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Review

Biochemical properties and biotechnological applications of microbial enzymes involved in the degradation of polyester-type plastics

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ABSTRACT

Application of polyester-degrading enzymes should be considered as an eco-friendly alternative to chemical recycling due to the huge plastic waste disposal nowadays. Many hydrolases from several fungi and bacteria have been discovered and successfully evaluated for their activity towards different aliphatic polyesters (PHA, PBS, PBSA, PCL, PLA), aromatic polyesters (PET, PBT, PMT) as well as their *co*-polyesters (PBST, PBAT, PBSTIL). This revision gives an up-to-date overview on the main biochemical features and biotechnological applications of those reported enzymes which are able to degrade polyester-based plastics, including different microbial polyester depolymerases, esterases, cutinase-like enzymes and lipases. Summarized information includes available protein sequences with the corresponding accession numbers deposited in NCBI server, 3D resolved structures, and data about optimal conditions for enzymatic activity and stability of many of these microbial enzymes that would be helpful for researchers in this topic. Although screening and identification of new native polyester hydrolases from microbial sources is undeniable according to literature, we briefly highlight the importance of the design of improved enzymes towards recalcitrant aromatic polyesters through different approaches that include site-directed mutagenesis and surface protein engineering.

1. Introduction

Biodegradable plastics (BPs) have received a lot of interest, as an eco-friendly contribution to the solution of problems derived from plastic waste disposal. There are several biodegradable polyester-type plastics which show properties *co*mparable to conventional plastics, such as polyhydroxyalkanoates (PHAs), poly(propiolactone) (PPL), poly (ε-caprolactone) (PCL), poly(*L*-lactic acid) (PLA), poly(butylene succinate) (PBS), poly(butylene succinate)-*co*-(butylene adipate) (PBSA), poly(ethylene succinate) (PES), and poly(ester carbonate) (PEC), as well as *co*-polyesters containing aliphatic and aromatic components like poly(butylene adipate-*co*-terephthalate) (PBAT), poly(butylene succinate-*co*-terephthalate) (PBST), and poly(butylene succinate / terephthalate / isophthalate)-*co*-(lactate) (PBSTIL) (Fig. 1). However, polyesters containing only aromatic components are more recalcitrant to biodegradation, such as poly(ethylene terephthalate) (PET), poly (butylene terephthalate) (PBT) and poly(trimethylene terephthalate) (PTT). Polyesters may be produced from fossil fuel (such as PBS, PCL and PES), biomass (PLA and PHA), and both resources (PET and bio-PET). Their applications vary from mulch films, compost bags, transparent films for wrapping food, polyester fabrics, packaging, and other biodegradable resins. Polyester-degrading microorganisms can be screened using solid media containing emulsified polymers. In this sense, clear halo zones around the colonies are formed when microorganisms excrete extracellular enzymes that diffuse through the agar and degrade the polymer into water soluble compounds. Up to date, many microbial hydrolases have been described as effective biocatalysts for biodegradation of polyester-type plastics. Likewise, the same enzyme frequently shows degrading activity against different polyester substrates. Besides, enzymatic degradation of a polyester is influenced not only by their chemical structure (for instance, the presence of functional groups) but also by their physical properties (such as crystallinity, melting point (T_m), glass transition temperature (T_g), etc.). As a general rule, enzymatic degradation is less achieved for polyesters with side chains, aromatic components, higher molecular weight (Mw > 4.000), higher melting temperature (lower flexibility), and increased crystallinity (since enzymes mainly attack amorphous rather than crystalline regions of a polymer) [1,2]. This review gives a general

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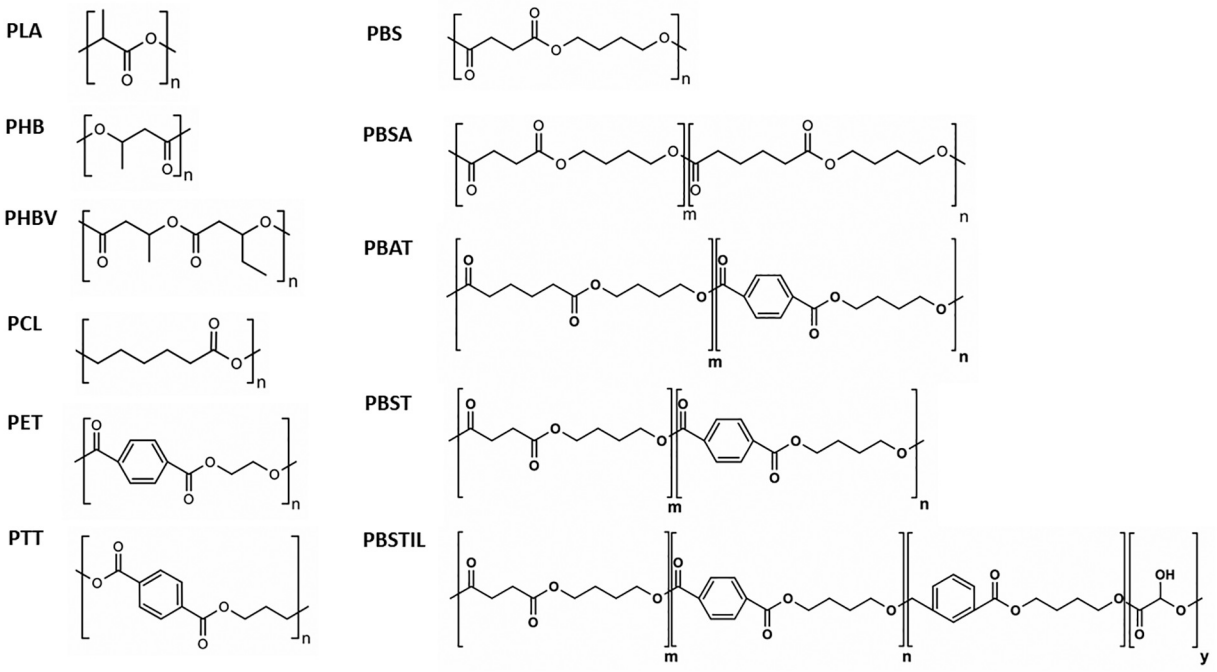

Fig. 1. Chemical structures of several polyester-type plastics that can be degraded by microbial enzymes. **PLA**: poly(lactic acid); **PHB**: poly(3-hydroxybutyrate); **PHBV**: poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate); **PCL**: poly(ε-caprolactone); **PET**: poly(ethylene terephthalate); **PTT**: poly(trimethylene terephthalate); **PBS**: poly(butylene succinate), **PBSA**: poly(butylene succinate)-*co*-(butylene adipate); **PBAT**: poly(butylene adipate-*co*-terephthalate); **PBST**: poly(butylene succinate-*co*-terephthalate); **PBSTIL**: poly(butylene succinate / terephthalate / isophthalate)-*co*-(lactate).

overview on the polyester-type degrading enzymes reported so far, taking into account that many novel enzymes remain to be discovered.

