacteria from the consortium were isolated by streaking on TSA agar (Difco, Detroit, MI). Each isolated colony was then recultured on IM with added transformer oil as the sole carbon source and for proving their growth capacity. Pure cultures were stored as 30% glycerol stocks at −70°C.

Degradation of PCB by the consortium

Cells were grown in IM containing 0.001% biphenyl and 0.02 ml transformer oil at 30°C until the exponential phase. The bacterial cells were harvested by centrifugation (15,000*g*, 15 min, 4°C), washed with 50 mmol sodium phosphate buffer (pH 7.5), and then resuspended in the same buffer. Two milliliters of cell suspension (final OD_{660} of 1.0) was dispensed into culture flasks with 50 ml IM. Cultures were incubated at 30°C in a shaker at 150 rev/min for 4 weeks.

Degradation experiments were performed adding different amounts of transformer oil (2.5, 5, and 10 µl), 0.1% Tween 80, and 0.001% biphenyl. The additives were incorporated individually or in combination, as shown in Table 1.

Degradation of PCB congeners by each bacterial consortium member

PCBs were identified and measured as isomer groups or homologues. A concentration of 2.5 µg ml^{-1} for each PCB isomer group was analysed; total PCB concentration in each sample extracted was obtained by the sum of the isomer group concentrations. Nine selected PCB congeners were used as calibration standards, and one internal standard, biphenyl, was used to calibrate the MS response to PCBs.

Experiments to study degradation of the PCB mixture congeners in the transformer oil were conducted in flasks containing 0.02% transformer oil, 0.1% Tween 80, and 0.001% biphenyl. All flasks were inoculated with the respective bacterial cultures; the inoculated cells were added as previously described, incubated horizontally under shaking at 30°C. Transformation reactions were stopped after 4 weeks and the residual PCB were extracted with hexane.

Percentage degradation was calculated as the decrease in the total summation of all electron capture detector area counts. The percentage of chloride released from the

transformer oil was determined based on percent composition of chlorine in the mixture.

GC/MS analysis

PCB were extracted from the pure cultures with hexane, the solvent extracts were concentrated to 5 ml. The condensed extract was passed through a Florisil column according to a protocol described previously (Haluka et al. 1995).

The identification and quantification from each PCB group was performed through GC/MS according to the EPA 680 method (Alford-Stevens et al. 1985), with a HP6890 GC system-linked HP 5973 mass selective detector (Hewlett Packard, Palo Alto, CA, USA) and a 30-m fused DB 5MS column [J&W Scientific, Folsom, CA (0.25-mm inside diameter by 30-m length: 0.25-μm film thickness)]. The injection was made at 70°C and held for 5 min; increasing 10°C/min to 250°C and holding for10 -min; increasing 5°C/min to 260°C and holding for 10 min. Independent experiments were analysed in triplicate.

The GC peaks were analysed by mass spectrometry and compared with a standard compounds library to recognize the composition of different molecules in each PCB congener. PCB congeners were identified by comparing the relative retention times of peaks corresponding to the PCB standard and corroborated with published chromatographs of Aroclors 1260 (USEPA 680 method). Relative amounts of PCB congeners were determined by area integration of experimental and internal standard peaks.

DNA extraction, amplification, cloning, and sequencing of the 16S rRNA gene fragment of the strains

Total bacterial DNA was extracted and purified using previously described protocols (Cullen and Hirsch 1998; Knaebel and Crawford 1995). The 16S rDNA genes amplifications were performed using bacteria universal primers (Relman 1993), and conditions previously described (Jan-Roblero et al. 2008). PCR products were purified using the QIAquick PCR Purification Kit (Qiagen Inc., Valencia, CA) and sequenced using the Thermo Sequenase Cy5.5 Dye Terminator Cycle Sequencing Kit (Amersham Pharmacia Biotech, Piscataway, NJ) and an ABI PRISM 310 Genetic Analyser (Perkin-Elmer Applied Biosystem, Inc., Boston, MA).

