Supplementary Material

1. Synthesis of Bte

Bte was synthesized according to the protocol from Perz et al. (Perz et al., 2016). Briefly, 23.1 g 1,4- butanediol (0.256 mol) and 12.4 g pyridine (0.157 mol) were combined and cooled to 0 °C. A solution of 6.13 g (0.03 mol) methyl 4-(chlorocarbonyl) benzoate (Sigma-Aldrich, USA) in 23.3 g of dichloromethane was added dropwise over 15 minutes. The reaction was stirred for 2 h at room temperature. The reaction was then poured over 200 mL water and brought to pH 1 with 150 mL of 1 M HCL. The aqueous phase was extracted three times with dichloromethane. The solvent was removed under reduced pressure to yield 6 g of methyl hydroxybutyl terephthalate. This product (6 g, 0.023 mol) was dissolved in 238 mL of a 1:1 mixture of methyl tert-butyl ether and chloroform. Dihydropyran (4.3 g, 0.06 mol) was added to the mixture dropwise over 15 minutes. The pH was adjusted to 4 with 0.22 g concentrated HCl. The reaction was stirred overnight at room temperature. It was then washed with 100 ml of saturated sodium bicarbonate solution and 100 ml water. The solvent was dried and removed under reduced pressure. Ten grams of crude product was purified by flash chromatography (hexane:ethyl acetate 8:1) to yield 5.3 g of the dihydropyranyl derivative of methyl hydroxybutyl terephthalate. 5.08 g of the dihydropyranyl derivative of methyl hydroxybutyl terephthalate and 2.67 g DABCO (23 mmol) were combined in a tube. The tube was sealed and heated to 100 °C for 4 h. The oil was taken up in 100 mL water at room temperature. Twenty five mL of 10% H₂SO₄ was added to this solution. The reaction was heated to 90°C, diluted with 75 mL of water and cooled to room temperature. After stirring at room temperature for a further two hours, the precipitate was filtered, washed twice with 20 mL of water and dried. Recrystallization from toluene yielded 2.1 g of monohydroxybutyl terephthalate. The structure was confirmed with ¹H NMR obtained on a Bruker Avance III platform (Bruker, USA). The purity was estimated to be 93% by ¹H NMR (500 MHz, DMSO-d6) δ 13.34 (s, 6H), 8.06 (s, 28H), 4.45 (s, 6H), 4.38 (s, 2H), 4.31 (t, J = 6.6 Hz, 13H), 3.89 (q, J = 1.0 Hz, 1H), 3.45 (d, J = 12.9 Hz, 13H), 3.31 (s, 3H), 1.93–1.87 (m, 1H), 1.82–1.67 (m, 13H), 1.61–1.50 (m, 13H) (Meyer-Cifuentes et al., 2020).

2. Supplementary Tables

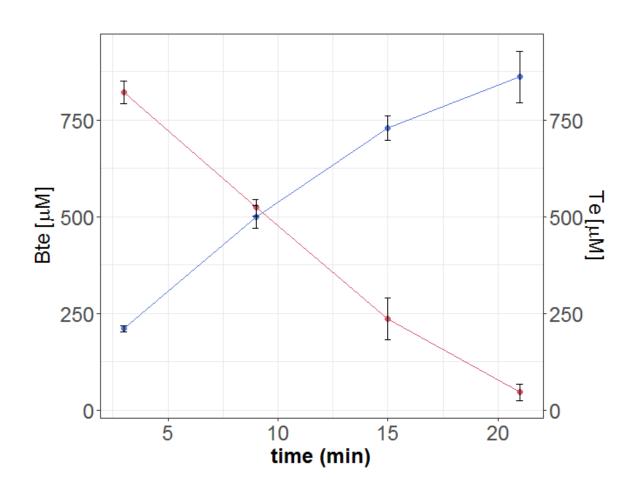
Supplementary Table 1. Gene sequence of the codon optimized *mle046*.

