Supplementary Information

Engineering microbes to bio-upcycle polyethylene terephthalate

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**Table S1 – Overview of PET hydrolyzing enzymes**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Enzyme | MW  kDa | Optimum | | Isolated  strain | Expression  strain | Tested substrate(s) | Conversion  conditions | products | Reference |
| **T** | **pH** |  |  |  |  |  |  |
| PETase (ISF6\_4831) | 28.6 | 30 °C | 9.0 | *Ideonella sakaiensis* 201-F6 | *E.coli* BL21 (DE3) CodonPlus | Low crystallinity PET film | 0.1 mM TPA and 0.2 mM MHET in 18 hours (30 °C, pH 7.0) | TPA, MHET, BHET |  |
| *Phaeodactylum tricornutum* | Industrially shredded PET | TPA and MHET produced in micromolar quantities (21 °C, pH 8.0) | TPA, MHET | (Moog et al., 2019) |
| *Chlamydomonas reinhardtii* CC-124 | Powdered commercial beverage PET bottle | 9.12 mg of TPA from 30 mg of PET powder in 4 weeks (30 °C) | BHET, TPA | (Kim et al., 2020) |
| *Pichia pastoris* | Bis(benzoyloxyethyl) terephthalate | Turnover rate of 1.5 sec-1 (30 °C, pH 7.0) | MHET, TPA, BHET | (Chen et al., 2020) |
| Commercial PET bottle | 99 to 109 nM of MHET in 18 hours (30 °C, pH 9.0) |
| BhrPETase | 27.5 | 80 °C | 6.0-8.0 | *Bacillus subtilis* HR29 | *Bacillus subtilis* strain CBS2 | Amorphous PET powder | More than 6 mM total products in 20 hours (70 °C, pH 8.0) | TPA, MHET, BHET | (Xi et al., 2021) |
| MHETase (ISF6\_0224) | 63.1 | 30 °C | 7.0 | *Ideonella sakaiensis* 201-F6 | *E. coli* Rosetta-gami B(DE3) | MHET | MHET turnover rate of 31 ± 0.8 s−1 | TPA, EG | (Yoshida et al., 2016) |
| Ple611  Ple628  Ple629  Ple200  Ple201  Ple453 | NA | 30 °C | 7.0 | Marine microbial consortium | NA | PBAT-based blend film (PF) | Highest degradation within 15 days (11-16% mineralized per day) (22 °C, pH 7.0) | ADPA, TPA, SA, TB | (Meyer-Cifuentes et al., 2020) |
| Tcur1278 | 35 | 60 °C | 8.5 | *Thermomonospora curvata* DSM43183 | Escherichia coli TOP10 | PET nanoparticles | Maximum hydrolysis rate of 3.3 × 10-3 min-1 with 80 μg/mL of enzyme in 60 minutes (50 °C, pH 8.5) | NA | (Wei et al., 2014) |
| Tcur0390 | 35 | 55 °C | 8.5 | *Thermomonospora curvata* DSM43183 | Escherichia coli TOP10 | PET nanoparticles | Maximum hydrolysis rate of 5.9 × 10-3 min-1 with 20 μg/mL of enzyme in 60 minutes (50 °C, pH 8.5) | NA | (Wei et al., 2014) |
| PET2 (lipIAF5-2) | NA | 70 °C | 8.0 – 9.0 | Uncultured bacterium | E. coli T7 | Low-crystallinity PET film | 900 μM terephthalic acid with 100 μg of PET 2 after 24 h of incubation (60 °C, pH 7.5) | TPA | (Danso et al., 2018) |
| PET5 (lipA OLEAN\_C07960) | NA | NA | NA | *Oleispira antarctica* RB-8 | E. coli T7 | Low-crystallinity PET film | Halos in agar plates containing PET nanoparticles after overnight incubation | NA | (Danso et al., 2018) |
