Supplementary Materials

Diversity of Organohalide Respiring Bacteria and Reductive Dehalogenases that Detoxify Polybrominated Diphenyl Ethers in E-waste Recycling Sites

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Key words:

Polybrominated diphenyl ethers (PBDEs), reductive debromination, PBDE RDases, debromination pathways, *Dehalococcoides*

Supplementary Figures

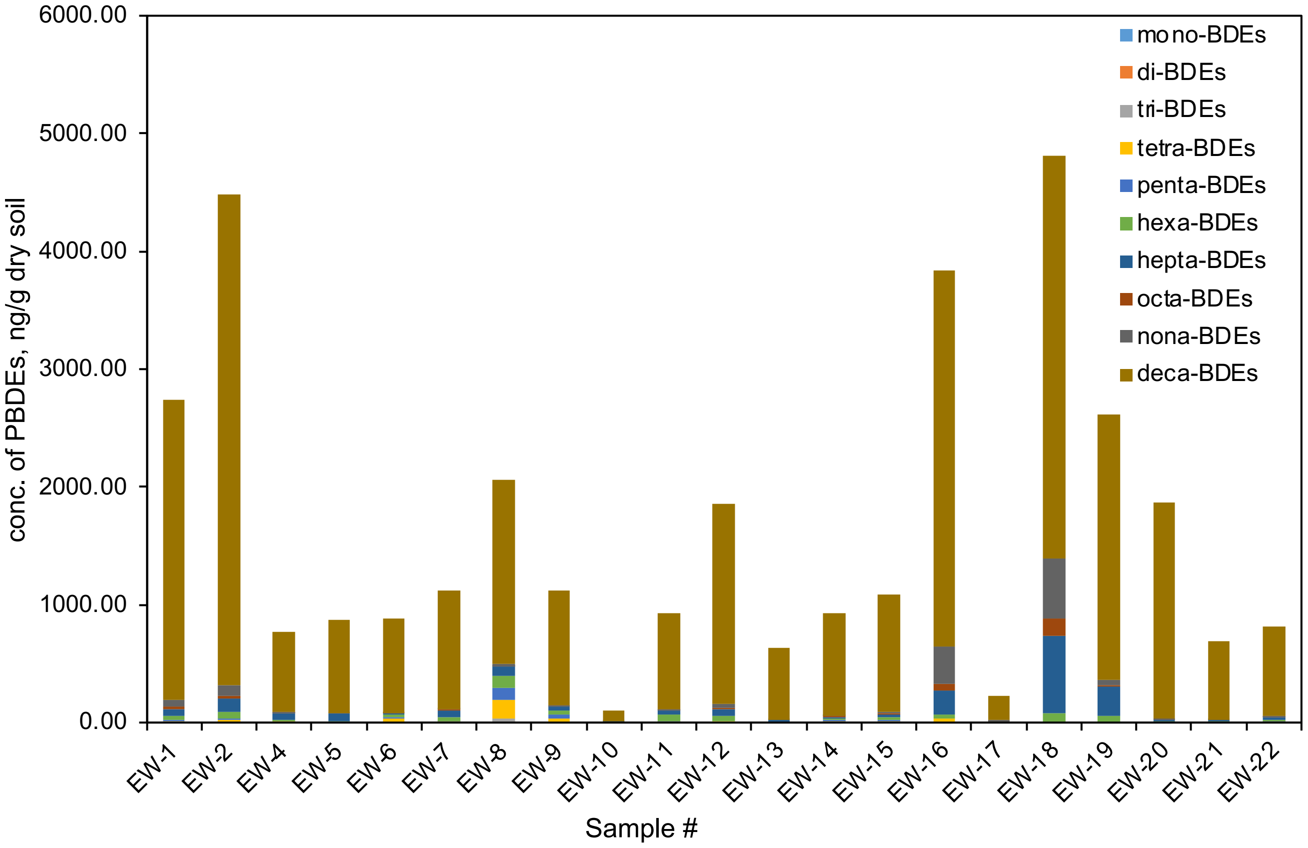

Figure S1. Concentration of PBDE homolog groups in e-waste soils collected in this study. Concentrations of individual PBDE congeners are available in Supplementary Dataset Table S10.

