Review Article

Microbial ecology: A new perspective of plastic degradation

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Abstract

Plastic pollution has emerged as an inevitable socio-environmental cause of pollution with ever increasing loads of plastic in everyday life. Extensive use of plastics especially disposable plastic (single-use) and presence of these non-degradable plastics in environment posing great menace to biodiversity in many habitats by disturbing ecosystem functioning. The purpose of this review is to explore the role of free living or symbiotic microbes in plastic degradation documented in the literature. Millions of plastic bags, dispensing containers, packaging and drinking bottles are thrown into the environment after single use every day. Polyethylene terephthalate (PET), Low density polyethylene (LDPE), Polypropylene (PP), Polystyrene (PS) and Expanded polystyrene (EPS) are major types of plastics used in the manufacture of millions of plastic bags, dispensing containers, packaging, food packaging film and drinking bottles are thrown into the environment after single use every day. These plastics accumulate in aquatic reservoirs and affects feeding guilds; ultimately disturbing community structure and ecosystem productivity. Five bacterial strains (belonging to genera *Pseudomonas* and *Bacillus*) have been isolated and found effective in degrading plastic. Recently, few insect larvae have been explored for their ability to feed on plastics; numerous bacterial strains, showing potential for degrading plastics, have been isolated from the guts of *Tenebrio molitor*, *Tenebrio obscurus* and *Plodia interpunctella* larvae. Biodegradation of plastics can prove to be an environment friendly way of eliminating plastics waste from environment. Elaborative work leading to the identification of specific enzymes and degradation pathways can help in plastic degradation at industrial level.

Keywords: Biodegradation; Insect larvae; Microbial species; Microplastics

Introduction

Urbanization over the past few centuries has been associated with increased demand for plastic products not only in industry but daily life as well. Until now, about 63000 million metric tons of plastic litter has been produced globally. This increased accumulation of plastics is impending threat to environment

as well as the living organisms inhabiting it [1]. Plastics are synthesized from different polymers like PVC, polyethylene, nylon etc and these polymers give specific properties to the plastic products [2]. Plastics are generally considered to be resistant to degradation [3]. However, the discovery of few insect and microbial species capable of degrading

plastics, is a revolutionary step towards biodegradation of plastics. Light and mechanical factors also play some role in overall process of plastics degradation. Different plastic types degraded by microbes have been summarized in (Table 1).

Microplastics

Plastics that are less than 5mm in length are called microplastics and are highly diverse in their properties [4]. As compared to aquatic habitats, microplastics are predominantly found in soils [5, 6]. The effect of microplastics on natural systems is similar to other chemical pollutants [7, 8]. Plastics are harmful due to the toxic chemicals used in composition as well as the hazardous wastes that accumulate on plastic surface [9, 10]. Suspension and deposit feeders are mainly affected by microplastics floating in water [11]. Microplastics can change the natural distribution ranges of species that drift with water currents [12, 13]. In soil, microplastics block the spaces which causes hinderance in the locomotion of soil arthropods [14]. Microbes use these microplastics as a source of energy [15]. When animals feed on microplastics, harmful chemicals get accumulated in their tissues [16]. Plastic scrap has been found in the intestinal tract of various fish species [17]. When microplastics are exposed to radiations, they become suitable for further decomposition [18].

Hazardous effects of plastic wastes on living organisms

Accumulation of plastic waste is continuously reducing the quality of aquatic ecosystems [19, 20]. The threats linked to plastic waste are because of its chemical composition as well as because of chemicals in the environment that get deposited over the surface of plastic [9, 10]. Plastic contamination in marine and freshwater habitats is continuously increasing [21]. *Rhodobacterales*, *Rhizobiales*, *Streptomycetales* and *Cyanobacteria* are the keystone species that inhabit the plastic

surface. Aquatic plastic debris is a possible source of transporting pathogenic *Vibrio* species. Polyethylene terephthalate and Polystyrene are dominated by *Alphaproteobacteria* and *Gammaproteobacteria*. Majority of the microplastics are inhabited by *Betaproteobacteria* [22]. Bacterial communities found on plastic are distinct from those found in surrounding water [23]. After being ingested by organisms, these harmful compounds get accumulated in the tissues [24]. This leads to bioaccumulation of harmful chemicals in food webs [25, 26] and causes dysfunction of the endocrine system, reduced diversity and decreased survival rate of aquatic species [27, 28, 29].

