Bull Environ Contam Toxicol (2008) 81:12–18
DOI 10.1007/s00128-008-9437-2

Characterization of Pentachlorophenol Degrading Bacterial Consortium from Chemostat

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Received: 23 April 2007 / Accepted: 14 April 2008 / Published online: 26 May 2008

Abstract A microbial consortium was developed by continuous enrichment of bacterial population isolated from sediment core of pulp and paper mill effluent in mineral salts medium (MSM) supplemented with pentachlorophenol (PCP) as sole source of carbon and energy in the chemostat. The enriched consortium contained three bacterial strains identified as *Escherichia coli* (PCP1), *Pseudomonas aeruginosa* (PCP2) and *Acinetobacter* sp. (PCP3) by morphological and biochemical tests, further confirmation was done using 16S rDNA sequence analysis. The potency of bacterial isolates in degradation of PCP was monitored in terms of growth and utilization of PCP as substrate with spectrophotometer and gas chromatograph-mass spectrometer (GC-MS) analysis. The strains were tested for their utilization of various organic compounds. The strain PCP3, showed higher potency to utilize PCP as sole source of carbon and energy than PCP1 and PCP2. The bacterial strain were able to utilize PCP through an oxidative and reductive route as indicated with the formation of tetrachloro-*p*-hydroquinone (TeCH), 2-chloro-1,4-benzenediol and 2,3,4,6-tetrachlorophenol, respectively.

Keywords Biodegradation · Chemostat · Consortia · GC-MS · Pentachlorophenol

The chlorophenolic compounds are major environmental contaminants giving global concern mainly due to use of these compounds as wide spectrum biocides in industry and agriculture (Vallecillo et al. 1999). The most common sources of chlorinated phenols in the environment include production of chlorine from bleaching of pulp, combustion of organic matter, partial transformation of phenoxy pesticides such as 2,4-dichlorophenoxy acetic acid and 2,4,6-trichlorophenoxyacetic acid, treatment of wood against fungi and insects and preservation of raw hides in leather tanning industries (Shukla et al. 2001). The toxicity of these compounds tends to increase with relative degree of chlorination (Reineke and Knackmuss 1988; Fetzner and Lingens 1994). Among chlorinated phenols pentachlorophenol (PCP) and its sodium salt have been widely used as wood and leather preservative owing to their toxic effect on bacteria, mould, algae and fungi (Kaoa et al. 2004). PCP is toxic to all forms of life since it is an inhibitor of oxidative phosphorylation (Shen et al. 2005; Yang et al. 2006). The US Environmental protection agency (EPA) has listed PCP as a priority contaminant because of its proven carcinogenicity and toxicity (Bock et al. 1996). PCP may be washed into streams and lakes due to surface runoff or may infiltrate and contaminate groundwater. Its large amount finally gets deposited onto sediments thus persisting in the environment (Shiu et al. 1994; Thakur et al. 2001). Despite widespread pollution observed, few indigenous bacterial strains capable of degrading PCP have been isolated (Chanama and Crawford 1997). Moreover, PCP is recalcitrant to degradation because of its stable aromatic ring system and high chloride content, thus persisting in the environment (Saber and Crawford 1985; Okeke et al. 1997; Copley 2000).

In case of environmental contamination by PCP, traditional clean-up methods have not been proved successful due to their higher treatment costs and possibilities of causing secondary pollution. The biodegradation of PCP has been studied in both aerobic and anaerobic systems. Anaerobic biodegradation of PCP in aquatic, sludge and

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soil environment has been studied by various researchers (Rensnick and Chapman 1994; McAllister et al. 1996; Wang et al. 1998; Vallecillo et al. 1999; Tartakovsky et al. 2001; Thakur et al. 2001). Reductive dechlorination has been suggested as the primary PCP biodegradation mechanism. The aromatic ring is thus totally dechlorinated prior to ring cleavage (Wang et al. 1998; Tartakovsky et al. 1999, 2001). Aerobic degradation of PCP has also been studied extensively and several bacterial strains capable of degradation have been reported such as *Flavobacterium*, *Arthrobacter*, *Pseudomonas*, and *Sphingomonas* (Edgehill and Finn 1983; Crawford and Mohn 1985; Saber and Crawford 1985; Xun and Orser 1991; Orser et al. 1993; Edgehill 1994; Miethling and Karlson 1996; Chanama and Crawford 1997; Leung et al. 1999; Thakur et al. 2002; Yang et al. 2006; Dams et al. 2007).

