

Supplemental materials for
Hydrolase CehA and monooxygenase CfdC are responsible for the
degradation of carbofuran in *Sphingomonas* sp. strain CDS-1

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The supplemental materials include one table and two figures.

TABLE S1 Highly conserved ORFs ($\geq 95\%$ nucleotides similarity) between carbofuran-degrading strains *Sphingomonas* sp. CDS-1 and *Novosphingobium* sp. KN65.2

Gene locus tags ¹	Scaffold No.	Product size ²	Database used in BLASTP search	Homologous protein ³	GenBank accession no.	Amino acid Identity (%)
CBW6_4_RS00060	1	702	NR	<i>Novosphingobium</i> sp. KN65.2 hypothetical protein	WP_054945771.1	97%
			SwissProt	Quinohemoprotein alcohol dehydrogenase ADH IIB	Q8GR64.1	32%
CBW6_4_RS00065	1	174	NR	<i>Novosphingobium</i> sp. KN65.2 hypothetical protein	WP_054945770.1	98%
			SwissProt	Protein ROS1	Q9SJK6.2	43%
CBW6_4_RS00070	1	272	NR	<i>Novosphingobium</i> sp. KN65.2 hypothetical protein	WP_054945769.1	96%
			SwissProt	Probable phytanoyl-CoA dioxygenase	O62515.2	25%
CBW6_4_RS00075	1	449	NR	<i>Novosphingobium</i> sp. KN65.2 hypothetical protein	WP_054945768.1	95%
			SwissProt	Probable feruloyl esterase ARB_07085	D4AS70.1	33%
CBW6_4_RS19125	122	63	NR	<i>Sphingomonadaceae</i> MULTISPECIES: hypothetical protein	WP_048575073.1	100%
			SwissProt	NA		
CBW6_4_RS19130	122	275	NR	<i>Novosphingobium</i> sp. KN65.2 hypothetical protein	CDO34422.1	100%
			SwissProt	Mitochondrial distribution and morphology protein 10	Q0TWV0.1	33%
CBW6_4_RS19135	122	92	NR	<i>Sphingomonadaceae</i> MULTISPECIES: hypothetical protein	WP_048575071.1	100%
			SwissProt	NA		
CBW6_4_RS19140	122	80	NR	<i>Sphingomonadaceae</i> MULTISPECIES: hypothetical protein	WP_053085602.1	100%
			SwissProt	NA		
CBW6_4_RS19145	122	108	NR	<i>Novosphingobium</i> sp. KN65.2 hypothetical protein	CDO34419.1	100%
			SwissProt	NA		
CBW6_4_RS19150	122	412	NR	<i>Sphingomonadaceae</i> MULTISPECIES: hypothetical protein	WP_053085601.1	100%
			SwissProt			

			SwissProt	Probable conjugal transfer protein TrbI	P55406.1	45%
CBW6 4_RS1 9155	122	340	NR SwissProt	<i>Sphingomonas</i> sp.MEA3-1 hypothetical protein Probable conjugal transfer protein TrbG	WP_053085600.1 P55404.1	100% 34%
CBW6 4_RS1 9160	122	228	NR SwissProt	<i>Sphingomonadaceae</i> MULTISPECIES: conjugal transfer protein TrbF Conjugal transfer protein TrbF	WP_030091789.1 P54914.1	100% 29%
CBW6 4_RS1 9165	122	570	NR SwissProt	<i>Sphingomonadaceae</i> MULTISPECIES: hypothetical protein NA	WP_048575069.1	100%
CBW6 4_RS1 9175	122	331	NR SwissProt	<i>Sphingomonadaceae</i> MULTISPECIES: hypothetical protein Mediator of RNA polymerase II transcription subunit 20a	WP_082165950.1 Q6NPF4.1	100% 23%
CBW6 4_RS1 9180	122	827	NR SwissProt	<i>Sphingomonadaceae</i> MULTISPECIES: conjugal transfer protein TrbE Conjugal transfer protein TrbE	WP_030091793.1 P54910.1	100% 39%
CBW6 4_RS1 9185	122	89	NR SwissProt	<i>Sphingomonadaceae</i> MULTISPECIES: hypothetical protein EVI5-like protein	WP_030091794.1 Q96CN4.1	100% 52%
CBW6 4_RS1 9190	122	107	NR SwissProt	<i>Sphingomonadaceae</i> MULTISPECIES: conjugal transfer protein TrbC Probable conjugal transfer protein TrbC	WP_030091795.1 P55396.1	100% 39%
CBW6 4_RS1 9195	122	318	NR SwissProt	<i>Sphingomonadaceae</i> MULTISPECIES: conjugal transfer protein TrbB Probable conjugal transfer protein TrbB	WP_030091796.1 P55395.1	100% 45%
CBW6 4_RS1 9200	122	653	NR SwissProt	<i>Sphingomonas</i> sp. MEA3-1 hypothetical protein DNA topoisomerase 3	WP_048575096.1 P96583.1	100% 30%
CBW6 4_RS1 9205	122	198	NR SwissProt	<i>Sphingomonas</i> sp. MEA3-1 conjugal transfer protein TraG Transcription initiation factor	WP_053085605.1 Q91857.3	100% 34%

