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Identification and characterization of integron mediated antibiotic resistance in pentachlorophenol degrading bacterium isolated from the chemostat

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Abstract

A bacterial consortium was developed by continuous enrichment of microbial 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 consortia contained three bacterial strains. They were identified as *Escherichia coli*, *Pseudomonas aeruginosa* and *Acinetobacter* sp. by 16S rRNA gene sequence analysis. *Acinetobacter* sp. readily degraded PCP through the formation of tetrachloro-*p*-hydroquinone (TecH), 2-chloro-1,4-benzenediol and products of *ortho* ring cleavage detected by gas chromatograph/mass spectrometer (GC-MS). Out of the three acclimated PCP degrading bacterial strains only one strain, *Acinetobacter* sp. showed the presence of integron gene cassette as a marker of its stability and antibiotic resistance. The strain possessed a 4.17 kb amplicon with 22 ORF's. The plasmid isolated from the *Acinetobacter* sp. was subjected to shotgun cloning through restriction digestion by *Bam*HI, *Hin*dIII and *Sal*I, ligated to pUC19 vector and transformed into *E. coli* XLBlue1α, and finally selected on MSM containing PCP as sole source of carbon and energy with ampicillin as antibiotic marker. DNA sequence analysis of recombinant clones indicated homology with integron gene cassette and multiple antibiotic resistance genes.

Key words: *Acinetobacter* sp.; antibiotic resistance gene; chemostat; integron gene cassette; pentachlorophenol; recombinant clone
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Introduction

Over recent decades, significant quantities of industrial, agricultural and domestic chemicals have been released into the environment. Halogenated aromatic compounds constitute one of the largest groups of chemicals used in industrial application and preservation of biological materials (Yang *et al*., 2007). Chlorinated phenols and their derivatives are inert, hydrophobic, stay longer in the environment and cause toxicity to flora and fauna (Chu *et al*., 2008). The toxicity of these compounds tends to increase with their degree of chlorination (Fetzner and Lingens, 1994; Reineke and Knackmuss, 1988). Pentachlorophenol (PCP) is general biocide used primarily as preservative of wood, leather, textile and related commercial products (Chanama and Crawford, 1997). Preservative materials containing PCP may be washed into streams and lakes by surface runoff or may infiltrate to contaminate ground water. In case of environmental contamination by PCP, traditional clean-up methods have not been proved successful. The major difficulties encountered in biological treatment methods are paucity of knowledge concerning PCP-degrading bacterial population and adverse environmental conditions (Thakur *et al*., 2001; Kaoa *et al*., 2004). Bacteria with the ability to degrade PCP are widespread in soil and sediment, however, most studies of PCP biodegradation have been carried out under laboratory conditions with arbitrarily selected PCP-degrading bacteria (Xun and Orser, 1991; Orser *et al*., 1993; McAllister *et al*., 1996; Thakur *et al*., 2002; Kaoa *et al*., 2004; Suegara *et al*., 2005).

Microbes are often found in consortia bound to surfaces, such as in biofilms or granules. Under these conditions bacteria are positioned in a heterogeneous environment with gradients of nutrients and waste products as a consequence of diffusion and mass transport processes, and it is therefore to be expected that this heterogeneity be reflected in the physiology of individual cells. The development of structurally organized communities may argue for the presence of overall regulatory elements, which control the formation of the community structures (Senior *et al*., 1976). An important factor in understanding community level processes is the relationship between structure and function in microbial communities, and its genetic stability which may be governed by antibiotic resistance (Hochhut *et al*., 2001; Holmes *et al*., 2003).

The genomics era has clearly indicated that a large proportion of bacterial genes have been acquired by horizontal gene transfer. Horizontal gene transfer is facilitated