However, in solving serious problem of PCP contamination, it is important to assess the potential of bacterial strains indigenous to PCP contaminated sites for its degradation. Bacterial isolates obtained from nature have not been proved effective in complete degradation of PCP and other related metabolites at contaminated sites. Consequently a microbial consortium is usually required to provide all the metabolic capabilities for complete degradation of PCP. In light of these facts, it is important to elucidate such biological interactions in a continuously operating system such as chemostat in order to develop a potential microbial consortium for the degradation of PCP. Therefore objectives of present study at initial stage are to develop a stable bacterial consortium by continuous enrichment in the chemostat, characterize the members of the bacterial consortium, identification of products released during degradation of PCP and evaluation of utilization potential of PCP in the presence of intermediary metabolites. The study could help in developing strategies for enhancing degradation and large scale removal of PCP from the environment.

Materials and Methods

Sediment samples together with liquid effluent (1:10 w/v) were collected from the drainage sites of pulp and paper mill located at Lalkuan, Uttaranchal state, India. The unsieved soil or sediment (4 g) and mineral salts medium (100 mL) was mixed by the waring blender for about 3 min. The mixture (8 mL) from waring blender was added to 100 mL of mineral salts medium containing 10 mg L^{-1} of PCP as the sole source of carbon and energy (Tartakovsky et al. 1999). The mixture was placed on a shaker (120 rpm) in dark at 30°C. The mineral salts medium (MSM) contained the following components at the specified concentrations (in mg L^{-1}): KH_2PO_4, 800; Na_2HPO_4, 800; $Mg_2SO_4 \cdot 7H_2O$, 200; $CaCl_2 \cdot 2H_2O$, 10; NH_4Cl, 500; plus 1 mL of trace metal solution which include $FeSO_4 \cdot 7H_2O$, 5; $ZnSO_4 \cdot 7H_2O$, 4; $MnSO_4 \cdot 4H_2O$, 0.2; $NiCl \cdot 6H_2O$, 0.1; H_3BO_3, 0.15; $CoCl_2 \cdot 6H_2O$, 0.5; $ZnCl_2$, 0.25; and EDTA, 2.5.

After 14 days of incubation at 30°C (120 rpm) in a Gyratory Shaker, 1 mL of enrichment culture was transferred to 100 mL of MSM containing PCP (20 mg L^{-1}). This medium was incubated at 30°C (120 rpm) for 14 days. The above procedures were repeated until the concentration of PCP in the medium reached 40 mg L^{-1}. The bacterial strains enriched by utilization of PCP were used as inoculum in the chemostat, and continuous enrichment was performed using mineral salts medium and PCP (100 mg L^{-1}) as described above. The chemostat culture was run in 2 L glass vessel (effective volume 1 L) provided by stirring, 250 rpm, temperature, 30°C; pH, 7.2–7.4; an air flow of 500 mL min^{-1} and MSM flow rate of 10 mL h^{-1}. Samples of the culture were collected under aseptic conditions. The growth of the bacterial community was determined by measuring the optical density at 600 nm. The total number of bacterial cells was determined by colony forming units (c.f.u.) by serial dilution of the culture medium and plated (0.1 mL per plate) on MSM agar plates.

The utilization of PCP in the chemostat was monitored by extraction of PCP from culture medium and analysis by GC-MS. The bacterial strains from mixed culture were diluted and were spread on MSM agar plates having PCP as sole carbon source at a concentration of 100 mg L^{-1} to obtain single colonies. The plates were incubated at 30°C for 12 h. Single colonies were picked up and streaked on to MSM agar plates containing PCP (100 mg L^{-1}) to check for their purity. Isolates were identified by morphological, physiological and chemotaxonomic properties in accordance with Bergy's Manual of Systematic Bacteriology (Palleroni 1984). Further identification was performed using 16S rDNA sequence analysis. 1.4 kb fragment of 16S rDNA was amplified using a Gene Amp 2400 PCR System (PE, USA). A 5 μL purified genomic DNA served as a template in the PCR reaction mixture with the following set of primers: upstream primer 27F (GAGAGTTTGATCCTGGCTCAG), and downstream primer 1495R (CTACGGCTACCTTGTTACGA). Each 200 μL PCR micro-tube contained 5 μL purified extracted DNA; 2 μL of dNTP at 2.5 mM; 2.5 μL of 10× *Taq* DNA polymerase buffer; 11.3 μL of sterile MQ water and 2 μL of amplification primers (10 pmol each). The tubes were then subjected to the following thermal cycling programme: denaturation at 94°C for 1 min, primer annealing at 54°C for 1 min, and chain extension at 72°C for 2 min with an additional extension time of 7 min on the final cycle, for a total of 30 cycles. The 16S rDNA sequence was compared against the Gene Bank database using National Centre for Biotechnology Information (NCBI) BLAST program.