				TFIID subunit 6		
CBW6 4_RS2 0215	139	524	NR SwissProt	<i>Novosphingobium</i> sp. KN65.2 Conjugal transfer protein TraG, partial Conjugal transfer protein TraG	WP_054944397.1 Q00185.1	94% 33%
CBW6 4_RS2 0220	139	509	NR SwissProt	<i>Sphingomonadaceae</i> MULTISPECIES: hypothetical protein Conjugal transfer protein TraA	WP_048575095.1 Q44349.2	100% 36%
CBW6 4_RS2 0225	139	142	NR SwissProt	<i>Sphingomonadaceae</i> MULTISPECIES: hypothetical protein Homeobox protein MOX-1	WP_048575094.1 P32442.1	100% 35%
CBW6 4_RS2 0230	139	299	NR SwissProt	<i>Sphingomonas</i> sp. MEA3-1 hypothetical protein L-lactate dehydrogenase B chain	WP_053085604.1 Q9XT86.3	100% 48%
CBW6 4_RS2 0235	139	176	NR SwissProt	<i>Sphingomonadaceae</i> MULTISPECIES: hypothetical protein Plasmid transfer protein TraF	WP_048575093.1 Q03450.1	100% 32%
CBW6 4_RS2 0240	139	109	NR SwissProt	<i>Sphingomonadaceae</i> MULTISPECIES: hypothetical protein NA	WP_048575092.1	100%
CBW6 4_RS2 0245	139	226	NR SwissProt	<i>Sphingomonas</i> sp. MEA3-1 Chromosome partitioning protein ParA Protein ParA	WP_048575091.1 P07175.1	100% 38%
CBW6 4_RS2 0250	139	85	NR SwissProt	<i>Sphingomonadaceae</i> MULTISPECIES: Antitoxin Antitoxin PemI	WP_048575090.1 P13975.1	100% 53%
CBW6 4_RS2 0255	139	108	NR SwissProt	<i>Sphingomonadaceae</i> MULTISPECIES: pemk protein Endoribonuclease PemK	WP_048575089.1 P13976.1	100% 58%
CBW6 4_RS2 0260	139	101	NR SwissProt	<i>Sphingomonadaceae</i> MULTISPECIES: transcriptional regulator Uncharacterized protein HI_1420	WP_030091807.1 P44191.1	100% 49%
CBW6 4_RS2	139	102	NR	<i>Sphingomonadaceae</i> MULTISPECIES:	WP_030091808.1	100%