| PET6 | NA | 55 °C | 8.0 – 9.0 | *Vibrio gazogenes* | E. coli T7 | Low-crystallinity PET film | Halos in agar plates containing PET nanoparticles after overnight incubation | NA | (Danso et al., 2018) |
| PET12 (AAW51\_2473) | NA | NA | NA | *Polyangium brachysporum* | E. coli T7 | Low-crystallinity PET film | Halos in agar plates containing PET nanoparticles after overnight incubation | NA | (Danso et al., 2018) |
| PE-H | 32 | NA | NA | *Pseudomonas aestusnigri*VGXO14T | *E. coli* BL21(DE3) | Amorphous PET film | 4 mg/l MHET produced within 48 hours (30 °C, pH 7.4) | MHET | (Molitor et al., 2020) (Bollinger et al., 2020) |
| HiC | NA | 70-  80 °C |  | *Humicola insolens* | NA | 7% crystalline PET film | 97±3% weight loss in 96 hours (70 °C, pH 8.0) | TPA | (Ronkvist et al., 2009) |
| PmC | NA |  |  | *Pseudomonas mendocina* | NA | 7% crystalline PET film | 5% weight loss in 96 hours (50 °C, pH 8.0) | TPA | (Ronkvist et al., 2009) |
| FsC | NA |  |  | *Fusarium solani* | NA | 7% crystalline PET film | 5% weight loss in 96 hours (40 °C, pH 8.0) | TPA | (Ronkvist et al., 2009) |
| Thc\_Cut1 | 29.4 | 50°C | 7.0 | *Thermobifida cellulosilytica* DSM44535 | *Escherichia coli* BL21-Gold (DE3) | 37% crystalline PET film | More than 50mmol TPA and less than 10mmol MHET per mol of enzyme in 72 hours (50°C, pH 7.0) | TPA, MHET  TPA, MHET | (Herrero Acero et al., 2011) |
|  |  |  | *Pichia pastoris* | 24% crystalline PET powder | More than 50mM soluble released products from 5µM enzyme in 96 hours (65°C, pH 8.0) | (Gamerith et al., 2017) |
| Thc\_Cut2 | 29.7 | 50°C | 7.0 | *Thermobifida cellulosilytica* DSM44535 | *Escherichia coli* BL21-Gold (DE3) | 37% crystalline PET film | Less than 10mmol TPA and more than 10mmol MHET per mol of enzyme in 72 hours (50°C, pH 7.0) | TPA, MHET | (Herrero Acero et al., 2011) |
| Thf42\_Cut1 | 29.6 | NA | NA | *Thermobifida* *fusca* DSM44342 | *Escherichia coli* BL21-Gold (DE3) | 37% crystalline PET film | More than 40mmol TPA and less than 10mmol MHET per mol of enzyme in 72 hours (50°C, pH 7.0) | TPA, MHET | (Herrero Acero et al., 2011) |
| Tha\_Cut1 | 28.1 | NA | NA | *Thermobifida alba* | *Escherichia coli* BL21-Gold (DE3) | Bis(benzoyloxyethyl) terephthalate (3PET) | More than 15mmol TPA and more than 30mmol MHET per mol of enzyme in 2 hours (50°C, pH 7.0) | TPA, MHET, BA, HEB | (Ribitsch et al., 2012a) |
| Cut190 | 30.3 | 65– 75 °C | 6.0 – 8.5 | *Saccharomonospora viridis* AHK190 | *Escherichia coli* Rosetta-gami B (DE3) | NA | Hydrophilzed PET film overnight (50°C) | NA | (Kawai et al., 2014) |
| LCC | 28 | 50-70°C | 8.0 | Leaf branch compost | *E. coli* BL21-CodonPlus(DE3) | Amorphous PET film | ≤ 25% weight loss in 24 hours (70°C, pH 8.5) | TPA, EG | (Sulaiman et al., 2012) |
