

TABLE S1: Bacterial strains and plasmids used in this work.

Strain	Properties	Reference/source
<i>E. coli</i> DH5α	<i>supE44 ΔlacU169 (Φ80 lacZ ΔM15) hsdR17 recA1 endA1 gyrA96 thi-1 relA1</i>	Invitrogen (Karlsruhe, Germany)
<i>E. coli</i> BL21 (DE3)	F ⁻ , <i>ompT</i> , <i>hsdS</i> B (r _B ⁻ m _B ⁻) <i>gal</i> , <i>dcm</i> , λDE3	Novagen/Merck (Darmstadt, Germany)
<i>E. coli</i> SHuffle® T7	<i>huA2 lacZ::T7 gene1 [lon] ompT ahpC gal</i> λatt::pNEB3-r1-cDsbC (SpecR, lacIq) <i>ΔtrxB sulA11</i> R(mcr-73::miniTn10--TetS)2 [dcm] R(zgb-210::Tn10 - -TetS) <i>endA1 Δgor Δ(mcrC-mrr)114::IS10</i>	NEB (Frankfurt am Main, Germany)
Vector	Properties	Reference/source
pET21a(+)	Expression vector, <i>lacI</i> , Amp ^R , T7- <i>lac</i> - promoter, C-terminal His ₆ -tag coding sequence	Novagen/Merck (Darmstadt, Germany)
pET21a(+)::PET27	1026 bp insert in pET21a(+) coding for PET27	This work
pET21a(+)::PET28	1029 bp insert in pET21a(+) coding for PET28	This work
pET21a(+)::PET29	1029 bp insert in pET21a(+) coding for PET29	This work
pET21a(+)::PET30	1032 bp insert in pET21a(+) coding for PET30	This work
pET21a(+)::PET30_Δ300-366	831 bp insert in pET21a(+) coding for PET30, truncated protein lacking PorC	This work
pET21a(+)::PET38	1341 bp insert in pET21a(+) coding for PET38	This work
pET21a(+)::PET53	1072 bp insert in pET21a(+) coding for PET53	This work
pET21a(+)::PET57	969 bp insert in pET21a(+) coding for PET57	This work
pET21a(+)::PET58	1014 bp insert in pET21a(+) coding for PET58	This work
pET21a(+)::PET59	1035 bp insert in pET21a(+) coding for PET59	This work
pMAL-p4x::IsPETase	795 bp insert in pMAL-p4x coding for the wildtype IsPETase from <i>Ideonella sakaiensis</i>	This work

TABLE S2: Primers used in this work.

Primer	Sequence (5' → 3')	Length (bp)	T _m (°C)	Source
T7_prom	TAATACGACTCACTATAGGG	20	53	Eurofins MWG (Elsberg, Germany)
T7_term	CTAGTTATTGCTCAGCGGT	19	54	Eurofins MWG (Elsberg, Germany)
PET_for	ATATAGGCGCCAGCAACC	18	59	Novagen/Merck (Darmstadt, Germany)
PET_rev	TCCGGATATAGTTCCTC	17	52	Novagen/Merck (Darmstadt, Germany)

TABLE S3: Identifiers and GenBank entries employed in the phylogenetic clustering of PET active enzymes in FIGURE 4.

