

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	ACGTGTGCAGCCAAGACA	16S rDNA PCR, sequencing	[23]
pA	AGAGTTGATCCTGGCTCAG	16S rDNA PCR, sequencing	[24]
pH	AAGGAGGTGATCCAGCCGCA	16S rDNA PCR, sequencing	[24]
16SInL	AGCATTAGAGATAGTGCCCCC	16S rDNA sequencing	This study
16SInR	TACCGTCACTTGCCTCTT	16S rDNA sequencing	This study
T3	AATTAAACCTCACTAAAGGG	16S rDNA and <i>phaZ</i> sequencing	Eurofins MWG Operon
T7	TAATACGACTCACTATAGGG	16S rDNA and <i>phaZ</i> sequencing	Eurofins MWG Operon
M13 Forward	TGTAAAACGACGCCAGT	<i>phaZ</i> sequencing	Eurofins MWG Operon
M13 Reverse	CAGGAAACAGCTATGACC	<i>phaZ</i> sequencing	Eurofins MWG Operon
PHAD-8	ATGCACACSTACGTSCCSGA	<i>phaZ</i> -specific, PCR for cloning	This study
PHADIN-3 ^a	GTNCCNACNGGNNGCNGC	<i>phaZ</i> -specific, PCR for cloning	This study
PHAZL2	CATCTGCCTGATCGACAGC	<i>phaZ</i> -specific, Inverse PCR	This study
PHAZR1A	ACACCTGCATGTACAGCCC	<i>phaZ</i> -specific, ssDNA ligation PCR	This study
ARB-B ^b	Pi-AGTTCACACTGGCGAGGCA	ssDNA ligation PCR	[35]
ARB-C	TGCCTCGCCAGTGTGAAC	ssDNA ligation PCR	[35]
GSP1	TACGAAGGTGGGCACCACCGTCGA	<i>phaZ</i> -specific, GenomeWalker kit	This study
GSP2	TGCTACACGGCTAACAACTACCAGCACA	<i>phaZ</i> -specific, GenomeWalker kit	This study
AP1	GTAATACGACTCACTATAGGC	adaptor-specific, GenomeWalker kit	Clontech
AP2	ACTATAAGGGCACCGTGTT	adaptor-specific, GenomeWalker kit	Clontech
PHAZF9	ATATATGGATCCTAAGGAGATATACCATGGGACAGCCGTACCC	<i>phaZ</i> -specific, PCR for cloning	This study
PHAZR9	ATATATAAGCTTCAAGGCCGAGCAGCCGGA	<i>phaZ</i> -specific, PCR for cloning	This study
PHAZL3	CGCCAACCTCCTGCTAAC	<i>phaZ</i> sequencing	This study
PHAZInF	CGATCTGGCAGGGCACATCG	<i>phaZ</i> sequencing	This study
PHAZInR	AGAAGCGGGCGGTGTGGTAG	<i>phaZ</i> sequencing	This study
PHAZR4	CGGTCAACCGAGGAGGAGAC	<i>phaZ</i> sequencing	This study

