

40 the *ribL*10 transcript (14) were used (lower panel). For primer sequences see Table S1. In a

- 41 negative control, RNA was used that was not reverse transcribed to exclude contamination by
- 42 genomic DNA. L, base pair ladder.



Fig. S3. Generation of the *ptfC* deletion mutant and verification of in-frame deletion strains by Southern blot analysis. (A) Construction of the *ptfC* deletion mutant. Wild type *ptfC* (long arrow), $\Delta ptfC$ (short arrow), flanking regions and restriction sites (*PciI* and *EcoRV*) are shown. The hybridisation probe is indicated by a bar. Successful deletion was verified by Southern blot analysis after cleavage with the restriction enzymes indicated. The *pfK* (B) and *fba* (C) deletion mutants were analyzed in the same way. For restriction enzymes and primer sequences see Table S2.

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Fig. S4. Growth of *H. volcanii* wild type (•) and deletion strains (A) $\Delta ptfC$, (B) $\Delta pfkB$ and (C) Δfba (each \blacksquare) on 25 mM glucose. Precultures were grown in complex medium containing 1 % Casamino Acids (A) and (B) or in synthetic medium containing 25 mM glucose (C).



FIG. S5. Growth of *H. volcanii* wild type, *fba* mutant and complementation strain on different substrates. The cells were grown on minimal medium with (A) xylose, (B) acetate (each 25 mM) or (C) 1% Casamino Acids. With each substrate, wild type (•), Δfba (•) and the complementation strain (•) were grown. Precultures were grown on synthetic medium with 20 mM glucose. Growth was measured by determining the optical density at 600 nm.

