Journal of Applied Biology & Biotechnology

Available online at http://www.jabonline.in

Genome analysis of *Streptomyces nigra* PRNP1-1W reveals enzymes and metabolic pathways associated with mesophilic poly(butylene succinate) biodegradation

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Doi: http://doi.org/10.7324/jabb.2027.333099

SUPPLEMENTRY MATERIAL

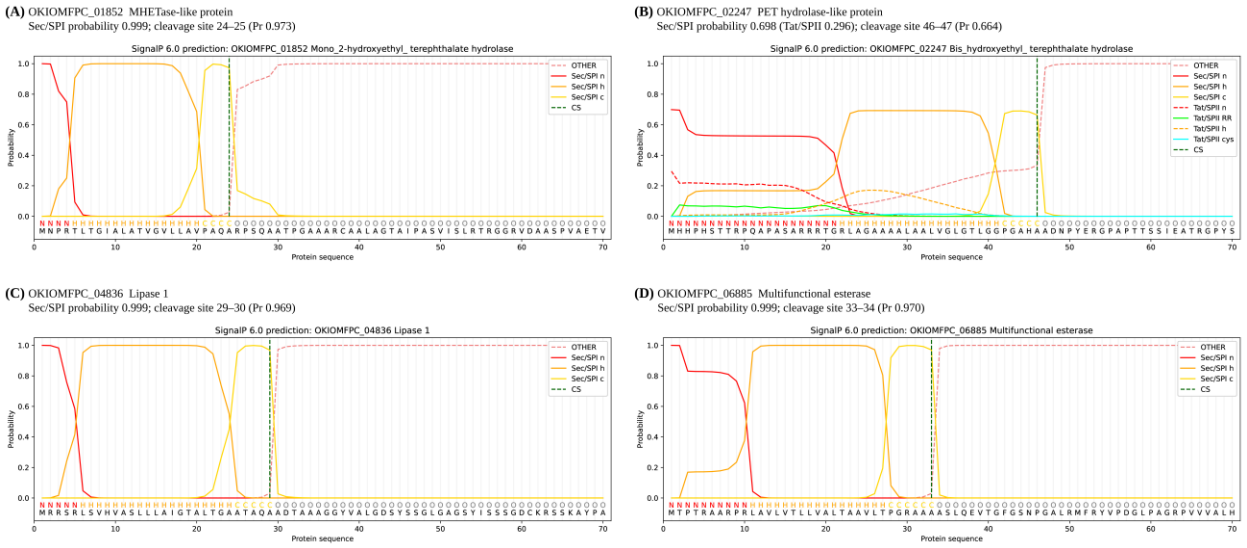

Figure S1. SignalP 6.0 predictions for the four candidate hydrolases of *S. nigra* PRNP1-1W that carry an N-terminal signal peptide: (A) OKIOMFPC_01852, (B) OKIOMFPC_02247, (C) OKIOMFPC_04836 and (D) OKIOMFPC_06885. Plot titles inside each panel show the original Prokka product names; panel

headings give the names used in Figure 4. Lines show per-residue probabilities of the n-, h- and c-regions, and the dashed vertical line marks the predicted cleavage site (CS).