Name	GenBank or PDB entry	Phylogenetic Affiliation	Reference
BsEstB	ADH43200.1	<i>Bacillus subtilis</i> 4P3-11	Herrero Acero <i>et al.</i> 2011
CalB	4K6G_A	<i>Candida antarctica</i>	Andersen <i>et al.</i> 1999; Xie <i>et al.</i> 2014
Cut190	BAO42836.1	<i>Saccharomonospora viridis</i> AHK190	Kawai <i>et al.</i> 2014
FsC	1CEX	<i>Fusarium solani</i> pisi	Silva <i>et al.</i> 2005
HiC	4OYY	<i>Humicola insolens</i>	Ronkvist <i>et al.</i> 2009
IsPETase	GAP38373.1	<i>Ideonella sakaiensis</i> 201-F6	Yoshida <i>et al.</i> 2016
LCC	AEV21261.1	uncultured bacterial species (leaf-branch compost)	Sulaiman <i>et al.</i> 2012
PE-H	A0A1H6AD45	<i>Pseudomonas aestusnigri</i> VGXO14T	Bollinger <i>et al.</i> 2020
PET2	C3RYL0	uncultured bacterium	
PET5	R4YKL9	<i>Oleispira antarctica</i> RB-8	Danso <i>et al.</i> 2018
PET6	A0A1Z2SIQ1	<i>Vibrio gazogenes</i>	
PET12	A0A0G3BI90	<i>Polyangium brachysporum</i>	
Tcur0390	WP_012850775.1	<i>Thermomonospora curvata</i> DSM43183	Wei <i>et al.</i> 2014
Tcur1278	WP_012851645.1	<i>Thermomonospora curvata</i> DSM43183	Chertkov <i>et al.</i> 2011
TfH	WP_011291330.1	<i>Thermobifida fusca</i> DSM43793	Müller <i>et al.</i> 2005
Thc_Cut1	ADV92526.1	<i>Thermobifida cellulosilytica</i> DSM44535	
Thc_Cut2	ADV92527.1	<i>Thermobifida cellulosilytica</i> DSM44535	Herrero Acero <i>et al.</i> 2011
Thf42_Cut1	ADV92528.1	<i>Thermobifida fusca</i> DSM44342	
Tha_Cut1	ADV92525.1	<i>Thermobifida alba</i> DSM43185	Ribitsch <i>et al.</i> 2012
Thh_Est	AFA45122.1	<i>Thermobifida halotolerans</i> DSM44931	

TABLE S4: Data collection and refinement statistics for PET30

	PET30
Wavelength	0.9795
Resolution range	38.8 - 2.1 (2.175 - 2.1)
Space group	P 43 21 2
Unit cell	109.755 109.755 41.803 90 90 90
Total reflections	267429 (24691)
Unique reflections	15447 (1505)
Multiplicity	17.3 (16.4)
Completeness (%)	99.95 (100.00)
Mean I/sigma(I)	13.95 (5.38)
Wilson B-factor	24.09
R-merge	0.1746 (0.7031)
R-meas	0.1799 (0.7255)
R-pim	0.04277 (0.1772)
CC1/2	0.997 (0.945)
CC*	0.999 (0.986)
Reflections used in refinement	15443 (1507)
Reflections used for R-free	716 (78)
R-work	0.1619 (0.1655)
R-free	0.2187 (0.2460)
CC(work)	0.962 (0.901)
CC(free)	0.932 (0.840)
Number of non-hydrogen atoms	2208
macromolecules	2006
solvent	202
Protein residues	273
RMS(bonds)	0.011
RMS(angles)	1.21
Ramachandran favored (%)	95.57
Ramachandran allowed (%)	3.69
Ramachandran outliers (%)	0.74
Rotamer outliers (%)	0.00
Clashscore	5.01
Average B-factor	27.12
macromolecules	26.45
solvent	33.82

Statistics for the highest-resolution shell are shown in parentheses.

TABLE S5: Homologs of bacteroidetal PET27 and PET30 hydrolases in metagenomes.