2. PHA-degrading enzymes

Polyhydroxyalkanoates (PHAs) are intracellular biopolymers, which are produced by a wide range of bacteria under unbalanced growth conditions [3,4]. PHAs are built from chiral (*R*)-3-hydroxy fatty acid monomers where the carboxyl group of one monomer forms an ester bond with the hydroxyl group of the adjacent monomer. Interestingly, PHAs have been employed as bioplastics for packaging and coating, even as carriers for slow drug delivery. Since PHAs are biodegradable and can be produced from renewable sources, these polyesters have become an green alternative to pollutant traditional plastics [5]. In the past few decades, PHAs have also attracted commercial attention due to numerous biomedical applications [6,7]. According to the number of carbon atoms of the monomers, PHAs are classified as scl-PHAs (short-chain length PHAs, from 3 to 5 carbon atoms) and mcl-PHAs (medium-chain length PHAs, from 6 to 14 carbon atoms) (Fig. 1) [8,9]. Poly-(*R*)-3-hydroxybutyrate [P(3HB)] is the most *co*mmon scl-PHA, although there are copolymers that may contain (*R*)-4-hydroxybutyrate and (*R*)-3-hydroxyvalerate, such as poly(3-hydroxy-butyrate-*co*-4-hydroxybutyrate) [P(3HB-co-4HB)] and poly(3-hydroxybutyrate-*co*-hydroxyvalerate) [P(3HB-*co*-3 HV)]. P(3HB-*co*-3 HV) is more flexible than P(3HB), and was initially developed by Imperial Chemical Industries (ICI), under the trade name Biopol®. Similarly, poly-(*R*)-3-hydroxyoctanoate (P(3HO)) is the most well-studied mcl-PHA, but some copolymers may include other medium chain length (*R*)-3-hydroxyacids in their structure such as (*R*)-3-hydroxyhexanoate (3HHx), 3-hydroxyheptanoate (3HHp), 3-hydroxynonanoate (3HN), and so on. Moreover, some of these copolymers may even include (*R*)-3-hydroxybutyrate, such as the case of the PHA-based biopolymer Nodax® developed by Procter & Gamble (P&G). Other commercial PHAs are produced by Bio-on (MINERV-PH®), Metabolix (Mvera®, Mirel®), PHB Industrial (BIOCYCLE®) and TianAn Biopolymer (ENMAT®), among other manufacturers. PHAs can be catabolized by many microorganisms depending on their localization by extracellular or intracellular PHA depolymerases [10]. In this sense, PHA is an amorphous polymer in vivo that becomes partially crystalline after cell lysis, showing a T_m value of 160–175 °C and a T_g value around 2 °C in the case of semi-crystalline P(3HB). Crystalline PHA, also referred as denatured PHA, can only be degraded by PHA-degrading microorganisms that secrete extracellular PHA depolymerases [8,11], and these enzymes have become relevant in biotechnology in the production of chiral hydroxyalkanoates (HAs) [12]. For instance, application of P(3HB) depolymerases to produce chiral (*R*)-3-hydroxybutyrate, which has proven to exhibit antimicrobial, insecticidal, and antiviral activities [13], and can be used as a chiral building block for the synthesis of fine chemicals such as antibiotics, vitamins, flavors, pheromones, and a wide range of other fine chemicals [14]. Numerous bacteria, streptomycetes, and fungi isolated from different terrestrial and aquatic environment (soil, sludge, compost, and seawater or lake water) produce extracellular PHA depolymerases in order to hydrolyze solid PHA into water-soluble monomers or oligomers to be used as nutrients. In this sense, many scl-PHA depolymerases (EC 3.1.1.75) have been purified and characterized (Table 1) in contrast to a limited number of mcl-PHA depolymerases (EC 3.1.1.76) (Table 2). Actually, few mcl-PHA-degrading microorganisms have been found in environment including Gram-negative bacteria (predominantly *Pseudomonas* species, as well as the obligated predator *Bdellovibrio bacteriovorus* HD100), and Gram-positive actinobacteria (predominantly *Streptomyces* species).

The primary structures of many extracellular PHA depolymerases (e-PhaZs) have been elucidated and deposited in the NCBI server (Tables 1 and 2). Sequence homology analysis has allowed to detect two functionally domains in these enzymes: (1) a catalytic domain, which includes the catalytic triad S-H-D in their active site (serine, histidine, and aspartate residues like serine hydrolases) where the catalytic serine is positioned in the consensus lipase-box pentapeptide Gly-X_1-Ser-X_2-Gly), and (2) a substrate binding domain (SBD), which is generally located at the C-terminal region [32]. Two types of catalytic domains may be distinguished according to the order of the active amino acids in the catalytic domain: type I [47,64], where the sequential order is H

Table 1
List of extracellular scl-PHA depolymerases with their biochemical features.

Microorganism (source)	Substrate specificity	MW (kDa)	Optimal conditions		pI	Km for P(3HB) (μg/mL)	Activation by		Protein NCBI accession number	Ref.
			pH	T (°C)			Ca^{2+}	Mg^{2+}		
Acidovorax sp. TP4	P(3HB), PPL, P(3HB-*co*-3 HV)	50.0	8.5	NR	NR	139.0	NR	NR	BAA35137.1	[15,16]
Acidovorax sp. HB01	P(3HB), P(3HB-*co*-3 HV), P(3HB-*co*-4HB), PCL	43.4	7.0	50.0	NR	NR	Yes	No	NR	[17]
Agrobacterium sp. K-03 (E1)	P(3HB), P(3HB-*co*-3 HV), PES, PCL	46.0	8.1	45.0	9.0	17.8	No	No	NR	[18]
Agrobacterium sp. K-03 (E2)	P(3HB), P(3HB-*co*-3 HV), PES, PCL	44.0	7.9	45.0	8.9	70.5	No	No	NR	[18]
Agrobacterium sp. DSGZ (sewage)	P(3HB), P(3HB-*co*-3 HV), P(3HB-*co*-4HB), PCL	34.0	7.0	50.0	NR	NR	No	No	NR	[19]
Alcaligenes faecalis AE122 (seawater)	P(3HB)	62.5	9.0	55.0	NR	NR	Yes	Yes	AAB40611.1	[20,21]
Arthrobacter sp.W6 (soil)	P(3HB), P(3HB-*co*-3 HV)	47.0	8.5	50.0	NR	NR	No	No	NR	[22]
Aspergillus fumigatus 202 (soil)	P(3HB)	63.7	9.0	45.0	4.2	NR	Yes	Yes	NR	[23]
Aspergillus fumigatus M2A (leaf compost)	P(3HB), P(3HB-*co*-4HB), PEA, PES, PTMA	57.0	8.0	70.0	7.2	NR	NR	NR	NR	[24]
Aspergillus fumigatus Pdf1	P(3HB), P(3 HV), P(3HB-*co*-3 HV)	40.0	8.5	45.0–60.0	NR	120.0	NR	NR	NR	[25]
Aspergillus fumigatus 76 T-3 (soil)	P(3HB), PES, PBS	57.0	6.4	55.0	NR	NR	NR	NR	EAL84505.1	[26]
Aureobacterium saperdae (soil)	P(3HB)	42.7	8.0	45.0	8.5	NR	NR	NR	NR	[27]
Bacillus megaterium N-18-25-9 (compost)	P(3HB)	NR	9.0	65.0	NR	NR	Yes	Yes	BAF35850.1	[28]
Caldimonas manganoxidans (formerly *Leptothrix* sp. HS) (hot spring)	P(3HB)	46.0	8.0	70.0	8.5	18.0	NR	NR	BAA92354.1	[29,30]
Comamonas acidovorans YM1609	P(3HP), P(4HB), P(3HB), PEA, PES	45.0	9.0	37.0	NR	NR	NR	NR	BAA19791.1	[31,32]
Comamonas sp. DSM6781	P(3HB), P(3HB-*co*-3 HV)	53.0	9.4	29.0–35.0	NR	NR	NR	NR	AAA87070.1	[33,34]
Comamonas testosteroni ATSU (soil)	P(3HB)	49.0	8.5	70.0	9.2	NR	NR	NR	NR	[35]
Comamonas testosteroni YM1004 (seawater)	P(3HB), P(3HB-*co*-3 HV), P(3HB-*co*-4HB)	50.0	10.0	NR	8.4	NR	NR	NR	BAA22882.1	[35–38]
Diaphorobacter sp. PCA039 (sludge)	P(3HB-*co*-3HV)	50.0	8.0	40.0	NR	NR	NR	NR	ACI48814.2	[39]
Emericellopsis minima W2	P(3HB), P(3HB-*co*-3 HV)	48.0	9.0	55.0	4.4	NR	NR	NR	NR	[40]
Fusarium solani Thom (wastewater)	P(3HB), P(3HB-*co*-3 HV)	85.0	7.0	55.0	NR	100.0	NR	NR	NR	[41]
Marinobacter sp. NK-1 (deep sea floor)	P(3HB), P(3HP), P(4HB)	70.0	8.0	NR	NR	NR	Yes	Yes	BAC15574.1	[42,43]
Paecilomyces lilacinus D218	P(3HB)	48.0	7.0	45.0	NR	130.0	No	No	NR	[44]
Paecilomyces lilacinus F4–5	P(3HB), P(3HB-*co*-3 HV)	45.0	7.0	50.0	NR	NR	NR	NR	NR	[45]
Paucimonas lemoignei (formerly *Pseudomonas lemoignei*) (PhaZ1)	P(3HB), P(3 HV), P(3HB-*co*-3 HV)	44.0	NR	62.0	NR	NR	NR	NR	P52090.1	[46]
Paucimonas lemoignei (formerly *Pseudomonas lemoignei*) (PhaZ2)	P(3HB), P(3HB-*co*-3 HV), P(3HB-*co*-3MP)	67.0	8.0	51.0	9–10	NR	Yes	NR	AAB17150.1	[47,48]
Paucimonas lemoignei (formerly *Pseudomonas lemoignei*) (PhaZ3)	NR	NR	NR	NR	NR	NR	NR	NR	AAB48166.1	[47]
Paucimonas lemoignei (formerly *Pseudomonas lemoignei*) (PhaZ4)	P(3HB), P(3 HV), P(3HB-*co*-3 HV), P (4HB)	65.5	8.0	55.0	NR	NR	NR	NR	AAA65703.1	[48]
Paucimonas lemoignei (formerly *Pseudomonas lemoignei*) (PhaZ5)	P(3HB), P(3HB-*co*-3 HV), P(3HB-*co*-3MP)	54.0–67.0	8.0	65.0	7.6	NR	Yes	NR	AAA65705.1	[47]
Paucimonas lemoignei (formerly *Pseudomonas lemoignei*) (PhaZ7)	Native P(3HB) and P(3 HV)	36.0	9.5–10-0	65.0	9.2	NR	Yes	Yes	AAK07742.1	[49]
Penicillium citrinum S2 (wastewater)	P(3HB)	240–250	6.0	50.0	NR	1250.0	No	No	NR	[50]
Penicillium expansum (wastewater)	P(3HB), P(3HB-*co*-3 HV)	20.0	5.0	50.0	NR	1.04	No	No	NR	[51]
Penicillium funiculosum ATCC9644	P(3HB)	37.0	6.0	NR	5.8	14,000.0	NR	NR	NR	[52]
Penicillium funiculosum IFO6345	P(3HB), P(3HB-*co*-3 HV)	33.0	6.5	37.0	6.5	3000.0	NR	NR	BAG32152.1	[53]
Penicillium pinophilum ATCC 9644	P(3HB)	35.0	6.0	50.0	NR	NR	No	No	NR	[54]
Penicillium simplicissimum LAR13	P(3HB)	36.0	5.0	45.0	NR	NR	No	No	NR	[55]
Penicillium sp. DS9701-09a	P(3HB)	44.8	5.0	50.0	6.7	1350.0	NR	NR	NR	[56]
Penicillium sp. DS9701-D2	P(3HB)	46.8	5.0	30.0	7.6	690.0	NR	No	NR	[57]
Penicillium sp. DS9713a	P(3HB)	15.1	8.6	50.0	NR	NR	No	Yes	NR	[58]
Pseudomonas mendocina (E1)	P(3HB), P(3HB-*co*-3 HV), P(3HB-*co*-4HB), PCL	59.4	8.5	50.0	NR	NR	No	No	WP_012020128.1	[59]
Pseudomonas mendocina (E2)	P(3HB), P(3HB-*co*-3 HV), P(3HB-*co*-4HB), PCL	33.8	8.0	50.0	NR	NR	No	No	NR	[59]

