SUPPLEMENTAL FIGURES AND TABLES

Functional Characterization of Reductive Dehalogenases Using Blue Native Polyacrylamide Gel Electrophoresis

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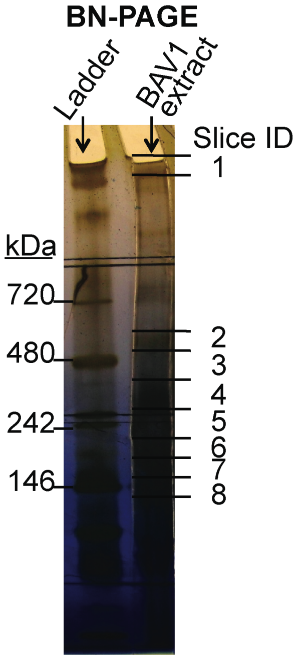
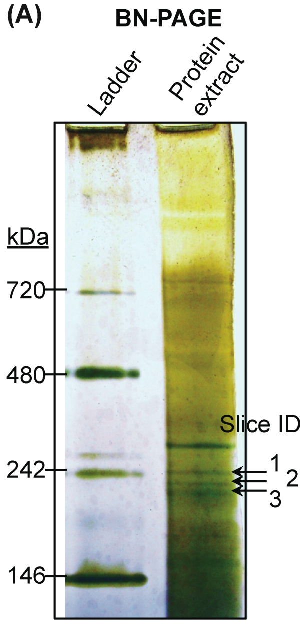


Activity assay on 1,2-DCA

Gel slices	Ethene (nmol)
1	2.1
2	0.14
3	0.72
4	8.1
5	5.5
6	0.09
7	0
8	0
+	11

FIG. S1. Distribution of 1,2-DCA dechlorinating activity on a BN-PAGE gel lane using protein extracts from a BAV1 culture grown on 1,2-DCA. Horizontal lines in the gel indicate positions where the gel was cut. The numbers show nmoles of ethene produced from 1,2-DCA in the activity assay. "+": positive control with 10 µL crude protein extract. Fig. S1 reports similar data as in Fig. 2, but in this experiment we obtained a more comprehensive distribution of activity along the lane compared to the data shown in Fig. 2. However gel slices from the samples shown in Fig. 2 were sent for LC-MS/MS analysis, therefore that data was kept in the body of the paper.

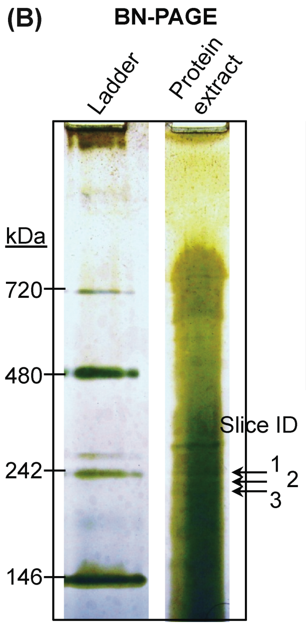


Activity assay on VC

Protein sample	Ethene (nmol)
1	0.66
2	52
3	87
+	380
-	0.00
Buffer	0.00

Activity assay on TCE

Protein sample	*cis*-DCE (nmol)	VC (nmol)	Ethene (nmol)
1	19	0.80	0
2	94	20	1.8
3	83	18	1.7
+	500	400	57
-	0	0.07	0
Buffer	0	0.54	0



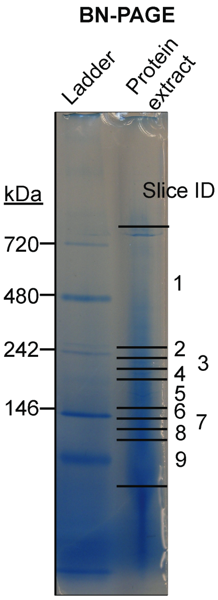
Activity assay on VC

Protein sample	Ethene (nmol)
1	88
2	55
3	68
+	350
-	0
Buffer	0

Activity assay on TCE

Protein sample	*cis*-DCE (nmol)	VC (nmol)	Ethene (nmol)
1	160	19	0.95
2	100	10	0.37
3	140	20	1.2
+	1100	130	2.8
-	1.5	0	0
Buffer	0	0.54	0

FIG. S2. Dechlorination assays with protein extracts and gel slices from the KB-1 culture: (A) of the VC-induced KB-1 culture and (B) of the TCE-induced KB-1 culture. "+": positive control with 25 μL crude protein extract; "-": negative control with a gel slice from a region without visible bands; "Buffer": negative control with only the assay buffer but without cell extract.



Activity assay on 1,2-DCA

Gel slice	Ethene (nmol)
1	0.40
2	0.22
3	0.27
4	0.08
5	0.02
6	0.02
7	0.02
8	0.02
9	0.02
+	63