Locus Tag	Gene ID	Genome Name (GREEN=Bacteroidetes; YELLOW=FCB)	NCBI Biosample Accession	Pubmed ID /GenBank entry
Ga0266410_112824	2790477168	<i>Aequorivita</i> sp. CPC67	SAMEA2621812	25999513
Ga0267287_12740	2786516536	<i>Marinimicrobia</i> bacterium SP4388	SAMEA2621812	25999513
Ga0214074_111348	2758370761	<i>Psychroflexus torquis</i> ATCC 700755	SAMN02603919	24391155 , 32431677
Ga0441744_095_43538_44635	2893010289	<i>Psychroflexus gondwanensis</i> ACAM 365	SAMN10790446	24391155, 32431677
Ga0452869_05_68007_69104	2890419200	<i>Brumimicrobium glaciale</i> IC156	SAMN10779751	24391155, 32431677
Ga0125537_104199	2776033716	<i>Altibacter lentus</i> JL2010	SAMN02988278	25342673
Ga0101260_1142	2663974789	<i>Bacteroidetes</i> bacterium SCGC AD-308-D03 - v2	SAMN06314724	373136
Ga0266415_110121	2790487960	<i>Aequorivita</i> sp. SAT106	SAMEA2620929	25999513
Ga0266677_12858	2778555477	<i>Flavobacteriaceae</i> bacterium SAT1509	SAMEA2620929	25999513
Ga0267170_100812	2814895288	<i>Gemmatimonadetes</i> bacterium RS373	SAMEA2620929	25999513
Ga0267250_100918	2786410539	<i>Marinimicrobia</i> bacterium RS418	SAMEA2620929	25999513
Ga0267252_10536	2786413511	<i>Marinimicrobia</i> bacterium RS816	SAMEA2620929	25999513
Ga0267252_10542	2786413517	<i>Marinimicrobia</i> bacterium RS816	SAMEA2620929	25999513
Ga0266410_112825	2769034576	<i>Aequorivita</i> sp. CPC68	SAMEA2621812	25999513
Ga0267287_12741	2767134878	<i>Marinimicrobia</i> bacterium SP4389	SAMEA2621812	25999513
Ga0214074_111349	2765235181	<i>Psychroflexus torquis</i> ATCC 700756	SAMN02603919	24391155 , 32431678
Ga0441744_095_43538_44636	2763335483	<i>Psychroflexus gondwanensis</i> ACAM 366	SAMN10790446	24391155, 32431677
Ga0452869_05_68007_69105	2761435786	<i>Brumimicrobium glaciale</i> IC157	SAMN10779751	24391155, 32431677
Ga0267200_1431	2786528832	<i>Marinimicrobia</i> bacterium EAC25	SAMEA2620855	25999513, 29337314
Ga0267200_1544	2786528992	<i>Marinimicrobia</i> bacterium EAC25	SAMEA2620855	25999513, 29337314
Ga0416765_13_4825_5925	2860314059	<i>Chryseobacterium</i> sp. 16F	SAMN14915977	25824943
PI23P_05397	639004873	<i>Polaribacter irgensii</i> 23-P	SAMN02436114	9542092 /NZ_CH724148.1
Aeqsu_2514	2509583556	<i>Aequorivita sublithicola</i> QSSC9-3, DSM 14238	SAMN02232006	28604660
Ga0266584_11073	2788314349	<i>Cryomorphaceae</i> bacterium CPC63	SAMEA2622923	25999513
Ga0266589_10312	2787698016	<i>Cytophagia</i> bacterium NAT375	SAMEA2622923	25999513
Ga0267262_14611	2786499724	<i>Marinimicrobia</i> bacterium SAT24	SAMEA2622923	25999513
Ga0267263_11113	2786500508	<i>Marinimicrobia</i> bacterium SAT2619	SAMEA2622923	25999513
Ga0267267_12326	2786498443	<i>Marinimicrobia</i> bacterium SP108	SAMEA2622923	25999513
Ga0267268_10328	2786441642	<i>Marinimicrobia</i> bacterium SP173	SAMEA2622923	25999513
Ga0267268_1112	2786442127	<i>Marinimicrobia</i> bacterium SP173	SAMEA2622923	25999513
Ga0267270_13120	2786484524	<i>Marinimicrobia</i> bacterium SP276	SAMEA2622923	25999513
Ga0267272_1173	2786478779	<i>Marinimicrobia</i> bacterium SP3060	SAMEA2622923	25999513
Ga0267273_13417	2786477564	<i>Marinimicrobia</i> bacterium SP3097	SAMEA2622923	25999513
Ga0267276_13625	2786480397	<i>Marinimicrobia</i> bacterium SP3117	SAMEA2622923	25999513
Ga0267279_10440	2786489803	<i>Marinimicrobia</i> bacterium SP328	SAMEA2622923	25999513
Ga0267280_12433	2786520859	<i>Marinimicrobia</i> bacterium SP359	SAMEA2622923	25999513
Ga0267282_10178	2786519018	<i>Marinimicrobia</i> bacterium SP4039	SAMEA2622923	25999513
Ga0063505_10623	2606052547	<i>Roseivirga seohaensis</i> aquiponti D-25	SAMN03145748	27107724/ JSVA01000000

