

**Application of stable isotope peptide mass spectrometry
to decipher amino acid metabolism in *Dehalococcoides*
strain CBDB1**

Ernest Marco-Urrea, Jana Seifert, Martin von Bergen, Lorenz Adrian

Supplementary Information

FIG. S1. Derivatized amino acids and fragmented species identified by GC-MS. a) Derivatized amino acid; b) fragment $[M-59]^+$ that contained the entire amino acid after releasing a 59 Da fragment from the carboxy terminus; c) fragment $[M-87]^+$ that was obtained after a 87 Da fragment with the the carboxylic group of the amino acid was removed from the amino acid; d) fragment $[f153]^+$ that released the side-chain of the amino acid and the isopropyl group; this fragment contained the C1 and the C2 of the amino acid e) fragment $[f126]^+$ that was freed after a 87 Da fragment containing the carboxylic group was removed from the carboxy-terminus and the side-chain of the amino acid was removed; this fragment contained only the C2 and the amino group of the amino acid; and f) fragment $[f139]^+$ which was only found for derivatized glutamine. The fragmentation sites are indicated with red lines.

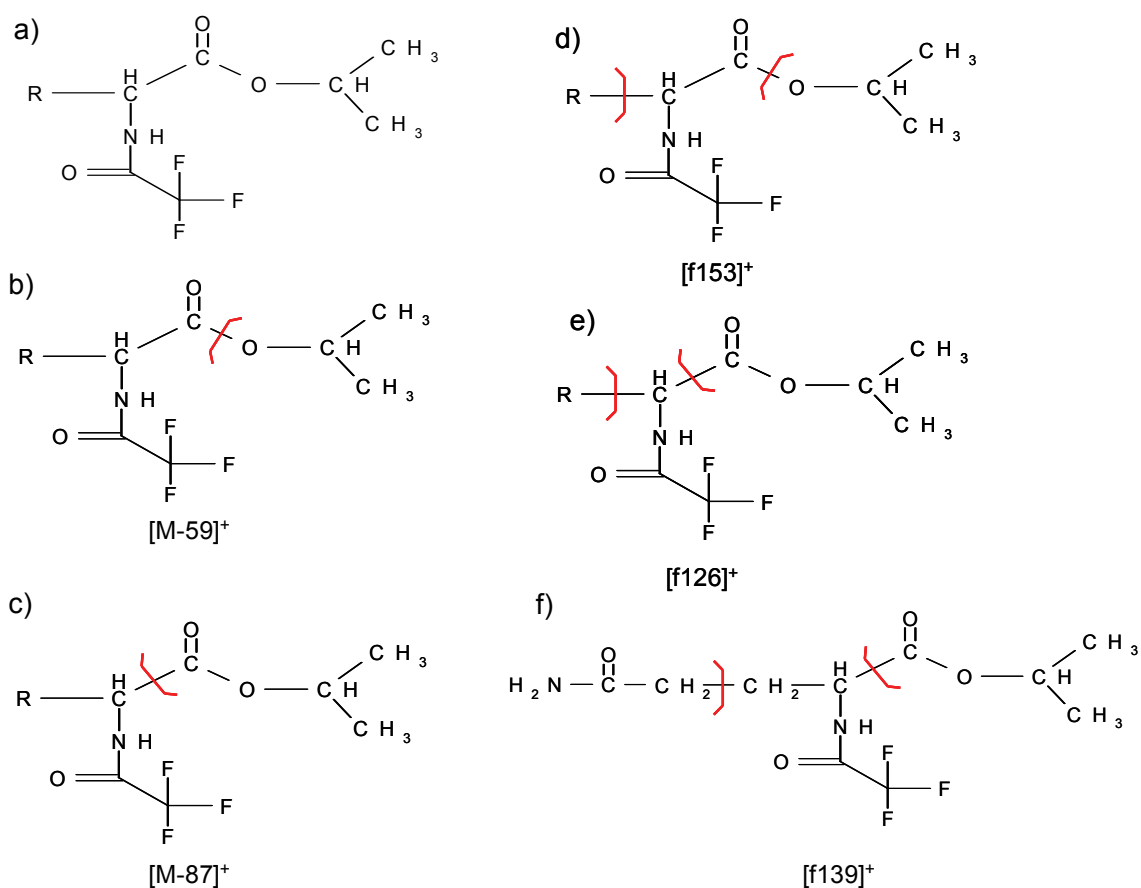


FIG. S2. The pentose phosphate pathway in *Dehalococcoides* CBDB1. Highlighted carbons indicate that they were synthesized from the C1 of acetate (green), the C2 of acetate (red) or from bicarbonate (blue). For the sake of clarity, dotted boxes are used to highlight the transfer of C1/C2 units. Double-headed arrows indicate reversible reactions.

