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Screening of Pentachlorophenol (PCP) Degrading Bacterial strains Isolated from the Tannery Effluent Sludge of Kanpur, India

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Abstract

Pentachlorophenol is a toxic compound mainly use as preservative in leather and wood industries and also as disinfectant in various sectors such as agriculture, food, wood, oil and paints industries. In present investigation fifteen bacterial strains were isolated from sediment core of tannery effluent sludge (Kanpur, U.P., India). These strains were screened on mineral salt agar medium, containing sodium pentachlorophenate (Na-PCP) as a sole source of carbon and energy and bromothymol blue (0.1%) as screening agent. Among fifteen isolates, eight strains showing PCP degrading capability were characterized morphologically as well as biochemically. The strains showed similarities with *Pseudomonas* species, *Arthrobacter* species (three strains of each), *Proteus* species and *Bacillus* species (one strains of each). The PCP degrading potential of each individual species was performed in lab scale bioreactor. It was observed that the *Arthrobacter* species has highest PCP degrading potential as it degraded 55 percent within 30 days followed by *Pseudomonas* species and *Bacillus* species degrading 47 and 44 percent respectively. Whereas, *Proteus* species showed lowest degrading potential as it degrades only 38 percent within same time.

Key words: Pentachlorophenol, Tannery effluent, Screening, Bacterial biodegradation, Bioreactor.

Introduction

In Indian economy tannery industries occupy place of pride, due to its higher potential for employment, export and growth. The major portion, i.e. 70 percent to 80 percent of processing occurs at small cottage–scale sectors. Export of leather goods has reclaimed new height of $2.8 billion (Rs.14,000 corers) in 2007-08 comparing to 1965-66 which was $65.5 million (Rs.32 corers), (Natesh, 2009). To mitigate huge demand rapid growth of tanneries were taken place around the nation. There are of about 3000 major

tanneries in India, which are mainly located at Kanpur (U.P.), Punjab, Maharashtra, Kolkata (W.B.) and Chennai (Tamil Nadu) (Hammer *et al.,* 1998; Elisa *et al.,* 2000; Shukla *et al.,* 2001). In Kanpur 407 tanneries were located near by river Ganga.were discharging their improperly treated effluent in nearby water bodies causing collateral damages to aquatic ecosystem.The production of leather goes through a process known as tanning. In this process variety of chemicals are used at different stages. Due to biocidal property, PCP is used for curving and preservation of leather in tannery industries (Fisher, 1991; Premlata and Rajkumar 1994; Shukla *et al.,* 2001). Pentachlorophenol is highly recalcitrant xenobiotic chlorinated hydrocarbon. It has ubiquitous occurrence, from ambient air of mountains to rural areas (0.25-0.93 mg/m^3) from urban areas (5.7-7.8 mg/m^3) to groundwater (3-23 mg/l) and surface water (0.07-31.9 mg/l). The maximum level of pentachlorophenol contamination has been set at 0.001 mg/l for drinking water (McAllister *et al.,* 1996; Yang *et al.,* 2006).

Pentachlorophenol has both acute and chronic effect on human beings as well as on aquatic environment. The major sites of action are liver, kidneys, plasma protein, brain and spleen. In acute toxicity, pentachlorophenol causes elevated temperature, profuse sweating, dehydration, loss of appetite, decreased body weight, nausea and neurological effect such as tremor, leg pain, muscle twitching and coma. In chronic response, pentachlorophenol inhaled by workers at the working place causes abdominal pain, fever, respiratory irritation as well as eye, skin and throat irritation. In high concentration PCP causes obstruction of circulatory system in lungs, heart failure and damage to central nervous system (U.S. Department of Health and Human Services, 2001).

It is rapidly absorbed through the gastrointestinal tract following ingestion, with a biological half-life of only 10 hours and its bioaccumulation may result significant. Several species of fish, invertebrates and algae have high levels of pentachlorophenol that were significantly higher (up to 10,000 times) than the concentration in the surrounding waters. Accumulation is not common, but if it does cause teratogenic, mutagenic, carcinogenic (Jain *et al.,* 2005).