Phylogenetic analysis of the bacteria

The isolated bacteria were identified by similarity and phylogenetic analysis of the 16S rRNA gene partial sequence in a similar manner to a previous work (Jan-Roblero et al. 2008). The sequence data reported in this paper have been deposited in the GenBank database, under accession numbers from EU249926 to EU249930.

Results and discussion

One of the best ways to achieve isolation of microorganisms with specific metabolic capabilities is through acclimation and enrichment cultures inoculated with the contaminated samples in a minimal medium containing the target compound as sole source of carbon. In the present study, a consortium was found, capable of growing on 0.001% biphenyl, and 0.02% transformer oil. It was obtained from PCB-contaminated soil and the culture reached a maximum growth rate of 0.0031 h^{-1}. Five isolated strains, four Gram-negative bacilli and other Gram-positive bacilli, were able to grow in PCB as a sole source of carbon. The high similarity to known species (>98.9%) and phylogenetic analysis of 16S-rDNA sequence based on 1,367 nucleotides indicated that the degrading-PCB consortium was formed by five cultivable species assigned to: *Brevibacterium antarcticum*, IPN16 strain; *Pandoraea pnomenusa*, IPN15 strain; and *Ochrobactrum intermedium*, IPN2, IPN9, and IPN20 strains, Fig. 1. The consortium and all strains were able to degrade or transform at least the lower-chlorinated PCB congeners under aerobic conditions. Apparently, the importance of adding biphenyl as a cosubstrate that induces biphenyl dioxygenase enzyme to the culture medium was demonstrated, an effect previously observed in former studies of PCB degradation (Kohler et al. 1988). Also, the addition of surfactants is especially important, because it enlarges the surface area of the hydrophobic substrates and their bioavailability (Ron and Rosenberg 2001).

Degradation of transformer oil by the consortium was evaluated by GC, Table 1. Thus, the consortium's PCB-degrading capacity diminished with the increase of PCB concentration in the medium, but no significant degradation was detected with 152 μmol l^{-1}. When biphenyl or Tween 80 was added independently, the extent of biodegradation was not significantly different from the control without additives. However, the highest level of biodegradation was obtained with the simultaneous addition of biphenyl and Tween 80.

The ability of each bacterial strain to degrade PCB congeners with different numbers of chlorine atoms is shown in Table 2. The degradation patterns of the compounds were analysed according to PCB category (tetra-, penta-, hexa-, hepta-, octa-, and nona chlorobiphenyls). Thus, *B. antarcticum* degraded 31.9% of tetrachlorobiphenyls, 0.7% heptachlorobiphenyls, and 30.5% octachlorobiphenyls; *P. pnomemusa* degraded 31.9% of tetrachlorobiphenyls and 30.5% octachlorobiphenyls, and *O. intermedium* degraded 31.9% tetrachlorobiphenyls,

