

Isolation and characterization of novel *Serratia marcescens* (AY927692) for pentachlorophenol degradation from pulp and paper mill waste

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Received: 29 November 2006 / Accepted: 28 February 2007 / Published online: 18 May 2007
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Abstract Seven aerobic bacterial strains were isolated from pulp paper mill waste and screened for pentachlorophenol (PCP) tolerance on PCP containing mineral salt agar medium (MSM). The organism was characterized by 16S rDNA sequencing which showed 99.7% sequence similarity with *Serratia marcescens*. PCP degradation was routinely monitored with spectrophotometric analysis and further confirmed by HPLC analysis. Among seven strains, ITRC S₇ was found to degrade up to 90.33% of 1.127 mM (300 mg/l) of PCP and simultaneous release of chloride ion (2.435 mM) emphasized the bacterial dechlorination in the medium in presence of glucose as an additional carbon and energy source under optimized condition within 168 h incubation. In absence of glucose bacterium was unable to utilize PCP indicating the phenomenon of co-metabolism. Bacterium was identified as *S. marcescens* (AY927692), was a novel and potential aerobic bacterial strain capable of degrading PCP in axenic condition. Further, this strain may be used for bioremediation of PCP containing pulp paper mill waste in the environment.

Keywords Dechlorination · Degradation · Pentachlorophenol · *Serratia marcescens* · 16S rDNA

Introduction

Pentachlorophenol (PCP) is a major environmental pollutant discharged from pulp and paper mills, tanneries, distilleries, dye and paint manufacturing and pharmaceutical industries (Crosby 1981). Among chlorophenols (CPs), PCP is a wide spectrum biocide with applications in agriculture, industry and public health. The US Environmental Protection Agency has registered PCP in the list of priority pollutants. They have had now restricted the sale and use of pesticide products containing PCP (EPA 1987).

PCP from the pulp bleaching process is found both in free (hexane extractable) and bound (extractable with strong alkali) forms in dissolved organic matter and particles (Schnell et al. 2000). High and low molecular weight chlorinated compounds are produced by complex reactions between chlorine and lignin in the wood pulp (Yeber et al. 2000). Bleached chemical pulp mill effluents have been identified as toxic according to the Canadian Environmental Protection Act due to the presence of high quantity of CPs (Schnell et al. 2000). In addition, PCP is very harmful to microorganisms because it destroys membrane function (Copley 2000) due to its ability to uncouple oxidative phosphorylation (Ito and Ohnishi 1982). It has been reported as mutagenic even at low level of concentration. It has inherent toxicity to be bioaccumulated in various food chains of biological system. PCP is toxic to living organisms and has a high half-life in environment because of the lack of biodegradative enzymes in the indigenous organisms (Bellinaso et al. 2001).

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Different researchers have reported PCP degrading microorganisms isolated from the natural environment. Saber and Crawford (1985) reported bacteria of the genus *Flavobacterium* utilizing 100 mg/l of PCP as sole source of carbon and energy. Schenk (1989) found the *Arthrobacter* sp. ATCC 33790 capable of utilizing PCP. Shah and Thakur (2002) have also observed that a *Pseudomonas fluorescens* could degrade PCP up to 100 mg/l in 120 h. However, the reported concentration of PCP degradation is less than that of PCP discharged by pulp paper industry. Therefore, there is need to isolate more potent bacterial strain which can degrade higher concentrations of PCP. There could still be a number of unidentified bacterial strains having capability of PCP degradation at higher concentrations. Therefore, this study is aimed to isolate and characterize more potent aerobic bacterial strains, which have the capacity to degrade PCP at higher concentrations than reported earlier.

Materials and methods

Chemicals

All reagents used were of analytical grade. Synthetic PCP (FW 266.30) was purchased from Sigma chemicals (USA). All solutions were prepared in Milli-Q water (Elix, Millipore purification system, France).

