

Microbial degradation of aliphatic and aliphatic-aromatic co-polyesters

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Abstract Biodegradable plastics (BPs) have attracted much attention since more than a decade because they can easily be degraded by microorganisms in the environment. The development of aliphatic-aromatic co-polyesters has combined excellent mechanical properties with biodegradability and an ideal replacement for the conventional nondegradable thermoplastics. The microorganisms degrading these polyesters are widely distributed in various environments. Although various aliphatic, aromatic, and aliphatic-aromatic co-polyester-degrading microorganisms and their enzymes have been studied and characterized, there are still many groups of microorganisms and enzymes with varying properties awaiting various applications. In this review, we have reported some new microorganisms and their enzymes which could degrade various aliphatic, aromatic, as well as aliphatic-aromatic co-polyesters like poly(butylene succinate) (PBS), poly(butylene succinate)-*co*-(butylene adipate) (PBSA), poly(ϵ -caprolactone) (PCL), poly(ethylene succinate) (PES), poly(L-lactic acid) (PLA), poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHB/PHBV),

poly(ethylene terephthalate) (PET), poly(butylene terephthalate) (PBT), poly(butylene adipate-*co*-terephthalate (PBAT), poly(butylene succinate-*co*-terephthalate) (PBST), and poly(butylene succinate/terephthalate/isophthalate)-*co*-(lactate) (PBSTIL). The mechanism of degradation of aliphatic as well as aliphatic-aromatic co-polyesters has also been discussed. The degradation ability of microorganisms against various polyesters might be useful for the treatment and recycling of biodegradable wastes or bioremediation of the polyester-contaminated environments.

Keywords Aliphatic polyesters · Aliphatic-aromatic co-polyesters · Biodegradation · Depolymerases

Introduction

A tremendous increase in production and consumption of plastics in various industries since a couple of decades has led to some serious environmental concerns. The persistence of synthetic polymers in the environment poses a major threat to natural ecological systems (Russell et al. 2011). Therefore, production of biodegradable plastics (BPs) is the only way to reduce environmental pollution due to plastic waste, since BPs are environmental friendly as well as susceptible to microbial attack (Narayan 2001). Some polyester-based BPs, both aliphatic and aromatic, have been developed. The aliphatic polyesters and their co-polymers proved to be easily degradable but insufficient for them to replace conventional plastics due to their poor physical and mechanical properties. On the other side, however, aromatic polyesters have excellent physical and mechanical properties as compared to aliphatic polyesters, but the rate of degradability is low due to their strong resistance to microbial attack in the environment (Chen et al. 2008). It is important to design polymers having both satisfactory mechanical properties and biodegradability; therefore,

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the researchers focused on the development of aliphatic-aromatic co-polyesters. A number of aliphatic as well as aliphatic-aromatic biodegradable co-polyesters have been developed and commercialized during the past two decades under different trade names such as GS Pla™ (Mitsubishi Chemical Co.), Ecoflex® (BASF), Apexa® (DuPont), Mater-Bi® (Novamont), Novon® (Warner-Lambert Co.), and several others (Hu et al. 2010). These are used in manufacturing compost bags, agricultural materials (mulch film), lamination materials, transparent films for wrapping food, packaging and containers, polyester fabrics, and other biodegradable resins (Kasuya et al. 2009). The global production of BPs has reached up to more than 1 million tons per year; only 60 % of this amount is properly disposed of, while approximately 400,000 tons of waste is still introduced into the environment annually (Scharathow 2009).

Biodegradable plastics (BPs) have received global attention since they are totally eco-friendly as well as they decompose completely in nature (Upreti and Srivastava 2003). BPs, both aliphatic as well as aliphatic-aromatic co-polyesters such as poly(butylene succinate) (PBS), poly(butylene succinate-*co*-adipate) (PBSA), poly(ethylene succinate) (PES), poly(ϵ -caprolactone) (PCL), poly(lactic acid) (PLA), poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHB/PHBV), poly(butylene adipate-*co*-terephthalate) (PBAT), and poly(butylene succinate-*co*-terephthalate) (PBST) have been produced so far and received great attention because of their degradability in the natural environment, hence a possible solution to the environmental pollution caused by the use of synthetic plastics (Table 1). The degradability of polymers is influenced not only by the chemical structure of the polymers, especially the presence of functional groups and hydrophilicity–hydrophobicity balance, but also by the ordered structure such as crystallinity, orientation, and other morphological properties (Chen et al. 2008). BPs can be degraded into water and CO₂ by microorganisms in bioactive environments (Gross and Kalra 2002; Song et al. 2009).

Recycling is one of the most environmentally suitable processing methods for the recovery of plastic wastes which usually includes mechanical recycling (material recycling) and feedstock recovery (chemical recovery). Monomer recovery is particularly effective in the recycling of biodegradable plastics because most of the commercially available biodegradable plastics are polyesters that are easily susceptible to hydrolysis and decompose into related monomers such as organic acids and polyols. In our previous report, we proposed biochemical monomer recycling process using specific enzymes for the depolymerization of plastics for monomer recovery (Nakajima-Kambe et al. 2009a). Since enzymes have substrate specificities, it is possible to produce pure monomers from mixed plastic wastes without distinction through the sequential use of specific plastic-depolymerizing enzymes.