Activity assay on VC

Gel slice	Ethene (nmol)
1	2.4
2	1.9
3	1.1
4	0.26
5	0.01
6	0
7	0
8	0
9	0
+	16

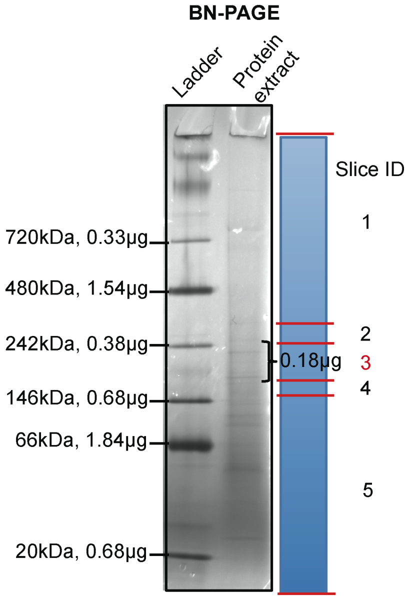
Activity assay on *cis*-DCE

Gel slice	VC (nmol)	Ethene (nmol)
1	3.4	0
2	3.8	0
3	1.6	0
4	0.26	0
5	0.02	0
6	0.03	0
7	0.02	0
8	0.01	0
9	0.01	0
+	240	1.5

Activity assay on TCE

Gel slice	*cis*-DCE (nmol)	VC (nmol)	Ethene (nmol)
1	3.8	0	0
2	2	0	0
3	0.9	0	0
4	0.67	0	0
5	0.53	0	0
6	0.39	0	0
7	0.38	0	0
8	0.38	0	0
9	1.5	0	0
+	55	67	0.95

FIG. S3. The distribution of dechlorination activity on BN-PAGE gel lanes using protein extracts of the 1,2-DCA KB-1 subculture. Horizontal lines in the gel indicate positions where the gel was cut. The numbers show amounts of product(s) formed from different electron acceptors in the activity assay. “+”: positive control with 20 µL crude protein extract.



Activity assay on VC

Gel slice	Ethene (nmol)
1	0.80
2	1.24
3	20
4	0
5	0
+	55
Buffer	0

Activity assay on TCE

Gel slice	*cis*-DCE (nmol)	*trans*-DCE (nmol)	1,1-DCE (nmol)	VC (nmol)	Ethene (nmol)
1	1.0	0.04	0.02	0.03	0
2	1.2	0.03	0.02	0.03	0
3	20	0.25	0.09	3.0	0.15
4	0.12	0	0.01	0.01	0
5	0.06	0.04	0.01	0	0
+	50	1.5	1.3	19	1.45
Buffer	1.0	0.01	0.03	0.16	0

Activity assay on 1,2-DCA

Gel slice	Ethene (nmol)
1	0.07
2	0.10
3	1.8
4	0.02
5	0.02
+	16
Buffer	0.04

Specific activity and purification factor

Substrate of dechlorination assay	Specific activity (nmol/min/mg protein)		Purification factor
	Crude protein extract	Gel slice 3	
VC	5.2	77	14.8
TCE	6.8	90	13.2
1,2-DCA	1.5	6.9	4.6

FIG. S4. Enrichment of RDase activity after separation by BN-PAGE. The crude protein extract from a KB-1 culture grown on TCE was applied to the gel. The results of dechlorination activity assays are shown as nmoles of dechlorination product(s). "+": positive control with 20 µL crude protein extract (7.4 µg total protein); "Buffer": negative control with assay buffer but no cell extract. The specific activity and fold purification was determined by dividing the nmoles dechlorinated in 24 hours by the concentration of protein in the respective samples.

TABLE S1. Specific dechlorination rates determined by methyl viologen assays with crude protein extracts.

	Estimated Specific Dechlorinating Activity[a] (nmol · min^{-1} · mg protein^{-1})		
	Culture (growth substrate)		
Enzyme assay substrate:	BAV1 (*cis*-DCE)	KB-1 (TCE)	KB-1 subculture (1,2-DCA)
PCE	0	0.47 ± 0.02	0.14 ± 0.03
TCE	1.2 ± 0.1	7.0 ± 1.0	6.6 ± 1.2
cis-DCE	23 ± 4	16 ± 2	11 ± 2
trans-DCE	12 ± 1	0.57 ± 0.04	0.24 ± 0.04
1,1-DCE	32 ± 3	N/A	N/A
VC	0.94 ± 0.08	3.6 ± 0.8	0.56 ± 0.10
1,2-DCA	4.7 ± 0.5	6.0 ± 1.1	2.9 ± 0.5

[a] Calculated as nmol dechlorination products formed after 24 hours divided by the time (1440 min) and divided by the protein content. Shown are results of triplicate samples ± SD. N/A: not measured

TABLE S2. Peptide hits and coverage of the RDases identified from the three KB-1 cultures[a].

Culture: TCE-Induced KB-1

RDases	Accession Number	Peptide Hits			Coverage (%)		
		TCE1	TCE2	TCE3	TCE1	TCE2	TCE3
KB1_VcrA	DCKB1_96900[b]	39	59	61	36	36	42
KB1_GeobRD	393716494[c,d]	8	15	17	17	28	26
KB1_BvcA	77176863[c]	5	9	19	5.4	15	30
KB1_TceA	DCKB1_14890[b]	3	3	0	5.4	5.5	0
KB1_RdhA5	DCKB1_110110[b]	0	3	2	0	7.9	5.8

Culture: VC-Induced KB-1

RDases	Accession Number	Peptide Hits			Coverage (%)		
		VC1	VC2	VC3	VC1	VC2	VC3
KB1_VcrA	DCKB1_96900[b]	48	64	108	42	45	48
KB1_BvcA	77176863[c]	24	14	13	36	24	27
KB1_TceA	DCKB1_14890[b]	4	6	9	7.9	7.9	8
KB1_GeobRD	393716494[c,d]	3	4	9	8.2	11	18
KB1_RdhA5	DCKB1_110110[b]	9	6	5	17	14	11
KB1_RdhA1	DCKB1_110270[b]	3	3	0	7.3	7.3	0

