

Response of indigenously developed bacterial consortia in progressive degradation of polyvinyl chloride

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Abstract Thermoplastic-based materials are recalcitrant in nature, which extensive use affect environmental health. Here, we attempt to compare the response of indigenously produced bacterial consortium-I and consortium-II in degrading polyvinyl chloride (PVC). These consortia were developed by using different combination of bacterial strains of *Pseudomonas otitidis*, *Bacillus cereus*, and *Acanthopleurobacter pedis* from waste disposal sites of Northern India after their identification via 16S rDNA sequencing. The progressive degradation of PVC by consortia was examined via scanning electron microscopy, atomic force microscopy, UV–vis, FT-IR spectra, gel permeation chromatography, and differential scanning calorimetry analysis at different incubations and time intervals. The consortium-II was superior over consortium-I in degrading the PVC. Further, the carbon source utilization

analysis revealed that the extensive use of consortia has not any effect on functional diversity of native soil microbes.

Keywords Indigenous consortia · Polyvinyl chloride · In situ biodegradation · Microbial community

Introduction

The rising exploitation of thermoplastics especially polyvinyl chloride (PVC) and low-density polyethylene (LDPE) in household practice, scientific activity, and technological functions has become a major environmental concern to rescue the natural ecosystem (Anwar et al. 2013). The frequent consumption of thermoplastics led to accumulation of polyvinyl chloride (PVC) in the environment (Barnes et al. 2009). It badly affects soil fauna, flora, and environment health and causes health hazards to human and animal (Yadav et al. 2014). In India, up to 3.1 million metric ton was increased per year by 2016–2017 (Yadav et al. 2014). The potentiality of microorganisms to degrade xenobiotics, particularly recalcitrant plastics has been of great investigations over past couple of decades (Magan et al. 2010; Anwar et al. 2013). Recently, two bacterial strains viz., *Enterobacter asburiae* YT1 and *Bacillus* sp. YP1 presented potential evidence for the biodegradation of polyethylene in the environment (Yang et al., 2014). Interestingly, bio-degradation offers a mild mean for its disposal and recycling with an advantage of greater control over the degree of degradation (Booth and Robb 2007).

Pseudomonas aeruginosa and *Aureobasidium pullulans*, *Rhodotorula aurantiaca*, and *Kluyveromyces* sp. have been reported to cause in situ colonization and substratum deterioration of PVC (Webb et al. 2000; Kawai 2010). *Curvularia* sp., *Trogia buccinalis*, and *Phanerochaete chrysosporium* have been recorded to degrade the blend of PVC/polycaprolactone (Sandra et al. 2010). Bacterial biodegradation of plasticizer content of

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PVC has been observed in the presence of *Pseudomonas fluorescens* FS1 (Zeng et al. 2002), *Coryneform bacterium*, and *Mycobacterium* sp. (Nakamiya et al. 2005). The enhanced colonization and microbial succession was observed during biodegradation of a variety of polymers by using consortia of bacterial strains (Roy et al. 2008). Recently, we have investigated the biodegradation of low density polyethylene in the presence of the consortia (Anwar et al. 2013). In this study, the differential response of indigenously produced bacterial consortium-I and consortium-II in polyvinyl chloride degradation was evaluated to keep the environment healthy, dynamic, and secure.

Materials and methods

Starting materials

The commercially available PVC film of 2.5×2.5 cm of exact size of dimension was and $3.0 \pm 0.01 \times 10^{-2}$ mm of average thickness was used as a primary carbon source for biodegradation studies using indigenously developed consortia. For the study of isolation, screening of bacteria, and PVC toxicity, a total of 10.0 g PVC beads were prepared through digesting them in boiling xylene (50 mL), followed by evaporating the solvent under ambient conditions. These samples were thoroughly washed with ethanol (70%) and dried at 50 ± 1 °C (dry oven) for 1 h. To study the in situ biodegradation of PVC, a total of 4.0 PVC film for each defined consortium as well as control was taken. The optimum concentration of PVC for individual bacterial strains was 5.0 mg/mL (Supplementary Table 1).

Bacteria culture and their screening

The bacterial strain was chosen for this investigation is based on utilization of PVC as carbon source. A total of 17 bacterial strains (SPT1, SPT2, SPT3, SPT4, SPT5, SPT6, SPA1, SPA2, SPA3, SPA4, SPK1, SPK2, SPM1, SPM2, SPM3, SPM4, and SPM5) were isolated from different plastic waste disposal sites viz., Thakurdwara, Aligarh, Kashipur, and Moradabad of North India. For preliminary nutritional screening, the collected different soil sample was autoclave at 121.5 °C for 30 min, and then, it was inoculated to PVC-enriched media for 10–12 days. After completing the incubation period, the bacterial cultures were isolated with the help of spread plate or pour plate method and sub-culturing method. Out of 17, six bacterial strains were screened for utilizing PVC as a primary carbon source by enrichment culture method. For this purpose, active cultures were prepared in nutrient broth by incubation at 37 °C for 24 h with continuous shaking at 120 rpm. Afterwards, aliquots (20 µL) were added to Minimal broth Davis w/o dextrose (5 mL) in distilled water with concentration (g/L): K_2HPO_4 (7.0), KH_2PO_4 (2.0), sodium citrate (0.50), $MgSO_4 \cdot 7H_2O$ (0.10), and $(NH_4)_2SO_4$ (1.0). The pH

of the medium was adjusted to 7.2. The PVC was added to the medium at the rate of 1.0–10.0 mg/mL. The bacterial strain on individual basis in the presence or in the absence of PVC was evaluated. A total of six bacterial isolates showed higher optical density indicating higher growth in the presence of PVC as compared to their growth in the absence of PVC were selected for further studies (Supplementary Table 2).

