JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Nov 2015. Vol. **9**(Spl. Edn. 2), p. 201-208

Biotransformation of Pentachlorophenol to Intermediary Metabolites by Bacteria Isolated from Pulp and Paper Mill Effluent Irrigated Cultivable Land

Dharmendra Kumar, Rajeev Kaushik, Lata and Anil K. Saxena

Division of Microbiology, Indian Agricultural Research Institute, New Delhi- 110012, India.

(Received: 09 August 2015; accepted: 01 October 2015)

Bacteria capable of utilizing Pentachlorophenol as sole C source in minimal medium were analysed for their capability to biotransform PCP to intermediate metabolites. Selected isolates were also assessed, alone and in combination, for their ability to biotransform PCP in sterile soil. HPLC studies revealed decrease in the residual PCP and formation of intermediates such as tetrachlorohydroquinone (TeCH) and dichlorophenols (DCP) by the isolates. Removal of PCP from sterile soil by selected isolates, *viz. Ensifer adhaerens* (LK 4), *Pseudomonas putida* (LK 39) and *Lysinibacillus fusiformis* (LK156), was studied in a 30 d experiment under controlled condition. These isolates were used alone and in combination. PCP removal by mixed inoculum was significantly higher (68.81%) than single inoculation after 30 d of incubation. The results of this study indicate that the enriched mixed inoculum has better potential in scavenging PCP from soil as compared to individual isolates. The potential isolates obtained in the present study can be used for developing consortium for bioremediation of sites contaminated with PCP.

Key words: Pentachlorophenol degradation, tetrachlorohydroquinone, Dichlorophenols, HPLC studies, *Ensifer adhaerens*, *Lysinibacillus fusiformis*.

Pulp and paper mills utilize huge amount of lignocellulosic components and chemicals during manufacturing processes. These are regarded as polluting industries owing to discharge of wastewater which enter into the environment (Pokhrel and Viraraghavan, 2004)[14]. Regardless of the manufacturing process used, effluents discharged from pulp and paper mills are complicated mixtures consisting of several compounds, among which chlorinated phenolics are an important class of toxicant (Schnell et al. 2000)[17]. Among the chlorophenols released, pentachlorophenol (PCP) is recalcitrant to aerobic biodegradation due to its high chlorinated ring structure (Anandrajah et al. 2000)[1]. It is listed as priority pollutants with 0.30 μgL^{-1} concentration as safe permissible limit in water (US EPA, 1999)[7]. It is very harmful to microorganisms as it destroys membrane function due to its ability to uncouple oxidative phosphorylation (Copley, 2000)[5].

The pulp and paper mill effluent contains far above the permissible limit of PCP even after the treatment at industrial scale. India has 406 registered pulp and paper mills and generates large quantity of effluent (72–225 m^3 ton^{-1} paper) with residual PCP in it (Tewari et al. 2009)[22]. Century Pulp and Paper Mill, Lal Kuan, Uttrakhand discharge its primary treated effluent in local water channel. During the last 25-30 years, farmers of this region use effluent from these channels to irrigate their

* To whom all correspondence should be addressed.
E-mail: dharmendra851@gmail.com

crops *viz* sugarcane, rice and wheat (Tripathi et al. 2011)[23]. The residual PCP in effluent irrigated soil poses significant health hazards (Chandra et al. 2009)[4]. In our earlier studies, we found that these effluent irrigated soils at Lal Kuan have accumulated PCP @ 113.34 mg Kg^{-1} soil (unpublished report) (Research Paper I). To treat PCP in such soils, the biological treatment is superior to the physicochemical methods, because the latter ones have higher treatment cost and possibility of causing a secondary pollution. Several strains of bacteria that are able to completely mineralize polychlorinated phenols have been described and applied to bioremediate many PCP-contaminated sites (Edgehill 1982)[6]. However, in solving the serious problems of pollution caused by PCP, it is important to assess the potential of bacterial strains indigenous to sites contaminated with PCP for their ability to degrade PCP. Bioremediation takes advantage of the activity of naturally occurring microorganisms to degrade or transform a contaminating compound or compounds. In our earlier studies, we identified eight bacterial isolates from pulp and paper mill effluent irrigated soils of Lal Kuan, Uttrakhand. These isolates were able to grow by using PCP as sole C source in minimal salt (MS) medium having PCP @ 500 mg L^{-1}. In present study, these 8 PCP utilizing isolates were analysed for their capability to biotransform PCP to intermediate metabolites such as tetrachlorohydroquinone (TeCH) or dichlorophenols (DCP). Selected isolates were also assessed, alone and in combination, for their ability to biotransform PCP in sterile soil.