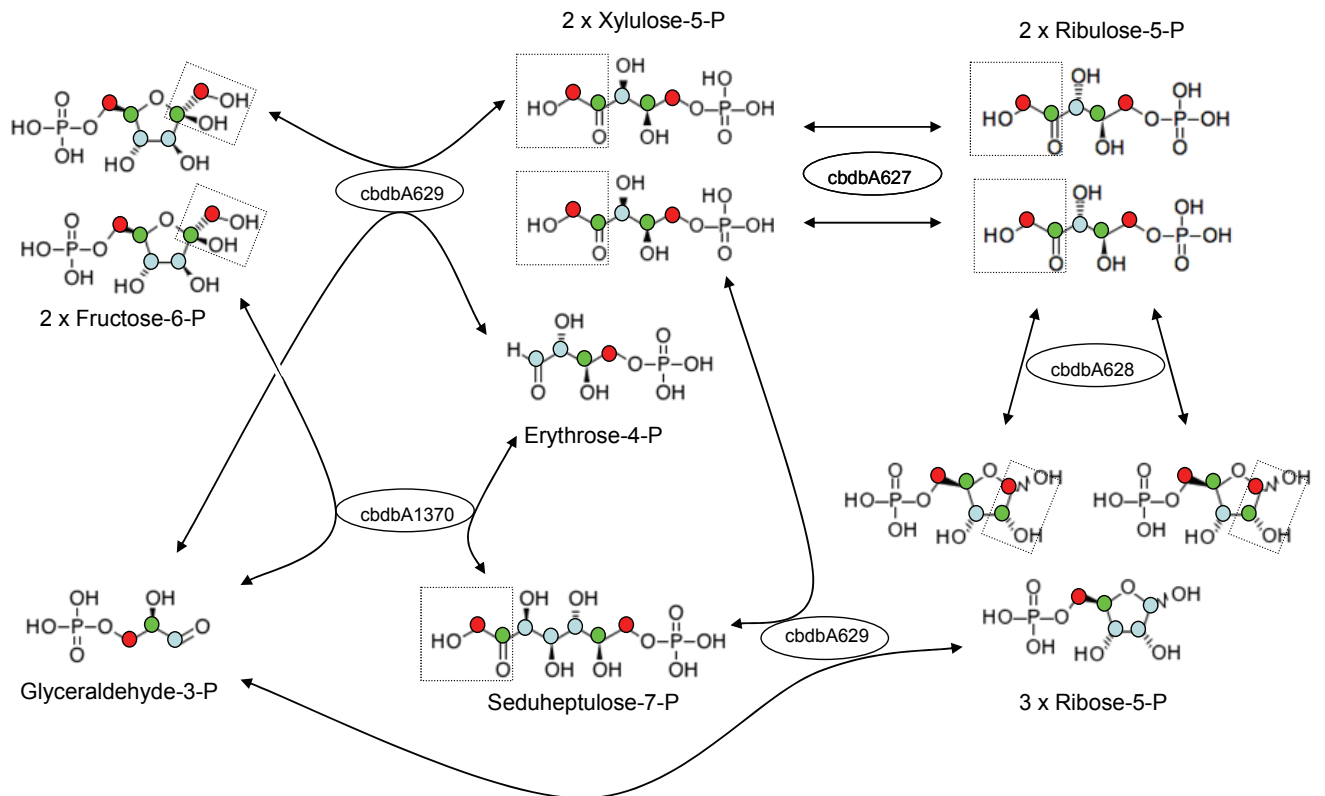


TABLE S1. List of peptides grouped by gi-numbers of the protein from which they are stemming, selected to calculate the specific isotope incorporation from labeled carbon sources into amino acids by peptide mass spectrometry. The experiment was done in triplicate and the selected peptides are shown in replicate 1, 2 and 3, respectively. Abbreviations for amino acids according to the one-letter code.