Identification of bacteria

The six potential PVC-utilizing strains such as SPT1, SPT2, SPT3, SPA1, SPA2, and SPK1 were selected and characterized based on 16S rDNA sequencing. Polymerase chain reaction (PCR) amplification of partial 16S rRNA gene was carried out with the bacterial primer set 16F27 (5'-CCAGAGTTTGATCMTGGCTCAG-3') and 16R1525XP (5'-TTCTGCAGTCTAGAAGGAGGTGWTCCAGGC-3') in an automated Gene amplification PCR system 9700 thermal cycler (Applied Biosystem, Foster City, USA). The amplicons were sequenced using a Big Dye terminator cycle sequencing kit (V3.1) in an ABI Prism 3730 Genetic Analyzer (Applied Biosystem, USA) employing primer 16F27 to yield a 700-base 5' end sequence. The sequences were then analyzed by basic local alignment search tool (BLAST) at National Center for Biotechnology Information, USA (NCBI) database. The isolates were identified based on their similarity scores, and the sequences were submitted to NCBI GenBank to get accession numbers. Further, the phylogenetic tree was constructed using the neighbor-joining tree method, and the phylogenetic data were obtained by aligning the different sequences of the 16S rRNA of closely related strains ($\geq 99\%$ identity).

Further, the identification of PVC-degrading bacterium was carried out according to Bargey's Manual on systematic Bacteriology (Holt et al. 1994). Catalase activity was determined by detective bubble formation with 3% H_2O_2 solution. Oxidase test was determined by using Bacteriological differentiation Oxidase Disc (Hi-Media Laboratories, India). Starch hydrolysis was determined on starch hydrolyzing agar by detecting clear zone formation around the colonies. Different enzyme activity and carbohydrate utilization were determined by biochemical test kits (Hi-Media Laboratories, India), having different test of nitrate reduction, deamination, urease, ornithine decarboxylase, lysine decarboxylase, H_2S production, glucose, adonitol, lactose, sorbitol, arabinose, xylose, galactose, ribose, mannose, fructose, and galactosamine.

Development of consortia

Based on preliminary nutritional screening, the selected bacterial strains were used for developing two consortia: consortium-I comprising SPA1 and SPA2 strains and consortium-II comprising SPT1, SPT2, SPT3, and SPK1 strains. For the preparation of bacterial consortia, first of all, each individual bacterial

strain was inoculated separately in a flask containing PVC-enriched media and incubate them for 2 or 3 days and check their growth by taking optical density (O.D) with the help of spectrophotometer. After that randomly chosen different combination of two or four bacterial strain and inoculated them with calculated amount into the PVC-enriched media and check their growth for 4 or 5 days. The combination, which shows better results for growth as compare to individual one is selected as consortia while the ones have lesser growth are leftover (Supplementary Table 3). A single colony from each selected culture was inoculated into 100-mL flasks containing 50-mL nutrient broth (pH 7.0±0.02) and incubated at 37 °C for 12 h with continuous shaking at 120 rpm. The calculated amount (cfu mL⁻¹) of each strain was mixed for the development of the consortia (~1.10×10⁸ to 2.48×10¹⁰).

Biodegradation assay

The top-soil (500 g) was dug from a barren land at Pantnagar, India and was filled into 600-mL glass beakers. The PVC film coupons (1.0 in.²) were sterilized with ethanol (70 %) for 10 min and subsequently mounted vertically inside the beakers at a depth of 1.0 in. below the soil surface and at the same distance apart. Further, the beakers were inoculated with active consortium (150 mL) and kept at 28±2 °C. Proper moisture and aeration conditions were maintained by adding 20 mL of autoclaved distilled water and shoveling (addition of distilled water at regular basis to all the experimental basin for better aeration via scooping it either from the side of the basin or from the top of the basin to a particular depth) the soil at regular intervals of 2 weeks. The sample is always kept in the middle and in a particular depth of the experimental basin to avoid mechanical destruction. Uninoculated soil with PVC film coupons was taken as a control. PVC film coupons were kept in soil for the period of 3, 6, and 9 months, and thereafter, it recovered from the soil, sterilized with ethanol (70 %) for 10 min and investigated for biodegradation studies. A total of four PVC samples were analyzed in biodegradation study for each defined treatment, and three biological replicate and each with three repeats were taken for each treatment.

Characterization of biodegraded PVC samples

A change in morphology of biodegraded PVC was determined through microscopy studies. For this purpose, scanning electron micrographs (SEM) of gold-coated samples have been observed at ×3000 magnification over LEO, 435VF at 15.00 kV EHT. UV-visible spectra were recorded in tetrahydrofuran (THF) over Genesis-10 Thermospectronic spectrophotometer. The FT-IR spectra were recorded on PerkinElmer FT-IR spectrophotometer in KBr. The change in the molecular weight of the biodegraded PVC samples has been recorded through gel permeation chromatography (GPC) over Varian Pro-Star 210 HPLC/GPC

system equipped with 355 refractive index detector using THF as mobile phase at 1.0 mL/min with reference to polystyrene standard. The heat of fusion of biodegraded PVC sample was determined by differential scanning calorimetry (DSC) data over Universal V4.5A Thermal Analyzer.

Microbial metabolic diversity using biolog

Biolog GN, Eco, and MT plates (Biolog, Inc., Hayward, CA, USA) were used to determine the carbon source utilization pattern of the soil samples recovered after 9 months of in situ assay (Campbell et al. 1997). The Biolog MT plates were prepared using the manufacturer's instructions (Biolog Inc., Hayward, CA 94545, U.S.A.). Individual soil samples (1.0 g) were shaken in 9.0 mL of sterile 0.85 % saline MQW for 60 min and made up to a final dilution of 10⁻³. After incubation, 150 µL of samples were inoculated into each well of Biolog Eco plates and incubated at 30 °C. The rate of utilization was indicated by the reduction of tetrazolium, a redox indicator dye, which changes from colorless to purple. The data were recorded after 15 days at 590 nm described earlier (Mishra and Nautiyal, 2009). Microbial activity in each microplate, expressed as average well color development (AWCD), was determined as described by Garland (1996). Diversity and evenness indices were calculated via adopting the method by Mishra and Nautiyal (2009). Principal component analysis (PCA) was performed on 15th day's data divided by the AWCD, as depicted by Garland and Mills (1991).