Types of plastics on basis of biodegradation

Biodegradable plastics

These are the group of synthetic polymers that can be degraded by living organisms [30, 31]. The microbes responsible for plastics degradation can be found in aerobic as well as anaerobic environment [32]. These include members of archaea, bacteria and some eukaryotic organisms as well [33, 34]. These polymers are firstly degraded into monomers by heat and electromagnetic radiations and then, the monomers are consumed by the microbes [35]. The rate of degradation in aerobic conditions is measured by the amount of carbon dioxide produced [36].

Polyhydroxyalkanoates (PHA)

These are produced within the microbes as a reservoir of energy and they make up about 90% of the dry weight of cells [37, 38]. Many genera of bacteria are capable of producing polyhydroxyalkanoates (PHAs) [39, 40]. Synthetic PHAs are also biodegradable and are non-toxic to living organisms [41]. The diverse properties of PHAs are due to a large number of monomers- about 150 [42]. Depolymerases from different microbes are responsible for the degradation of PHAs [43]. Polyhydroxybutyrate can be completely

degraded in soil [44, 45]. Anaerobic decomposition of PHAs results in the production of methane as a byproduct [46]. *Streptovorticillium kashmeriense* strain AFI can degrade polyhydroxybutarate [47]. *Penicillium simplicissimum* LAR 13 and *Paecilomyces farinosus* LAR 10 degrade PHB at 28°C-37°C [48].

Polyvinyl chloride (PVC)

Chloroethyl groups are linked to form PVC which consists of 57% chlorine and 43% carbon [49]. Information about the degradation of PVC is scarce and only few PVC degrading microbes have been discovered [50]. Extensive research has been done on the physical decomposition of PVC [51]. White-rot fungi (*Phanerochaete chrysosporium*) can be a possible biodegrading agent of PVC because of its low molecular weight [52]. Polymer degrading fungi are the major contributors of carbon to carbon cycle [53, 54].

Polyester polyurethane (PU)

It is synthesized by the condensation of polyol and isocyanate which results in the formation of urethane linkages [55]. If polyurethane is buried in soil for 5 months, there is a 95% reduction in its tensile strength. Fungi are mainly responsible for degrading PU in laboratory conditions. *Geomyces pannorum*, *Nectaria* and *Phoma spp* are the fungal species capable of degrading polyurethane [56]. The ester and urethane linkages are hydrolyzed by the microbes due to which tensile strength decreases. Polyurethane esterase is a polyurethane degrading enzyme derived from *Comamonas acidovorans* TB-35 [57]. A bacterial species *Pseudomonas chlororaphis* utilizes polyurethane as a source of carbon and energy [58]. Diethylene glycol, trimethylolpropane and adipic acid are the metabolites derived from the breakdown of

ester linkages in polyurethane [59]. Polyurethane degrading enzymes have been classified as esterases [59], lipases [60], ureases and proteases [61]. Polyurethane degrading enzyme, PueB lipase has been isolated from *Pseudomonas chlororaphis* [62] and *Comamonas acidovorans* [63, 64].

Polyethylene (PE)

Polyethylene is composed of a straight chain of carbon atoms that serves as the backbone of this polymer. It is the most abundant plastic waste because its production is four times more than other types of plastics [65]. It was previously believed to be non-degradable because of high molecular weight and water repelling property [66]. Burning of PE releases cancer causing compounds and greenhouse gases into the atmosphere [67]. PE in soil negatively affects drainage, soil organisms and minimizes soil quality [68]. Many species of bacteria and fungi have the ability to decompose PE [69]. It can be slowly degraded by treating with bacterium *Nocardia asteroides* [70]. *Alcanivorax borkumensis* forms thick films on low-density polyethylene and degrades it [71].