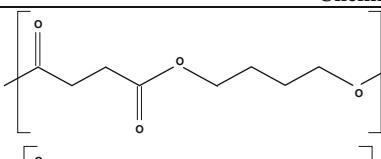
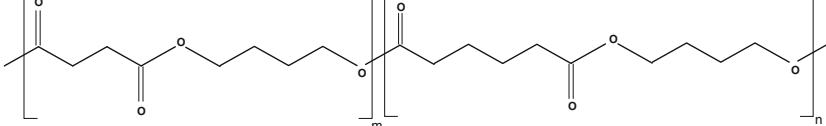
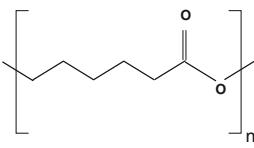
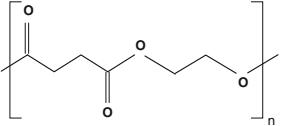
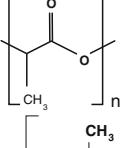
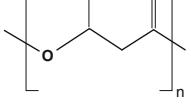
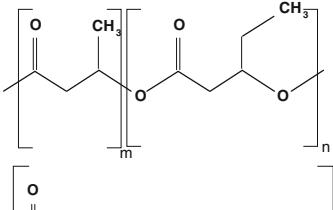
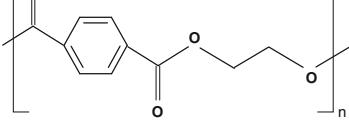
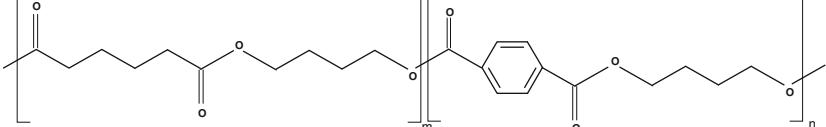
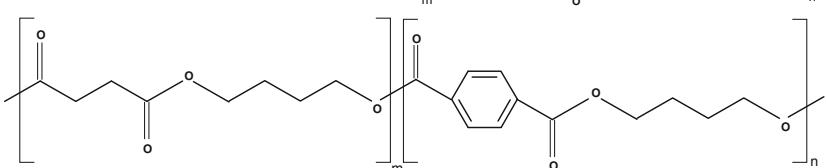
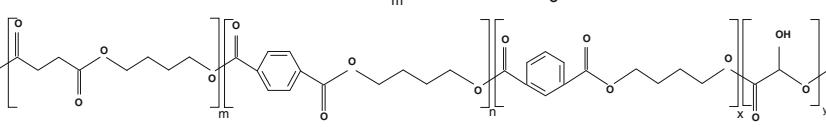
Understanding the substrate specificities and plastic depolymerization dynamics of the enzymes from microbes will help create new enzymes suitable for monomer recycling using molecular techniques. It may also help in the development of novel biodegradable plastics suitable for enzymatic monomer recycling (Nakajima-Kambe et al. 2009a).

Biodegradation of aliphatic polyesters

Various microorganisms such as bacteria, actinomycetes, and fungi could degrade BPs in diverse environmental conditions (Gross and Kalra 2002; Song et al. 2009; Akutsu-Shigeno et al. 2003; Maeda et al. 2005; Seo et al. 2007; Li et al. 2008; Nakajima-Kambe et al. 2009a, b). Recently, a new bacterium *Roseateles depolymerans* strain TB-87 has been isolated from fresh water, which could degrade various aliphatic polyester-type BPs such as PBS, PBSA, PES, PCL except PLA, and PHBV (Fig. 1) (Shah et al. 2013a). Some novel esterases with wide substrate specificity and high activity against aliphatic co-polyester, both in liquid and solid state, have been reported from strain TB-87 (Fig. 2) (Shah et al. 2013a). Previously, we reported another bacterium, *Leptothrix* sp. strain TB-71 with wide substrate specificity but with a slight difference in activity, against various aliphatic polyesters as compared to strain TB-87. Strain TB-71 could effectively degrade co-polyester PBSA but could not degrade PBS. An esterase from strain TB-71 played a major role in degrading these polymers, in emulsion as well as in film form, and these polyesters acted as inducers for enzyme production (Nakajima-Kambe et al. 2009a). A novel bacterial strain, *Pseudomonas* sp. strain AKS2, reported by Tribedi et al. (2012) from soil could degrade aliphatic polyesters. PHAs degrading various bacterial and actinomycetes strains, such as *Bacillus* sp. AF3 (Shah et al. 2007), *Pseudomonas lemoignei* (Schober et al. 2000), *Acidovorax facilis*, *Variovorax paradoxus* (Mergaert et al. 1993), *Pseudomonas fluorescens* (Schirmer et al. 1993), *Comamonase* sp. (Molitoris et al. 1996), *Nocardiopsis aegyptia* (Ghanem et al. 2005), *Streptomyces* sp. SNG9 (Mabrouk and Sabry 2001), *Streptomyces exfoliates* (Klingbeil et al. 1996), *Streptomyces venezuelae* (Santos et al. 2013), and *Actinomadura* sp. AF-555 (Shah et al. 2010), have been reported so far from different natural environments such as soils, sludges, composts, and marine water.

Besides bacteria, fungi are also potential degraders of polyesters. A fungal strain *Aspergillus oryzae* RIB40 have been reported that could degrade PBS and PBSA under mesophilic condition (Maeda et al. 2005). Shinozaki et al. (2013) reported a fungus (yeast), *Pseudozyma antarctica* strain JCM 10317, which exhibited strong degradation activity against various BPs. A cutinase-like enzyme from strain JCM 10317 could actively degrade not only emulsified but also solid films of PBS, PBSA, PCL, and PLA. PHAs degrading fungal strains

Table 1 Name and chemical structures of aliphatic, aromatic, and aliphatic-aromatic co-polyester-type biodegradable plastics