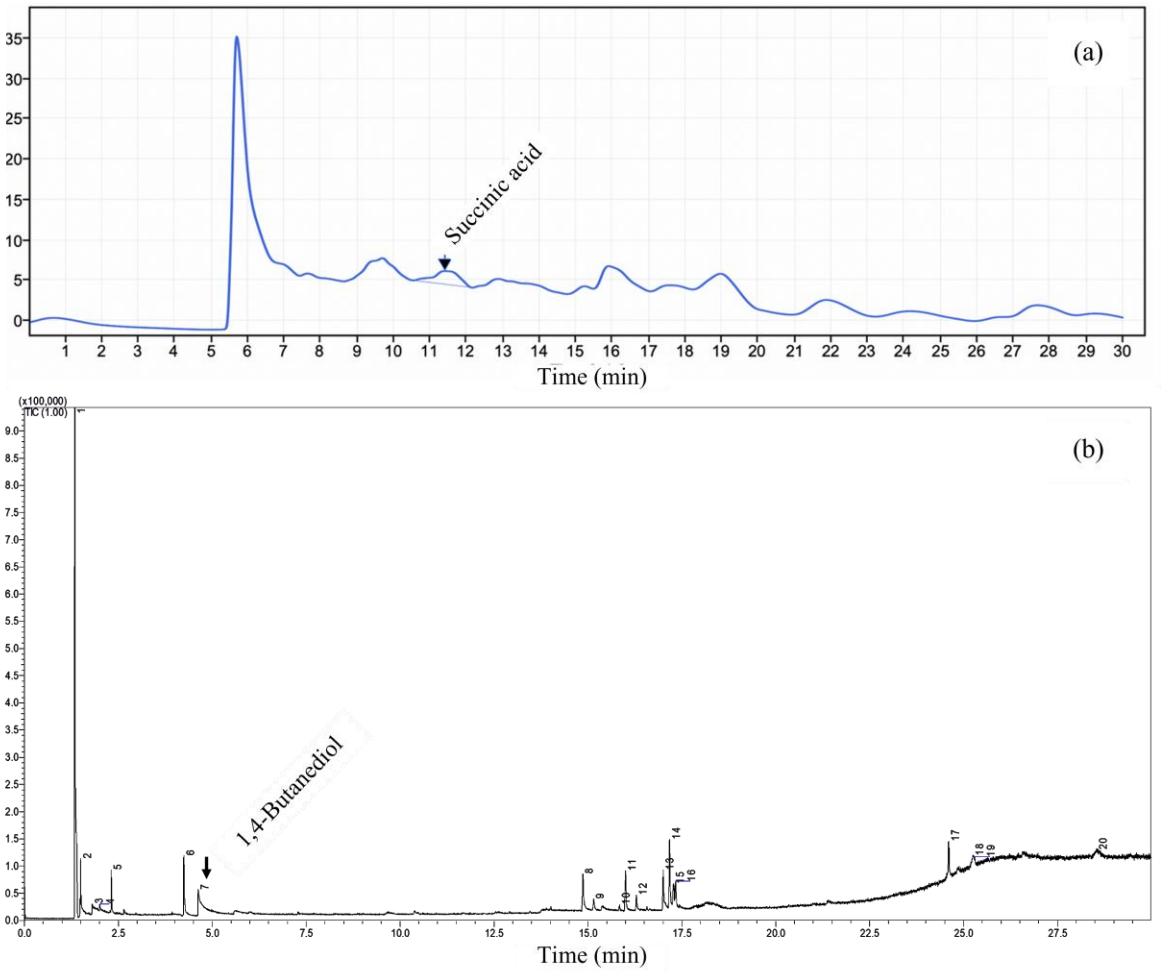

Figure S2. Chromatographic analyses of PBS degradation products: (a) HPLC chromatogram of the external standard showing the characteristic peak of succinic acid at a retention time of 11.393 min, and (b) GC-MS total ion chromatogram (TIC) profile of the culture sample indicating the detection of metabolic intermediates.

Table S1. Candidate genes for PBS hydrolysis and for the proposed metabolism of PBS-derived monomers in *Streptomyces nigra* PRNP1-1W.