In extraction of metabolites, the cell suspension was clarified by centrifugation at 8,000 rpm for 3 min. The cell free supernatant fractions were extracted three times with an equal volume of n-hexane by shaking vigorously for 15 min in a standard separating funnel. The organic layer was dried with anhydrous sodium sulphate, and the solvent was removed by gently blowing under a steam of N_2. The residue was finally dissolved in 50 μL mixture of n-hexane: ethyl acetate (10:1) and analyzed immediately on a GC-MS. The GC-MS analyses were performed in electron ionization (EI) mode (70 eV) with an Agilent 6890N gas chromatograph, equipped with 5973 MSD (Agilent Technologies, Palo Alto, CA, USA). A HP-5MS (Agilent, USA) capillary column (5% phenyl 95% methylpolysiloxane; 30 m length × 0.025 mm i.d. × 0.25 μm film thickness) was used at a temperature programme of 50°C (2 min), then raised to 10°C min^{-1} to 280°C, where it was held for 10 min. Helium was used as the carrier gas at a constant flow of 1.2 mL min^{-1}. The samples were analyzed in split less mode at an injection temperature of 250°C, an EI source temperature of 230°C and a quadrupole analyzer temperature of 150°C, unit mass resolution, scan range m/z 35–500, with a scan cycle of 3 scans s^{-1}. The injected volume was 0.1 μL.

Utilization of various chlorinated compounds by each bacterial strain was assessed on MSM agar plates with the test compound. Growth of bacterial strains at 30°C was monitored daily by visual inspection for 3 days. The bacterial cells were then washed from the plate with saline and absorbance (A_{600}) values of the suspension were determined. The bacterial strains were inoculated in Erlenmeyer flasks containing mineral salts medium supplemented with test compounds (0.5 mM) as sole source of carbon and energy, and incubated at 30°C on an orbital shaker at 150 rpm in dark. The samples were removed after 0, 6 and 12 h, and growth of bacterial strains and utilization of carbon source was determined. All experiments were performed in triplicates and data represents are a mean of values obtained.

Results and Discussion

Sediment core along with liquid effluent from sampling sites of pulp and paper mill initially yielded 18 bacterial strains capable of utilizing pentachlorophenol tested on MSM agar plates containing PCP (100 mg L^{-1}) as sole carbon and energy source. The bacterial cells were extracted and enriched in the chemostat in MSM with PCP by continuous process. Figure 1 shows the growth pattern of the bacterial strains enriched in the presence of PCP. Initially there was an increase in turbidity, but after day 5 it declined, which persisted up to day 20. The rate of nutrient supply in the culture vessel was decreased from 10 mL h^{-1} to 5 mL h^{-1}. The fluctuation in turbidity continued till day 120, with an almost constant growth rate. After day 140 there was an increase in turbidity up to day 200, followed by almost constant growth rate even with increased flow rate (10 mL h^{-1}) till the end of the run (280 days). The growth of the bacterial cells in the chemostat determined by the c.f.u. on MSM agar plates was also concomitant to the turbidity. The initial fluctuations in the turbidity of the medium may be due to inability of the bacterial strains to utilize PCP, formation of intermediary metabolites released during its degradation and also because cells were not well acclimatized to the new environment containing PCP. The growth of bacterial cells in the chemostat was constant as the cell became physiologically adapted to PCP, and was probably due to nutritional interaction between the members of the community. Results of the study indicated a significant increase in utilization of carbon source from 51% on day 140 to 82.5% at the end of the run i.e. day 280.