MATERIALS AND METHODS

Chemicals, reagents and standard stocks

The standards of PCP including their possible intermediates such as TCHQ and DCP were purchased from Sigma Aldrich (St, Louis, MO, USA). Ethyl acetate and methanol (HPLC grade) were obtained from Merck (Darmstadt, Germany). The stock solution (100 ìg ml^{-1}) of the above compounds was prepared in ethyl alcohol. Stock solutions were diluted to obtain 10 ìg ml^{-1} working solutions. All chemicals, aqueous solutions and standards were filtered through 0.22 ìm filter (Millipore, USA) and stored at 4°C.

Procurement of PCP utilizing bacterial isolates and growth medium

Bacterial isolates capable of utilizing PCP @ 300 and 500 mg L^{-1} as sole C source were obtained from microbial germplasm of Division of Microbiology, Indian Agricultural Research Institute, New Delhi. These isolates were isolated in our earlier studies (Unpublished report; Research Paper 1) from PCP contaminated soils of Lal Kuan, Uttrakhand. The isolates were *Pseudomonas aeruginosa* strain LK1, *Ensifer adhaerens* strain LK4, *Pseudomonas putida* strain LK39, *Pseudomonas citronellolis* strain LK81, *P. plecoglossicida* strain LK124, *P. citronellolis* strain LK141, *P. plecoglossicida* strain LK147 and *Lysinibacillus fusiformis* strain LK156. The cultures were maintained in mineral salt (MS) medium with PCP @ 50 µg ml^{-1} (Dams et al., 2007). The composition of MS medium (in gmL^{-1}) was KH_2PO_4, 0.68; K_2HPO_4, 1.73; $MgSO_4.7H_2O$, 0.1; $CaCl_2.2H_2O$, 0.02; NH_4SO_4, 0.017; and 1 ml of trace metal solution which includes (in mgL^{-1}) $FeSO_4.7H_2O$, 200; $ZnSO_4.7H_2O$, 10.0; $MnSO_4.4H_2O$, 3.0; $NiCl.6H_2O$, 2.0; H_3BO_3, 30.0; $CoCl_2.6H_2O$, 1.0; $ZnCl_2$, 10.0; and EDTA, 2.5. PCP was added to the medium after autoclaving. The pH was adjusted to 7.3 ± 0.2 prior to autoclaving.

PCP removal and formation of intermediates by selected isolates

PCP removal and formation of intermediates by the isolates was carried out in a batch culture. MS broth with 300 mg L^{-1} PCP was inoculated with inoculum of selected 8 isolates (6.0 x 10^5 mL^{-1} - 2.87 x 10^6 cfu mL^{-1}) in three replications and incubated at 30 °C at 200 rpm for 10 d. Initial pH of broth was maintained at 7.3±0.2. Un-inoculated control was also maintained. After 5 and 10 days of incubation, pH of the culture broth was measured before estimating residual PCP. The % PCP utilization or residual PCP and formation of intermediates was detected in the culture fluid as described earlier by Chandra et al. (2006)[3]. The dried PCP extracted sample was dissolved in 2.0 ml methanol (HPLC grade) and a 10 µl sample was analysed with a Waters 2487 HPLC equipped with a UV-VIS detector set at wavelength of 254 nm using reverse phase C-18 column (size 250 x 4.6 mm) with particle size 5 mm was used to carry out separation. The isocratic mobile phase was