Locus tag	BV-BRC ID	Contig	Position (bp)	Strand	Length (aa)	Product (Prokka)	EC number
Candidate hydrolases							
OKIOMFPC_00784	1883.5681.peg.787	1	807,164–808,345	-	393	Serine protease I	3.4.21.-
OKIOMFPC_00982	1883.5681.peg.992	2	166,542–167,741	-	399	Serine protease II	3.4.21.-
OKIOMFPC_01852	1883.5681.peg.1901	3	354,346–355,998	+	550	MHETase-like esterase	3.1.1.102
OKIOMFPC_02765	1883.5681.peg.2847	5	156,183–157,757	-	524	Carboxylesterase	3.1.1.1
OKIOMFPC_04836	1883.5681.peg.4947	9	372,848–373,657	-	269	Lipase 1	3.1.1.3
OKIOMFPC_05360	1883.5681.peg.5472	11	27,205–27,984	-	259	Thermostable monoacylglycerol lipase	3.1.1.23
OKIOMFPC_06885	1883.5681.peg.7048	18	21,438–22,421	-	327	Multifunctional esterase	3.1.1.-
PET hydrolase-like protein							
OKIOMFPC_02247	1883.5681.peg.2308	4	155,624–156,550	+	308	PET hydrolase-like (BdeA homolog) (bdeA)	3.1.1.-
Proposed downstream pathway genes							
OKIOMFPC_01685	1883.5681.peg.1710	3	154,678–155,700	-	340	Alcohol dehydrogenase	1.1.1.1
OKIOMFPC_03749	1883.5681.peg.3854	7	183,360–184,322	-	320	Alcohol dehydrogenase	1.1.1.1
OKIOMFPC_02358	1883.5681.peg.2420	4	265,108–266,160	-	350	putative zinc-binding alcohol dehydrogenase	1.1.1.1
OKIOMFPC_05697	1883.5681.peg.5808	11	389,212–390,255	+	347	putative zinc-binding alcohol dehydrogenase	1.1.1.1
OKIOMFPC_06107	1883.5681.peg.6230	13	92,830–94,020	-	396	putative zinc-binding alcohol dehydrogenase	1.1.1.1
OKIOMFPC_06543	1883.5681.peg.6675	15	86,679–87,662	-	327	Zinc-type alcohol dehydrogenase-like protein	–
OKIOMFPC_01786	1883.5681.peg.1826	3	277,947–279,521	-	524	Alpha-ketoglutaric semialdehyde dehydrogenase 2	1.2.1.26
OKIOMFPC_05646	1883.5681.peg.5757	11	337,897–339,363	+	488	Alpha-ketoglutaric semialdehyde dehydrogenase 2	1.2.1.26
OKIOMFPC_03434	1883.5681.peg.3532	6	382,630–384,024	+	464	Succinate-semialdehyde dehydrogenase [NADP(+)] 1 (gabD1)	1.2.1.79
OKIOMFPC_04346	1883.5681.peg.4453	8	296,482–298,095	+	537	Putative succinate-semialdehyde dehydrogenase [NADP(+)] 2 (gabD2)	1.2.1.79

Genes were predicted with Prokka v1.15.6 in Proksee; BV-BRC IDs are from the RASTtk annotation (genome 1883.5681) and were matched by contig coordinates.

OKIOMFPC_05646 overlaps BV-BRC peg.5757 with a different start codon (337,834–339,363). Product names follow the Figure 4 labels. Prokka names: OKIOMFPC_00784 and 00982, 'Serine protease'; OKIOMFPC_01852, 'Mono(2-hydroxyethyl) terephthalate hydrolase'; OKIOMFPC_02247, 'Bis(hydroxyethyl) terephthalate hydrolase'.

Table S2. Signal peptide prediction for candidate hydrolases of *S. nigra* PRNP1-1W.

Locus tag	Protein	Signal peptide type	Probability	Cleavage site (aa)	Cleavage-site probability
Candidate hydrolases (Figure 4)					
OKIOMFPC_00784	Serine protease I	None	1	–	–
OKIOMFPC_00982	Serine protease II	None	1	–	–
OKIOMFPC_01852	MHETase-like esterase	Sec/SPI	0.999	24–25	0.973
OKIOMFPC_02765	Carboxylesterase	None	1	–	–
OKIOMFPC_04836	Lipase 1	Sec/SPI	0.999	29–30	0.969
OKIOMFPC_05360	Monoacylglycerol lipase	None	1	–	–
OKIOMFPC_06885	Multifunctional esterase	Sec/SPI	0.999	33–34	0.97
PET hydrolase-like protein (Figure 4)					
OKIOMFPC_02247	PET hydrolase-like (BdeA homolog)	Sec/SPI	0.698	46–47	0.664

Prediction with SignalP 6.0 (organism group 'Other', slow model mode). Sec/SPI, secretory signal peptide cleaved by signal peptidase I. For OKIOMFPC_02247 the second most likely type was Tat/SPII (0.296). None of the ten proposed pathway genes listed in Table S1 had a predicted signal peptide (probability of no signal peptide ≥ 0.9999).