Culture: 1,2-DCA KB-1 subculture

RDases	Accession Number	Peptide Hits			Coverage (%)		
		DCA1	DCA2	DCA3	DCA1	DCA2	DCA3
KB1_VcrA	DCKB1_96900[b]	8	16	9	24	33	29
KB1_TceA	DCKB1_14890[b]	10	9	7	21	18	13
KB1_BvcA	77176863[c]	2	2	0	5.2	4.7	0
KB1_RdhA5	DCKB1_110110[b]	0	2	0	0	7.4	0

[a] For each culture, three adjacent BN-PAGE gel slices from the region of high dechlorinating activity were analyzed by LC-MS/MS. For the TCE-Induced KB-1 culture, the slices were labeled TCE1, TCE2 and TCE3; for VC-induced KB-1 culture, they were labeled VC1, VC2 and VC3; for the 1,2-DCA KB-1 subculture, they were labeled DCA1, DCA2 and DCA3.

[b] IMG gene locus tag.

[c] NCBI GI number.

[d] The KB1-GeobRD (NCBI GI 393716494) was assembled from contigs from the metagenome, including the partial sequences with IMG locus tags DCKB1_87010 and DCKB1_86980.

TABLE S3. Proteins identified from the active BN-PAGE gel slices from the three KB-1 cultures. The MS spectra were searched against the KB-1 metagenome. For each culture, the results were produced from three consecutive gel slices covering the active region and each slice was analyzed by LC-MS/MS separately. RDase-related entries are highlighted in red.

Culture: TCE-Induced KB-1

Annotation	**Putative Organism**	**IMG Gene Locus Tag**	**Peptide Hits**			**Coverage**		
			1	**2**	**3**	**1**	**2**	**3**
reductive dehalogenase, KB1_VcrA	*Dehalococcoides*	DCKB1_96900	24	35	35	36%	34%	39%
chaperonin GroEL	*Dehalococcoides*	DCKB1_11070	20	21	18	35%	32%	31%
formate dehydrogenase alpha subunit	*Dehalococcoides*	DCKB1_107840	15	19	18	19%	23%	22%
ATPases with chaperone activity, ATP-binding subunit	*Dehalococcoides*	DCKB1_10910	6	12	9	6%	16%	11%
chaperone protein DnaK	*Dehalococcoides*	DCKB1_174500	7	11	9	15%	22%	17%
Acyl-CoA synthetase	*Dehalococcoides*	DCKB1_13860	7	10	15	9%	12%	18%
acetyl-coenzyme A synthetase	*Dehalococcoides*	DCKB1_13470	7	8	4	13%	17%	9%
NADPH-dependent glutamate synthase beta chain and related oxidoreductases	*Dehalococcoides*	DCKB1_108280	6	7	7	10%	15%	13%
argininosuccinate synthase	*Dehalococcoides*	DCKB1_175820	3	7	8	8%	17%	21%
D-3-phosphoglycerate dehydrogenase	*Dehalococcoides*	DCKB1_40850	2	6	10	5%	12%	20%
2-oxoacid:acceptor oxidoreductase, gamma subunit, pyruvate/2-ketoisovalerate family	*Dehalococcoides*	DCKB1_41690	6	6	0	31%	30%	0%
DNA polymerase III, beta subunit	*Dehalococcoides*	DCKB1_114230	4	6	7	13%	18%	20%
glyceraldehyde-3-phosphate dehydrogenase	*Dehalococcoides*	DCKB1_40760	2	5	5	6%	23%	19%
ATPases with chaperone activity, ATP-binding subunit	*Dehalococcoides*	DCKB1_108770	0	5	0	0%	8%	0%
IMP dehydrogenase family protein	*Dehalococcoides*	DCKB1_131130	3	5	7	12%	14%	19%
Formate-tetrahydrofolate ligase	*Acetobacterium*	DCKB1_134100	5	5	6	16%	16%	18%
translation elongation factor 2	*Dehalococcoides*	DCKB1_13440	0	4	3	0%	9%	6%
F420-non-reducing hydrogenase subunit A	*Dehalococcoides*	DCKB1_40990	0	4	2	0%	9%	5%
pyruvate ferredoxin oxidoreductase, alpha subunit	*Dehalococcoides*	DCKB1_41710	5	4	2	15%	12%	7%
Partial sequence of a reductive dehalogenase KB1_GeobRD	*Geobacter*	DCKB1_86980	3	4	4	13%	17%	17%
hypothetical protein	*Dehalococcoides*	DCKB1_105500	2	4	2	13%	22%	13%
Trypsin-like serine proteases, typically periplasmic, contain C-terminal PDZ domain	*Dehalococcoides*	DCKB1_175550	4	4	3	10%	9%	6%
hypothetical protein	*Dehalococcoides*	DCKB1_10850	0	3	5	0%	6%	8%
ATPase components of various ABC-type transport systems, contain duplicated ATPase	*Dehalococcoides*	DCKB1_11590	0	3	2	0%	13%	8%
ABC-type multidrug transport system, ATPase component	*Dehalococcoides*	DCKB1_42380	0	3	0	0%	8%	0%
Partial sequence of a reductive dehalogenase KB1_GeobRD	*Geobacter*	DCKB1_87010	4	3	5	16%	13%	18%