methanol and 1% acetic acid in the ratio of 90:10 (v/v) and flow rate was 1 ml min^{-1}. PCP, TeCH and DCP standard were run under the same conditions. The % PCP degradation was estimated with the help of following equation:

$$Residual\ PCP = \frac{Peak\ area\ of\ extracted\ sample \times concentration\ of\ stand. \times vol.\ of\ extract}{Peak\ area\ of\ standard \times volume\ of\ sample}$$

$$\%\ PCP\ Utiliation/Degradation = \frac{Ao - An}{Ao} \times 100$$

where, A_n = Amount recovered (ìg mL^{-1}or ìg g^{-1}) on n days; A_0 = Amount recovered (ìg mL^{-1}or ìg g^{-1}) on 0 day; n = Time interval after fortification (i.e. 5, 10, 20, 30……days)

Degradation of PCP in sterile soil

Selected isolates, alone and in combination, were studied for degradation of PCP in 500 gm of sterile soil. The soil from IARI farm with no history of PCP accumulation was used for the study. The soil was sandy loam in texture having 0.52% total organic carbon, 58.06 kg ha^{-1} available N, 10.38 kg ha^{-1} available P and 105.25 kg ha^{-1} exchangeable K. The soil was passed through 0.2 mm sieve and sterilized in autoclave. The sterile soil was fortified with analytical grade PCP (SIGMA-ALDRICH Inc) under aseptic conditions so as to make final concentration of HŠ300 mg Kg^{-1} in each pot. Recovery of PCP from fortified soil was analysed by HPLC for estimating exact quantity of PCP in soil before inoculation with cultures as per the method described below. The fortified soil was filled in sterile pots @ 500 gm pot^{-1}. Three best performing isolates based on earlier results were selected. Carrier based inoculum of all three isolates and their mixture was prepared. Liquid inoculum of selected 3 isolates (cell population of $10^7 - 10^8$ cells ml^{-1}) was raised in nutrient broth. Thirty ml broth of each culture was properly mixed with 100 gm of sterile charcoal under aseptic conditions. For making consortium, 10 ml of each of culture was mixed together in 100 gm of sterile charcoal under aseptic conditions. The mixture was air dried in shade and to each pot, containing sterile soil with 300 ppm of PCP, the carrier based inoculum was mixed @ 1% under aseptic conditions. The final treatments with three replications were; T1: Culture 1 + soil; T2: Culture 2 + Soil; T3: Culture 3 + Soil; T4: Culture 1 +2 + 3 + Soil; T5: Un-incoculated control. The HPLC analysis for estimating residual PCP was carried out at 10, 20 and 30 d interval.

HPLC analysis for PCP degradation by selected isolates

After 10, 20 and 30 d of incubation, the residual PCP and intermediates formed were quantified as described earlier by Chandra et al. (2006)[3]. Five gram of soil sample from each pot was extracted with 10 ml ethyl acetate and separated by centrifugation. The upper organic phase was collected and evaporated to dryness in rotary evaporator at 45-50 °C. The dried extract were processed for analysis in same way as described above for bacterial isolates.

Data Analysis

Statistical analyses of the data was performed using STATISTICA 10. Analysis of variance and separation of means by least significant differences were performed by using the general linear models (GLM). Unless indicated otherwise, differences were considered only when significant at P = 0.05.

RESULTS AND DISCUSSION

PCP removal and formation of intermediates by selected isolates

Table 1 shows the % PCP utilization by the 8 isolates from MS medium with 300 mg L^{-1} PCP as sole C source after 5 and 10 days of incubation in a batch culture. The results indicated that all the 8 isolates grew and utilized PCP as an energy source. Reduction in pH of culture broth to 6.1 - 6.3 was observed in all the flasks except un-inoculated control. No reduction in pH and %PCP was observed in un-inoculated control. However, significant variation in % PCP utilization was observed by the isolates at 5 and 10 days of incubation. At 5 days of incubation, all the isolates could utilize 36.65 to 55.65 % PCP. Isolate *Lysinibacillus fusiformis* (LK 156) showed significantly higher % PCP removal than other isolates after 5 and 10 days of incubation followed by *Pseudomonas putida* (LK 39) and *Ensifer adhaerens* (LK 4) (Table 1).