The excessive use of this chemical has resulted in environmental nuisance and immensely demands its remediation (Tewari *et al*., 2010). Several physico-chemical methods are available for the degradation of PCP but the most feasible way is the bioremediation technique. A number of aerobic and anaerobic cultures of fungi and bacteria have been applied for the degradation of pentachlorophenol by different workers at nation as well as international level (Stanlake and Finn, 1982; Brown *et al.*, 1986; Hammer *et al.*, 1998; Schie and Young, 1998; Nagyun *et al.*, 2002; El-Syed *et al.,* 2003; Chandra *et al.*, 2006; Rahman and Anuar, 2009; Tripathi and Garg, 2010). The workers have got effective results during their study but complete mineralization of this xenobiotic compound is still unstated.

The present study emphasizes on screening the PCP degrading bacterial strains, characterization and identification of these strains and assessment of PCP degrading potential of each individual strain. This investigation may further be helpful in designing

the stable bacterial consortium which can effectively and efficiently degrade PCP.

Materials and Methods

Sampling

The present investigation was conducted on tannery effluent released from tannery industries of Jazmau, Kanpur (U.P.) India. Here 402 tanneries are situated on both sides of the road. The effluent of these industries is discharged into river Ganga through a main channel. Samples were collected randomly from the main channel and sediment along with the effluent in the ratio of 1:10 (w/v) was collected. The samples were then brought to laboratory for further analysis.

Isolation and screening of PCP degrading bacterial strains

For the isolation of bacterial strains, the sample after filtration with the help of muslin cloth was serially diluted from 10^{-1} to 10^{-10} and each dilution was spread over nutrient agar plates and incubated overnight at 29^0C.

The screening of PCP degrading bacterial strains was performed by culturing the isolates on mineral salt agar medium. The medium consist of (L^{-1}): $Na_2HPO_4.2H_2O$, 7.8 g; KH_2PO_4, 6.8 g; MgSO4, 0.02 g; Fe $(CH_3COO)_3$ NH_4, 0.01 g; Ca $(NO_3)_2$, $4H_2O$, 0.05 g; $NaNO_3$, 0.085 g; Agar 14 g; PCP 0.5 g; Bromothymol blue 0.1% and pH was maintained at 7 and 1 ml trace element solution was added to the medium (Shukla *et al.,* 2001; Sharma and Thakur, 2008). All the isolates were cultured on mineral salt agar medium separately and kept for five days incubation at 29^0C. The results were observed on the basis of change in color of medium. The screened colonies were re-cultured on mineral salt agar medium alternatively for standardizing the process.

Estimation of PCP

Pentachlorophenol was extracted by acidifying 10ml effluent sample with 5N HCL. Then PCP from sample was extracted three times by Dichloromethane (10 ml). The organic phase was reextracted with 0.5N NaOH. Now, aqueous phase was taken and optical density of pentachlorophenol was analyzed by spectrophotometer at 320nm (Edgehill and Finn 1983).

Morphological and Biochemical characterization

The screened bacterial colonies were cultured on nutrient agar plates for morphological characterization depending upon their shape, size, color, opacity, texture, elevation, spreading nature and margin (Seeley and Van Demark, 1972). The biochemical characterization of the strains was performed by the methods described by Aneja, (2001).

Application of Bacterial strains in bioreactor

The PCP utilizing capacity of the bacterial strains was assessed by applying them in 5 liter lab-scale bioreactor. The stabilized and enriched bacterial consortia were applied for PCP removal in a lab scale bioreactor, fabricated by using a glass column of 5 L with effective volume of 2 L**.** The first chamber contained tannery effluent (Chamber I), from outlet at its base the effluent was supplied to the second reactor chamber (Chamber II). The uppermost part of the reactor chamber was provided with three opening for stirring, aeration and inlet for effluent, the stirring was made possible by fixing a motor, oxygen was provided by passing sterile air through aerator. For application of consortium in bioreactor, 20 ml bacterial culture was added in reactor chamber which contains 2 L effluent and the retention time was set for 12 hrs. A layer of gravel (150 g) and sand (100 g) was placed in the lower portion of the reactor chamber to avoid immobilization of bacterial culture. The lowermost part of the reactor chamber was connected to Chamber III for collection of treated effluent. The samples were collected at different time intervals for PCP estimation.