Statistical analysis of the experimental data

The data obtained in this study were analyzed statistically by one-way analysis of variance (ANOVA) to determine the variation between the treatments, and their significance levels ($p < 0.05$) were determined using SPSS 16.0 (SPSS, Inc., now IBM, <http://www-01.ibm.com/software/analytics/spss>).

Results

Development of PVC-degrading consortia

Six bacterial strains viz., SPT1, SPT2, SPT3, SPA1, SPA2, and SPK1 has been selected based on their relatively higher growth and utilization of PVC as sole source of carbon and energy. The actual meaning of higher growth is that when we investigate each bacteria strain on individual basis, in the presence or in the absence of PVC by inoculating them on PVC-enriched media, the result shows peculiarities that in the presence of PVC, the optical density of each bacterial strain was found higher compare to in absence of PVC (Supplementary Table 2). In the study of PVC toxicity on growth statistics of individual bacterial strain, a total six bacterial strains out of 17 was screened on

PVC enriched to evaluate the effect of toxicity of PVC upon bacterial strains survival at different concentration of PVC (mg/mL). The result obtained revealed their tolerance upto PVC at 5 mg/mL concentration, and beyond 5 mg/mL PVC concentration, it shows toxic effects (Supplementary Table 1). Further, subsequently, at random by choosing different combination of two or four bacterial strain, inoculated them into the PVC-enriched media and check their growth. The combination, which shows better results of growth as compare to individual one is selected as consortia for further experiment while the ones have lesser growth are leftover (Supplementary Table 3). The biological basis for composing of two different consortia was to perceive the evaluation of comparative effect of biodegradation of PVC by two separate combinations of bacteria, i.e., consortia. The morphological and biochemical characterization of the bacterial strains viz., SPT1, SPT2, SPT3, SPA1, SPA2, and SPK1 were performed, and the results obtained was shown in Table 1. The partial sequence data of the isolates were analyzed by BLAST search that shows unambiguous similarities (97–100 %) with *Pseudomonas otitidis* strain SPT1 (GU598256), *Bacillus aerius* strain SPT2 (GU598257), *Acanthopleurobacter pedis* strains SPT3 (GU598258),

Table 2 Bacterial strains exploited in the present investigation

Bacterial strains	Accession number
<i>Pseudomonas otitidis</i> strain SPT1	GU598256
<i>Bacillus aerius</i> strain SPT2	GU598257
<i>Acanthopleurobacter pedis</i> strain SPT3	GU598258
<i>Bacillus cereus</i> strain SPK1	GU598261
<i>Acanthopleurobacter pedis</i> strains SPA1	GU598259
<i>Bacillus cereus</i> strains SPA2	GU598260
<i>Bacillus cereus</i> strains SPK1	GU598261

Acanthopleurobacter pedis strains SPA1 (GU598259), *Bacillus cereus* strains SPA2 (GU598260), and *Bacillus cereus* strains SPK1 (GU598261), respectively (Table 2). The potential of developed consortium-I and consortium-II towards biodegradation of PVC with incubation period in soil has been confirmed through surface roughness analysis using SEM. Further, a characteristic shift of maximum absorption in UV spectra, wave number in Fourier transform infrared spectroscopy (FT-IR), lowering of heat of fusion in differential scanning calorimetry (DSC), and molecular mass reduction by gel

Table 1 Morphological and biochemical characterization of isolated bacterial strains

Test	Bacterial strains					
	SPT1	SPT2	SPT3	SPA1	SPA2	SPK1
Colony shape	Punctiform	Punctiform	Irregular	Circular	Circular	Circular
Colony surface	Smooth	Smooth	Wrinkle	Smooth	Smooth	Smooth
Gram stain	–	+	+	+	+	+
Catalase test	+	+	+	+	+	+
Starch hydrolysis	–	+	+	+	+	+
Oxidase test	+	–	–	–	–	–
Nitrate reduction	–	+	+	+	+	+
Deamination	–	–	–	–	–	–
Urease	–	–	–	–	–	–
Ornithine decarboxylase	–	+	+	–	+	+
Citrate utilization	+	–	–	+	+	+
Lysine decarboxylase	–	–	–	+	+	+
H ₂ S production	–	–	–	–	–	–
Glucose	+	–	–	–	–	–
Adonitol	–	–	–	–	–	–
Lactose	–	–	–	–	–	–
Arabinose	+	+	+	+	+	+
Sorbitol	–	–	–	–	–	–
Xylose	–	–	–	+	+	–
Dextrose	–	–	+	+	+	–
Glactose	–	–	–	+	+	–
Mannose	–	–	+	+	+	–
Glucosamine	–	–	–	–	–	+
Ribose	+	+	+	+	+	–
Fructose	–	–	+	–	+	–

permeation chromatography (GPC) indicates the macromolecular degradation of PVC which was induced by consortia (Fig. 1, 2, and 3).

Effect of incubation period on the morphology of PVC film in the absence/presence of consortia: a scanning electron microscopy study

The comparative account of the effect of incubation period on the surface characteristics of PVC was performed in the absence of consortia over 0.0, 3.0, 6.0, and 9.0 months through SEM. The SEM revealed the un-incubated (0.0 month) PVC showing homogeneous morphology of PVC film (Fig. 1a), which followed by either negligible or slight change after 3.0, 6.0, and 9.0 months of incubation as shown in Fig. 1b–d). The relative potential of the consortium-I and consortium-II in degrading PVC film was examined under different intervals of incubation periods viz., 3, 6, and 9 months via SEM (Fig. 1i–n). The SEM study revealed that the exposure of consortium-I over 3 months imparts heterogeneous morphology of PVC film (Fig. 1i), which subsequently increases upon incubation to 6 months (Fig. 1j) and further to 9 months (Fig. 1k). However, consortium-II showed elevated heterogeneous morphology of PVC film as compared to consortium-I at the incubation period of 3 months (Fig. 1l), which followed by further increase biodegradation of PVC film by proceeding from 6 to 9 months of incubation periods (Fig. 1m–n). In order to have further insight into the relative potentials of the consortia, the average roughness of the PVC film with incubation periods has been investigated. It was 3.22, 3.83, and 4.4 nm after 3 months of

incubation, which further increased at 6 months (3.26, 5.1, and 5.89 nm) and 9 months (3.4, 5.6, and 6.6 nm) (Fig. 1o).