Significant variation in %PCP utilization by the isolates was observed between 5 and 10 days of incubation. However, PCP utilization was incomplete (41.21 to 68.37%) even after 10 days of incubation. The plausible reason could be that the

growth of isolates must have attained stationery phase after 5 d of incubation in batch culture. Karn et al. (2010)[12] observed growth of pure isolates attaining stationery phase after 120 to 144 h of incubation in MS medium amended with 600 mgL^{-1} PCP. Chandra et al. (2006)[3] reported that *Bacillus cereus* ITRCS degraded 67% of 300 mg L^{-1} PCP after 168 h. The aerobic bacterial strain *Serratia marcescens* could utilize up to 300 mg L^{-1} PCP within 168 h (Singh et al. 2007).[20] Shah and Thakur (2002)[18] have observed that *Pseudomonas ûuorescens* could degrade PCP up to 100 mg L^{-1}.

The other possible reason for the observed incomplete degradation of PCP by the isolates in batch culture at 300 mg L^{-1} PCP could be that the lower pH (6.1 – 6.3) inhibited the activity of the organism. The aerobic pathway of PCP degradation is: $C_6Cl_5OH + 4.5O_2 + 2H_2O$ '! $6O_2 +$ 5HCl. The equation showed that PCP degradation leads to a decrease in pH and it affects the growth of organism. Yang et al. (2006)[24] reported that *Sphingomonas chlorophenolica* removed 90% of PCP when the initial pH was 9.2 and that it could not remove PCP when the pH value was below 6.0. Barbeau et al. (1997)[2] found that bacterial activity was apparently reduced when the pH was less than 6.0. Edgehill (1994)[6] also found that the growth rate for *Arthrobacter* sp at pH 7.4 was higher than under acidic conditions in the presence of PCP.

Formation of intermediates

The batch culture experiments performed revealed the decrease in PCP concentration of the MS medium and formation of tetrachlorohydroquinone (TeCH) and dichlorophenols (DCP) by all the eight isolates