translation elongation factor 1A	*Dehalococcoides*	DCKB1_105110	2	3	4	7%	10%	10%
Myo-inositol-1-phosphate synthase	*Dehalococcoides*	DCKB1_105280	2	3	2	6%	9%	6%
acetolactate synthase, small subunit	*Dehalococcoides*	DCKB1_106470	0	3	0	0%	18%	0%
Polysulphide reductase	*Dehalococcoides*	DCKB1_107850	3	3	2	9%	7%	5%
Polyferredoxin	*Dehalococcoides*	DCKB1_109740	2	3	2	6%	9%	6%
serine hydroxymethyltransferase	*Dehalococcoides*	DCKB1_131610	0	3	0	0%	10%	0%
chorismate synthase	*Dehalococcoides*	DCKB1_131870	2	3	0	4%	8%	0%
2-oxoglutarate ferredoxin oxidoreductase, beta subunit	*Geobacter*	DCKB1_138890	7	3	5	21%	10%	18%
2-oxoglutarate ferredoxin oxidoreductase, alpha subunit	*Geobacter*	DCKB1_138900	0	3	5	0%	18%	24%
Partial sequence of a reductive dehalogenase KB1_BvcA	*Dehalococcoides*	DCKB1_247960	4	3	6	11%	11%	23%
carbamoyl-phosphate synthase large subunit	*Dehalococcoides*	DCKB1_13540	0	2	0	0%	2%	0%
transketolase, bacterial and yeast	*Dehalococcoides*	DCKB1_41270	0	2	2	0%	5%	5%
pyruvate ferredoxin oxidoreductase, beta subunit	*Dehalococcoides*	DCKB1_41720	3	2	0	14%	8%	0%
NADH:ubiquinone oxidoreductase, NADH-binding	*Dehalococcoides*	DCKB1_41740	3	2	3	5%	4%	5%
threonyl-tRNA synthetase	*Dehalococcoides*	DCKB1_41970	0	2	3	0%	5%	6%
vacuolar-type H(+)-translocating pyrophosphatase	*Dehalococcoides*	DCKB1_42280	4	2	0	6%	4%	0%
LSU ribosomal protein L10P	*Dehalococcoides*	DCKB1_105160	5	2	0	27%	17%	0%
bacterial translation initiation factor 2	*Dehalococcoides*	DCKB1_105240	0	2	0	0%	4%	0%
histidinol-phosphate aminotransferase	*Dehalococcoides*	DCKB1_106350	2	2	2	6%	6%	6%
Adenylosuccinate lyase	*Dehalococcoides*	DCKB1_106380	11	2	0	24%	5%	0%
3-isopropylmalate dehydratase, large subunit	*Dehalococcoides*	DCKB1_106510	2	2	3	6%	6%	10%
3-isopropylmalate dehydrogenase	*Dehalococcoides*	DCKB1_106530	3	2	2	8%	7%	8%
ABC-type transport system involved in Fe-S cluster assembly, permease component	*Dehalococcoides*	DCKB1_106620	0	2	0	0%	7%	0%
protein-export membrane protein SecD	*Dehalococcoides*	DCKB1_107190	0	2	2	0%	5%	5%
hydrogenases, Fe-only	*Dehalococcoides*	DCKB1_108050	0	2	0	0%	4%	0%
ABC-type multidrug transport system, ATPase component	*Dehalococcoides*	DCKB1_108350	2	2	0	6%	6%	0%
formate dehydrogenase beta subunit	*Dehalococcoides*	DCKB1_108390	0	2	2	0%	8%	8%
reductive dehalogenase, KB1_RdhA5	*Dehalococcoides*	DCKB1_110110	0	2	2	0%	6%	6%
prolyl-tRNA synthetase, family II	*Dehalococcoides*	DCKB1_130980	2	2	0	4%	4%	0%
uridylate kinase	*Dehalococcoides*	DCKB1_131050	0	2	2	0%	12%	12%
methionine adenosyltransferase	*Dehalococcoides*	DCKB1_132330	2	2	0	4%	6%	0%
phosphoenolpyruvate synthase	*Dehalococcoides*	DCKB1_132740	5	2	0	6%	3%	0%
2-oxoglutarate ferredoxin oxidoreductase, alpha subunit	*Geobacter*	DCKB1_138910	0	2	0	0%	19%	0%
Dehydrogenases with different specificities	*Dehalococcoides*	DCKB1_164270	0	2	0	0%	12%	0%
ABC-type multidrug transport system, ATPase component	*Dehalococcoides*	DCKB1_174980	0	2	0	0%	9%	0%
protein-export membrane protein, SecD/SecF family	*Geobacter*	DCKB1_242250	0	2	0	0%	12%	0%