Plastics	Abr.	Chemical structure
Poly(butylene succinate)	PBS	
Poly(butylene succinate- <i>co</i> -adipate)	PBSA	
Poly(ϵ -caprolactone)	PCL	
Poly(ethylene succinate)	PES	
Poly(L-lactic acid)	PLA	
Poly(3-hydroxybutyrate)	PHB	
Poly(3-hydroxybutyrate- <i>co</i> -hydroxyvalerate)	PHBV	
Poly(ethylene terephthalate)	PET	
Poly(butylene adipate- <i>co</i> -terephthalate) (Ecoflex TM)	PBAT	
Poly(butylene succinate- <i>co</i> -terephthalate)	PBST	
Poly(butylene succinate/terephthalate/isophthalate- <i>co</i> -lactate)	PBSTIL	

Nakajima et al. (2009a, b); Shah et al. (2013a, b)

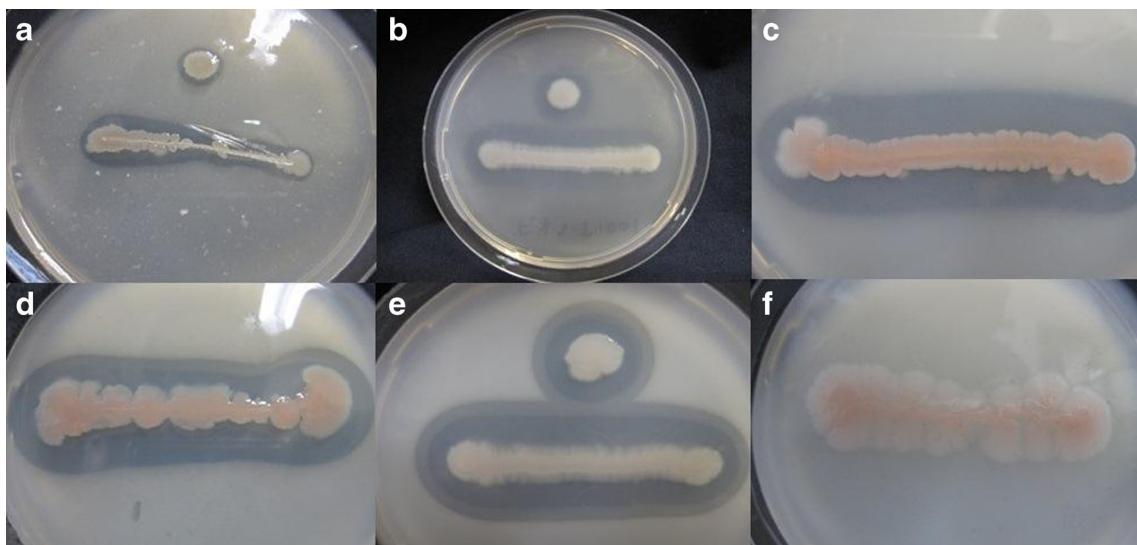


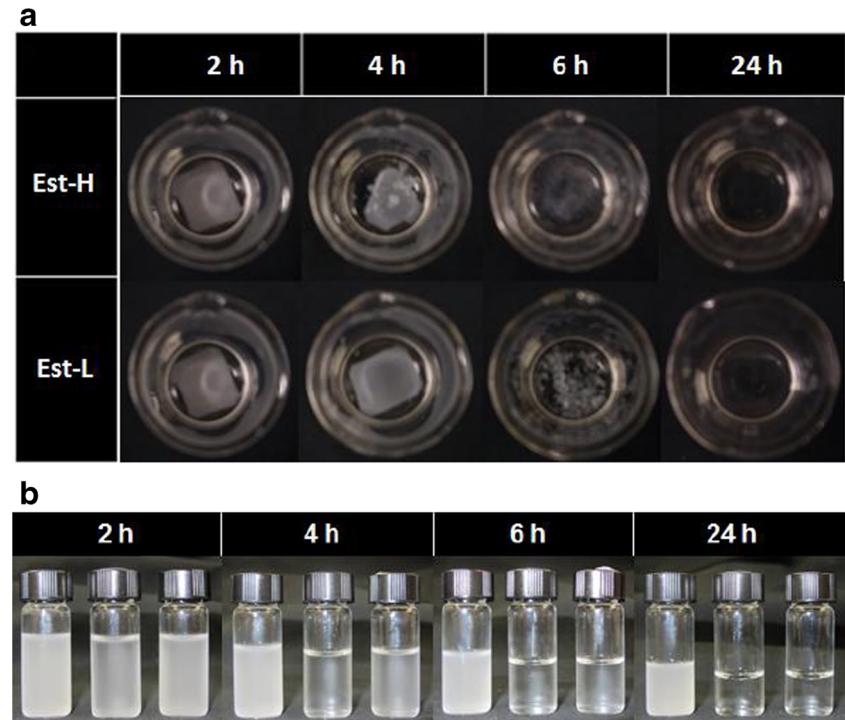
Fig. 1 Clear zones around growth of strain TB-87 on various plastic emulsion-overlaid NB agar plates: **a** PBSTIL, **b** PBS, **c** PCL, **d** PBSA, **e** PES, and **f** PLA

such as *Aspergillus fumigatus* and *Penicillium funiculosum* have also been reported (Nadhaman et al. 2012; Mergaert et al. 1993). Li et al. (2012) reported a new fungal strain, *Penicillium oxalicum* strain DSYD05-1, from soil which showed a wide range of substrate specificity, with degradation ability against various polyesters PCL, PHB, and PBS, except PLA.

The enzymes must be in close proximity to its substrate for a specific period of time for efficient activity against it. The enzymes can easily come in contact with water-soluble substrates as compared to the insoluble ones. However, plastic

films have extremely low contact efficacy with enzyme molecules, and they possess certain properties that enable them to adsorb to the surface of substrates, which allow the enzymes to be in close proximity to its substrate and thus facilitates the degradation process (Shinozaki et al. 2013). The esterase activity and cell surface hydrophobicity are contributing factors to PES degradation ability of *Pseudomonas* sp. strain AKS2, hence confirming the hypothesis (Tribedi et al. 2012). Ohtaki et al. (2006) also reported comprehensively the mechanism of adherence of polyester-degrading enzyme to the surface of polyester.

