Supplementary Data

D-Ribose catabolism in archaea:

Discovery of a novel oxidative pathway in *Haloarcula* species

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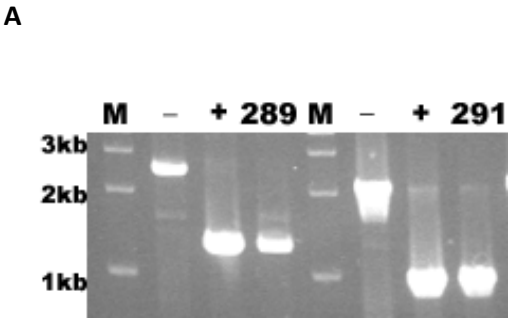
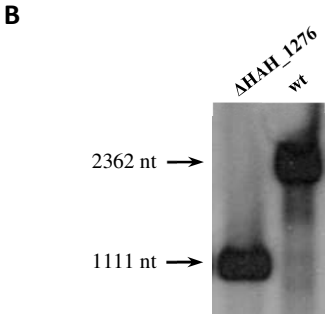
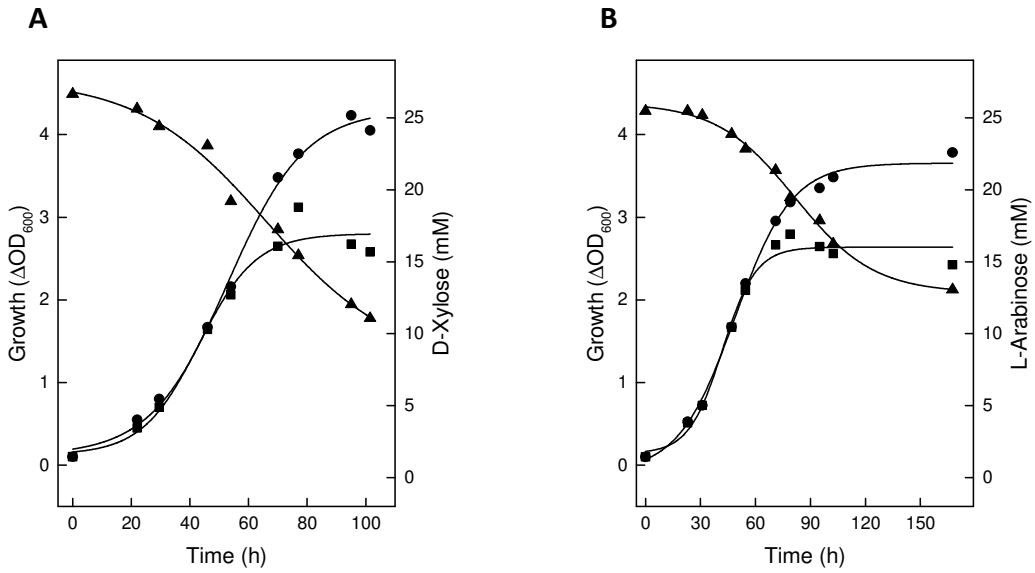
Supplemental Figure S1 Verification of successful deletion of HAH_0289, HAH_0291 and HAH_1276 in *Haloarcula hispanica* DF60. (**A**) ΔHAH_0289 and ΔHAH_0291 mutants were analyzed by PCR with the forward primer of fragment 1 and the reverse primer of fragment 2; wild type (-) and plasmid (+) were used as control. (**B**) ΔHAH_1276 mutant was analyzed by Southern blot analysis with EcoR1 treated genomic DNA of wild type (wt) and the ΔHAH_1276 mutant. Length of specific signals were determined with the DNA molecular weight marker III, DIG-labeled (Roche Diagnostics). The probe was amplified with the PCR DIG Probe Synthesis Kit (Roche Diagnostics, Germany).

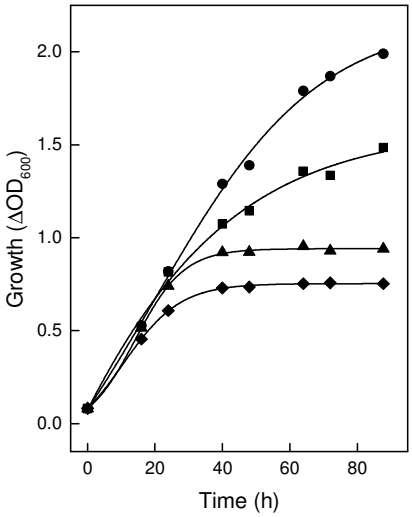




Supplemental Figure S2 Growth of *Haloarcula marismortui* on D-xylose and L-arabinose. Growth was performed at 37°C on 25 mM D-xylose (**A**) and L-arabinose (**B**) in the presence of 0.25% yeast extract and 0.5% casamino acids (circles); growth without the addition of pentoses (squares) and consumption of xylose or arabinose (triangles).