Results and Discussion

The bacterial communities were isolated from depth of 1mm sediment core from effluent sludge of tannery industries Kanpur. Fifteen bacterial colonies were isolated from tannery effluent sludge. As, the effluent of tannery industries contain chlorinated phenols, so there is high probability of getting pentachlorophenol degrading bacterial strains. Several workers have also isolated bacterial strains for the degradation of pentachlorophenol from different sources (Shukla *et al.,* 2001; Visvanathan *et al.,* 2005; Khadijah, 2009). Isolated strains were cultured on mineral salt agar medium containing sodium pentachlorophenol (0.5 g/l) as sole source of carbon and energy and bromothymol blue (0.1%) as indicator and kept for 5 days at 29^0 C in bacteriological incubator. No supplementary co-substrate was provided in the medium. After five days of incubation, out of fifteen isolated strains eight strains showed growth on the medium that was differentiated on the basis of color change. The color of bromothymol blue in basic medium changes to yellow in acidic medium. Due to the degradation of PCP, chlorine was released resulting change in the pH of the medium. This acidic property of the medium resulted in yellow coloration. Thus, the bacterial strains showing color change of the medium indicated there ability for PCP degradation without any co-substrate. Reports are available which show that PCP is utilized as sole source of carbon and energy by different bacterial strains (Shukla *et al.,* 2001).

The PCP degrading bacterial strains were identified on the basis of morphological and biochemical characteristics. The results of morphological and biochemical characteristics of the bacterial strains is showed in table 1 and 2 respectively. The strains were morphologically characterized depending upon their shape, size, color, opacity, texture, elevation, spreading nature and margin and for biochemical testing various tests viz., Gram staining, starch hydrolysis, casein, lipid hydrolysis, citrate utilization and urease test. The strains were identified as one species each of *Proteus* and *Bacillus* and three species each of *Pseudomonas* and *Arthrobacter* species.

Pentachlorophenol degrading capabilities of these strains is also reported by different workers. In 1982, Stanlake and Finn, isolated and characterized Arthrobacter species from soil that showed degradation of PCP. Utilization of PCP in form of sole source of carbon by Pseudomonas species and Arthrobacter species was reported by Shukla *et al.*, in 2001 and Shah and Thakur, 2002. Sharma and Thakur, (2008) characterized the Pseudomonas species from paper mill and studied the potency of the isolated strains for PCP reduction in sequential bioreactor. Kotresha and Vidyasagar, 2008 also reported the PCP reduction by Pseudomonas species. PCP degrading Proteus species and Bacillus species was isolated from rhizosphere and characterized by Azaj *et al.*, in 2004. Tripathi and Garg, 2010 also isolated and characterized Bacillus species form the tannery effluent.

Table 1 Morphological characterization of PCP degrading bacterial strains isolated from tannery sludge

Characteristics	Bacterial strains							
	PSKI	PSKII	PSKIII	PSKIV	PSKV	PSKVI	PSKVII	PSKVIII
Shape	I	C	C	C	C	I	I	C
Size (mm)	0.05	0.25	0.05	0.1	0.1	0.05	0.25	0.05
Color	Y	CR	Y	CR	OR	Y	OR	W
Opacity	T	T	O	T	O	T	O	O
Texture	V	NV	NV	V	NV	V	NV	V
Spreading nature	Y	N	N	Y	Y	N	Y	N
Elevation	F	F	F	CO	CO	F	F	F
Margin	SR	SR	S	SR	SR	S	S	SR

C- Circular, I-Irregular, W-Whitish, CR- Creamish, OR-Orange, P-Pinkish, Y-Yellowish, GW-Grayish white, O-Opaque, T-Transparent, V-Viscous, NV- Not viscous, Y- Yes, N-No, F-Flat, CO-Convex, S-Smooth, SR –Serrated.