Codon-optimized *mle046*

catatgg cagttg caggtg acttcccg a at cagttcctg agctgcttcg at g cagcca at ctgacegaa attgaactgccggcagatgttcagggcttccgtctgattgaaattgccgaacatgccggtgataaaggcatgccggcccattgtgaaattgtgggcgccattaatgatcgcattagtcc ggtggatggccagcattatagcattaaattccgtctgcgtctgccgcaggattggaatggtcg cttctatatggaaggcggtggcggtagcaatggtgttctgaaagatgcaatgggcccgaccggcetgaatcaggaagatagtgccetggaacgcggcttcgcagtggtgaccaccgatagc ggccatgataatgataccaatagcgatagcaatgccagcggcgcagcgccttcggtatgg atccgcaggcacgcctggacttcggttatatgagttatgatattgttacccgtgtgggtaaagc aattgtggaaaaatattatggtgcagcccggaaaaaagttacttcattggttgtagtgaagg cggtcgtgaagcagccctgatgacccagcgctatccggatctgtatgatggtgtggttgccg gtgcccgggtattcacttcagctatagtgcagcctatgcccgttcctgctgcgcatcttcgg taatetggccgaaaccagaaatcagagcggtccggatggtattccgctgctgaataaactgt atagtgataatgatgtgcagctgattgccgatgccgttgtgggcgcctgtgatgccctggatg gcctggaagatcgtatgagcaataatattgaagcctgtaccaccgttaccgtgctgccgcgc ctgcgcgctctgacatgcagtggcgccaaagaagatggctgctgctggaagatcagattg at gcct tcgt ggccgg tat ggcagg tccggt gaccagt gat ggtacccgtct gtat ccgggtcatccgtgggatccgggtattggcggtcgtattggtgatagtgttaatgatggcttccgtagttggtggttcggtagctatgatagcgatcagaataatgcccgcaaagtgaccctgagcacccc geageatgecatgetgtggcagaccccgccggttccgctgctgctgatgaatatgtgcgct tcgaaatgaacttcaatattgatgaaacacctgccctggcatacgctaccaccgatctgtatcc ggtgagcagtgcagaactgggcaatgccgatagtccggatctgagcgacttcgcaagccg cggcggtaaactggtgatctatcatggtgccgcagatgcagcattcagtgcactggatacca tcattattccgggtatgaatcattgccagggtggtccggccaccgatgatgttgatctgctgaccccgctgatggcatgggttgaagatgatctgccgattgaacgcctggaagccaccgtgagc aatccggattacttcggtggtaaaaatctgagtcgcccgctgtgtccgtatccgctgtatgcc gaatatgatggtgaaggtgatccgagtagcgcagaatcattcacctgtgtggccaataagctt

Supplementary Table 2. Kinetic parameters of Mle046. The kinetic parameters were determined in pH 7.5 buffer at 30°C. Parameters were calculated with GraphPad

Best-fit values								
Et	0.0365 μΜ							
kcat	80,87 s ⁻¹ (±15,84)							
Km	2,638 (±797)							
Vmax	2.952 μMs ⁻¹							
95% CI (profile likelihood)								
kcat	47.92 to 113.8 s ⁻¹							
Km	980.8 to 4296							
Goodness of Fit								
Degrees of Freedom	21							
R squared	0.8960							
Sum of Squares	0.2590							
Sy.x	0.1110							
Number of points analyzed	23							



Supplementary figure S1. Bte degradation by Mle046. Bte degradation by Mle046 is shown in red on the left y-axis and Te formation in blue on the right y-axis. Four sampling points are shown: 3, 9, 15 and 21 min. Error bars indicate standard deviation (n=3).