ABC-type dipeptide/oligopeptide/nickel transport system, ATPase component	*Dehalococcoides*	DCKB1_11600	0	0	2	0%	0%	8%
ATPases with chaperone activity, ATP-binding subunit	*Dehalococcoides*	DCKB1_23500	3	0	3	10%	0%	9%
aspartyl-tRNA synthetase	*Dehalococcoides*	DCKB1_41570	0	0	2	0%	0%	5%
vacuolar-type H(+)-translocating pyrophosphatase	*Dehalococcoides*	DCKB1_42090	4	0	0	6%	0%	0%
Acetyl-CoA hydrolase	*Geobacter*	DCKB1_61160	3	0	0	8%	0%	0%
translation elongation factor 2	*Geobacter*	DCKB1_91560	0	0	2	0%	0%	11%
protein translocase subunit secF	*Dehalococcoides*	DCKB1_107200	0	0	2	0%	0%	8%
Isopropylmalate/homocitrate/citramalate synthases	*Dehalococcoides*	DCKB1_109560	0	0	3	0%	0%	8%
membrane protease FtsH catalytic subunit	*Dehalococcoides*	DCKB1_131180	0	0	2	0%	0%	3%
malate dehydrogenase	*Geobacter*	DCKB1_144110	0	0	2	0%	0%	11%
Inorganic pyrophosphatase	*Geobacter*	DCKB1_294670	2	0	0	9%	0%	0%
Pyruvate:ferredoxin oxidoreductase and related 2-oxoacid:ferredoxin oxidoreductases, gamma subunit	*Acetobacterium*	DCKB1_309350	3	0	0	12%	0%	0%

Culture: VC-Induced KB-1

Annotation	**Putative Organism**	**IMG Gene Locus Tag**	**Peptide Hits**			**Coverage**		
			1	**2**	**3**	**1**	**2**	**DCA3**
reductive dehalogenase, KB1_VcrA	*Dehalococcoides*	DCKB1_96900	28	36	61	39%	40%	47%
chaperonin GroEL	*Dehalococcoides*	DCKB1_11070	18	14	23	37%	31%	37%
ATPases with chaperone activity, ATP-binding subunit	*Dehalococcoides*	DCKB1_10910	8	10	9	11%	12%	12%
Acyl-CoA synthetase	*Dehalococcoides*	DCKB1_13860	9	8	13	12%	10%	16%
chaperone protein DnaK	*Dehalococcoides*	DCKB1_174500	7	8	8	15%	17%	17%
pyruvate ferredoxin oxidoreductase, alpha subunit	*Dehalococcoides*	DCKB1_41710	8	7	6	22%	22%	19%
Partial sequence of a reductive dehalogenase KB1_BvcA	*Dehalococcoides*	DCKB1_247960	4	7	6	18%	25%	23%
LSU ribosomal protein L10P	*Dehalococcoides*	DCKB1_105160	10	6	0	46%	39%	0%
2-oxoacid:acceptor oxidoreductase, gamma subunit, pyruvate/2-ketoisovalerate family	*Dehalococcoides*	DCKB1_41690	6	5	4	30%	21%	22%
reductive dehalogenase, KB1_RdhA5	*Dehalococcoides*	DCKB1_110110	7	5	3	14%	13%	8%
IMP dehydrogenase family protein	*Dehalococcoides*	DCKB1_131130	3	5	7	13%	18%	23%
phosphoenolpyruvate synthase	*Dehalococcoides*	DCKB1_132740	8	5	0	11%	7%	0%
Formate-tetrahydrofolate ligase	*Acetobacterium*	DCKB1_134100	0	5	11	0%	16%	31%
D-3-phosphoglycerate dehydrogenase	*Dehalococcoides*	DCKB1_40850	4	4	7	10%	10%	15%
ABC-type transport system involved in Fe-S cluster assembly, permease component	*Dehalococcoides*	DCKB1_106620	6	4	2	14%	12%	6%
chorismate synthase	*Dehalococcoides*	DCKB1_131870	4	4	0	10%	10%	0%
acetyl-coenzyme A synthetase	*Dehalococcoides*	DCKB1_13470	4	3	3	8%	8%	6%
Partial sequence of a reductive dehalogenase KB1_GeobRD	*Geobacter*	DCKB1_86980	3	3	3	13%	13%	8%