(*continued on next page*)

Table 1 (*continued*)

Microorganism (source)	Substrate specificity	MW (kDa)	Optimal conditions		pI	Km for P(3HB) (μg/mL)	Activation by		Protein NCBI accession number	Ref.
			pH	T (°C)			Ca^{2+}	Mg^{2+}		
Pseudomonas pickettii YM-b	P(3HB-*co*-4HB), P(3HB), P(3HB-*co*-3 HV)	40.0	5.5	40.0	NR	NR	NR	NR	NR	[60]
Pseudomonas stutzeri YM1006 (seawater)	P(3HP), P(3HB), PES, P(4HB), PEA	57.5	NR	NR	NR	NR	NR	NR	BAA32541.1	[32,61]
Pseudomonas stutzeri YM1414 (lake wáter)	P(3HB), P(4HB)	48.0	9.5	55.0	9.2	NR	NR	NR	NR	[62]
Ralstonia pickettii T1 (formerly *Alcaligenes faecalis* T1 from sludge)	P(4HB), P(3HP), P(3HB), P(3HB-*co*-3 HV), PEA, PES	50.0	7.5	NR	8.6	13.3	No	No	AAA21974.1	[16,32,63,64]
Schlegelella sp. KB1a (hot compost)	P(3HB)	49.0	10.0	76.0	NR	NR	NR	NR	AAT09963.1	[65]
Schlegelella thermodepolymerans DSMZ 15344	P(3HB), P(3HB-*co*-3MP)	40.0	8.2	75.0–90.0	NR	45.0	NR	NR	NR	[66]
Streptomyces ascomycinicus DSMZ 40822	P(3HB), P(3HB-*co*-3 HV)	48.4	6.0	45.0	NR	268.0	Yes	Yes	AAF86381.1	[67]
Streptomyces exfoliatus DSMZ 41693	P(3HB), P(3HB-*co*-3 HV)	48.4	8.0	40.0	NR	125.0	Yes	Yes	AAB02914.1	[68,69]
Streptomyces lydicus MM10 (wastewater)	P(3HB)	45.0	8.0	45.0	NR	NR	NR	NR	NR	[70]
Streptomyces sp. MG	P(3HB), P(3HB-*co*-3 HV), PPL, PEA, PES	41.0	8.5	60.0	NR	NR	No	No	NR	[71]
Streptomyces sp. IN1 (soil)	P(3HB), P(3HB-*co*-3 HV)	62.0	12.0	80.0	NR	NR	No	No	NR	[72]
Streptomyces sp. AF-111 (sludge)	P(3HB-*co*-3 HV)	51.0	7.0–8.0	35.0–55.0	NR	NR	No	No	NR	[73]
Thermus thermophilus HB8	P(3HB)	42.0	8.0	70.0	NR	53,0	Yes	Yes	BAD70022.1	[74]

NR: not reported; P(2HP): poly(2-hydroxypropionate; P(3HB): poly(3-hydroxybutyrate); P(3 HV): poly(3-hydroxyvalerate); P(3HP): poly(3-hydroxypropionate); P(4HB): poly(4-hydroxybutyrate); P(3HB-*co*-3MP): poly(3-hydroxybutyrate-*co*-3-mercaptopropionate); P(3HB-*co*-3 HV): poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate); PCL:poly(ε-caprolactone); PPL: poly(propiolactone); PEA: poly(ethylene adipate); PES: poly(ethylene succinate); P(3HB-*co*-4HB): poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate); PBA: poly(butylene adipate); PBS: poly(butylene succinate); PTMA: poly(1,4-tetramethylene adipate).

(oxyanion hole)-S-D-H from the N-terminal to the C-terminal region (thus, lipase-box serine is located in the middle of the catalytic domain), and type II [15,38,39] where the order is S-D-H-H (oxyanion hole). There is also a region that links these two domains which may be a sequence enriched with threonine, a fibronectin type III like sequence, or a cadherin-like sequence [32]. Interestingly, PhaZs from *Bacillus* sp. NRRL B-14911 [75] and *Shewanella* sp. JKCM-AJ-6,1α [76] contain two distinct types of substrate binding domains (SBD1 and SBD2) which may provide an advantage to more easily gain access to PHA in the environment. However, there are also small e-PhaZs lacking the SBD, and only contain the catalytic domain, and this feature has allowed their crystallization and structure elucidation. So far, these small enzymes are PhaZ from *Penicillium funiculosum* [77] and PhaZ7 from *Paucimonas lemoignei* [78] (Fig. 2), and both of them possess a large number of different solvent-exposed hydrophobic residues forming a putative polymer-attachment site [79,80].

A novel strategy for protein micropatterning has been developed by using the SBD as an anchoring motif for specific binding on to PHA coated glass [81]. Since any protein can be fused to the SBD motif, this strategy has been also useful for the immobilization of fusion proteins on PHA microbeads [82] or the specific immobilization of pathogen-specific biotin-labelled DNA probes via core streptavidin fused to the SBD [83]. The reported biochemical properties of many extracellular scl-PHA depolymerases have permitted to conclude that these enzymes share several common features. On one hand, they are mainly monomeric, and show a molecular weight ranging from 15.1 to 85.0 kDa. Exceptionally, PHB depolymerase from *Penicillium citrinum* S2 is comprised of three homodimers with a total molecular weight of approximately 240–250 kDa [50]. Likewise, some of these enzymes exhibited glycosylations that increase their apparent molecular weight when estimated by SDS-PAGE in comparison to those deduced by their amino acid sequence, such as PhaZs from *Paucimonas lemoignei* (formerly known as *Pseudomonas lemoignei*) [48] and PhaZ from *P. funiculosum* [52]. Glycosylation has been proposed as a protection strategy of these enzymes against extracellular proteases secreted by a variety of bacteria. In contrast to scl-PHA depolymerases, all reported mcl-PHA depolymerases show a small molecular weight (about 20–30 kDa), and some of them have been described as dimers (PhaZs from *Pseudomonas fluorescens* GK13) or tetramers (PhaZ from *Pseudomonas* sp. RY-1). In addition, hydrophobic resins (Dowex 50, butyl-Toyopearl, phenyl-Toyopearl, octyl-Sepharose, among others) have been routinely used for their purification by protein chromatography, due to their strong affinity to these materials. On the other hand, scl-PHA depolymerases displayed their optimal temperature from 30 °C to 90 °C, whereas their optimal pH has been established between 5.0 and 12.0, depending on the microbial source. In fact, there are some examples of highly tolerant enzymes in harsh conditions such as PhaZs depolymerases from *Comamonas testosteroni* YM1004 [35], *Schlegelella* sp. KB1a [65], *Schlegelella thermodepolymerans* [66], *Streptomyces* sp. IN1 [72] and *Thermus thermophilus* HB8 [74,84]. These biocatalysts could be employed in degradation of polymers from industrial wastes that may require high pH and/or temperatures since polymer solubility is increased and microbial contamination is reduced. Likewise, mcl-PHA depolymerases displayed their optimal temperature from 35 °C to 70 °C, whereas their optimal pH has been established in the alkaline range between 8.5 and 12.0. Most of scl-PHA depolymerases showed their pI value in the alkaline pH range (Table 1), whereas the majority of mcl-PHA depolymerases have an acidic pI value (Table 2). Some scl-PHA depolymerases are activated by divalent ions such as Ca^{2+} and Mg^{2+} (Table 1), while increasing amounts of other compounds inhibit their activity such as other divalent ions (Cu^{2+} [43,44], Fe^{2+} [55], Mn^{2+} [68], and Hg^{2+} [23]). Addition of detergents (such as Tween 20, Triton X-100, and SDS) also inhibits the hydrolysis of PHAs by most of the PhaZs, indicating that a hydrophobic region might be located near or at their active site. Maintenance of disulfide bonds is critical in protein structure of several PHA depolymerases, since their enzymatic activity is