Among the isolated 18 strains, only three bacterial strains (PCP1, PCP2 and PCP3) with relatively high degradation ability were selected and discussed in this work. The bacterial strains were identified morphologically and biochemically as *Pseudomonas* sp. (PCP2 and PCP3) and *Enterobacter* sp. (PCP1). The morphological and biochemical characteristics of selected bacterial strains have

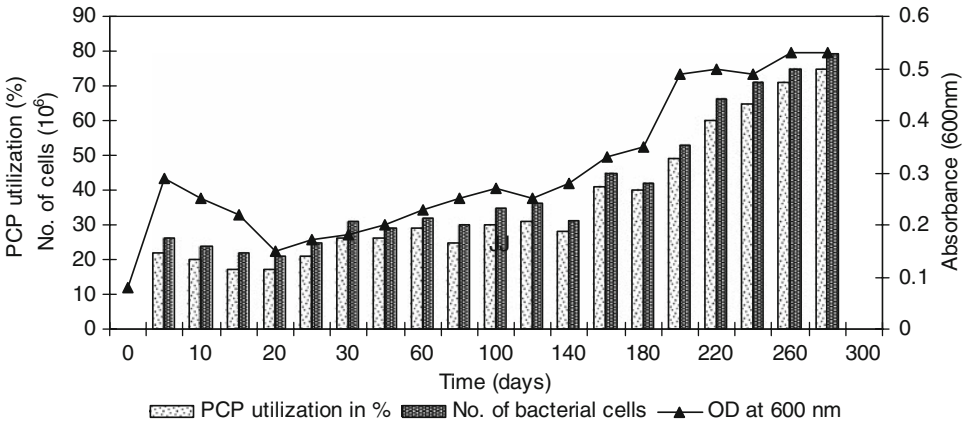

Fig. 1 Growth pattern of bacterial consortium in MSM-PCP medium

been shown in Table 1. The strains were also identified by method based on 16S rDNA gene sequence analysis. The analysis of 16S rDNA gene sequence confirmed that the strain PCP1 as *E. coli*; PCP2 as *Pseudomonas aeruginosa* and PCP3 identified as *Acinetobacter* sp.

Figure 2 shows degradation of PCP by individual strain investigated in terms of growth and utilization of PCP as substrate. Growth of bacterial strains exhibited by increase in optical density (OD) was observed up to 48 h but after that it declined (till 96 h). Utilization of PCP was determined by spectrophotometeric and GC-MS analysis. It was observed that PCP3 had higher potency to utilize PCP, more than 20% within 6 h, followed by PCP2, which could utilize 15% PCP and PCP1 which utilized 10% PCP at the same time interval. The most significant result observed in this study was utilization of more than 80% pentachlorophenol by PCP3 by 96 h followed by PCP2 and PCP1, which utilized almost 60% and 45%. The utilization potency of PCP1 was less than the other two strains of the consortium. A positive Rothera test for *Pseudomonas aeruginosa* (PCP2) and *Acinetobacter* sp. (PCP3) confirmed *ortho*-ring cleavage. During utilization of carbon source a stoichiometric amount of chloride was accumulated in the culture broth. The release of chloride was highest in the case of isolate PCP3 followed by PCP2 and PCP1, indicating greater degradation of PCP by strain PCP3 (data not shown).

The utilization of chlorinated phenols and intermediary metabolite of PCP by the bacterial strains, PCP1, PCP2 and PCP3, was tested on mineral salts medium containing various carbon sources under aerobic conditions (Table 2). The strain PCP3 was able to grow well on plates containing pentachlorophenol, tetrachlorohydroquinone, chlorohydroquinone and catechol. The growth was observed minimum when grown on 2,4,6-trichlorophenol. The utilization potential of PCP2 and PCP1 was good on catechol. Normal

Table 1 Morphological and biochemical characteristics of bacterial strains

Characters	Bacterial strains and their responses		
	PCP1	PCP2	PCP3
Gram staining	−	−	+
Morphology	Rod, single	Rod, single	Cocci, in pair
Color	Light yellow	Cream	Cream
Gelatin hydrolysis	+	+	+
Urease production	−	−	−
Oxidase production	−	+	+
Indole production	+	−	−
Catalase production	−	−	+
Starch hydrolysis	−	−	−
NaCl tolerance	>10%	>18%	>22%
Spreading nature	−	−	−

+, Positive; −, Negative

Table 2 Growth of bacterial strains on various carbon sources (20 mg L^{-1}) on MSM agar plate