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by a number of genetic elements in bacteria, including plasmids, transposons, and integron gene cassette (Hansson *et al*., 2002; Machado *et al*., 2005). The dispersal of genes underlying complex cellular processes, including photosynthesis, nitrogen fixation, carbon fixation, sulfate reduction, bioremediation and pathogenicity has been implicated (Nemergut *et al*., 2004; Machando *et al*., 2005) The units of DNA captured by integron gene cassettes are the simplest known mobile elements and consist of a site-specific recombinase (*Int*I) that belongs to a distinct tyrosine-recombinase super family, responsible for the insertion of gene cassettes at *att*I, and also provides the promoter responsible for the expression of cassette-encoded genes (Jones *et al*., 2005; Machado *et al*., 2005). Integron-like structures have been amplified from soil DNA samples and have been found in database searches of available genome sequences, including the sequences of *Nitrosomonas europaea*, *Geobacter sulfurreducens*, *Vibrio cholerae*, *Shewanella oneidensis*, *Acidithiobacillus ferrooxidans*, and *Treponema denticola* (Collis *et al*., 1993; Riley *et al*., 2001; Leon and Roy, 2002). It has been shown that the net pool of gene cassettes present in bacterial communities contains unprecedented levels of genetic novelty. Therefore, the possibility that all integron may share the flexible gene cassette acquisition and their expression properties has a wide range of implications (Nemergut *et al*., 2004; Jones *et al*., 2005). In this study we focused on the development of stable bacterial consortium by continuous enrichment in the chemostat, and tried to evaluate the integron-gene cassette system and antibiotic resistance gene that may influence the movement of gene which would helped in stability of the consortium that may be applied for *in situ* bioremediation of sites contaminated with chlorinated phenols.

1 Materials and methods

1.1 Bacterial strains and growth media

Bacteria were routinely cultured in Luria-Bertani (LB) broth or LB agar supplemented with ampicillin (100 μg/mL), chloramphenicol (25 μg/mL) or kanamycin (5 μg/mL). *Escherichia coli* XLBlue1α and DH5α cells were used as host for construction and maintenance of all plasmid clones and recombinant clones.

1.2 Sediments sample and culture condition

Sediment samples together with liquid effluent (1:10, *W/V*) were collected from three sites of the main channel of M/s. Century pulp and paper mill located at Lalkua, Nainital, Uttarakhand State, India. The bacterial cells were extracted and plated on MSM-PCP agar containing bromothymol blue (0.1%) and PCP (100 mg/L) as sole source of carbon. The mineral salts medium contained the following components at the specified concentrations (in mg/L): KH_2PO_4, 800; Na_2HPO_4, 800; $MgSO_4{\cdot}7H_2O$, 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. Samples used for isolation attempts were serially diluted in sterile media and spread plated onto 1.5% agar plates containing the same growth medium. The cultures were incubated for up to seven days at 30°C. Isolated colonies that were representative of morpho-types observed on the plates were picked off and inoculated onto fresh plates to obtain pure cultures. The formation of yellow colored colonies on MSM-PCP agar containing bromothymol blue indicated the utilization of PCP by the bacteria.

1.3 Bacterial community

The mixed microbial population indicating utilization of PCP was used as inoculum in chemostat, and continuous enrichment was performed using MSM and PCP (0.1 g/L). The chemostat culture was run in 2-L glass vessel (effective volume 1 L) by stirring, 250 r/min; temperature, 30°C; pH 7.2–7.4; an air flow of 500 mL/min and medium flow rate of 10 mL/h. 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. Samples were diluted and plated on nutrient agar plate (0.1 mL/plate). The bacterial colonies appeared on nutrient agar plates were morphologically characterized and purified by repeated culturing. The bacterial strains were again enriched in Erlenmeyer flasks containing MSM supplemented with PCP (0.1 g/L) as sole source of carbon and energy and incubated at 30°C on an orbital shaker at 150 rev/min. Aliquots were transferred weekly from culture to fresh medium. Subcultures were streaked on MSM-PCP agar plates containing the above carbon source and Bromothymol Blue. The strains showing the highest utilization of PCP on agar plates were selected for further experiments.

1.4 Structural analysis of bacterial community

The morphologically distinct isolates were identified by physiological and chemotaxonomic properties in accordance with Bergy's Manual of Systematic Bacteriology (Palleroni, 1984). In 16S rRNA gene sequencing, genomic DNA from individual bacterial strains was isolated with the Genome DNA Kit (Qiagen Inc., USA) as per manufacturer's instruction. The 16S rRNA gene was selectively amplified from genomic DNA by using Gene Amp 2400 PCR System (PE, USA) with the following set of primers: upstream primer 27F (5'-GAGTTTGATC(A/C)TGGCTCAG-3'), and downstream primer 1492R (5'-TACGG(CT)TACCTTGTTACGACTT-3') (Weisburg *et al*., 1991). The amplification product was gel purified using QIA gel extraction kit (Qiagen, Germany) and was sequenced. The 16S rRNA gene sequences were compared against known sequences available in GenBank using National Centre for Biotechnology Information (NCBI) BLAST program.