Sample collection

Pulp paper mill sludge samples contain significant amount of PCP (50.31 mg/l) (Raj et al. 2005) were collected from M/s Century pulp and paper mill, Lalkuan, Nainital, Uttaranchal (India) located at foothills of Himalayas (79° 10' E and 29° 3' N). The samples were taken into pre-sterilized test tube and immediately preserved at 4 °C.

Isolation, purification and screening of PCP degrading bacteria

Screening of PCP tolerant bacteria were done using nutrient enrichment technique by adding one gram pulp paper mill effluent sludge containing (50.31 mg/l of PCP) in mineral salt medium (MSM) of following composition (mg/l): K₂HPO₄, 85; KH₂PO₄, 17; MgSO₄, 30; FeSO₄·7H₂O, 30; CaSO₄, 30; MnSO₄·H₂O, 30; (NH₄)₂SO₄, 17 and purified agar, 18 g/l (pH 7.0 ± 0.2), trace element solution, 1 ml/l amended with different concentrations of PCP (0.376, 0.752, 1.127 and 1.503 mM) along with glucose 1% (w/v) as additional carbon source (Pfennig and Lippert 1966). The flask containing culture was incubated at 30 ± 1 °C and 120 rev/min for 5 days. Thereafter, the

bacterial suspension was plated out on to MSM agar with and without glucose. Seven phenotypically different bacterial colonies were picked and purified by repeated streaking on the same medium. These strains were designated as ITRC S₁, ITRC S₂, ITRC S₃, ITRC S₄, ITRC S₅, ITRC S₆ and ITRC S₇. The Gram staining of aforesaid strains was done as per standard protocol following Williams et al. (1993). The confirmatory test for the tolerance and accumulation of PCP by these bacteria were performed using bromocresol purple (BCP-16 mg/l) containing MSM (Martins et al. 1997) and control were used without inoculum. The most potent strain capable of degrading PCP was used for further studies and was identified by 16S rDNA sequencing.

Isolation of genomic DNA and amplification of 16S rDNA

Genomic DNA was isolated following the method described by Ausubel et al. (1992) with some minor modifications.

16S rDNA was polymerase chain reaction (PCR) amplified from the genomic DNA template with the help of universal primers 27f (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492r (5'-TACGGYTACCTTGTTACGACTT-3') (*Escherichia coli* 16S rDNA numbering system (Brosius et al. 1978; Weisburg et al. 1991) using a personal thermocycler (Eppendorf, Hamburg, Germany). Reaction mixture contained 70–100 ng of genomic DNA, 1 U of Deep Vent DNA polymerase, 1× Thermopol reaction buffer (10 mM KCl, 20 mM Tris-HCl [pH 8.8]), 10 mM (NH₄)₂SO₄, 2 mM MgSO₄ and 0.1% Triton X-100), 200 (µM of each deoxynucleoside triphosphate (New England Biolabs, Beverly, USA) and 20 pmol of each primer (Biobasics, Canada). PCR cycling parameters included an initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min and extension at 75 °C for 2 min and a final extension for 10 min at 75 °C.

Sequencing of 16S rDNA and phylogenetic analysis

Approximately 1.5 kb amplicon was separated by gel electrophoresis, eluted by Qiaquick gel extraction kit (Qiagen) and was sequenced using the following four forward and one reverse primers, viz. 27f, 357f, 704f, 926f and 1492r (Johnson 1994). The 16S rDNA sequence was determined following the dideoxy chain-termination method using ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit as directed in the manufacturer's protocol. Sequence reactions were electrophoresed and analysed by ABI 310 Genetic Analyzer (Applied Biosystems, USA). Sequences of closely related taxa of the isolate were retrieved from the GenBank database using

BLAST (www.ncbi.nlm.nih.gov/BLAST) and Ribosomal Database Project II (<http://rdp.cme.msu.edu/index.jsp>) (Altschul et al. 1997; Larsen et al. 1993). For the construction of phylogenetic tree, only sequences from the type strains of species whose names have been validly published were taken into account. All the sequences were aligned using Clustalx program (Thompson et al. 1997) and the alignment was manually corrected. The evolutionary distance dendrogram was constructed using neighbor-joining analysis (Saitou and Nei 1987) and the distances between the sequences were calculated from the models of Jukes and Cantor (1969). Bootstrap analysis was performed to assess the confidence limits of the branching (Felsenstein 1985).