| Thh\_Est | NA | NA | NA | *Thermobifida halotolerans* DSM44931 | *E. coli* BL21-Gold (DE3) | Bis(benzoyloxyethyl) terephthalate (3PET) | More than 15mmol TPA and more than 30mmol MHET per mol of enzyme in 2 hours (50°C, pH 7.0) | TPA, MHET, BA, HEB | (Ribitsch et al., 2012b) |
| BsEstB | NA | 40°C | 7.0 | *Bacillus subtilis* | *Escherichia coli* BL21-Gold (DE3) | Bis(benzoyloxyethyl) terephthalate (3PET) | 800 µg TPA in 36 hours (30°C, pH 7.0) | TPA, MHET, BA, HEB | (Ribitsch et al., 2011) |
| TfH | 28 | 65 – 70 °C | 6.0 – 7.0 | Thermobifida fusca DSM43793 | *E.coli*  *Escherichia coli* BL21(DE3) | Amorphous PET film  Semi crystalline PET film | 50% weight loss in 3 weeks (55°C, pH 7.0)  ≤ 14% weight loss in the presence of 10 mM CaCl2 in 48 hours (65 °C, pH 8.5) | TPA, EG | (Kleeberg et al., 2005;Müller et al., 2005)  (Then et al., 2015) |
| Est119 | 30 | 50 °C | 6.0 | Thermobifida alba AHK119 | *Escherichia coli* Rosetta-gami B (DE3) | Poly(caprolactone) (PCL) | TPA acid detected by HPLC (50 °C, pH 7.0) | TPA | (Hu et al., 2008;Thumarat et al., 2012) |
| TfCut1 | NA | 55-65 °C | 8.5 | Thermobifida fuscaKW3 | *Escherichia coli* BL21(DE3) | Semi crystalline PET film | ≤ 11% weight loss in the presence of 10 mM CaCl2 in 48 hours (65 °C, pH 8.5) | NA | (Then et al., 2015) |
| TfCut2 | 30.8 | 55-65 °C | 8.5 | Thermobifida fusca KW3 | *Escherichia coli* BL21(DE3) | Semi crystalline PET film | ≤ 12% weight loss in the presence of 10 mM CaCl2 in 48 hours (65 °C, pH 8.5) | NA | (Roth et al., 2014;Then et al., 2015) |
| Cbotu\_EstA | NA | 50 – 60 °C | 7.0 | Clostridium botulinumATCC3502 | *E. coli* BL21-Gold (DE3) | PET film | ≤ 5 U-1 mol enzyme in 72 hours (50 °C, pH 7.0) | MHET | (Biundo et al., 2018) |
| BTA-2 | 32.5 | 50 °C | 8.5 | *Thermobifida fusca* DSM43793 | *Escherichia coli* BL21(DE3) | Semi crystalline PET film | ≤ 4% weight loss in the presence of 10 mM CaCl2 in 48 hours (65 °C, pH 8.5) | NA | (Kleeberg et al., 2005;Müller et al., 2005;Then et al., 2015) |
| Tfu\_0882 | 34.4 | 55 - 65 °C | 8.0 | *Thermobifida fusca*YX (*T. fusca* WSH03-11) | *Escherichia coli* BL21(DE3) | Semi crystalline PET film | ≤ 5% weight loss in the presence of 10 mM CaCl2 in 48 hours (65 °C, pH 8.5) | NA | (Lykidis et al., 2007;Chen et al., 2008;Su et al., 2013;Then et al., 2015) |
| Tfu\_0883 | 32.2 | 60 °C | 8.0 | *Thermobifida fusca*YX (*T. fusca* WSH03-11) | Escherichia coli JM109 | NA | NA | NA | (Lykidis et al., 2007;Chen et al., 2008;Su et al., 2013) |
| TfAXE | 28 | 60 °C | 7.5 | *Thermobifida fusca*NTU22 | E. coli DH5α | NA | NA | NA | (Huang et al., 2010) |
| Cut1 | 30.1 | 55 °C | 8.0 | *Thermobifida fusca*NRRL B-8184 | *Escherichia coli* BL21 (DE3) | NA | NA | NA | (Hegde and Dasu, 2013) |
| Cut2 | 29.6 | 55 °C | 8.0 | *Thermobifida fusca*NRRL B-8184 | *Escherichia coli* BL21 (DE3) | NA | NA | NA | (Hegde and Dasu, 2013) |