Study of effect of consortia in PVC degradation via UV and FT-IR spectra

The relative potentials of the consortia towards degradation of PVC have further been supported with UV and FT-IR spectra. A remarkable shift has been observed in the absorption of the PVC in the presence of the each of the consortia via UV spectra, which revealed that the shift in the degraded PVC has been found to be higher in the presence of the consortium-II (224 nm) (iv) over consortium-I (227 nm) (iii). To compare the results, the UV spectra have been scanned under identical concentrations of the PVC films dissolved in tetrahydrofuran (THF). UV spectrum reveals the characteristic absorption of untreated PVC at 236 nm (i). The absorption of PVC has been retained in the sample isolated from the soil bed devoid of consortia even after 9 months of the incubation (ii) (Fig. 2a). Further, FT-IR spectra for the potential of consortia towards degradation of PVC films have been reflected through shifts in the wave numbers (cm^{-1}) to lower values, in comparison to the untreated PVC. The degradation studies of PVC incubated in the presence of consortia over 9 months has been divided under two regions. The first region deals with investigation of the degradation of macromolecular chain and the plasticizer content of PVC in the spectral range 4000 to 1000 cm^{-1} . This has been ascertained through introduction of the additional wave numbers corresponding to ν OH with simultaneous reduction in the

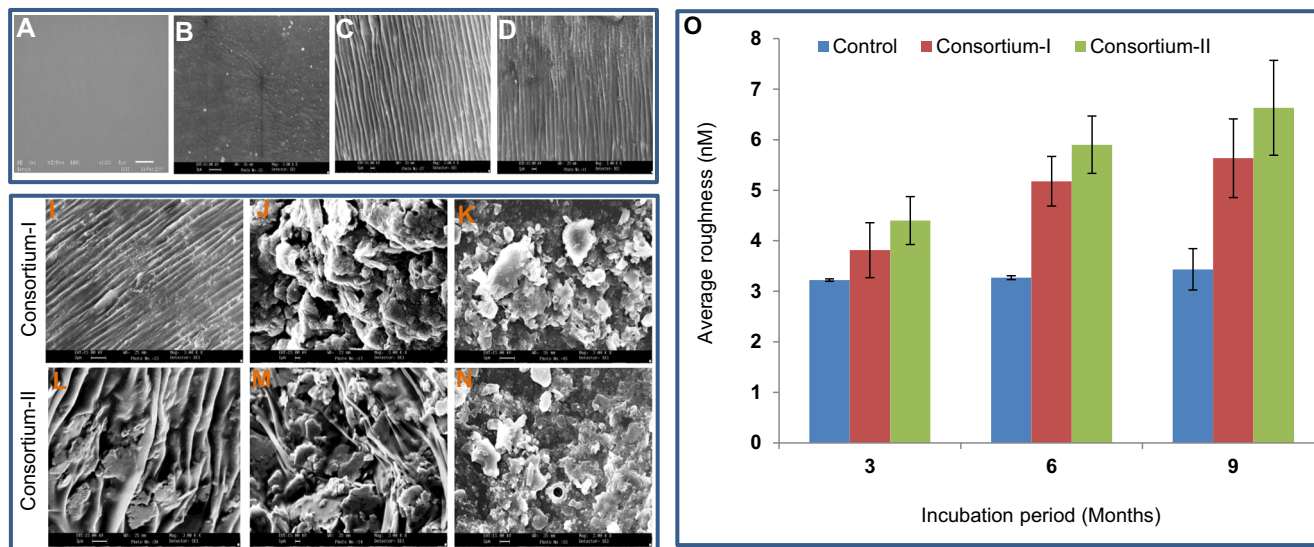
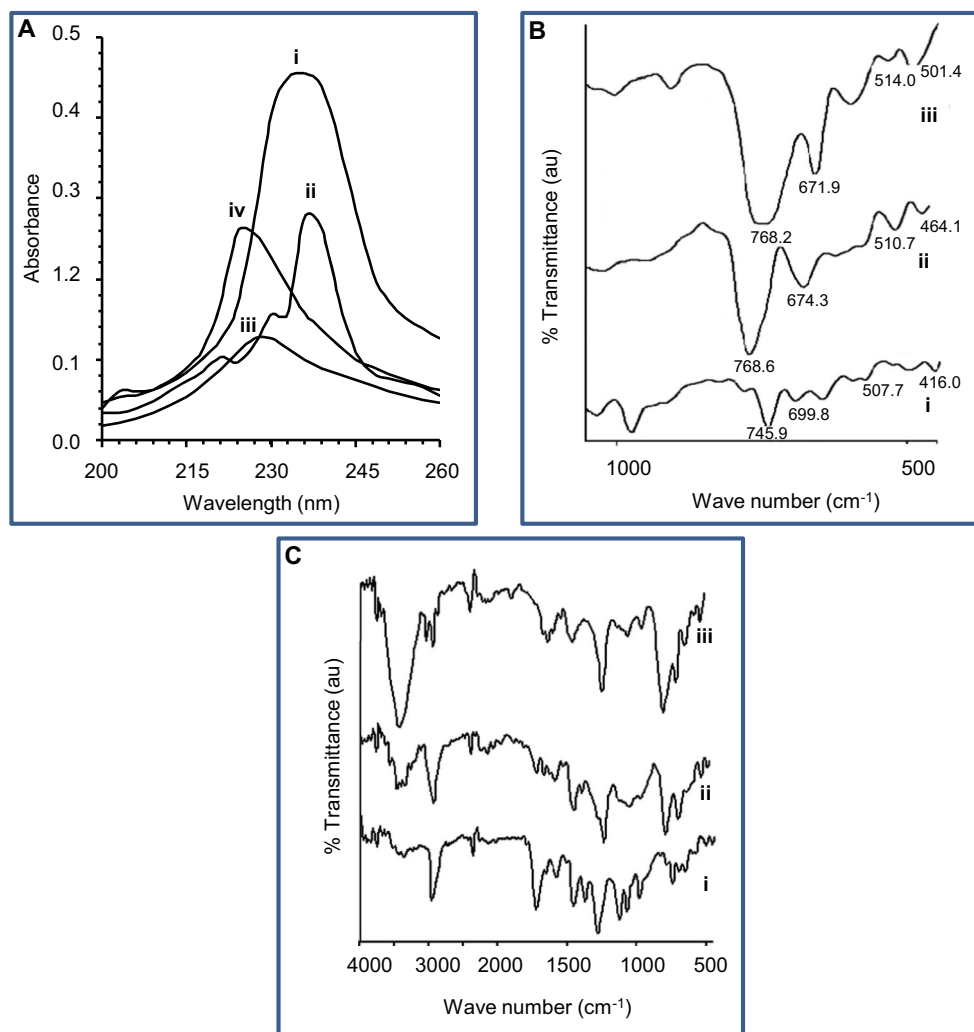