Partial sequence of a reductive dehalogenase KB1_GeobRD	*Geobacter*	DCKB1_87010	2	3	4	8%	13%	18%
translation elongation factor 2	*Acetobacterium*	DCKB1_152190	4	3	0	11%	8%	0%
Arabinose efflux permease	*Dehalococcoides*	DCKB1_175400	3	3	3	6%	6%	6%
argininosuccinate synthase	*Dehalococcoides*	DCKB1_175820	2	3	7	4%	8%	18%
reductive dehalogenase, KB1_TceA	*Dehalococcoides*	DCKB1_14890	3	2	3	5%	4%	5%
threonyl-tRNA synthetase	*Dehalococcoides*	DCKB1_41970	4	2	3	10%	3%	8%
translation elongation factor 1A	*Dehalococcoides*	DCKB1_105110	3	2	2	10%	7%	7%
Pyruvate:ferredoxin oxidoreductase and related 2-oxoacid:ferredoxin oxidoreductases, gamma subunit	*Dehalococcoides*	DCKB1_105590	0	2	0	0%	11%	0%
indolepyruvate ferredoxin oxidoreductase, alpha subunit	*Dehalococcoides*	DCKB1_105600	4	2	0	8%	5%	0%
acetolactate synthase, large subunit	*Dehalococcoides*	DCKB1_106460	0	2	0	0%	5%	0%
Hydrogenase maturation factor	*Dehalococcoides*	DCKB1_109770	0	2	4	0%	8%	16%
reductive dehalogenase, KB1_rdhA1	*Dehalococcoides*	DCKB1_110270	3	2	0	7%	5%	0%
DNA polymerase III, beta subunit	*Dehalococcoides*	DCKB1_114230	0	2	2	0%	9%	9%
hypothetical protein	*Dehalococcoides*	DCKB1_10850	2	0	0	3%	0%	0%
Co-chaperonin GroES	*Dehalococcoides*	DCKB1_11060	2	0	0	31%	0%	0%
ABC-type dipeptide transport system, periplasmic component	*Dehalococcoides*	DCKB1_11630	2	0	0	5%	0%	0%
translation elongation factor 2	*Dehalococcoides*	DCKB1_13440	0	0	2	0%	0%	2%
ATPases with chaperone activity, ATP-binding subunit	*Dehalococcoides*	DCKB1_23500	3	0	0	9%	0%	0%
transketolase, bacterial and yeast	*Dehalococcoides*	DCKB1_41270	0	0	3	0%	0%	6%
pyruvate ferredoxin oxidoreductase, beta subunit	*Dehalococcoides*	DCKB1_41720	8	0	0	21%	0%	0%
Acetyl-CoA hydrolase	*Geobacter*	DCKB1_61160	4	0	0	10%	0%	0%
Myo-inositol-1-phosphate synthase	*Dehalococcoides*	DCKB1_105280	2	0	2	7%	0%	6%
hypothetical protein	*Dehalococcoides*	DCKB1_105500	2	0	0	13%	0%	0%
Adenylosuccinate lyase	*Dehalococcoides*	DCKB1_106380	7	0	0	16%	0%	0%
3-isopropylmalate dehydrogenase	*Dehalococcoides*	DCKB1_106530	0	0	5	0%	0%	16%
hydrogenases, Fe-only	*Dehalococcoides*	DCKB1_108050	0	0	2	0%	0%	4%
hypothetical protein	*Dehalococcoides*	DCKB1_110130	2	0	0	10%	0%	0%
Pyruvate:ferredoxin oxidoreductase and related 2-oxoacid:ferredoxin oxidoreductases, alpha subunit	*Acetobacterium*	DCKB1_120030	3	0	0	9%	0%	0%
Pyruvate:ferredoxin oxidoreductase and related 2-oxoacid:ferredoxin oxidoreductases, beta subunit	*Acetobacterium*	DCKB1_120620	2	0	0	17%	0%	0%
phospho-2-dehydro-3-deoxyheptonate aldolase	*Dehalococcoides*	DCKB1_131930	2	0	0	6%	0%	0%
methionine adenosyltransferase	*Dehalococcoides*	DCKB1_132330	0	0	2	0%	0%	6%
2-oxoglutarate ferredoxin oxidoreductase, beta subunit	*Geobacter*	DCKB1_138890	2	0	3	9%	0%	13%
2-oxoglutarate ferredoxin oxidoreductase, alpha subunit	*Geobacter*	DCKB1_138900	0	0	2	0%	0%	9%
2-oxoglutarate ferredoxin oxidoreductase, alpha subunit	*Geobacter*	DCKB1_138910	0	0	2	0%	0%	11%

Pyruvate:ferredoxin oxidoreductase and related 2-oxoacid:ferredoxin oxidoreductases, gamma subunit	*Acetobacterium*	DCKB1_309350	4	0	0	20%	0%	0%

Culture: KB-1 1,2-DCA subculture

Annotation	**Putative Organism**	**IMG Gene Locus Tag**	**Peptide Hits**			**Coverage**		
			1	**2**	**3**	**1**	**2**	**3**
chaperonin GroEL	*Dehalococcoides*	DCKB1_11070	50	58	56	55%	56%	61%
chaperone protein DnaK	*Dehalococcoides*	DCKB1_174500	19	19	23	34%	28%	32%
reductive dehalogenase, KB1_VcrA	*Dehalococcoides*	DCKB1_96900	8	14	8	24%	29%	25%
ATPases with chaperone activity, ATP-binding subunit	*Dehalococcoides*	DCKB1_10910	6	12	7	8%	17%	12%
translation elongation factor 2	*Dehalococcoides*	DCKB1_13440	6	12	11	11%	24%	21%
pyruvate ferredoxin oxidoreductase, beta subunit	*Dehalococcoides*	DCKB1_41720	9	11	6	27%	27%	19%
Formate-tetrahydrofolate ligase	*Acetobacterium*	DCKB1_134100	7	11	21	19%	19%	30%
D-3-phosphoglycerate dehydrogenase	*Dehalococcoides*	DCKB1_40850	4	10	12	11%	19%	22%
ABC-type Fe3+-hydroxamate transport system, periplasmic component	*Dehalococcoides*	DCKB1_41330	9	10	13	17%	26%	25%
translation elongation factor 1A	*Dehalococcoides*	DCKB1_105110	8	10	9	15%	22%	19%
ABC-type transport system involved in Fe-S cluster assembly, permease component	*Dehalococcoides*	DCKB1_106620	7	9	5	20%	26%	14%
argininosuccinate synthase	*Dehalococcoides*	DCKB1_175820	6	9	15	14%	20%	20%
acetyl-coenzyme A synthetase	*Dehalococcoides*	DCKB1_13470	5	8	7	7%	16%	15%
reductive dehalogenase, KB1_TceA	*Dehalococcoides*	DCKB1_14890	9	8	8	19%	16%	14%
F420-non-reducing hydrogenase subunit A	*Dehalococcoides*	DCKB1_40990	8	8	5	20%	16%	13%
pyruvate ferredoxin oxidoreductase, alpha subunit	*Dehalococcoides*	DCKB1_41710	6	8	4	15%	22%	10%
NADH:ubiquinone oxidoreductase, NADH-binding	*Dehalococcoides*	DCKB1_41740	7	8	6	16%	15%	9%
L-glutamine synthetase	*Dehalococcoides*	DCKB1_114930	3	8	9	8%	14%	17%
phospho-2-dehydro-3-deoxyheptonate aldolase	*Dehalococcoides*	DCKB1_131930	3	8	7	8%	17%	20%
Acyl-CoA synthetase	*Dehalococcoides*	DCKB1_13860	6	7	13	9%	9%	19%
nicotinate-nucleotide--dimethylbenzimidazole phosphoribosyltransferase	*Dehalococcoides*	DCKB1_41400	2	6	7	11%	13%	16%
Isopropylmalate/homocitrate/citramalate synthases	*Dehalococcoides*	DCKB1_109560	2	6	8	6%	12%	19%
Pyruvate:ferredoxin oxidoreductase and related 2-oxoacid:ferredoxin oxidoreductases, alpha subunit	*Acetobacterium*	DCKB1_120030	4	6	0	11%	20%	0%
hypothetical protein	*Dehalococcoides*	DCKB1_10850	5	5	4	6%	6%	6%
cysteine synthase	*Dehalococcoides*	DCKB1_13970	4	5	3	22%	22%	22%
2-oxoacid:acceptor oxidoreductase, gamma subunit, pyruvate/2-ketoisovalerate family	*Dehalococcoides*	DCKB1_41690	6	5	4	34%	34%	26%
Leucyl aminopeptidase	*Dehalococcoides*	DCKB1_105960	2	5	0	7%	12%	0%

