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Selection and screening of microbial consortia for efficient and ecofriendly degradation of plastic garbage collected from urban and rural areas of Bangalore, India

Sinosh Skariyachan · M. Megha · Meghna Niranjan Kini · Kamath Manali Mukund · Alya Rizvi · Kiran Vasist

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Abstract Industrialization and urbanization have led to massive accumulation of plastic garbage all over India. The persistence of plastic in soil and aquatic environment has become ecological threat to the metropolitan city such as Bangalore, India. Present study investigates an ecofriendly, efficient and cost-effective approach for plastic waste management by the screening of novel microbial consortia which are capable of degrading plastic polymers. Plastic-contaminated soil and water samples were collected from six hot spots of urban and rural areas of Bangalore. The plastic-degrading bacteria were enriched, and degradation ability was determined by zone of clearance method. The percentage of polymer degradation was initially monitored by weight loss method, and the main isolates were characterized by standard microbiology protocols. These isolates were used to form microbial consortia, and the degradation efficiency of the consortia was compared with individual isolate and known strains obtained from the Microbial Type Culture Collection (MTCC) and Gene Bank, India. One of the main enzymes responsible for polymer degradation was identified, and the biodegradation mechanism was hypothesized by bioinformatics studies. From this study, it is evident that the bacteria utilized the plastic polymer as a sole source of carbon and showed 20–50 % weight reduction over a period of 120 days. The two main bacteria responsible for the degradation were microbiologically characterized to be *Pseudomonas* spp. These bacteria could grow optimally at 37 °C in pH 9.0 and showed 35–40 % of plastic weight reduction over 120 days. These isolates were showed better degradation ability than known strains from MTCC. The current study further revealed that the microbial consortia formulated by combining *Psuedomonas* spp. showed 40 % plastic weight reduction over a period of 90 days. Further, extracellular lipase, one of the main enzymes responsible for polymer degradation, was identified. The computational docking studies suggested that polyethylene glycol and polystyrene present in the plastics might have good interaction towards the microbial lipase with stable binding and interacting forces which probably could be one of the reasons for the degradative mechanisms.

Keywords Polymer degradation · Plastic-degrading bacteria · Ecological threat · Effective approach · Microbial consortia · Zone of clearance · Weight loss method · Extracellular lipase · *Pseudomonas* · Computational docking

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S. Skariyachan (✉) · M. Megha · M. N. Kini · K. M. Mukund · A. Rizvi · K. Vasist
R & D Centre, Department of Biotechnology Engineering, Dayananda Sagar Institutions, Bangalore, Karnataka 560 078, India
e-mail: sinoshskariya@gmail.com

Introduction

Plastics are an integral part of our day to day life and are being used in packaging, building materials and for

many other purposes (Gnanavel et al. 2012). The speed of technological change is increasing remarkably such that life in 2030 will be unrecognizable compared to that of today; plastics will play a substantial role in this change (Andrady and Neal 2009). Despite the advantages that plastics have over many other polymers, the fact remains that it is nondegradable and not eliminated from the environment efficiently (Zubris and Richards 2005). Due to their stable nature, they tend to accumulate in the environment causing severe pollution (Hemashenpagam et al. 2013; Tserki et al. 2006; Kim et al. 2007; Ray et al. 2007). Improper recycling and waste management systems in developing countries are majorly responsible for plastic pollution (Jayasiri et al. 2013). Many studies showed that several efforts have been made to eradicate plastic wastes in the different parts of India (Kathiresan 2003; Hemashenpagam et al. 2013; Jayasiri et al. 2013); however, the success rate is not satisfactory. Bangalore considered as the one of the cosmopolitan cities in India, with nearly eight million people and generates about 4000 tonnes of garbage every day, major portions of them are plastic wastes. Due to the unscientific disposal of plastics and other waste, the pollution has risen to unprecedented level in urban and rural areas of Bangalore, India. Recent report suggested that the plastic waste management has become a major environmental concern along with industrial and vehicular pollution (Hemashenpagam et al. 2013). The industries which manufacture plastic bags and other materials are bound to release waste products containing both solid and liquid forms of plastics, and these wastes are directly dumped in soil and aquatic ecosystems. The long-chain polymers released from such xenobiotic compounds are persisted in the environment for a very long time and accumulated in living tissues and undergoes biomagnifications (Tokiwa et al. 2009; John et al. 2012). Hence, there is a high priority to address these issues and identify novel ecofriendly strategies to detoxify or degrade the plastic materials from the ecosystem.

With the increase in usage of plastics by human and increasing pressure being on the facilities available for plastic waste disposal, the need for biodegradation of plastic wastes has alarming importance (Zheng et al. 2005). There are reports which revealed that various soil bacteria, fungi and actinomycetes in the plastic-contaminated environment eventually tend to adapt to the extreme conditions and utilize these waste products as carbon and nitrogen sources. The utilization of various microorganisms for plastic degradation has a profound scope because such approaches are considered to be highly economical and ecofriendly (Narancic et al. 2012; Gu et al. 2000; Shimao 2001). These microorganisms are associated in the form of consortia and degrade the polymeric compounds by co-metabolism or gratuitous degradation (Bhardwaj et al. 2012). Enzymatic degradation is one of the main approaches that exists for plastic waste management by microorganisms. These microbial enzymes are successful in increasing the rate of biodegradation of plastic polymers effectively without any detrimental impact to the ecosystem (Bhardwaj et al. 2012; Wu et al. 2000; Shah et al. 2008). The microbial degradation is proven to be ecofriendly because of its non-toxic end products such as CO_2, H_2O and CH_4 (Shah et al. 2008). The screening of microbial consortia from highly polluted areas has profound scope and applications towards the degradation of plastics. Advanced biotechnology approaches emphasize the selective isolation and screening of such microorganisms for the effective degradation of various polymeric materials from the ecosystem (Bhardwaj et al. 2012). By considering all the issues caused due to plastic, current study aimed to find a safe, efficient and ecofriendly method for plastic degradation. This issue if neglected in the future, as it has been in the past, threatens to have a severe global impact, which will hamper the life of every individual on the earth. Though the degradation of plastic by microorganisms have been widely known to the scientific community, only few reports are available about the utility of novel types of microbial consortia towards plastic waste management and detoxification of long-chain polymers. This study provides an ecofriendly explanation by microbial consortia for the elimination of plastic from the environment, thus making Bangalore City a better place to live in.