Table S3. Conserved domains and transmembrane segments of the candidate proteins (InterProScan).

Locus tag	Length (aa)	Pfam domain(s) (accession; position)	Other family / signature matches	Transmembrane segments (Phobius)
Candidate hydrolases				
OKIOMFPC_00784	393	Colicin V production protein (PF02674; 4–146); Trypsin-like peptidase domain (PF13365; 215–351)	MarP family serine protease (NF033740)	3 (29–49, 61–83, 103–129)
OKIOMFPC_00982	399	Colicin V production protein (PF02674; 4–146); Trypsin-like peptidase domain (PF13365; 220–358)	MarP family serine protease (NF033740)	4 (6–21, 28–49, 61–81, 102–121)
OKIOMFPC_01852	550	Tannase and feruloyl esterase (PF07519; 78–548)	–	0
OKIOMFPC_02765	524	Carboxylesterase family (PF00135; 7–458)	Carboxylesterases type-B serine active site (PS00122)	0
OKIOMFPC_04836	269	GDSL-like Lipase/Acylhydrolase family (PF13472; 40–257)	–	0
OKIOMFPC_05360	259	Alpha/beta hydrolase family (PF12697; 19–237)	–	0
OKIOMFPC_06885	327	Esterase PHB depolymerase (PF10503; 54–251)	extracellular catalytic domain type 1 short-chain-length polyhydroxyalkanoate depolymerase (NF050058); PHB depolymerase family esterase (TIGR01840)	0
PET hydrolase-like protein				
OKIOMFPC_02247	308	PET hydrolase-like (PF12740; 46–305)	Twin arginine translocation (Tat) signal profile (PS51318); bis(hydroxyethyl) terephthalate hydrolase (NF051441)	0
Proposed downstream pathway genes				
OKIOMFPC_01685	340	Alcohol dehydrogenase GroES-like domain (PF08240; 24–136); Zinc-binding dehydrogenase (PF00107; 175–298)	Zinc-containing alcohol dehydrogenases signature (PS00059); alcohol dehydrogenase AdhP (NF006940)	0
OKIOMFPC_03749	320	Alcohol dehydrogenase GroES-like domain (PF08240; 30–124); Zinc-binding dehydrogenase (PF00107; 165–283)	–	0
OKIOMFPC_02358	350	Alcohol dehydrogenase GroES-like domain (PF08240; 25–142); Zinc-binding dehydrogenase (PF00107; 182–289)	Zinc-containing alcohol dehydrogenases signature (PS00059)	0
OKIOMFPC_05697	347	Alcohol dehydrogenase GroES-like domain (PF08240; 25–132); Zinc-binding dehydrogenase (PF00107; 180–303)	–	0
OKIOMFPC_06107	396	Alcohol dehydrogenase GroES-like domain (PF08240; 25–146); Zinc-binding dehydrogenase (PF00107; 189–272)	Zinc-containing alcohol dehydrogenases signature (PS00059)	0
OKIOMFPC_06543	327	Alcohol dehydrogenase GroES-like domain (PF08240; 24–113); Zinc-binding dehydrogenase (PF13602; 187–324)	Quinone oxidoreductase / zeta-crystallin signature (PS01162)	0
OKIOMFPC_01786	524	Aldehyde dehydrogenase family (PF00171; 18–454)	–	0
OKIOMFPC_05646	488	Aldehyde dehydrogenase family (PF00171; 2–425)	–	0
OKIOMFPC_03434	464	Aldehyde dehydrogenase family (PF00171; 3–460)	Aldehyde dehydrogenases cysteine active site (PS00070); NADP-dependent succinic semialdehyde dehydrogenase (NF006915)	0
OKIOMFPC_04346	537	Aldehyde dehydrogenase family (PF00171; 46–501)	Aldehyde dehydrogenases glutamic acid active site (PS00687); succinic semialdehyde dehydrogenase (NF006916)	0

Domains were identified with InterProScan (EMBL-EBI web service, job R20261016-080744-0939-44528382-p2m) using all member databases. Positions are amino acid residues.