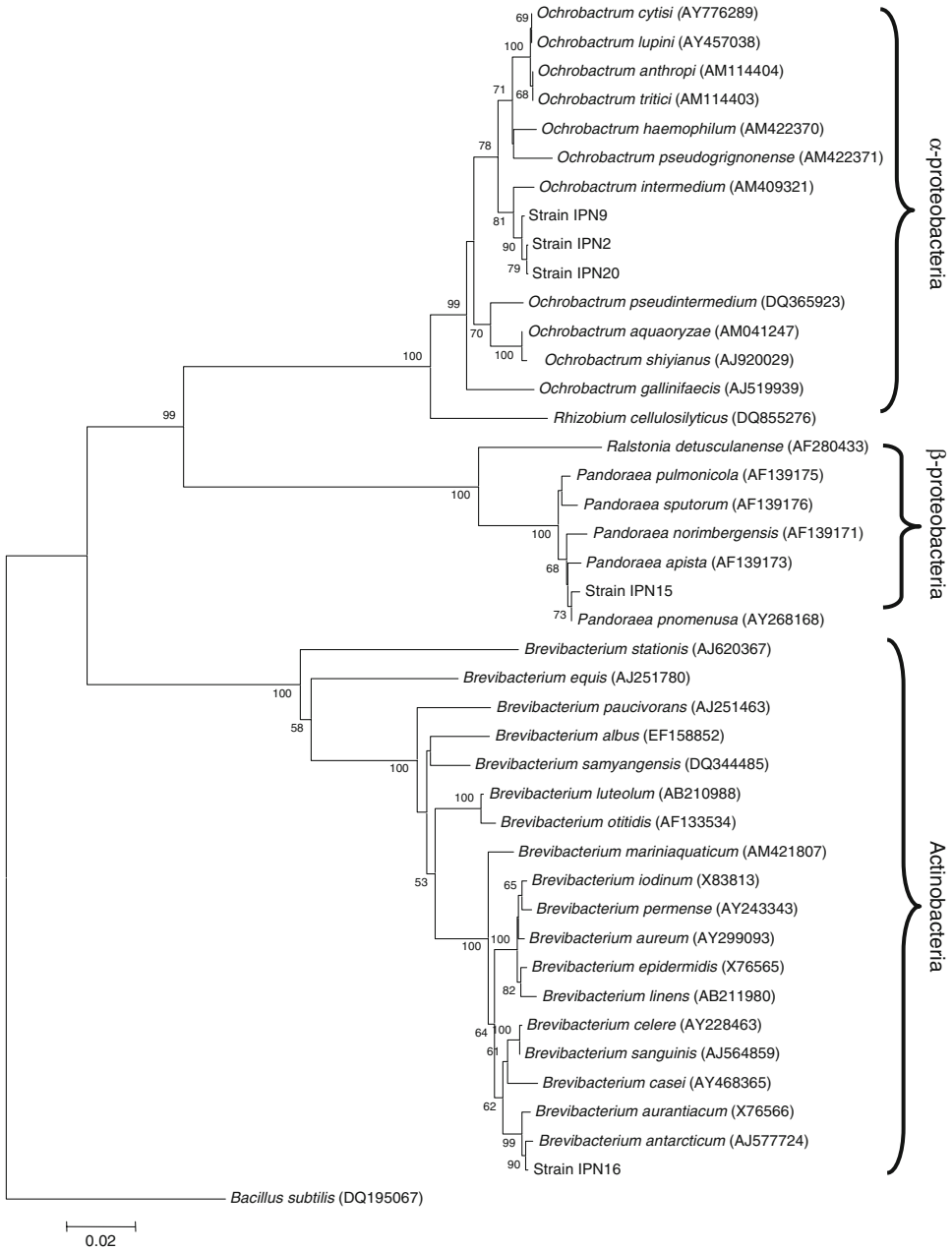

Fig. 1 Phylogenetic relationship between the 16S rRNA sequences of the isolated strains and the related bacterial genus. Bootstrap values >50% are indicated in the main nodes in a bootstrap analysis of 1,000 replicates, the scale bar represents the expected number of substitutions averaged over all sites analysed. *Bacillus subtilis* was used as a bacterial outgroup. A total of 1,367 nucleotides were included in the phylogenetic reconstruction

Table 1 Extent of biodegradation of PCB by the bacterial PCB-degrading consortium

Treatment				
PCB concentration μM	Without biphenyl Without Tween 80	With biphenyl Without Tween 80	Without biphenyl With Tween 80	With biphenyl With Tween 80
152	0	23.8 ± 2	25.7 ± 2	44.7 ± 3
129	35.7 ± 4	43.1 ± 3	58.4 ± 6	53.5 ± 4
55	49.7 ± 5	33.5 ± 2	33.8 ± 2	67.5 ± 4