The current study has also made an attempt to suggest the probable degradation mechanism by microbial lipase, a major enzyme responsible for the degradation of plastic and similar polymeric substances, by in silico approaches. Bioinformatics and computational biology approaches pave insight to understand the enzyme substrate binding which aids in suggesting the probable mechanism of enzymatic degradation of hydrocarbons (Rengachari et al. 2013). The binding property of various xenobiotic compounds and identification of main amino acid residues present in the binding cavity of enzyme could be used for site-directed mutagenesis

and similar studies. This may be supportive in understanding their role in the activity and substrate specificity which will be probably helpful for engineering novel microbial enzymes capable of degrading various non-degradable compounds.

Methodology

Description about the study area

Six sampling spots were identified based on the extent of plastic contaminations which cover the urban and rural areas of Bangalore (Fig. 1). The first sampling spot was Vrishabhavati Reservoir in Byramangala, Bangalore Rural District, India, situated between north latitude 12° 45′ 50.8 ″ and east latitude 77° 25′ 43.3″. It is located at a distance of 37 km from the research centre. The area was known to be a water reservoir, previously, which was used by people living in the vicinity. The main pollution includes plastic waste and garbage that gets thrown into the area by the residents (The Time of India 2007).

The second sampling spot was Mallathalli Lake, situated between the north latitude 12° 57′ 57.5″ and east latitude 77° 29′ 43.1″. It is located at a distance of 11.1 km from the research lab. This lake is situated in the Nagarbhavi area of west Bangalore. Previous studies have shown that Mallathalli Lake is a freshwater which has been contaminated by sewage and plastic garbage from the neighbouring residential areas in the last few years (Ravikumar et al. 2013).

The third sampling spot was Hebbal Lake, situated in the Hebbal industrial area in the north Bangalore, India, bearing the coordinates 13° 02′ 48.1″ north latitude and 77° 35′ 07.9″ east latitude. This spot is located 24 km away from the research centre. According to the report, this lake was once a thriving water body but now, has been heavily polluted with truckloads of plastic, weeds and silts (The Time of India 2007).

The fourth sampling spot was Vartur Lake, situated in the area Marathalli in the eastern part of Bangalore, India. This spot is located between north latitude 12° 57′ 02.5″ and east latitude 77° 44′ 26.6″ and at a distance of 26.1 km from the research centre. This lake serves as major water sources for the surrounding croplands. Recent studies revealed that this area has been excessively polluted by sewage, plastic wastes, garbage and industrial effluents (Ramachandra et al. 2011).

The fifth spot was the Bellandur Lake, situated in the south-eastern part of Bangalore, India between north latitude 12° 56′ 03.0″ and east latitude 77° 39′ 43.4″. This spot is located 14.7 km away from the research centre. Studies indicated that the water samples collected from this lake contained high content of municipal solid wastes, especially plastics, which were dumped in the lake (Pattusamy et al. 2013). The present study also observed an enormous content of plastic-derived waste materials embedded deep in the soil below the surface of the water and also in the soil surrounding the lake.

The sixth spot was the Yelachenahalli Lake in Bangalore, India bearing the coordinates 12° 53′ 58.9″ north latitude and 77° 33′ 56.5″ east latitude, located 1.3 km away from the research centre. There are plastic industries located a few metres away from the lake. The houses are situated at a distance of approximately 100 m from the lake. The lake was heavily contaminated with plastic and toxic chemicals that appeared as blackish colour with foul odour. The soil around the lake was also found to be embedded with enormous quantity of plastic bags.

Sample collection and transportation

The water and soil samples which were highly contaminated with plastic materials were collected from each hot spot with all necessary precautions. Approximately 500 g of soil samples in sterile zip lock bags and 2 L of water samples were collected in 5-L sterile containers by aseptic techniques (Sanders 2012). The samples were sealed properly and transported to the laboratory in ice boxes and stored at 4 °C. All the samples were processed within 24 h after collection. The environmental parameters such as temperature and pH were recorded at the time of sample collection from each spots which was found to be ranged from 20 to 26 °C and 7.0–8.0, respectively (Supplementary file, Table 1).

Isolation and screening of plastic-degrading bacteria

Both soil and water samples were enriched in nutrient broth (Hi-media, Mumbai, India) and serially diluted by standard methods (Geldreich et al. 1972). The serially diluted samples were used for the isolation of plastic-degrading bacteria. In all the experiments carried out in this study, plastic in its various forms were used as the sole source of carbon (Nanda et al. 2010). The various forms of plastic used in our study were UV-sterilized