Transmembrane segments were predicted by Phobius within InterProScan. For OKIOMFPC_02247, the PROSITE Tat signal profile (PS51318) matched residues 1–46, whereas SignalP 6.0 favoured a Sec/SPI signal peptide (Table S2).

Table S4. Closest homologs of the candidate proteins in UniProtKB/Swiss-Prot.

Locus tag	Accession	Protein	Organism	Identity (%)	Query coverage (%)	E-value
Candidate hydrolases						
OKIOMFPC_00784	P9WHR8.1	Serine protease MT3772	*Mycobacterium tuberculosis* CDC1551	38.89	99	8e-51
OKIOMFPC_00982	P9WHR8.1	Serine protease MT3772	*Mycobacterium tuberculosis* CDC1551	38.64	99	7e-63
OKIOMFPC_01852	Q8UK62.1	Uncharacterized esterase Atu5261	*Agrobacterium fabrum* str. C58	34.99	99	2e-95
OKIOMFPC_02765	P86325.1	Carboxylesterase	*Thermobifida fusca*	46.6	92	4e-129
OKIOMFPC_04836	Q9S2A5.1	Lipase 1	*Streptomyces coelicolor* A3(2)	73.51	100	5e-131
OKIOMFPC_05360	P82597.3	Thermostable monoacylglycerol lipase	*Bacillus* sp. H-257	40.24	95	2e-53
OKIOMFPC_06885	P52090.1	Poly(3-hydroxyalkanoate) depolymerase C	*Paucimonas lemoignei*	52.48	98	1e-119
PET hydrolase-like protein						
OKIOMFPC_02247	Q9L2J6.1	Bis(hydroxyethyl) terephthalate hydrolase	*Streptomyces coelicolor* A3(2)	87.87	88	6e-177
Proposed downstream pathway genes						
OKIOMFPC_01685	P42328.1	Alcohol dehydrogenase	*Geobacillus stearothermophilus*	54.9	99	3e-130
OKIOMFPC_03749	O35017.1	Uncharacterized zinc-type alcohol dehydrogenase-like protein YogA	*Bacillus subtilis* subsp. subtilis str. 168	37.3	94	6e-59
OKIOMFPC_02358	P47734.2	S-(hydroxymethyl)glutathione dehydrogenase	*Methylobacter marinus*	34.36	99	3e-71
OKIOMFPC_05697	P77316.1	Uncharacterized zinc-type alcohol dehydrogenase-like protein YbdR	*Escherichia coli* K-12	33.59	100	1e-54
OKIOMFPC_06107	P47734.2	S-(hydroxymethyl)glutathione dehydrogenase	*Methylobacter marinus*	43.1	100	1e-107
OKIOMFPC_06543	Q9ZUC1.2	NADPH-dependent alkenal/one oxidoreductase, chloroplastic	*Arabidopsis thaliana*	36.72	99	1e-47
OKIOMFPC_01786	Q08IC0.1	Alpha-ketoglutaric semialdehyde dehydrogenase 2	*Azospirillum brasilense*	52.1	99	2e-172
OKIOMFPC_05646	Q08IC0.1	Alpha-ketoglutaric semialdehyde dehydrogenase 2	*Azospirillum brasilense*	48.83	94	1e-117
OKIOMFPC_03434	Q73TP5.1	Succinate-semialdehyde dehydrogenase	*Mycobacterium avium* subsp. paratuberculosis K-10	63.66	100	0.0
OKIOMFPC_04346	A0R4Q0.1	Putative succinate-semialdehyde dehydrogenase	*Mycolicibacterium smegmatis* MC2 155	52.49	90	3e-169

BLASTp (NCBI) against UniProtKB/Swiss-Prot with default parameters (RID D3MN197C016); values are for the top hit.