PCP degradation assay

The degradation studies were performed by inoculating 1% inoculum (2.5×10^3 cfu/ml) of the most PCP tolerant bacterial strain to 250 ml Erlenmeyer shake flask batch culture containing 99 ml MSM supplemented with 1.127 mM (300 mg/l) PCP in presence of 1% (w/v) glucose as carbon source, incubated at 30 ± 1 °C in a refrigerated incubator shaker (Innova 4230, NJ, USA) at 120 rev/min up to 168 h. The stability of organism in experimental and control (without inoculum) conditions was optimized at different environmental conditions (i.e. temperature, pH and aeration) to ensure that the disappearance of PCP was caused by biodegradation. The culture sample was removed under aseptic conditions. In order to estimate the PCP concentration cells were centrifuged at 5,000 g for 20 min (Remi C-24) and the supernatant was collected. Growth of the bacterial cells was determined in culture medium by measuring absorbance at 620 nm and PCP concentration in the supernatant was quantified by measuring absorbance at 320 nm at every 24 h interval up to 168 h by using UV-Vis spectrophotometer (GBC Cintra-40, Australia). For HPLC analysis the supernatant was initially acidified to pH 2.0 using 1N HCl and subsequently extracted three times using ethyl acetate (99.5%) in 1:1 ratio in a separating funnel by intermittent shaking. The extracted upper organic layer containing residual PCP was filtered through sodium sulphate to absorb excess water. Filtered samples were evaporated to dryness at elevated temperature (50–60 °C) in hot air oven, subsequently resuspended in 5.0 ml acetonitrile (HPLC grade) and analyzed using Waters 515 model equipped with UV-Vis (Waters-2487, Milford, USA) detector operating at 320 nm. Separation was carried out with a reverse phase watersmake 5 µm C-18 column (250 × 4.6) mm and the isocratic mobile phase was acetonitrile and water (70:30, v/v) with a flow rate of 1 ml/min. PCP standard was analyzed under the same conditions and the utilization of PCP was

Table 1 Screening for tolerance pattern of bacterial isolates in presence of bromocresol purple with glucose and without glucose

PCP (in mM)	Stains number		ITRC S ₁ (Gram – ve)		ITRC S ₂ (Gram –ve)		ITRC S ₃ (Gram – ve)		ITRC S ₄ (Gram – ve)		ITRC S ₅ (Gram – ve)		ITRC S ₆ (Gram + ve)		ITRC S ₇ (Gram –ve)		Control (No reaction)	
	Glucose (%)	Glucose (%)	Glucose (%)	Glucose (%)	Glucose (%)	Glucose (%)	Glucose (%)	Glucose (%)	Glucose (%)	Glucose (%)	Glucose (%)	Glucose (%)	Glucose (%)	Glucose (%)	Glucose (%)	Glucose (%)	Glucose (%)	Glucose (%)
0.376	0.5	1	0	0.5	1	0	0.5	1	0	0.5	1	0	0.5	1	0	0.5	1	0
	++	(B)	++	(B)	++	(B)	++	(B)	++	(B)	++	(B)	++	(B)	++	(B)	++	(B)
0.752	0.5	1	0	0.5	1	0	0.5	1	0	0.5	1	0	0.5	1	0	0.5	1	0
	++	(B)	++	(Y)	++	(Y)	++	(B)	++	(B)	++	(B)	++	(Y)	++	(Y)	++	(Y)
1.127	0.5	1	0	0.5	1	0	0.5	1	0	0.5	1	0	0.5	1	0	0.5	1	0
	++	(B)	++	(B)	++	(B)	++	(B)	++	(B)	++	(B)	++	(B)	++	(B)	++	(B)
1.503	0.5	1	0	0.5	1	0	0.5	1	0	0.5	1	0	0.5	1	0	0.5	1	0
	++	(B)	++	(B)	++	(B)	++	(B)	++	(B)	++	(B)	++	(B)	++	(B)	++	(B)