Iron-regulated ABC transporter ATPase subunit SufC	*Dehalococcoides*	DCKB1_106630	2	5	0	8%	16%	0%
DNA polymerase III, beta subunit	*Dehalococcoides*	DCKB1_114230	2	5	7	10%	13%	19%
IMP cyclohydrolase	*Dehalococcoides*	DCKB1_10960	5	4	7	14%	10%	17%
hypothetical protein	*Dehalococcoides*	DCKB1_14310	0	4	0	0%	35%	0%
glyceraldehyde-3-phosphate dehydrogenase	*Dehalococcoides*	DCKB1_40760	4	4	6	18%	19%	22%
LSU ribosomal protein L10P	*Dehalococcoides*	DCKB1_105160	2	4	0	19%	19%	0%
Myo-inositol-1-phosphate synthase	*Dehalococcoides*	DCKB1_105280	9	4	6	26%	11%	18%
histidinol dehydrogenase	*Dehalococcoides*	DCKB1_106340	3	4	4	9%	13%	9%
ATPase components of various ABC-type transport systems, contain duplicated ATPase	*Dehalococcoides*	DCKB1_11590	3	3	0	12%	8%	0%
aspartyl-tRNA synthetase	*Dehalococcoides*	DCKB1_41570	2	3	3	5%	8%	6%
threonyl-tRNA synthetase	*Dehalococcoides*	DCKB1_41970	0	3	5	0%	8%	10%
Pyruvate:ferredoxin oxidoreductase and related 2-oxoacid:ferredoxin oxidoreductases, gamma subunit	*Dehalococcoides*	DCKB1_105590	0	3	0	0%	12%	0%
histidinol-phosphate aminotransferase	*Dehalococcoides*	DCKB1_106350	2	3	0	8%	13%	0%
3-isopropylmalate dehydratase, small subunit	*Dehalococcoides*	DCKB1_106520	0	3	4	0%	18%	18%
3-Cys thioredoxin peroxidase	*Dehalococcoides*	DCKB1_109870	0	3	3	0%	11%	11%
Pyruvate:ferredoxin oxidoreductase and related 2-oxoacid:ferredoxin oxidoreductases, beta subunit	*Acetobacterium*	DCKB1_120630	3	3	0	16%	16%	0%
IMP dehydrogenase family protein	*Dehalococcoides*	DCKB1_131130	4	3	5	17%	6%	17%
degV family protein	*Dehalococcoides*	DCKB1_131470	0	3	5	0%	13%	26%
serine hydroxymethyltransferase	*Dehalococcoides*	DCKB1_131610	0	3	3	0%	10%	10%
translation elongation factor 2	*Dehalococcoides*	DCKB1_131960	0	3	4	0%	6%	7%
Pyruvate:ferredoxin oxidoreductase and related 2-oxoacid:ferredoxin oxidoreductases, gamma subunit	*Acetobacterium*	DCKB1_309350	0	3	0	0%	20%	0%
ABC-type oligopeptide transport system, periplasmic component	*Dehalococcoides*	DCKB1_14180	0	2	6	0%	5%	13%
transketolase, bacterial and yeast	*Dehalococcoides*	DCKB1_41270	0	2	0	0%	6%	0%
trigger factor	*Dehalococcoides*	DCKB1_41580	0	2	0	0%	5%	0%
triosephosphate isomerase	*Dehalococcoides*	DCKB1_41880	0	2	4	0%	10%	21%
hypothetical protein	*Dehalococcoides*	DCKB1_46220	3	2	5	16%	13%	17%
LSU ribosomal protein L12P	*Dehalococcoides*	DCKB1_105170	0	2	0	0%	20%	0%
dihydrodipicolinate synthase	*Dehalococcoides*	DCKB1_105340	0	2	3	0%	11%	14%
Adenylosuccinate lyase	*Dehalococcoides*	DCKB1_106380	5	2	0	9%	6%	0%
3-isopropylmalate dehydratase, large subunit	*Dehalococcoides*	DCKB1_106510	0	2	4	0%	8%	14%
ribonucleoside-diphosphate reductase, adenosylcobalamin-dependent	*Dehalococcoides*	DCKB1_107080	3	2	0	4%	4%	0%
Hydrogenase maturation factor	*Dehalococcoides*	DCKB1_109770	3	2	0	9%	9%	0%
Uncharacterized conserved protein	*Dehalococcoides*	DCKB1_109830	0	2	2	0%	12%	13%
hypothetical protein	*Dehalococcoides*	DCKB1_110130	3	2	3	10%	10%	10%