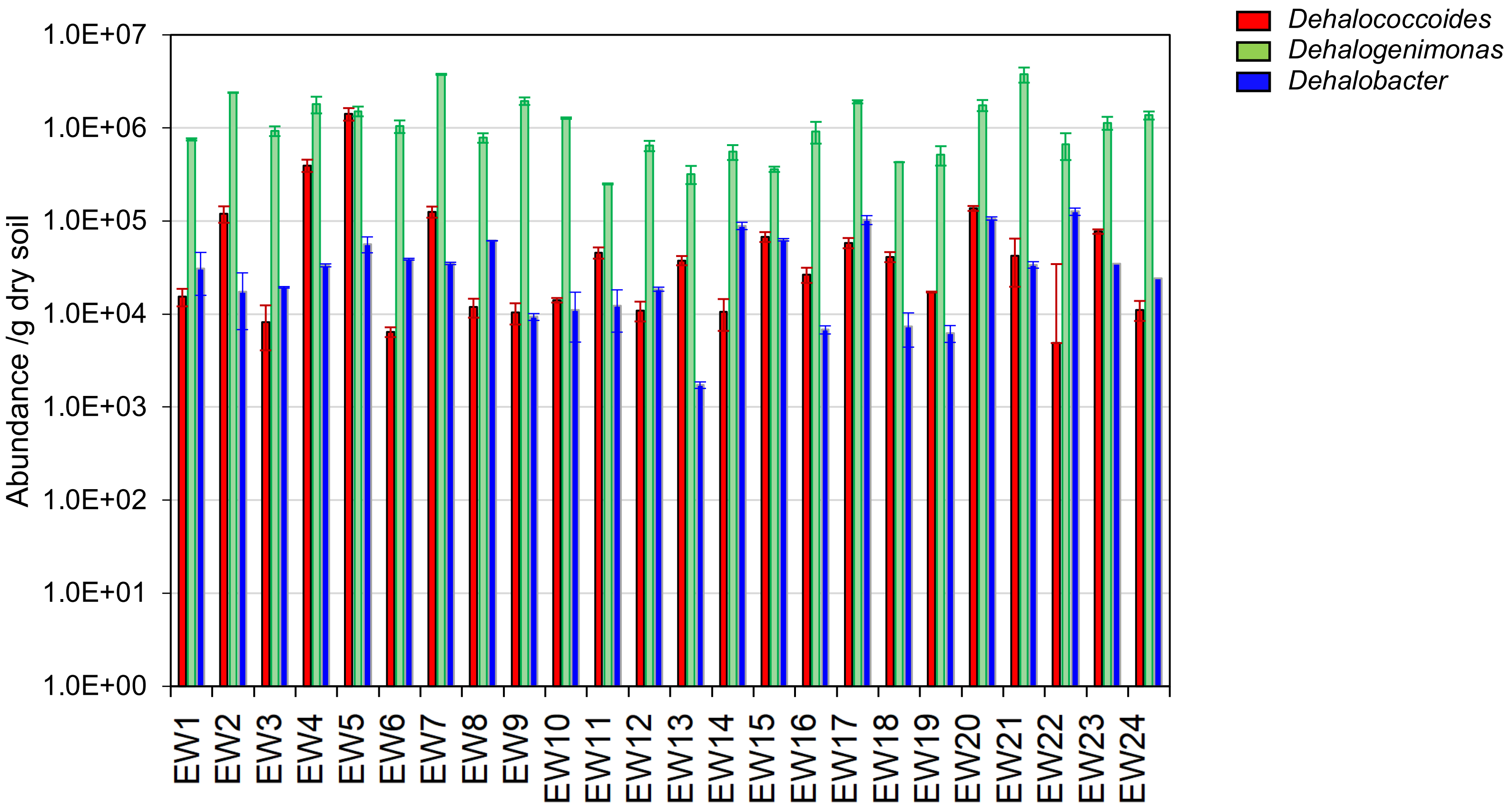

Figure S2. Abundance of obligate OHRB (*Dehalococcoides*, *Dehalogenimonas* and *Dehalobacter*) in soils and sediments collected from 24 e-waste contaminated sites.

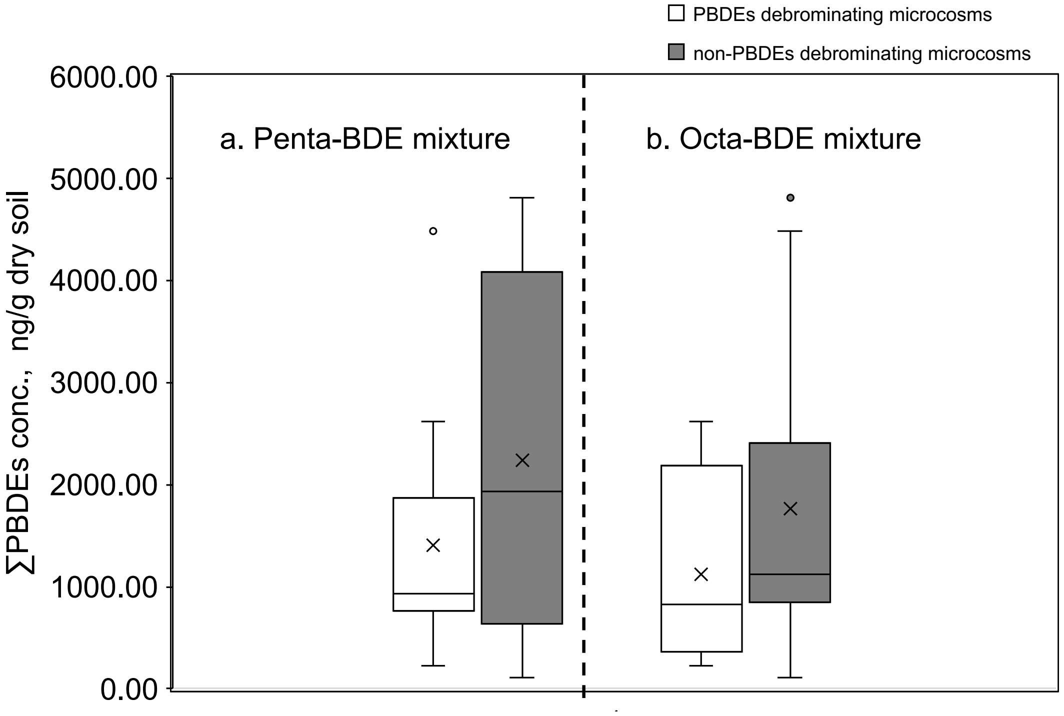

Figure S3. Boxplots of *in situ* ∑ PBDEs in microcosms. There was no significant difference in the mean ∑ PBDEs in soil samples which showed debromination of either the penta- or octa-BDE mixture (t-test, $p<0.05$). The penta-BDE mixture debrominating microcosms comprised EW-2, -3, -4, -5, -6, -7, -8, -11, -12, -13, -14, -15, -17, -19, -20, -21, and the octa-BDE mixture debrominating group comprised EW-4, -6, -17, -19. The concentrations of PBDEs in original soils, water content, and organic content of the original soils are available in Supplementary Dataset Table S10.