Polyethylene tetrathalate (PET)

PET is formed by the bonding of terephthalic acid and ethylene glycol [72]. Only few PET degrading microbes have been discovered [72]. Genomic database of five PET degrading bacterial strains i.e., *Bacillus thuringiensis* strain C15, *Pseudomonas* sp. B10, *Pseudomonas* sp. SWI36 and *Bacillus albus* strain PFYN01, have been studied in detail [73, 74]. Bacterium *Iadonella sakaiensis* produces PETase enzyme to degrade ester linkages in PET [75]. It has been reported that, the diatom *Phaeodactylum tricornutum* can be converted into a chassis for PET degradation [76]. *Ideonella sakaiensis* can degrade PET at a faster rate [75].

Table 1. Microbes involved in degradation of plastics

Type of plastic	Bacteria	Fungi
Polyhydroxybutyrate	<i>Streptovorticillium kashmeriense</i> strain AF1 [47]	<i>Penicillium simplicissimum</i> LAR13 and <i>Paecilomyces farinosus</i> LAR 10 [48]
Polyurethane	<i>Pseudomonas chlororaphis</i> [58], <i>Comamonas acidovorans</i> TB-35 [57]	<i>Geomyces pannorum</i> and <i>Nectaria</i> [56]
Polyvinyl chloride		<i>Phanerochaete chrysosporium</i> [52]
Polyethylene	<i>Nocardia asteroides</i> [70] and <i>Alcanivorax borkumensis</i> [71]	
Polylactic acid (PLA)	<i>Amycolatopsis</i> sp. strain SCM_MK2-4, <i>Amycolatopsis thailandensis</i> , <i>A. orientalis</i> , <i>Bacillus pumilus</i> , <i>B. cereus</i> , <i>B. brevis</i> , <i>B. licheniformis</i> [88]	
Polyethylene terephthalate (PET)	<i>Idonella sakaiensis</i> [75], <i>Bacillus thuringiensis</i> strain C15, <i>Pseudomonas</i> sp. B10, <i>Pseudomonas</i> sp. SWI36, <i>Bacillus albus</i> strain PFYN01 [73, 74] and <i>Phaeodactylum tricornutum</i> [76]	
Nylon (Polyamide)	<i>Arthrobacter</i> sp. Strain KI72, <i>Bacillus cereus</i> , <i>B. sphaericus</i> , <i>Vibrio furnissi</i> , <i>Brevundimonas vesicularis</i> , <i>Pseudomonas aeruginosa</i> strain PAO1 [82, 84, 85]	
Low density polyethylene (LDPE)	<i>Alcanivorax borkumensis</i> [71]	

Polystyrene (PS)

It consists of many styrene molecules that link together to form polystyrene [77]. Mealworms (larvae of *Tenebrio molitor*) are able to decompose PS into CO₂ and other metabolic byproducts [78]. Within 12-14 hours, half of the PS in the gut of mealworms gets digested [79]. The anaerobic bacteria in anterior gut of mealworms include *Lactococcus* and *Pantoea* while in the posterior gut, *Enterobacter* and *Clostridium* are abundant [80].

Polyamide

The compounds that contain repeating amide linkages –CO–NH– are called polyamides [81]. Proteins, silk and nylon are few examples of polyamides. *Arthrobacter* sp. Strain KI72 can grow on 6-aminohexanoate (a derivative of nylon). Three enzymes

responsible for decomposing 6-aminohexanoate are cyclic-dimer hydrolase, dimer hydrolase and oligomer hydrolase [82]. *nylD1* and *nylE1* genes are responsible for the metabolism of secondary 6-aminohexanoate [83]. *Bacillus cereus*, *B. sphaericus*, *Vibrio furnissi* and *Brevundimonas vesicularis* are potential degraders of nylon [84]. *Pseudomonas aeruginosa* strain PAO1 can efficiently degrade 6-aminohexanoate linear dimers [85].