1.5 Utilization of chlorinated compounds by bacterial strains

Utilization of various chlorinated compounds by each bacterial strain was assessed on plates of agar solidified

mineral salts with the test compound spread over one side of the plate after streaking as described earlier (Thakur *et al*., 2001). Growth of bacterial strains at 30°C was monitored daily by visual inspection for 3 d. The bacterial cells were then washed from the plate with saline and the A_{600} (600 nm) values of the suspension were determined. All the tests were repeated three times. The bacterial strains were inoculated in Erlenmeyer flasks containing MSM supplemented with test compounds (0.1 g/L) as sole source of carbon and energy, and incubated at 30°C on an orbital shaker at 150 r/min. The samples were taken after 0, 6, and 12 h, and the growth of bacterial strains and the utilization of carbon source were determined.

1.6 Functional analysis of bacterial community

The biodegradation of PCP was determined by extraction of metabolites from the culture medium. In extraction of metabolites, the cell suspension was clarified by centrifugation at 8000 r/min 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 glass 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, 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. The injected volume was 0.5 μL.

1.7 Isolation and detection of plasmid DNA

Total DNA was isolated as per standard laboratory protocol. A overnight grown culture of LB (500 mL) was centrifuged, and pellet was suspended in 10 mL of Glucose-Tris-EDTA solution containing glucose (50 mmol/L), Tris (25 mmol/L, pH 8.0) and EDTA (10 mmol/L). One milliliter of lysozyme (10 mg/mL in 10 mmol/L Tris, pH 8.0) was added. After 30 min, 20 mL lysis solution containing NaOH (0.2 mol/L) and SDS (1%) was added, and after 5 min ice cold 15 mL solution of potassium acetate (5 mol/L, pH 4.8) was added. The DNA was extracted by precipitation with 0.6 volume of iso-propanol. Pellet was washed by ethanol (70%) and dissolved in TE (25 mmol/L Tris and 10 mmol/L EDTA, pH 8.0). The DNA was purified by EtBr-CsCl density gradient method. Both genomic (upper layer) and plasmid (lower layer). DNA band were removed carefully and decontaminated by isopropyl alcohol.

1.8 Ligation and transformation

Isolated plasmid DNA and pUC19 vector were digested with *Bam*HI, *Hin*dIII and *Sal*I according to manufacturer's instruction. Plasmid DNA fragments were cloned into vector pUC19 by ligation (2 μg of plasmid DNA and 0.5 μg of pUC19 DNA), followed by phenol extraction, ethanol precipitation and ligation in buffer supplemented with 2U of T4 DNA ligase. The ligation mixture was used to transform competent cells of *E. coli* XLBlue1α cells. The hybrid plasmid in transformed clone was initially selected on LB-agar plate containing isopropyl-beta-thio galactopyranoside (IPTG) and X-gal. The white colonies were transferred on LB-agar plates and patched on MSM agar plate containing PCP (50 mg/L) and Bromothymol Blue as indicator. The recombinant clones having better potentiality to utilize PCP were tested for the degradation of PCP on mineral salt medium shake flask culture. The growth of the cells in terms of colony forming units and percentage utilization of PCP were determined as described earlier. In addition, clones were also selected on antibiotics for integron gene cassette analysis. DNA from recombinant clones having a higher utilization of PCP and resistant to antibiotics was extracted from Qiagen GmbH plasmid mini kit, and digested by *Sal*I restriction enzyme. The DNA was purified by agarose gel (0.8%) and probe was prepared using 32PdCTP by random primer method. The sequence of the bacterial recombinant clone was obtained by PCR-mediated chromosome walking method (Microsynth, Switzerland).

2 Result and discussion

2.1 Isolation and characterization of PCP degrading bacterial strains from the chemostat

The bacterial population from the sediment core of pulp and paper mill were extracted and enriched in the chemostat in presence minimal salt medium and PCP by continuous enrichment process. A microbial consortium capable of using PCP was isolated after four months of selective enrichment by repeated subcultures. The consortium was able to grow on and degrade PCP when the compound was supplied as sole source of carbon and energy. Figure 1 shows the growth pattern of the microbial population enriched in the presence of PCP. Initially, there was an increase in turbidity, but after day 20, it was declined which persisted up to day 80. The rate of nutrient supply in the culture vessel was decreased from 10 to 5 mL/h, and after that the growth was increased from day 120. The fluctuation in turbidity was observed till day 220, and then growth was constant till the end of the run (280 d).

