Table S1: Oligonucleotides and PCR primers used in this study.

Name	5'-3' Sequence	Description or Use	Reference
Strep B	ACAAGCCCTGGAAACGGGGT	16S rDNA PCR, sequencing	[23]
Strep F	ACGTGTGCAGCCCAAGACA	16S rDNA PCR, sequencing	[23]
pA	AGAGTTTGATCCTGGCTCAG	16S rDNA PCR, sequencing	[24]
pH	AAGGAGGTGATCCAGCCGCA	16S rDNA PCR, sequencing	[24]
16SInL	AGCATTAGAGATAGTGCCCCC	16S rDNA sequencing	This study
16SInR	TACCGTCACTTGCGCTTCTT	16S rDNA sequencing	This study
T3	AATTAACCCTCACTAAAGGG	16S rDNA and *phaZ* sequencing	Eurofins MWG Operon
T7	TAATACGACTCACTATAGGG	16S rDNA and *phaZ* sequencing	Eurofins MWG Operon
M13 Forward	TGTAAAACGACGGCCAGT	*phaZ* sequencing	Eurofins MWG Operon
M13 Reverse	CAGGAAACAGCTATGACC	*phaZ* sequencing	Eurofins MWG Operon
PHAD-8	ATGCACACSTACGTSCCSGA	*phaZ*-specific, PCR for cloning	This study
PHADIN-3[a]	GTNCCNACNGGNGCNGC	*phaZ*-specific, PCR for cloning	This study
PHAZL2	CATCTGCCTGATCGACAGC	*phaZ*-specific, Inverse PCR	This study
PHAZR1A	ACACCTGCATGTACAGCCC	*phaZ*-specific, ssDNA ligation PCR	This study
ARB-B[b]	Pi-AGTTCACACTGGCGAGGCA	ssDNA ligation PCR	[35]
ARB-C	TGCCTCGCCAGTGTGAACT	ssDNA ligation PCR	[35]
GSP1	TACGAAGGTGGGCACCACCACGTCGA	*phaZ*-specific, GenomeWalker kit	This study
GSP2	TGCTACACGGCTAACAACTACCAGCACA	*phaZ*-specific, GenomeWalker kit	This study
AP1	GTAATACGACTCACTATAGGGC	adaptor-specific, GenomeWalker kit	Clontech
AP2	ACTATAGGGCACGCGTGGT	adaptor-specific, GenomeWalker kit	Clontech
PHAZF9	ATATATGGATCCTAAGGAGATATACCATGGGACAGCCGTACCCT	*phaZ*-specific, PCR for cloning	This study
PHAZR9	ATATATAAGCTTTCAGGCCGAGCAGCCGGA	*phaZ*-specific, PCR for cloning	This study
PHAZL3	CGCCAACTCCTGCTTCAAC	*phaZ* sequencing	This study
PHAZInF	CGATCTGGCAGGGCACATCG	*phaZ* sequencing	This study
PHAZInR	AGAAGCGGGCGGTGTGGTAG	*phaZ* sequencing	This study
PHAZR4	CGGTCACCGAGGAGGAGAC	*phaZ* sequencing	This study