Polylactic acid (PLA)

It is a certified polyester used mainly for the packing of various industrial products and it can be degraded at industrial level [86]. Higher temperature around 60°C is needed for biodegradation of PLA in industries [87]. Proteases obtained from *Amycolatopsis* sp.

strain SCM_MK2-4, *Amycolatopsis thailandensis*, *A. orientalis* and lipases extracted from *Bacillus pumilus*, *B. cereus*, *B. brevis*, *B. licheniformis* species can degrade PLA [88]. An increase in the molecular weight of PLA decreases its susceptibility to microbial degradation [89]. Synthesis of low molecular weight PLA is a possible way for increasing its biodegradability.

Non-biodegradable plastics

Starch containing plastics

These plastics contain cornstarch (40% dry weight) in combination with polyethylene and polyethylene-co-acrylic acid (EAA). When exposed to the aquatic environment, starch present in these plastics is decomposed by amylolytic microbes [90]. Degradation of starch from these plastic films reduces the tensile strength due to which further disintegration of plastic film is possible [91]. Few studies indicate that polyethylene present in the starch containing plastic films can also degrade at a slower rate due to various microbial and photochemical degradation processes [92].

Symbiotic microbes in insect guts

Many insect species have been found to have microbial association for plastic degradation. This symbiotic relationship shown by different insect species with different microbes has been summarized in (Table 2).

Darkling beetle (*Tenebrio molitor*)

Yellow mealworms (larvae of *T. molitor*) are omnivores and they can feed on a variety of food items due to the adaptability of gut microbiota [93]. Microbes present in the gut of mealworms are able to degrade polyurethane, polystyrene, polyvinyl chloride and polylactic acid. After 24 hour retention in gut, the larvae effectively decompose styrofoam [93]. A polyurethane and polystyrene degrading bacterial species, *Exugibacterium* sp. YT2 has been isolated from the gut of *T. molitor* larvae [94]. Two operational taxonomic units of bacteria i.e.

Citrobacter and *Kosakonia* are associated with polyethylene and polystyrene degradation [95]. Mixing polystyrene with bran, increases the degradation rate of PS [95]. Gentamicin (antibiotic) fed mealworms lose the ability to depolymerize polystyrene because it inhibits the growth of gut bacteria [96].

Darkling beetle (*Tenebrio obscurus*)

Dark mealworms (larvae of *T. obscurus*) can efficiently degrade polystyrene at a faster rate as compared to yellow mealworms [97]. Both *Tenebrio* species associated with the degradation of polystyrene contained three predominant families of gut microbes i.e. *Enterobacteriaceae*, *Spiroplasmataceae* and *Enterococcaceae* [97].

Greater wax moth (*Galleria mellonella*)

Waxworms (larvae of wax moth) inhabit the honey bee hives where they feed on the wax of hive [98]. Complex compounds like alkanes, fatty acids and esters are present in the beeswax [99]. Hundred waxworms can degrade 92mg of PE within 12 hours [100]. Spreading of worm homogenate on plastic film results in 13% loss in mass of PE [100]. PE degradation by waxworms is higher than the degradation rate of PE by other microbes [101]. Glycol is produced as a byproduct of LDPE degradation. A recent study has confirmed the LDPE degradation activity of symbiotic bacterial genus *Acinetobacter*, found in the gut of waxworms [102]. However, waxworms that lack intestinal microbes successively degrade long chain fatty acids. The presence of long chain fatty acid degradation gene products enable these larvae to degrade long chain hydrocarbons without the assistance of intestinal microbes [103].

Lesser wax moth (*Achroia grisella*)

Hundred lesser waxworms can decompose 90% wax comb (WC) and 43% PE within a period of 8 days [104]. Worms feeding on PE have low weight and survival rate because of

low nutrients in PE as compared to WC [105].