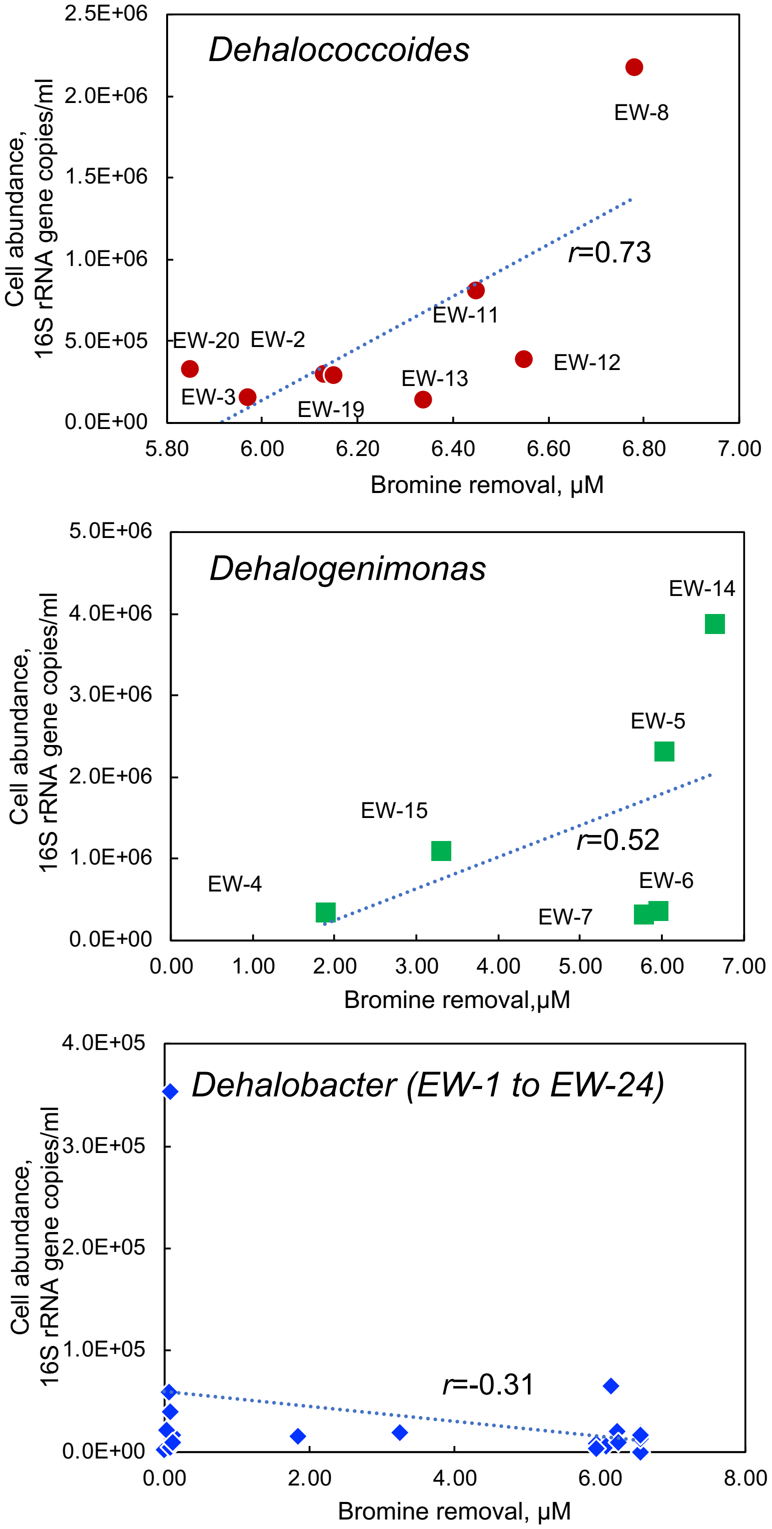

Figure S4. Correlations between the abundance of obligate OHRB and debromination of a penta-BDE mixture in microcosms.

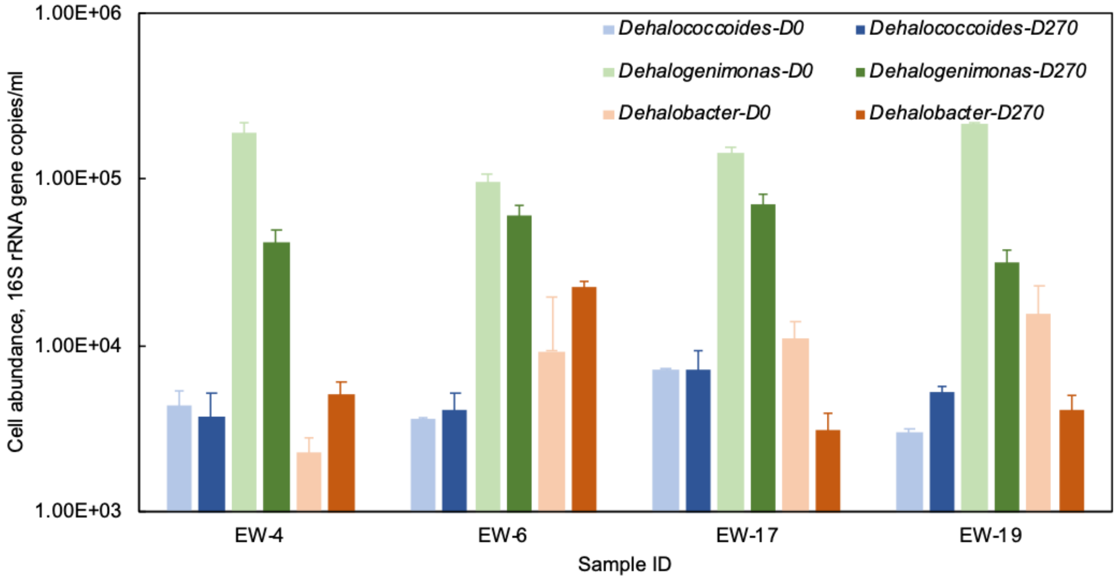

Figure S5. Change in abundance of obligate organohalide respiring bacteria genera (i.e., *Dehalococcoides*, *Dehalogenimonas*, and *Dehalobacter*) in four microcosms that showed debrominating activiity of an octa-BDE mixture after 270 days' incubation. The microcosms were established from soil and sediments collected from geographically distinct e-waste contaminated sites. The cell abundance was measured on day0 (D0) and day270 (D270).