Tween 80 and biphenyl were incorporated in concentrations of 0.1% and 0.001%, respectively. The degradation experiments were performed after a 4-weeks incubation period. Values are the means ± SD of three independent experiments

Table 2 Degradation of PCB congeners by the isolated strains

Number of chlorine substitutions in PCB	PCB degradation (%)			
	Control	*B. antarcticum*	*P. pnomenusa*	*O. intermedium*
4	-	31.9 ± 3	31.9 ± 2	31.9 ± 3
5	-	-	-	-
6	-	-	-	-
7	-	0.7 ± 0.03	-	64.5 ± 4
8	-	30.5 ± 2	30.5 ± 3	28.9 ± 1
9	-	-	-	-

-, No-effect-detected

Data are average ± SD from experiments performed in triplicate. Biphenyl-grown cells of each strain were incubated in IM with 0.1% Tween 80 and 0.001% biphenyl at 30°C for 4 weeks. The chlorinated compounds were analysed by gas chromatography. A control, without microorganisms, was included. The concentration of individual congeners in the reaction mixture was 2.5 μg ml^{-1}

64.5% heptachlorobiphenyls, and 28.9% octachlorobiphenyls. No treatment using the individual strains appreciably degraded the penta- and nona-chlorobiphenyl fractions. However, all strains exhibited some level of degradation when using some of the PCB fractions.

Pandoraea species have been associated with biodegradation of hexachlorocyclohexane and alpha-hexachlorocyclohexane pesticides, as well as with endosulfan (a cyclodiene organochlorine) (Okeke et al. 2002; Siddique et al. 2003). To our knowledge, only one recent study has recognized the PCB-degrading capacity of a *Pandoraea* species. In particular, the biphenyl dioxygenase from *P. pnomenusa* B-356 was recognized as an enzyme responsible for dihydroxylation of the biphenyl component of di-, tri, -tetra- PCB congeners used as substrate (Gomez-Gil et al. 2007), similarly to *P. pnomenusa* IPN15 isolated in this work.

No information concerning PCB degradation by *B. antarcticum* was detected, however, several *Brevibacterim* members have been isolated from petroleum-contaminated soils and indirectly associated with the degradation of saturated hydrocarbons (*n*- and isoalkanes, isoprenoids), dibenzofuran, and particularly cyclohexylamine (CHAM), a compound with a high structural similarity with phenyl moiety of PCBs (Engesser et al. 1989; Iwaki et al. 1999).

Finally, no information about the PCB-degrading capacity of *O. intermedium* stains is available. Thus, this is the first report on the degradation of a fraction of PCB congeners by *Ochrobactrum*. However, this genus has been associated with the degradation a variety of compounds, such as phenolic and chlorophenol compounds, *N*-acylhomoserine lactones, organophosphate pesticides, nonylphenols, nicotine, and nitroaromatic compounds (Müller et al. 1998; El-Sayed et al. 2003; Jafra et al. 2006; Qiu et al. 2006; Yuan et al. 2006; Chang et al. 2007).

In this work, a consortium composed of *B. antarcticum, P. pnomenusa,* and *O. intermedium* was obtained from a contaminated soil and showed a moderate degradation capability towards tetra-, hepta-, and octa-chlorobiphenyls, and no effect on penta-, hexa-, and nona-chlorobiphenyls. This consortium may have a potential role in the bioremediation of soil contaminated with PCBs.

Acknowledgments This work was supported by grants SIP20070651 and SIP20070164 Instituto Politécnico Nacional (IPN). C. Hernández-Rodríguez and J. Jan-Roblero appreciate the fellowship of Comisión de Operación y Fomento de Actividades Académicas (COFAA) and Estímulo al Desempeño Académico (EDI), IPN, and Sistema Nacional de Investigadores (SNI), CONACyT.