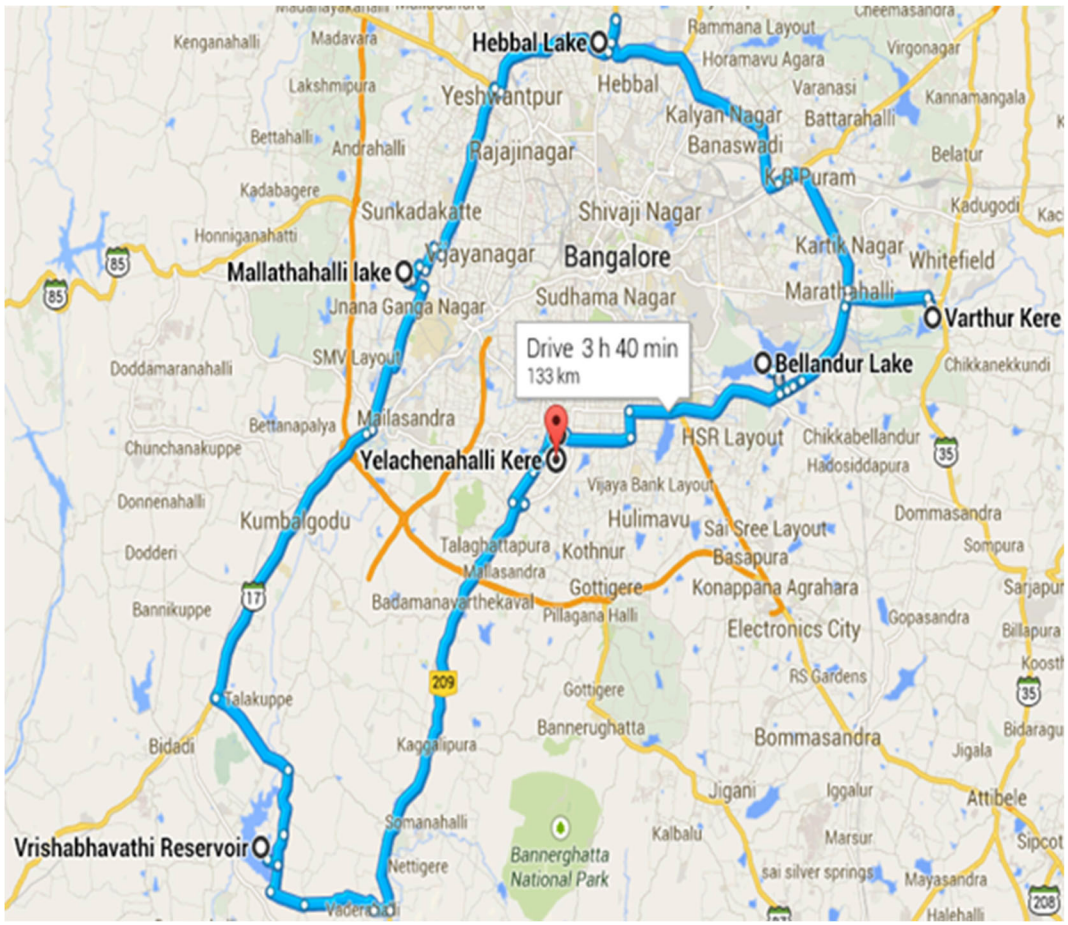

Fig. 1 Route map showing the place where the plastic-contaminated samples collected from urban and rural areas of Bangalore, India. Vrishabhavati reservoir is a part of Bangalore rural region. Mallathahallilake, Hebbal Lake, Vartur lake, Bellandur lake and Yelachenahallikere are parts of Bangalore urban region. (*Curtsey*: *Google maps*)

polyethylene glycol (PEG;(1.0 g/L), low density polyethylene powder (LDPE;(1.0 g/L), polyethylene pellets (1.0 g/L) and polyethylene strips (2 cm×15 cm size). For all the plastic forms, the isolation and screening were performed simultaneously.

The PEG-degrading bacteria were isolated by growing them in modified minimal salt media (Hemashenpagam et al. 2013). The minimal media contain ammonium sulphate (1.0 g/L), di-potassium phosphate (7.0 g/L), potassium phosphate (2.0 g/L), sodium citrate (0.5 g/L), magnesium sulphate (0.1 g/L) and bacteriological agar (2.0 g/L) and polyethylene glycol (1.0 g/L). The polyethylene glycol is replaced by dextrose as the sole source of carbon in all the experimental setup. The screening of PEG-degrading bacteria was performed by zone of clearance method (Dey et al. 2012). Isolation and screening of plastic-degrading bacteria were performed using modified minimal salt agar plates containing LDPE powder (1.0 g/L) as carbon source. The control media for all the sets were prepared by incorporating dextrose and usual carbon sources to the minimal media instead of plastic materials. The isolation and polymer degradation by microorganisms were carried out in control media to provide quality control assurance. The enriched samples were grown on agar plates for screening of plastic-degrading bacteria and the colonies thus observed were isolated and characterized.

Determination of the rate of plastic degradation by weight loss method

The isolated bacteria were inoculated in a minimal salt broth containing plastic strips (2 cm×15 cm size) and pellets (1.0 g/L), which were prepared from the

collected samples. The degradation was determined periodically by weighing the strips and pellets for a period of 120 days (Nanda et al. 2010).

The current study further focussed on a comparative analysis of the rate of plastic degradation of bacterial isolates with known strains. The type strains which were previously known to degrade hydrocarbons were obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India, for comparative analysis. The three bacterial cultures obtained from MTCC were *Pseudomonas putida* MTCC 2445, *Bacillus subtilis* MTCC 9447 and *Pseudomonas stutzeri* MTCC 2643. The three strains were independently tested for plastic degradation, and the efficiency of the isolates screening in the present study was compared with known type strains.

Microbial characterization of isolated bacteria

The morphological characteristics of isolated bacteria were determined by Gram staining (Beveridge 2001). The isolates were plated on MacConkey's agar (Hi-media) for the selective isolation of Gram-negative bacteria. The isolates were further plated on milk agar with cetrimide (Hi-media, India), a selective medium for *Pseudomonas* spp. This is performed based on the fact that previous studies revealed most of the hydrocarbon-degrading bacteria belonged to *Pseudomonas* spp. (Kyaw et al. 2012; John et al. 2012). The bacterial isolates were further characterized by standard physiological and biochemical tests. The various biochemical tests performed were IMViC (Lee et al. 2003), triple sugar iron (Stager et al. 1983), mannitol motility nitrate (Titters and Sancholzer 1936), urease (Klein et al. 1991), catalase (Taylor and Achanzar 1972) and oxidase (Jurtshuk and Mcquitty 1976) tests.