[a]N = 53.2% G, 38.l% C, 4.6% T, and 4.1% A

[b]Pi = added 5' phosphate group

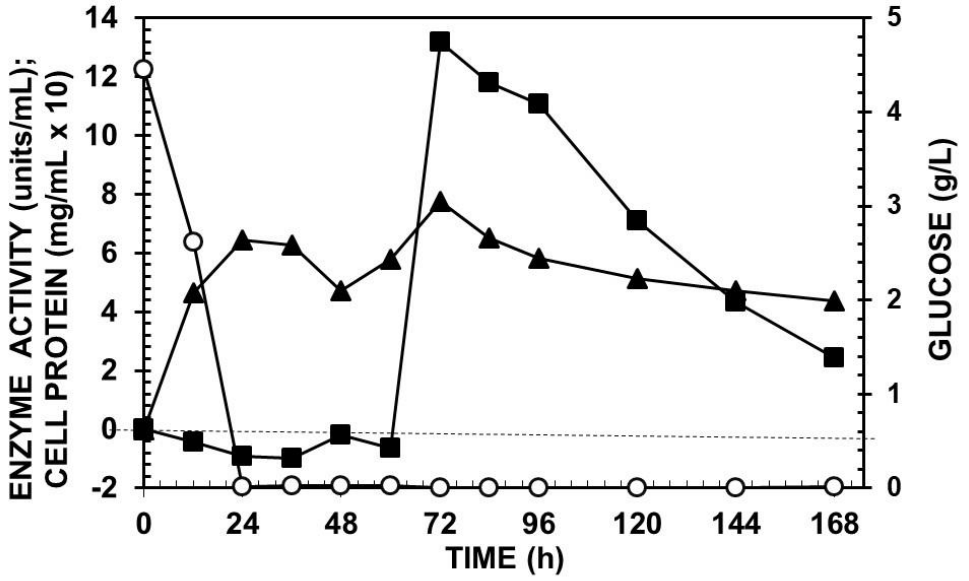


Figure S1: PHA depolymerase synthesis in the presence of glucose and PHB. Cells from a 40 mL overnight culture in NB were pelleted by centrifugation, and then resuspended without washing in 400 mL of SNC broth containing 0.5% glucose and 0.2% PHB. Samples (2.0 ml) were removed at intervals and assayed for PHA depolymerase activity by the turbidometric method (■), glucose (○), and cell protein (▲). Values of e-PHB depolymerase activity from 0 to 60 hours were below zero, since turbidity changes in those samples were lower than that of a no enzyme control, possibly due to clumping of PHB granules. The dashed line indicates the zero point for enzyme activity and protein. Values are averages from duplicate assays.

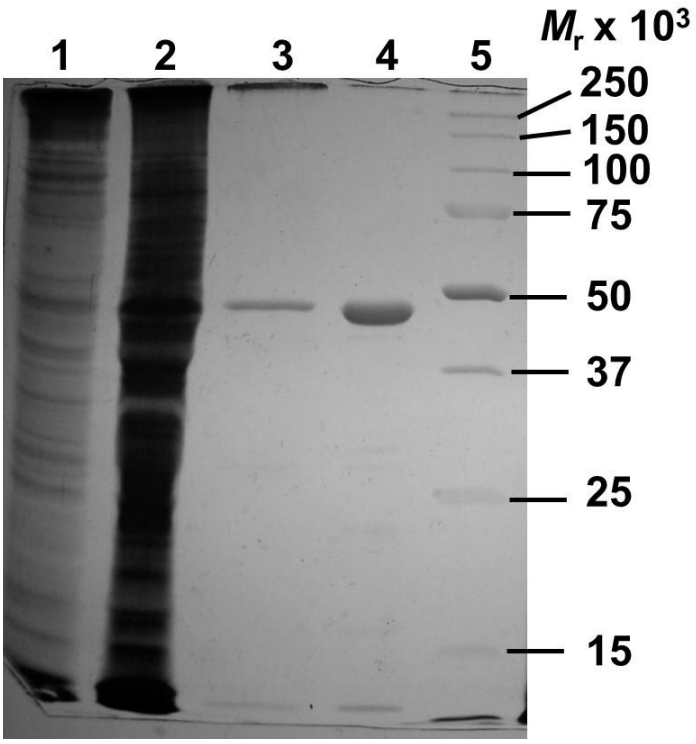


Figure S2: SDS-PAGE analysis of samples from $PhaZ_{Ssp5A}$ purification. Protein amounts loaded are indicated. Lanes: (1), culture supernatant, 3.2 μg; (2), ammonium sulfate (55% saturation) precipitate, 12.2 μg; (3), sample from Phenyl Sepharose® CL-4B step, 0.18 μg; (4) sample from Sephacryl® S-100-HR step, 0.73 μg; (5) Bio-Rad Precision Plus Protein™ Unstained Standards (10 μl loaded), with M_r x 10^3 indicated at the right.