References

Alford-Stevens A, Bellar TA, Eichelberger JW, Budde WL (1985) Method 680: Determination of pesticides and PCBs in water and soil/sediment by gas chromatography/mass spectrometry. US EPA Office of Research and Development, Cincinnati, Ohio

Arensdorf JJ, Focht DD (1994) Formation of chlorocatechol meta cleavage products by a *Pseudomonas* during metabolism of monochlorobiphenyls. Appl Environ Microbiol 60:2884–2889

Beaudette LA, Davies S, Fedorak PM, Ward OP, Pickard MA (1998) Comparison of gas chromatography and mineralization experiment for measuring loss of selected polychlorinated biphenyl congeners in cultures of white rot fungi. Appl Environ Microbiol 64:2020–2025

Bedard DL, Unterman R, Bopp LH, Brennan MJ, Haberl M, Johnson C (1986) Rapid assay for screening and characterizing microorganisms for the ability to degrade polychlorinated biphenyls. Appl Environ Microbiol 51:761–768

Borlakoglu JT, Haegeles KD (1991) Comparative aspects in the bioaccumulation, metabolism and toxicity with PCBs. Comp Biochem Physiol C 100:327–338. doi:10.1016/0742-8413(91)90004-D

Chang BV, Chiang BW, Yuan SY (2007) Biodegradation of nonylphenol in soil. Chemosphere 66:1857–1862. doi:10.1016/j.chemosphere.2006.08.029

Cullen DW, Hirsch PR (1998) Simple and rapid method for direct extraction of microbial DNA from soil to PCR. Soil Biol Biochem 30:983–993. doi:10.1016/S0038-0717(98)00001-7

El-Sayed WS, Ibrahim MK, Abu-Shady M, El-Beih F, Ohmura N, Saiki H, Ando A (2003) Isolation and identification of a novel

strain of the genus *Ochrobactrum* with phenol-degrading activity. J Biosci Bioeng 96:310–312

Engesser KH, Strubel V, Christoglou K, Fischer P, Rast HG (1989) Dioxygenolytic cleavage of aryl ether bonds:1, 10-dihydro-1, 10-dihydroxyfluoren-9-one, a novel arene dihydrodiol as evidence for angular dioxygenation of dibenzofuran. FEMS Microbiol Lett 65:205–209. doi:10.1111/j.1574-6968.1989.tb03623.x

Finette BA, Subramanian V, Gibson DT (1984) Isolation and characterization of *Pseudomonas putida* PpF1 mutants defective in the toluene dioxygenase enzyme system. J Bacteriol 160:1003–1009

Furukawa K, Tonomura K, Kamibayashi A (1978) A effect of chlorine substitution on the biodegradability of polychlorinated biphenyls. Appl Environ Microbiol 35:223–227

Gomez-Gil L, Kumar P, Barriault D, Bolin JT, Sylvestre M, Eltis LD (2007) Characterization of biphenyl dioxygenase of *Pandoraea pnomenusa* B-356 as a potent polychlorinated biphenyl-degrading enzyme. J Bacteriol 189:5705–5715. doi:10.1128/JB.01476-06

Haluka L, Barancikova G, Balaz S, Dercova K, Vrana B, Paz-Weisshaar M, Furciova E, Bielek P (1995) Degradation of PCB in different soils by inoculated *Alcaligenes xylosoxidans*. Sci Total Environ 175:275–285. doi:10.1016/0048-9697(95)04927-4

Hou L, Dutta SK (2000) Phylogenetic characterization of several para- and meta-PCB dechlorinating *Clostridium* species: 16s rDNA sequence analyses. Lett Appl Microbiol 30:238–243. doi:10.1046/j.1472-765x.2000.00709.x

Iwaki H, Shimizu M, Tokuyama T, Hasegawa Y (1999) Biodegradation of cyclohexylamine by *Brevibacterium oxydans* IH-35A. Appl Environ Microbiol 65:2232–2234