Table 2
List of extracellular mcl-PHA depolymerases with their biochemical features.

Microorganism	Substrate specificity	MW (kDa) (quaternary structure)	Optimal conditions		pI	Protein NCBI accession number	Ref.
			pH	T (°C)			
Bdellovibrio bacteriovorus HD100	P(3HO-*co*-3HHx)	30.0 (NR)	12.0	37.0	NR	CAE81078.1	[89]
Pseudomonas sp. RY-1	P(3HO), P(3HN)	115 (tetramer)	8.5	35.0	5.9	NR	[90]
Pseudomonas alcaligenes LB19	P(3HHp-*co*-3HN-*co*-3HUD), P(3HHx-*co*-3HO-*co*-3HD), P(3HHp-*co*-3HN), P(3HHp-*co*-3HN-*co*-3HUD)	27.6 (monomer)	9.0	45.0	5.7	AAO73963.1	[91,92]
Pseudomonas alcaligenes M4–7	P(3HO), P(3HN), P(HPV)	28.0 (NR)	9.0	35.0	5.9[a]	AAQ72538.1	[92]
Pseudomonas fluorescens GK13	P(3HO), P(3HD-*co*-3HO)	48.0 (dimer)	8.5	45.0	5.7[a]	AAA64538.1	[93,94]
Pseudomonas indica K2	P(3HO-*co*-3HHx)	28.0 (NR)	8.5	35.0	NR	NR	[66]
Pseudomonas luteola M13–4	P(3HB-*co*-3 HV 60%), P(3HO), P(3HHp)	28.0 (monomer)	10.0	40.0	NR	AAV51817.1	[95]
Streptomyces sp. KJ-72	P(3HHx), P(3HHp), P(3HO), P(3HN), P(3HD), P (3HUD), PCL	27.6 (monomer)	8.5	50.0	4.7	NR	[86]
Streptomyces exfoliatus DSMZ 41693	P(3HO-*co*-3HHx), PHACOS	27.6 (monomer)	10.0	30.0	NR	WP_024761024.1	[85]
Streptomyces roseolus SL3	P(3HO), PCL, PLA	28.0 (NR)	9.5	NR	5.2	AFQ93688.1	[87]
Streptomyces venezuelae SO1	P(3HP), P(3HB), P(3HB-*co*-3 HV), P(3HO), PCL	27.0 (NR)	9.5	50.0	5.9	AFQ93689.1	[88]
Thermus thermophilus HB8	P(3HO), P(3HO-*co*-3HHx)	28.0 (NR)	8.5	70.0	NR	NR	[84]
Xanthomonas sp. JS02	P(HPV), P(5POHV-*co*-7POHH)	41.7 (monomer)	8.5	60.0	NR	NR	[96]

[a] Theorical pI value calculated from amino acid sequence. NR: not reported; 3HHx: 3-hydroxyhexanoate; 3HHp: 3-hydroxyheptanoate; 3HO: 3-hydroxyoctanoate; 3HN: 3-hydroxynonanoate; 3HD: 3-hydroxydecanoate; 3HUD, 3-hydroxyundecanoate; HPV: 3-hydroxy-5-phenylvalerate; 5POHV: 3-hydroxy-5-phenoxyvalerate; 7POHH: 3-hydroxy-7-phenoxyheptanoate; PCL: poly(ε-caprolactone); PLA: poly(l-lactic acid); PHACOS: polymers containing thioester groups in the side chain.

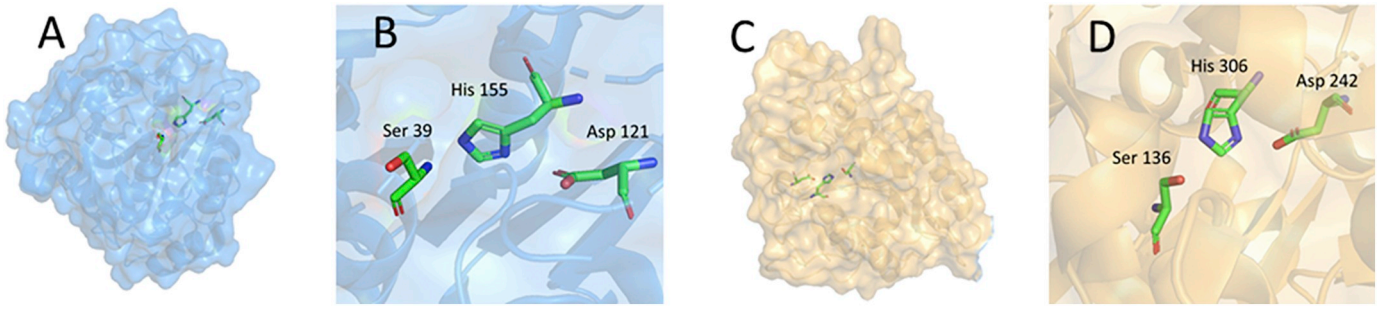

Fig. 2. Three-dimensional structures of PhbZ from *Penicillium funiculosum* (PDB code 2D80) (panel A) and PhabZ7 de *Paucimonas lemoignei* (PDB code 4BRS) (panel C), and the catalytic triad in their active site (panel B and D, respectively).

completely abolished by dithiothreitol (DTT). Likewise, a serine esterase inhibitor such as phenylmethyl sulfonyl fluoride (PMSF) is able to significantly reduce the enzymatic activity of the majority of e-PhaZs, confirming the presence of a catalytic serine in the active site of these enzymes. Kinetic parameters of PhaZs have been barely reported, although there are some articles that indicate the Km for P(3HB) ranging from 13.3 to 14,000 μg/mL (Table 1). The lack of the SBD in PhaZ from *Penicillium funiculosum* would explain the larger Km value for P(3HB) of this fungal enzyme compared to the other PhaZs [53]. Finally, PHA depolymerases may release monomers [24,25,53], dimers [40,57,63], or even a mixture of monomers and 3HA oligomers [27,43,44,67,68] as hydrolytic products, indicating that these enzymes might be classified as *exo*- (only monomers) or endo-type hydrolases (oligomers). Interestingly, mcl-PHA depolymerase form *Streptomyces exfoliatus* is able to degrade functionalized polymers containing thioester groups in the side chain, releasing functional thioester-based monomers and oligomers [85]. In addition to PHAs, some PhaZs can degrade other biodegradable plastics such as PCL [17,18,21,59,86–88], PLA [87], PPL [15,71], PEA [24,32,71], PES [18,24,26,32,71], PTMA [24], and PBS [26] (see Tables 1 and 2).

