Biochemical Characterization of the First Haloalkane Dehalogenase from the Cold-Adapted Bacterium

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Supplementary material

Table S1. Putative haloalkane dehalogenases from psychrophilic organisms

Protein Accession No.[a]	Source Organism	Temperature Range		Temperature Optimum [°C]	
		EGP[b]	GOLD[c]	EGP[b]	GOLD[c]
YP_268879	*Colwellia psychroerythraea* 34H	Psychrophilic	Psychrophile	8	8
YP_066368	*Desulfotalea psychrophila* LSv54	Psychrophilic	Psychrophile	7	10
ZP_01736514	*Marinobacter* sp. ELB17	Psychrophilic	Psychrophile	12-15	12-15
ZP_01897865	*Moritella* sp. PE36	Cryophilic	Mesophile	-	-
ZP_01221858	*Photobacterium profundum* 3TCK	Psychrophilic	Psychrophile	15	15
YP_129676	*Photobacterium profundum* SS9	Psychrophilic	Psychrotolerant	15	15
YP_580518	*Psychrobacter cryohalolentis* K5	Psychrophilic	Psychrophile	-	-
YP_943362	*Psychromonas ingrahamii* 37	Psychrophilic	Psychrophile	-	-
ZP_01216824	*Psychromonas* sp. CNPT3	Psychrophilic	Psychrophile	-	-
YP_523535	*Rhodoferax ferrireducens* T118	Mesophilic	Psychrotolerant	25	25
YP_001365757	*Shewanella baltica* OS185	Mesophilic	Psychrotolerant	-	4
YP_002358709	*Shewanella baltica* OS223	Mesophilic	Psychrotolerant	-	4
YP_001675030	*Shewanella halifaxensis* HAW-EB4	Psychrophilic	Psychrophile	10	10
YP_001093840	*Shewanella loihica* PV-4	Mesophilic	Psychrophile	-	-
YP_002312624	*Shewanella piezotolerans* WP3	Mesophilic	Psychrotolerant	15-20	15 – 20
YP_001473250; YP_001473516	*Shewanella sediminis* HAW-EB3	Psychrophilic	Psychrophile	10	10

[a]Accession number to Protein database of NCBI

[b]Information from the Entrez Genome Project database (3)

[c]Information from the Genomes OnLine Database (1)