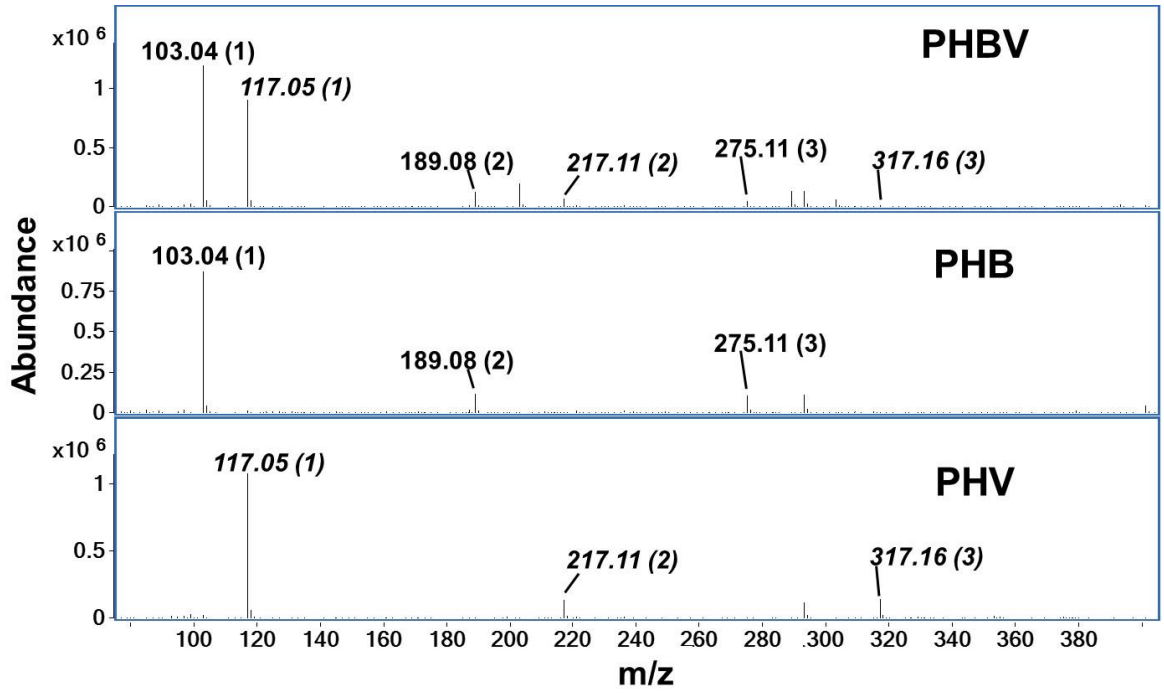


Figure S3: Representative ESI-TOF-MS profiles for products of PHA thin film degradation by $PhaZ_{Ssp5A}$ after 4 h. m/z ratios for degradation products are shown above the spectral peaks. Numbers in parentheses indicate the monomer (1), dimer (2), and trimer (3) forms of 3HB (regular type) or 3HV (italics).