^aN = 53.2% G, 38.1% C, 4.6% T, and 4.1% A

^bPi = added 5' phosphate group

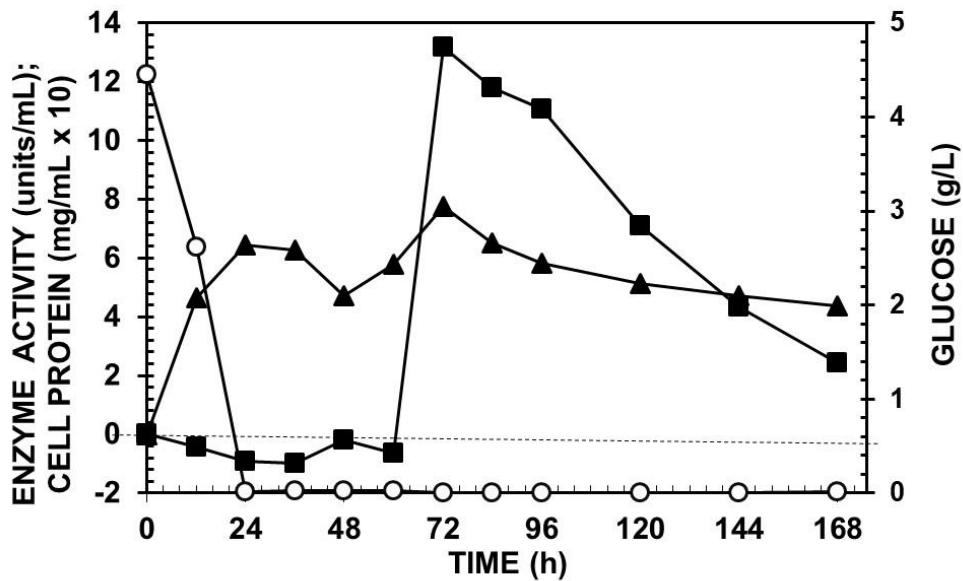


Figure S1: PHA depolymerase synthesis in the presence of glucose and PHB. Cells from a 40 mL overnight culture in NB were pelleted by centrifugation, and then resuspended without washing in 400 mL of SNC broth containing 0.5% glucose and 0.2% PHB. Samples (2.0 ml) were removed at intervals and assayed 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.

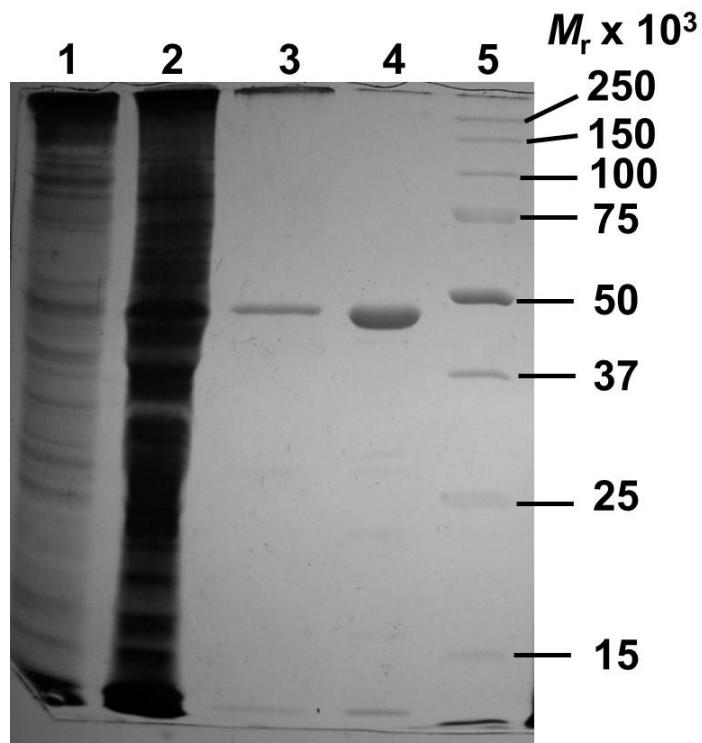


Figure S2: SDS-PAGE analysis of samples from $\text{PhaZ}_{\text{Ssp5A}}$ purification. Protein amounts loaded are indicated. Lanes: (1), culture supernatant, 3.2 μg ; (2), ammonium sulfate (55% saturation) precipitate, 12.2 μg ; (3), sample from Phenyl Sepharose® CL-4B step, 0.18 μg ; (4) sample from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