Carbon source	Bacterial strains		
	PCP1	PCP2	PCP3
Pentachlorophenol	++	++	+++
2,4,6-trichlorophenol	+	++	+
2,4-dichlorophenol	+	++	++
p-chlorophenol	+	+	++
Tetrachlorohydroquinone	−	++	+++
Chlorohydroquinone	−	+	+++
Catechol	+++	+++	+++
4-chlorosalicylic acid	−	+	++
4-chlorobenzoic acid	−	−	++
Dibenzofuran	−	−	−

+++, Good growth; ++, normal growth; +, minimum growth; −, no growth

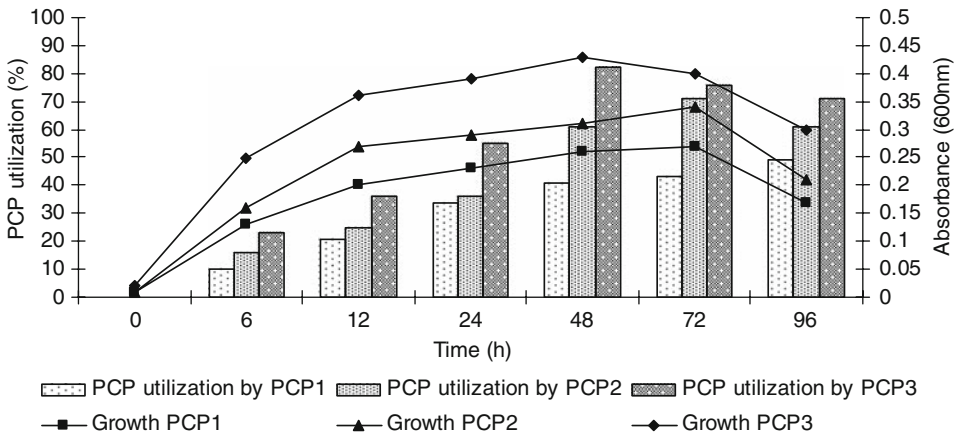

Fig. 2 Utilization of PCP by bacterial isolates (PCP1, PCP2 and PCP3) in terms of growth and percent reduction of substrate at different time interval

growth of strain PCP2 was observed on substrate such as pentachlorophenol, tetrachlorohydroquinone, trichlorophenol and dichlorophenol. None of the isolated strains were able to grown on dibenzofuran as substrate. An apparent lag in PCP utilization was greatly reduced by supplementation of readily degradable carbon source present along with PCP in MSM. Dextrose, sodium acetate and sodium citrate lead to significant growth. However, cellobiose and inulin were proved poor substrates compared to dextrose. Nitrogen source supplements included yeast extract, glutamate and aspartate as organic nitrogen and ammonium chloride and ammonium nitrate as inorganic nitrogen in MSM containing PCP. No PCP utilization was observed in the presence of readily available carbon source and yeast extract.

Figure 3 shows the GC-MS profile of PCP degradation by bacterial strains PCP1, PCP2 and PCP3. Peaks were identified using documented data from National Institute of Standards and Technology (NIST) library. The results of the study indicated degradation of PCP only after 48 h by PCP3 and emergence of two new peaks, tetrachloro-*p*-hydroquinone (TeCH) [m/z (% relative intensity)]: 250 (16), 248 (81), 230 (21), 211 (17), 147 (34), 111 (18), 87 (53), 49 (11) and 2-chloro-1,4-benzenediol [m/z