The growth of the bacterial cells in the chemostat determined by colony forming units (CFU) on agar plates was also concomitant to the turbidity (Shah and Thakur, 2003). The initial fluctuations in the turbidity of the medium may be due to the inability of the bacterial strains to utilize PCP, formation of intermediary metabolites released during the degradation of PCP and also because cells were not well acclimatized to the new environment containing PCP. Results indicated a significant increase in the utilization of carbon source from day 140 to day 280 (Fig. 1). It was observed that after day 20 the utilization of carbon source was decreased, but once the growth of bacterial cells was increased the utilization of carbon source was

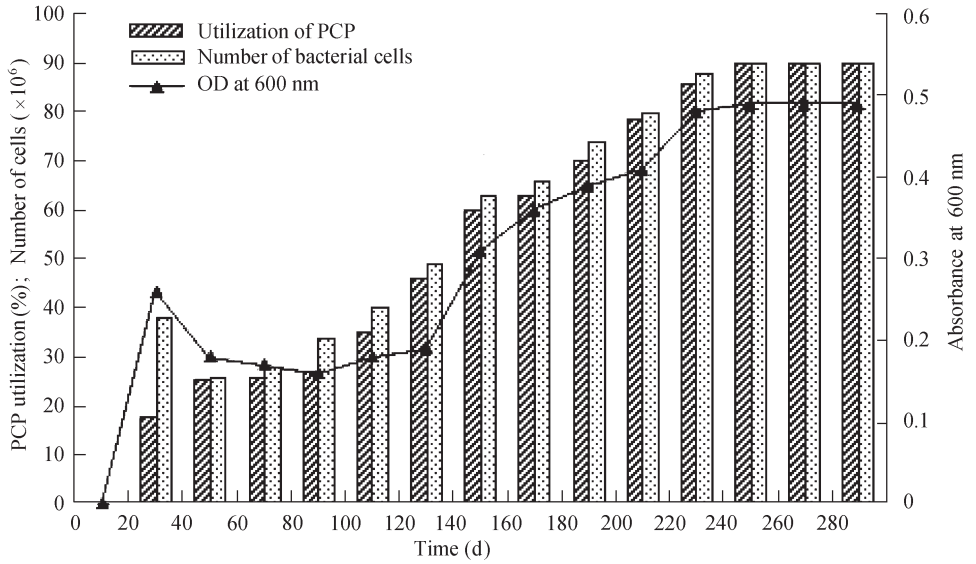

Fig. 1 Growth pattern of bacterial community in the chemostat in presence of mineral salts medium containing PCP (0.1g/L) as sole source of carbon and energy.

also increased. The result of the study indicated that more than 50% carbon source was utilized from day 140 which reached to 90% from day 220 till the end of the run. The significant finding emerged in this study was more than 90% utilization of PCP by the enriched bacterial cells of the pulp and paper mill effluent. The reason behind the stabilization of bacterial cell growth in the chemostat from day 220 is not known. This may be due to the physiological adaptation of the bacterial community to PCP, and probably due to the nutritional interaction among the members of community in chemostat (Senior *et al*., 1976). Results indicated a significant increase in utilization of carbon source from 45% on day 140 to 79.1% at the end of the run, i.e., day 240. One important factor for optimal performance of a chemostat is the homogeneous distribution of the suspended cells, and it is considered important to prevent surface attachment of the cells to the reactor walls. Under such optimal conditions, in which only one nutrient is limiting for growth, it may be expected that an organism will totally outcompete all others if it has an improved efficiency of substrate utilization relative to the others. The concentration of the particular nutrient will always be below the threshold level for the less efficient cells, and the rate of washout is therefore higher than the rate of cell proliferation for these cells.

Samples of the enrichment culture were plated onto nutrient agar plates and incubated at 30°C. Three distinct round and convex colony types were observed on LB-agar plates whereas colonies obtained in MSM agar plates under the same conditions were very small and undistinguishable. Subsequent plating of the repeated subcultures of the degrading consortium in liquid medium revealed that only three of the observed colonial types were consistently recovered.