+, Indication very slow growth; ++, Indication slow growth; +++, Fast and luxuriant growth; –, No growth; (Y), Yellow showed PCP utilization and tolerant; (B), Blue colour; no PCP utilization and tolerant; Gram +ve, Indicate Gram positive bacteria; Gram –ve, indicate Gram negative bacteria

estimated by measuring the peak area of the compound. PCP degradation was also determined by estimation of the chloride ion released in aqueous media at every 24 h interval up to 168 h following the method described by Bergmann and Sanik (1957). Comparing respective standard curves quantitated amount of PCP degraded and chloride ion released.

Statistical analysis

Data were statistically analyzed by an overall one-way analysis of variance (ANOVA) and when differences observed were significant, the mean were compared by Tukey–Kramer Multiple Comparison Test. Linear regression analysis was used to determine the relation between the PCP degradation and chloride ion release were performed using Microsoft Excel 2000. All the experiments in this study were performed in triplicates and values are mean of three ($n = 3$) \pm SD.

Results and discussion

Screening of bacteria for PCP tolerance

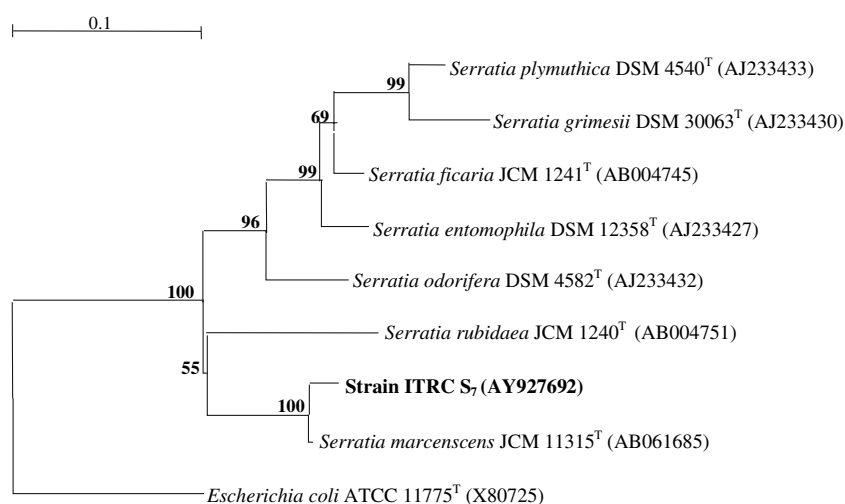
Out of the seven strains, ITRC S₁, ITRC S₂, ITRC S₃, ITRC S₄, ITRC S₅, ITRC S₇ were Gram negative and ITRC S₆ was found Gram positive. Out of these seven aerobic bacterial isolates, ITRC S₇ showed most potent for growth on PCP amended MSM agar in presence of glucose 0.5 and 1% (w/v) during the screening procedure whereas growth was negligible in absence of glucose (Table 1). The strain showed fast and luxuriant growth at various concentrations of PCP such as 0.376, 0.752 and 1.127 mM; however, no growth and change in colour of bromocresol purple were

observed at a concentration of 1.503 mM PCP. Strain ITRC S₇ was found to be the most potent isolate capable of degrading up to 1.127 mM PCP amended MSM agar in presence of 1% (w/v) glucose (Table 1). Change in colour of bromocresol purple from blue to yellow was due to the acidic pH resulting from release of chloride ion as well as hydrogen from PCP aromatic ring during dechlorination in the medium (Martins et al. 1997).

