Supplemental Figures

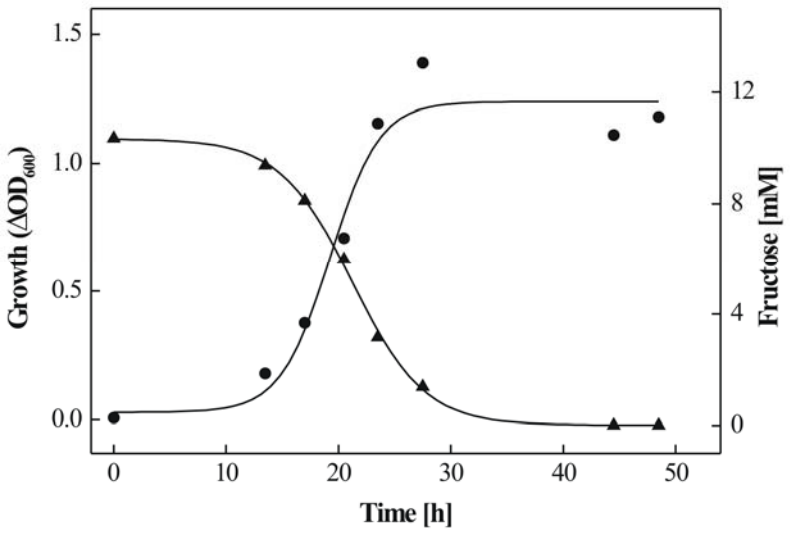


Fig. S1. Growth of *H. volcanii* H26 on fructose at 42 °C in minimal medium with fructose as sole carbon and energy source. Growth (●) and fructose consumption (▲) was followed over time. The preculture was grown in minimal medium with 10 mM fructose.

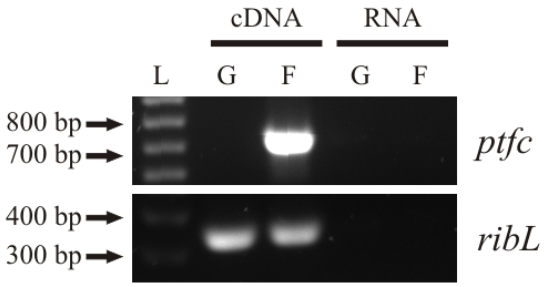


FIG. S2. RT-PCR of PTS component *ptfC* coding for EIIC of *H. volcanii* after growth on glucose and fructose. Total RNA of cells, grown on minimal medium with glucose (G) or fructose (F) was isolated, digested with DNAse and reverse transcribed to cDNA with random hexamers. From cDNA, PCR was performed with primers specific for *ptfC* encoding fructose specific PTS component EIIC (upper panel). As an unregulated control, specific primers for the *ribL*10 transcript (14) were used (lower panel). For primer sequences see Table S1. In a negative control, RNA was used that was not reverse transcribed to exclude contamination by genomic DNA. L, base pair ladder.