TPA – Terephthalic acid, EG – Ethylene glycol, MHET - mono-2-hydroxyethyl terephthalate, HEB - 2-hydroxyethyl benzoate BA - Benzoic acid, TB – Terephthalate butanediol monoester, SA – Sebacic acid, ADPA – Adipic acid, NA – Data not available

**Table S2 – Engineered PET hydrolyzing enzymes**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Original Enzyme** | **Mutations** | **Host strain** | **Expression vector** | **Affinity tag** | **Tested**  **Substrate(s)** | **PET conversion & conditions** | **Reference** |
| PETase | S121E/D186H/R280A | *E. coli* strain  Rosetta gami - B | pET15b | N-terminal His6-tag | Commercial PET film | 83.3 μm MHET and 37.6 μm TPA within 72 hours (40 °C, pH 9.0) | (Son et al., 2019) |
| PETase | S214H-I168R-W159H-S188Q-R280A-A180I-G165A-Q119Y-L117F-T140D | *E. coli* TOP10 | pET21b-PETase | C-terminal His6-tag | Semicrystalline PET film | More than 2.5 mM total compounds released in 10 days (37 °C, pH 9.0) | (Cui et al., 2019) |
| PETase | R280A | *E. coli* Rosetta gami-B | pET15b | C-terminal His6-tag | BHET  Commercial PET film | Similar activity to wild type PETase (30 °C, pH 7.0)  Increased activity by 22.4% relative to wild type PETase (30 °C, pH 9.0) | (Joo et al., 2018) |
| PETase | S238F/W159H | *E. coli* C41(DE3) | pET- 21b(+) | C-terminal His6-tag | PET coupons with an initial crystallinity of 14.8 ± 0.2% | 4.13% higher crystallinity loss in 96 hours (30 °C, pH 7.2) | (Austin et al., 2018) |
| PETase | I179F | E. coli BL21 (DE3) | pET28a | His tag | PET film | 6 mmol.L-1 TPA in 48 hours, degradation rate of 22.5 mg per μmol·L−1  (30 °C, pH 8.5) | (Ma et al., 2018) |
| PETase | Y58A | *E. coli* BL21-CodonPlus (DE3) RIPL | pET-21b | C-terminal His6-tag | Commercial PET drinking bottle | More than 30 nM MHET and more than 20 nM TPA in 20 hours (30 °C, pH 9.0) | (Liu et al., 2018) |
| PETase | R53E | *E. coli* BL21-CodonPlus (DE3) | pET-21b | C-terminal His6-tag | Low crystallinity PET | More than 0.2 nmol min-1cm-2 (30 °C, pH 8.0 & 9.0) | (Furukawa et al., 2018) |
| PETase | Y58A  T59A | *E. coli* BL21trxB (DE3) | pET32a | His tag | PET film | More than 80% and 90% MHET production respectively in 42 hours (30 °C, pH 9.0) | (Han et al., 2017) |
| MHETase | R411K/S416A/F424I | *E. coli* BL21 (DE3) -T1R | pET22b (+) | C-terminal His6-tag | Amorphous PET film | Enzyme activity of 8 μm in 72 hours (30 °C, pH 8.0) | (Sagong et al., 2020) |
| MHETase | S416A\_F424N  R411A\_S419G\_F424N  W397A | *E. coli* Shuffle T7 | pColdII | C-terminal His6-tag | BHET | More than 0.12 s-1 turnover rate of BHET.  More than 20 s-1 turnover rate of MHET with W397A (30 °C, pH 7.5) | (Palm et al., 2019) |
| LCC | LCC variant | *E. coli* BL21 (DE3) | pET26b | C-terminal His6-tag | Amorphized bottle grade PET | 90% PET depolymerization in 10 hours (72 °C, pH 8.0) | (Tournier et al., 2020) |
| LCC | DSM1313::pHK‐LCC \* | *Clostridium thermocellum* | pHK | NA | Amorphous PET film | 62% weight loss in 14 days (60 °C, pH 7.4) | (Yan et al., 2020) |