16S rDNA sequencing of strain ITRC S₇ and phylogenetic analysis

The results of the conventional identification techniques were supported by phylogenetic analysis. Hagstrom et al. (2000) found that 16S rDNA sequence having similarity of $\geq 97\%$ is a reasonable level of grouping bacteria into species. Using universal primers 1442 bp of 16S rDNA of strain ITRC S₇ was amplified, sequenced and submitted to the GenBank and the accession number AY927692 was obtained. Nucleotide–nucleotide BLAST searches of the obtained sequence showed 99% sequence similarity with the first hit of the search result i.e. *Serratia marcescens* strain AU736. Therefore, the most potent PCP-degrading isolate could be characterized as a strain of *S. marcescens* and was designated as *S. marcescens* strain ITRC S₇. To understand the taxonomic position of strain ITRC S₇ among other type strains of the genus *Serratia*, a detailed phylogenetic study was performed as shown in (Fig. 1). The evolutionary distance dendrogram revealed that all the sequences obtained from the database related to strain ITRC S₇ fell into the class of γ -Proteobacteria, a lineage of domain Bacteria. The phylogenetic analysis showed 99% similarity with *S. marcescens* at the level of 16S rDNA sequence and also revealed its phylogenetic affiliation with some of the other members of *Serratia* species.

Fig. 1 Phylogenetic neighbour-joining tree based on 16S rDNA sequences showing type strains of *Serratia* species related to strain ITRC S₇. *Escherichia coli* ATCC 11775^T (X80725) was used as the outgroup. Numbers at nodes indicate levels of bootstrap support $\geq 50\%$ based on a neighbour-joining analysis of 1000 resampled datasets. GenBank accession numbers are given in parentheses. Bar, 10 nucleotide substitution per 100 nucleotides



Degradation of PCP by strain ITRC S₇

Figure 2a representing the growth curve of strain ITRC S₇ indicated a prolonged lag phase followed by an exponential phase at approximately 140 h in presence of glucose. Up to 90.33% of PCP was significantly (ANOVA, $P < 0.05$) degraded within 168 h of incubation at 30 ± 1 °C, pH 7.0 and 120 rev/min (optimized condition) (Table 2). The simultaneous removal of PCP and release of chloride ion (2.435 mM) in the medium also emphasized the significant (ANOVA, $P < 0.05$) bacterial dechlorination and degradation of PCP (Table 2, Fig. 2b). In addition, no growth

and degradation was observed in control sample during experiment (Fig. 2b).

The rate of PCP degradation was not favoured by the variation at different environmental conditions when compared to the optimized condition as shown in Table 2 and Fig. 2 (c, d and e). Further, HPLC analysis confirmed the degradation of PCP (approx 90.33%) by showing disappearance of the peak of PCP as compared to control (Fig. 3).

There was no change in colour of bromocresol purple on PCP amended MSM agar medium in absence of glucose (Table 1). This phenomenon indicated that PCP