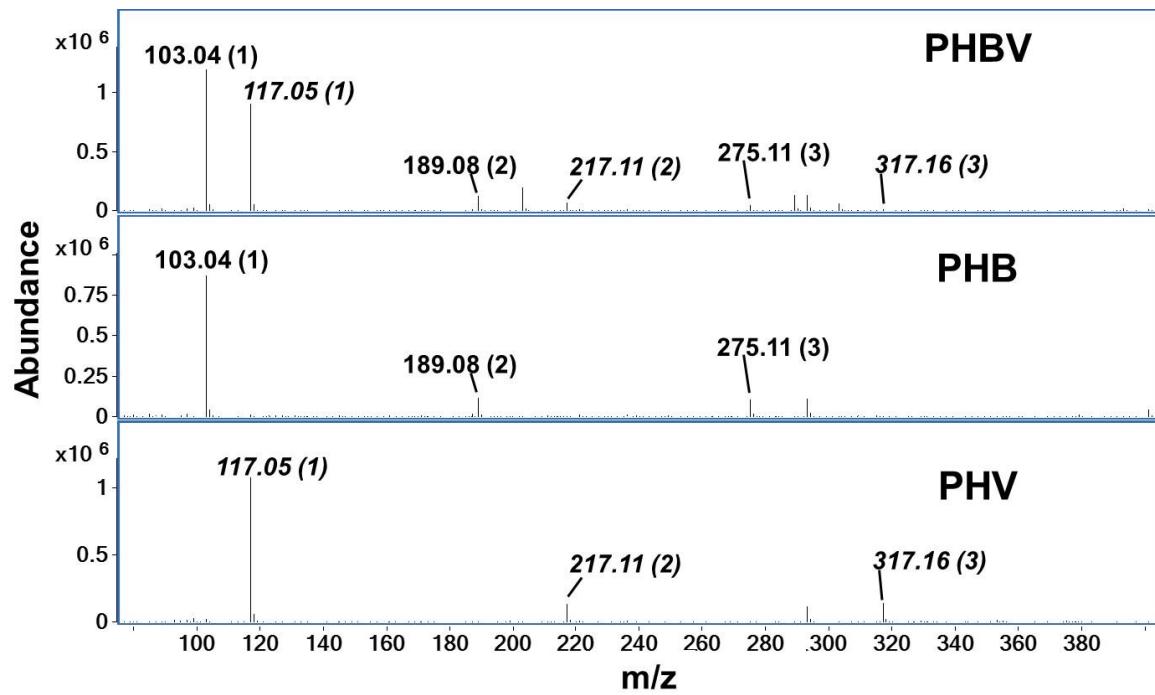


Figure S3: Representative ESI-TOF-MS profiles for products of PHA thin film degradation by $\text{PhaZ}_{\text{Ssp5A}}$ after 4 h. m/z ratios for degradation products are shown above the spectral peaks. Numbers in parentheses indicate the monomer (1), dimer (2), and trimer (3) forms of 3HB (regular type)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

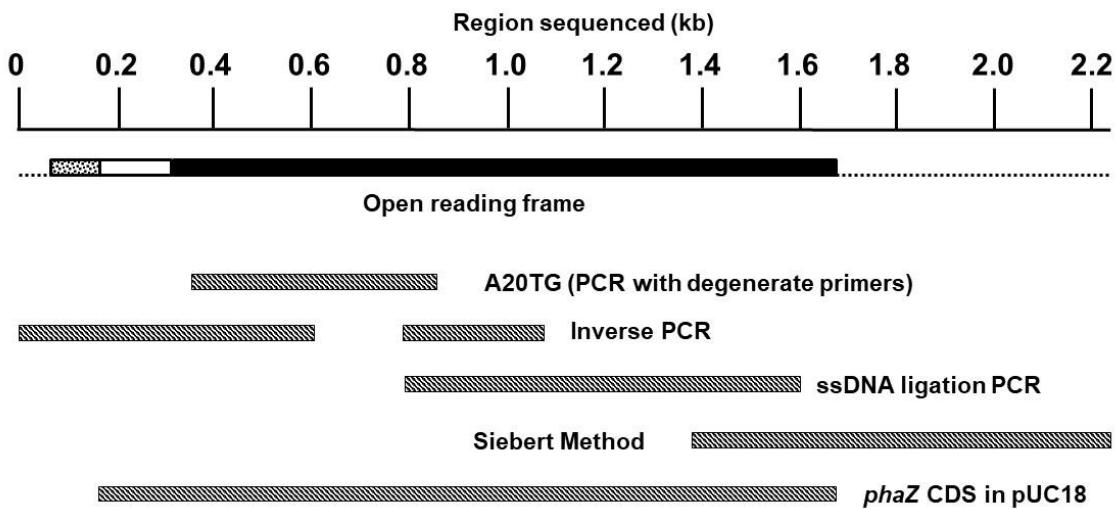


Figure S4: Cloning strategy for *phaZ*. The open reading frame consists of the secreted protein coding sequence (solid bar) and signal peptide coding sequence (open bar); the stippled box line indicates a putative promoter region. The cross hatched bars indicate sequences obtained from the cloning methods 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