| LCC | LCC-G | *Pichia pastoris* | PET28 | NA | 7% crystalline PET film | ∼95% weight loss in 48 hours (70 °C, pH 8) | (Herrero Acero et al., 2011;Shirke et al., 2018) |
| Thc\_Cut2 | Thc\_Cut2 variant | *Eschericha coli* BL21- Gold (DE3) | pET26b(+) | C-terminal His6-tag | PET film | ≤ 0.45 mM TPA in 2 days (50 °C, pH 7.4) | (Herrero Acero et al., 2013) |
| Thc\_Cut1 | Thc\_Cut1\_ko\_Asn | *Pichia pastoris* | pPICZαB | C-terminal His6-tag | 24% crystalline PET powder | More than 60mM soluble released products from 5µM enzyme in 96 hours (65°C, pH 8.0) | (Gamerith et al., 2017) |
| Thc\_Cut1 | Thc\_Cut1\_ko\_ST | *Pichia pastoris* | pPICZαB | C-terminal His6-tag | 24% crystalline PET powder | More than 60mM soluble released products from 5µM enzyme in 96 hours (65°C, pH 8.0) | (Gamerith et al., 2017) |
| Est119 | A68V/S219P | *Eschericha coli* Roseta-gami B (DE3) | pQE80L | N-terminal His6-tag | p-nitrophenyl butyrate | 50-fold increase of activity over the wild type  (37 °C, pH 7.0) | (Thumarat et al., 2012) |
| Tfu\_0883 | Q132A/T101A | NA | pET20b | NA | PET fabric | 390 mM TPA produced in 48 hours (60 °C, pH 7.5) | (Silva et al., 2011) |
| TfH | rTfH\*\* | Escherichia coli TG1 (DSM 6056) | pCytexP1-OmpA-bta1 | C-terminal His6 tag | NA | NA | (Dresler et al., 2006) |
| Cut190 | S226P/R228S | *Escherichia coli* Rosetta-gami B (DE3) | pQE8oL | His6 tag | Amorphous PET  PET film from PET package | Degradation rate of 13.5 ± 0.5 % in 72 hours (63°C, pH 8.2)  Degradation rate of 27.0 ± 1.0 % in 72 hours (63°C, pH 8.2) | (Kawai et al., 2014) |
| Cut190 | Cut190\*/Q138A  /D250C-E296C | NA | NA | NA | PET microfiber | 24.8% degradation in 113 hours (30 °C, pH 8.5) | (Kawai et al., 2019) |
| Cut190 | Q138A/D250C-E296C  /Q123H/N202H | *Escherichia coli* Rosetta-gami B (DE3) | NA | His6 tag | Microfiber amorphous PET | More than 30% degradation rate in 3 days (70 °C, pH 8.5) | (Oda et al., 2018) |
| Cut190 | I224A/ Q138A | *Escherichia coli* Rosetta-gami B (DE3) | NA | N-terminal His6-tag | BHET | Catalytic activity of 150±0.2 s-1  (37 °C, pH 8.2) | (Kawabata et al., 2017) |
| PE-H | Y250S | E. coli DH5α | pET22b\_PE-Hc6H | His6 tag | Amorphous PET film | More than 5 mg/l MHET in 48 h (30 °C, pH 7.4) | (Bollinger et al., 2020) |
| TfCut2 | G62A/F209A | *E. coli* BL2 (DE3) | pET21-b | C-terminal His6 tag | Low crystallinity PET film | Degradation rate of 97 ± 1.8% within 30 hours (65 °C, pH 9.0) | (Furukawa et al., 2019) |
| TfCut2 | G62A/I213S | *E. coli* BL2 (DE3) | NA | NA | Amorphous PET film | ≤ 45% weight loss after 50 h  (65 °C, pH 8.0) | (Wei et al., 2016) |
| TfCut2 | D204C-E253C(γ), γ -D174L, γ-D174N, γ-D174R, γ-D174A, γ-D174R-G205D | *E. coli* BL2 (DE3) | NA | NA | Amorphous PET film | ≤ 25% weight loss after 48 h  (65 - 80 °C, pH 8.0) | (Then et al., 2016) |
| Cbotu\_EstA | Del171\_Cbotu\_EstA\_S119A | *E. coli* BL21-Gold (DE3) | NA | NA | PET film | ≤ 5 U-1 mol enzyme in 72 hours (50 °C, pH 7.0) | (Biundo et al., 2018) |