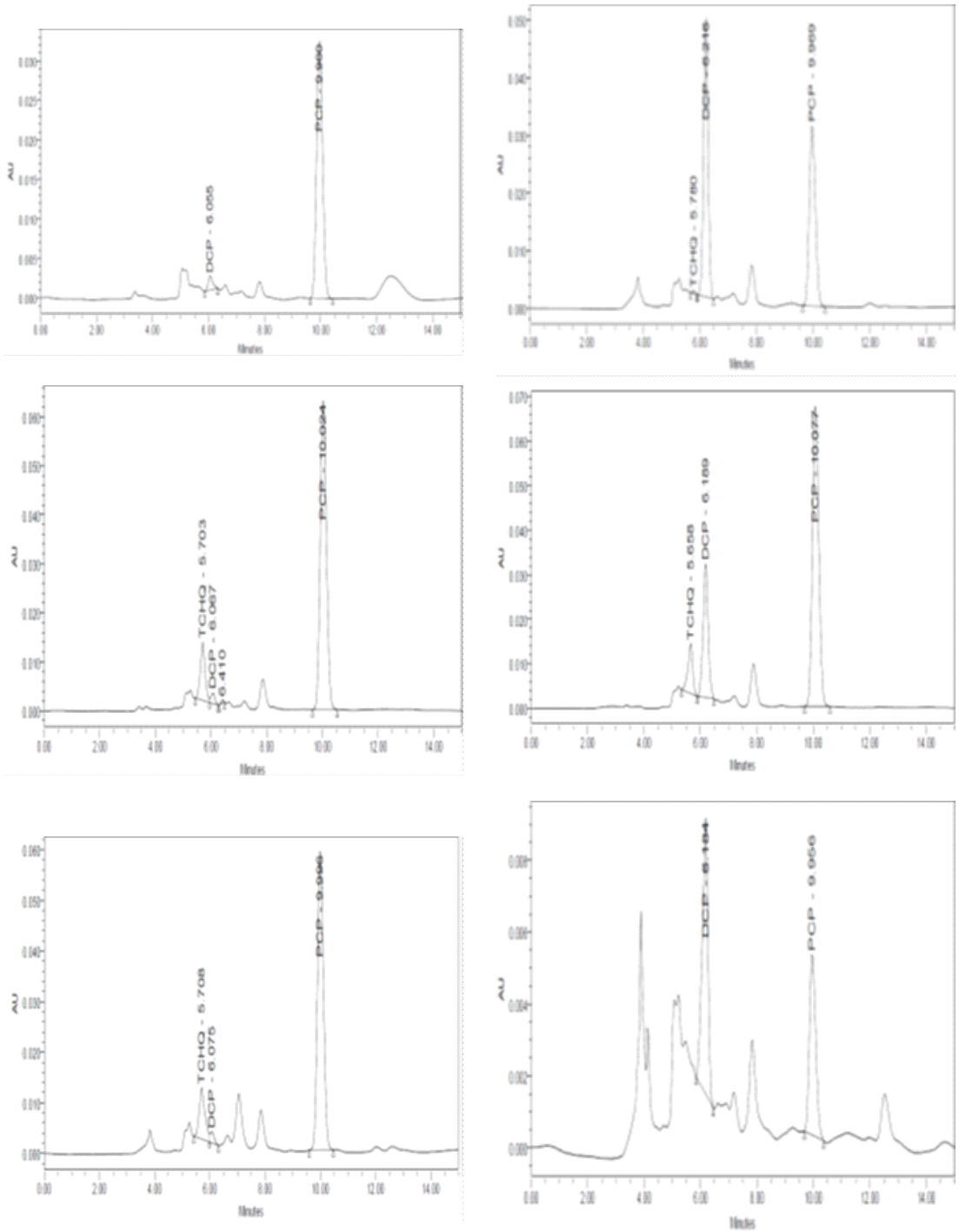
Figure 1 shows HPLC chromatogram of selected isolates at 5 and 10 days of incubation. PCP biodegradation occurs by dechlorination. Dechlorination can occur by one of the following mechanisms: reductive, hydrolytic, or oxygenolytic (McAllister, 1996)[13]. Reductive dechlorination results in the replacement of chlorines on the ring with a hydrogen by a reductive dehalogenase enzyme. Hydrolytic dechlorination is carried out by a PCP hydroxylase enzyme which replaces a chlorine on the ring with a hydroxyl group from water. During oxygenolytic dechlorination, a PCP monooxygenase enzyme mediates the replacement of chlorine by a hydroxyl group. Sharma and Thakur (2008)[18,19] observed the formation of TeCH and 2-chloro-1,4 benzenediol after 48 h of incubation. The results of the study clearly indicate the nature of pathway followed by bacterial strains. Formation of intermediates TeCH and DCP in the present study indicates that isolates were able to degrade PCP via reductive dehalogenation and hydrolytic dehalogenation. Dichlorophenols are produced naturally by certain fungi and insects (Gribble, 1991)[9], so it is to be expected that soil bacteria will be able to degrade these compounds through typical hydroxylation and ring cleavage reactions. The identification of dichlorophenol and tetrachlorohydroquinone during degradation suggests that these are produced by

Table 1. Percent PCP utilization by the selected isolates in MS medium with 300 mg L^{-1} PCP as sole C source after 5 and 10 days of incubation.