Three different bacterial isolates were obtained on nutrient agar plates depending upon the morphological differentiation from the stable microbial consortium. The size and shapes of the colonies of individual bacterial strains were in agreement with standard microbiological literature available. All members of the bacterial consortium were Gram-negative, rod-shaped, motile, non-sporulating, catalase positive and oxidase positive. The bacterial strains were identified biochemically and by method of 16S rRNA sequence analysis as *E. coli* (PCP1), *Pseudomonas aeruginosa* (PCP2) and *Acinetobacter* sp. (PCP3) (Table 1). The BLAST search of PCP1, PCP2 and PCP3 showed 99.9%, 99.6%, and 99.8% sequence homology with *E. coli*, *P. aeruginosa*, and *Acinetobacter baumani*, respectively.

Table 1 Identification of the members of the bacterial consortium obtained from the chemostat

Strain	16S rRNA gene sequence analysis	GenBank accession No.
PCP1	*Escherichia coli*	EF432789
PCP2	*Pseudomonas aeruginosa*	EF432790
PCP3	*Acinetobacter* sp.	EF432791

2.2 Growth of three member degrading consortium in batch culture

The utilization of PCP and related metabolites by members of the consortium, PCP1, PCP2, and PCP3 detected on MSM agar plates is summarized in Table 2. The result indicated that PCP3 (*Acinetobacter* sp.) had a higher potency to utilize PCP and its intermediary metabolites. The utilization of PCP was determined by spectrophotometeric analysis and GC-MS. It was observed that PCP3 had a higher potency to utilize PCP which degraded more than 50% PCP within 3 h, followed by PCP2 which utilized 45% PCP, and PCP1 which utilized only 30% of PCP at the same time interval. The most significant result observed in this study was utilization of more than 80% PCP by PCP3 within 5 h. The results indicated the disappearance of PCP only after 6 h by PCP3. The emergence of two new peaks, tetrachloro-*p*-hydroquinone (TecH) and 2-chloro-1,4-benzenediol at 48 h by PCP3, indicate their formation from PCP (Fig. 2). In other two strains, the disappearance

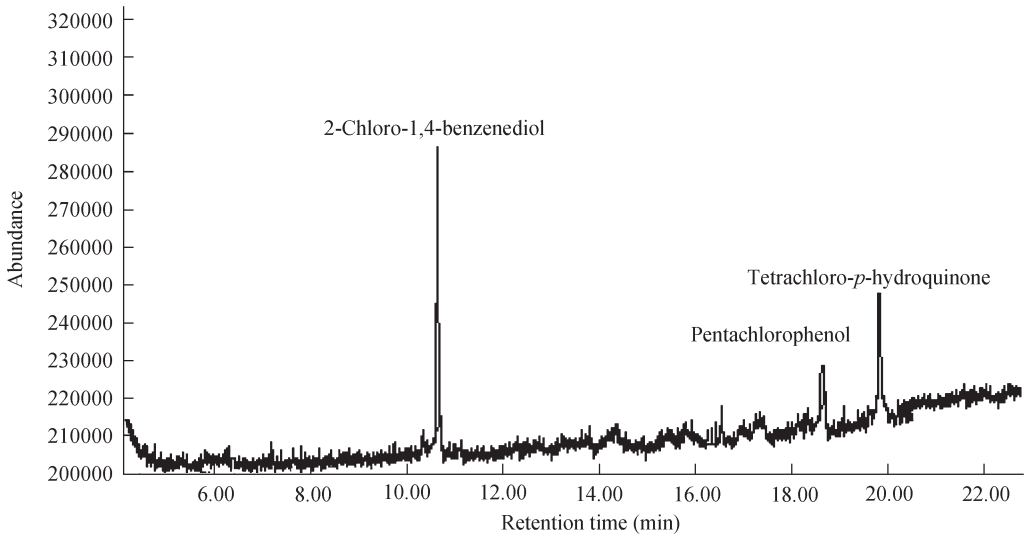

Fig. 2 Gas chromatography-mass spectrometric profile of PCP degradation by *Acinetobacter* sp. at 48 h of incubation.