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Haloferax volcanii	MPFYGGEELATVYDEALDEGFGLIASNIAEPNIMMGLMEGADRMDSDLLLQMSGGACRFAGD	62
Halogeometricum borinquense	MPFYRGNELAKVYDDALDEGFGLIASNIAEPNVMMGLMEGAARENSDLLLQLSGGAATFAGN	62
Mycoplasma pneumoniae	MLVNIKQMLQHAKQHHYAVPHININNYEWAKAVLTAAQQAKSPIIVSTSEGALKYMSG	58
Helicobacter pylori	MLVKGNEILLKAHKEGYGVGAFNFVNFEMLNAIFEAGNEENSPLFIQASEGAIKYMG	57
Borrelia burgdorferi	-MGVLDKIKPGVVYGKELHFLYEICKKEGFAIPSINCIGTNSINAVLEAAKEINSPIMIQFSNSGSAFISGKGLKMEKPQ	79
Escherichia coli	MSKIFDFVKPGVITGDDVQKVFQVAKENNFALPAVNCVGTDSINAVLETAAKVKAPVIVQFSNGGASFIAGKGVKSDVPQ	80
Haloferax volcanii	GDAVAGLKAMGNYIETIAEFYDIGVFLNMDHQTDLEFIEQQIELDIPSSIMIDASHEPFDENVATSRE	130
Halogeometricum borinquense	EDPVSGLKAMGTYIESIAEQYDIGVFLNMDHQTDMDFIEAQMDLGIPSSIMIDASHEPFEENVERSRE	130
Mycoplasma pneumoniae	HQVVVPMVKGLVDALEITVPVALHLDHCSYEGCKAALQAGFSS-IMFDGSHLPFQENFTKSKE	120
Helicobacter pylori	IDMAVGMVKTMCERYE-HIPVALHLDHGTTFESCEKAVKAGFTS-VMIDASHHAFEENLELTSK	119
Borrelia burgdorferi	GVSIVGAISGAMHVHLMAEHYGVPVVLHTDHCAKNLLPWVEGLLEYGEKYYSQHKKPLFSSHMLDLSEEPIKENIEISKK	159
Escherichia coli	GAAILGAISGAHHVHQMAEHYGVPVILHTDHCAKKLLPWIDGLLDAGEKHFAATGKPLFSSHMIDLSEESLQENIEICSK	160
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Haloferax volcanii	VVEMVEAAGSDVIIEAELGQIKGVEDEIEAEEAFYTDPEQAVEFVDKTGADLLAISVGTQHGVAKGKDLELR	202
Halogeometricum borinquense	VVQMKEDKGADVIIEAELGQIKGVEDEIEAEEAFYTDPEQAVEFVDRTGCDLLAISVGTQHGVAKGKDLELR	202
Mycoplasma pneumoniae	LIELAKQTNASVELEVGTLGGEEDGIVGQGELANIEECKQIATLKP-DALAAGIGNIHGLYPDNWKGLN	188
Helicobacter pylori	VVKMAHNAGVSVEAELGRLMGIEDNISVDEKDAVLVNPKEAEQFVKESQVDYLAPAIGTSHGAFKFKGE	188
Borrelia burgdorferi	FLERMAKIEMELEIELGITGGEEDGVDNSDRALHELFSTPEDIYYGYSELLKVSPNFQIAAAFGNVHGVYKPGNVKLT	237
Escherichia coli	YLERMSKIGMILEIELGCTGGEEDGVDNSHMDASALYTQPEDVDYAYTELSKISPRFTIAASFGNVHGVYKPGNVVLT	238
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Haloferax volcanii	PDLANDIRQALRDHGLDTPLVLHGSSGVQPDQLQEMLKHG-ICKVNKDTRYQYEYTRTAYDRYNEEPNAIVPPEG	276
Halogeometricum borinquense	PDLARD IRVALRDHG LDTPLVLHGSSGVQPDQLQEMLQHG - ICKVNKDTRYQYEYTRTAFDHYREDPEAIVPPEG	276
Mycoplasma pneumoniae	YELIEAIAKATNLPLVLHGGSGIPEADVKKAIGLG-ISKLNINTECQLAFAKAIREYVEAKKDLDTHNKG	257
Helicobacter pylori	PKLDFERLQEVKRLTNIPLVLHGASAIPDDVRKSYLDAGGDLKGSKGVPFEFLQESIKGGINKVNTDTDLRIAF	262
Borrelia burgdorferi	PKVLKDGQDYVISKTGVNMAKPVSYVFHGGSGSTIDEINEALSYG-VVKMNIDTDTQWAAWEGVLNYYKKNESRLQGQLG	316
Escherichia coli	PTILRDSQEYVSKKHNLPHNS-LNFVFHGGSGSTAQEIKDSVSYG-VVKMNIDTDTQWATWEGVLNYYKANEAYLQGQLG	316
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Haloferax volcanii	VADARDTFFNETDWSPNKDVFDPRVAGRDIRERIADVHADLTEVSGSAGQSLFK 330	
Halogeometricum borinquense	VEDARDTFFNETEWSPNKDVFDPRVVGRKIRERIADVHADLTHVSGSANQSRYV 330	
Mycoplasma pneumoniae	288	
Helicobacter pylori	IAEVRRVANEDKSQFDLRKFFSPAQLALKNVVKERMKLLGSANKI 307	
Borrelia burgdorferi	DGKDIDIPNKKFYDPRVWLREAEVSMKDRVKIACKNLNNINRN 359	
Escherichia coli	NPKGEDQPNKKYYDPRVWLRAGQTSMIARLEKAFQELNAIDVL 359	

178 FIG. S6. Multiple amino acid sequence alignment of Class II fructose-1,6-bisphosphate 179 aldolases from the haloarchaea H. volcanii and H. borinquense and from selected bacteria. 180 The alignment was generated with ClustalX using the Gonnet Matrix (47). Consensus patterns 181 (ALDOLASE CLASS II 1 and 2) are marked by boxes. The ALDOLASE CLASS II 1 182 consensus pattern is [FYVMT]-x(1,3)-[LIVMH]-[APNT]-[LIVM]-x(1,2)-[LIVM]-H-x-D-H-[GACH] (PS00602) and the ALDOLASE CLASS II 2 pattern is [LIVM]-E-x-E-[LIVM]-G-183 184 x(2)-[GM]-[GSTA]-x-E (PS00806). Conserved amino acids are marked by asterisks. 185 Additionally, the important catalytic residues R300, E155 and D92 (
) and histidines that 186 ligate the divalent cation H93, H191, H225, (•) are depicted (22). Accession numbers for the 187 aldolases are as follows: Borrelia burgdorferi (AAB91507), Escherichia coli (BAE76989), 188 volcanii (YP 003535543), Halogeometricum borinquense (ADQ67306), Haloferax 189 Helicobacter pylori (CBI66947) and Mycoplasma pneumoniae (BAL21593).