Fig. 1 Evaluation of effect of incubation period on the morphology of PVC film present in the soil in the absence/presence of consortia via scanning electron microscope (SEM) (a–d). In the absence of consortia, the morphology of the PVC film was changed slightly under SEM (a–d) after 0.0, 3.0, 6.0, and 9.0 months. However, in the presence of consortia, the degradation of PVC film was more obvious. The SEM images

indicating in situ biodegradation of PVC in consortium-I (i–k) and consortium-II (l–n) treatments after 3.0, 6.0, and 9.0 months of incubation. Relative potentials of consortia to degrade PVC film in soil in control (untreated) treated with consortium-I and consortium-II after 3.0, 6.0, and 9.0 months of incubation periods was shown as an average roughness of PVC film (o)

Fig. 2 Estimation of effect of consortia in PVC degradation via UV and FT-IR spectra. UV spectra (a) of untreated PVC film (without incubation and in the absence of consortia) (i), PVC film ((9 months of incubation and in the absence of consortia) (ii), in the presence of consortium-I (iii), and consortium-II (iv). FTIR spectra to examine the effect consortia on PVC film degradation under defined conditions (i–iii) (b). The specific spectral range in FT-IR spectra (1000–450 cm^{-1}) indicating the effect of consortia on degradation of PVC film under the same treatments (c)



transmittance of the $>\text{C}=\text{O}$ group (Fig. 2b). The second region of the spectral range has been investigated beyond 1000 cm^{-1} corresponding to the degradation of C-Cl bonds of PVC (Fig. 2c). Untreated PVC shows characteristic wave numbers (cm^{-1}) at 2962.8 (νCH_2 , asymmetric), 1726.3 ($\nu\text{C}=\text{O}$), 1463.0 (δCH_2), 1376.3 (δCH), 1280.8 (γCH), 1127.6 ($\delta\text{C}-\text{C}$), and 1073.2 ($\nu\text{C}-\text{O}-\text{C}$). The PVC treated with consortium-I shows wave number corresponding to 3110.3–3755.7 (νOH), 2925.2 (νCH_2 , asymmetric), 2374.7 (νCH_2 , symmetric), 1712.6 ($\nu\text{C}=\text{O}$), 1440.6 (δCH_2), 1218.9 (γCH), and 1028.3 ($\nu\text{C}-\text{O}-\text{C}$). A comparative account indicates that the consortium-I has introduced the absorption corresponding to the formation of hydroxyl group (3110.3–3755.7 cm^{-1}) on the macromolecular chain of the PVC with simultaneous shift in the wave numbers to lower values. This has further reduced the transmittance of the $>\text{C}=\text{O}$ group of the PVC incubated in presence of the consortium-I over the untreated PVC. The FT-IR spectrum of the PVC degraded in the presence of consortium-II shows characteristic wave number corresponding to 3413.3 (νOH), 3020.8 Ar (CH), 2928.1 (νCH_2 ,

asymmetric), 2372.1 (νCH_2 , symmetric), 1436.5 (δCH_2), 1217.6 (γCH), and 1028.9 ($\nu\text{C}-\text{O}-\text{C}$). The absence of the wave number corresponding to $\nu\text{C}=\text{O}$ may be attributed to the higher potential of the consortium-II over consortium-I towards degradation of the plasticizer content of PVC. Further, the relative potentials of the consortia have been reflected in the comparative FT-IR of the biodegraded PVC in spectral range 1000 to 450 cm^{-1} (Fig. 2c).

Evaluation of effect of consortia on PVC degradation: heat of fusion by differential scanning calorimeter, molecular mass, and microbial community analysis

The degraded PVC samples have been investigated after 9 months of incubation for their onset temperature, peak temperatures ($^{\circ}\text{C}$), and heat of fusion (ΔH , J/g) in a differential scanning calorimeter (DSC) study. Thermal data revealed that degradation of control PVC has been started with onset temperature 276.28. This has been supported with peak temperature 281.76 and heat of fusion 914.1 (Fig. 3a–c). The relative