PhaZSsp5A	1	M GQPYPPHP P-----F RPVR -----G RVFGGI R RWLTAAG -A ALATGGLVAVNP -----	44
PhaZSas	1	M OPPPFR -G ILTPLPLSS S PPVGSLSRPGRGV ---L TRVLAVVA -L VLG -A ALLGPAPTAHAA	59
PhaZSpr	1	M RVRTRV -R I -----R RA ---A GRILGAALA -V VAG -G LLISPAPV ---	34
PhaZTfu	1	M LHLTRR -I P -----A RVW ---V ALTAVLG -L GAALLGTTALAP ---	34
PhaZTsp.	1	-----	0
PhaZJsp.	1	M ARTRTV -L-----G WM -S ALAVAAAATLGIV -T AQ -----	28
PhaZSgr	1	M A -----A GAGQPGPAASAA -----	15
PhaZ3Ple	1	M NKYL -----K NLCF -A AAATVTLM -A S -----	20
PhaZ2Ple	1	M MSSQTT -Q S -----S KF ---S FLKRGLLL -A AAP -L LA -M S -----	31
cons	1	-----	66

CATALYTIC DOMAIN

		H _{ox}	
PhaZSsp5A	45	-----Q ATAAGLT Q VTGFGSNPGNLT M HTYVPDGLAAGAPLVVA H G C TQSASDYYAHSGWPKF A	104
PhaZSas	60	AGLAKP G LTKADL T EVADFGTNPGRLN MYV YVRPASLP A EPAVVFA H G C TQDAQGYADNSGLLSFA	125
PhaZSpr	35	-----A HAAAVVLEHVADFGADPGN LN MYVYRPASL A PDPA L VALV HAH G C TQSAQVYADNSGLTLA	94
PhaZTfu	35	-----R AEAATLTQVS A FGSNPGN LN MYVYRPATLP D NAPLVV LL HG C SQDAATYAHSGWAKYA	94
PhaZTsp.	1	-----T LTVQSAFGSNPGN LN MYVYRPATLP D NAPLVV LL HG C SQDAATYAHSGWAKYA	55
PhaZJsp.	29	-----G ASAATLQVTSFGSNPGN AL TMWSYRPDNAAAGAPLVIA H G C TQEASTYLNGSGWRDLA	88
PhaZSgr	16	-----P RAAASLERVTAFGANPGNLAMYVYRPAGL PAGAPV VVVA H G C TQSARVYSDNAGLDTFA	75
PhaZ3Ple	21	-----A PSAFALSEVTGFTNPGALKMFKHVTSMPTN APL IVAM H G C TQSASAYE -G GWSALA	79
PhaZ2Ple	32	-----A SSALAATQVTGFSNPGNLL MYK HVPSSMPANAPLVIA H G C TQSASAYE -A TGWTQLA	90
cons	67	-----	132

CATALYTIC DOMAIN

		LB	
PhaZSsp5A	105	DAYGFALVFPQTTSANNANS C FNWFDSGDSTR RG GEALSIRQMVDAA VARY GSDTRVYIT G LSAG	170
PhaZSas	126	DRYGFLLVFAETTSNNANR C FNWFQSSDNRR Q GEASIRQMAA HTVSAY GADPQRTYIT G LSAG	191
PhaZSpr	95	DRHGFLVVLAGTTTSANNANS C FNWFQTS DNRR QGEASVRQMVA HAESAY GADAGRTFVT G LSAG	160
PhaZTfu	95	DSLGFALVYAEQ KSANNSSS C FNWFQKS DTARG SGEAQSI RMSV DYAVRTYS LD EERVYIS G LSAG	160
PhaZTsp.	56	DSLGFALVYAEQ KSANNSSS C FNWFQKS DTARG SGEAQSI RMSV DYAVRTYS LD EERVYIS G LSAG	121
PhaZJsp.	89	DRNGITVVLPQQSTANNMN T C FNWFQAGDVTR R QGEVASI ASMRHAITTY SANPARVYVT G LSGG	154
PhaZSgr	76	DRHGFLVLYAETTAANNANT C FNWFQPGDTR R QGEAASIRQMVA HAASAY GAD-GRVH G LSAG	140
PhaZ3Ple	80	NNYKFYVVYPEQQSGNNNSNK C FNWFESGDI ARG QGEALSIKQMVDKMKADHSIDT NRVY VT G LSAG	145
PhaZ2Ple	91	NTYKFYVVYPEQQSSNNQN K FNWFEPGDI ARG QGEALSIKQMVDKMKADHSIDT NRVY VT G LSAG	156
cons	133	-----	198