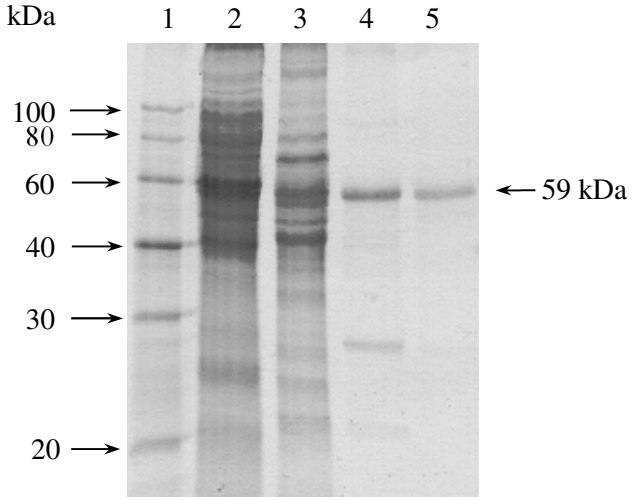


Supplemental Figure S3 Growth of *Haloarcula hispanica* on D-xylose, D-ribose and L-arabinose. Growth was performed at 37°C on 25 mM D-xylose (circles), D-ribose (squares) and L-arabinose (triangles) in the presence of 1 g/l yeast extract; growth without the addition of pentoses (diamonds).



Supplemental Figure S4 Purification of pentose dehydrogenase from *Haloarcula marismortui* cells grown on D-ribose. (**A**) Purification as analyzed by SDS-PAGE, Coomassie blue stained; Lanes: 1, molecular mass standard; 2, cell extract; 3, after Sepharose CL 4B; 4, after Phenyl-Sepharose; 5, after Superdex 200; (**B**) Enzyme activity was measured at 37°C as $NADP^+$ dependent oxidation of D-ribose. The assay mixture contained 1.5 M KCl, 1 mM $NADP^+$ and 10 mM D-ribose in 100 mM Tris-HCl, pH 8.8.