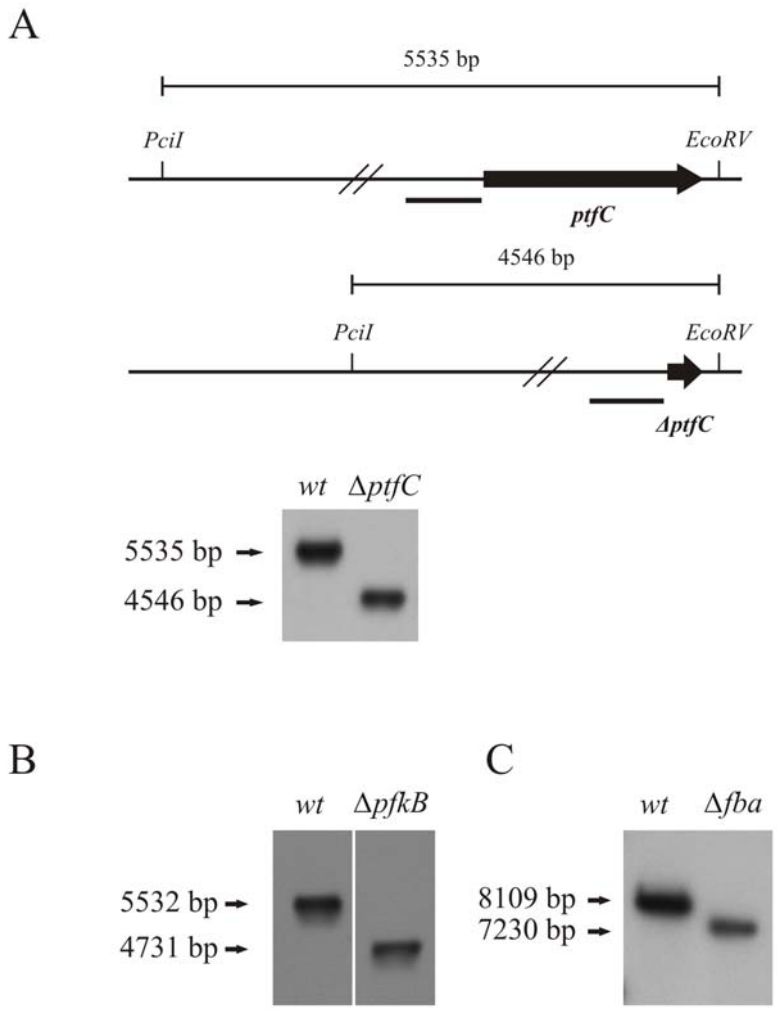


Fig. S3. Generation of the *ptfC* deletion mutant and verification of in-frame deletion strains by Southern blot analysis. (A) Construction of the *ptfC* deletion mutant. Wild type *ptfC* (long arrow), Δ*ptfC* (short arrow), flanking regions and restriction sites (*PciI* and *EcoRV*) are shown. The hybridisation 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.

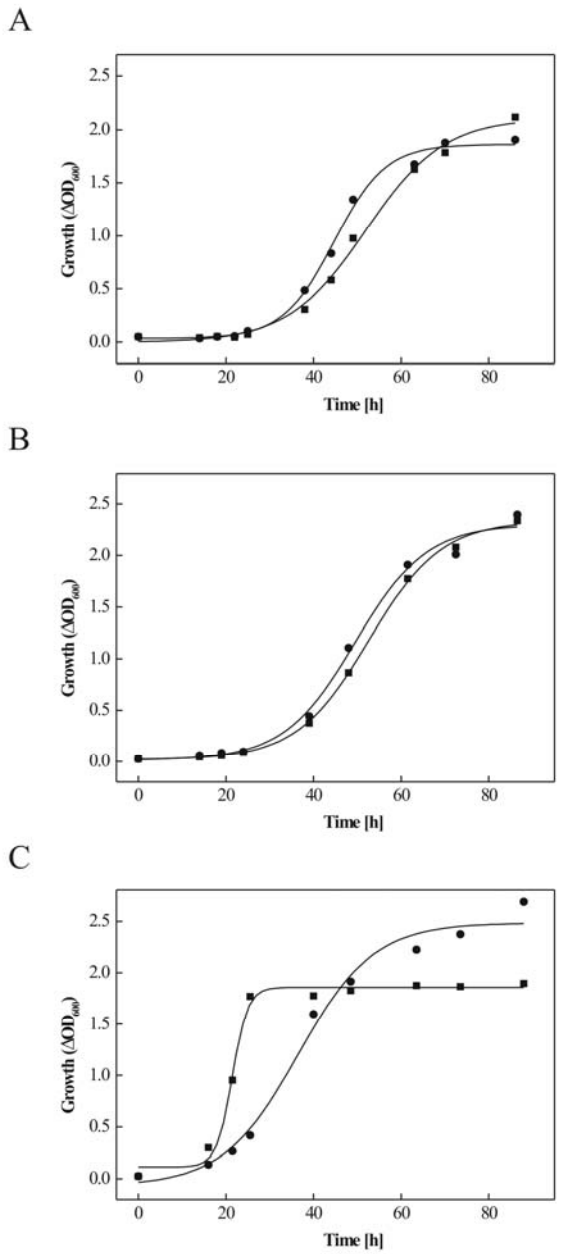


Fig. S4. Growth of *H. volcanii* wild type (●) and deletion strains (A) Δ*ptfC*, (B) Δ*pfkB* and (C) Δ*fba* (each ■) on 25 mM glucose. Precultures were grown in complex medium containing 1 % Casamino Acids (A) and (B) or in synthetic medium containing 25 mM glucose (C).