Fig. 2 Degradation of PBSA by purified Est-H and Est-L in 100 mM phosphate buffer at 30 °C on shaker incubator. **a** Film; **b** emulsion (bottles: left, control; center, Est-H; right, Est-L) (Shah et al. 2013a)



Biodegradation of aliphatic-aromatic co-polyesters

Many researchers have reported on degradation of aliphatic polyesters (Akbar et al. 2013; Li et al. 2012; Tribedi et al. 2012; Shinozaki et al. 2013), while information about the degradation of aliphatic-aromatic co-polyesters is still insufficient. Since a couple of years, several researchers have focused their attention towards degradation of these hybrid types of polyesters in variable environmental conditions. Microbial activities against aliphatic-aromatic co-polyesters in compost environments at high temperature and pH have been reported (Kijchavengkul et al. 2010). A thermophilic bacterium, *Thermomonospora fusca*, has been reported that could monomerize an aliphatic-aromatic co-polyester PBAT (Ecoflex™) at high temperature within 3–4 weeks (Witt et al. 2001). Kleeberg et al. (2005) reported that PBAT acts as an inducer for production of a novel aliphatic-aromatic co-polyester degrading thermophilic hydrolase from *Thermobifida fusca* (TfH).

Generally, plastic waste is disposed of as open landfills; therefore, it is necessary to study the process of degradation under mesophilic condition. In contrast, Tan et al. (2008) reported various bacteria from soil which could degrade Ecoflex™ under ambient environmental conditions. We have reported a bacterium *R. depolymerans* strain TB-87 that could degrade not only aliphatic but also aliphatic-aromatic co-polyesters such as PBAT, PBST, and PBSTIL except PLA and PHBV (Shah et al. 2013a). It indicates that the biodegradation of polyesters by this strain may not only depend on their chemical structures but also on their physico-chemical properties such as surface hydrophobicity or crystal structure (Nakajima-Kambe et al. 2009b). The depolymerases from strain TB-87 could degrade aliphatic-aromatic co-polyesters, both in emulsion and film form (Fig. 3), but the rate of degradation was slower, since polyesters with aromatic constituent might exhibit resistance to biological attack (Shah et al. 2013a). Similarly, *Leptothrix* sp. strain TB-71 could also degrade various aliphatic-aromatic co-polyesters such as PBSTIL, PBST, and Ecoflex™ but at a slower rate than strain TB-87 (Shah et al. 2013a; Nakajima-Kambe et al. 2009b). Kasuya et al. (2009) isolated three fungal and two bacterial strains from soil, out of which a fungal strain NKCM 1712, closely related to *Isaria fumosorosea* could degrade PBAT at faster rate as compared to others.

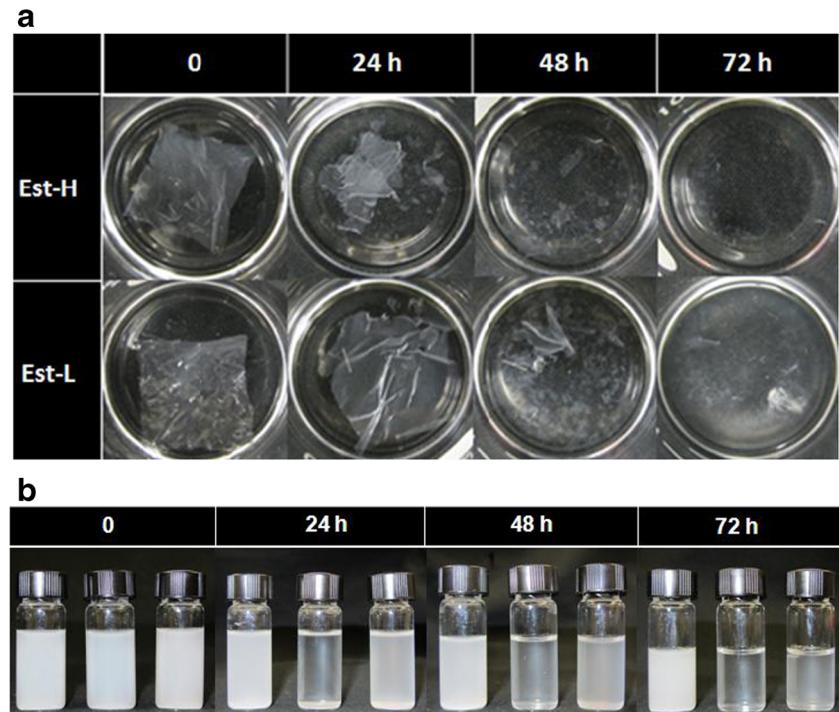
Polyester-degrading enzymes

All biodegradable polymers contain hydrolyzable bonds, such as glycosides, esters, orthoesters, anhydrides, carbonates, amides, urethanes, ureas, etc. (Göpferich 1996; Williams and Zhong 1994). Polymers with strong covalent bonds in the backbone (like C–C) and with no hydrolyzable groups require

longer times to degrade (Hasirci et al. 2001). Hydrolysis reactions may be catalyzed by enzymes known as hydrolases, which include proteases, esterases, lipases, glycosidases, phosphatases, etc. This class of enzymes comprises cell-derived proteins that are responsible for the catalysis of several reactions in the human body. For example, hydrolytic enzymes are present in the plasma and interstitium, in the brush border membrane and lumen of the gastrointestinal tract, and in the tubular epithelium of the kidneys, where they ensure the efficient hydrolysis of different substrates to facilitate absorption of nutrients and solutes (Shalaby and Park 1994). Some other hydrolases like pectinases, cellulases, and xylanases are responsible for the hydrolysis of pectin, cellulose, and xylane, respectively. In this sense, it is expected that some of these enzymes may play an important role in the degradation of biomaterials by catalyzing their hydrolysis (Hasirci et al. 2001).