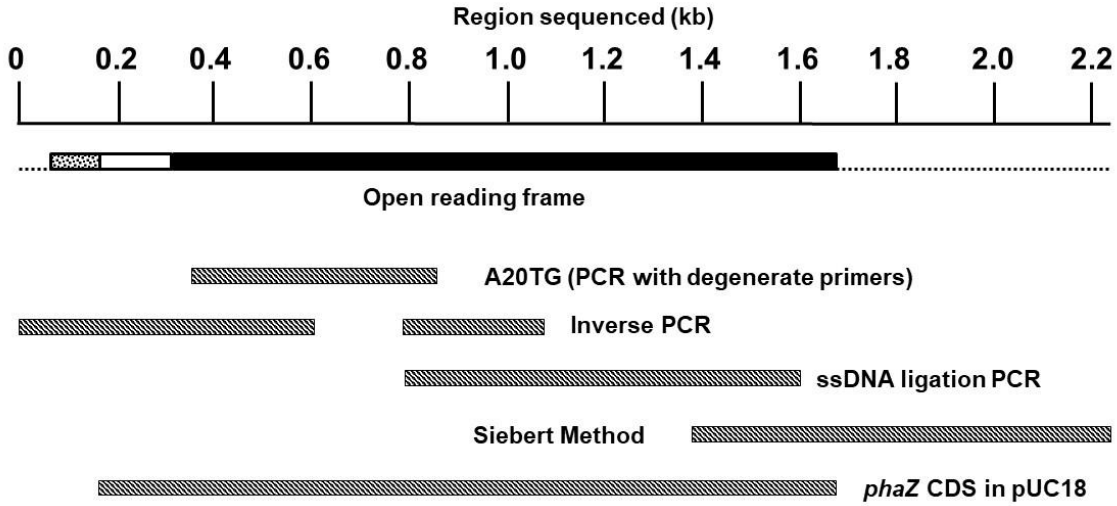
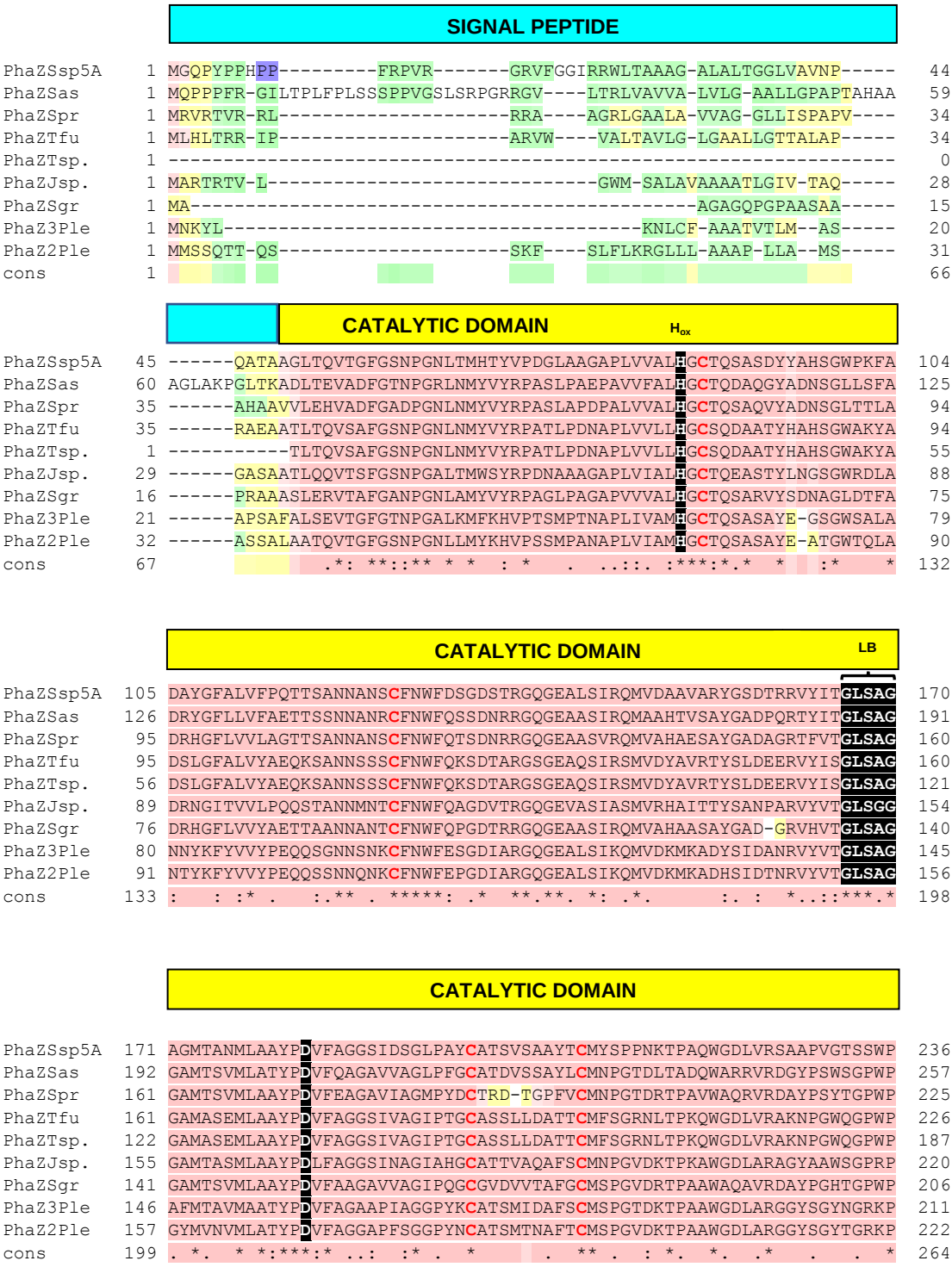
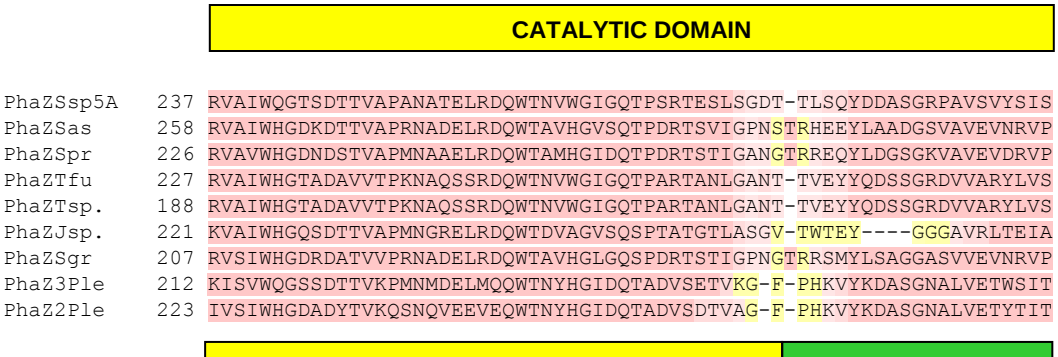