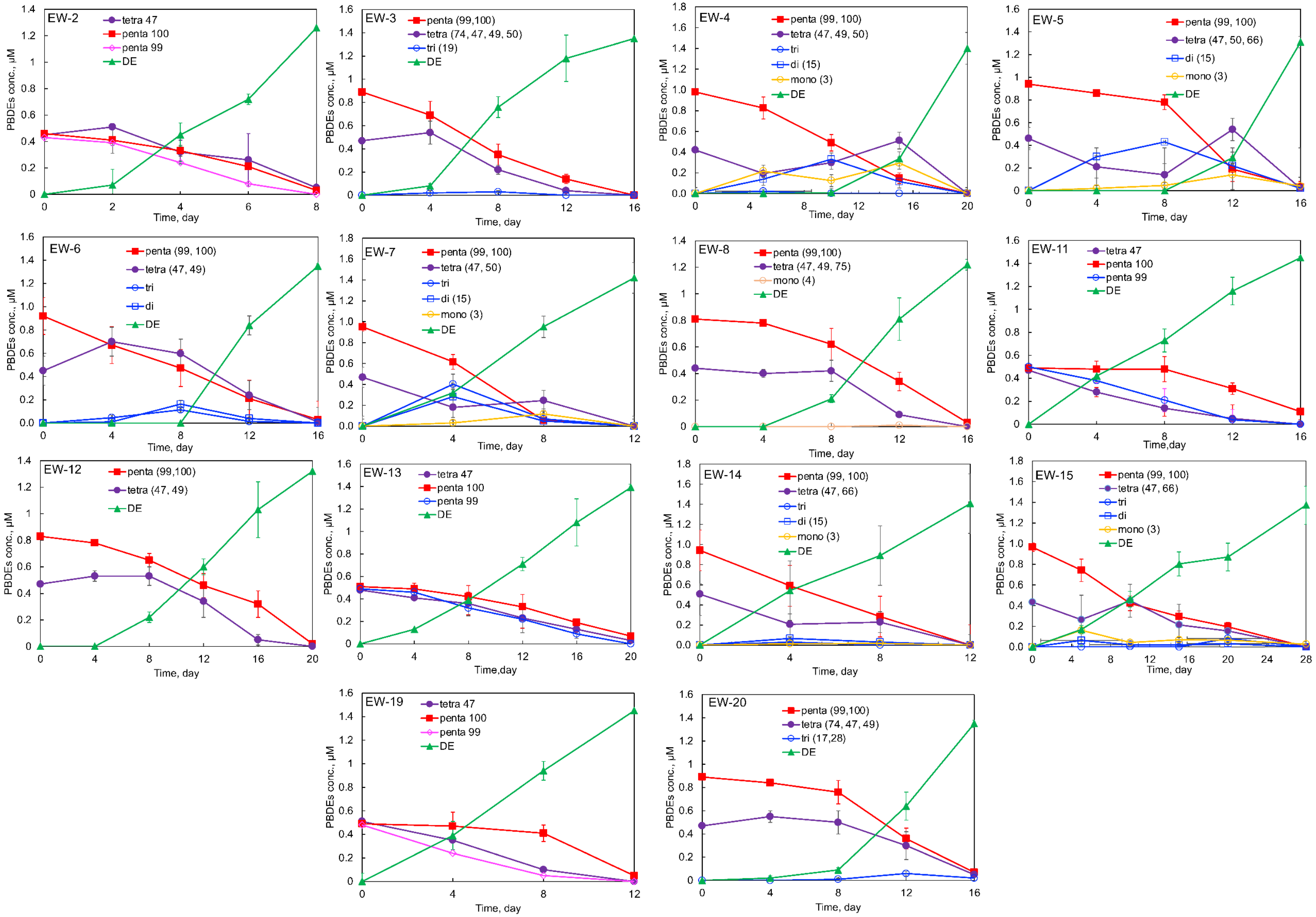

Figure S6. Debromination of a tetra- and penta-BDE mixture by enrichment cultures derived from PBDE debrominating microcosms. Kinetics data is available in Supplementary Dataset Table S11.