0265			SwissProt	addiction module antitoxin RelB Uncharacterized protein HI_1419	P44190.1	58%
CBW6 4_RS2 0270	139	217	NR SwissProt	<i>Sphingomonas</i> sp. MEA3-1 hypothetical protein Imidazole glycerol phosphate synthase subunit HisF	WP_048575088.1 Q7SIB9.1	100% 48%
CBW6 4_RS2 0275	139	299	NR SwissProt	<i>Sphingomonadaceae</i> MULTISPECIES: hypothetical protein Antitoxin HicB 2	WP_048575087.1 Q7MZD8.1	99% 28%
CBW6 4_RS2 0280	139	96	NR SwissProt	<i>Novosphingobium</i> sp. KN65.2 hypothetical protein Enolase	WP_054944302.1 Q5GTG4.1	100% 36%
CBW6 4_RS2 0285	139	108	NR SwissProt	<i>Sphingomonadaceae</i> MULTISPECIES: single-stranded DNA-binding protein NA	WP_048575085.1	100%
CBW6 4_RS2 0290	139	150	NR SwissProt	<i>Novosphingobium</i> sp. KN65.2 hypothetical protein, partial Serine recombinase gin	WP_054947562.1 P03015.2	100% 43%
CBW6 4_RS2 2300	177	313	NR SwissProt	<i>Sphingomonas</i> -like bacterium B12 RNA polymerase subunit sigma ECF RNA polymerase sigma factor SigJ	WP_051035096.1 L0TCG5.1	98% 37%
CBW6 4_RS2 2305	177	146	NR SwissProt	<i>Novosphingobium</i> sp. KN65.2 hypothetical protein Undecaprenyl phosphate-alpha-4-amino-4-de oxy-L-arabinose arabinosyl transferase	WP_054948431.1 Q48HY9.1	97% 37%
CBW6 4_RS2 2310	177	253	NR SwissProt	<i>Novosphingobium</i> sp. KN65.2 NAD-dependent epimerase/dehydratase Uncharacterized protein YbjT	CDO38883.1 P75822.2	100% 24%
CBW6 4_RS2 2315	177	148	NR SwissProt	<i>Sphingomonas</i> -like bacterium B12 Alkylhydroperoxidase Uncharacterized protein YdfG	WP_026095316.1 P96684.1	97% 34%
CBW6	180	109	NR	<i>Novosphingobium</i> sp. KN65.2	CDO34166.1	100%

4_RS2 2410			SwissProt	hypothetical protein NA		
CBW 64_RS 22415 (<i>cehA</i>)	180	794	NR SwissProt	<i>Rhizobium</i> sp. AC100 carbaryl hydrolase NA	WP_032490241.1	99%
CBW6 4_RS2 2420	180	798	NR SwissProt	<i>Novosphingobium</i> sp. KN65.2 hypothetical protein Pesticin receptor	WP_054944686.1 P0C2M9.1	99% 23%
CBW6 4_RS2 2430	180	226	NR SwissProt	<i>Streptomyces</i> pluripotens TetR family transcriptional regulator, partial Transposon Tn10 TetC protein	WP_043419395.1 P28815.1	41% 42%
CBW6 4_RS2 2435	180	94	NR SwissProt	<i>Novosphingobium</i> sp. KN65.2 hypothetical protein Anthranilate 3-monoxygenase oxygenase component	WP_054944687.1 A4IT51.1	100% 36%
CBW6 4_RS2 4070	228	86	NR SwissProt	<i>Brevundimonas</i> diminuta aromatic amino acid transferase Probable 5-dehydro-4-deoxyglucarate dehydratase	WP_032495125.1 Q3KI36.1	100% 45%
CBW6 4_RS2 4075	228	478	NR SwissProt	<i>Novosphingobium</i> sp. KN65.2 putative N6 adenine-specific DNA methyltransferase Ribosomal RNA small subunit methyltransferase A	CDO34218.1 A1WVT7.1	100% 34%
CBW6 4_RS2 4085	228	82	NR SwissProt	<i>Sphingomonadaceae</i> MULTISPECIES: hypothetical protein NA	WP_030541643.1	100%
CBW6 4_RS2 4090	228	269	NR SwissProt	<i>Sphingomonadaceae</i> MULTISPECIES: hypothetical protein Holliday junction ATP-dependent DNA helicase RuvA	WP_008831890.1 Q5PBM0.1	100% 24%
CBW6 4_RS2 4095	228	160	NR SwissProt	<i>Sphingomonadaceae</i> MULTISPECIES: hypothetical protein Uncharacterized protein YfjM	WP_008831891.1 O31547.1	100% 32%