Isolate No.	Name	% PCP utilization at 5 and 10 d of incubation	
		5 d	10 d
LK 1	*Pseudomonas aeruginosa*	39.70	42.03
LK 4	*Ensifer adhaerens*	48.04	62.86
LK 39	*Pseudomonas putida*	50.17	63.62
LK 81	*Pseudomonas citronellolis*	38.00	41.21
LK 124	*P. plecoglossicida*	37.13	42.25
LK 141	*P. citronellolis*	36.65	40.60
LK 147	*P. plecoglossicida*	39.45	43.40
LK 156	*Lysinibacillus fusiformis*	55.65	68.37

LSD $_{(p=0.05)}$= Between isolates (I): 4.14; between days (D): 2.07; Interaction (I x D): 5.85

*Un-inoculated control did not show any reduction in PCP and concentration remained 300 ppm (±3.3)



5 Days **10 Days**

Fig. 1. HPLC chromatogram showing biotransformation of PCP by selected isolates to intermediate metabolites at 5 and 10 days of incubation in MS medium amended with 300 mg L^{-1} PCP. *LK 4: Ensifer adhaerens; LK 39: Pseudomonas putida; LK 156: Lysinibacillus fusiformis*

dechlorination as intermediates prior to ring cleavage.

Degradation of PCP in sterile soil by selected isolates

The residual PCP and % PCP degradation in sterile soil after 10, 20 and 30 days incubation with selected microbial isolates is given in table 2 and fig 2, respectively. Based on results of % PCP utilization (Table 1) and its biotransformation (Fig 1), three isolates *viz. LK 4: Ensifer adhaerens; LK 39: Pseudomonas putida; LK 156: Lysinibacillus fusiformis* were selected for degradation of PCP in sterile soil. The carrier based inoculum of all the 3 isolates was inoculated, alone and as a mixed inoculum (MI) in 500 gm of sterile soil having average PCP concentration of 278.24 ±5.36 mg Kg^{-1} at 0 day of incubation.

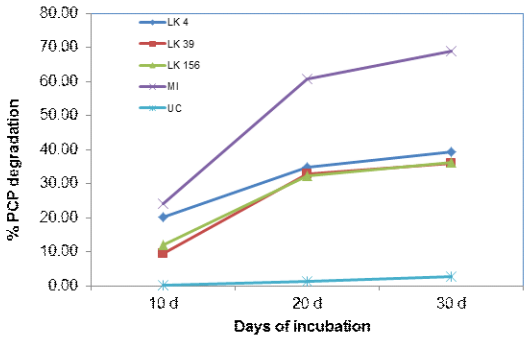


Fig. 2. Percent degradation of PCP in sterile soil after 10, 20 and 30 days of incubation with selected isolates individually and in combination. (*LK 4: Ensifer adhaerens; LK 39: Pseudomonas putida; LK 156: Lysinibacillus fusiformis*; MI: Mixed inoculum; UC: Un-inoculated control

Significant reduction in PCP was observed as result of bacterial inoculations in soil after 10, 20 and 30 days of incubation as compared with un-inoculated control (Table 2). The % PCP degradation (Fig 2) was calculated from values of residual PCP (Table 2) with reference to initial PCP concentration of 278.24 ±5.36 mg Kg^{-1} at 0 day. As expected, mixed inoculum showed significantly highest % degradation of PCP (68.81%) as compared to single inoculum (35.87 to 39.22%) and un-inoculated control (2.60%).Many reported a mixed culture degraded 70% of PCP within 50 days and 98% by 130 days.

Conversely, degradation with pure culture was significantly lower (Table 2 and Fig 2). Saber and Crawford (1985)[16] reported that PCP biodegradation rate reached 40-90 mg/l/d with pure *Flavobacterium.* Reduction in residual PCP in un-inoculated control (2.60%) over the period of 30 days (Table 2 and Figure 2) can be attributed to the abiotic factors. PCP removal can occur by abiotic processes such as volatilization, photodecomposition, and absorption (MacAllister et al. 1996)[13].

The degradation of PCP for the first 10 days was slow and it was in the range of 9.56 to 24.20. The possible reason for this could be the priority given by inoculum to utilize readily available nutrients present in the soil. It is only under nutrient limitation condition the inoculated cultures started utilizing PCP. It is known that microbial degradation of PCP in soil is carried out by acclimatized cultures (MacAllister et al. 1996)[13].