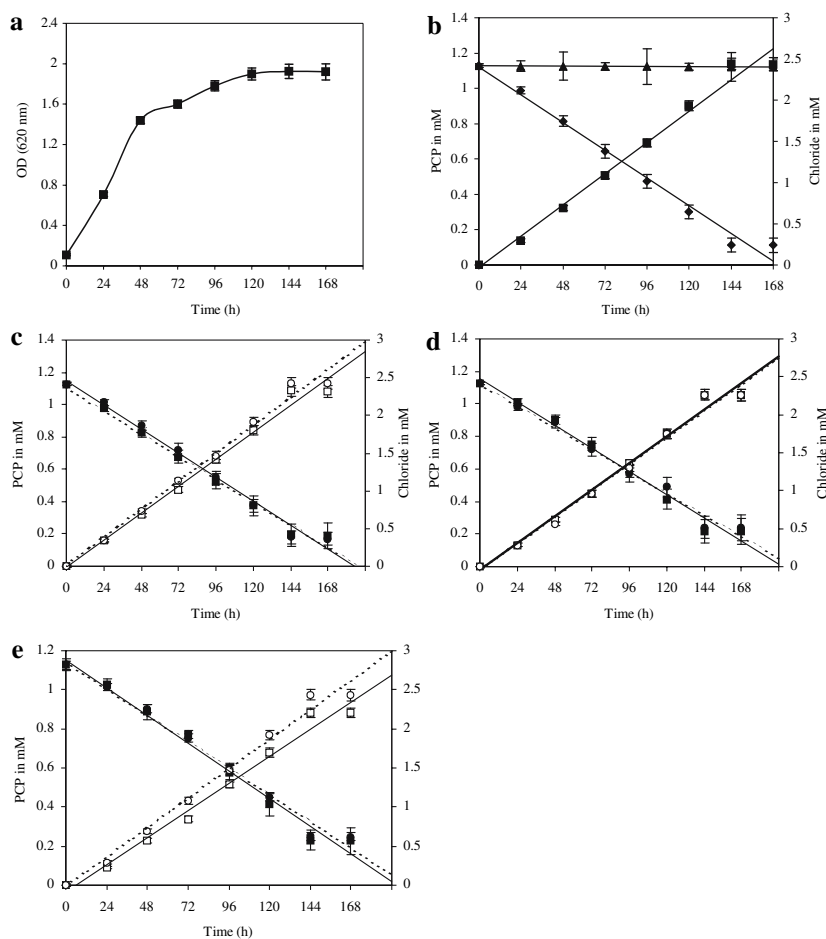


Fig. 2 (a) Growth curve (■—■) of *Serratia marcescens* (AY927692) in 1% glucose containing MSM, in the presence of 1.127 mM (300 mg/l) of PCP at optimized condition (30 ± 1 °C, pH 7.0 and 120 rev/min). All the experiments in this study were performed in triplicate and values are mean of three replicates. (b) PCP degradation and chloride ion release of *Serratia marcescens* (AY927692) in 1% glucose containing MSM, in the presence of 1.127 mM (300 mg/l) of PCP and its control (without inoculum) at optimized condition (Temperature 30 ± 1 °C, pH 7, 120 rev/min): (◆—◆) indicates PCP degradation ($R^2 = 0.986$), (■—■) indicates chloride ion release ($R^2 = 0.9863$) and (▲—▲) indicates control ($R^2 = 0.9837$). (c) Effect of temperature on PCP degradation and chloride ion release of *Serratia marcescens* (AY927692). PCP degradation: (■—■) indicates

20 °C ($R^2 = 0.9871$), (●—●) indicates 37 °C ($R^2 = 0.9873$) and chloride ion release: (□—□) indicates 20 °C ($R^2 = 0.9869$), (O—O) indicates 37 °C ($R^2 = 0.9871$). (d) Effect of pH on PCP degradation and chloride ion release of *Serratia marcescens* (AY927692). PCP degradation: (■—■) indicates pH 6.0 ($R^2 = 0.9895$), (●—●) indicates pH 9.0 ($R^2 = 0.9851$) and chloride ion release: (□—□) indicates pH 6.0 ($R^2 = 0.9862$), (O—O) indicates pH 9.0 ($R^2 = 0.9847$). (e) Effect of aeration on PCP degradation and chloride ion release of *Serratia marcescens* (AY927692). PCP degradation: (■—■) indicates 50 rev/min ($R^2 = 0.983$), (●—●) indicates 200 rev/min ($R^2 = 0.9862$) and chloride ion release: (□—□) indicates 50 rev/min ($R^2 = 0.9844$), (O—O) indicates 200 rev/min ($R^2 = 0.9863$)

Table 2 PCP degradation of *Serratia marcescens* (AY927692) in 1% glucose containing MSM, in the presence of 1.127 mM (300 mg/l) of PCP at optimized condition (Temperature 30 ± 1 °C, pH 7, 120 rev/min) and temperature, pH, aeration rate effect on PCP degradation