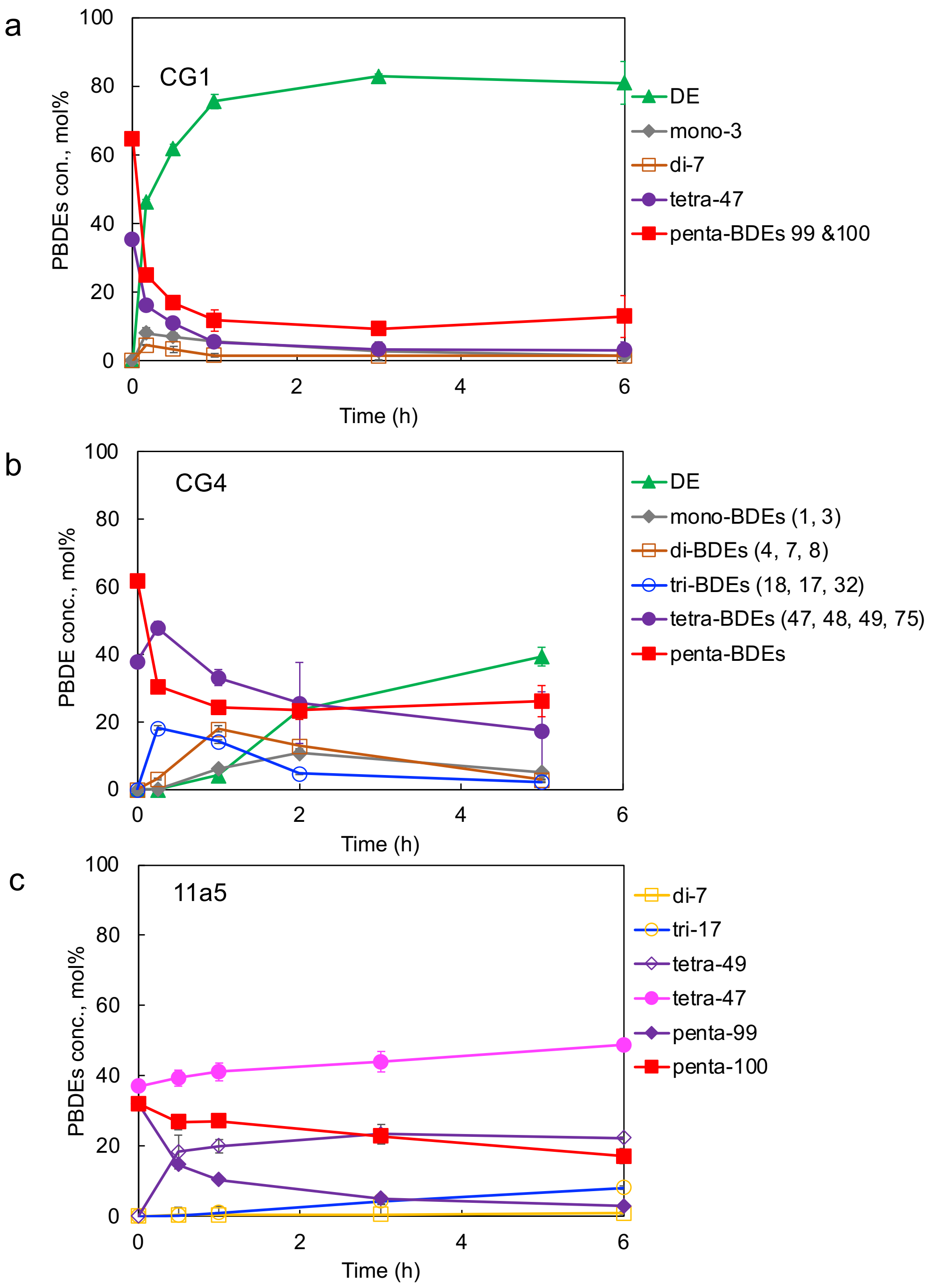

Figure S7. Debromination of penta- and tetra-BDEs in *in vitro* enzymatic assays using crude cell extracts from cultures of (a) *Dehalococcoides mccartyi* strain CG1 amended with tetrachloroethene (PCE), (b) *D. mccartyi* strain CG4 amended with PCE, and (c) *D. mccartyi* strain 11a5 amended with trichloroethene. DE, diphenyl ether

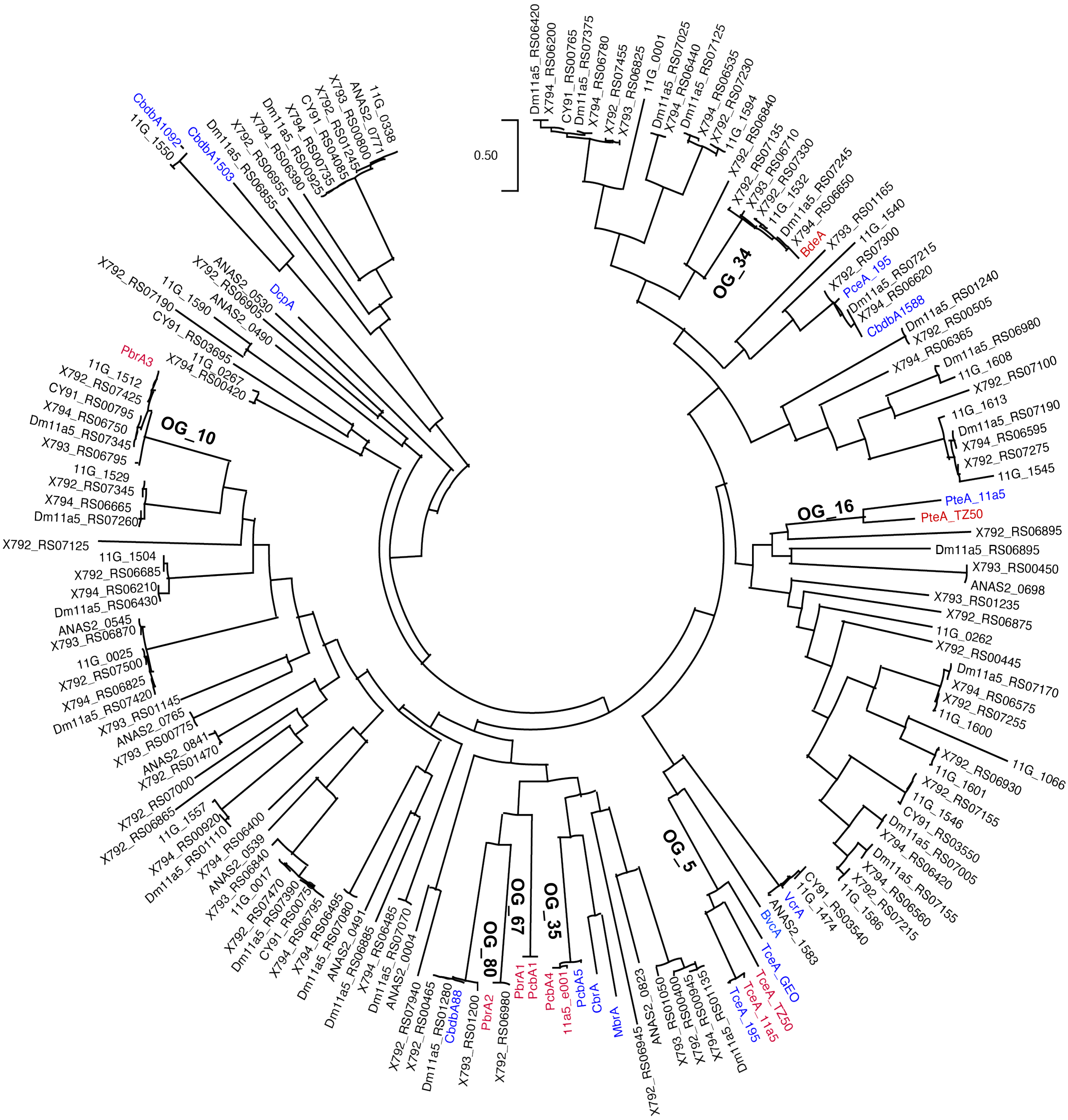

Figure S8. Dendrogram of putative and characterized reductive dehalogenases (RDases) encoded on the genomes of *Dehalococcoides mccartyi* isolates used in this study (GEO12, 11a, 11G, 11a5, ANAS2, CG1, CG4, and CG5). Blue color font indicates functionally characterized non-PBDE RDases; red color font indicates PBDE RDases. The amino acid sequences and NCBI protein database accession numbers of sequences used to construct this phylogeny are listed in Supplementary Dataset Table S13.