3. PBS, PBSA, PCL and PLA-degrading enzymes

Poly(butylene succinate) (PBS) is a semi-crystalline thermoplastic which is chemically synthesized by polycondensation of succinic acid with 1,4-butanediol [97]. With a melting point of 112–114 °C [98], the mechanical properties of PBS are similar to low-density polyethylene (LDPE) or polypropylene (PP) [98,99], but it can be biodegraded in contrast to LDPE and PP [100]. PBS is one of the most suitable materials to be processed into injection molded products, films, paper laminates, and sheets [101]. When adipic acid is used as additive, poly(butylene succinate-*co*-adipate) (PBSA) is synthetized, showing a reduced melting point (T_m = 95 °C) and higher flexibility (320–380 MPa of flexural modulus) in comparison to PBS (60–70 MPa) [102]. Besides, PBSA copolymer is better degraded due to its lower crystallinity and glass transition temperature (T_g value of - 45 °C) compared to PBS homopolymer (T_g = − 28 °C) [103]. PBS and PBSA were manufactured under the trademark Bionolle® (series 1000 and 3000, respectively) by Showa Highpolymer Co., Ltd. but their production was discontinued; nowadays PBS is produced by Mitsubishi Chemical. On other hand, poly (ε-caprolactone) (PCL) is another synthetic aliphatic polyester which is produced by polycondensation of 6-hydroxyhexanoic acid, or by ring-opening polymerization of ε-caprolactone [104]. Despite its slow biodegradability [105], PCL is an alternative to recalcitrant petroleum based-plastics due to its low melting point (T_m = 58–63 °C), low glass transition temperature (T_g value around - 65 °C), hydrophobicity, and high crystallinity [106]. Environmentally advantageous, PCL has been also chosen in biomedicine for controlled drug delivery and tissue engineering [107]. Finally, poly(lactic acid) (PLA) is a thermoplastic which can be produced by fermentation (from biomass) or chemical synthesis (direct condensation polymerization of lactic acid, or ring opening polymerization of lactide). Biodegradable PLA has been

gradually commercialized under different trademarks such as LACEA® (Mitsui Chemicals, Inc.) and INGEO® (NatureWorks LLC). PLA behaves like PET but also performs like PP, showing excellent strength properties and biocompatibility that have allowed this unique polymer to be used in the manufacture of stents, sutures, dialysis media, and drug delivery devices. Moreover, PLA possesses high transparency and is an excellent material for packaging. There are three stereoisomers of PLA: poly(*L*-lactic) (PLLA) acid, poly(*D*-lactic acid) (PDLA) and racemic poly (*DL*-lactic acid) (PDLLA). PLA can be either amorphous glassy polymers (PDLLA) or semi-crystalline products (PLLA and PDLA) in the solid state, depending on the sequence of enantiomeric repeating units (L and D) in the polymer backbone. Enantiomerically pure PLA is more difficult to degrade in natural environments than other BPs, mainly due to its high melting point and glass transition temperature. In fact, semi-crystalline PLLA shows a T_g = 50–80 °C and a T_m = 173–178 °C, whereas amorphous racemic PDLLA presents a T_g = 55–60 °C. Many microorganisms expressing enzymes that are able to accomplish PBS and PBSA degradation have been found in compost [101]. Generally, the same enzyme can hydrolyze both polyesters, and this specificity has been attributed to their similar chemical and physical properties [108–110]. Likewise, degradation of PCL and PLA may be accomplished by the same microbial enzymes that also degrade PBS and PBSA. As shown in Table 3, there are several microorganisms, including bacteria and fungi, that express different hydrolases able to decompose all these polyesters. Some of these enzymes have been subjected to several studies that are summarized below.

3.1. Lipases for PBS(A), PCL and PLA degradation

Firstly, lipases may catalyze ester bond hydrolysis present in different polyesters [128], including PBS and PBSA [129,130]. Nevertheless, several available lipases such as those from *Burkholderia cepacia* (lipase PS) [131,132], *Aspergillus niger* (lipase A), *Candida rugosa* (lipase AY) or *Rhizopus oryzae* (lipase F) [117] cannot degrade PBS nor PBSA. Consequently, novel lipases with degrading activity towards these polyesters have been sought. In this sense, it was found that PBS could be completely degraded by lipase Asahi (from *Chromobacterium viscosum*) at 37 °C and pH 7.0 after 17 days of incubation, whereas PBSA hydrolysis was fully completed by lipase Asahi, and lipases F (from *Rhizopus niveus*), QL (from *Alcaligenes* sp) and F-AP15 (from *R. oryzae*) after 4, 6, 11 and 22 days of incubation, respectively. Longer incubation times were required for lipase AY30 from *C. rugosa* and lipase M10 from *Mucor miehei* that degraded PBSA at 21.9% and 36.1% after 100 days of incubation, respectively [133]. In the same study, PCL films were completely degraded after 100 days at under the same conditions by lipases Asahi, F, and QL after 6, 11 and 14 days of incubation, respectively. Other microbial PCL-degrading lipases have been found in fungi (like *Aspergillus oryzae* [134] and *M. miehei* [133,135]) and bacteria (like *Pseudomonas* and *Lactobacillus* [136,137]). It is worth mentioning that *M. miehei* lipase was able to catalyze the degradation of PCL in toluene with a maximum conversion degree of about 70% only after 1 h, within the temperature range of 40–60 °C [138]. Regarding PLA degradation, lipases show preference towards PDLA stereoisomer and racemic PDLLA. In this sense, lipases from *Paenibacillus amylolyticus* strain TB-13 (PlaA) [122,139] and *A. niger* MTCC 2594 [140] can degrade both PDLA and PDLLA, as well as other BPs such as PCL, PBS or PBSA. As a rule of thumb, biodegradability of different polyesters by lipases tends to be PBSA > PCL > PBS ≥ P(3HB)/V = PLA [133]. In general, polymer chains are randomly hydrolyzed by lipases to high-molecular weight oligomers and finally to monomers and dimers.

3.2. Cutinase-like enzymes for PBS(A), PCL and PLA degradation

Acidovorax delafieldii BS-3 is bacterial strain isolated from soil [108] that was reported to secrete an extracellular enzyme (named PbsA) with degrading activity against solid and emulsified PBSA, as well as emulsified PBS. In contrast, PbsA displayed no activity with poly(3HB-*co*-4HB) and PLA. Although PbsA showed some similarities to lipases from *Streptomyces* sp. M11 and *Mollaxella* sp. TA144 [108,111], this enzyme was finally considered as a cutinase-like enzyme due its higher sequence homology to proteins belonging to the cutinase family [141]. Cutinases (EC 3.1.1.74) [142] are produced by phytopathogenic fungi, hydrolyzing the biopolyester cutin in plant surfaces during the initial stage of the fungal infection [143]. In addition to natural cutin substrate, cutinases are also active on soluble as well as on emulsified triglycerides, although they do not exhibit interfacial activation like classical lipases [144]. Besides, cutinases catalyze the hydrolysis of esters with chain lengths lower than 10 carbon atoms, whereas lipases hydrolyze acyl esters with higher than 10 carbon atoms. These properties have been exploited for reactions with small esters and synthetic polyesters [115,145,146] and, consequently, several cutinase-like enzymes (CLEs) for PBS(A) degradation have been gradually found. For instance, a BP-degrading enzyme from *Cryptococcus* sp. S-2 was formerly classified as a lipase [118], but it was also described as a CLE [117]. CLE from *Cryptococcus* sp. S-2 (*Cs*CLE) could effectively degrade PBS and other polyester-type bioplastics, such as PCL, P(3HB) and PDLA [147]. Previously, *Cs*CLE biochemical characterization was carried out using lipase substrates such as *p*-NPL (*p*-nitrophenyl laurate) and olive oil, and maximum activity was observed at pH 7.0 and 37 °C, while the enzyme was stable at pH values ranging from 5.0 to 9.0 and temperatures up to 50 °C [118]. Another BP-degrading enzyme was also isolated and purified from *Pseudozyma antarctica* JCM10317, and it was named PaE [148]. PaE degraded solid films of PBS, PBSA, PCL and PLLA [125], and it showed a PBSA-degrading activity that was 6500 times higher than the one displayed by lipase B from *Candida antarctica* (Lipozyme CALB-L) [148]. Likewise, high identity was observed between PaE protein sequence and other CLEs [125]. Similarly, extracellular enzyme from *Paraphoma*-like fungus B47–9 (named PCLE) was able to degrade films of PBS, PBSA, PBAT, PCL, and PDLLA, but did not degrade PLLA and PHB at all [123,149]. Production of PCLE enzyme was enhanced with 0.27% PBSA concentration in jar-fermentor cultures [124]. Likewise, yeast *Cryptococcus flavus* GB-1 produces an extracellular CLE (hereafter *Cf*CLE) with PBSA-degrading activity (0.58 $U.mL^{-1}$), whereas its UV-induced mutant strain GB-1-DMC1 showed improved enzyme production (1.53 $U.mL^{-1}$). Due to its broad substrate specificity, *Cf*CLE could degrade several cast films composed by PBSA, PBS, PCL, PBAT, and it degraded PDLA more efficiently than PLLA. The deduced amino acid sequence of *Cf*CLE was found to be 93% identical to *Cs*CLE [119]. Another BP-degrading enzyme was produced by the fungus *Cryptococcus magnus*, isolated from the larval midgut of a stag beetle, *Aegus laevicollis*. The partial amino acid sequence of this enzyme showed similarity to two previously described members of the CLE family (*Cs*CLE and PaE), and it was named *Cm*Cut1. Enzymatic degradation of PBSA, PBS, PCL, PDLA, and PLLA films by *Cm*Cut1 was confirmed, whereas P(3HB) films were not degraded [120]. Other microbial CLEs with PBS-degrading activity have been isolated and characterized so far, such as CutL1 from *Aspergillus oryzae* RIB40 [103] and *Tt*cutA from *Thielavia terrestris* CAU709 [127]. On one hand, CutL1 can degrade emulsified PBS, PBSA, and PLA (no stereoisomer was indicated) [103], and its specific activity with these substrates was 0.42 $U.mg^{-1}$, 11 $U.mg^{-1}$ and 0.067 $U.mg^{-1}$, respectively. On the other hand, *Tt*cutA was able to degrade PBS, PCL and PET films at a hydrolytic rate of 56.4, 203.0, and 1.1 $mg.h^{-1}.mg^{-1}$ protein, respectively [127]. Finally, an extracellular PBS depolymerase was purified from the culture supernatant of the fungal strain *Fusarium* sp. FS1301, and its characterization revealed that this depolymerase could degrade PBS and PCL, but not PHB nor PLA (no stereoisomer was indicated). Optimal conditions for enzyme activity were established at 50 °C and pH 8.0, and presence of Na^+ and K^+ increased significantly PBS depolymerase activity. Analysis of the amino acid sequence of the enzyme by protein fingerprinting indicated that PBS depolymerase from *Fusarium* sp. FS1301 was identical to cutinase from *Fusarium solani* (FsC)