CBW6 4_RS2 4100	228	85	NR SwissProt	<i>Sphingomonadaceae</i> MULTISPECIES: hypothetical protein NA	WP_008831892.1	99%
CBW6 4_RS2 4330	238	95	NR SwissProt	<i>Sphingomonadaceae</i> MULTISPECIES: hypothetical protein Leucine--tRNA ligase	WP_030541545.1 Q3J7S8.1	100% 33%
CBW6 4_RS2 4335	238	88	NR SwissProt	<i>Sphingomonadaceae</i> MULTISPECIES: hypothetical protein Uroporphyrinogen-III synthase	WP_008831641.1 Q59335.1	100% 57%
CBW6 4_RS2 4340	238	69	NR SwissProt	<i>Sphingomonadaceae</i> MULTISPECIES: hypothetical protein NA	WP_004213310.1	100%
CBW6 4_RS2 4345	238	177	NR SwissProt	<i>Sphingomonas</i> sp. LH128 hypothetical protein NA	WP_037520606.1	100%
CBW6 4_RS2 4350	238	96	NR SwissProt	<i>Sphingomonadaceae</i> MULTISPECIES: hypothetical protein Peroxisomal acyl-coenzyme A oxidase 3	WP_008831821.1 Q9EPL9.2	100% 53%
CBW6 4_RS2 4355	238	78	NR SwissProt	<i>Sphingomonadaceae</i> MULTISPECIES: hypothetical protein Oxidized low-density lipoprotein receptor 1	WP_008831822.1 Q9EQ09.2	100% 48%
CBW6 4_RS2 4690	254	477	NR SwissProt	<i>Novosphingobium</i> sp. KN65.2 putative conjugal transfer protein; traA Probable conjugal transfer protein TraA	CDO37889.1 P55418.1	95% 39%
CBW6 4_RS2 4695	254	245	NR SwissProt	<i>Sphingomonadaceae</i> MULTISPECIES: hypothetical protein NA	WP_017501675.1	100%
CBW6 4_RS2 4700	254	89	NR SwissProt	<i>Novosphingobium</i> sp. KN65.2 Conserved hypothetical protein Probable S-adenosyl-L-methionine-dep	CDO37891.1 P45134.1	100% 32%

				endent methyltransferase TehB		
CBW6 4_RS2 5130	277	211	NR SwissProt	<i>Sphingomonadaceae</i> MULTISPECIES: phosphopantetheine--protein transferase Protein ParA	WP_017501684.1 P07175.1	100% 27%
CBW6 4_RS2 5135	277	94	NR SwissProt	<i>Sphingomonadaceae</i> MULTISPECIES: hypothetical protein Telomerase reverse transcriptase	WP_017501683.1 Q1PS67.2	100% 35%
CBW6 4_RS2 5140	277	367	NR SwissProt	<i>Sphingomonadaceae</i> MULTISPECIES: hypothetical protein Putative replication protein	WP_004213366.1 P60119.1	100% 38%
CBW6 4_RS2 5205	283	51	NR SwissProt	<i>Sphingobium</i> MULTISPECIES: transcription elongation protein SprT NA	WP_020817759.1	100%
CBW6 4_RS2 5290	288	459	NR SwissProt	<i>Sphingomonas</i> sp. MEA3-1 transposase, partial Transposase for transposon Tn4430	WP_048578782.1 P10021.1	100% 27%
CBW6 4_RS2 5295	288	288	NR SwissProt	<i>Sphingomonas wittichii</i> RW1 Resolvase, N-terminal domain (plasmid) Integrase-like protein y4IS	ABQ71233.1 P55559.1	100% 48%
CBW 64_RS 25315 (<i>cf</i>dC)	290	395	NR SwissProt	<i>Novosphingobium</i> sp. KN65.2 hypothetical protein Pigment production hydroxylase	WP_054944612.1 P26698.1	99% 37%
CBW6 4_RS2 5320	290	220	NR SwissProt	<i>Novosphingobium</i> sp. KN65.2 hypothetical protein Fatty acid metabolism regulator protein	WP_054944613.1 P94548.1	99% 24%
CBW6 4_RS2 5460	301	209	NR SwissProt	<i>Sphingomonas</i> sp. DC-6 conjugal transfer protein TraG, partial Protein virD4	WP_030092925.1 P13464.1	100% 35%
CBW6 4_RS2 5465	301	143	NR	<i>Sphingomonadaceae</i> MULTISPECIES: hypothetical protein	WP_017501681.1	100%