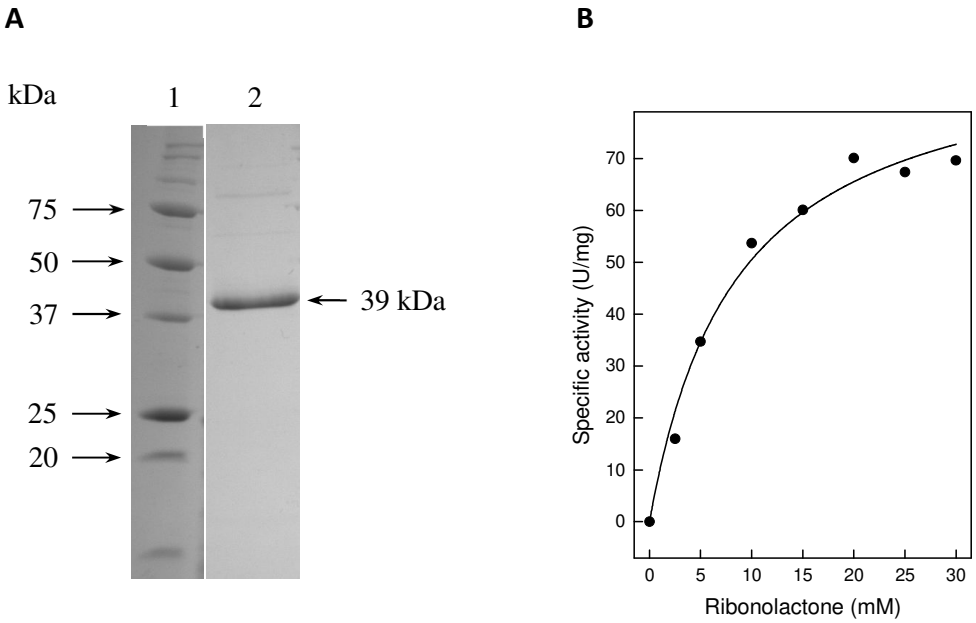
A



B

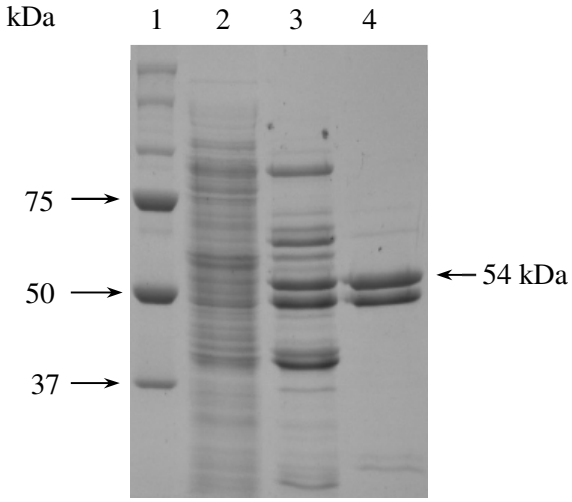
	Protein (mg)	Activity (U)	Sp. activity (U/mg)	Yield (%)	Purification (fold)
Cell extract	985	232	0.235	100	1
Sepharose CL 4B	22	124	5.61	54	24
Phenyl-Sepharose	1.14	40	36	17	153
Superdex 200	0.12	7.3	62	3.2	264

Supplemental Figure S5 Pentonolactonase of *Haloarcula hispanica.* (**A**) Coomassie blue stained SDS-PAGE. Lanes: 1, molecular mass standard; 2, protein after Superdex 200. (**B**) Rate dependence of pentonolactonase on the concentration of ribonolactone.



Supplemental Figure S6 Purification of xylonate dehydratase from *Haloarcula marismortui*. (**A**) Purification as analyzed by SDS-PAGE, Coomassie blue stained. Lanes: 1, molecular mass standard; 2, cell extract; 3, after Phenyl-Sepharose; 4, after Superdex 200. (**B**) Enzyme activity was measured at 37°C, the assay mixture contained 1.0 M KCl, 20 mM $MgCl_2$ and 10 mM D-xylonate in 100 mM Tris-HCl, pH 8.5.

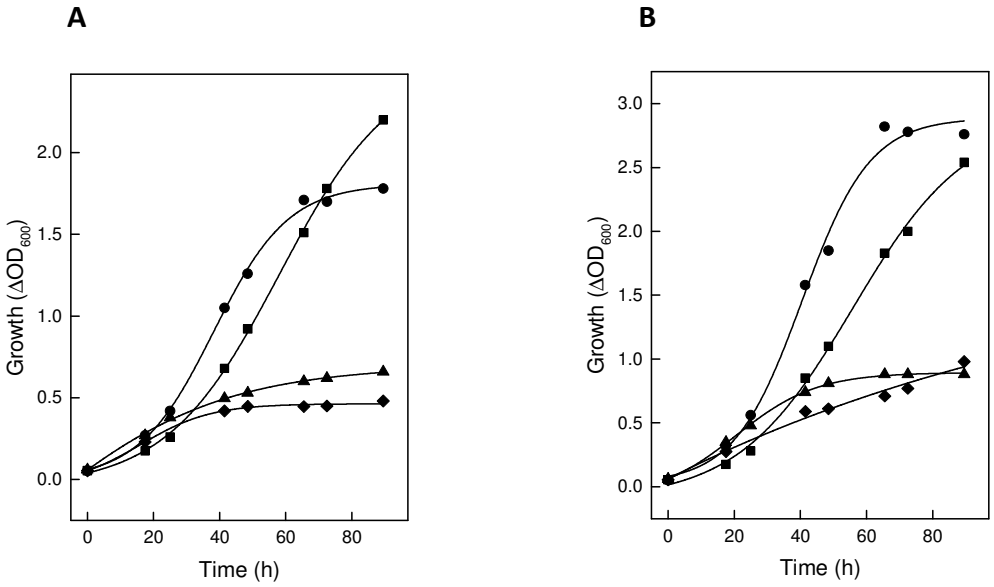
A



B

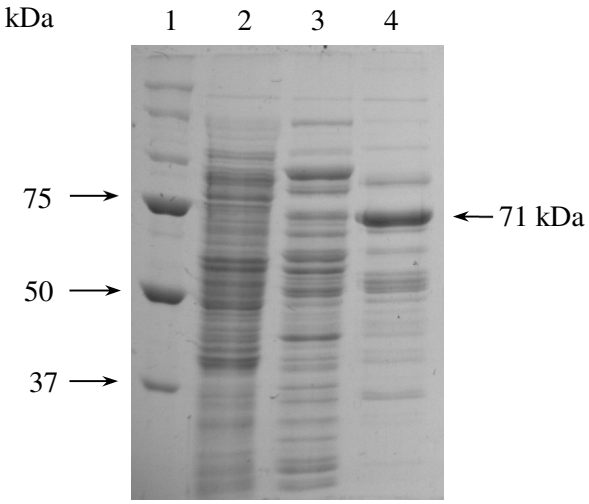
	Protein (mg)	Activity (U)	Sp. activity (U/mg)	Yield (%)	Purification (fold)
Cell extract	601	0.145	0.00024	100	1
Phenyl-Sepharose	2.81	0.0302	0.0108	20.8	45
Superdex 200	0.121	0.0072	0.06	5	250

Supplemental Figure S7 Growth of the ΔHVO_B0038A mutant from *Haloferax volcanii* that was complemented with rrnAC0575 and rrnAC3032 from *Haloarcula marismortui.* Growth on 20 mM L-arabinose (**A**) and 20 mM D-xylose (**B**). ΔHVO_B0038A mutant (diamonds); wild type (squares), and the ΔHVO_B0038A mutant complemented with rrnAC0575 (circles) or rrnAC3032 (triangles). Expression of the *Haloarcula marismortui* genes was induced by the addition of 0.1 mM tryptophan.