Table 2 Growth of bacterial strains on various carbon sources (20 mg/L) on MSM agar plate

Carbon source	Bacterial strain		
	PCP1	PCP2	PCP3
Pentachlorophenol	++	++	+++
2,4,6-Trichlorophenol	+	++	+
2,4-Dichlorophenol	+	++	++
p-Chlorophenol	+	+	++
Tetrachlorohydroquinone	–	++	+++
Chlorohydroquinone	–	+	+++
Catechol	+++	+++	+++
4-Chlorosalicylic acid	–	+	++
4-Chlorobenzoic acid	–	–	++
Dibenzofuran	–	–	–

Relative growth of colonies in term of utilization of substrates is expressed as: +++, good growth; ++, normal growth; +, minimum growth; –: no growth

of PCP was observed at 72 h which was accompanied by the appearance of the two new peaks identified as 3,5-di-methoxyphenol and 2,3,4,6-tetrachlorophenol by PCP1 and PCP2 respectively detected by GC-MS (data not shown). The study has shown transformation of PCP in the chemostat during enrichment in a continuous culture by microbial population of pulp and paper mill effluent. The microbial community selected by enrichment is characterized based on the structure and physiology of the individual strains. The relationship between the structural and functional properties of bacterial strains in PCP transformation provided in this study may be useful in predicting environmental persistence as a function of site-specific conditions, as well as provide insight about potential enrichment of *in situ* microbial population.

2.3 Molecular characterization of integron gene cassette

Detection of integrons in either the environment or bacterial isolates is complicated by the considerable diversity in the core integron sequence among classes, the associated gene cassette arrays, and the genomic context of the integron-gene cassette system (Ramirez *et al.*, 2005). Generally, integrases belonging to different classes show only 50%–60% sequence identity. However, in PCR experiments aimed at generic detection of integrons, new integron classes and new gene cassettes have been recovered from environmental DNA. The nature and possible role of plasmid and genomic DNA in degradation of PCP has been assayed. As can be seen in Fig. 3A, high molecular size plasmids were observed on gel surface of PCP3. The restriction digest with *Eco*RI and *Hin*dIII indicated at least seven restriction fragments in PCP3 with molecular size found to be 85 kb (data not shown). Presence of integron gene cassette was investigated by shotgun cloning, and characterization and sequencing of genes. Shotgun cloning was performed by partial digestion of total plasmid DNA of *Acinetobacter* sp., by three restriction enzymes (*Bam*HI, *Hin*dIII and *Sal*I). Approximately 1700 clones (white colonies) were isolated from LB-agar plate containing ampicillin and IPTG and X-gal. The colonies selected from plates were streaked on mineral salt agar plates containing Bromothymol Blue indicator changed into yellow color. Recombinant clones were removed, and transferred into shake flask culture

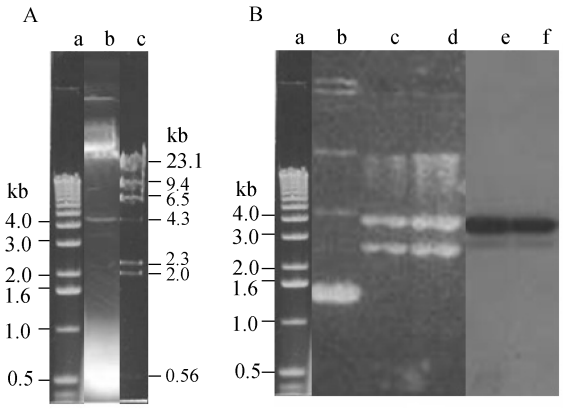

Fig. 3 Agarose gel electrophoresis and Southern blot analysis of recombinant clone for the characterization of integron gene cassette. (A) a and c are DNA molecular weight markers, b is plasmid DNA; (B) a is molecular weight markers; b is DNA of recombinant clone; c and d are DNA of recombinant clone digested by *Sal*I; e and f are Southern blot analysis of DNA of recombinant clone digested by *Sal*I.

containing PCP as the sole carbon source and ampicilline as antibiotic. The significant finding emerged from the study was the utilization of PCP by recombinant clones of DNA fragments obtained by *Sal*I. Preliminary results indicate that size of the gene was approximately 4.17 kb. Restriction fragments pattern generated by *Sal*I digested DNA were analyzed by Southern blot analysis (Fig. 3B) (Thakur *et al*., 2001). Intensity of hybridization with restriction fragments of plasmid and genomic DNA and DNA digested by *Sal*I indicated the cross-reaction of DNA probe with the recombinant clones of 4.2 kb size genes present in plasmid DNA (Fig. 3B). Such cross-reaction was not observed with genomic DNA.