			SwissProt	Ragulator complex protein LAMTOR3	Q4SSF5.1	37%
CBW6 4_RS2 5505	305	288	NR	<i>Sphingomonas wittichii</i> RW1 Resolvase,N-terminal domain (plasmid)	ABQ71679.1	100%
			SwissProt	Integrase-like protein y4IS	P55559.1	45%
CBW6 4_RS2 5510	305	249	NR	<i>Sphingomonas</i> sp. SRS2 Transposase	KKC25847.1	100%
			SwissProt	Transposase for transposon Tn1721	P51565.1	30%
CBW6 4_RS2 5555	309	195	NR	<i>Mastigocladus laminosus</i> hypothetical protein	WP_044449036.1	30%
			SwissProt	Argininosuccinate synthase	A9IQ90.1	48%
CBW6 4_RS2 5560	309	377	NR	<i>Exiguobacterium</i> sp. S17 hypothetical protein	EPE61704.1	37%
			SwissProt	Glutamyl-tRNA(Gln) amidotransferase subunit B, mitochondrial	C5JUE6.1	51%
CBW6 4_RS2 5595	312	69	NR	<i>Sphingobium</i> MULTISPECIES: conjugal transfer protein TraG	WP_020817742.1	100%
			SwissProt	Protein virD4	P13464.1	41%
CBW6 4_RS2 5615	314	456	NR	<i>Sphingomonadaceae</i> MULTISPECIES: transposase	WP_013039223.1	100%
			SwissProt	NA		
CBW6 4_RS2 5665	321	179	NR	<i>Novosphingobium</i> sp. KN65.2 Putative outer membrane phospholipase A (fragment)	CDO38161.1	100%
			SwissProt	Phospholipase A1	P0A922.1	34%
CBW6 4_RS2 5745	331	93	NR	<i>Sphingomonadaceae</i> MULTISPECIES: DNA damage-inducible protein	WP_017501687.1	100%
			SwissProt	Antitoxin DinJ	Q47150.1	56%
CBW6 4_RS2 5750	331	106	NR	<i>Sphingomonadaceae</i> MULTISPECIES: translation repressor RelE	WP_026109291.1	100%
			SwissProt	mRNA interferase YafQ	Q47149.1	39%
CBW6 4_RS2 5865	346	254	NR	<i>Gammaproteobacteria</i> MULTISPECIES: Transposase	WP_001375121.1	100%
			SwissProt	Transposase for insertion sequence-like element IS431mec	P0A043.1	42%

CBW6 4_RS2 5900	352	138	NR SwissProt	<i>Novosphingobium</i> sp. KN65.2 DNA-invertase from lambdoid prophage e14 Transposon Tn552 resolvase	CDO38162.1 P18358.1	100% 32%
CBW6 4_RS2 5905	352	69	NR SwissProt	<i>Sphingomonadaceae</i> MULTISPECIES: hypothetical protein Pseudouridine kinase	WP_017501689.1 P30235.2	100% 48%
CBW6 4_RS2 6050	376	68	NR SwissProt	<i>Sphingomonadaceae</i> MULTISPECIES: conjugal transfer protein TraD NA	WP_017501678.1	100%
CBW6 4_RS2 6055	376	88	NR SwissProt	<i>Sphingomonadaceae</i> MULTISPECIES: hypothetical protein Arylsulfatase A	WP_026109288.1 P50428.2	100% 52%

¹ Gene locus tags in GenBank database.

² Number of amino acids.

³ The top BLASTP hit was selected.