Jafra S, Przysowa J, Czajkowski R, Michta A, Garbeva P, Van der Wolf JM (2006) Detection and characterization of bacteria from the potato rhizosphere degrading N-acyl-homoserine lactone. Can J Microbiol 52:1006–1015. doi:10.1139/W06-062

Jan-Roblero J, Posadas A, de la Serna JZ, García R, Hernández-Rodríguez C (2008) Phylogenetic characterization of bacterial consortia obtained of corroding gas pipelines in Mexico. World J Microbiol Biotechnol 24:1775–1784. doi:10.1007/s11274-008-9674-x

Knaebel DB, Crawford RL (1995) Extraction and purification of microbial DNA from petroleum contaminated soils and detection of low number of toluene, octane and pesticide degraders by multiple polymerase chain reaction and Southern analysis. Mol Ecol 4:579–591. doi:10.1111/j.1365-294X.1995.tb00258.x

Kohler HP, Kohler-Staub D, Focht DD (1988) Cometabolism of polychlorinated biphenyls: enhanced transformation of Aroclor 1254 by growing bacteria cells. Appl Environ Microbiol 54:1940–1945

Lindner AS, Whitfield C, Chen N, Semrau JD, Adriaens P (2003) Quantitative structure-biodegradation relationships for ortho-substituted biphenyl compounds oxidized by *Methylosinus trichosporium* OB3b. Environ Toxicol Chem 22:2251–2257. doi:10.1897/02-387

Müller RH, Jorks S, Kleinsteuber S, Babel W (1998) Degradation of various chlorophenols under alkaline conditions by gram-negative bacteria closely related to *Ochrobactrum anthropi*. J Basic Microbiol 38:269–281. doi:10.1002/(SICI)1521-4028(199809)38:4<269::AID-JOBM269>3.0.CO;2-P

Okeke BC, Siddique T, Arbestain MC, Frankenberger WT (2002) Biodegradation of gamma-hexachlorocyclohexane (lindane) and alpha-hexachlorocyclohexane in water and a soil slurry by a *Pandoraea* species. J Agric Food Chem 50:2548–2555. doi:10.1021/jf011422a

Pieper DH (2005) Aerobic degradation of polychlorinated biphenyls. Appl Microbiol Biotechnol 67:170–191. doi:10.1007/s00253-004-1810-4

Qiu XH, Bai WQ, Zhong QZ, Li M, He FQ, Li BT (2006) Isolation and characterization of a bacterial strain of the genus *Ochrobactrum* with methyl parathion mineralizing activity. J Appl Microbiol 101:986–994. doi:10.1111/j.1365-2672.2006.03016.x

Relman DA (1993) Universal bacterial 16S rRNA amplification and sequencing. In: Persing DH, Smith TF, Tenover FC, White TJ (eds) Diagnostic molecular microbiology: principles and applications. ASM Press, Washington DC, pp 489–495

Robinson GK, Lenn MJ (1994) The bioremediation of polychlorinated biphenyls (PCBs): problems and perspectives. Biotechnol Genet Eng Rev 12:139–188

Ron EZ, Rosenberg E (2001) Natural roles of biosurfactants. Environ Microbiol 3:229–236. doi:10.1046/j.1462-2920.2001.00190.x

Siddique T, Okeke BC, Arshad M, Frankenberger WT Jr (2003) Enrichment and isolation of endosulfan-degrading microorganisms. J Environ Qual 32:47–54

Wiegel J, Wu Q (2000) Microbial reductive dehalogenation of polychlorinated biphenyls. FEMS Microbiol Ecol 32:1–15. doi:10.1111/j.1574-6941.2000.tb00693.x

Yuan YJ, Lu ZX, Huang LJ, Bie XM, Lu FX, Li Y (2006) Optimization of a medium for enhancing nicotine biodegradation by *Ochrobactrum intermedium* DN2. J Appl Microbiol 101:691–697. doi:10.1111/j.1365-2672.2006.02929.x