Optimization of growth parameters

The isolated bacteria were grown under varying condition of pH and temperature to optimize the growth parameters. The pH variation includes 5.54, 6.13, 7.27, 8.55 and 9.02. Similarly, temperature variations used in the study were 4, 25, 37 and 45 °C. The minimal salt media with LDPE as the sole source of carbon was used for further optimization. Moreover, the isolated bacteria grown in the minimal media with plastic strip as the carbon source under optimized conditions were performed, and the degradation ability was determined by weight loss method over a period of 90 days.

Enzyme extraction

The literature survey suggested that lipase might be one of the major enzymes responsible for plastic degradation (Uchida et al. 2000; Bhardwaj et al. 2012). The identification of the presence of lipase in the isolated bacteria was initially performed by zone of clearance method (Gupta et al. 2004) where the bacterial isolates were grown in a Luria Bertani agar (Hi-media, India) containing 1 % Tween-80 as the substrate for lipase (Gupta et al. 2004). Once the zone of clearance was observed around the colonies, the isolated bacteria were grown in minimal salt media containing olive oil as substrate for lipase (Gupta et al. 2004). This medium was incubated at 37 °C for 8 days in a shaker incubator. After incubation, the bacterial culture was subjected to centrifugation where the pellet was discarded and the supernatant was subjected to ammonium sulphate precipitation (20–40 % saturation) (Kukreja and Bera 2005). The solution was further centrifuged, the supernatant discarded and the pellets were suspended in phosphate buffer and stored at 4 °C. This was further subjected to dialysis (Gupta et al. 2004). The dialyzed samples were run on SDS-PAGE (Al-Tubuly 2000) to identify the presence of enzyme. Lowry's test was also performed to check for presence of protein (Sengupta and Chattopadhyay 1993).

Formulation of microbial consortia

Studies have shown that the interaction between various microorganisms in consortia enhances the rate of plastic degradation (Tribedi et al. 2012; Roy et al. 2008). Previously weighed plastic strips were incorporated in the minimal media broth as the sole source of carbon. The pure cultures of two isolated bacteria which showed better plastic degradation ability were used to formulate the consortia. Roughly, 100 µl of each of the bacterial isolates were inoculated into a conical flask containing nutrient media. The pure isolates were inoculated individually in separate nutrient media for comparative degradation analysis. The degradation of plastic strips was determined by weight loss method over a period of 90 days (Nanda et al. 2010).

Study of the enzyme-ligand interaction by computational docking

Computer-aided studies were carried out to hypothezise the probable mechanism and the interaction between microbial lipase and major polymeric substances present in plastics. Polyethylene and polystyrene were identified as major polymeric substances present in plastic materials based on extensive literature survey (Tokiwa et al. 2009). The three-dimensional structures of lipase (open conformation; PDB: 2LIP) (Schrag et al. 1997) and extracellular lipase (PDB: 2Z8X) (Angkawidjaja et al. 2007) from *Pseudomonas* spp. were retrieved from PDB (http://www.rcsb.org/pdb/home/home.do) (Sussman et al. 1998). Similarly, the three-dimensional structures of polystyrene and polyethylene were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/) (Sussman et al. 1998) database. The receptor-ligand interactions were studied by molecular docking using PatchDock Server (http://bioinfo3d.cs.tau.ac.il/PatchDock/) (Schneidman-Duhovny et al. 2005) server. The docked complexes were visualized using PyMOL (http://www.pymol.org) (Seeliger and de Groot 2010).

Results and discussion

The sampling spots were identified in such a manner that it covered all the four corners of Bangalore District including urban and rural areas (Fig. 1). The hot spots that were highly contaminated with huge portion of plastic garbage which directly enters into surrounding fresh water and agricultural farms were observed during the survey and sampling of this study. The environmental parameters observed during the sample collection have been shown in supplementary file (Supplementary file, Table 1). After the selective enrichment (El-Fantroussi 2000), the bacteria were allowed to grow for 72 h and stored at 4 °C for further studies.

The present study focused on the degradation of polyethylene glycol, a major polymer present in most of the plastic products. The screening of polyethylene glycol (PEG)-degrading bacteria was carried out by zone of clearance method (Dey et al. 2012). White coloured, small, circular, opaque and translucent bacterial colonies were observed in the agar plates which contain PEG. The plates were stained with coomassiee brilliant blue, and the colonies were observed for zone of clearance. Upon staining, very tiny distinct zones of clearance around most of the colonies were observed in the medium. These colonies indicated that they have the ability to utilize the PEG as sole sources of carbon in the absence of such sources in the medium. Previous studies revealed that molecular weights of PEG ranging from 200 to 1500 were capable of being degraded by many bacteria, fungi and actinomyces (Obradors and Aguilar 1991). The current study showed that PEG could be utilized by the microorganisms isolated from all the samples collected. The growth of colonies was observed within 72 h of incubation.

This study further tested the degradation of plastic polymers by the bacterial isolates using weight loss method (Nanda et al. 2010). The collected plastics from the sites were made in the form of pellets (1 g/L) and strips (2×15 cm long) and incorporated in the minimal media as carbon source. This study observed a 20–50 % reduction in the weight of plastic strips (Fig. 2) and a 3–5 % reduction in weight of plastic pellets (Fig. 3) over an incubation period of 120 days. From these results, it is clear that the rates of degradation for plastic strips by microorganisms are slightly faster than that of the plastic pellets. This is probably due to difference in the surface area of plastic strips and pellets. In plastic strip, more numbers of bacteria can colonize because of wide surface areas. Hence, present study suggests that the plastic materials with more surface area and planar geometry may degrade in faster rate. Similarly, other probable parameter responsible for the degradation efficiency of microorganisms is the position of the functional groups in the hydrocarbon chain. There are reports which revealed that the degradation of plastics by bacteria and fungi are very slow processes and the degradation efficiency is mainly depending on the position functional groups and other side chains in the aromatic ring. If the side chain is in meta-position, microbial degradation may take place in faster rate than the ortho and para-positions. Recent studies reported that there was 20 % reduction in the weight of polythene films over an incubation period of 120 days (Kyaw et al. 2012). Another study suggested that there was 25–45 % reduction in the weight of plastic strips incorporated as carbon source in liquid media inoculated with the samples collected from waste disposal sites, drainage sites and soil from sewage sludge (Nanda et al. 2010).