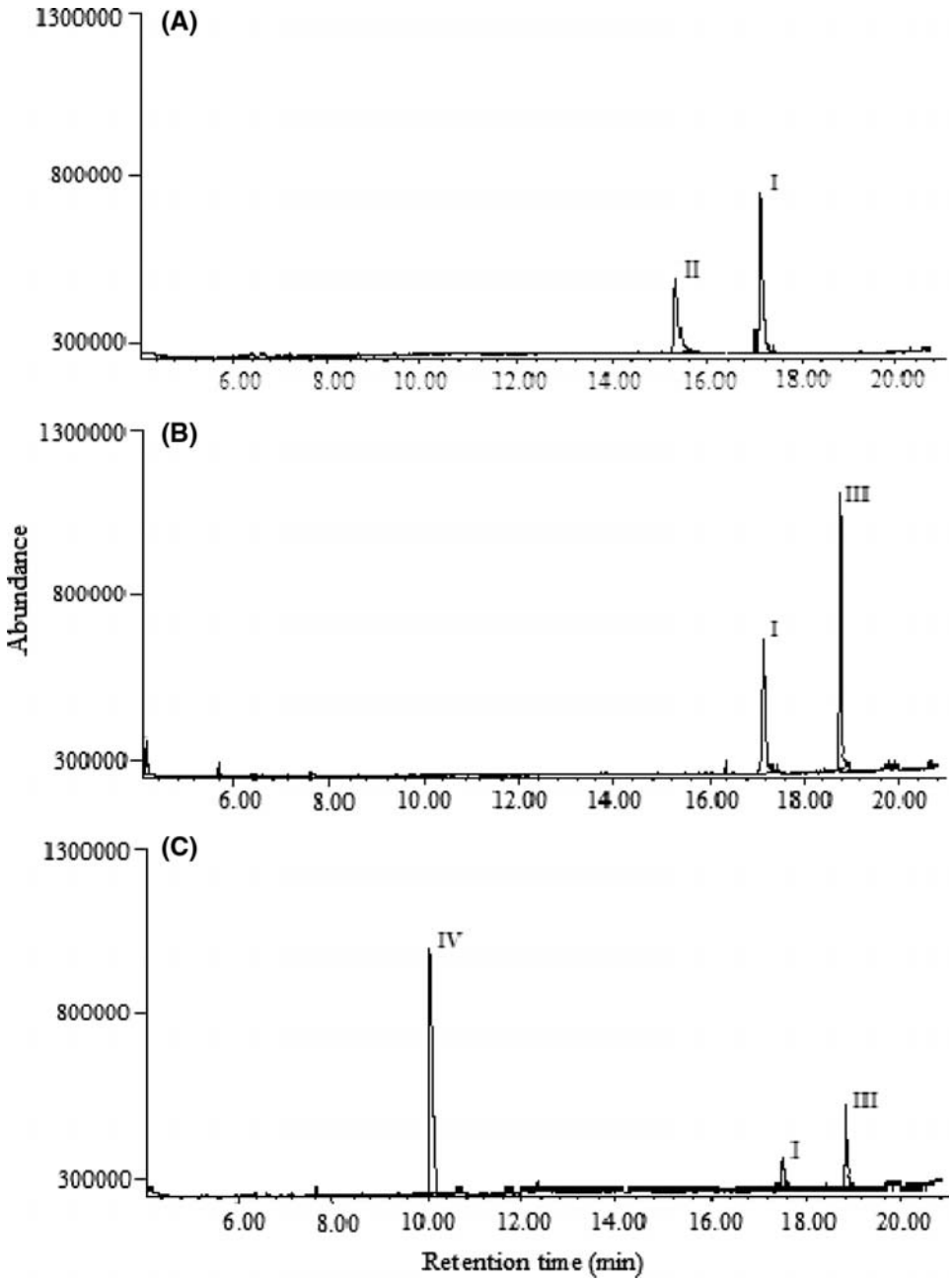

Fig. 3 GC-MS chromatograms of metabolites of PCP degradation after 48 h of incubation by (**a**) *E. coli* (PCP1) (**b**) *Pseudomonas aeruginosa* (PCP2) (**c**) *Acinetobacter* sp. (PCP3); I: Pentachlorophenol; II: 2,3,4,6-tetrachlorophenol; III: Tetrachlorohydroquinone; IV: 2-chloro-1,4-benzenediol

(% relative intensity)]: 207 (31), 144 (68), 80 (46), 52 (15). A decrease in the concentration of PCP was observed in case of PCP2 at 48 h with emergence of single peak of TeCH was observed. However, PCP1 showed little decrease in PCP concentration with the emergence of a new peak of 2,3,4,6-tetrachlorophenol [m/z (% relative intensity)]: 232 (82), 203 (13), 194 (24), 153 (32), 116 (11), 96 (38), 71 (9), 48 (7). So far, two types of pathways for aerobic degradation of PCP have been described; one is via chloro-catechols and other is via hydroquinone. In the pathway via chloro-catechols, these chlorophenols are further metabolized via ortho or modified ortho ring cleavage pathways (Thakur et al. 2002). In the hydroquinone pathway, subsequent dechlorination leads to the formation of hydroquinone, which is subsequently cleaved by ortho ring cleavage enzyme (Chanama and Crawford 1997; Copley 2000; Hu et al. 2006). The results of the study clearly indicate the nature of pathway followed by individual bacterial strains. PCP2 and PCP3 were able to degrade PCP via hydroquinone pathway. However, detection of tetrachlorophenol as a metabolite of PCP1 indicates a novel degradation pathway followed by this strain in aerobic condition.