Ga0267161_105313	2814952585	<i>Gemmatimonadetes bacterium</i> EAC635	SAMEA2623295	25999513
Ga0267164_1577	2814955360	<i>Gemmatimonadetes bacterium</i> NAT196	SAMEA2623295	25999513
Ga0267234_12226	2786460090	<i>Marinimicrobia bacterium</i> NAT495	SAMEA2623295	25999513
Ga0267237_1518	2786446587	<i>Marinimicrobia bacterium</i> NAT62	SAMEA2623295	25999513
Ga0267238_1416	2786464404	<i>Marinimicrobia bacterium</i> NAT74	SAMEA2623295	25999513
Ga0267239_13724	2786462435	<i>Marinimicrobia bacterium</i> NP104	SAMEA2623295	25999513
Ga0215720_101116	2756777030	<i>Marinirhabdus gelatinilytica</i> DSM 101478	SAMN08776299	QRAO00000000
Ga0266586_11614	2788308996	<i>Cryomorphaceae bacterium</i> SP53	SAMEA2619927	25999513
Ga0267165_10476	2814943193	<i>Gemmatimonadetes bacterium</i> NP105	SAMEA2619818	25999513
SCB49_04680	641143976	<i>Ulvibacter</i> sp. SCB49	SAMN02981237	ABCO00000000
G440DRAFT_00359	2524126300	<i>Aequorivita capsosiphonis</i> DSM 23843	SAMN02440880	AUBG00000000
Ga0266370_16423	2789796087	<i>Altibacter</i> sp. EAC109	SAMEA2619376	25999513
Ga0266560_154	2825980380	<i>Crocinitomicaceae bacterium</i> NAT165	SAMEA2619376	25999513
Ga0267217_1484	2786425266	<i>Marinimicrobia bacterium</i> MED806	SAMEA2619376	25999513
Ga0267217_16831	2786425767	<i>Marinimicrobia bacterium</i> MED806	SAMEA2619376	25999513
Ga0267218_1219	2786426885	<i>Marinimicrobia bacterium</i> MED808	SAMEA2619376	25999513
Ga0267219_10943	2786427199	<i>Marinimicrobia bacterium</i> MED812	SAMEA2619376	25999513
Ga0267220_10096	2786428744	<i>Marinimicrobia bacterium</i> MED829	SAMEA2619376	25999513
Ga0267220_10106	2786428754	<i>Marinimicrobia bacterium</i> MED829	SAMEA2619376	25999513
Ga0267224_14310	2786401069	<i>Marinimicrobia bacterium</i> NAT217	SAMEA2619376	25999513
Ga0267226_12918	2786404156	<i>Marinimicrobia bacterium</i> NAT220	SAMEA2619376	25999513
Ga0267227_13041	2786403315	<i>Marinimicrobia bacterium</i> NAT224	SAMEA2619376	25999513
Ga0267228_1422	2786399119	<i>Marinimicrobia bacterium</i> NAT230	SAMEA2619376	25999513
Ga0267208_10372	2786507568	<i>Marinimicrobia bacterium</i> MED586	SAMEA2619667	25999513
Ga0267208_10412	2786507636	<i>Marinimicrobia bacterium</i> MED586	SAMEA2619667	25999513
Ga0267209_1018	2786508441	<i>Marinimicrobia bacterium</i> MED589	SAMEA2619667	25999513
Ga0267212_106014	2786417419	<i>Marinimicrobia bacterium</i> MED648	SAMEA2619667	25999513
Ga0267212_10712	2786417608	<i>Marinimicrobia bacterium</i> MED648	SAMEA2619667	25999513
Ga0267212_110812	2786418228	<i>Marinimicrobia bacterium</i> MED648	SAMEA2619667	25999513
Ga0267213_15817	2786422578	<i>Marinimicrobia bacterium</i> MED757	SAMEA2619667	25999513
Ga0267214_1214	2786420423	<i>Marinimicrobia bacterium</i> MED764	SAMEA2619667	25999513
Ga0267214_12210	2786420472	<i>Marinimicrobia bacterium</i> MED764	SAMEA2619667	25999513
Ga0267214_1225	2786420467	<i>Marinimicrobia bacterium</i> MED764	SAMEA2619667	25999513
Ga0267214_14025	2786421083	<i>Marinimicrobia bacterium</i> MED764	SAMEA2619667	25999513
Ga0344934_2405	2839770976	<i>Dokdonia sinensis</i> SH27	SAMN10250232	32228747
Ga0441973_01_775875_776969	2890600833	<i>Ulvibacter</i> sp. KK4	SAMD00166796	32539909
LY87DRAFT_1302	2597312806	<i>Dokdonia</i> sp. Hel_I_5	SAMN05661066	32539909
Ga0267202_1226	2786524372	<i>Marinimicrobia bacterium</i> EAC649	SAMEA2620230	25999513
Ga0267202_13210	2786524561	<i>Marinimicrobia bacterium</i> EAC649	SAMEA2620230	25999513
Ga0267202_13216	2786524567	<i>Marinimicrobia bacterium</i> EAC649	SAMEA2620230	25999513
Ga0350409_3222	2848310855	<i>Psychroflexus</i> sp. MES1-P1E	SAMN08125772	PJBS00000000, CM009131
Ga0066802_10753	2623289129	<i>Aequorivita viscosa</i> DSM 26349	SAMN04487908	FQYV00000000
Ga0077144_1092	2641186029	<i>Chryseobacterium jeonii</i> DSM 17048	SAMN03145167	JSYL00000000
Ga0079842_10854	2668214597	<i>Aequorivita viscosa</i> CGMCC 1.11023	SAMN05216556	FNNS00000000