The screening of plastic-degrading bacteria was also performed using low density plastic powder. In the present study, plastic powder incorporated in solid minimal

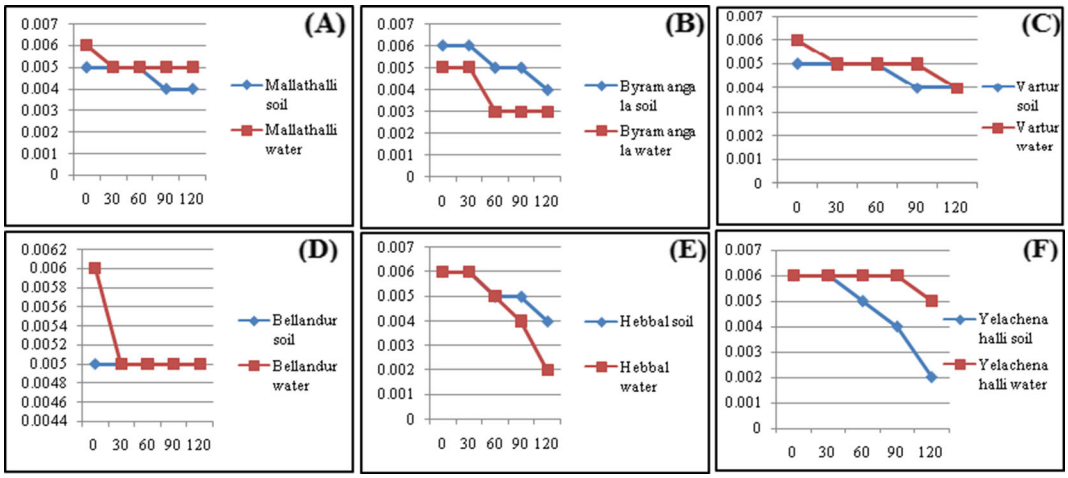

Fig. 2 The graphs show the biodegradation of plastic (in strip form) from the selected hotspots in minimal media by one of our isolate using weight reduction method. The graphs were plotted by considering the time (in days) along the *x*-axis and weight the plastic (in gram) along the *y*-axis

media as sole carbon source showed the growth of colonies upon 72 to 96 h of incubation. This clearly showed that the isolates screened in the present study were capable of utilizing plastic as their sole carbon source. Studies carried out in Seoul, Korea, revealed that the samples collected from crude oil-contaminated soil showed positive activity against PE powder under similar growth conditions (Yoon et al. 2012). Another study suggested that zone of clearances were observed around the bacterial colonies inoculated in suitable conditions at 37 °C for a period of 10 days (Usha et al. 2011).

From the recent literatures, it is evident that most of the bacteria capable of degrading long-chain polymers present in the plastics are Gram-negative bacilli (Kyaw et al. 2012; Nanda et al. 2010; John et al. 2012; Usha et al. 2011). Further, studies have revealed that among Gram-negative bacilli, *Pseudomonas* spp. are the major plastic-degrading bacteria (Kyaw et al. 2012; John et al. 2012; Tribedi et al. 2012). Reports suggested that there is a direct correlation between cell surface hydrophobicity of an organism and its potential role in degradation of many non-polar polymers. It is reported that the cell

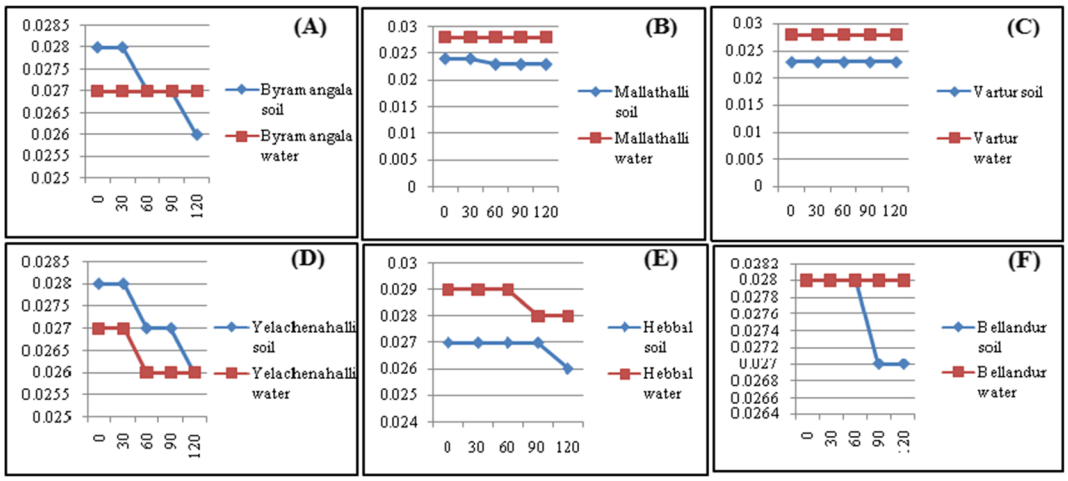

Fig. 3 The set of graphs showing weight reduction of plastic pellets in the six soil and water samples. *x*-axis: time in days; *y*-axis: weight in grams. **a** Reduction observed in Byramangala soil sample. **b** No appreciable reduction observed. **c** No reduction observed during uncubation period. **d** Reduction observed in both soil and water samples. **e** Little reduction observed in both soil and water samples. **f** Little reduction observed in water sample