Table 2. Insect species involved in plastic degradation

Type of plastic	Insect larvae	Microbes in the gut of insect larvae
Polyurethane, Polystyrene, Polyvinyl chloride and polylactic acid	Yellow mealworms (<i>T. molitor</i>)	<i>Exugibacterium</i> sp. YT2 [94], <i>Citrobacter</i> and <i>Kosakonia</i> [95], <i>Lactococcus</i> , <i>Pantoea</i> , <i>Enterobacter</i> and <i>Clostridium</i> [80]
Polystyrene	Dark mealworms (<i>T. obscurus</i>)	<i>Enterobacteriaceae</i> , <i>Spiroplasmataceae</i> and <i>Enterococcaceae</i> [97]
Polyethylene, LDPE	Greater waxworms (<i>G. mellonella</i>)	<i>Acinetobacter</i> [102]
Polyethylene	Lesser waxworms (<i>A. grisellaa</i>)	Not discovered yet
Polyethylene	Indian mealmoth larvae (<i>P. interpunctella</i>)	<i>Bacillus</i> sp. YP1 and <i>Enterobacter asburiae</i> YT1 [106]
LDPE	Rice moth larvae (<i>C. cephalonica</i>)	Not discovered yet
Polyester polyurethane	Giant mealworms (<i>Z. morio</i>)	<i>Aspergillus flavus</i> G8 [108]

Indian mealmoth (*Plodia interpunctella*)

The larvae of *P. interpunctella* can degrade polyethylene due to the activity of gut microbes i.e., *Bacillus* sp. YP1 and *Enterobacter asburiae* YT1 [106].

Rice moth larvae (*Corcyra cephalonica*)

These larvae can degrade low density polyethylene (LDPE). It has been reported that, half of the larvae were fed with an antibiotic to kill any gut microbes that may be responsible for degrading LDPE. The results indicated that larvae fed with antibiotic degraded 21% LDPE while the larvae unfed with antibiotic degraded 25% of LDPE within a period of 20 days [107]. It can be interpreted that enzymes for decomposition of LDPE may be produced by the gut tract of these larvae.

Darkling beetle (*Zophobas morio*)

Superworms or giant mealworms (larvae of *Z. morio*) can feed on polyester polyurethane. A number of microbes are found in the gut of these larvae however, a fungal strain *Aspergillus flavus* G8 has been found to be actively involved in the degradation of polyester polyurethane [108].

Conclusion and recommendations

Biodegradation of plastics need to explored extensively especially identification of bacterial and fungal species and their symbiotic hosts (insects) associated with plastic degradation. Those insect groups which have been reported for plastic degradation indicate the presence of potential microbes in more related insect species. Insects have been reported for their direct or indirect association with plastic degradation. Larvae of darkling beetle (*T. molitor*), greater waxmoth (*G. mellonella*), lesser waxmoth (*A. grisella*), Indian meal moth (*P. interpunctella*) and rice moth (*C. cephalonica*) have been reported for their potential to consume plastics as a source of energy. Similarly extensively studied, *Bacillus* and *Pseudomonas* strains for which complete genome has been sequenced may be utilized for producing genetically modified organisms having ability to degrade plastics. Bacterial strains inhabiting the gut of some insect larvae have been reported for their ability to degrade plastics i.e. *T. molitor* and *P. interpunctella* whereas *C. cephalonica*

larvae degrade LDPE by enzymes produced by their gut tract. More focused and elaborative work on species associated with plastics degradation is direly needed. Further research should be conducted at molecular level to identify the degradation pathways as well as the enzymes involved in degradation process so that plastics can be degraded at industrial level.

Authors' contributions

Conceived idea: S Liaqat, M Hussain & MF Malik, Performed literature review: A Aslam & K Mumtaz, Organised data: S Liaqat, A Aslam & K Mumtaz, Wrote the paper: S Liaqat, M Hussain.

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