Top hits for OKIOMFPC_01852, 03749, 04346 and 05697 are uncharacterized or putative proteins. First annotated hits: 01852, MHETase (A0A0K8P8E7), 30.24%, see Table S5; 03749, zinc-type alcohol dehydrogenase-like protein C1773.06c (O94564), 29.97%; 04346, succinate-semialdehyde dehydrogenase (P94428), 36.60%; 05697, S-(hydroxymethyl)glutathione dehydrogenase (P47734), 31.39%.

Table S5. Comparison of terephthalate-related candidate proteins with characterized PET-pathway enzymes.

Locus tag	Protein	Reference protein (UniProt)	Identity (%)	Query coverage (%)	E-value	Catalytic triad in query (reference)
OKIOMFPC_02247	PET hydrolase-like (BdeA homolog)	BHETase, *S. coelicolor* A3(2) (Q9L2J6)	87.87	88	6e-177	–
		IsPETase, *Ideonella sakaiensis* (A0A0K8P6T7)	46.78	93	4e-71	Ser177–Asp223–His255 (IsPETase Ser160–Asp206–His237)
OKIOMFPC_01852	MHETase-like esterase	Uncharacterized esterase Atu5261, *Agrobacterium fabrum* (Q8UK62)	34.99	99	2e-95	–
		MHETase, *I. sakaiensis* (A0A0K8P8E7)	30.24	86	3e-43	Ser211–Asp443–His481 (MHETase Ser225–Asp492–His528)

Identity, coverage and E-values from BLASTp against UniProtKB/Swiss-Prot. Catalytic residues were located by pairwise alignment with the reference sequences.

Neither protein has been tested for activity on PET, BHET or MHET.

Table S6. BLASTp comparison of *S. nigra* PRNP1-1W candidate hydrolases with polyester hydrolases reported to degrade polybutylene succinate (PBS)

Locus tag (protein)	Reference enzyme, organism (accession)	Evidence of PBS hydrolysis (reference)	Identity (%)	Query coverage (%)	Aligned region in query (aa)	E-value
OKIOMFPC_02247 (PET hydrolase-like, BdeA homolog)	Cutinase AML, *Amycolatopsis mediterranei* U32 (ADJ49206.1)	Purified enzyme hydrolysed PBS and PCL (Tan et al., 2021)	73.26	84	49–306	8e-142
	Cutinase, *Saccharomonospora viridis* (WP_015787089.1)	Listed as PBS-degrading by Hu et al. (2024); activity of this sequence not verified	68.06	85	46–306	5e-121
	Cutinase 1 (Thc_Cut1), *Thermobifida cellulosilytica* (ADV92526.1)	93% mass loss of PBS films (Gamerith et al., 2017)	63.98	83	49–305	1e-125
	Est119 (= cutinase Est2, UniProt F7IX06), *Thermobifida alba* AHK119 (BAK48590.1)	Halo formation on PBS and PBSA agar; PBSA turbidity assay (Thumarat et al., 2012)	62.84	83	49–305	7e-119
	PBS(A) depolymerase PbsA, *Acidovorax delafieldii* BS-3 (BAB86909.1)	Recombinant enzyme degraded PBS and PBSA (Uchida et al., 2002)	51.34	82	49–301	4e-79
	Lipase 1, *Moraxella* sp. TA144 (P19833.1)	No PBS activity reported (Feller et al., 1990); included because it was used by Hu et al. (2024)	42.91	78	67–305	1e-67
	Hypothetical esterase HP, *Pseudomonas mendocina* (ATH80290.1)	Purified enzyme caused PBS film weight loss (Hu et al., 2024)	29.41	61	114–302	3e-07
OKIOMFPC_05360 (Thermostable MAG lipase)	Best hit: Thc_Cut1 (ADV92526.1)	—	28.07	42	11–118	1e-05
OKIOMFPC_06885 (Multifunctional esterase)	Best hit: Thc_Cut1 (ADV92526.1)	—	30.91	16	120–172	0.005
OKIOMFPC_00784, 00982, 01852, 02765, 04836	All seven reference enzymes	—	No significant similarity			