surface hydrophocity features are the key component in the degradation of various hydrocarbons by *Pseudomonas* spp. (Tribedi and Sil 2013). Hence, the present study focuses to identify the presence of similar type of bacteria from the collected samples. The isolates were grown on MacConkey agar plates (Mossel et al. 1962), a selective medium for Gram-negative bacteria, for an incubation period of 48 h. Pale yellow, circular, non-lactose fermenting colonies were observed. All the collected samples showed the presence of Gram-negative bacilli and coccobacilli after Gram staining (Beveridge 2001). Similarly, the isolated bacteria were also grown in milk agar with cetrimide for the preliminary detection of *Pseudomonas* spp. (Szita et al. 1998). The isolates were showed green pigmented, circular and opaque colonies after the incubation; these are the physiological features of *Pseudomonas* spp.

Characterization of Gram-negative isolates were performed by standard microbiology techniques. Among various isolates, two showed affirmative results for all the biochemical tests and these isolates were screened from the water and soil samples collected from Hebbal, Bangalore, India. The microbiology features of these two isolates have shown in Table 1. From these results, present study concludes that the isolates were characterized to be two different species of *Pseudomonas* because they showed variations in mannitol fermentation (Table 1). The comparative analysis of the degradation efficiency of the bacterial isolates with known isolates from MTCC was further performed. Previous studies revealed that these bacteria showed significant reduction in polymer degradation by weight loss method (Obradors and Aguilar 1991; Tosin et al. 2012). However, it was found that these bacteria were unable to grow in the modified minimal media used in this study. Moreover, MTCC strains showed less rate of degradation in comparison with the isolates screened in the present study (Fig. 4). Hence, it is clear that the isolates have better ability to degrade polyethylene glycol and similar types of polymers than known strains.

When both the strains of *Pseudomonas* were grown at a wide range of pH and temperature, this study observed that these bacteria showed maximum growth under extremely alkaline conditions of pH 9.0 and an optimum temperature of 37 °C. Further, the weight reduction of plastic strips under the optimum conditions was found to be roughly 35–40 % over a period of 120 days (Fig. 4).

The microbial degradation of plastic materials is mainly due to enzymatic activities. Many studies revealed that microbial lipases are one of main enzymes responsible for hydrocarbon degradation (Uchida et al. 2000; Bhardwaj et al. 2012). Hence, this study focused on the identification of microbial lipases from the bacterial isolates. To test the presence of lipase activity of *Pseudomonas* spp., the growth of the screened isolates was observed in a minimal media within 48 h of incubation with Tween-80. Further, to obtain a fair quantity of the enzyme, isolates were grown on minimal media containing olive oil as the sole source of carbon. As lipase from *Pseudomonas* spp. is known to be extracellular in nature, Lowry's test was carried out for the determination of protein content (Sengupta and Chattopadhyay 1993). The protein content was found to be 960 μg/ml of crude extract. After ammonium sulphate precipitation followed by dialysis, when the protein content was estimated again, there was a reduction in protein content, 120 μg/ml of sample. Previous studies revealed that lipase as its extracellular forms, extremely smaller amount, was present in the culture broth (Shabtai and Daya-Mishne 1992). The decrease of protein content after dialysis, hence, attributed to the fact that the lipase content in the culture broth might be less. Further, SDS-PAGE analysis showed extremely light band of protein, indicating less enzyme content in the broth supernatant.

There are reports that the microbial consortia have better degradation efficiency than individual isolates (Tribedi et al. 2012; Cosgrove et al. 2007; Hanson et al. 1999). Thus, the two isolates of *Pseudomonas* spp. were formulated as consortia and grown in the medium containing plastic materials. Along with the consortium, cultures of the individual isolates were also incubated with the minimal medium in presence of plastic polymers under similar conditions for comparison. The results revealed that the microbial consortia showed 40 % weight reduction upon 90 days of incubation period. However, the degradation rates of the individual isolates were found to be less compared with the consortia (Fig. 5). Hence, it is clear that when the isolates formulated as microbial consortia, it showed better degradation competence than the microorganisms employed independently. Previous studies reported that potential bacterial consortia formulated by randomly combining bacteria such as *Microbacterium* spp.,

Table 1 Microbiological characterization of the isolate showed best degradation

Morphological features		Physiological characteristics				Biochemical characteristics													Organisms
Morphology	Motility	Milk agar with Cetrimide	MacConkey agar	pH	Tem[n]	I[a]	Mr[b]	Vp[c]	C[d]	TS[e]	Hs[f]	G[g]	M[h]	Nr[i]	O[j]	C[k]	U[l]	Sh[m]	
Gram-negative long rods	+	Light greenish coloured pigmented colonies	NLF[o], light yellow colonies	9.0	37 °C	−	−	−	+	−	−	−	−	+	+	+	+	−	*Pseudomonas* spp.
Gram-negative short rods	+	Light greenish coloured pigmented colonies	NLF, light yellow colonies	9.0	37 °C	−	−	−	+	−	−	−	+	+	+	+	+	−	*Pseudomonas* spp.