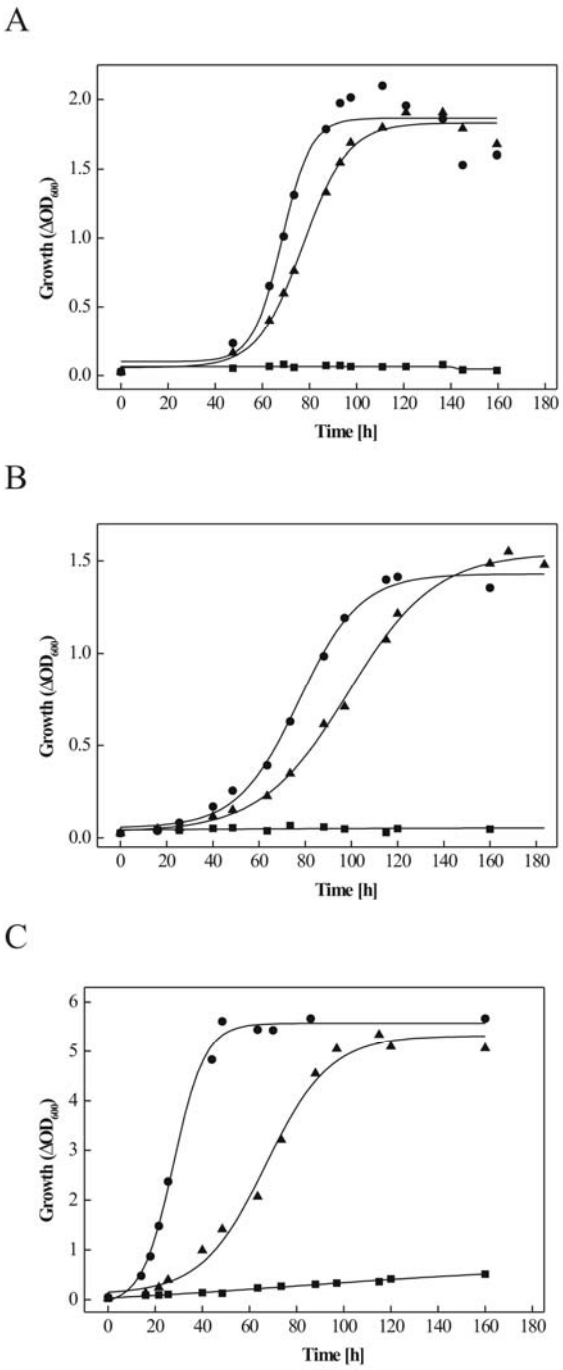
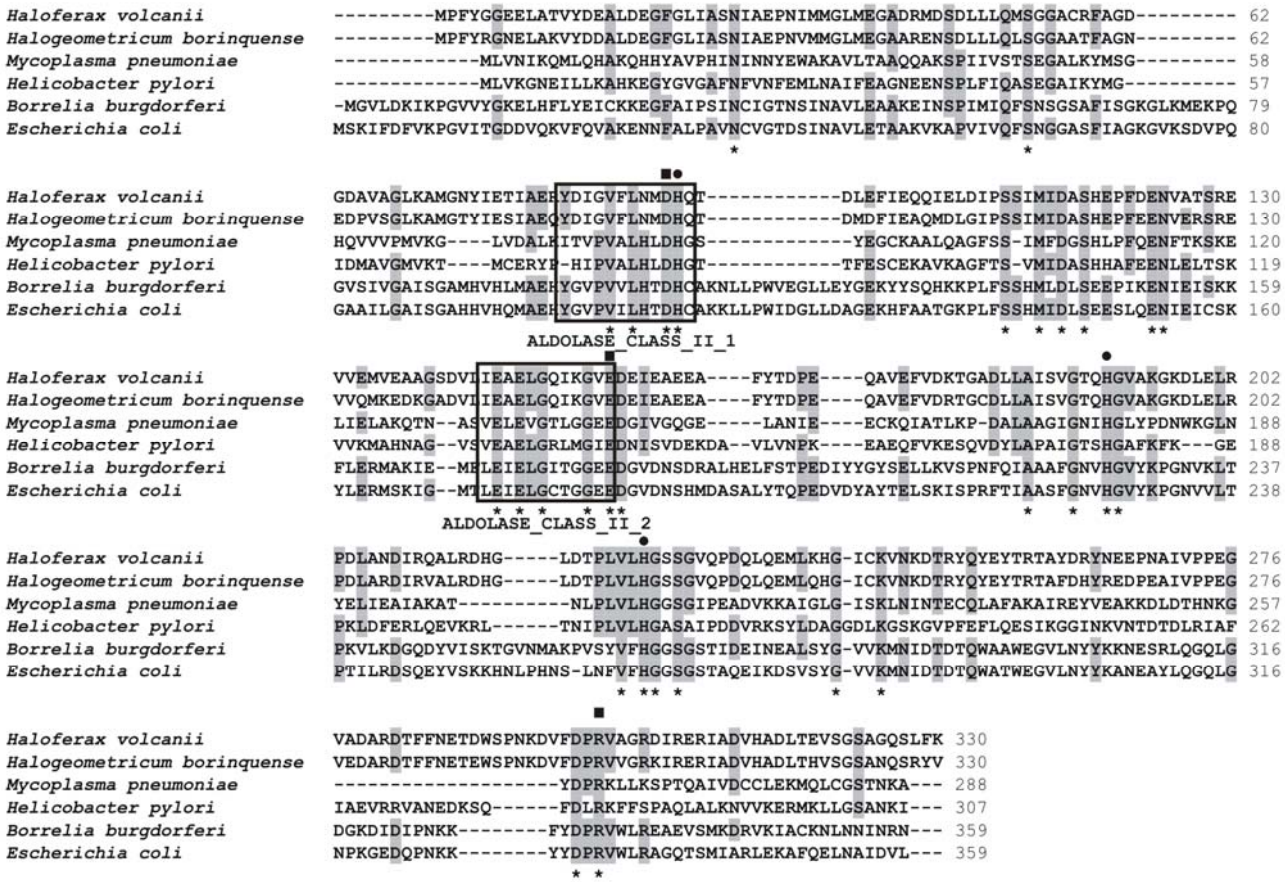