10 70 80 80 80 10 10 10 10 10 10 10 10 10 10 10 10 10	MONTAR AND RAY PLANDER TO THE SAQCANVS VWITS SVPPLRE HMDRRVTR ROLMOTRING THE AND LAGGES FFLT GVDHAD-AAVAGDEP PROPERED PR	NWPNIXXWASXXYW-AXANASAAXXNE	TEBELBADWQGFRLIELAEHAGDKGMBAHGEBVGAHNDRISPVDGQHYSHKFRLRUPQDWNGREYMEGGGGSNGVHKDAMGPTGLNOEDSBLERGENVN GROWNWRATTVURGAATVAURAATTVERAATTGE		SCHIDINDTND STARSCHESMINGTONE CANNEY DUTRY CRANDEN TO EXCRANDING THE TOTAL CONTINUENT CONTINUENT TO THE TOTAL CONTINUENT	KHURHQXINĞQ CDANDG LX D GREDPXAÇÇPWFXXXANXÇTĞARTTDONXGAXXAXKRAMBGPVGSÂGNANYPRW	V	₩VVFSARX™LWDFXTPYXP-XXXX-XXMXF-≣DWDPAK∥AATXGP®TXSSX-XXXATSTDDSAF#DRGGKLLLWHGMYDAAFSALDTKXYYFRW	SARSWILMD FATP PUR LY DE YVRE EMN RID DETPALAYATTOLWPUS SAELGNADSP DELSGEGGKIN WINGHAMDAFSALDEN SARSWILMD FATP PEPMPHTQVARAMMENDEN SARSWILMD FOR STRUKKAN WEGGSTER SARS SAESTEDEN SARSWILMD FATP PEPWPLHQVARAMMEN FOR DOTPOP RIBERTS OF SARSWILMD FATS PINIA AND SARSWILMD FATP PEPWPLHQVARAMMEN DISDOVERSTER SAEGKSCK WIN WEGGSTER SAEGKSCK WEGGSTER SAEGKSTER SAEGKSCK WEGGSTER SAEGKSTER SAEGKSTER SAEGKSTER SAEGKSTER SAEGKSTER SAEGKSTER SAEGKSTER SAEGKSTER SAEGKSTER SA	EXXXIXILILIPGINITGSGGPGTDRFDWTTPLWAWVIXGKADXXWXAXKXTPGVFGXVXXIIRPLGPYDQIARYKGXG	ETAD——GQAADEARLELLEPGNINGCOGREATD————————————DVDNUTTPINGWARDDIPLERTUSNED FEGEKANDINGS OF FOR A REPORTEGIES SAESETTOWAN AAMP——GAAGEARLELWOOTH RESCONDENT FOR A REPORTED FOR
Consensus Mai	1. MIRO46 2. LSMHTT3es AOAOKRPBE7 3. C. thioxydans WP. 080747404.1 4. Hydrogenophaga sp. PML113 WP_083293388.1 5. Aspergilus oryzae Q2UMK6.1 6. Aspergilus oryzae Q2UWS.1 7. Aspergilus niger Q8WZI8	Consensus	162	•	1. Mileda6 2. IsMHETASE ADADKREPET7 3. C. Thioxydans WP (80977404.1 4. Hydrogenophaga Sp. PML113 WP_083293388.1 5. Kapergillus oryzae (2.2UWKs1 1 6. Aspergillus oryzae (2.2UWKs1 1 7. Aspergillus niger Q8WZI8	Consensus X – Identity	Metodic Company Comp	Consensus NQI	1. MieCd6 2. LiSMHT73es AOA0K8P8E7 N C 2. LiSMHT73es AOA0K8P8E7 3. C. thioxydans WP. 080747404.1 N Q 4. Hydrogenophaga sp. PML113 WP_083293388.1 S. Aspergillus onyzae Q2UMX6.1 Y N 6. Aspergillus niger Q8WZ18 7. Spergillus niger Q8WZ18	Consensus ×AMX Identity	1. Miedde 2. LisMHFTase A0A0K8P8E7 AAI 3. C. Linoxydans WP. 080747404.1 AAI 3. C. thioxydans WP. 080747404.1 AAI AAI 4. Lydrogenopiag as, punchi13 WP.083293388.1 TAAI 5. Aspergilus oryzae Q2UMX6.1 ETT 6. Aspergilus niger Q8WZI8

Supplementary figure S2. Pairwise alignment of Mle046 to MHETase homologs. Pairwise alignment of Mle046 homologues was performed with Clustal Omega. The NCBI/UniProt accession number for each homolog is given. The blue circle depicts the oxyanion hole. The red stars depict the catalytic triad. The blue star is the position S131 in IsMHETase.

References

- Meyer-Cifuentes, I.E., Werner, J., Jehmlich, N., Will, S.E., Neumann-Schaal, M., and Öztürk, B. (2020). Synergistic biodegradation of aromatic-aliphatic copolyester plastic by a marine microbial consortium. *Nat. Commun.* 11, 5790.doi: 10.1038/s41467-020-19583-2
- Perz, V., Bleymaier, K., Sinkel, C., Kueper, U., Bonnekessel, M., Ribitsch, D., et al. (2016). Substrate specificities of cutinases on aliphatic—aromatic polyesters and on their model substrates. *New Biotechnol.* 33, 295-304.doi: 10.1016/j.nbt.2015.11.004