\* Signal peptide sequence of Cel48S used for secretory production of LCC

\*\* Express as a fusion protein using the OmpA leader sequence and a His6 tag

TPA- Terephthalic acid, MHET – Monohydroxyethyl terephthalate, BHET – Bis (2-hydroxyethyl terephthalate), NA- Data not available

|  |
| --- |
| **Table S3 – Compound list of Figure 1** |

|  |  |
| --- | --- |
| Number | Compound |
| 1 | Polyethylene terephthalate |
| 2 | Bis(2-Hydroxyethyl) terephthalate |
| 3 | Ethylene glycol |
| 4  5 | Terephthalic acid  Sodium terephthalate |
| 6 | (3*S*,4*R*)-3,4-Dihydroxy-1,5-cyclohexadiene-1,4-dicarboxylic acid |
| 7 | Protocatechuate |
| 8 | (*Z*)-(*E*)-4-Formylmethylidene-2-hydroxy-2-pentenedioate |
| 9 | 4-Carboxy-2-hydroxymuconate-semialdehyde |
| 10 | 2-Pyrone-4,6-dicarboxylic acid |
| 11 | 4-Oxalomesaconic acid (enol form) |
| 12 | 4-Oxalomesaconic acid (Keto form) |
| 13 | 4-carboxy-4-hydroxy-2-oxoadipic acid |
| 14 | Pyruvate |
| 15 | β-Carboxy-cic,cis-mucinic acid |
| 16 | ϒ-Carboxymuconolactone |
| 17 | Muconolactone |
| 18 | β-Ketoadipic acid |
| 19 | 3-oxoadipyl-CoA |
| 20 | Succinyl-CoA |
| 21 | Acetyl-CoA |
| 22 | 5-Carboxy-2-hydroxymuconate-semialdehyde |
| 23 | 2-Hydroxymuconate-semialdehyde |
| 24 | 4-Oxalocrotonic acid (enol form) |
| 25 | 4-Oxalocrotonic acid (keto form) |
| 26 | 4-Hydroxy-2-oxovaleric acid/2-Oxo-4-pentenoate |
| 27 | 4-Hydroxy-2-oxovaleric acid |
| 28 | Acetaldehyde |
| 29 | Glycolaldehyde |
| 30 | Glycolate |
| 31 | Glyoxalate |
| 32 | Tartronate semialdehyde |
| 33 | Hydroxypyruvate |
| 34 | Glycerate |
| 35 | 2-phosphoglycerate |
| 36 | Phosphoenolpyruvate |
| 37 | Gallic acid |
| 38 | Pyrogallol |
| 39 | Vanillin |
| 40 | Catechol |
| 41 | *cis,cis*-muconate |
| 42 | Malonyl-CoA |
| 43 | Malonyl-ACP |
| 44 | Acetoacyl-ACP |
| 45 | 3-Ketoacyl-ACP |
| 46 | (R)-3-Hydroxyacyl-ACP |
| 47 | Enoyl-ACP |
| 48 | Acyl-ACP |
| 49 | Malondialdehyde |
| 50 | 3-Hydroxypropionic acid |
| 51 | (R)-3-Hydroxyfatty acid |
| 52 | (R)-3-Hydroxyacyl-CoA |
| 53 | Medium chain length polyhydroxyalkanoate (mcl-PHA) |
| 54 | 2-Trans-Enoyl--CoA |
| 55 | Citrate |
| 56 | Isocitrate |
| 57 | α-Ketoglutarate |
| 58 | Succinyl-CoA |
| 59 | Fumarate |
| 60 | Malate |
| 61 | Oxaloacetate |
| 62 | Adipic acid |