Most of the biodegradable plastics are polyesters; their degradation may be catalyzed by esterolytic enzymes such as esterases, lipases, or proteases with some differences in substrate preference or interfacial activation (Bornscheuer 2002). All enzymes tested for polyester degradation have the same, so called catalytic triad in their active site, which is typical for all serine hydrolases. However, the form, depth, and the surrounding of the catalytic center seem to differ significantly (Mueller et al. 2007). Cutinase belongs to a family of enzymes that exhibits lipase as well as esterase characteristics. Cutinases have been exploited for degradation of various synthetic polyesters. Recently, cutinases from various fungal species, such as *Alternaria brassicicola* (AbC), *A. fumigatus* (Afc), *A. oryzae* (AoC), *Hemicoloma insolens* (HiC), and *Fusarium solani* (FsC) have been studied, which demonstrated enhanced PCL hydrolysis at high temperature and wide pH range. The enzymes' overall neutral surface charge and additional disulfide bond formation were the main reasons behind their stability (Baker et al. 2012). The results indicated that re-engineering of such enzymes may lead to improved function and stability over a range of temperature and pH conditions suitable for biotransformation reactions (Bornscheuer and Pohl 2001). Kawai et al. (2013) have expressed two tandem cutinases (Est1 and Est119) from *Thermobifida alba* AHK119 in *Escherichia coli* Rosetta-gami (DE3). Recombinant enzymes showed wide substrate specificity towards aliphatic and aliphatic-aromatic co-polyesters, whereas the activity and thermostability were improved through random and site-directed mutagenesis (Kawai et al. 2013). Acero et al. (2013) reported that the pronounced differences in hydrolysis efficiencies between two closely related cutinases Thc-Cut1 and Thc-Cut2 from *Thermobifida cellosilytica* DSM44535 against aromatic polyester polyethylene terephthalate (PET) are mainly due to dissimilarities in their electrostatic and hydrophobic surface properties in the vicinity to the active site.

Fig. 3 Degradation of PBSTIL by purified Est-H and Est-L in 100 mM phosphate buffer at 30 °C on shaker incubator. **a** Film; **b** emulsion (bottles: left, control; center, Est-H; right, Est-L) (Shah et al. 2013a)



Lipases and esterases are differentiated on the basis of hydrolytic cleavage of acyl glycerols with different acyl chain lengths. Lipases hydrolyze acyl esters with >10 carbon atoms, whereas esterases catalyze breakdown of esters with chain lengths <10 carbon atoms (Rhee et al. 2005; Bornscheuer 2002). Polyesterases preferentially attach the amorphous region of the polymer; consequently, the degree of crystallinity of the polymer increases on enzyme treatment (Herzog et al. 2006; Mueller 2006). Kawai et al. (2011) reported two types of PLA-degrading enzymes, protease (proteinase K) and lipase (cutinase-like enzyme) (CLE) from *Cryptococcus* sp. strain S-2. A PLA-degrading lipase has also been reported from *Paenibacillus amylolyticus* strain TB-13 (Akutsu-Shigeno et al. 2003). PCL- and PBS-degrading enzymes have been confirmed as lipases or cutinases (Masaki et al. 2005; Maeda et al. 2005), and PHB and PHBV depolymerases are recognized as enzymes specific for PHB and PHBV hydrolysis (Tseng et al. 2006; Akbar et al. 2013).

The extracellular PHA depolymerases (e-PHA depolymerases) are carboxyesterases and generally consist of three domains in the following sequential order as indicated by the primary amino acid sequences of all available PHA depolymerases: (1) a catalytic domain including a catalytic triad of serine, aspartate, histidine, and the oxyanion histidine; (2) a linking domain between the catalytic domain and the C-terminal domain; and (3) a C-terminal substrate-binding domain (Schober et al. 2000; Jendrossek and Handrick 2002). The PHA-binding domain in most PHA depolymerases is located at the C terminus of the polypeptide chain, and the active and surface-binding domains are linked by a flexible

linker, which is a threonine-rich region (Jendrossek et al. 1996). The extracellular poly(3-hydroxybutyrate) depolymerases of *Alcaligenes faecalis*, *P. lemoignei*, and *Comamonas* sp. (Jendrossek et al. 1993), poly(3-hydroxyvalerate) depolymerases of *P. lemoignei* (Muller and Jendrossek 1993), poly(3-hydroxyoctanoate) depolymerase of *P. fluorescens* (Schirmer et al. 1993), and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) of *Streptomyces* sp. AF-111 (Akbar et al. 2013) have been isolated and characterized.