Tables.

Table S1. Mass spectra peak area response factors for PBDE homolog groups. Response factors were determined empirically from a mixture of 39 mono- to hepta-BDEs; response factors for each homolog group were calculated as the mean of the peak area response factor for all congeners in a given homolog group in the congener PBDE mixture. The number of congeners in each homolog group in the congener mixture is indicated (n).

	Response factors, $\times 10^{-7}$ ppm		
	n	mean	95% CI
Mono-BDEs	3	2.13	2.12–2.14
Di-BDEs	7	2.08	2.05–2.11
Tri-BDEs	8	2.51	2.48–2.54
Tetra-BDEs	6	1.66	1.63–1.68
Penta-BDEs	7	2.38	2.37–2.39
Hexa-BDEs	5	24.16	24.07–24.25
Hepta-BDEs	3	10.45	10.05–10.58
Octa-BDEs	3	12.91	12.80–13.02
Nona-BDE	1	155.20	153.12–157.28
Deca-BDE	1	292.80	290.12–295.48

Table S2. Primers used in this study.

Targets	**Primer ID**	**Sequence 5′-3′**	**Product size (bp)**	**Tm**	**Reference**
Dehalococcoides	Dhc-qF2	GGTAATACGTAGGAAGCAAGCG	98	60	[1]
	Dhc-qR	CCGGTTAAGCCGGGAAATT			
Dehalogenimonas	mod-BL-DC-1243f	GGYACAATGGGTTGCCACCGG	127	63	[2]
	BL-DC-1351r	AACGCGCTATGCTGACACGCGT			
Dehalobacter	Dre441F	GTTAGGGAAGAACGGCATCTGT	203	58	[3]
	Dre645R	CCTCTCCTGTCCTCAAGCCATA			
Acetobacterium	Aceto572f	GGCTCAACCGGTGACATGCA	212	63	[4]
	Aceto784r	ACTGAGTCTCCCCAACACCT			
Sulfurospirillum	Sulfuro114f	GCTAACCTGCCCTTTAGTGG	307	59	[5]
	Sulfuro421r	GTTTACACACCGAAATGCGT			
Desulfitobacterium	Dsb1299F	GTGGGGATGACGTCAAATCA	149	58	[6]
	Dsb1448R	CATGTTCTCGAGTTGCAGAGAA			
pbrA1/pcbA1	GY50_1350F	TTTTGGGTAGGACAGGTTGGA	127	60	[7]
	GY50_1350R	AGGAACAGAGTGCCATTGACG			
pbrA2	GY50_1352F	GCGCCTTCATTGATTGCTTC	155	60	[7]
	GY50_1352R	GTTGCCCAACCTGGTCTCG			
pbrA3	GY50_1427F	GTTAAAGATCGGCGTGGTTG	94	60	[7]
	GY50_1427R	TCTGGACGGATGGTGTTGC			
pteA_TZ50	pteA848F	CCATAGGCACCTTGGTAGCA	150	60	[8]
	pteA998R	TCACCAATGCCTGCTAACGT			
tceA_TZ50/tceA_11a5	tceA1279F	ATCCAGATTATGACCCTGGTGAA	58	58	[9]
	tceA1336R	GCGGCATATATTAGGGCATCTT			
bdeA	bdeA628F	CTGCGTCAGGTAGTCCGTTT	131	60	[8]
	bdeA758R	GCCGCTTCGTCTACGTTTTC			
pcbA4/11a5_e001	CG4-F (759F)	TGAAGATGTTGACGCAGCCT	175	60	[10]
	CG4-R (843R)	GCGCATACTCCAGGTGAAGA			

Table S3. Sensitivity and amplification metrics for real-time quantitative PCR (qPCR) primers used in this study. LOD, limit of detection; LOQ, limit of quantification; Efficiency, amplification efficiency; r, correlation coefficient of six-point standard curve (range: 5E+02–5E+07 /ml).

	LOD, /ml	LOQ, /ml	Efficiency, %	*r*
pbrA1/pcbA1	5.77E+01	1.14E+02	102.5	99.8
pbrA2	1.01E+01	3.20E+01	104.3	99.7
pbrA3	2.10E+01	5.20E+01	94.7	99.9
pteA_TZ50	8.40E+01	2.13E+02	105.6	99.7
tceA_TZ50/tceA_11a5	5.69E+01	1.57E+02	99.7	99.9
bdeA	3.40E+01	8.90E+01	104.2	99.8
pcbA4/11a5_e001	2.96E+01	5.80E+01	103.2	99.5

Table S4. *Dehalococcoides mccartyi* strains used in this study .