[a] Indole

[b] Methyl red

[c] Voges Proskauer

[d] Citrate

[e] Triple sugar

[f] Hydrogen sulphide production

[g] Gas production

[h] Mannitol fermentation

[I] Nitrate reduction

[j] Oxidase

[k] Catalase

[l] Urease

[m] Starch hydrolysis

[n] Temperature

[o] Non-lactose fermenter

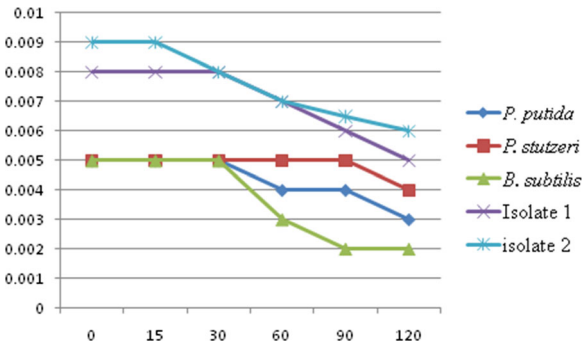

Fig. 4 Comparative analysis of the biodegradation efficiency of our isolate over MTCC cultures. The graph plotted by considering the time (in days) along the *x*-axis and weight of the plastic (in gram) along the *y*-axis. Figure revealed that our *isolates 1 and 2* showed 35–40 % degradation in a span of 120 days under optimized conditions in comparison with known isolates

Pseudomonas putida, *Pseudomonas aeruginosa*, *Pseudomonas otitidis*, *Bacterium* Te68R, *Bacillus aerius*, *Bacillus cereus* and *Acanthopleurobacter pedis* showed successful degradation of low density polyethylene (Sah et al. 2011; Anwar et al. 2013).

This study further focused to predict the probable mechanisms of polymer degradation by microbial enzymes. The enzyme-ligand interaction studies were carried out by molecular docking. The three-dimensional structure of the ligand, oxidized polyethylene, which was retrieved from PubChem database, was docked with lipase (open conformation, PDB: 2LIP) from *Pseudomonas* spp. (Schrag et al. 1997). The score of the docked complex was found to be 4008, and its best minimum binding energy was determined to be −140.92 kcal/J. The main interacting residues found in the active site were Asp 228, Thr 231, Ile 232, leu 234, Asn 239 and Phe 249, and the interactions are stabilized by network of hydrogen bonding (Fig. 6a). Similarly, the ligand polystyrene was docked with the enzyme with best minimum binding energy of −372.91 kcal/J, and the docking score was found to be 5324. Here, Val 26 and Leu 27 were found to be interacted with the ligand by covalent bonding (Fig. 6b).

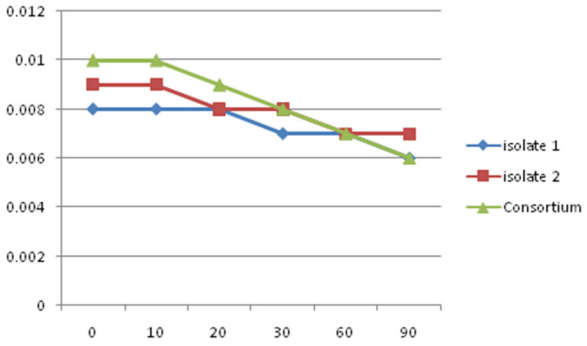

Fig. 5 Comparative analysis of the biodegradation efficacy of bacterial consortia over individual isolates. The graph plotted by considering the time (in days) along the *x*-axis and weight of the plastic (in gram) along the *y*-axis. Figure revealed that microbial consortia showed 40 % degradation in 90 days which is higher in comparison with individual isolate

When oxidized polyethylene was docked with extracellular lipase (PDB: 2Z8X) from *Pseudomonas* (Angkawidjaja et al. 2007), the best minimum binding energy and the score was obtained to be −166.94 kcal/J and 4022, respectively. The main residues responsible for the ligand binding identified to be Tyr 29, His 30, Asn 31, Leu 32, Gly 60, Thr 61, Gln 64, Gly 65, Ile 70, Arg 106, Gln 120 and Arg 141. The interactions are stabilized by a network of hydrogen bonding and covalent interactions. This docked complex showed the maximum interaction between the ligand and receptor (Fig. 6c). The ligand polystyrene was further docked with the extracellular lipase which showed a minimum energy of −235.96 kcal/J and score of 5382. The main interacting residues present in the active site were found to be Ser 28, Tyr 29, Leu 32, Asn 62, Ser 63, Gly 65, Asn 104, Arg 106, Thr 108, Gln 120 and Arg 147 and the interactions were stabilized by hydrogen and covalent bonding (Fig. 6d). There are many studies reported that molecular docking and computational simulations have crucial role to understand the biodegradation mechanism of various xenobiotic compounds by microbial enzymes. These approaches pave significant insight towards detoxification mechanism of various lead molecules and thereby understand the mechanism of stable binding of hydrocarbon derivatives to the active site of the enzymes (Rengachari et al. 2013; Cao et al. 2011; Bosma et al. 2002). In the present study, the probable mechanism of interaction between microbial lipase and selected polymeric compounds were suggested based on computational prediction. Hence, further experimental analysis is required to validate the hypothesis. The conclusion drawn from the present in silico studies may provide crucial insights for understanding the degradation mechanism of plastic polymers by microbial consortia.

From this study, it is clear that bacteria screened from Bangalore urban and rural areas of plastic pollution showed high ability to degrade polymers such as