**Table S4: Description of enzymes of the biofunneling pathways represent in Figure 01**

|  |  |  |
| --- | --- | --- |
| Protein | Enzyme name | EIC number |
| AceE | Pyruvate dehydrogenase E1 component | EC:1.2.4.1 |
| AcnA | Aconitate hydratase | EC:4.2.1.3 |
| AlkK | Acyl-CoA synthetase | EC:6.2.1.3 |
| AroY | Protocatechuate decarboxylase | EC:4.1.1.68 |
| CatA | Catechol 1,2-dioxygenase | EC:1.13.11.1 |
| CatBC | Muconate cycloisomerase 1/Muconolactone Delta-isomerase | EC:5.5.1.1/EC:5.3.3.4 |
| Eno | Enolase | EC:4.2.1.11 |
| ER | Enoate reductase | EC:1.3.1.31 |
| AccA | Acetyl-CoA carboxylase | EC:6.4.1.2 |
| FabAZ | 3-hydroxydecanoyl-[acyl-carrier-protein] dehydratase/3-hydroxyacyl-[acyl-carrier-protein] dehydratase FabZ | EC:4.2.1.59 |
| FabBF | 3-oxoacyl-[acyl-carrier-protein] synthase 1/3-oxoacyl-[acyl-carrier-protein] synthase 2 | EC:2.3.1.41/EC:2.3.1.179 |
| FabD | Malonyl CoA-acyl carrier protein transacylase | EC:2.3.1.39 |
| FabG | 3-oxoacyl-[acyl-carrier-protein] reductase | EC:1.1.1.100 |
| FabH | 3-oxoacyl-ACP synthase | EC:2.3.1.180 |
| FabIV | Enoyl-[acyl-carrier-protein] reductase [NADH] | EC:1.3.1.9 |
| GalB | 4-oxalmesaconate hydratase | EC:4.2.1.83 |
| GalC | 4-carboxy-4-hydroxy-2-oxoadipic acid aldolase | EC:4.1.3.17 |
| GalD | 4-oxalomesaconate tautomerase | EC:5.3.2.8 |
| Gcl | Glyoxylate carboligase | EC:4.1.1.47 |
| GlcDEF | Glycolate oxidase, putative FAD-linked subunit/Glycolate oxidase, putative FAD-binding subunit/ | EC:1.1.99.14 |
| gltA | Citrate synthase | EC:2.3.3.16 |
| GlxR | Tartronate semialdehyde reductase | EC:1.1.1.60 |
| HsoMT | Catechol O-methyltransferase | EC:2.1.1.6 |
| Hyi | Hydroxypyruvate isomerase | EC:5.3.1.22 |
| lcd | Isocitrate dehydrogenase [NADP] | EC:1.1.1.42 |
| LigAB | Type II extradiol dioxygenases/ protocatechuate 4,5-dioxygenase | [EC:1.13.11.](https://enzyme.expasy.org/EC/6.5.1.2)8 |
| LigC | 4-carboxy-2-hydroxymuconate-6-semialdehyde dehydrogenase | EC:1.1.1.312 |
| LigI | 2-pyrone-4,6-dicarboxylate hydrolase | EC:3.1.1.57 |
| Lpdc | Gallate decarboxylase | EC:4.1.1.59 |
| Mcr | malonyl-CoA reductase | EC 1.1.1.298 |
| Mdh | Probable malate dehydrogenase | EC:1.1.1.37 |
| PcaB | 3-carboxy-cis,cis-muconate cycloisomerase | EC:5.5.1.2 |
| PcaC | 4-carboxymuconolactone decarboxylase | EC:4.1.1.44 |
| PcaD | 3-oxoadipate enol-lactonase 2 | EC:3.1.1.24 |
| PcaF | 3-oxoadipyl-CoA thiolase | EC:2.3.1.174 |
| PcaHG | Protocatechuate 3,4-dioxygenase beta chain/Protocatechuate 3,4-dioxygenase alpha chain | EC:1.13.11.3 |
| PcaIJ | 3-oxoadipate CoA-transferase | EC:2.8.3.6 |
| PedEH | PQQ-dependent dehydrogenase | EC:1.1.2.8 |
| PedI | Aldehyde dehydrogenase | EC:1.2.1.3 |
| PhaC | Poly(3-hydroxyalkanoate) polymerase 2 | EC:2.3.1.- |
| PhaG | (R)-3-hydroxydecanoyl-ACP:CoA transacylase | EC:2.4.1.- |
| PhaJ | (R)-specific enoyl-CoA hydratase | EC:4.2.1.119 |
| AceA-D | Isocitrase | EC:4.1.3.1 |
| PobA | p-hydroxybenzoate hydroxylase | EC:1.14.13.2 |
| PP\_0897 | Fumarate hydratase class I | EC:4.2.1.2 |
| PP\_4300 | Putative hydroxypyruvate reductase | EC:1.1.1.81 |
| PraA | Protocatechuate 2,3-dioxygenase | EC:1.13.11.8 |
| PraH | 5-carboxy-2-hydroxymuconate-6-semialdehyde decarboxylase | EC:[4.1.1.45](https://www.genome.jp/dbget-bin/www_bget?ec:4.1.1.45) |
| PykAF | Pyruvate kinase | EC:2.7.1.40 |
| SdhB | Succinate dehydrogenase iron-sulfur subunit | EC:1.3.5.1 |
| SucAB | Oxoglutarate dehydrogenase (succinyl-transferring)/Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex | EC:1.2.4.2/ EC:2.3.1.61 |
| SucCD | Succinate--CoA ligase [ADP-forming] subunit beta/ | EC:6.2.1.5 |
| TphAabc | Terephthalate 1,2-dioxygenase | EC:1.14.12.15 |
| TphB | 4-hydroxythreonine-4-phosphate dehydrogenase | EC:1.1.1.262 |
| TpiABC | Triosephosphate isomerase/Small transmembrane protein of the aromatic acids transporter | EC:5.3.1.1 |
| XylG | 2-hydroxymuconic semialdehyde dehydrogenase | EC:1.2.1.85 |
| XylH | 2-hydroxymuconate tautomerase | EC:5.3.2.6 |
| XylI | 4-oxalocrotonate decarboxylase | EC:4.1.1.77 |
| XylJ | 2-oxopent-4-enoate hydratase | EC:4.2.1.80 |
| XylK | 4-hydroxy-2-oxovalerate aldolase | EC:4.1.3.39 |
| XylQ | Acetaldehyde dehydrogenase | EC:1.2.1.10 |