Ga0104531_1111	2676887423	<i>Chryseobacterium jeonii</i> DSM 17048	SAMN05421876	FOLA00000000
Ga0114183_10545	2656209723	<i>Roseivirga seohaensis</i> SW-152	SAMN04423148	28077207
Ga0170448_3258	2729662814	<i>Ulvibacter antarcticus</i> DSM 23424	SAMN06264851	REFC00000000
Ga0310487_0626	2799156613	<i>Roseivirga ehrenbergii</i> DSM 102268	SAMN10864729	SMGS00000000
Ga0336340_1074	2835308220	<i>Winogradskyella</i> sp. KYW1333	SAMN09667312	QPHL00000000
Ga0336670_2825	2837407865	<i>Aequorivita lipolytica</i> CIP 107455	SAMEA4644770	30225207
Ga0336672_3534	2835103747	<i>Aequorivita antarctica</i> CIP 107457	SAMEA4644771	PMC6139392
Ga0443045_01_3096381_3097484	2884436497	<i>Nonlabens</i> sp. Ci31	SAMN12697551	CP043633
pgond44_05165	2533771942	<i>Psychroflexus gondwanensis</i> ACAM 44	SAMN02471957	24391155
Ga0077372_102327	2628626758	<i>Aequorivita vladivostokensis</i> KMM 3516	SAMN03084320	JSVU00000000
Ga0114184_10616	2654412999	<i>Roseivirga echinicomitans</i> KMM 6058	SAMN04382068	LRDB00000000.1
Ga0248413_145580	2812952679	<i>Roseivirga ehrenbergii</i> KMM 6017	SAMN03084331	LQZQ00000000
Ga0373279_1152	2830035069	<i>Lewinella antarctica</i> DSM 105096	SAMN13172327	PMC6139392
Ga0336671_791	2835108492	<i>Aequorivita</i> sp. CIP 111184	SAMEA4704834	UEFQ00000000
Ga0399719_270	2836794304	<i>Aequorivita</i> sp. H23M31	SAMN10518960	CP034951
Ga0441974_01_521424_522518	2890652824	<i>Ulvibacter marinus</i> KCTC 32322T	SAMD00166797	BKCG00000000.1