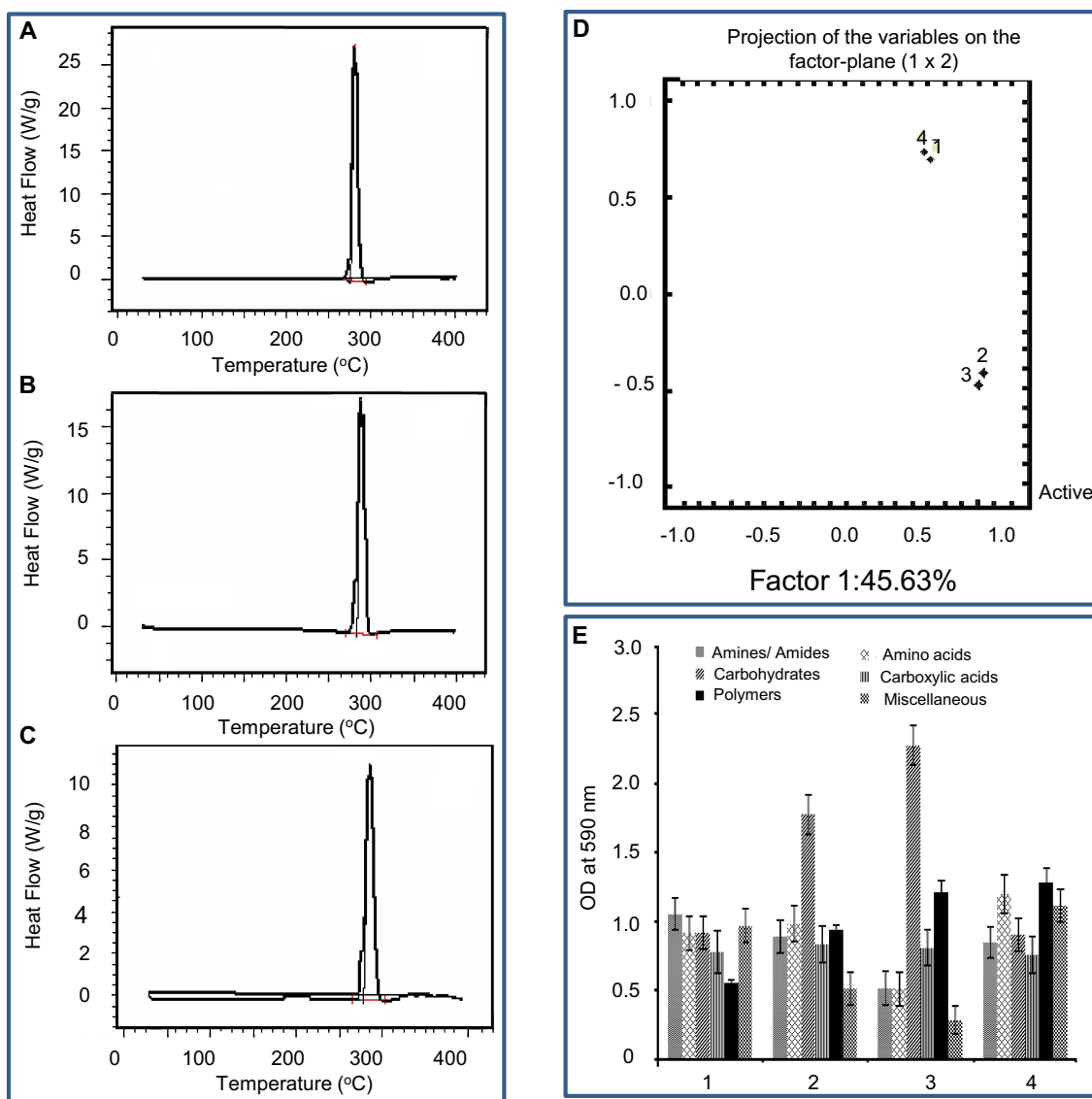


Fig. 3 The differential scanning calorimeter (DSC) indicating effect of consortia on the heat of fusion of PVC after incubation of 9 months under in situ conditions: untreated control (a), treated with consortium-I (b), and consortium-II (c). Principal component analysis based on carbon source utilization by microflora of soils under different conditions of treatment

such as un-inoculated soil without PVC (1), soil plus PVC (2); soil plus PVC plus consortium-I (3) and soil plus PVC plus consortium-II (4) (d). Categorized carbon substrate utilization pattern by soil microflora in different treatments: un-inoculated soil without PVC (1), soil plus PVC (2); soil plus PVC plus consortium-I (3) and soil plus PVC plus consortium-II (4) (e)

potentials of the consortium-II over consortium-I towards the degradation of PVC has been clearly reflected through a regular decrease in the number average molecular mass with variations in the polydispersity index (PDI) over untreated PVC film. The control PVC has been progressed with a narrow decrease in the number average molecular mass (KD) ranging 69.88 to 66.61 with subsequent period of incubation. The degradation of PVC in presence of consortium-I has been progressed with a remarkable decrease in the number average molecular mass ranging 49.98 to 31.57. The decrease in the number average molecular mass of PVC has been found more remarkable in the presence of consortium-II over consortium-I with time (Table 3). Microbial community structure in the soil

Table 3 Effect of incubation period on structural degradation of PVC (commercial PVC=72.65 (1.001))

Incubation (month)	Number average molecular mass in KD (PDI)		
	Control	C-1	C-II
3	69.88 (1.00)	49.98 (1.00)	29.52 (1.00)
6	67.80 (1.03)	41.68 (1.10)	24.07 (2.82)
9	66.61 (1.02)	31.57 (1.00)	15.63 (4.70)

KD kilo Dalton, PDI polydispersity index

of the four treatments was assessed using Biolog GN, Eco, and MT plates (Fig. 3d, e) (Supplementary Table 4). PCA of carbon source utilization pattern on Biolog Eco plates demonstrated that the treatments 1 and 2 were distributed separately among others at 45.63 and 35.83 % on factor 1 and 2 axis (Fig. 3d). The PCA results were substantiated by the McIntosh, Shannon, and Simpson indices, whereby variable significant differences were noted among treatments (Supplementary Table 4). Significant differences were observed in uninoculated soil (control) and consortium-I treated soil on the basis of Shannon, Simpson, and McIntosh diversity indices. Moreover, treatment 2 and 3 differed significantly from each other on the basis of all indices, fortifying the differential influence of the two consortia upon functional microbial community. The categorized C-substrate utilization pattern of the soil microflora was also found to exhibit notable diversity across the different treatments with respect to the uninoculated soil (Fig. 3e).