Strain	Origins	Halogenated compounds reduced	End Products/ Dehalogenation pathway	Characterized *rdh*	References
11a	Discharge area of an industrial wastewater treatment station of a chemical plant, China	TCE, *trans*-DCE, *cis*-DCE, 1,1,-DCE, VC, 1,2-DCA	Ethene	*vcrA*	[11, 12]
11a5	Discharge area of an industrial wastewater treatment station of a chemical plant, China	PCE, TCE, *trans*-DCE, *cis*-DCE, 1,1,-DCE	VC (ethene)	*pteA* *tceA*	[11, 13]
11G	Sungei Mandai mangrove, Singapore;	TCE, *trans*-DCE, *cis*-DCE, 1,1,-DCE, VC, 1,2-DCA	Ethene	*vcrA*	[14]
ANAS2	a contaminated site at Alameda Naval Air Station in California, USA	TCE, *cis*-DCE, 1,1,-DCE, VC, 1,2-DCA	Ethene	*vcrA*	[15]
GEO	A TCE and 1,1,1-TCA dechlorinating anaerobic bioreactor	TCE, *trans*-DCE, *cis*-DCE, 1,1,-DCE, VC, 1,2-DCA	Ethene	*tceA* *vcrA*	[16]
CG1	E-waste contaminated sites, Guiyu, China	PCBs,	33′-meta removal	*pcbA1*	[10]
		PCE	DCEs		
		TBBPA	BPA		[17]
CG4	E-waste contaminated sites, Guiyu, China	PCBs,	44′-para > 55′-meta > 33′-meta removal	*pcbA4*	[10]
		PCE	DCEs		
CG5	E-waste contaminated sites, Guiyu, China	PCBs	33′- and 55′-meta; flanked para removal	*pcbA5*	[10]
		PCE	DCEs		

Table S5. Relative abundance of *Dehalococcoides*, *Dehalogenimonas*, and *Dehalobacter* in microbial communities of original soil samples, microcosms (day 60), and enrichments.

	Dehalococcoides			*Dehalogenimonas*			*Dehalobacter*		
	Soil	Microcosms	Enrichments	Soil	Microcosms	Enrichments	Soil	Microcosms	Enrichments
EW-1	0.001%	0.005%	0.000%	0.040%	0.067%	0.000%	0.002%	0.182%	0.000%
EW-2	0.017%	0.160%	1.200%	0.339%	0.207%	0.000%	0.002%	0.010%	2.952%
EW-3	0.000%	0.048%	1.900%	0.024%	0.045%	0.000%	0.000%	0.002%	0.000%
EW-4	0.136%	0.019%	0.000%	0.617%	0.144%	0.594%	0.011%	0.011%	0.000%
EW-5	0.497%	0.015%	0.000%	0.532%	3.373%	2.068%	0.020%	0.026%	0.652%
EW-6	0.022%	0.010%	0.000%	0.669%	0.566%	0.325%	0.006%	0.021%	2.284%
EW-7	0.022%	0.008%	0.000%	0.659%	0.257%	0.774%	0.006%	0.031%	0.217%
EW-8	0.002%	1.734%	6.700%	0.124%	0.234%	0.000%	0.010%	0.009%	0.000%
EW-9	0.001%	0.010%	0.000%	0.197%	0.119%	0.000%	0.001%	0.008%	0.000%
EW-10	0.003%	0.012%	0.000%	0.263%	0.220%	0.000%	0.002%	0.090%	0.000%
EW-11	0.035%	1.289%	5.500%	0.189%	0.106%	0.000%	0.009%	0.042%	0.000%
EW-12	0.005%	0.745%	3.800%	0.292%	0.133%	0.000%	0.008%	0.053%	0.000%
EW-13	0.027%	0.395%	5.400%	0.231%	0.155%	0.000%	0.001%	0.083%	0.000%
EW-14	0.005%	0.014%	0.000%	0.261%	3.279%	2.453%	0.042%	0.023%	0.205%
EW-15	0.010%	0.021%	0.000%	0.052%	0.163%	2.632%	0.009%	0.037%	0.675%
EW-16	0.001%	0.004%	0.000%	0.045%	0.077%	0.000%	0.000%	0.009%	0.000%
EW-17	0.017%	0.004%	0.000%	0.549%	0.123%	0.000%	0.030%	0.039%	0.000%
EW-18	0.002%	0.003%	0.000%	0.025%	0.515%	0.000%	0.000%	0.017%	0.000%
EW-19	0.006%	0.202%	4.700%	0.166%	0.056%	0.000%	0.002%	0.018%	0.000%
EW-20	0.074%	0.488%	2.800%	0.955%	0.091%	0.000%	0.058%	0.041%	0.000%
EW-21	0.008%	0.001%	0.000%	0.719%	0.077%	0.000%	0.032%	0.012%	0.000%
EW-22	0.002%	0.007%	0.000%	0.271%	1.176%	0.000%	0.010%	0.027%	0.000%
EW-23	0.006%	0.001%	0.000%	0.089%	0.001%	0.000%	0.003%	0.000%	0.000%
EW-24	0.002%	0.001%	0.000%	0.240%	0.000%	0.000%	0.004%	0.000%	0.000%

Table S6. Pearson's correlations (*r*) between bromine removal and cell abundance of obligate OHRB in enrichment cultures.

	Dehalococcoides	*Dehalogenimonas*	*Dehalobacter*
EW-2	0.66	N.A.	0.99
EW-3	0.87	N.A.	N.A.
EW-8	0.93	N.A.	N.A.
EW-11	0.98	N.A.	N.A.
EW-12	0.99	N.A.	N.A.
EW-13	0.98	N.A.	N.A.
EW-19	0.96	N.A.	N.A.
EW-20	0.98	N.A.	N.A.
EW-4	N.A.	0.92	N.A.
EW-5	N.A.	0.78	0.85
EW-6	N.A.	0.92	0.99
EW-7	N.A.	0.99	0.97
EW-14	N.A.	0.95	0.97
EW-15	N.A.	0.92	0.91

Table S7. Metabolites produced by debromination of BDE 183 by *Dehalococcoides mccartyi* strains CG1, CG4, and 11a5 after cultivation for 9 months (*in vivo*), as well in enzymatic assays of crude cell extracts (*in vitro*) of cultures supplied a penta- and tetra-BDE mixture as an electron acceptor and cultivated for 7 d at 30 ºC. Abundance of BDE congeners is indicated as the fraction of total BDE composition.