FIG. S5. Growth of *H. volcanii* wild type, *fba* mutant and complementation strain on different substrates. The cells were grown on minimal medium with (A) xylose, (B) acetate (each 25 mM) or (C) 1% Casamino Acids. With each substrate, wild type (●), Δ*fba* (■) and the complementation strain (▲) were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



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-
183 [GACH] (PS00602) and the ALDOLASE_CLASS_II_2 pattern is [LIVM]-E-x-E-[LIVM]-G-
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 *Haloferax volcanii* (YP_003535543), *Halogeometricum borinquense* (ADQ67306),
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	337
	RibL10-RT_R	GTCCTCGACGTCGCAGTAGATGG	
HVO_1499	HVO_1499RT_s	CAAACGACGCAGAAGACGCAGTTC	706
	HVO_1499RT_as	CCCTCGGAGATGAGGCCGAC	

	primers for probe		
HVO_1499	HVO_1499Northern_s	CAAACGACGCAGAAGACGCAGTTC	706
	HVO_1499Northern_as	CCCTCGGAGATGAGGCCGAC	
HVO_1500	HVO_1500Northern_s2	GACCGCCTCGGGCTTTCTCG	707
	HVO_1500Northern_as2	CGTCTTCGAGGTCCGGGACG	
HVO_1494	HVO_1494Northern_s	GGACCACCAGACGGACCTCG	718
	HVO_1494Northern_as	CTTGAACAGCGACTGGCCGGC	

	primers for overexpression and complementation		
HVO_1500	HVO_1500_OE_s	CGGAGTCGCATCATGATTCTCAC	1000
	HVO_1500_OE_as	GTGCTCAGGATCCCTTTCGAATATG	
HVO_1494	HVO_1494_OE_s	TTGCGCATATGCACCACCACCACCACCACA TGCCGTTCTACGGCGGGGAG	1077
	HVO_1494_OE_as	CGCGATGCGGATCCGACGAGC	
HVO_1499	HVO_1499_OE_s	GTGGCAACACCATGGCAAACGAC	1248
	HVO_1499_OE_as	GCCGGCGGATCCTCGAGCG	

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/Δ [bp/bp]
ΔHVO_1500	HVO_1500frgt1_s	GTGGGCTCATCGAGCGGTCG	697 bp	1347 bp	HVO_1500 probe_s	GGCAACACTATG GCAAACGACGC	506 bp	*EcoRV, PciI*	5532 / 4731
	HVO_1500frgt1_as	CACGTCTTCCGGTTCGTCGA AGTGAATCGTGTG							
	HVO_1500frgt2_s	CTTCGACGAACCGGAAGACG TGCTGACGAACGAAACG	672 bp						
	HVO_1500frgt2_as	GCGACCGGGATGAGGAGCAC							
ΔHVO_1499	HVO_1499frgt1_s	GGATGTCGCTTTCCGACGTGGAC	688 bp	1356 bp	HVO_1499 probe_as	GTTCGTCAGCAC GTCTTCGAGGTC	464 bp	*EcoRV, PciI*	5535 / 4546
	HVO_1499frgt1_as	GCGATGACGGCCCCGGTCAT CAGGTCCTCCTTC							
	HVO_1499frgt2_s	GATGACCGGGGCCGTCATCGCG ACGGCCATC	689 bp						
	HVO_1499frgt2_as	GAGCTGTACGTCGGCGTCGAAC							
ΔHVO_1494	HVO_1494frgt1_s	CACTAGACCAATGAAAC TCGTCGCAG	712 bp	1368 bp	HVO_1494 probe_s	CCCACGTCGTTTA AGGCGTCACG	441 bp	*PstI, SmaI*	8109 / 7230
	HVO_1494frgt1_as	CACGTCGGCGAGCGCCTCGTCG TACACTGTG							
	HVO_1494frgt2_s	CGAGGCGCTCGCCGACGTGC ACGCCGACC	675 bp						
	HVO_1494frgt2_as	CGGCGGCAAGAA AGGTGAACAGG							

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