Replicate 1

gi73748963	VSVEQLGAEK		
gi73749113	AQIEETESAFDR	TTIIDGEGSAEAIKDR	IETVAELLPALEK
	LEGDEATGVNIVR	GVDTLANTVR	GNLNILAVK
	NIAAGAEPINLK	VEDALAATR	GYISAYFVTDPR
	QQIVQVATITGK	TTIIDGEGSAEAIKDR	DGNVIVDK
gi73748309	ILIGSDLLSETATLLR	LGLCENETALR	SIGLNLSGR
	LVIADTGCLK	LIADFGLPTNPK	ASIVCQDETDR
gi73748401	FLEANFASVLTAIAK	TAIALDTIINQK	DALVVYDDLTK
gi73749084	TTQELSTALQSVGSAVYK	NQAILNPENTVYSIK	NVADNLAYNAEK
	IPAELNTELESK	VIQGNNEVR	
gi73748746	LEVWDSPNSAGVVIDAVR	TNAVTSQLDYK	
gi73748078	QWIIQAQNTNPEAGIAR	LESLLGELDGAK	ALVIFGSAPGGR
gi73748120	TEAIASLGATLDNEECYLLSK	SELEVISSLLSR	TLTNPNCVFQLLK
	LSWTYSTNPSAADVAK	ALGIVYLDLSQAR	SGSEIAFIGGLIK
	LSTASSLEALAASFGR	NAPFNQSGGLPFK	ISSLQQLESPEDLR
	AGDQSAASLLK		
gi73749328	ANVSGYGNIDR		
gi73748484	INGNIYSINTDLIGR	LVGVTTYCNYPEAAK	IVEALEQLAK
gi73748315	ILDQSALQIVEALER		
gi73748444	AGQQLTDGSINPDILSILGR	AVVQSDQNVVAR	LSIQAFEPVLIDGSALR
gi73748193	VFGCGGGGCNAVTR	VDTLIIPNDR	TEDELDPVPSFLR
gi73748855	AACPAANIEGVTLQR	FLQAYGIDATVPFLAR	DAAQIASALR
gi73748473	VIGCGGAGSNAVTR		
gi73748913	LYGAGQDLLCDAFSGNVK		
gi73748779	SDIDLICELLADAK		
gi73748310	SIPFAYFSSLPGVK	YNLDLAAVPIALK	TIIGLIGDEK
gi73748490	VGGYEIGGLAGVILGAAANR	AISLNNPDAK	
gi73748328	LFFITLPR		
gi73748567	LAGENAAAVAK		
gi73748652	ATSQISQTTLR	NVLGQSELDILLSQR	
gi73748430	LGDLIAYIAK	IPSASVSLVDFVGELAR	
gi73748429	ALFLISAPAK	INELGAEIAR	SISSVADAVILVENQR
gi73748806	QGNVATFSPIR		
gi73748066	ADLLAEAYER		
gi73748403	IFNVLGEALDNR	SLAEQALYPAVDPLANSR	TVLIQELIR