Biological recycling of polyesters through composting is one of the promising techniques, and a number of polyester-degrading thermophilic bacteria and their enzymes have been reported from compost environment. Two kinds of thermoactive esterases have been reported from the genus *Thermobifida*, one from *T. fusca* strain 43793 (Chen et al. 2008; Kleeberg et al. 2005) and the other from *T. alba* strain Est119 (Hu et al. 2010), that could depolymerase aliphatic-aromatic co-polyesters. A cutinase and a lipase from *T. fusca* and *Thermomyces lanuginosus*, respectively, hydrolyzed the aromatic polyester poly(trimethylene terephthalate) (PTT), based on the detection of water-soluble hydrolysis products (Eberl et al. 2008). The process of degradation is affected by the medium's temperature, as the rate of enzymatic hydrolysis of polyesters increases with the enhancement of the temperature. In spite of this fact, it is necessary to study the degradation of synthetic co-polyesters under more moderate environmental conditions and with microorganisms other than thermophiles, because it is likely that the synthetic co-polyesters might be disposed in the environment as landfills. Therefore,

it is important to screen some novel depolymerases which function under mesophilic conditions rather than in compost at elevated temperature. Recently, we reported some novel enzymes from *Leptothrix* sp. strain TB-71 and *R. depolymerans* strain TB-87, designated as esterases on the bases of their activity against various *p*-nitrophenyl acyl esters with maximum activity against *p*-nitrophenyl acetate (pNPA) and *p*-nitrophenyl butyrate (pNPB), respectively (Table 2). Esterases from both TB-71 and TB-87 could degrade various polyesters with a slight difference in activity against them, especially nondegradability of PBS and PLA, respectively (Nakajima-Kambe et al. 2009b; Shah et al. 2013a). The degradation ability of enzymes from strains TB-87 and TB-71 against various aliphatic and aliphatic-aromatic co-polyesters might be helpful in their application against mixed plastic wastes for monomer recycling.

Mechanism of degradation of aliphatic and aliphatic-aromatic co-polyesters

Since a couple of decades, biodegradable plastics have become of remarkable interest because they undergo a controlled biological degradation by microorganisms. However, most of biodegradable plastics are polyesters with potentially hydrolyzable ester bonds, and these are susceptible to hydrolysis by depolymerases, such as esterases and lipases, and decompose into related monomers such as organic acids and polyols. Primarily, the microorganisms secrete depolymerases that attach to the polyester and catalyze the enzymatic hydrolysis of ester, amide, or urethane bonds in the polymers. In response,

the polymer breakdown into water-soluble intermediates, i.e., monomers as well as short and long chain oligomers, which are assimilated and subsequently metabolized by microbial cells. However, in many cases, the primary degradation step may also be achieved nonenzymatically (hydrolysis), but the intermediates are finally mineralized by microorganisms (Chen et al. 2008).

The BPs require the flexibility of their polymer chain for zymolysis by microorganisms and their enzymes. The aliphatic polyesters degrade easily as compared to aromatic as well as aliphatic-aromatic co-polyesters due to their flexible polymer chain (Lucas et al. 2008). We have recently studied the mechanism of degradation of aliphatic as well as aliphatic-aromatic co-polyesters by depolymerases from *R. depolymerans* strain TB-87. The enzymes degrade the aliphatic polyester PBSA into its respective monomers. The succinic acid monomer is detected as the first degradation product followed by 1,4-butandiol and adipic acid (Shah et al. 2013b). This is the point of differentiation between the degradation mechanism of depolymerases from strains TB-71 and TB-87, where the enzyme from the former specifically adsorbs to adipic acid segments of the PBSA polymer chain, resulting into the formation of adipic acid as a degradation product prior to succinic acid (T. Nakajima-Kambe, unpublished data). We propose one speculation that the depolymerization of PBSA by strain TB-87 begins at the points of succinic acid segments that may cause rumpling of the polymer chain crystal at first, and degradation of adipic acid segments may occur after shredding the polymer chain. Another possibility is that the enzymes specifically adhere to succinic acid segments (BS) rather than adipic acid (BA). We have also detected a number

Table 2 List of microbial enzymes with their substrate specificities

Enzymes	Microorganisms	Substrate specificity	Reference
Esterases	<i>Thermobifida alba</i> Est119	PET	Hu et al. (2010)
	<i>R. depolymerans</i> strain TB-87	PES, PCL, PBS, PBSA, PBST, PBAT, PBSTIL	Shah et al. (2013a, b)
	<i>Leptothrix</i> sp. strain TB-71	PES, PCL, PLA, PBSA, PBST, PBAT, PBSTIL	Nakajima-Kambe et al. (2009a, b)
Cutinase	<i>P. antarctica</i> JCM10317	PCL, PLA, PBS, PBSA	Shinozaki et al. (2013)
	<i>T. cellulosilytica</i> DSM44535	PET	Acero et al. (2013)
	<i>T. fusca</i>	PTT	Eberl et al. (2008)
Lipases	<i>A. oryzae</i> RIB40	PBS	Maeda et al. (2005)
	<i>Cryptococcus</i> sp. strain S2	PCL, PLA	Masaki et al. (2005), Kawai et al. (2011)
	<i>Paenibacillus amylolyticus</i>	PLA	Akutsu-Shigeno et al. (2003)
Protease	<i>T. lanuginosus</i>	PTT	Eberl et al. (2008)
	<i>Cryptococcus</i> sp. strain S2	PLA	Kawai et al. (2011)
	<i>P. lemoignei</i>	PHV	Shober et al. (2000)
PHA depolymerases	<i>B. thuringiensis</i>	PHB/PHBV	Tseng et al. (2006)
	<i>Streptomyces</i> sp. strain AF-111	PHBV	Akbar et al. (2013)
	<i>Alcaligenes faecalis</i>	PHB	Jendrossek et al. (1993)
	<i>P. fluorescens</i>	PHO	Schirmer et al. (1993)

of short and long chain oligomers as degradation products through LC-MS (Fig. 4) (Shah et al. 2013b).