Supplemental Tables

Gene	primers for RT-PCR	sequence 5'-3'	length PCR product [bp]
RibL	RibL10-RT_F	ACAGCACAATATGGGCGACCTGC	227
	RibL10-RT_R	GTCCTCGACGTCGCAGTAGATGG	557
HVO_1499	HVO_1499RT_s	CAAACGACGCAGAAGACGCAGTTC	706
	HVO_1499RT_as	CCCTCGGAGATGAGGCCGAC	/00

	primers for probe		
HVO 1499 HVO 1499Northern s HVO 1499Northern as		CAAACGACGCAGAAGACGCAGTTC	706
		CCCTCGGAGATGAGGCCGAC	700
HVO_1500	HVO_1500Northern_s2	GACCGCCTCGGGCTTTCTCG	707
	HVO_1500Northern_as2	CGTCTTCGAGGTCCGGGACG	/0/
HVO_1494	HVO_1494Northern_s	GGACCACCAGACGGACCTCG	719
	HVO_1494Northern_as	CTTGAACAGCGACTGGCCGGC	/18

	primers for overexpression and complementation				
HVO_1500 HVO_1500_OE_s		CGGAGTCGCA <u>TCATGA</u> TTCTCAC	1000		
	HVO_1500_OE_as	GTGCTCA <u>GGATCC</u> CTTTCGAATATG	1000		
HVO_1494	HVO_1494_OE_s	TTGCG <u>CATATG</u> CACCACCACCACCACCACA TGCCGTTCTACGGCGGGGGGG	1077		
	HVO_1494_OE_as	CGCGATGC <u>GGATCC</u> GACGAGC			
HVO_1499	HVO_1499_OE_s	GTGGCAACA <u>CCATGG</u> CAAACGAC	1248		
	HVO_1499_OE_as	GCCGGC <u>GGATCC</u> TCGAGCG	1240		

Table S1 Primers used for RT-PCR, Northern blot probe generation, overexpression and complementation. Restriction enzyme cleavage sites were introduced in primers for overexpression and complementation (underlined). For HVO_1500 *BspHI* and *BamHI* sites were included, primers for HVO_1494 contain *NdeI* respectively *BamHI* sites and for HVO_1499 *NcoI* and *BamHI* sites were used.

H. volcanii	primers for deletion	sequence 5'-3'	length PCR product	length PCR fusion product	additional primer for Sth. Blot	sequence 5'-3'	length of probe [bp]	used enzymes	length in Sth. blot WT/∆ [bp/bp]
ΔHVO_1500	HVO_1500frgt1_s HVO_1500frgt1_as	GTGGGCTCATCGAGCGGTCG CACGTCTTCCGGTTCGTCGA AGTGAATCGTGTG	697 bp 13 672 bp	- 1347 bp	HVO_1500 probe_s	GGCAACACTATG GCAAACGACGC	506 bp	EcoRV, PciI	5532 / 4731
	HVO_1500frgt2_s	CTTCGACGAACCGGAAGACG TGCTGACGAACGAACGAACG							
<u>ΔHVO_</u> 1499	HVO_1499frgt1_s HVO_1499frgt1_as	GGATGTCGCTTTCCGACGTGGAC GCGATGACGGCCCCGGTCAT CAGGTCCTCCTTC	- 688 bp	– 1356 bp	HVO_1499 probe_as	GTTCGTCAGCAC GTCTTCGAGGTC	464 bp	EcoRV, PciI	5535 / 4546
	HVO_1499frgt2_s HVO_1499frgt2_as	GATGACCGGGGGCCGTCATCGCG ACGGCCATC GAGCTGTACGTCGGCGTCGAAC	— 689 bp						
<u>ΔHVO_1494</u>	HVO_1494frgt1_s	CACTAGACCAATGAAAC TCGTCGCAG CACGTCGGCGAGCGCCTCGTCG TACACTGTG	- 712 bp 675 bp	1368 bp	HVO_1494 probe_s	CCCACGTCGTTTA AGGCGTCACG	441 bp	PstI, SmaI	8109 / 7230
	HVO_1494frgt2_s	CGAGGCGCTCGCCGACGTGC ACGCCGACC CGGCGGCAAGAA AGGTGAACAGG							

 Table S2: Primers used for synthesis of deletion mutants and Southern probes.