Table 3
Biochemical properties of several enzymes involved in PBS(A), PCL and PLA degradation.

Microorganism (enzyme)	Substrate specificity	Molecular weight (kDa)	Optimal conditions		Enzymatic activity expressed in U.mg^{-1} (substrate)	Protein NCBI accession number	Ref.
			pH	T (°C)			
Acidovorax delafieldii BS-3 (pbsA)	PBSA	NR	NR	NR	NR	BAB86909.1	[108,111]
Amycolatopsis orientalis PLAase I	PLA, casein	24.0	9.5	60.0	4.5 (PLA)	NR	[112]
ssp. orientalis PLAase II		19.5	10.5	50.0	2.0 (PLA)	ABY53108.1	
PLAase III		18.0	9.5	60.0	8.75 (PLA)	ABY67151.1	
Amycolatopsis sp. K104–1 (PLD)	PLA	24.0	9.5	55.0–60.0	25.7 (PLA)	BAD02196.1	[113,114]
Aspergillus oryzae (CutL1)	PBS, PBSA, PCL PLA	21.6	9.0	33.0–55.0	0.42 (PBS), 11.0 (PBSA)	BAM28634.1	[103,115]
Brevundimonas sp. MRL-AN1	PCL, PES, PLA, P(3HB), P(3HB-*co*-3 HV)	63.5	6.0	30.0	31.2 (p-NPB)	NR	[116]
Cryptococcus sp. S-2 (*Cs*CLE)	PBS, PCL, P(3HB), PDLA	22.0	7.0	37.0	865 (p-NPL)	BAC67242.1	[117,118]
Cryptococcus flavus GB-1 (*Cf*CLE)	PBS, PBSA, PCL, PBAT, PDLA, PLLA	22.0	7.8	45.0	326.6 (PBSA)	BAT32793.1	[119]
Cryptococcus magnus (*Cm*Cut1)	PBS, PBSA, PCL, PDLA, PLLA	21.0	7.5	40.0	44.4 (PBSA)	BAN42607.1	[120]
Fusarium sp. FS1301	PBS, PCL	20.0	8.0	50.0	71.8 (PBS)	AAB05922.1	[121]
Paenibacillus amylolyticus TB-13 (PlaA)	PDLLA PBS, PBSA, PES, PCL	22.0	10.0	45.0–55.0	184.6 (p-NPB)	BAC67195.1	[122]
Paraphoma-related fungus B47–9 (PCLE)	PBS, PBSA, PCL, PBAT, PDLLA	19.7	7.2	45.0	363 (PBSA)	BAN51852.1	[123,124]
Psudozyma antarctica JCM 10317 (PaE)	PBS, PBSA, PCL, PLLA	20.4	9.5	40.0	58.8 (PBSA)	BAN66731.1	[125]
Ralstonia sp. MRL-TL	PCL, PES, PLA, P(3HB), P(3HB-*co*-3 HV)	50.0	7.0	50.0	34.3 (p-NPB)	NR	[126]
Thielavia terretris CAU709 (*Tt*cutA)	PBS, PCL, PET	25.3	4.0	50.0	983.0 (p-NPB)	NR	[127]

NR: not reported; PCL: poly(ε-caprolactone); PLA: poly(lactic acid); PLLA: poly(*L*-lactic acid); PDLA: poly(*D*-lactic acid); PDLLA: poly(*DL*-lactic acid); P(3HB): poly(3-hydroxybutyrate); P(3HB-*co*-3 HV): poly(3-hydroxybutyrate-co-3-hydroxyvalerate); PES: poly(ethylene succinate); PBS: poly(butylene succinate), PBSA: poly(butylene succinate)-*co*-(butylene adipate); PBST: poly(butylene succinate-*co*-terephthalate); PBSTIL: poly(butylene succinate / terephthalate / isophthalate)-*co*-(lactate); p-NPL: *p*-nitrophenyl laurate. p-NPB: *p*-nitrophenyl butyrate.

[121]. This enzyme possesses hydrophobic areas on its molecular surface, such as a hydrophobic binding loop and a flap close to the active site [121]. Although the structures of *A. oryzae* (CutL1) and *F. solani* (FsC) cutinases have a similar fold (Fig. 3), comparison of the two sequences revealed that CutL1 possessed an additional disulfide bond, and a topologically favored catalytic triad with a continuous and deep groove. Such structural features were proposed to result in improved hydrolytic activity of CutL1, as well as enhanced thermostability and remarkable reactivity towards PCL degradation [115]. As shown in Table 3, all reported CLEs with PBS(A)-degrading activity show a small molecular weight (about 20–30 kDa). In addition, different strategies have been used for their purification, mainly ion-exchange [118,148], affinity [119,120,123] or hydrophobic [103] chromatography. Depending on the microbial source, CLEs displayed their optimal temperature from 30 °C to 50 °C, whereas their optimal pH was established between 4.0 and 9.5. Interestingly, PCLE activity was improved by Ca^{2+} but inhibited by Mg^{2+} [123,149]. However, *Cm*Cut1 activity was enhanced in presence of Ca^{2+} and Mg^{2+} [120], whereas *Cf*CLE was inhibited by the same divalent ions [119]. Interestingly, best specific activities were obtained with *Cf*CLE (326.6 U.mg^{-1}) and PCLE (363.0 U.mg^{-1}), employing emulsified PBSA as substrate.

As indicated before, several cutinases show degrading activity towards PCL in addition to other polyester-type BPs (Table 3). An interesting study about activity and stability of different fungal PCL-degrading cutinases indicated that *Thermomyces insolens* (formerly *Humicola insolens*) produced the most active cutinase (HiC) under all pH conditions [150]. Remarkably, complete PCL hydrolysis in 3 h was achieved by HiC at pH 8.0 and 40 °C. Moreover, HiC was the most stable and active under extreme temperature and acidic pH conditions due to its unique surface charge, followed by cutinases from *A. oryzae* and *A. fumigatus* which both possessed additional disulfide bonds. In this sense, recombinant *A. fumigatus* was able to completely degrade PCL films after 12 h at 40 °C (or 6 h at 60 °C), but it also showed synthetic activity in organic solvents using ω-hydroxy-hexadecanoic acid as substrate, yielding polymers with a molecular weight of 25,000 [151]. Results from these studies suggest that some specific cutinases might be candidates for further protein engineering in order to obtain highly active and stabilized biocatalysts suitable for biotechnological and industrial applications. In this sense, a recent approach has been based on the construction of a bi-functional enzyme (Lip-Cut) formed by thermostable *Thermomyces lanuginosus* lipase and *T. terrestris* cutinase (*Tt*cutA) in order to develop a more efficient biocatalyst for PCL degradation and recycling [152]. The fusion enzyme was expressed in *Pichia pastoris* and displayed significantly enhanced capacity for PCL degradation, since weight loss of PCL film at 6 h with Lip-Cut was 13.3, 11.8 and 5.7 times higher than that obtained with lipase, *Tt*cutA and lipase/*Tt*cutA mixture, respectively.