In case of aliphatic-aromatic co-polyester PBSTIL, the rate of degradation is slow as compared to aliphatic polyesters due to the inclusion of aromatic segments into aliphatic backbone. PBSTIL was depolymerized into monomers by enzymes from strain TB-87 (Shah et al. 2013b). We propose one speculation that the depolymerization of PBSTIL begins at the point of succinic acid that may cause formation of long chain oligomers at first, and degradation of terephthalic/isophthalic acid segments may occur after shredding the polymer chain. The aliphatic segment (BS) has been found to be broken first followed by aromatic segment (BT/BI), and it reveals that the succinic acid segment is the point of initiation of degradation of the polymer chain. A number of oligomers in the form of pentamer, tetramers, trimers, and dimers were detected which indicates that the polymer chain has broken down

gradually from long to short chain oligomers before final monomerization. Lactic acid was detected as water-soluble oligomer rather than as monomer (Fig. 5) (Shah et al. 2013b). The accumulation of terephthalic acid (T)-containing fragments in higher concentration as compared to T-free fragments suggests that the enzymatic hydrolysis of terephthalate ester might be slower than that of succinate ester (Honda et al. 2003; Shah et al. 2013b). In our previous report, we discussed the mechanism of degradation of PBSTIL, PBST, and EcoflexTM by *Leptothrix* sp. strain TB-71, and the degradation products were analyzed by HPLC. The bacterium depolymerized these polymers into monomers as well as some water-soluble oligomers as intermediates. The small peaks of monomers from aliphatic segments indicated that these might be utilized by strain TB-71 as the carbon source for its growth, whereas no change in the amount of aromatic constituents was observed (Nakajima-Kambe et al. 2009b). Kasuya et al.

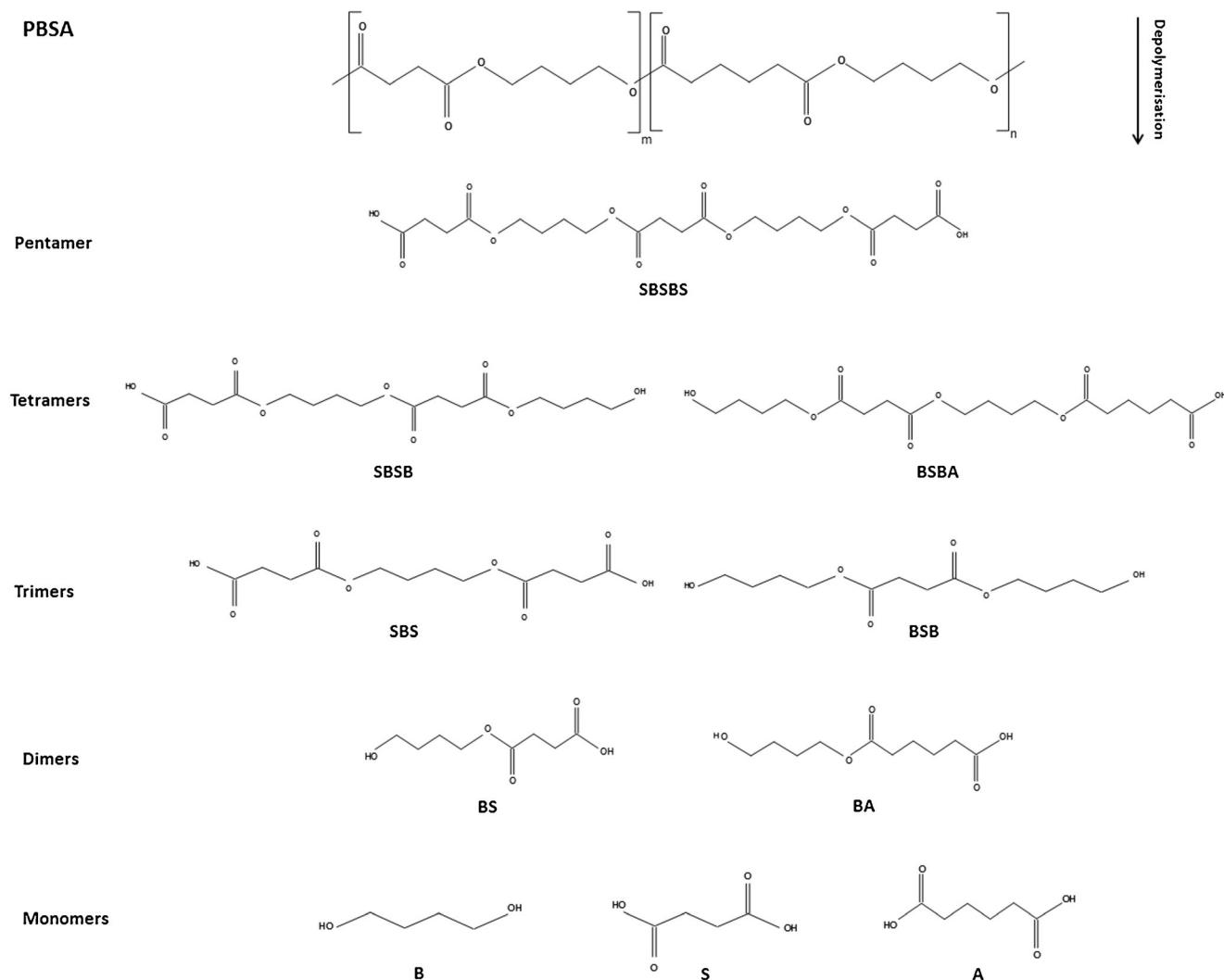


Fig. 4 Mechanism of degradation of poly(butylene succinate-*co*-adipate) by enzymes from *Roseateles depolymerans* strain TB-87. PBSA disintegrates into long chain water-soluble oligomers (pentamers), which further

breakdown into short chain oligomers (tetramers, trimers, and dimers) as the incubation time with esterases proceeds, then finally monomerizes (Shah et al. 2013b)

