

# Supplementary Materials for

## Biodegradation of Poly (Ethylene Terephthalate) by *Bacillus safensis* YX8

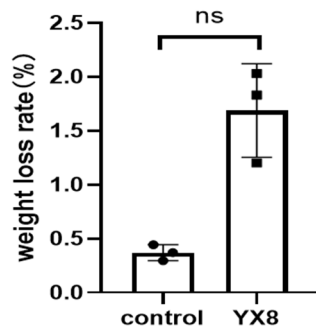
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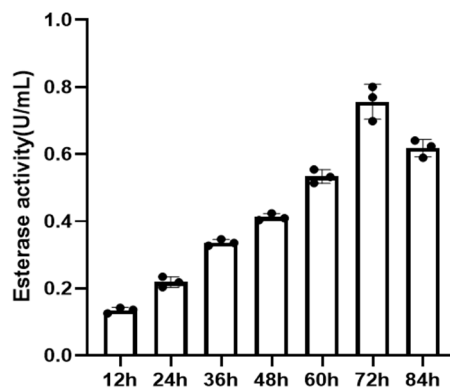
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**Figure S1.** The weight loss rate of PET film after incubated with or without strain YX8. One (2 cm×2 cm) PET film was incubated in 50mL LB broth with or without strain YX8 at 45°C and 200 rpm for 2 months.



**Figure S2.** The extracellular esterase activity of strain YX8 grown in LB broth. The esterase assay system included 10  $\mu$ L of 10 mM *p*-nitrophenol acetate, 10  $\mu$ L of crude enzyme, 980  $\mu$ L of 50 mM Tris-HCl (pH 8.0 and 300 mM NaCl). The reaction was carried out at 25°C for 3 min, and then OD<sub>405</sub> was detected by spectrophotometer.



**Figure S3.** Detection the PCL-hydrolyzing activity of concentrated crude enzymes. Strain YX8 was grown in LB broth for 72 h. The extracellular enzymes were concentrated and dropped on LB plate containing 0.44% PCL.

Table S1. Reported PET-degrading strains and enzymes.

Enzyme	Source	Substrate	PET degradation ability and temperature	Reference
PET2	Uncultured bacterium (marine metagenome)	nanoparticle ager	Zone of clearance, 50°C	[1]
PET5	<i>Oleispira antarctica</i> RB-8	nanoparticle ager	Zone of clearance , 50°C	[1]
PET12	<i>Polyangium brachysporum</i>	nanoparticle ager	Zone of clearance , 50°C	[1]
PET6	<i>Vibrio gazogenes</i>	nanoparticle ager	Zone of clearance , 50°C	[1]
PE-H	<i>Pseudomonas aestusnigri</i> VGXO14 <sup>T</sup>	amorphous PET film	MHET released in 48 h, 30°C	[2]
CALB	<i>Candida antarctica</i>	Low-crystallinity and biaxially oriented PET films	TPA, BHET, MHET were released	[3]
LCC	Metagenome from leaf branch compost	amorphous PET film	50% weight loss, 50-70°C	[4]
LCC <sup>iccg</sup>	Engineered LCC	amorphous PET film	90% weight loss in 9.3 h, 72°C	[5]
Hic	<i>Humicola insolens</i>	lcPET (crystallinity 7%)	97 ± 3% weight loss in 6 day, 70°C	[6]
FsC	<i>Fusarium solani pisi</i>	lcPET (crystallinity 7%)	5% weight loss in 4 days, 30-60°C	[6]
Cut190	<i>Saccharomonospora viridis</i> AHK190	Amorphous PET film and package-grade PET	TPA and MHET released in 3 days,60-65°C	[7]
TfH	<i>Thermobifida fusca</i> DSM43793	bottle-grade PET (crystallinity 10 %)	50% weight loss, 55°C	[8]
Thc_Cut1	<i>Thermobifida cellulosilytica</i> DSM44535	PET film (crystallinity 37%)	MHET, TPA, and HEB released in 5 days, 50°C	[9]
Thc_Cut2	<i>Thermobifida cellulosilytica</i> DSM44535	PET film (crystallinity 37%)	MHET, TPA, and HEB released in 5 days, 50°C	[9]
Thf42_Cut1	<i>Thermobifida fusca</i> DSM44342	PET film (crystallinity 37%)	MHET, TPA, and HEB released in 5 days, 50°C	[9]
TfCut2	<i>Thermobifida fusca</i> KW3	PET film	12.6% weight loss in 48 h, 55-65°C	[10]
Tcur0390	<i>Thermomonospora curvata</i> DSM43183	PET nanoparticle	20 µg/mL substrate degrade rate 5.9×10 <sup>-3</sup> min <sup>-1</sup> ,50°C	[11]
TfCa	<i>Thermobifida fusc</i> KW3	cyclic PET trimers	EMT, MHET, and BHET were released, 50-60°C	[12]
BsEstB	<i>Bacillus subtilis</i>	3PET	TPA, MHET and BA were released, 40-45°C	[13]
Thh_Est	<i>Thermobifida halotolerans</i> DSM44931	3PET	TPA, BA HEB, and MHET were released, 50°C	[14]