This study has shown transformation of PCP in a chemostat during enrichment in a continuous culture by microbial population isolated from sediment core of pulp and paper mill effluent. The microbial community was characterized based on structure and physiological characteristics of individual bacterial strains. Out of three predominant strains present in the acclimated mixed bacterial consortia, the most efficient pentachlorophenol degrader was *Acinetobacter* sp. (PCP3). The strain manifests high degradation capabilities. The strain *Pseudomonas aeruginosa* (PCP2) was able to degrade pentachlorophenol fairly well. However, the utilization potency of *E. coli* (PCP1) was less than PCP3 and PCP2. Results of the study indicated significance of PCP3 and PCP2 in degradation of PCP and acted as a potential member of the consortium, however, PCP1 may have played as associated members of the consortium in degradation of pentachlorophenol. The relationship between the structural and functional properties of bacterial strains in PCP transformation provided in this study may provide insight about potential enrichment of in situ microbial population.

Acknowledgement We thank Department of Biotechnology, Ministry of Science and Technology, Government of India, New Delhi, for providing financial assistance to carry out this work.

References

Bock C, Kroppenstedt RM, Schmidt U, Diekmann H (1996) Degradation of prochloraz and 2,4,6-trichlorophenol by environmental bacterial strains. Appl Microbiol Biotechnol 45:257–262. doi:10.1007/s002530050680

Chanama S, Crawford RL (1997) Mutational analysis of pcpA and its role in pentachlorophenol degradation by *Sphingomonas (Flavobacterium) chlorophenolica* ATCC 39723. Appl Environ Microbiol 63:4833–4838

Copley SD (2000) Evolution of metabolic pathway for degradation of a toxic xenobiotic: the patchwork approach. Trends Biochem Sci 25:261–265. doi:10.1016/S0968-0004(00)01562-0

Crawford RL, Mohn WW (1985) Microbial removal of pentachlorophenol from soil using a Flavobacterium. Enzyme Microb Technol 7:617–620. doi:10.1016/0141-0229(85)90031-6

Dams RI, Paton GI, Killham K (2007) Rhizomediation of pentachlorophenol by *Sphingobium chlorophenolicum* ATCC 39723. Chemosphere 68:864–870. doi:10.1016/j.chemosphere.2007.02.014

Edgehill RU (1994) Pentachlorophenol removal from slightly acidic mineral salts, commercial sand, and clay soil by recovered Arthrobacter strain ATCC 33790. Appl Microbiol Biotechnol 41:142–148. doi:10.1007/BF00166097

Edgehill RU, Finn RK (1983) Microbial treatment of soil to remove pentachlorophenol. Appl Environ Microbiol 45:1122–1125

Fetzner S, Lingens F (1994) Bacterial dehalogenase: biochemistry, genetics and biotechnological applications. Microbiol Rev 58:641–685

Hu Z, Wu YR, Lin BK, Maskaoui K, Zhuang DH, Zheng TL (2006) Isolation and characterization of two phenol degrading yeast strains from marine sediment. Bull Environ Contam Toxicol 76:899–906. doi:10.1007/s00128-006-1003-1

Kaoa CM, Chaib CT, Liub JK, Yehc TY Chena KF, Chend SC (2004) Evaluation of natural and enhanced PCP biodegradation at a former pesticide manufacturing plant. Water Res 38:663–672. doi:10.1016/j.watres.2003.10.030

Leung KM, Campbell S, Gan Y, White DC, Lee H, Trevors T (1999) The role of the *Sphingomonas* species UG30 pentachlorophenol-4-monooxygenase in *p*-nitrophenol degradation. FEMS Microbiol Lett 173:247–253. doi:10.1111/j.1574-6968.1999.tb13509.x

McAllister KA, Lee H, Trevors JT (1996) Microbial degradation of pentachlorophenol. Biodegradation 7:1–40. doi:10.1007/BF00056556