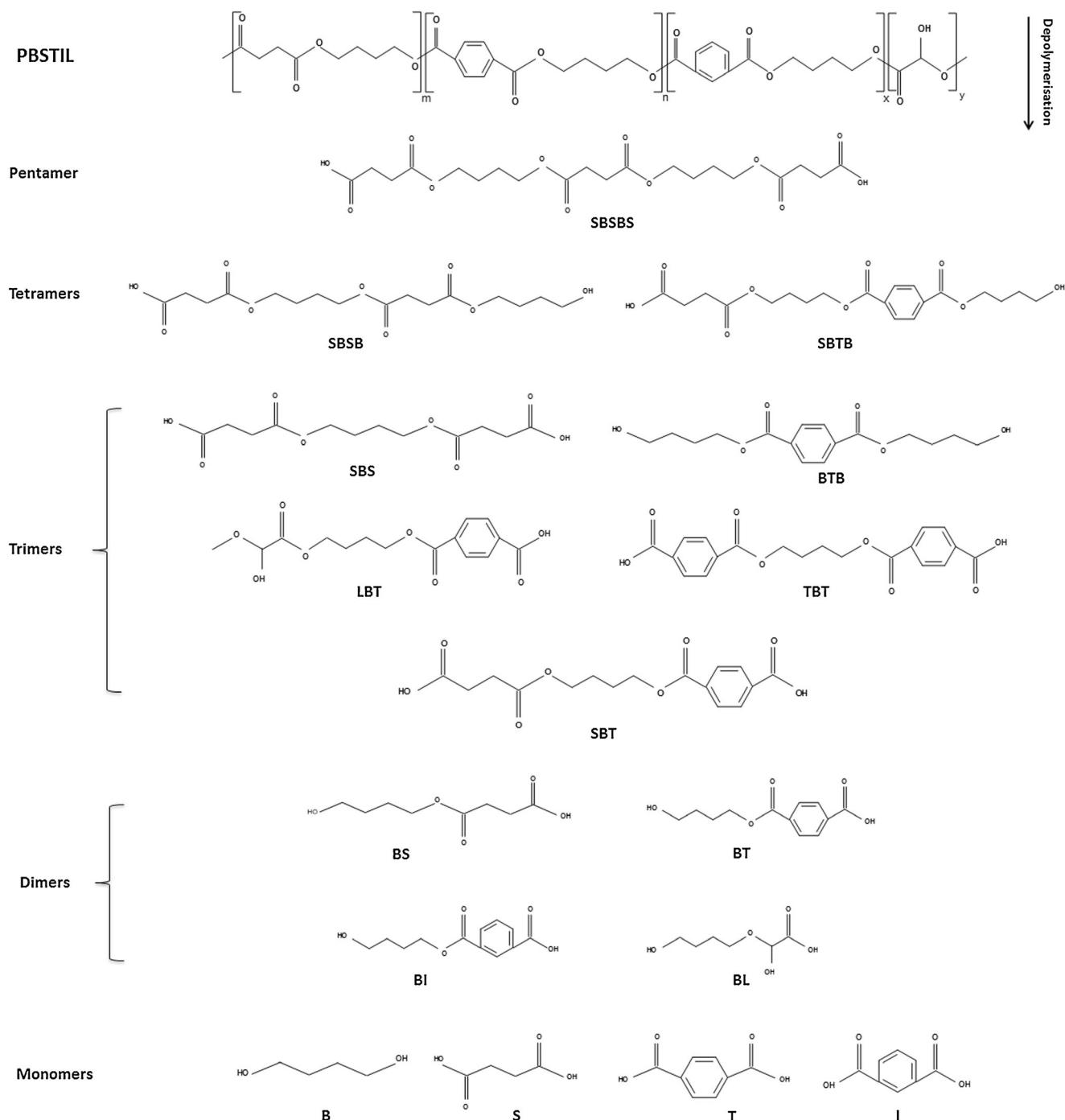


Fig. 5 Mechanism of degradation of poly(butylene succinate/terephthalate/isophthalate)-co-(lactate) by enzymes from *Roseateles depolymerans* strain TB-87. These enzymes first attack the aliphatic segments, leading to the formation of succinic acid (S) containing water-soluble oligomers. Later on, the aromatic segments (terephthalic/

isophthalic acid) (T/I) is broken down by these enzymes and the defragmented polymer chain results into the formation of T and I containing oligomers. Finally, aliphatic and aromatic monomer subunits are generated (Shah et al. 2013b)

(2009) analyzed the degradation products by LC/ESI-MS after culturing the PBAT film with a fungal strain NKCM1712 for 14 days and reported various oligomers with aliphatic as well as aromatic constituents. They also reported that the fungal strain utilized the monomers from aliphatic segments at a faster rate than those from aromatic segments; therefore,

adipic acid and 1,4-butanediol in the supernatant could not be detected except terephthalic acid (Kasuya et al. 2009). Marten et al. (2005) reported that it is more difficult for the enzymes such as some lipases and *T. fusca* PBAT hydrolase (TfH) to cleave esters near to T of PBAT, compared to esters containing only aliphatic sequences. Later on, it was

confirmed by Kijchavengkul and co-workers who found that the soft aliphatic domain (BA) consisting of dimer, adipic acid, and 1,4-butanediol was more susceptible to hydrolysis and biodegradation than the rigid aromatic domain (BT) with dimer, terephthalic acid, and 1,4-butanediol (Kijchavengkul et al. 2010).

Conclusion

In this review, we have concluded that the environment is rich of potential microorganisms, which can degrade polyester-type biodegradable plastics. These microorganisms produce enzymes, which can degrade various types of biodegradable plastics; therefore, such kind of enzymes might be helpful in the treatment of environments rich in mixed plastic wastes. It also encourages the industries to develop novel biodegradable plastics suitable for enzymatic monomer recycling.

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