		***in vivo*, %**			***in vitro* assay, %**		
Name	IUPAC	CG1	CG4	11a5	CG1	CG4	11a5
DE						0.6	
mono-1	2					0.2	
di-7	24			3.0			
di-8	2-4					0.6	
tri-30	246			2.4			
tri-17	24-2					2.2	
tri-28	24-4			7.3			
tetra 53	25-26						
tetra-49	24-25					0.7	
tetra-71	26-34					0.7	
tetra-47	24-24					1.4	
tetra-74	245-4			6.2			
tetra-66	24-34					0.5	
penta-95	236-25					19.5	20.9
penta-103	246-25			9.7		0.4	1.8
penta-99	245-24					1.2	
penta-118	245-34			4.9			
penta-85	234-24					2.6	2.4
hexa-154	245-246					3.5	10.4
hexa-144	2346-25					2.4	0.9
hexa-138	234-245					0.5	
hepta-183	2346-245	100	100	66.4	100	63.1	63.5

Table S8. (a) Proteins detected in strain CG1 during PBDE debromination.

Accession ID	Locus_tag	Protein annotation	Averaged abundance	Relative abundance, (%)
WP_012882149.1	X792_RS04400	elongation factor Tu	3.66E+07	48.39
WP_012881820.1	X792_RS02605	F0F1 ATP synthase subunit alpha	1.14E+07	15.04
WP_023651701.1	X792_RS00130	TrkA family potassium uptake protein	4.85E+06	6.41
WP_041331460.1	**X792_RS06970**	**reductive dehalogenase**	**5.66E+06**	**7.48**
WP_041330877.1	X792_RS03735	2-isopropylmalate synthase	4.70E+06	6.21
WP_041330976.1	X792_RS04385	50S ribosomal protein L11	3.36E+06	4.44
WP_012882465.1	X792_RS06430	co-chaperone GroES	2.08E+06	2.75
WP_012881860.1	X792_RS02820	DNA-directed RNA polymerase subunit beta	1.63E+06	2.16
WP_041331321.1	X792_RS06325	hypothetical protein	1.38E+06	1.83
WP_012882718.1	X792_RS07740	recombinase RecA	1.28E+06	1.70
WP_012882466.1	X792_RS06435	chaperonin GroEL	7.59E+05	1.00
WP_041330550.1	X792_RS01275	formate dehydrogenase-N subunit alpha	7.41E+05	0.98
WP_041331598.1	X792_RS07490	DUF3068 domain-containing protein	5.64E+05	0.75
WP_041330621.1	X792_RS01790	ATP-dependent metallopeptidase FtsH/Yme1/Tma family protein	3.53E+05	0.47
WP_041330465.1	X792_RS00960	AbrB/MazE/SpoVT family DNA-binding domain-containing protein	3.11E+05	0.41

(b) Proteins detected in strain CG4 during PBDE debromination.

Accession ID	Locus_tag	Protein annotation	Averaged abundance	Relative abundance, (%)
WP_041340852.1	**X793_RS00330**	**reductive dehalogenase**	**2.03E+07**	**50.77**
WP_041343058.1	X793_RS06320	chaperonin GroEL	3.39E+06	8.47
WP_052470928.1	X793_RS01255	response regulator transcription factor	4.18E+06	10.44
WP_080768397.1	X793_RS06730	PAS domain S-box protein	4.60E+06	11.50
WP_041341847.1	X793_RS03180	sodium-translocating pyrophosphatase	1.99E+06	4.97
WP_041343158.1	X793_RS06590	winged helix-turn-helix transcriptional regulator	1.18E+06	2.94
WP_041341828.1	X793_RS03125	hypothetical protein	9.55E+05	2.39
WP_041342412.1	X793_RS04675	leucyl aminopeptidase	6.95E+05	1.74
WP_010937112.1	X793_RS06315	co-chaperone GroES	5.61E+05	1.40
WP_041340780.1	X793_RS00140	Trk system potassium transporter TrkA	3.69E+05	0.92
WP_010936996.1	X793_RS05745	DUF1508 domain-containing protein	4.16E+05	1.04
WP_041342363.1	X793_RS04470	inositol-3-phosphate synthase	2.92E+05	0.73
WP_010936699.1	X793_RS04550	elongation factor Tu	1.65E+05	0.41
WP_041341240.1	X793_RS01385	hypothetical protein	1.21E+05	0.30
WP_010936411.1	X793_RS02720	cell division protein FtsZ	7.98E+05	1.99

(c) Proteins detected in strain 11a5 during PBDE debromination.

Accession ID	Locus_tag	Protein annotation	Averaged abundance	Relative abundance, (%)
WP_011309438.1	Dm11a5_RS04460	elongation factor Tu	4.53E+07	35.90
WP_011309787.1	Dm11a5_RS06170	chaperonin GroEL	1.60E+07	12.66
WP_034375880.1	**Dm11a5_RS07840**	**reductive dehalogenase**	**1.52E+07**	**12.05**
WP_062900263.1	**Dm11a5_RS06655**	**reductive dehalogenase**	**1.12E+07**	**8.85**
WP_034376728.1	Dm11a5_RS01605	hypothetical protein	1.01E+07	8.02
WP_034376260.1	Dm11a5_RS06060	hypothetical protein	7.80E+06	6.18
WP_011309441.1	Dm11a5_RS04490	GNAT family N-acetyltransferase	5.87E+06	4.65
WP_011309786.1	Dm11a5_RS06165	co-chaperone GroES	3.23E+06	2.56
WP_011308770.1	Dm11a5_RS00480	HAMP domain-containing protein	2.81E+06	2.22
WP_011929022.1	Dm11a5_RS03230	sodium-translocating pyrophosphatase	2.05E+06	1.62
WP_011309118.1	Dm11a5_RS02630	DNA-directed RNA polymerase subunit beta	1.45E+06	1.15
WP_011309989.1	Dm11a5_RS07410	DUF3068 domain-containing protein	1.42E+06	1.12
WP_015407039.1	Dm11a5_RS03180	hypothetical protein	8.73E+05	0.69
WP_034376825.1	Dm11a5_RS00840	DUF4186 family protein	1.28E+06	1.01
WP_011309757.1	Dm11a5_RS06025	molecular chaperone DnaJ	9.06E+05	0.72
WP_011929073.1	Dm11a5_RS03575	glutamine-hydrolyzing GMP synthase	8.83E+05	0.70

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