CATALYTIC DOMAIN

PhaZSsp5A	171	AGMTANMLAAY P D V FAGGS I D SGLPAY C AT SV AA Y T C MYSP PN KTPAQW GD LVR AA PVG T SSWP	236
PhaZSas	192	GM ATSVMLAAY P D V FQAGAVVAGL PF G C AT D VSSAYL C MNP G TDLTAD Q WARRVR D GYPSWGPWP	257
PhaZSpr	161	GM ATSVMLAAY P D V FEAGAVIAG MP YD C TRD-T GPFV C MNP G TDRTPA V WQVR D DAYPSYTGPWP	225
PhaZTfu	161	GM ASEM LAAY P D V FAGGS I VAG IP TC CASS LL DATT C MFSGRN L TPKQW GD LVR AK NP GW QGPWP	226
PhaZTsp.	122	GM ASEM LAAY P D V FAGGS I VAG IP TC CASS LL DATT C MFSGRN L TPKQW GD LVR AK NP GW QGPWP	187
PhaZJsp.	155	GM ATSM LAAY P D L FAGGS I NAG I A HG C ATT V QA F C MNP G VDKTPKAW GD LAR GY AA WG PRP	220
PhaZSgr	141	GM ATSVMLAAY P D V FAAGAVVAGI P QG C GV D VVTA F GC MS P GDRT P AA QA VR D AY P G H TGPWP	206
PhaZ3Ple	146	AFMTAVMAAT Y P D V FAGA API AGGPYK C AT SM IDAF S C MS P GTDKTPA AW GL D LARG GY SG Y NGRKP	211
PhaZ2Ple	157	GYMVNVMLAAY P D V FAGGAP FSGGPY NC CAT SM TNAFT C MS P GDKTPA AW GL D LARG GY SG Y GTGRKP	222
cons	199	-----	264

CATALYTIC DOMAIN

PhaZSsp5A	237	RVAIWQGTSDDTVPANATELRDQWTVNWGIGQTPSRTESLSGDT-TLSQYDDASGRPAVSYSIS	301
PhaZSas	258	RVAIWHGDKDTTVPANADELRDQWTAHVGSQTPDRTSIVGPNSTRHEEYLAADGSVAVEVNRPV	323
PhaZSpr	226	RVAVWHGDNDSTVAPMNAELRDQWTAMHGISDQTPDRTSTIGANGTRREQYLDGSGKVAVEVDVRP	291
PhaZTfu	227	RVAIWHTGTDAAVTPPKNAQSSRDQWTVNWGIGQTPARTANLIGANT-TVEYYQDSSGRDVVARLYLV	291
PhaZTsp.	188	RVAIWHTGTDAAVTPPKNAQSSRDQWTVNWGIGQTPARTANLIGANT-TVEYYQDSSGRDVVARLYLV	252
PhaZJsp.	221	KVAIWHGQSDDTVPAMNGRELRDQWTDAGVGSQSPATGTLASGV-TWTEY---GGGAUVRLEIA	281
PhaZSgr	207	RVSIWHGDRDATTVPVRNADELRDQWTAHVGLQSQSPDRTSTIGPNGTRRSYMLSAAGGAVVEVNRPV	272
PhaZ3Ple	212	KISVWQGSSDTVVKPMNMDELMOQWNTNYHGIDQTADEVKG-F-PHKVYKDASGNALVETWSIT	275
PhaZ2Ple	223	IISIWHGDADETVKQSNQVEEVEQWNTNYHGIDQTADEVSDTVAG-F-PHKVYKDASGNALVETYTIT	286