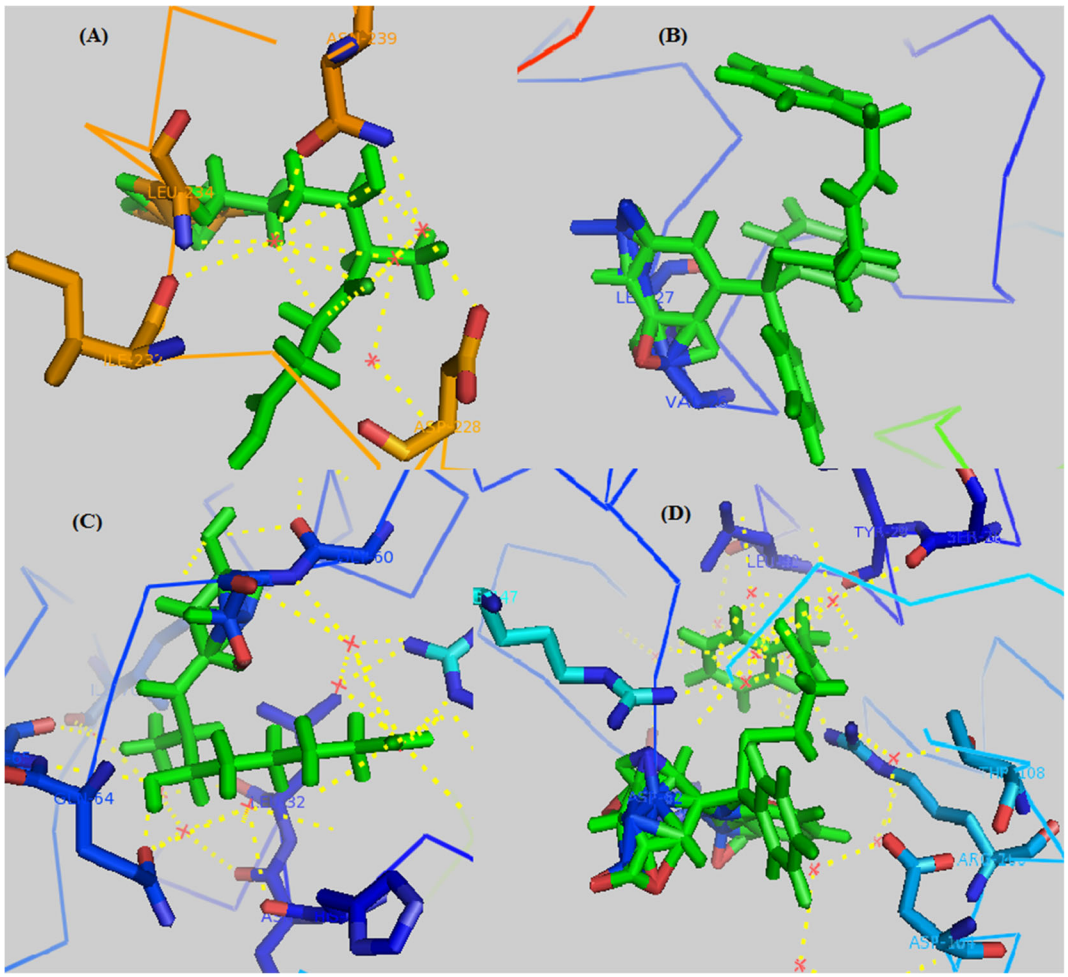

Fig. 6 Molecular interaction between microbial lipase of *Pseudomonas* spp. and selected polymeric compounds analysed by docking studies. The ligand (shown in green coloured stick figures) binds to the (shown in various coloured sticks) active site of enzyme by networks of hydrogen bonding (shown in *dotted lines*) and other interactions. **a** Interaction between lipase open conformation (PDB: 2LIP) and oxidized polyethylene. **b** Interaction between lipase open conformation (PDB: 2LIP) and polystyrene. **c** Interaction between extracellular lipase (PDB: 2Z8X) and oxidized polyethylene. **d** Interaction between extracellular lipase (PDB: 2Z8X) and polystyrene

polyethylene glycols. These bacteria if formulated in a consortium would be a reliable approach for plastic degradation. Thus, the present study concludes that biodegradation of polymeric materials by microbial consortia serve as a promising approach for environmental monitoring and elimination of plastic garbage. Present study focuses the formulation of microbial consortia using two isolates of *Pseudomonas* spp. which showed better degradation abilities than the known strains. Hence, there are many other polymer-degrading microorganisms that can also be characterized and formulated as consortia and their degradation efficiency can be tested. Furthermore, consortia can be formulated with actinomycetes and fungi which may probably give better results for plastic and polymer degradation.

Recent report suggested that the microbial consortia formulated from highly diverse environmental microflora which share similar composite phylum showed high degradation of lignocellulolytic and other types of polymers (Wongwilaiwalin et al. 2013). The current study has drawn conclusion based on experimental analysis performed for a limited period of time. Hence, the consistency of the finding, especially in the graphs, is not satisfactory. The study can be further extended for a long period with many replicates which may give more consistent results. However, it is assured that the present

study paves foundations to carry out such kinds of long-term analysis.

Conclusion

The current study aimed to devise an approach of long-chain hydrocarbon degradation by microorganisms such as bacteria which were screened from plastic garbage collected from urban and rural areas of Bangalore, India. The screening and characterization of the best two plastic-degrading isolates identified to be *Pseudomonas* spp. To achieve a higher rate of degradation, the growth parameters for the isolated bacteria were optimized at which 40 % reduction in weight of plastic was observed in a span of 120 days. Further, a consortium of the isolated bacteria was formulated to study the symbiotic effects of bacteria on plastic polymers. A steady reduction in the weight of plastic was observed. A comparative analysis of the percentage of plastic degradation between known strains and the isolates screened in the present study were carried out, and the result indicated that the latter have better ability to degrade polyethylene glycol. One of the major enzymes responsible for hydrocarbon degradation was preliminarily carried out and which was identified to be microbial lipases. Bioinformatic analysis showed that polystyrene and polyethylene have good interaction with lipases enzymes which hypothesize degradation mechanisms. Thus, the present study suggests that novel microbial consortia screened from various polluted areas can be used as cost-effective, ecofriendly and safe approach for the elimination of plastic polymers and thereby find an effective method for plastic waste management. However, in the present study, the conclusions were drawn based on a short-term study with limited observations. As biodegradation by microorganisms is a slow process, this study need to be extended further for long periods. In the current study, the consortia were formulated using two isolates which showed better degradation than known strains; hence, other microorganisms responsible for plastic degradation are also needed to be screened, characterized and formulated. These organisms can be used as effective microbial consortia for better degradation of various types of long-chain polymers. Present data provide crucial insights for many future concerns and perspectives.

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