Table 2. Residual PCP (mg Kg^{-1}) in soil after 10, 20 and 30 days of incubation with selected isolates, alone and in combination

Isolate Number	Residual PCP (mg kg^{-1}) in soil at different days of incubation[1]		
	10 days	20 days	30 days
LK 4 (*Ensifer adhaerens)*	222.18	181.07	169.11
LK 39 (*Pseudomonas putida)*	251.64	186.90	178.43
LK 156 (*Lysinibacillus fusiformis)*	244.76	188.20	177.70
LK 4 + LK 39 + LK 156 (Mixed inoculum)	210.90	109.11	86.78
Un-inoculated control	277.53	274.43	271.01

$LSD_{(p=0.05)}$= Between days of incubation (D): 4.63; Between isolates (I): 5.98; Interaction (D x I): 10.36

[1]Average recovery of PCP in soil at 0 day of incubation: 278.24 mg Kg^{-1} (±5.36)

In the absence of microbial degradation the half-life of PCP in soil can be several months. However, in case of microbial degradation of PCP by cultures the acclimatized half-life of PCP is reduced significantly to 3-4 weeks and account for 26-46% removal (Fisher, 1997)[8]. In general, studies which used acclimatized inocula, demonstrated enhanced degradation in shorter times and minimized lag phases (MacAllister et al. 1996). The mixed inoculum of bacteria (LK, LK 39 and LK 156) performed well in consortium to degrading PCP in soil. Aerobic PCP degradation by mixed microbial cultures is important since most PCP-contaminated sites are surface soil or sediments which may support growth and activity of aerobic microbial consortia. PCP degradation can occur by the combined efforts of microorganisms in consortia (Kaoa et al. 2004; Pu and Cutright, 2007)[11,15], but these microbes should overcome the initial toxicity of PCP (Kaoa et al. 2004)[11] before it can activate genes of PCP degrading pathway PCP. Our isolates were tolerant to 700 ppm of PCP in growth medium (research paper I) and can overcome PCP toxicity.

In the present study, the consortium has shown potential to mineralize 68.81% PCP within 30 days for a PCP concentration of more than 275 mg Kg^{-1}. Previous studies reported that both pure and mixed cultures of fungi and/or bacteria are able to degrade PCP; however pure cultures are often only responsible for one step in the degradation pathway (Szweczyk et al. 2003)[21]. It is important to note that complete mineralization to innocuous end-products is only capable with a consortia of organisms (Becaert et al. 2000, Hansen et al. 2004)[2,10]. The most effective consortia were microorganisms isolated from PCP-contaminated soils. In present study, bacteria isolated from soils irrigated with PCP containing pulp and paper mill effluent and the enriched mixed inoculum has proved as better scavenger of PCP from soil as compared to individual isolates. The potential isolates obtained in present study can be used for developing consortia for bioremediation of sites contaminated with PCP.