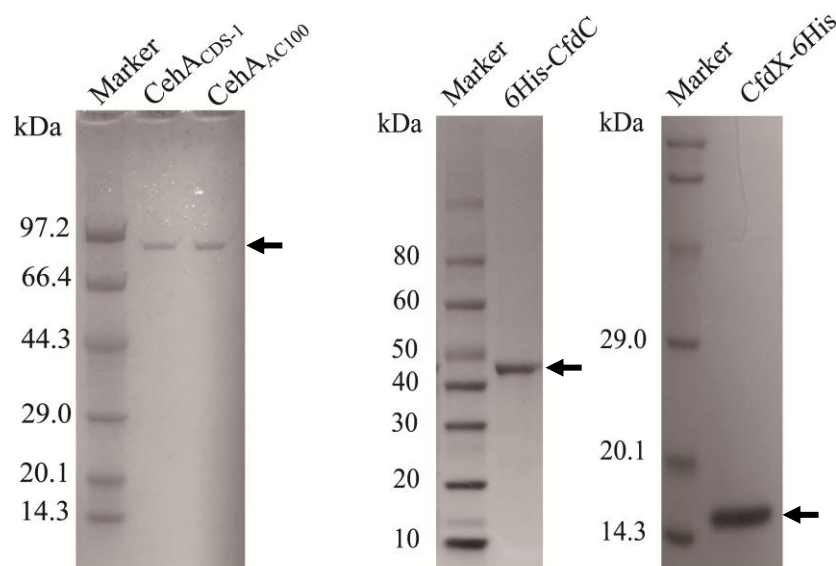


FIG S1 SDS-PAGE results for purified 6His-CehA_{CDS-1}, 6His-CehA_{AC100}, 6His-CfdC and CfdX-6His. 6His-CehA_{CDS-1}, 6His-CehA_{AC100}, 6His-CfdC and CfdX-6His were synthesized in *E. coli* and purified using Ni-NTA gravity column. The purified proteins were detected by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

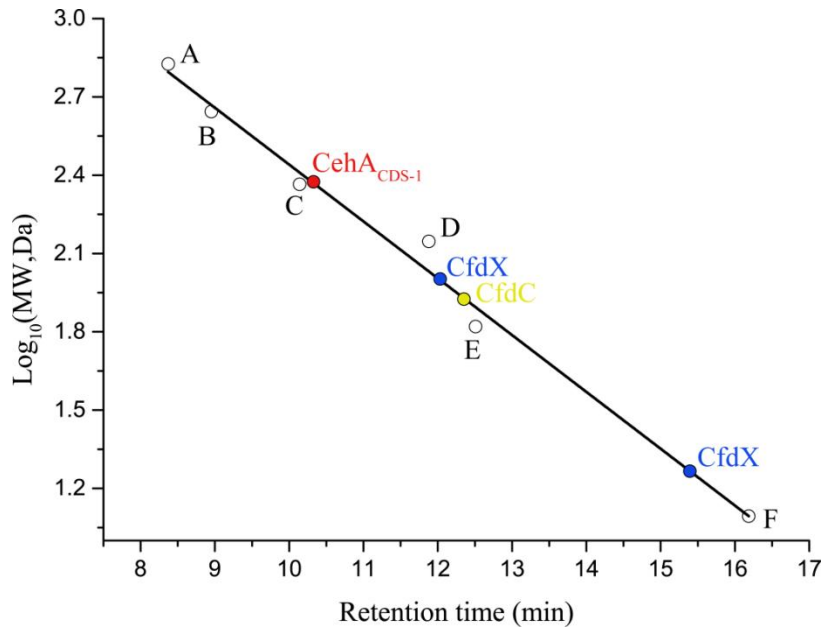


FIG S2 Determination of native molecular mass of 6His-CehA_{CDS-1}, 6His-CfdC and CfdX-6His based on a standard curve using gel filtration calibration kit. The standard proteins include: thyroglobulin from porcine thyroid (A, 669 kDa), ferritin from equine spleen (B, 440 kDa), catalase from bovine liver (C, 232 kDa), lactate dehydrogenase from bovine liver (D, 140 kDa), bovine serum albumin (E, 66 kDa), Cytochrome C (F, 12.4 kDa). According to the standard curve, native 6His-CehA_{CDS-1} is 221 kDa, native 6His-CfdC is 88 kDa, and native CfdX-6His has two forms, 106 and 18 kDa.