Discussion

A polyethylene material has become a major environmental concern owing to its nondegradable nature. The degradation of plastic-based materials by means of microbial strain may possibly recommend a solution to the problem. Recent reports have shown that it can be degraded at a very slow rate by activity of different beneficial microbes, for example, degradation of low-density polyethylene by *Pseudomonas* sp. AKS2 (Tribedi and Sil 2013). There are indications that the growth sustainability of bacteria in the absence and presence of the polymers (PVC and LDPE) could be achieved (Anwar et al. 2013). Further, Kapri et al. (2010) and Sah et al. (2010) reported that an elevated bacterial biomass with respect to change in the polymer can be observed under different incubation periods. The presence of more than one bacterial strain in comparison to single isolates may enhance the degradation efficiency of the bacterial culture due to their cooperative activity (Soni et al. 2009). Here, the compatibility test of selected bacterial strains performed in different combinations reveals that two combinations does enhance higher growth as compared to the individual one. These combinations of bacterial strains were called as consortium-I and consortium-II, which role towards biodegradation of PVC was validated through scanning and atomic force microscopy, UV spectra, FT-IR, DSC, and GPC indicates macromolecular degradation of PVC induce by consortia (Figs. 1, 2, and 3). By using bacterial consortia, a similar kind of observation was documented for surface degradation of PVC and LDPE (Anwar et al. 2013; Sah et al. 2011), surface degradation of HDPE (Kapri et al. 2010). A parallel category of surface degradation of PVC was observed by using SEM (Ali et al. 2014). In this study, in the absence of consortia, the incubation periods ranging from 0.0

to 9.0 months showed either no change or negligible change in the morphology of the PVC film present in the soil in SEM (Fig. 1a–d). Further, in the presence of consortia, the SEM study showed that consortium-I imparts the remarkable surface degradation of PVC with incubation period ranging from 6 to 9 months (Fig. 1i–k), whereas consortium-II commences PVC degradation from 3 months (Fig. 1l–n). The substantial increase in the average roughness of PVC ranging 3.88 to 6.0 in presence of consortium-I and 4.38 to 6.63 through consortium-II in comparison to control (3.25 to 3.4) (Bhattacharyya and Klapperich, 2007). A subsequent group of surface degradation of polycarbonate was observed (Arefian et al. 2013). Here, in-depth comparative potentials of the consortia, the typical roughness of the PVC film with incubation periods has been observed (Fig. 1o). A roughness change of 3.4–6.6 nm of film during 9 months of incubation was in respect to film size of 2.5×2.5 cm and $3.0 \pm 0.01 \times 10$ –2 mm of average thickness (Fig. 1o).

The biodegradability potential of *Micrococcus* species to degrade PVC has been realized in which PVC as a sole carbon source was confirmed. An increase their cell density of bacteria in test media, carbon dioxide production and its growth on the surface of PVC film along with the release of chloride was reported (Patil and Bagde 2012). The variations in wave numbers of PVC in virtue of respective consortia reveal that the PVC has been used as sole carbon source during incubation period. Further, the consortium-II imparts increasing shift in the ν C-Cl, ν C-C-Cl, and δ C-C-C with simultaneous reduction in the wave number of ν C-C-C groups of PVC over consortium-I. Such shift in the wave numbers of the functional group (C-Cl) and backbone of PVC (C-C-C) indicates that both of the consortia have been found effective towards degradation of PVC (Ramesh and Yi, 2008; Anwar et al. 2013). In this study, the relative potentials of the consortia towards degradation of PVC have further been supported with UV and FT-IR spectra and DSC (Fig. 2a–c). The control PVC shows higher onset and peak temperatures over the PVC degraded in presence of consortia. The values of onset and peak temperature of the PVC degraded by both the consortia were found to be similar. A higher potential of the consortium-II over consortium-I in degrading PVC structure was evidenced via heat of fusion data. The heat of fusion was lowest for the PVC degraded with consortium-II followed by consortium-I with reference to control PVC reveal the clear insight of the higher potential of consortium-II. The change in the heat of fusion of polylactides was reported (Ahmed et al. 2009). Here, the degraded PVC was examined with peak temperature 281.76 and heat of fusion 914.1 (Fig. 3a–c). In the presence of consortium-II, the degradation of PVC has been progressed with a sharp decrease in the number average molecular mass ranging 29.52 to 15.63 with simultaneous increase in the PDI. A remarkable increase in the PDI during 6 to 9 months in the presence of consortium-II indicates the degradation of PVC

with variations in the weight average molecular mass along with Mn (Agrawal et al. 2010). The reduced average molecular mass of PVC was remarkable in the presence of consortium-II over consortium-I with respect to time (Table 3). Biolog plates permit assessment of microbial metabolic diversity to differentiate community profiles from diverse habitats (Garland, 1996). Microbial community structure in the soil of the four treatments (Fig. 3d-e) was assessed using Biolog GN, Eco, and MT plates (Supplementary Table 1). This indicated that changes occurred in the soil microbial community by the addition of PVC film. Further, treatments 1 and 4 grouped together indicating that the changes caused by the PVC in the microbial diversity were neutralized by consortium-II. However, this effect was not observed in case of consortium-I as treatments 2 and 3 grouped together. Moreover, unlikely to the control, carbohydrate utilization was maximum in treatment 3 (consortium-I with PVC) the most discriminating substrate was D-trehalose (data not shown), followed by treatment 2 (PVC only), which was neutralized in treatment 4 (consortium-II). Nonetheless, in treatment 4, besides evenness in utilization of different substrates, preferential utilization of polymers was also documented followed by treatment 3 (consortium-I).

Conclusively, this study highlights the development of two suitable bacterial consortia which have the ability to degrade PVC in nature. The degradation was found to be better with consortium-II which comprised of four different bacterial strains unlikely to consortium-I which had only two strains. To the best of our knowledge, it is the first report which describes well-resolved surface degradation of PVC, a polymer which is otherwise considered as extremely inert. The above findings support the suitability of consortium-II in PVC degradation in terms of its efficiency and adaptability under natural conditions. Efforts are underway for optimization of conditions so that role of this consortium in technology development and commercialization can be attained.

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Conflict of interest The authors declare that they have no competing interest.