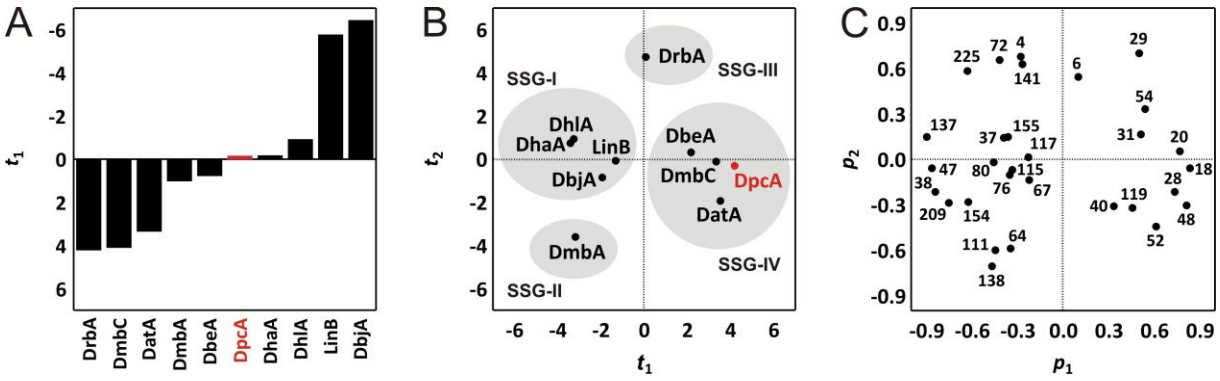

Figure S1. Quantitative comparison of activity and specificity of DpcA with the known biochemically characterized HLDs using the Principle Component Analysis (PCA) (4). The score plots (**A+B**) represent one-dimensional view and two-dimensional window into the multidimensional space, respectively. Enzymes (objects) with similar properties are collocated in these plots. A loading plot (**C**) quantifies contributions of original variables to newly created principal components (axes of the score plots). Variables localized further from the origin possess a stronger effect on the principal component than the variables localized closer to the origin of the plot. PCA was carried out as described previously (2). (**A**) The raw data concerning individual enzymes' specific activities towards thirty substrates were used as data matrix. The depicted t_1 score plot explains 45% of the variance in the primary dataset and shows differences in overall activities of individual HLDs. The overall activity of DpcA is average due to exceptionally high as well as poor activities with specific substrates. (**B**) For the comparison of specificity profiles, the raw data were log-transformed and weighted relative to the individual enzymes' activity towards other substrates prior to analysis. Individual substrate specificity groups (SSGs) of HLDs are marked by grey in the t_1/t_2 score plot. DpcA belongs to SSG-IV, together with DbeA, DatA and DmbC. The classification of DpcA into SSG-IV is further supported by the distribution of enzymes along the third principle component (*data not shown*). (**C**) Corresponding p_1/p_2 loading plot enables

identification of substrates preferentially converted by members of SSG-IV as 1-bromobutane (**18**), 1-iodopropane (**28**) and 1,3-dibromopropane (**48**) which can be found in the right part of the plot. Numbering of the substrates is provided in Table S2.

Table S2. Thirty halogenated substrates used for HLD substrate specificity testing.

Substrates	
1-chlorobutane (4)	1,2-dichloropropane (67)
1-chlorohexane (6)	1,2-dibromopropane (72)
1-bromobutane (18)	2-bromo-1-chloropropane (76)
1-bromohexane (20)	1,2,3-trichloropropane (80)
1-iodopropane (28)	bis(2-chloroethyl)ether (111)
1-iodobutane (29)	chlorocyclohexane (115)
1-iodohexane (31)	bromocyclohexane (117)
1,2-dichloroethane (37)	(1-bromomethyl)cyclohexane (119)
1,3-dichloropropane (38)	1-bromo-2-chloroethane (137)
1,5-dichloropentane (40)	chlorocyclopentane (138)
1,2-dibromoethane (47)	4-bromobutyronitrile (141)
1,3-dibromopropane (48)	1,2,3-tribromopropane (154)
1-bromo-3-chloropropane (52)	1,2-dibromo-3-chloropropane (155)
1,3-diiodopropane (54)	3-chloro-2-methylpropene (209)
2-iodobutane (64)	2,3-dichloropropene (225)

Table S3. Comparison of amino acid composition of DpcA with biochemically characterized HLDs from mesophilic organisms.

Enzyme	Accession no.[a]	Amino acid composition [%]																			
		A	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**K**	**L**	**M**	**N**	**P**	**Q**	**R**	**S**	**T**	**V**	**W**	**Y**
DpcA	YP_580518	7	1	9	4	5	8	3	5	4	9	2	4	8	5	6	5	6	5	2	3
DatA	BAJ23993	9	0	5	6	6	7	3	5	3	12	0	4	7	5	7	5	6	6	2	2
DbjA	BAC46352	15	1	4	8	6	7	4	5	2	9	2	1	8	3	8	5	4	6	1	3
DhaA	AAC15838	8	1	6	8	5	7	4	6	3	10	2	3	10	1	6	3	3	7	3	3
DhlA	P22643	10	1	8	6	7	6	2	5	4	9	4	3	7	4	5	4	5	5	2	4
DhmA	CAC41377	11	0	7	4	4	7	3	5	2	9	1	2	10	3	9	5	4	7	3	4
DmbA	AAK46969	8	0	6	7	4	9	3	6	2	8	3	3	7	4	8	5	3	7	3	3
DmbB	NP_216812	11	0	7	4	5	9	4	4	2	9	1	1	10	3	8	4	5	7	3	4
DmbC	NP_216349	10	1	6	5	6	7	3	6	2	8	3	2	8	2	10	5	4	7	2	3
DmlA	Q98C03	12	1	7	5	5	6	3	5	3	10	2	2	9	4	7	5	3	6	1	2
DppA	ZP_01908831	10	1	7	6	7	11	2	3	2	9	4	1	9	3	6	3	4	7	2	2
DrbA	CAM90600	11	2	7	4	3	6	3	6	3	10	3	3	7	2	9	8	5	5	2	2
Jann2620	YP_510562	13	0	7	6	4	7	2	7	1	8	3	4	8	4	5	4	7	6	2	2
LinB	BAA03443	12	1	7	7	4	8	2	7	2	9	3	2	7	3	8	4	3	4	3	3
Sav4779	NP_825956	12	0	7	6	3	9	4	5	2	10	3	1	7	1	7	5	4	6	3	2
Mean[b]	-	11	1	7	6	5	8	3	5	2	9	2	2	8	3	7	5	4	6	2	3
Min[b]	-	8	0	4	4	3	6	2	3	1	8	0	1	7	1	5	3	3	4	1	2
Max[b]	-	15	2	8	8	7	11	4	7	4	12	4	4	10	5	10	8	7	7	3	4