Table 2 Biochemical characterizations of PCP degrading bacterial strains isolated from tannery sludge

Characters	Bacterial strains and their responses							
	PSKI	PSKII	PSKIII	PSKIV	PSKV	PSKVI	PSKVII	PSKVIII
Gram staining	-	+	+	-	-	-	+	+
Starch Test	-	+	-	+	+	+	+	+
Casein Test	+	-	+	-	-	+	-	+
Lipid hydrolysis Test	-	+	+	-	+	+	+	+

Citrate utilization Test	-	-	-	+	+	+	+	+
Urease Test	+	-	-	-	-	+	+	-

+ Positive result; – Negative result

The figure 1 shows percentage reduction of pentachlorophenol was observed at 320 nm by applying the screened bacterial strains separately in lab scale bioreactor for 30 days, it was observed that, *Arthrobacter* species and *Pseudomonas* species showed 55 percent and 47 percent reduction in PCP concentration within 30 days whereas *Bacillus* species and *Proteus* species showed 44 percent and 38 percent reduction in PCP respectively.

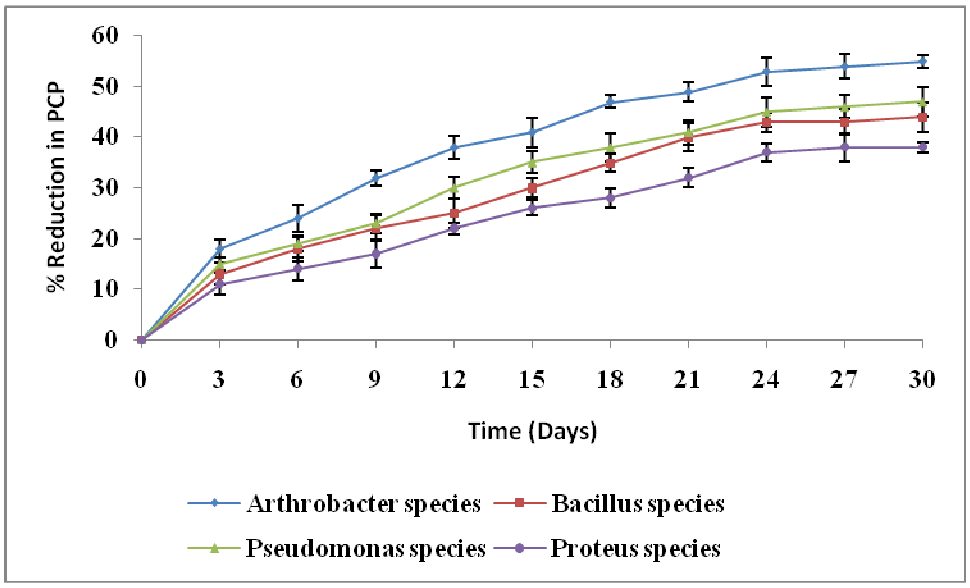

Figure 1 Reduction of PCP in bioreactor

Several workers have also reported reduction of PCP by these strains in different time intervals Brown *et al.*, in 1986 studied consortium of *Flavobacterium* and *Arthrobacter* in a fixed film bioreactor and reported that 60-80% of PCP reduction in 120 days, by same the consortium this was also reported by Stanlake and Finn, 1982. The consortium of *Pseudomonas* and *Arthrobacter* in a sequential bio reactor showed 80.8% of PCP reduction reported by Shukla *et al.*, in 2001. In another sequential bioreactor study, 65% of PCP reduction was reported in 300 hrs by Shah and Thakur, in 2002.

For further investigation, this standardized process is being use and the screened bacterial strains were enriched in lab scale chemostat for enhancement of degrading potentiality and effective bacterial consortium will be designed.

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