3.3. Depolymerases for PBS(A), PCL and PLA degradation

In addition to lipases and cutinases, there are other hydrolases that can depolymerize aliphatic polyesters such as PBS(A), PCL and PLA. On one hand, *Leptothrix* sp. TB-71 was identified as a microorganism that was able to degrade PBSA, PCL and PES, but not PBS, P(3HB-*co*-3 HV), nor PLA [103]. In this sense, a putative PBSA depolymerase was suggested to play a major role in PBSA degradation, but partially purified enzyme was not characterized so far [153]. Likewise, several extracellular PCL-degrading enzymes form different microbial sources have been reported. Most of them could degrade not only PCL but also other BPs. Firstly, a PCL depolymerase could be purified from culture broths of *Brevundimonas* sp. MRL-AN1, a strain isolated from soil. The enzyme was stable at wide range of temperature (from 20 °C to 45 °C) and pH (from 5.0 to 9.0). As expected, this PCL depolymerase could also degrade PES, PLA, P(3HB), and P(3HB-*co*-3 HV) [116]. On the other hand, two other PCL-degrading enzymes were purified from the culture supernatant of *Streptomyces thermoviolaceus* subsp. *thermoviolaceus* strain 76 T-2. The molecular weights of these extracellular PCL depolymerases were 25 kDa and 55 kDa, respectively. Interestingly, the 25-kDa protein could degrade chitin and its N-terminal sequence was identical to those amino acids located at 64–71 fragment position of chitinase Chi25 of *Streptomyces thermoviolaceus* OPC-520 [106]. Finally, an extracellular PCL depolymerase from the was also isolated and purified from the broth culture of

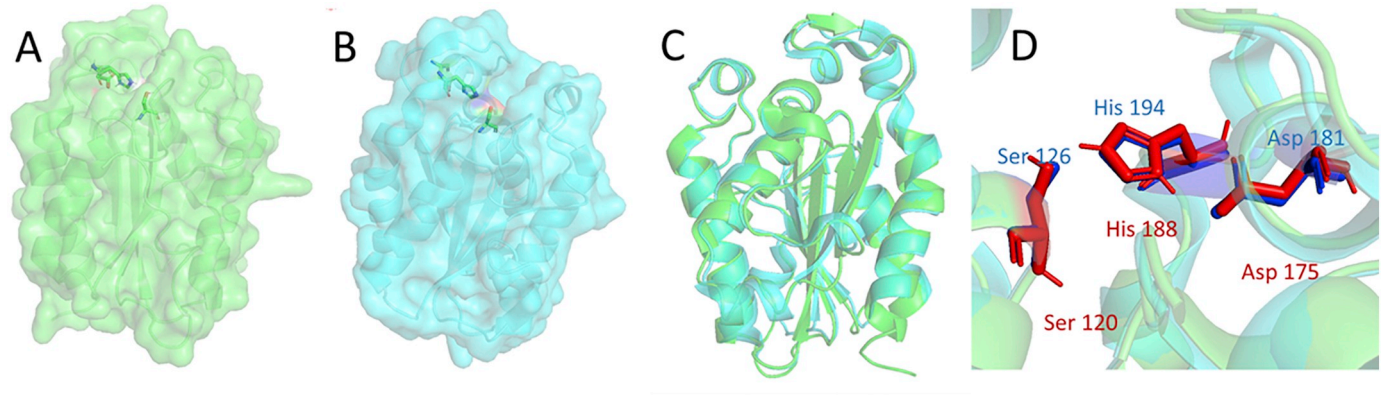

Fig. 3. Three-dimensional structures of cutinases from *Fusarium solani* (PDB code 1CUS) (panel A) and *Aspergillus oryzae* (PDB code 3GBS) (panel B). Superposition of *A. oryzae* (cyan) and *F. solani* (green) cutinases revealing nearly identical structural similarity (panel C). Superposition of catalytic triad of *A. oryzae* (blue) and *F. solani* (red) cutinases (panel D).

thermophilic bacteria *Ralstonia* sp. MRL-TL. In addition to PCL, different aliphatic polyesters like PES, PLA, P(3HB) and P(3HB-*co*-3 HV) could be efficiently degraded by this enzyme. Interestingly, PCL depolymerase from *Ralstonia* sp. MRL-TL was stable at wide temperature (from 37 to 50 °C) and pH (from 5.0 to 7.0) range, showing maximum stability at 50 °C and pH 7.0, so it might be useful for the bioremediation of polyester-contaminated environments under harsh conditions. This enzyme was active in the presence of metal ions such as Na^+, Ca^{2+}, Fe^{2+}, Zn^{2+}, Mn^{2+} in contrast to K^+ and Mg^{2+} that inhibited the enzyme activity [126]. According to their stereoisomer preference, PLA depolymerases may be categorized into lipase-type (mentioned in section 3.1) and protease-type enzymes. Within the second group, proteinase K from *Tritirachium album* [154], as well as several serine-proteases like trypsin, elastase, and subtilisin have demonstrated to hydrolyse PLLA [155]. Another member of this group is PLA depolymerase from *Amycolatopsis* sp. K104–1 (named PLD) which could also hydrolyze casein and fibrin [113,114]. Interestingly, PLD bound to P(3HB), PCL, and PBS as well as PLA, but it degraded only PLA. Similarly, three novel PLA-degrading enzymes (named PLAase I, II and III) were purified culture supernatant of *Amycolatopsis orientalis* ssp. *orientalis* [112], whose activities were significantly stronger than proteinase K. All PLAases degraded casein besides PLA, whereas they showed no activity for PHB. Results of sequence analysis indicated a relatively high similarity of PLAase II and PLAase III with serine proteases, but revealed no homology among PLAase II, PLAase III and PLD [114].

4. Enzymes for aromatic polyester degradation

Polyesters containing a high ratio of aromatic components (such as PET, PBT and PTT) are chemically synthetized using terephthalic acid which is derived from raw petroleum. At temperatures above 65 °C (glass transition temperature of PET), the amorphous parts of the polymer become flexible and more accessible to an enzymatic attack. Therefore, suitable enzymes for PET hydrolysis must present high thermal stability [156]. Within this particular context, microbial lipases display low activity against PET due to their lid structure covering the buried hydrophobic active site, whereas fungal and bacterial cutinases lack this lid and show an exposed catalytic center close to the protein surface that increase their accessibility for polymeric substrates. Unlike PHA depolymerases [157], a specific substrate binding domain (SBD) is absent in cutinases [142,158] and enzyme adsorption to PET surface is presumably mediated by hydrophobic regions surrounding the catalytic site [159]. Firstly, some examples of thermostable PET-degrading enzymes include fungal cutinase from *T. insolens* (HiC) that was able to hydrolyze a low crystalline PET film almost completely after 96 h at 70 °C [160], and bacterial LC-cutinase (from leaf-branch compost metagenome) that hydrolyzed approximately 25% of a low crystalline PET film after 24 h at 70 °C [161]. The presence of divalent ions (Ca^{2+} and Mg^{2+}) has proven to enhance the thermostability of several PET-degrading esterases produced by the thermophilic actinomycete *Thermobifida fusca* KW3, allowing PET degradation at 65 °C [162]. By substitution of the metal binding site with a salt bridge or a disulphide bridge, variants of TfCut2 from *T. fusca* KW3 (Fig. 4A), were able to degrade amorphous PET films at 70 °C in absence of divalent ions [162]. Other interesting cutinases with PET-degrading activity have been found, such as those from *T. fusca* DSM 44342 (Thf42_Cut1) and *Thermobifida cellulolysitica* (Thc_Cut1 and Thc_Cut2) (Fig. 4B). Enzyme adsorption to PET was increased when Thc_Cut1 was fused to the SBD of PHB depolymerase from *A. faecalis* T1 without affecting its k_{cat} value on pNP-butyrate as substrate and increasing its hydrolytic activity against PET substrates respect to the native enzyme. Adsorption to PET films was weaker with the fusion enzyme holding the SBD of 1,4-β-cellobiohydrolase I from *Hypocrea jecorina* (formerly *Trichoderma reesei*) [163]. As a matter of fact, protein engineering has been revealed as a promising approach to generate improved enzymes for PET degradation. One example is represented by the truncated esterase from *Clostridium botulinum* (Cbotu_EstA) (Fig. 4C) [164], whose hydrolytic activity was enhanced due to the emergence of an exposed hydrophobic patch (absent in the whole native enzyme) that facilitated its adsorption to the polyester [165]. Likewise, highest activity and thermostability have been also achieved by the double mutation S226P/R228S of the Ca^{2+}-activated cutinase from the thermophile *Saccharomonospora viridis* AHK190 (Cut190) (Fig. 4D) [166]. Besides PET, the mutant Cut190 showed hydrolytic activity towards different aliphatic polyesters including PBSA, PBS, PCL, P(3HB), PDLA and PLLA.