REFERENCES

1. Anandaraja K, Kiefer PM, Donhoe JBS, Copley SD, Recruitment of a double bond isomerase to serve as a reductive dehalogenase during biodegradation of pentachlorophenol. *Biochemistry*, 2000; **39**(18): 5303–5311
2. Becaert V, Deschenes L, Samson R, A simple method to evaluate the concentration of pentachlorophenol degraders in contaminated soils. *FEMS Microbiology Letters*, 2000; **184**: 261–264.
3. Chandra R, Ghosh A, Jain RK, Singh S, Isolation and characterization of two potential pentachlorophenol degrading aerobic bacteria from pulp paper effluent sludge. *J. Gen. Appl. Microbiol*. 2006; **52**: 125-130.
4. Chandra R, Abhay R, Yadav S, Patel DK, Reduction of pollutants in pulp paper mill effluent treated by PCP-degrading bacterial strains. *Environ. Monit. Assess*, 2009; **155**: 1-1.
5. Copley SD, Evolution of metabolic pathway for degradation of a toxic xenobiotic: the patchwork approach. *Trends Biochem. Sci*, 2000; **25**: 261-265.
6. Edgehill RU, Finn RK, Isolation characterization and growth-kinetics of bacteria metabolizing pentachlorophenol. *Eur. J. Appl. Microbiol. Biotechnol*. 1982; **16**: 179-184.
7. EPA (2010). "Average annual emissions, all criteria pollutants in MS Excel." National Emissions Inventory (NEI) Air Pollutant Emissions Trends Data. Office of Air Quality Planning and Standards 2009.
8. Fisher B, Pentachlorophenol:Toxicology and Environmental Fate. *Journal of pesticide reform*, 1997; **11**.
9. Griebler C, Adrian L, Meckenstock, RU, Richnow HH, Stable carbon isotope fractionation during aerobic and anaerobic transformation of trichlorobenzene. *FEMS Microbiol. Ecol*. 2004; **48**: 313-321.
10. Hansen L, Nestler C, Ringelberg D, Bajpai R, Extended bioremediation of PAH/PCP contaminated soils from the POPILE wood treatment facility. *Chemosphere*, 2004; **54**: 1481–1493.
11. Kaoa CM, Chaib CT, Liub JK, Yehc TY, Chena KF, Chend SC, Evaluation of natural and enhanced PCP biodegradation at a former pesticide manufacturing plant. *Water Res*. 2004; **38**: 663-672
12. Karn SK, Chakrabarty SK, Reddy MS, Pentachlorophenol degradation by *Pseudomonas stutzeri* CL7 in the secondary sludge of pulp and paper mill. *Journal of Environmental Sciences*. 2010; **22** (10): 1608-1612.
13. McAllister KA, Lee H and Trevors J T, Microbial degradation of Pentachlorophenol. Biodegradation 1996; **7**: 1-40.
14. Pokhrel D, Viraraghavan T, Treatment of pulp

and paper mill wastewater – a review. *Sci. Tot. Env*., 2004; **333**: 37-58.

15. Pu X, Cutright TJ, Degradation of Pentachlorophenol by Pure and Mixed Cultures in Two Different Soils. *Env. Sci. Pollut. Res.* 2007; **14** (4): 244–250.
16. Saber DL, Crawford RL, Isolation and characterization of *Flavobacterium* strain that degrade pentachlorophenol. *Appl. Environ. Microbiol.* 1985; **50**: 1512-1518.
17. Schnell A, Stell P, Melcer H, Hudson PV Carey, JH, Enhanced biological treatment of bleached kraft mill effluents removal of chlorinated organic compounds and toxicity. *Water Res*. 2000; **34**: 493–500.
18. Shah S, Thakur I S, Treatment of Tannery Effluent by Pentachlorophenol –Degrading Bacterial Community from the chemostat. Journal of Science and Industrial Research 2002; **61**, 1051-1055.
19. Sharma A, Thakur IS, Characterization of pentachlorophenol degrading bacterial consortium from chemostat. *Bull. Environ. Cont. Toxicol*. 2008; **81**(1): 12-18.
20. Singh S, Chandra R, Patel DK, Rai, Isolation and characterization of novel *Serratia marcescens* (AY927692) for pentachlorophenol degradation from pulp andpaper mill waste. *World Journal Microbiology Biotechnology*, 2007; **23**: 1747-1754.
21. Szewczyk R, Bernat P, Milczarek K, Dlugonski J, Application of microscopic fungi isolated from polluted industrial areas for polycyclic aromatic hydrocarbons and pentachlorophenol reduction. *Biodegradation*, 2003; **14**: 1–8.
22. Tewari PC, Andrabi SZA, Chaudhary CB, Shukla S, Screening of Pentachlorophenol (PCP) Degradin Bacterial strains Isolated from the Tannery Effluent Sludge of Kanpur, India. *Environ. Int. J. Sci. Tech*, 2011; **6**: 77-84.
23. Tripathi BM, Kaushik R, Kumari P, Saxena AK, Arora DK, Genetic and metabolic diversity of streptomycetes in pulp and paper mill effluent treated crop fields. *World J Microbiol Biotechnol* 2011; **27**(7): 1603–1613.
24. Yang CF, Lee CM, Wang CC, Isolation and physiological characterization of the pentachlorophenol degrading bacterium *Sphingomonas chlorophenolica. Chemosphere*, 2006; **62**: 709-714.