BLASTp (NCBI, Align two or more sequences, default parameters; RID D3ZHGPZH114). Query coverage is relative to the full-length query (OKIOMFPC_02247, 308 aa; 05360, 259 aa; 06885, 327 aa). For OKIOMFPC_02247 all alignments start at residues 46–49, immediately after the predicted signal-peptide cleavage site (positions 46–47; Table S2, Figure S1), i.e. they span the mature protein. Identity of 51–73% to four enzymes with demonstrated PBS hydrolysis supports OKIOMFPC_02247 as a putative PBS depolymerase; activity has not been tested experimentally. PCL, polycaprolactone; PBSA, poly(butylene succinate-co-adipate); MAG, monoacylglycerol.

Table S7. Sequences included in the multiple sequence alignment (Figure 5)

Label in Figure 5	Protein	Organism	Other identifiers	Evidence of PBS hydrolysis	Reference
PRNP1-1W_02247	PET hydrolase-like protein (BdeA homolog); NCBI name: bis(hydroxyethyl) terephthalate hydrolase	*Streptomyces nigra* PRNP1-1W	Prokka locus tag OKIOMFPC_02247; NCBI locus tag AC0NME_RS11330; RefSeq protein WP_474776732.1; NZ_JBWBCN010000004.1:155,624–156,550 (+)	Not tested (this study)	This study
ATH80290.1	Hypothetical protein HP (alpha/beta hydrolase)	*Pseudomonas (Ectopseudomonas) mendocina*	—	Purified enzyme caused PBS film weight loss	Hu et al. (2024) Int Biodeterior Biodegrad 195:105910
BAB86909.1	PBS(A) depolymerase PbsA	*Acidovorax delafieldii* BS-3	—	Recombinant enzyme degraded PBS and PBSA	Uchida et al. (2002) J Biosci Bioeng 93:245–247
ADV92526.1	Cutinase 1 (Thc_Cut1)	*Thermobifida cellulosilytica*	UniProt E9LVH8; PDB 5LUI	93% mass loss of PBS films	Gamerith et al. (2017) Front Microbiol 8:938
ADJ49206.1	Cutinase/lipase AML (AmCut)	*Amycolatopsis mediterranei* U32	UniProt P0DX29	Purified enzyme hydrolysed PBS and PCL	Tan et al. (2021) Comput Struct Biotechnol J 19:869
BAK48590.1	Polyesterase Est119 (cutinase Est2)	*Thermobifida alba* AHK119	UniProt F7IX06	Halo formation on PBS and PBSA agar; PBSA turbidity assay	Thumarat et al. (2012) Appl Microbiol Biotechnol 95:419–430
WP_015787089.1	Cutinase (PET hydrolase family protein)	*Saccharomonospora viridis*	GenBank ACU97777.1	Listed as PBS-degrading by Hu et al. (2024); activity of this sequence not verified	Hu et al. (2024); Kawai et al. (2014) Appl Microbiol Biotechnol
P19833.1	Lipase 1	*Moraxella* sp. TA144	GenBank CAA37220.1	No PBS activity reported; reference lipase used by Hu et al. (2024)	Feller et al. (1990) Nucleic Acids Res 18:6431

Sequences were retrieved from NCBI Protein. UniProt identifiers were confirmed by 100% identity in BLASTp against UniProtKB/Swiss-Prot; PDB 5LUI is the crystal structure of Thc_Cut1 (RCSB PDB). PBS, polybutylene succinate; PBSA, poly(butylene succinate-co-adipate); PCL, polycaprolactone; PDB, Protein Data Bank.