Cloning and sequencing of insert present in recombinant clones of *Acinetobacter* sp. implied that integron-containing bacteria were present in enrichment cultures in the chemostat. BLASTN searches of sequence databases revealed 22 ORFs in the 4.17 kb recombinant clone with representative of more than 59-base sequences of integron and antibiotic resistance gene (data not shown). All 22 ORFs characterized by BLASTN searches of sequence databases indicated three major integrons and antibiotic resistance gene sites with one of the sites containing promoter sequences. There were ten ORFs cassette-associated genes that gave database matches, but only three of the matches were to families containing antibiotic resistance gene. One of the initial cassette ORFs showed a relationship to previously described gene cassette of pKlebB, (*tetR*), *Tet*A, (*tetA*), Tn1331 transposase (*tnpA*) gene. An another significant ORF cassette showed homology with PKlebB, TraM (*traM*), TraJ (*traJ*), TraY (*traY*), TraA (*traA*), TraL (*traL*), RNA I, RNA II, rom and exc1 genes. The third significant ORF exhibited similarity to delta *tnp*A, ORF294, *tetA*, *tetR*, *blaTEM*, *tnpR* and *tnp* and fourth ORF was similar to RNA I, RNA II, *rom* and *exc1*, pKlebB-k17/80, *MobC* gene, alkaline phosphatase (*phoA*), lac repressor (*lacI*), and chloramphenicol acetyltransferase (*cat*) genes, transposon Tn5-OT182, pFPTB1 delta *tnpA*, ORF294, *tetA*, *tetR*, plasmid I Mob (*mobA*), retron reverse transcriptase (*rrt*), and cold shock protein (*csp*) genes. The inferred product of a cassette-associated gene from recombinant clone of *Acinetobacter* sp. indicated the presence of promoter and integron gene cassette with more than 59 base elements located downstream of the genes with most highly conserved 7-base core site with the consensus site GTTRRRY located at the right-hand end of the element and a core site with consensus site RYYYAAC at the left-hand end.

One observation arising from study is the recovered cassettes included a diverse range of genes, a vast majority of which have no known homologues in the databases. The importance of horizontal gene transfer for bacterial evolution and the potential negative effects (i.e., the spread of antibiotic resistance) and beneficial features (i.e., the potential for bioremediation of contaminated environments). It is imperative that we gain a better understanding of the role of the integron in nature. Most of the gene cassettes that sequenced so far are related to genes of integrase genes or integron recombination sites.

For each sequence that had a significant BLAST hit, we studied the DNA of antibiotic resistance and integrons are widespread features of bacterial populations. Collectively these data give cause to reconsider our ideas of bacterial genome flexibility and the diversity of proteins likely to be found in even well-known bacterial species. In addition to providing a means of tracking integron-mediated gene transfer in the environment, the PCR strategy represents a unique opportunity to prospect for new genes of biotechnological importance by culture-independent means (Boucher *et al*., 2006). The "floating genome" of the integron-gene cassette system is evidently extensive, exists across multiple species and environments, and includes highly diverse genes (Biskri *et al*., 2005). This diversity of genomic context, in conjunction with the ability of at least some integrons to act as a scaffold for multi-gene assembly, makes it increasingly likely that we will observe the impact of integrons at all scales of bacterial genome evolution in the stability of the bacterial consortium during bioremediation of chlorinated aromatic compounds.

3 Conclusions

In this study, a stable consortium was developed by continuous enrichment of the bacterial strains isolated from the sediment core of pulp and paper mill effluent. The consortia showed significant degradation of PCP, when supplied as sole source of carbon and energy. Out of three isolated bacterial strains *E. coli* (PCP1), *P. aeruginosa* (PCP2), and *Acinetobacter* sp. (PCP3), *Acinetobacter* sp. showed higher potency to degrade PCP. Out of the three acclimated bacterial strains, presence of integron gene cassette for multiple antibiotic resistances has been conferred in *Acinetobacter* sp. only. Molecular techniques were used to examine the diversity of integrons and antibiotic resistance gene in chlorinated compounds-contaminated pulp and paper mill sediment core. Shotgun cloning method was used because of heterogeneity in both published primers and newly designed primers to uncover new integron integrase genes and new gene cassettes from this environment. The integron-gene cassette and antibiotic resistance gene influence movement of gene which helped in stability of the consortium that may be applied for successful *in situ* bioremediation of PCP in the environment.

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