Figure S4: Cloning strategy for *phaZ.* The open reading frame consists of the secreted protein coding sequence (solid bar) and signal peptide coding sequence (open bar); the stippled box line indicates a putative promoter region. The cross hatched bars indicate sequences obtained from the cloning methods indicated. CDS, coding sequence of signal peptide and mature protein regions. See text and Table S1 for description of cloning methods and PCR primers.


SIGNAL PEPTIDE
CATALYTIC DOMAIN
Hox
CATALYTIC DOMAIN
LB
CATALYTIC DOMAIN

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PhaZSas     258 RVAIWHGDKDTTVAPRNADELRDQWTAVHGVSQTPDRTSVIGPNSTRHEEYLAADGSVAVEVNRVP 323
PhaZSpr     226 RVAVWHGDNDSTVAPMNAAELRDQWTAMHGIDQTPDRTSTIGANGTRREQYLDGSGKVAVEVDRVP 291
PhaZTfu     227 RVAIWHGTADAVVTPKNAQSSRDQWTNVWGIGQTPARTANLGANT-TVEYYQDSSGRDVVARYLVS 291
PhaZTsp.    188 RVAIWHGTADAVVTPKNAQSSRDQWTNVWGIGQTPARTANLGANT-TVEYYQDSSGRDVVARYLVS 252
PhaZJsp.    221 KVAIWHGQSDTTVAPMNGRELRDQWTDVAGVSQSPTATGTLASGV-TWTEY----GGGAVRLTEIA 281
PhaZSgr     207 RVSIWHGDRDATVVPRNADELRDQWTAVHGLGQSPDRTSTIGPNGTRRSMYLSAGGASVVEVNRVP 272
PhaZ3Ple    212 KISVWQGSSDTTVKPMNMDELMQQWTNYHGIDQTADVSETVKG-F-PHKVYKDASGNALVETWSIT 275
PhaZ2Ple    223 IVSIWHGDADYTVKQSNQVEEVEQWTNYHGIDQTADVSDTVAG-F-PHKVYKDASGNALVETYTIT 286
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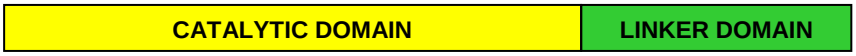


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cons        265 :::*:*  * .*   *  .  :***   *:.*:.  :  :     *    *   *    :. 330
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PhaZSsp5A   302 GMAHGLAVAPGSGPDQCGTTGT-YYLD-TICSSYHTARFWGLDGGDGGSGQNPGTLPA-PAGLTVG 364
PhaZSas     324 GIGHGTPVDPGTGAQQCGSTGAAYFLD-SICSSYWITQFFGLSGSAS----DPGSLPA-PSGLAAT 383
PhaZSpr     292 SIGHGTPVDPGSGPEQCGRTGTAHFID-SICSSHWVAGFFGLAGGDP----DPGGLPA-PAGLTVT 351
PhaZTfu     292 GMGHGTPVDPGSGTDQCGTAGA-YFLD-TICSTYHTIQFWELDGDPQ-----PSPSPS-PSPS--- 346
PhaZTsp.    253 GMGHGTPADPGSGTDQCGTAGA-YFLD-TICSTYHTIQFWELDGDPQ-----PSPSPS-PSPS--- 307
```


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PhaZJsp.    282 GMTHGTPVDPGSGLTQCGTAGA-YFID-TVCGAYYDAQFFGLIGSSP----SPTATAT-PTG---- 336
PhaZSgr     273 GIAHGTPVDPGGGGEQCGATGTQHFID-SICSSHWITRFFGLDGGT-----TPEPP---------- 322
PhaZ3Ple    276 GMAHGTPVDPGTGAEQCGTSGS-YILDVNICSSYHIAQFFGLTGAGT----TT-TTTVGSTS---- 331
PhaZ2Ple    287 GMGHGTPVDPGTGSLQCGTAGA-YILDVNICSSYYVAKFWGLIGGSG----TTTTTSAGTTT---- 343
cons        331 .: ** .. ** *  *** :*: : :* .:*.::    *: * *     .               396
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PhaZSsp5A   365 GTTDSTVSLSWNPVDGASSYTVYRGGTKVGTTTSTAYTDTGLATGTAYAYTVAAVDAAGAVGTVSS 430
PhaZSas     384 GATDTTISLTWKPVDGATDYAVHRDGAQITTSATTSYTDTGLRAGTSHTYAVAARDADGKAGPLSG 449
PhaZSpr     352 GSDDTSIALAWQAVDGASDYAVYRDGTRVATPLGPSFTDTPLAAGSTHAYRVAARDAAGTEGARSA 417
PhaZTfu     347 ------------------------------------------------------------------ 346
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PhaZTsp.    308 ------------------------------------------------------------------ 307
PhaZJsp.    337 ---------------------------------TP---------------------------TATPT 343
PhaZSgr     323 -------------VD------------------------------------------------------ 324
PhaZ3Ple    332 ---------------------------------TTTGYTST---------------SSAPVTTTTSV 350
PhaZ2Ple    344 ---------------------------------TTSAGTTT--------T------KASTTTTKVST 363
cons        397                                                                    462
```

