

Supplementary Materials: Characterisation of the first archaeal mannonate dehydratase from *Thermoplasma acidophilum* and its potential role in the catabolism of D-mannose

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Table S1. Overview of genes, potentially encoding for mannonate dehydratases located in the genomic neighbourhood of AldT (Ta0754). The gene product of Ta0753 is referred to as TaManD in the following and was further investigated as part of this study. MR/MLE, mandelate racemase/muconate lactonising enzyme.

old locus tag	new locus tag	NCBI gene annotation	protein family
Ta0752	TA_RS03865	L-rhamnonate dehydratase	MR/MLE enolase superfamily
Ta0753	TA_RS03870	D-mannonate dehydratase	uxuA xylose isomerase-like superfamily
Ta0756	TA_RS03885	mandelate racemase/muconate lactonizing enzyme family protein	MR/MLE enolase superfamily

Table S2. Oligonucleotides used in this study. Engineered restriction sites are displayed in lowercase. RE, restriction enzyme.

Expression plasmid	gene locus	oligonucleotide used	oligonucleotide sequence (5' → 3')	RE used
pETDuet-1-AldT	Ta0754	AldT-fw	TGATggatccCATGTTTCAGCGATCTAAGGGA	BamHI
	Ta0754	AldT-rev	ATTATgaattcCTCATTCTGGCGTGCTTATGG	EcoRI
pProEX HTa-Ta0753	Ta0753	ManD_woATG_fw	TGATggatccTAAGCTCAGAATAGCCGAGATC	BamHI
	Ta0753	ManD_HindIII_rev	TGATaagcttTCAGGAAATCTGTTTATGATTTG	HindIII

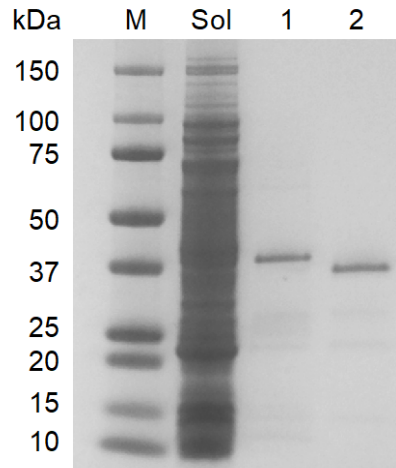


Figure S1. Purification of the Ta0753 gene product (TaManD) after expression in *E. coli*. M, Bio-rad Precision Plus Protein Standard; Sol, soluble cell extract; 1, enzyme after first His-tag purification (expected size 42 kDa); 2, enzyme after TEV cleavage and second His-tag purification (expected size 38 kDa).

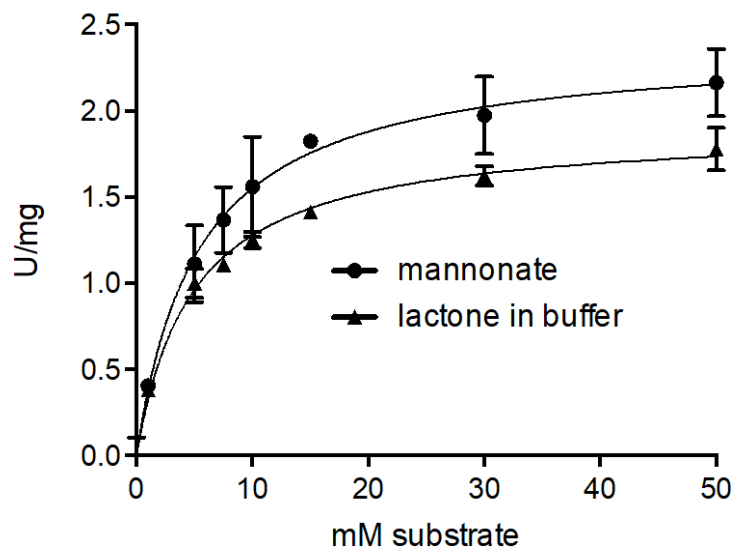


Figure S2 TaManD kinetic data. Kinetic measurements with D-mannonate (prepared by hydrolysis with NaOH) and with D-mannono-1,4-lactone prepared in buffer (pH 7). Non-linear fitting was applied using Prism 6 (GraphPad software). Error bars indicate standard error of mean values.