Similarly, the highly active and thermostable mutant esterase Est1 (A68V/T253P) from *Thermobifida alba* AHK119 hydrolyzed an amorphous PET film as well as other aliphatic-aromatic copolyesters (AAC) [167]. In this sense, AACs share the good material properties of aromatic polyesters, and may be used as a film or coating in disposable food packaging. Some commercial AACs are available from BASF (PBTA as Ecoflex®), DuPont (PBST as Apexa®, formerly Biomax®) and Eastman Chemicals (PTAT as EasterBio®), among other manufacturers. In contrast to aromatic polyesters which are quite recalcitrant, some aliphatic-aromatic copolyesters such as PBST, PBAT, and PBSTIL (Fig. 1) can be degraded more easily by some reported enzymes (Table 4). Formerly, it was observed that *R. delemar* lipase activity towards AACs was abruptly diminished when aromatic components were increased in the polyester [2], and therefore other hydrolases with improved AAC-degrading activity were searched. As a result, *Thermobifida fusca* DSM 43793 was reported to produce a PBAT-degrading enzyme (named BTA-1 or BTA hydrolase 2) that showed high similarity to lipases from *Streptomyces albus* G and *Streptomyces* sp. M11 [168], and 100% sequence identity to lipase Tfu_0883 from *T. fusca* YX [169]. BTA-1 was also able to

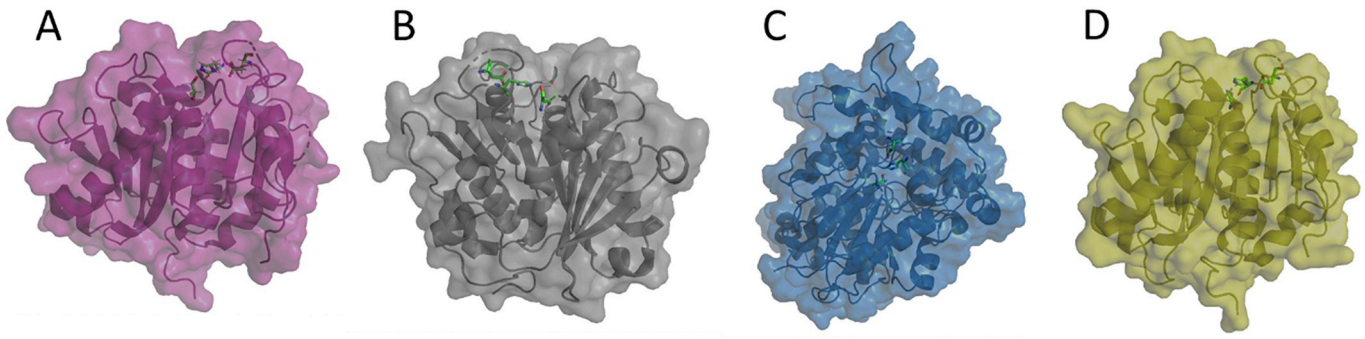

Fig. 4. Three-dimensional structures of cutinases with high hydrolytic activity towards PET. Panel A: TfCut2 from *Thermobifida fusca* (PDB code 4CG1); Panel B: Thc_Cut2 from *Thermobifida cellulolysitica* (PDB code 5LUK); Panel C: Cbotu_EstA from *Clostridium botulinum* (PDB code 5AH1); Panel D: Cut190 from *Saccharomonospora viridis* (PDB code 5ZNO).

Table 4
Biochemical properties of several enzymes involved in aromatic polyester and aliphatic-aromatic copolyester degradation.

Microorganism (enzyme)	Substrate specificity	Molecular weight (kDa)	Optimal conditions		Enzymatic activity expressed in $U.mg^{-1}$ (substrate)	Protein NCBI accession number	Ref.
			pH	T (°C)			
Bacillus pumilus ($PBATH_{Bp}$)	PBAT, PBSA, PBS, PCL, PES	19.0	NR	NR	2.7 (p-NPA)	BAV72205.1	[173]
Metagenome from leaf-branch compost (LC-cutinase)	PET, PCL	27.9	8.5	50.0	270.0 (p-NPB)	4EB0_A	[161]
Rosaeteles depolymerans TB-87 (Est-H)	PBSTIL, PBS, PBSA, PBST,	31.0	9.0	30.0	34.1 (PBSA)	NR	[175,176]
Rosaeteles depolymerans TB-87 (Est-L)	PCL	27.0	9.0	30.0	27.6 (PBSA)	NR	
Saccharomonospora viridis AHK190 (Cut190_ S226P_R228S)	PBSA, PBS, PCL, PBTA, P (3HB), PDLA, PLLA, PET	33.0	6.5–7.0	65.0	16.1 (PBSA), 14.2 (p-NPB)	BAO42836.1	[166]
Thermobifida alba AHK119 (Est119)	PET, PBAT, PBSA, PBS, PCL, PDLA, PLL	30.0	6.0	50.0	1.37 (p-NPB)	BAK48590.1	[172]
Thermobifida fusca DSM 43793 (BTA-1)	PBAT, PET, PTT	28.0	6.0–7.0	65.0–70.0	360.0 (tributyrin)	CAH17554.1	[168–171]

NR: not reported; PCL: poly(ε-caprolactone); PES: poly(ethylene succinate); PBS: poly(butylene succinate), PBSA: poly(butylene succinate)-*co*-(butylene adipate); PBST: poly(butylene succinate-*co*-terephthalate); PBSTIL: poly(butylene succinate / terephthalate / isophthalate)-*co*-(lactate); PET: poly(ethylene terephthalate), PTT: poly(trimethylene terephthalate); p-NPA: *p*-nitrophenyl acetate; p-NPB: *p*-nitrophenyl butyrate.

depolymerize PET, in contrast to lipases from *Pseudomonas* sp. and *C. antarctica* that did not degrade PET [170]. The same enzyme hydrolyzed both PTT fibres and films, whereas lipase from *Thermomyces lanuginosus* was only able to hydrolyze the fibres [171]. On the other hand, the thermoactive esterase Est119 from *Thermobifida alba* AHK119 was reported to display degrading activity towards PBST [172], whereas PBAT hydrolase from *Bacillus pumilus* ($PBATH_{Bp}$) degraded PBAT films at a rate of 14.3 mg/cm^2/day [173]. Interestingly, two different PBSTIL depolymerases from *Roseateles depolymerans* TB-87 (Est-H and Est-L) showed a broad substrate specificity since they could degrade emulsified PBSTIL and PBST [174]. It is worth mentioning that the activity of Est-H was remarkably very high as compared to Est-L [174]. Recently, a chaperone (est-Ch) have been suggested to help the correct folding and transport of Est-H [175]. As shown in Table 4, enzymes involved in aromatic polyesters and AACs were mainly small (about 19–33 kDa) and displayed their optimal temperature from 30 °C to 70 °C, whereas their optimal pH ranged from 6.0 to 9.0.

Although most of them display a broad substrate specificity towards different aliphatic and aromatic BPs, thermostable enzymes from thermophilic actinomycetes seem more attractive for their application in PET degradation [158], taking into account that temperatures above 60 °C are required for a complete hydrolysis of this recalcitrant polymer.

5. Conclusions

Large quantities of polyester-type plastics have been introduced into the environment through its production and disposal, resulting in their accumulation in ecosystems worldwide. Chemical recycling of these polyesters is environmentally harmful and energy-consuming, and application of polyester degrading enzymes is an eco-friendly alternative that should be seriously considered. Many hydrolases from several fungi and bacteria have been discovered and successfully evaluated for their activity against different aliphatic and aromatic polyesters and their *co*-polyesters. Likewise, research has been focused on screening of novel thermostable biocatalysts active on recalcitrant polyesters containing aromatic components that requires high temperatures for their enzymatic hydrolysis. Interestingly, many of the reported polyester hydrolases display a broad substrate specificity including microbial PHA depolymerases and cutinase-like enzymes as the most relevant examples. As summarized in this review, biochemical characterization of many of these enzymes has resulted in the identification of their protein sequences, amino acid residues involved in catalysis, presence or absence of structural domains involved in substrate binding according to resolved 3D structures, and kinetic analysis using chromogenic substrates (*p*-nitrophenyl esters) and polymers to determine their optimal conditions (pH and temperature) for enzymatic activity and stability. Knowledge of all these features has already enabled the tailoring of some improved enzymes through different approaches that include site-directed mutagenesis and surface protein engineering.

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