Time in hours	Degradation of PCP (%)						
	Optimized condition	Temperature (20 °C)	Temperature (37 °C)	pH 6	pH 9	Aeration rate 50 rev/min	Aeration rate 200 rev/min
0	0 ± 0	0	0	0	0	0	0
24	12.3 ± .02 ^{**}	13.36 ± .02 ^{***}	10.20 ± .02 ^{***}	10.36 ± .02 ^{***}	13.03 ± .02 ^{***}	8.86 ± .03 ^{***}	10.03 ± .02 ^{***}
48	27.66 ± .03 ^{***}	26.69 ± .03 ^{***}	22.69 ± .03 ^{***}	20.03 ± .03 ^{***}	21.53 ± .03 ^{***}	21.36 ± .04 ^{***}	20.03 ± .02 ^{***}
72	42.85 ± .04 ^{***}	40.02 ± .04 ^{***}	36.02 ± .04 ^{***}	33.36 ± .04 ^{***}	36.02 ± .04 ^{***}	31.69 ± .02 ^{***}	33.36 ± .02 ^{***}
96	58.01 ± .04 ^{***}	53.35 ± .05 ^{***}	51.18 ± .04 ^{***}	46.68 ± .05 ^{***}	49.68 ± .05 ^{***}	48.85 ± .05 ^{***}	46.68 ± .02 ^{***}
120	73.34 ± .04 ^{***}	66.67 ± .06 ^{***}	66.61 ± .04 ^{***}	63.34 ± .06 ^{***}	56.68 ± .06 ^{***}	63.34 ± .06 ^{***}	60.01 ± .02 ^{***}
144	90 ± .04 ^{***}	82.84 ± .07 ^{***}	84.17 ± .04 ^{***}	80.67 ± .07 ^{***}	78.67 ± .07 ^{***}	79.50 ± .05 ^{***}	77.87 ± .02 ^{***}
168	90.1 ± .04 [*]	83 ± .08 [*]	84.3 ± .04 ^{**}	80.839 ± .08 [*]	78.83 ± .08 [*]	79.76 ± .07 ^{**}	78 ± .02 ^{**}

All the values are mean ($n = 3$) ± SD and compared vertically. ^{***} highly significant, ANOVA $P < 0.001$; ^{**} significant, ANOVA $P < 0.01$; ^{*} less significant, ANOVA $P < 0.05$; ^{NS} non significant, ANOVA $P > 0.05$

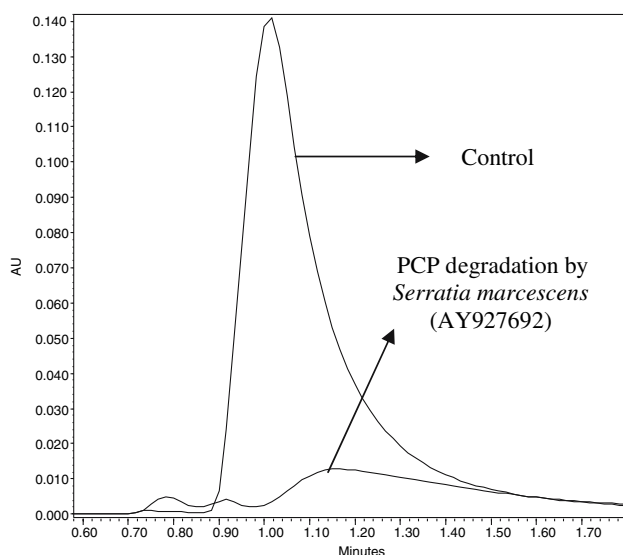


Fig. 3 HPLC chromatogram of PCP degradation by *Serratia marcescens* (AY927692) compared with control after 168 h incubation period at optimized condition (30 ± 1 °C, pH 7.0 and 120 rev/min)

degradation was a result of co-metabolism process, where glucose was utilized initially followed by PCP degradation. This finding was similar as reported earlier by Premalatha and Rajkumar (1994) for PCP degradation by *Pseudomonas aeruginosa*. The simultaneous release of chloride ion also corroborated the previous findings of dechlorination during PCP degradation as described by Mohn and Kennedy (1992). Variation in pH, temperature, aeration and increased PCP concentration did not favour the PCP degradation phenomenon. Alteration of optimized pH might be inhibitory to the activity of the enzymes responsible for PCP degradation in bacteria (Miller et al. 2004).