Replicate 2

gi73748120	TDNNTNYSYVNAIK TLTNPNCFVQLLK ALGIVYLDQAR ASNGEPVIDK	SELEVISSLLSR LSTASSLEALAASFGR LSWTYSTNPSAADVAK	SGSEIAFIGGLIK NAPFNQSGGLPFK AADGTVPTITGR
gi73748855	FLQAYGIDATVPFLAR DVAVGLPPLNQVLAR	SDVGGVILGLK IILQNLK	AACPAANIEGVTLQR
gi73749113	TTIIDGEGSAEAIK GVDLANTVR IETVAELLPALEK DGVITIEESR	AQIEETESAFDR VEDALAATR GNLNILAVK	LEGDEATGVNIVR NIAAGAEPINLK GYISAYFVTDPPGR
gi73748484	LVGVTTYCNYPEAAK	IVEALEQLAK	
gi73748065	LSEPYCLFLVGCK		
gi73748779	SDIDLICELLADAK		
gi73748807	LAEQCNQFLSK		
gi73749084	TTQELSTALQSVGSAVYK IPAELNTELESK VIQGNNEVR	NQAILNPENTVYSIK IIDWLIAEYK	NVADNLAYNAEK TALQGNDVEAIK
gi73748403	VIDLIAPFAR	TVLIQELIR	
gi73748567	AAIYAAEEATAK QTAEVAQSAR	LAGENAAVAK	LATAVDEAGR
gi73748343	TLAENIEAK	VISLPNAGDIDAR	FDAQTVVEYYSR
gi73748915	LLTEFVSSLPNDK	YLLDVLGVLK	
gi73748263	DGVEITQDQFFER	LSGTYSIAIQAK	
gi73748309	NIVGSFYQPR	ILIGSDLLSETATLLR	LIADFGLPTNPK
gi73749328	ANVSGYGNIDR		
gi73748430	IPSASVSLVDFVGELAR	AVALVPELK	LGDLIAYIAK
gi73748353	FVVGPPVSDTGFTGR	NLVAAGLADR	

Replicate 3

gi73748353	FVVGPPVSDTGFTGR	ILVDTYGGFAR	ICDQISDAVLDAIIAK
gi73748490	AISLNNPDAK		
gi73748650	AEDCYEVTDLIEDK	NAYEIANLIR	LGFEDELVR
gi73748017	QTFNQAQYDNR	LQNLITPEFGPLIR	
gi73749328	VEPVSGVPVYSDTTTTLK	ANVSGYGNIDR	
gi73748064	DGQGVYGPVEQALIGTK	DNDNPFELVR	
gi73748811	VEEVQQNTVQAVK DSQTAIDKADTTAK	ASESALLK	DNCEAAVGQTK
gi73748963	VSVEQLGAEK		
gi73748737	ANQELIDLQDEIR	LADAAITIR	VILNPNVPIISR
gi73748309	SAAIGSSEIFR SIGLNLSGR	LIADFGLPTNPK LLETNTQAVK	ILIGSDLLSETATLLR
gi73748193	VFGCGGGGCNAVTR		
gi73748278	NTVPFAENQTANVPAGIR		
gi73748443	GEDVLQPGVPESFK	VLAAGSTISEDSFK	IGAEVGPDDILVGK
gi73748764	ILDTAEPGDAVGLLLR	AYDQIDNAPEEK	
gi73749113	TTIIDGEGSAEAIKDR TTIIDGEGSAEAIK	WGAPTVIDDGVTIAR LEGDEATGVNIVR	AQIEETESAFDR VEDALAATR

	GVDTLANTVR	GNLNILAVK	IETVAELLPALEK
	NIAAGAEPINLK	NIAAGAEPINLKR	QQIVQVATITGK
	KIETVAELLPALEK		
gi73748944	LSPLALIQK	QDLDAFIDSSQR	
gi73748304	NNDPFNIQSDQIR	QNISQSDVNCPDLAASK	
gi73749084	IINEPTAAALAYGLDK	TTQELSTALQSVGSAVYGK	GVNPDEVVAIGAAIQAGVLK
	NQAILNPENTVYSIK	NVADNLAYNAEK	IPAEINTELESK
	VTEAVITVPAYFNDAQR	VIQGNNEVR	
gi73748401	ELAAFAQFGTSELDK	GQDIVSIK	
gi73748635	VIVACVEGGR		
gi73748855	VDNIGDLFSCAAILNTSNLPR	GLENLYETPEELAVDVPDPSK	DAAQIASALR
	ALLEGLSEESSR	AACPAANIEGVTLQR	
gi73749370	LAANDYFSQFIGR		
gi73748779	SDIDLICELLADAK	AAFYCPICNNEFVWSAK	
gi73748120	TLTNPNCVFQLLK	DGVTSQTEVFLLPTACSYEK	LSTASSLEALAASFGR
	LQTLYQGEGSGLNAEAITK	TDNNTNYSYVNAIK	LPGYLSAPNEDDTTFAAYASK
	ISSLQLESPEDLR	SGSEIAFIGGLIK	LSTASSLEALAASFGR
	AGDQSAASLLK	LSWTYSTNPSAADVAK	
gi73748452	SLQNAQFTPGR	SVCDTCPTIR	
gi73748066	YCQNACPFPIPK		
gi73748797	ILAVEPASCPSLTK		
gi73749066	IIDNACNEDFAR		
gi73748634	TPVICGLAR		
gi73748710	VGLSVCYTGPAAEK		
gi73748529	SESSLSATCPVCSAESTR		
gi73748430	IISNASCTTNCVAPLIK		
gi73748065	LSEPYCLFLVGCK		
gi73749289	TLEFGDVEECVETPR		
gi73749091	ALAGTAGLDCGVSYTADFGK		
gi73748637	LTGAQIVCESLLK		
gi73748075	NLGVSEVNLVCSR		
gi73748576	NAAVIADLVGDNVGDCAGR		
gi73748913	LYGAGQDLLCDAFSGNVK		
gi73748594	AADTGADIVGK		
gi73748429	ALFLISAPAK		
gi73749102	SNAIVLAK		
gi73748746	APGSTADIVK		
gi73748877	FNEFITSK		
gi73748567	LAGENAAAVAK		
gi73748327	NDNVLVIAGK		