Pyruvate:ferredoxin oxidoreductase and related 2-oxoacid:ferredoxin oxidoreductases, beta subunit	*Acetobacterium*	DCKB1_120620	0	2	0	0%	15%	0%
protein translocase subunit secA	*Dehalococcoides*	DCKB1_131590	0	2	0	0%	3%	0%
methionine adenosyltransferase	*Dehalococcoides*	DCKB1_132330	3	2	2	8%	8%	8%
phosphoenolpyruvate synthase	*Dehalococcoides*	DCKB1_132740	14	2	2	19%	3%	3%
transcriptional regulator, AsnC family	*Dehalococcoides*	DCKB1_175700	2	2	0	29%	29%	0%
nicotinate-nucleotide pyrophosphorylase	*Dehalococcoides*	DCKB1_10810	0	0	2	0%	0%	9%
DNA-directed DNA polymerase III	*Dehalococcoides*	DCKB1_11400	0	0	2	0%	0%	3%
carbamoyl-phosphate synthase large subunit	*Dehalococcoides*	DCKB1_13540	2	0	0	4%	0%	0%
endoribonuclease L-PSP	*Dehalococcoides*	DCKB1_14300	2	0	0	22%	0%	0%
Superoxide dismutase	*Dehalococcoides*	DCKB1_29830	2	0	0	15%	0%	0%
F420-non-reducing hydrogenase subunit G	*Dehalococcoides*	DCKB1_40980	3	0	3	17%	0%	10%
ABC-type cobalamin/Fe3+-siderophores transport systems, ATPase components	*Dehalococcoides*	DCKB1_41350	4	0	0	20%	0%	0%
CO dehydrogenase/acetyl-CoA synthase complex, beta subunit	*Dehalococcoides*	DCKB1_41490	2	0	2	4%	0%	4%
Uncharacterized metal-binding protein	*Dehalococcoides*	DCKB1_41530	2	0	0	6%	0%	0%
NADH dehydrogenase/NADH:ubiquinone oxidoreductase 75 kD subunit	*Dehalococcoides*	DCKB1_41750	0	0	2	0%	0%	16%
glycyl-tRNA synthetase	*Dehalococcoides*	DCKB1_42310	0	0	2	0%	0%	5%
hypothetical protein	*Acetobacterium*	DCKB1_43560	9	0	0	26%	0%	0%
Bacterial surface proteins containing Ig-like domains	*Acetobacterium*	DCKB1_43570	5	0	0	12%	0%	0%
Isoleucyl-tRNA synthetase	*Dehalococcoides*	DCKB1_46310	0	0	3	0%	0%	4%
hypothetical protein	*Desulfosporosinus*	DCKB1_59670	2	0	0	13%	0%	0%
Polyribonucleotide nucleotidyltransferase	*Dehalococcoides*	DCKB1_105370	2	0	0	4%	0%	0%
dihydroxyacid dehydratase	*Dehalococcoides*	DCKB1_106450	0	0	2	0%	0%	5%
acetolactate synthase, large subunit	*Dehalococcoides*	DCKB1_106460	2	0	0	7%	0%	0%
formate dehydrogenase alpha subunit	*Dehalococcoides*	DCKB1_107840	0	0	2	0%	0%	3%
ATPases with chaperone activity, ATP-binding subunit	*Dehalococcoides*	DCKB1_108770	0	0	2	0%	0%	4%
reductive dehalogenase, KB1_RdhA5	*Dehalococcoides*	DCKB1_110110	2	0	0	6%	0%	0%
prolyl-tRNA synthetase, family II	*Dehalococcoides*	DCKB1_130980	0	0	2	0%	0%	5%
uridylate kinase	*Dehalococcoides*	DCKB1_131050	0	0	2	0%	0%	12%
phosphoribosylformylglycinamidine synthase subunit II	*Dehalococcoides*	DCKB1_131090	2	0	0	5%	0%	0%
chorismate synthase	*Dehalococcoides*	DCKB1_131870	3	0	0	8%	0%	0%
argininosuccinate lyase	*Dehalococcoides*	DCKB1_175810	9	0	0	19%	0%	0%

TABLE S4. [Sequences in FASTA format] The custom RDase database (amino acid sequences) used as the reference database for RDase identification from the samples of the KB-1 cultures. The first 36 sequences belong to the RDases identified from KB-1 cultures. The remaining sequences include 117 putative RDases from other *Dehalococcoides* strains and 65 putative RDases from other organisms. The IMG gene locus tag, NCBI gene ID or NCBI GI number is attached to the end of each sequence identifier. For example, the identifier “KB1_RdhA1 77176848” indicates that the RDase called KB1_RdhA1 has the NCBI GI number 77176848.

```
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