Graphical user interface, application

Description automatically generated

**Figure S1:** Architecture of the proposed PETsome**.**

Most of the cellulosome's key components can be copied to construct PETsome, including cohesion, dockerin domains, the linkers (27 – 35 amino acid long flexible linkers), and the anchoring protein (Krasteva et al., 2017;Anandharaj et al., 2020;Dvořák et al., 2020b;Vita et al., 2020). Contrary to the cellulosome, the PET binding domain and PET hydrolyzing enzymes need to be engineered to form the PETsome. PETsome can be assembled either in vitro using purified components or in vivo on a suitable microbial host's surface.

The strategies suggested by Dvořák and coworker can be adopted to develop an efficient PETsome on *P. putida*, a popular workhorse for plastic upcycling (Dvořák et al., 2020b). They highlighted that surface engineering for removing non-essential outer membrane structures (fimbriae, pili, curli, adhesins, exopolysaccharides, and lipopolysaccharides) *P. putida* (i.e., *P. putida* EM71) enhances *in vivo* cellulosome formation relative to the parental strain. They used monomeric type V secretion pathway protein, Ag43 autotransporters to cell surface display of PETsome. The Ag43 gene encodes all three domains needed for display (a signal peptide, a surface-exposed passenger, and a transmembrane β-domain).

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