TABLE S2. Number of amino acids identified in peptides of cultures of strain CBDB1 grown with different labeled carbon sources. Numbers are given for all three replicates of each treatment. All amino acids matched with the labeling pattern model given in Table 2 and no differently labeled amino acids were found. The peptides analyzed for each replicate are listed in Table S1. Treatments: 1C: cultures containing 5 mM of [1-¹³C]acetate and 30 mM unlabeled bicarbonate; 2C: cultures with [2-¹³C]acetate and 30 mM unlabeled bicarbonate; Carb: cultures with 5 mM unlabeled acetate and 30 mM [¹³C]bicarbonate.

		Gly	Ala	Ser	Pro	Val	Thr	Cys	Ile	Leu	Asn	Asp	Gln	Lys	Glu	Phe	Arg	Tyr
Rep. 1	1C	22	46	26	10	16	17	6	19	33	21	18	13	4	24	10	6	8
	2C	33	69	35	18	29	29	20	35	34	30	33	21	4	35	15	8	13
	Carb	39	74	36	16	41	30	18	42	44	30	33	25	8	44	19	10	9
Rep. 2	1C	27	35	20	7	14	13	4	19	28	19	18	8	3	14	7	5	9
	2C	22	48	34	11	28	22	2	24	52	20	28	18	5	27	9	6	12
	Carb	23	45	20	8	27	23	5	25	49	20	19	16	4	29	6	7	6
Rep. 3	1C	12	34	8	9	11	15	3	13	15	19	8	10	4	15	12	6	13
	2C	15	31	15	7	18	12	1	13	28	13	11	7	2	16	7	7	8
	Carb	15	44	15	11	15	12	1	16	30	19	13	11	4	18	8	8	6

TABLE S3. Number of labeled amino acids identified in peptides from cultures that were grown with a labeled carbon source and unlabeled amino acids. All identified amino acids had isotopic labeling patterns as shown in Table 2. The peptides analyzed were the same as those shown in Table S1 for replicates 1 and 2. Treatments: 1C: cultures containing 5 mM of [1-¹³C]acetate and 30 mM unlabeled bicarbonate; 2C: cultures with [2-¹³C]acetate and 30 mM unlabeled bicarbonate; Carb: cultures with 5 mM unlabeled acetate and 30 mM [¹³C]bicarbonate.

		Gly	Ala	Ser	Pro	Val	Thr	Cys	Ile	Leu	Asn	Asp	Gln	Lys	Glu	Phe	Arg	Tyr
Rep. 1	1C	16	33	11	8	10	8	3	9	19	13	10	6	2	11	10	6	9
	2C	11	31	23	4	19	14	1	16	42	14	16	10	1	16	6	7	10
	Carb	29	58	23	9	27	20	5	25	58	19	22	17	4	33	13	10	4
Rep. 2	1C	9	21	9	3	4	10	2	10	13	7	7	4	0	10	7	4	8
	2C	8	22	11	4	11	9	1	5	16	8	7	3	0	7	3	4	6
	Carb	11	40	10	4	12	12	1	16	19	15	11	6	2	21	5	6	7