References

- Agrawal V, Vishnoi S, Zaidi MGH, Alam S, Rai AK (2010) Synthesis and properties of [60] fullerene-polymethyl methacrylate conjugates in supercritical carbon dioxide. *Int J Polym Anal Char* 15(5):267–276
- Ahmed J, Varshney SK, Zhang JX, Ramaswamy HS (2009) Effect of high pressure treatment on thermal properties of polylactides. *J Food Engg* 93:308–312
- Ali MI, Ahmed S, Robson G, Javed I, Ali N, Atiq N, Hameed A (2014) Isolation and molecular characterization of polyvinyl chloride (PVC) plastic degrading fungal isolates. *J Basic Microbiol* 54(1): 18–27
- Anwar MS, Negi H, Zaidi MGH, Gupta S, Goel R (2013) Biodeterioration studies of thermoplastics in nature using indigenous bacterial consortia. *Brazilian Arch Biol Technol* 56: 475–484
- Arefian M, Zia M, Tahmourespour A, Bayat M (2013) Polycarbonate biodegradation by isolated molds using clear-zone and atomic force microscopic methods. *Int J Environ Sci Technol* 10:1319–1324
- Barnes DAK, Galgani F, Thompson RC, Barlaz M (2009) Accumulation and fragmentation of plastic debris in global environment. *Phil Trans R Soc B* 364:1985–1998
- Bhattacharyya A, Klapperich CM (2007) Mechanical and chemical analysis of plasma and ultraviolet–ozone surface treatments for thermal bonding of polymeric microfluidic devices. *Lab on a Chip* 7:876–882
- Booth GH, Robb JA (2007) Bacterial degradation of plasticised PVC-effect on some physical properties. *Int J Appl Chem* 18: 194–197
- Campbell CD, Grayston SH, Hirst D (1997) Use of rhizosphere C sources in sole C source tests to discriminate soil microbial communities. *J Microbiol Methods* 30:33–41
- Garland JL (1996) Analytical approaches to the characterization of samples 6 of microbial communities using patterns of potential C source utilization. *Soil Biol Biochem* 28:213–221
- Garland JL, Mills A (1991) Classification and characterization of heterotrophic microbial communities on the basis of patterns of community level sole-carbon-source utilization. *Appl Environ Microbiol* 57:2351–2359
- Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST (1994) Gram-positive cocci. In: Hensyl WR (ed) *Bergey's manual of determinative microbiology*, 9th edn. Williams and Wilkins, Baltimore, pp 527–558
- Kapri A, Zaidi MGH, Goel R (2010) Implications of SPION and NBT nanoparticles upon in-vitro and insitu biodegradation of LDPE film. *J Microbiol Biotechnol* 20:1032–1041
- Kawai F (2010) The biochemistry and molecular biology of xenobiotic polymer degradation by microbes. *Biosci Biotechnol Biochem* 74: 1743–1759
- Magan N, Silvia F, Catarina B (2010) Environmental factors and bioremediation of xenobiotics using white rot fungi. *Mycobiol* 38:238–248
- Mishra A, Nautiyal CS (2009) Functional diversity of the microbial community in the rhizosphere of chickpea grown in diesel fuel-spiked soil amended with *Trichoderma reesei* using sole-carbon-source utilization profiles. *World J Microbiol Biotechnol* 25:1175–1180
- Nakamiya K, Hashimoto S, Ito H, Edmonds JS, Yasuhara A, Morita M (2005) Microbial treatment of bis (2-ethylhexyl) phthalate in polyvinyl chloride with isolated bacteria. *J Biosci Bioeng* 99:115–119
- Patil R, Bagde US (2012) Isolation of polyvinyl chloride degrading bacterial strains from environmental samples using enrichment culture technique. *Afr J Biotechnol* 11:7947–7956
- Ramesh S, Yi LM (2008) FT-IR spectra of plasticized high molecular weight PVC-LiCF₃SO₃ electrolytes. *Ionics* 15:413–420
- Roy PK, Titus S, Surekha P, Tulsi E, Deshmukh C, Rajagopal C (2008) Degradation of abiotically aged LDPE films containing pro-oxidant by bacterial consortium. *Polym Degrad Stab* 93:1917–1922
- Sah A, Kapri A, Zaidi MGH, Negi H, Goel R (2010) Implications of Fullerene-60 upon *in vitro* LDPE biodegradation. *J Microbiol Biotechnol* 20:908–916

- Sah A, Negi H, Kapri A, Anwar MS, Goel R (2011) Comparative shelf life and efficacy of LDPE and PVC degrading bacterial consortia under bioformulation. *Ekologija* 57:55–61
- Sandra M, Franchetti M, Egerton TA, White JR (2010) Morphological changes in poly (caprolactone)/poly (vinyl chloride) blends caused by biodegradation. *J Polym Environ* 18:79–83
- Soni R, Kapri A, Zaidi MGH, Goel R (2009) Comparative biodegradation studies of non-poritized and poritized LDPE using indigenous microbial consortium. *J Polym Environ* 17:233–239
- Tribedi P, Sil AK (2013) Low-density polyethylene degradation by *Pseudomonas* sp. AKS2 biofilm. *Environ Sci Pollut Res Int* 20: 4146–4153
- Webb JS, Nixon M, Ian ME, Malcolm G, Geoffrey DR, Pauline SH (2000) Fungal colonization and biodeterioration of plasticized poly-vinyl chloride. *Appl Environ Microbiol* 66:3194–3200
- Yadav A, Kumar A, Anupa M (2014) Retrospect and prospect of occupationally induced health hazards for plastic industry workers. *Weekly Sci Res J* 1:2321–7871
- Yang J, Yang Y, Wu W, Zhao J, Jiang L (2014) Evidence of polyethylene biodegradation by bacterial strains from the guts of plastic-eating waxworms. *Environ Sci Technol* DOI. doi:10.1021/es504038a
- Zeng F, Cui K, Fu J, Sheng G, Yang H (2002) Biodegradation of di(2-ethylhexyl) phthalate by *Pseudomonas fluorescens* FS1. *Water Air Soil Pollut* 140:297–305