[a]Accession number in the Protein Database of NCBI

[b]Data relates to HLDs from mesophilic organisms (without DpcA)

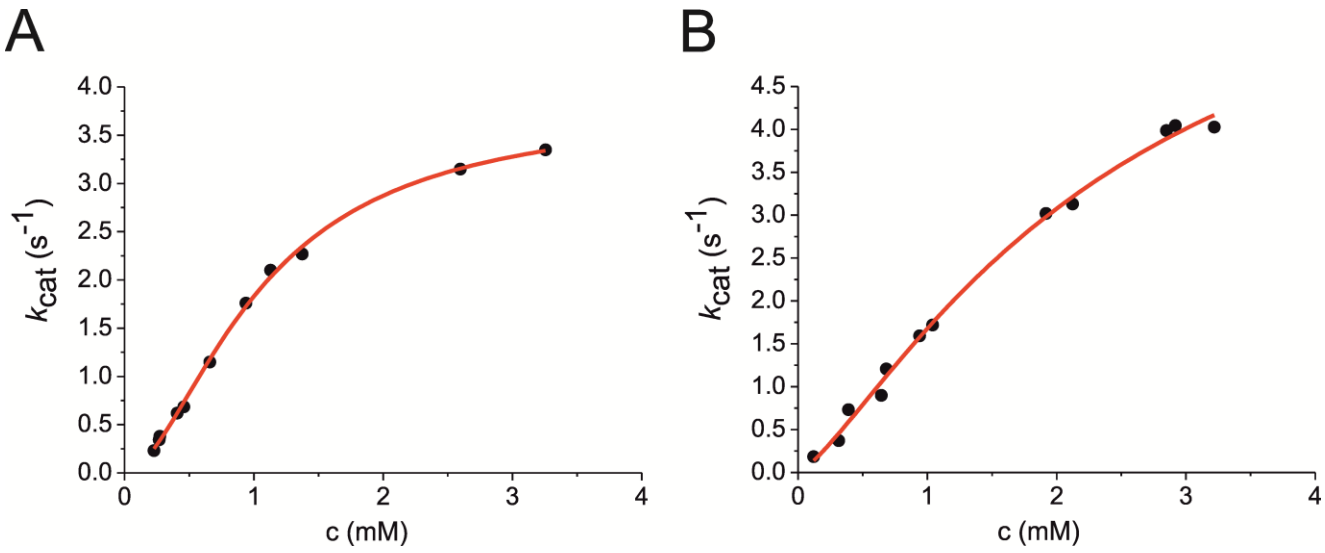

Figure S2. Steady-state kinetics of haloalkane dehalogenase DpcA with 1-bromobutane (A) and 1,3-dibromopropane (B). Red curve represents non-linear fit of Hill equation to the experimentally observed data.

References

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