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PhaZSsp5A   431 SV-TATTTGYTPTCYTANNYQHTTAGRAYQSGGYTYATGSGQAMGLYNTFTTHTLKQTAPGYYVLA 495
PhaZSas     450 AV-TAQTTGATAVCWTAGNYAHVQAGRATTSAGYTYAKGSGQNMGLYNTFVTTTLKESPTGYFTVA 514
PhaZSpr     418 QI-TASTTGAAAVCWTGSNYAHVRAGRATTTGGYTYAKGSGQNMGLYNTFVTHTLKESPAGHYVIA 482
PhaZTfu     347 PS-PSPTTPPAGECVTANNYQHVAEGRAYVSYGYAYSVGGNDLLGLWNIYVITSVQETSPGYWERV 411
PhaZTsp.    308 PS-PSPTTPPAGECVTANNYQHVAEGRAYVSYGYAYSVGGNDLLGLWNIYVITSVQETSPGYWERV 372
PhaZJsp.    344 P-TPTPTATSTPTCVTATNYAHVQAGRAHNVGGYALANGSNDNLGLYNTFYTSAVRETSPGYWQKC 408
PhaZSgr     325 -----PPT-EPAACWTASNYEHVRAGRATTTGGQVYAKGSAQHLGLYNTFVTHTLKESPAGHYVLA 384
PhaZ3Ple    351 A-STTTTTVAAGACYNASNYAHVTAGRAVNSMGYAKAKGSNQNMGLYNTFTTSKLREAPAGYFTID 415
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PhaZ2Ple    364 TTTASTTTTTAGACYNSSNYAHVTAGRAHDTGGYAYTNGSNQKMGLNNTFYTSKLRKTGTNYYVID  429
cons        463       .:  .  * .. ** *.  ***    * . : *. : :** * :    :::: ..::    528
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PhaZSsp5A  496 DSGCSA  501
PhaZSas    515 NDTCP-  519
PhaZSpr    483 DGNCP-  487
PhaZTfu    412 -SGC--  414
PhaZTsp.   373 -SGC--  375
PhaZJsp.   409 ------  408
PhaZSgr    385 DSTCP-  389
PhaZ3Ple   416 -STCP-  419
PhaZ2Ple   430 -TTCP-  433
cons       529        534
```

Figure S5: Amino acid sequence alignment of PHA depolymerase from *Streptomyces* sp. SFB5A with selected PHA depolymerases from extracellular denatured PHA-scl depolymerases with catalytic domain type 1, family 11 [4]. Enzyme abbreviations and organisms (accession numbers in parentheses): PhaZSsp5A, *Streptomyces* sp. SFB5A; PhaZSas; *Streptomyces ascomycinicus* sp. nov. DSMZ 40822 (formerly known as *Streptomyces hygroscopicus* subsp. *ascomyceticus*) (AAF86381.1); PhaZSpr, *Streptomyces pristinaespiralis* ATCC 25486 (EDY63333.1); PhaZTfu, *Thermobifida fusca* YX (AAZ54120.1); PhaZTsp., *Thermobifida* sp. BCC23166 (ACF17837.1); PhaZJsp., *Janibacter* sp. HTCC2649 (EAP98776.1); PhaZSgr, *Streptomyces griseus* NBRC 13350 (BAG18022.1); PhaZ2Ple and PhaZ3Ple, *Paucimonas lemoignei* PHA depolymerases PhaZ2 and PhaZ3 (AAB17150.1 and AAB48166.1 respectively). Essential conserved amino acids in the catalytic domain are in white type with black highlight. LB, lipase box; H_{OX}, conserved oxyanion hole histidine. Conserved cysteines are in bold red type. Putative domains are in color coded boxes. The degree of consensus (cons) is shown by shading:
BAD AVG GOOD