Our results suggest that the degradation of PCP by strain ITRC S₇ does not go to completion because of the slow degradation rate of the metabolic intermediate. Results obtained for degradation of PCP through HPLC analysis of the extractable products show formation of tetrachloro-*p*-hydroquinone and 6-chlorohydroquinol in the extracts. The metabolites released during degradation of PCP was extracted and analyzed by HPLC. The culture medium was extracted twice with ethyl acetate. The extracted solution was acidified to pH 2.0 and extracted twice with ethyl acetate (acidic extract). PCP (RT 1.014) was degraded at 168 h and two new peaks appeared which were detected as tetrachloro-*p*-hydroquinone (TeCH) (RT 0.787) and 6-chlorohydroquinol (RT 0.916). The result of the study indicated conversion of PCP into TeCH, which further converted to 6-chlorohydroquinol. The identification of intermediary metabolites is a significant step in complete mineralization of PCP. Extraction methods are critical steps and selection of time points for sampling is another step, which must be optimized for degradation of PCP. Two methods are adopted for extraction of PCP and its intermediary metabolites for the complete mineralization of PCP. The main reason for this was the lack of sufficient information on the inhibitory effect of metabolites on microbial growth (data not shown).

The bacterial enzyme PCP-4-monooxygenase from *Flavobacterium* sp. strain ATCC 39723 catalyzes the oxygenolytic removal of the first chlorine from PCP. PCP-4-monooxygenase is a FAD-binding, NADPH-requiring oxygenase with similar functional domains as other bacterial flavoprotein monooxygenase specific for phenolic substrate (Lange et al. 1996). PCP-4-monooxygenase converts PCP to 2, 3, 5, 6-tetrachloro-*p*-hydroquinone (TeCH) in the presence of oxygen and NADPH, which is further converted to trichlorohydroquinone by reductive dechlorination and

subsequently 2, 6-dichlorohydroquinone (DiCH) was formed by reductive dehalogenase enzyme (Xun and Orser 1991a; Xun et al. 1992a;). They showed that *pcp A* has novel ring-cleavage dioxygenase activity that cleaves aromatic rings with two hydroxyl group para to each other and that *pcp A* converts 2, 6-DCHQ to 2-chloromaleylacetate. This enzyme not only catalyzes dehalogenation but also removes hydrogen, nitro, amino and cyano groups from the benzene ring at the *para* position in relation to the hydroxyl of phenol (Xun et al. 1992b). Dechlorination of PCP by *Flavobacterium* sp. and PCP induces the presence of several proteins in the bacterium (Xun and Orser 1991b). Identification, purification, and characterization of PCP-4-monooxygenase that converts PCP to TeCH from *P. fluorescens* are also reported by Shah and Thakur (2003).

According to the reports (Xun and Orser 1991a; Cai and Xun 2002) of PCP degradation pathway, adding glucose facilitated the PCP metabolism by *Sphingomonas chlorophenolica*. The first possible reason was that some part of the metabolic pathway of PCP might overlap the metabolic pathway of glucose. PCP could be biotransformed into 2, 6-dichlorohydroquinone (2, 6-DCHQ). Subsequently, 2, 6-DCHQ entered the ring cleavage pathway to form maleylacetate and then maleylacetate entered the TCA cycle, which was an important metabolic pathway of glucose. Thus *S. chlorophenolica* might use some enzymes produced by metabolizing glucose to metabolize PCP. The second possible reason was that the glucose metabolism could provide more NADH to facilitate the first step. Therefore, *S. chlorophenolica* might use NADH produced by metabolizing glucose to metabolize PCP. The final conjecture could be that *S. chlorophenolica* used glucose, which were easily biodegraded to increase the biomass and thus increasing the total activity for metabolizing PCP. Further tests should be performed to determine the reason for facilitating PCP degradation using glucose.

Conclusions

S. marcescens strain ITRC S₇ is a novel and most potent aerobic bacterial isolate capable of degrading PCP in axenic condition. Therefore, this strain may be used for bioremediation of PCP containing industrial wastes.

Acknowledgements Authors are thankful to Director, ITRC, Lucknow, for his encouragement. The financial support from Council of Science and Technology, UP is also highly acknowledged.

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