Miethling R, Karlson U (1996) Accelerated mineralization of pentachlorophenol in soil upon inoculation with *Mycobacterium chlorophenolicum* PCP1 and *Sphingomonas chlorophenolica* RA2. Appl Environ Microbiol 62:4361–4366

Okeke BC, Paterson A, Smith JE, Watson-Craik IA (1997) Comparative biotransformation of pentachlorophenol in soils by solid substrate cultures of *Lentinula edodes*. Appl Microbiol Biotechnol 48:563–569. doi:10.1007/s002530051097

Orser CS, Lange CC, Xun L, Zahrt TC, Schneider BJ (1993) Cloning, sequence analysis, and expression of the *Flavobacterium* pentachlorophenol-4-monooxygenase gene in *Escherichia coli*. J Bacteriol 175:411–416

Palleroni NJ (1984) Pseudomonas. In: Krieg NR, Holt JG (eds) Bergey's manual of systematic bacteriology. Williams Wilkins, Baltimore, pp 141–198

Reineke W, Knackmuss HJ (1988) Microbial degradation of haloaromatics. Ann Rev Microbiol 42:263–287. doi:10.1146/annurev.mi.42.100188.001403

Rensnick SM, Chapman PJ (1994) Physiological properties and substrate specificity of a pentachlorophenol-degrading *Pseudomonas* species. Biodegradation 5:47–54

Saber DL, Crawford RL (1985) Isolation and characterization of *Flavobacterium* strain that degrade pentachlorophenol. Appl Environ Microbiol 50:1512–1518

Shen DS, Liu XW, Feng HJ (2005) Effect of easily degradable substrate on anaerobic degradation of pentachlorophenol in an upflow

anaerobic sludge blanket (UASB) reactor. J Hazard Mater B119:239–243. doi:10.1016/j.jhazmat.2004.12.024

Shiu WY, Ma KC, Varhanickova D, MacKay D (1994) Chlorophenols and alkylphenols: a review and correlation of environmentally relevant properties and fate in an evaluative environment. Chemosphere 29(6):1155–1224. doi:10.1016/0045-6535(94)90252-6

Shukla S, Sharma R, Thakur IS (2001) Enrichment and characterization of pentachlorophenol degrading microbial community from the treatment of tannery effluent. Pollut Res 20:353–363

Tartakovsky B, Levesque MJ, Dumortier R, Beaudet R, Guiot SR (1999) Biodegradation of pentachlorophenol in a continuous anaerobic reactor augmented with *Desulfitobacterium frappieri* PCP-1. Appl Environ Microbiol 65:4357–4362

Tartakovsky B, Manuel MF, Beaumier D, Greer CW, Guiot SR (2001) Enhanced selection of an anaerobic pentachlorophenol-degrading consortium. Biotechnol Bioeng 73:476–483. doi:10.1002/bit.1082

Thakur IS, Verma P, Upadhyaya KC (2001) Involvement of plasmid in degradation of pentachlorophenol by *Pseudomonas* sp. from a chemostat. Biochem Biophys Res Comm 286:109–113. doi:10.1006/bbrc.2001.5340

Thakur IS, Verma PK, Upadhaya KC (2002) Molecular cloning and characterization of pentachlorophenol-degrading monooxygenase genes of *Pseudomonas* sp. from the chemostat. Biochem Biophys Res Commun 290:770–774. doi:10.1006/bbrc.2001.6239

Vallecillo A, Garcia-Encina PA, Pena M (1999) Anaerobic biodegradability and toxicity of chlorophenols. Water Sci Technol 40:161–168. doi:10.1016/S0273-1223(99)00622-8

Wang YT, Muthukrishnan S, Wang Z (1998) Reductive dechlorination of chlorophenols in methanogenic cultures. J Environ Eng 124(3):231–238. doi:10.1061/(ASCE)0733-9372(1998)124:3(231)

Xun L, Orser CS (1991) Purification and properties of pentachlorophenol hydroxylase, a flavoprotein from *Flavobacterium* sp. strain ATCC 39723. J Bacteriol 173:4447–4453

Yang CF, Lee CM, Wang CC (2006) Isolation and physiological characterization of the pentachlorophenol degrading bacterium *Sphingomonas chlorophenolica*. Chemosphere 62:709–714. doi:10.1016/j.chemosphere.2005.05.012