Supplemental Figure S8 Purification of α-ketoglutarate semialdehyde dehydrogenase from *Haloarcula marismortui*. (**A**) Purification as analyzed by SDS-PAGE, Coomassie blue stained. Lanes: 1, molecular mass standard; 2, cell extract; 3, after Phenyl-Sepharose; 4, after Superdex 200. (**B**) Enzyme activity was measured at 37°C as $NADP^+$ dependent oxidation of glutardialdehyde. The assay mixture contained 1.5 M KCl, 1 mM $NADP^+$ and 5 mM glutardialdehyde in 100 mM Tris-HCl, pH 8.5.

A



B

	Protein (mg)	Activity (U)	Sp. activity (U/mg)	Yield (%)	Purification (fold)
Cell extract	695.4	44.4	0.056	100	1
Phenyl Sepharose	28.62	13	0.454	29.3	8.1
Superdex 200	0.581	1.29	2.2	2.9	39.3

Supplemental Table S1 Primer used in this study. F = forward; R = reverse; 5'-flanking region of target gene, fragment 1; 3'-flanking region of target gene, fragment 2.

Primer used for transcriptional analyses		
gene		**sequence 5'->3'**
rrnAC3032		F_ CAATCACGCGGAAACTATTCGGCG
		R_GTGTTCGGTCTGGAGAATCGGTG
rrnAC3033		F_CGACTGGTTCGTCCGCGACG
		R_TGGAGCACCTTCGGGTCGCC
rrnAC3034		F_GTACGAGGAGTTCCACGACG
		R_ACTCCGTCTCGGATGCTTCG
rrnAC3036		F_GGACGCGGACGAAGCGGTTG
		R_GCTCGGCATGACGACGGTCG
rrnAC1339		F_CTGGGGACGGTGTGTACGATTTG
		R_CTCACAGGTGCGTTTCATCTCGC
rrnAC0575		F_ GAAGAGGCTGACCCCGAGGC
		R_ CCCGGCTCCTCCGGAATCTC
Primer used for generation of deletion mutants		
gene	**flanking fragments**	**sequence 5'->3'**
ΔHAH_1276	fragment1	F_GCGTCGTGGACTGTCTCTGAATCG
		R_CTCGACGACCAGGTCAGCGTAGTCTACGTCG
	fragment2	F_CTGACCTGGTCGTCGAGGGCGACACCG
		R_CGGCAACTGTTCGAGAACCTCATCC
ΔHAH_0289	fragment1	F_CGCGGATCCCCGGTGGGGTACGTCATC
		R_CCCAAGCTTTTGCCGGACATACTGTGGTG
	fragment2	F_CCCAAGCTTCGGCACTGTTTTTCCCGGTA
		R_ATAGGGTACCCGCGGTCGCTTGCCAGATG
ΔHAH_0291	fragment1	F_CGCGGATCCGGCGAGAAACGGGACCGGTATC
		R_CCCAAGCTTGCCACTGCCATCGACTGGCT
	fragment2	F_CCCAAGCTTATGCGGTACGAGATTTTTTG
		R_CATGCCATGGCGGGGTCGTTGGTGCCAGTAA
Primer used for cloning of plasmids for overexpression and/or complementation		
gene		**sequence 5'->3'**
rrnAC0771		F_ATTGCGCATATGCACCACCACCACCACCACATGCATCTCGAAAAAGTAGACAC
		R_CCGGGTATCGGATCCGGCCTG
HAH_0290		F_GTTCGGCCCATGGTTCAG
		R_GCCACCGGATCCACGGTC
rrnAC3036		F_GAATTAAACTCATGACGCAGACG
		R_CTGTAACAAGGATCCACTTCACG

rrnAC0575	F_CAAGCGGGGCCATGGGAAAGGC
	R_ACGATGGGGGTGGATCCTTACGC
rrnAC3032	F_TATGTCCGGCACATGTCGCCAAC
	R_TCTATGACGGATCCGTACGCAGC
rrnAC3034	F_CGACAGTGTCATGAACGTTGAC
	R_CGTTGACGGATCCATGTGCC