CATALYTIC DOMAIN

LINKER DOMAIN

cons 265 : * ; * . * . * . ; *** * ; * . ; . ; . ; . ; . ; . ; . ; . ; . 330

PhaZssp5A	302	GMA H GLAVAPGSGPDQC T GTG-YYLD-TIC CSSYHTARFWGLDGGDGSGQNPCTLPA-PAGLTVG	364
PhaZsas	324	GIG H GTPVDPGTGAQQ C GSTGAAYFLD-SIC SSYWITQFFGLSGSAS -----DPGSLPA-PSGLAAAT	383
PhaZspr	292	SIG H GTPVDPGSGPE Q CGRTGTAHFID-SIC SSHWVAGFFGLAGGD P-----DPGGLPA-PAGLTVT	351
PhaZtfu	292	GM G HGTPVDPGSGTDQC T GTAGA-YFLD-TIC STYHTIQFWELDGPQ -----PSPSPS-PSPS---	346
PhaZtsp.	253	GM G HGTPADPGSGTDQC T GTAGA-YFLD-TIC STYHTIQFWELDGPQ -----PSPSPS-PSPS---	307

LINKER DOMAIN

PhaZJsp.	282	GMT H GTPVDPGSLT QCGTAGA-YFID-TV C GAYYDAQFFGLIGSSP---SPTATAT- PTG ---	336
PhaZSgr	273	GIA H GTPVDPGGGE QC CGATGTQHFID-S I CSSHWI T RFFGLDG G TT-TPEPP-----	322
PhaZ3Ple	276	GMA H GTPVDPGTGA E Q C GTSGS-Y I LDVNI C SSYHIAQFFGLTGAG T TT-TTTVGSTS-----	331
PhaZ2Ple	287	GM G HGTPVDPGTGSL QCGTAGA-Y ILDVNI C SSYYVAKF W GLIGGSS-TTTTTSAGTT T -----	343
cons	331	... : ** .. * ** * *** : * : * : * : : * : * *	396

PhaZSsp5A	365	GTTDSTVSLAWNPDGASSYTIVRGGTKVGGTTSTAYTDGLATGTAYAYTVAAVDAAGAVGVSS	430
PhaZSas	384	GATDTTISLTWKPDGATDYAVHRDGAQITTSATTSYTDGLRAGTSHTYAVAARDADGKAGPLSG	449
PhaZSpr	352	GSDDTSIALAWQAVDGASDYAVYRDGTRVATPLGPSFTDTPLAAGSTHAYRVAARDAAGTEGARSA	417
PhaZTfu	347	-----	346

SUBSTRATE BINDING DOMAIN

PhaZSsp5A	431	SV-TATTTGTYPTC CYTANNYQHTTAGRAYQSGGYTYATGSGQAMGLYNTFTTHTLQ TAPGGYVLA	495
PhaZSas	450	AV- TAQTTGATAVCWTAGNYAHVQAGRATTSAGTYAKGSGQNMGLYNTFVTTLKESPTGYFTVA	514
PhaZSpr	418	QI-TASTTGAAAV CWTGSNSYAHVRAAGRATTGGTYAKGSGQNMGLYNTFVTTLKESPAGHYVIA	482
PhaZTfu	347	PS- PSPTTPAGECVTANNYQHVAEGRAYVSYGYAYSVGGNDLLGLWLNIYVITSVQETSPGYWERV	411
PhaZTsp.	308	PS- PSPTTPPAGECVTANNYQHVAEGRAYVSYGYAYSVGGNDLLGLWLNIYVITSVQETSPGYWERV	372
PhaZJsp.	344	P-TPTPTATSTPT CVTATNYAHVQAGRAHNVGGYALANGSDNDLGLYNTFTYSAVRETSPGYWK	408
PhaZSgr	325	---- PPT-EPAACWTASNYEHVRAAGRATTGGQVYAKGSAQHGLYNTFVTTLKESPAGHYVLA	384
PhaZ3Ple	351	A-STTTTTVAAGA CYNA SNYAHVTAGRAVNSMGYAKAGSNQNMGLYNTFTTSKLREAPAGYFT D	415

PhaZ2Ple 364 TTTASTTTTAGACYNSSNYAHVTAGRAHDTGGYAYTNGSNQKMGLNNTFYTSKLRKTGTNYYVID 429
cons 463 .: .. * .. ** *. *** * . : *. : ;** * : :::: .::: 528

PhaZSsp5A	496	DSG C SA	501
PhaZSas	515	NDT CP -	519
PhaZSpr	483	DGN CP -	487
PhaZTfu	412	-SGC--	414
PhaZTsp.	373	-SGC--	375
PhaZJsp.	409	-----	408
PhaZSgr	385	DST CP -	389
PhaZ3Ple	416	-ST CP -	419
PhaZ2Ple	430	-TT CP -	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