IsPETase	<i>Ideonella sakaiensis</i> 201-F6	PET film (crystallinity 1.9%)	TPA, MHET, and EG released in 1 day, 20-45°C	[15]
Unknown	<i>Bacillus safensis</i> YX8	PET nanoparticles	TPA, MHET, and BHET released in 48h, 45°C	This study

## Reference

1. Danso D, Schmeisser C, Chow J, et al. New Insights into the Function and Global Distribution of Polyethylene Terephthalate (PET)-Degrading Bacteria and Enzymes in Marine and Terrestrial Metagenomes. *Appl. Environ. Microbiol.* 2018, 84(8): e02773-17.
2. Bollinger A, Thies S, Knieps-Grünhagen E, et al. A Novel Polyester Hydrolase From the Marine Bacterium *Pseudomonas aestusnigri* - Structural and Functional Insights. *Front. Microbiol.* 2020, 11: 114.
3. Carniel A, Valoni É, Nicomedes J, et al. Lipase from *Candida antarctica* (CALB) and cutinase from *Humicola insolens* act synergistically for PET hydrolysis to terephthalic acid. *Process Biochem.* 2017, 59: 84-90.
4. Sulaiman S, Yamato S, Kanaya E, et al. Isolation of a novel cutinase homolog with polyethylene terephthalate-degrading activity from leaf-branch compost by using a metagenomic approach. *Appl. Environ. Microbiol.* 2012, 78(5): 1556-62.
5. Tournier V, Topham C M, Gilles A, et al. An engineered PET depolymerase to break down and recycle plastic bottles. *Nature.* 2020, 580(7802): 216-219.
6. Ronkvist Å M, Xie W, Lu W, et al. Cutinase-Catalyzed Hydrolysis of Poly(ethylene terephthalate). *Macromolecules.* 2009, 42(14): 5128-5138.
7. Kawai F, Oda M, Tamashiro T, et al. A novel Ca<sup>2+</sup>-activated, thermostabilized polyesterase capable of hydrolyzing polyethylene terephthalate from *Saccharomonospora viridis* AHK190. *Appl. Microbiol. Biotechnol.* 2014, 98(24): 10053-64.
8. Müller R J, Schrader H C G, Profe J, et al. Enzymatic Degradation of Poly(ethylene terephthalate): Rapid Hydrolyse using a Hydrolase from *T. fusca*. *Macromol Rapid Commun.* 2005, 26: 1400-1405.
9. Herrero Acero E, Ribitsch D, Steinkellner G, et al. Enzymatic Surface Hydrolysis of PET: Effect of Structural Diversity on Kinetic Properties of Cutinases from *Thermobifida*. *Macromolecules.* 2011, 44(12): 4632-4640.
10. Then J, Wei R, Oeser T, et al. Ca<sup>2+</sup> and Mg<sup>2+</sup> binding site engineering increases the degradation of polyethylene terephthalate films by polyester hydrolases from *Thermobifida fusca*. *BIOTECHNOL J.* 2015, 10(4): 592-598.
11. Wei R, Oeser T, Then J, et al. Functional characterization and structural modeling of synthetic polyester-degrading hydrolases from *Thermomonospora curvata*. *AMB Express.* 2014, 4: 44.
12. Billig S, Oeser T, Birkemeyer C, et al. Hydrolysis of cyclic poly(ethylene terephthalate) trimers by a carboxylesterase from *Thermobifida fusca* KW3. *Appl. Microbiol. Biotechnol.* 2010, 87(5): 1753-64.

13. Ribitsch D, Heumann S, Trotscha E, et al. Hydrolysis of polyethyleneterephthalate by p-nitrobenzylesterase from *Bacillus subtilis*. *Biotechnol. Prog.* 2011, 27(4): 951-60.
14. Ribitsch D, Herrero Acero E, Greimel K, et al. A New Esterase from *Thermobifida halotolerans* Hydrolyses Polyethylene Terephthalate (PET) and Polylactic Acid (PLA). *Polymers*. 2012, 4(1): 617-629.
15. Yoshida S, Hiraga K, Takehana T, et al. A bacterium that degrades and assimilates poly(ethylene terephthalate). *Science*. 2016, 351(6278): 1196-9.

### 16s rRNA gene sequence

GGCGGCGTGCCTAATACATGCAAGTCGAGCGGACAGAAGGGAGCTTGCTCCCGGATGTTAGCGGCGGACGG  
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### *gyrA* gene sequence

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