

Table S1. Occurrence of TK0250 and His biosynthesis gene homologs in Thermococcales species.

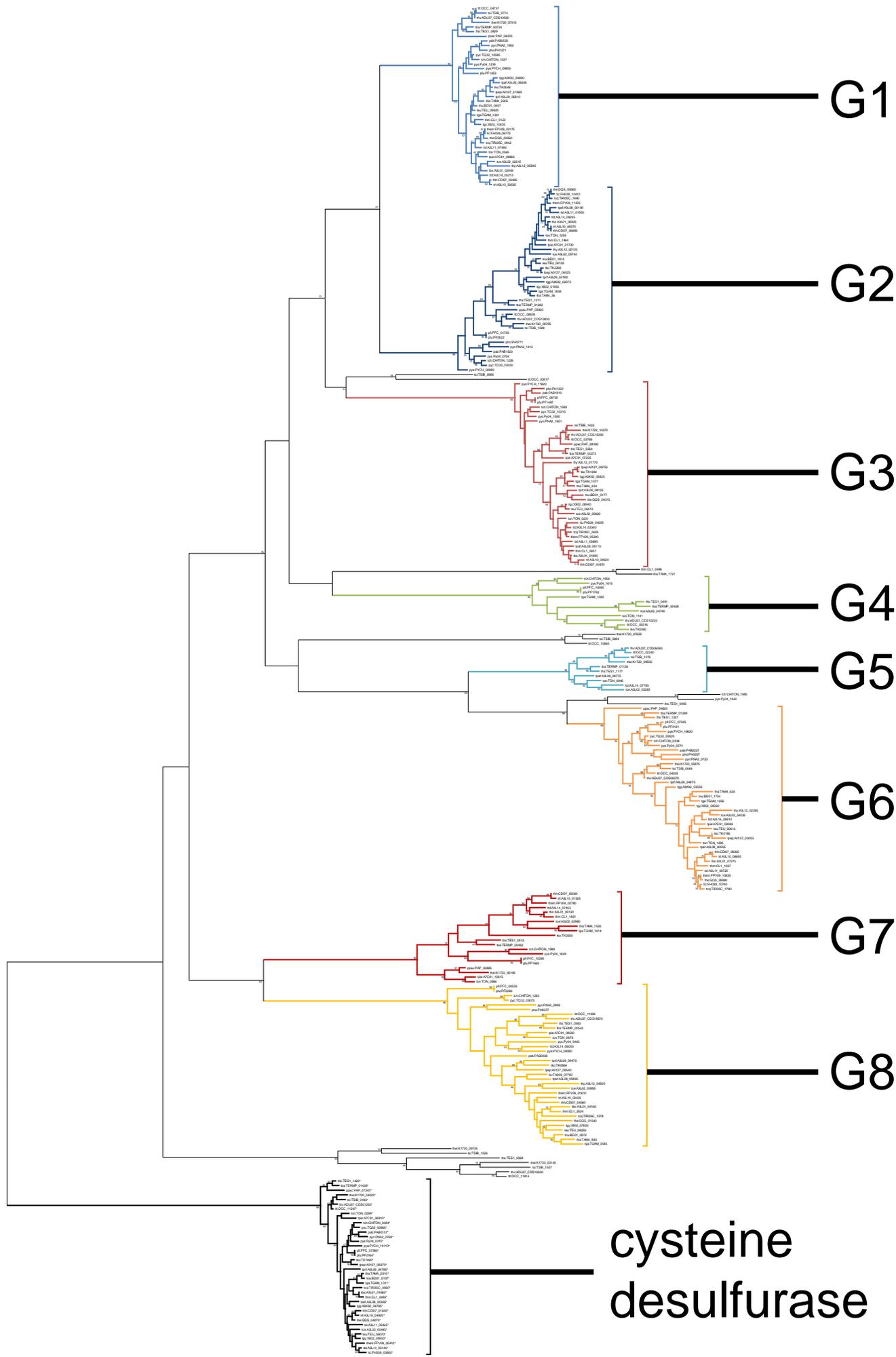
Organism	TK0250 homolog	His biosynthesis gene cluster
<i>Pyrococcus furiosus</i> DSM 3638	PF1665	
<i>Pyrococcus furiosus</i> COM1	PFC_10280	
<i>Pyrococcus horikoshii</i>		
<i>Pyrococcus abyssi</i>		
<i>Pyrococcus</i> sp. NA2		
<i>Pyrococcus yayanosii</i>		
<i>Pyrococcus</i> sp. ST04	Py04_1649	
<i>Pyrococcus kukulkanii</i>		
<i>Pyrococcus chitonophagus</i>	CHITON_1999	
<i>Thermococcus kodakarensis</i>	TK0250	
<i>Thermococcus onnurineus</i>	TON_0886	
<i>Thermococcus gammatolerans</i>	TGAM_1614	
<i>Thermococcus sibiricus</i>		
<i>Thermococcus barophilus</i>	TERMP_00432	
<i>Thermococcus</i> sp. 4557		
<i>Thermococcus</i> sp. AM4	TAM4_1525	
<i>Thermococcus cleftensis</i>	CL1_1821	
<i>Thermococcus litoralis</i>		
<i>Thermococcus paralvinellae</i>	TES1_0513	
<i>Thermococcus nautili</i>		
<i>Thermococcus eurythermalis</i>		
<i>Thermococcus guaymasensis</i>		
<i>Thermococcus</i> sp. 2319x1		
<i>Thermococcus peptonophilus</i>		
<i>Thermococcus piezophilus</i>	A7C91_10615	
<i>Thermococcus gorgonarius</i>		
<i>Thermococcus celer</i>	A3L02_02960	
<i>Thermococcus barossii</i>	A3L01_05120	
<i>Thermococcus</i> sp. 5-4	CDI07_05060	
<i>Thermococcus siculi</i>		
<i>Thermococcus thio-reducens</i>	A3L14_07455	
<i>Thermococcus profundus</i>		
<i>Thermococcus radiotolerans</i>	A3L10_01525	
<i>Thermococcus pacificus</i>		
<i>Thermococcus</i> sp. P6		
<i>Thermococcus indicus</i>		
<i>Thermococcus camini</i>		
<i>Thermococcus aciditolerans</i>	FPV09_02780	
<i>Thermococcus</i> sp. IOH2	K1720_06185	
<i>Palaeococcus pacificus</i>	PAP_03885	

The presence of homologs or predicted gene clusters is indicated in black.

Table S2. Occurrence of TK0260 and Phe/Tyr biosynthesis gene homologs in Thermococcales species.

Organism	TK0260 homolog	Chorismate mutase	Prephenate dehydrogenase	Prephenate dehydratase
<i>Pyrococcus furiosus</i> DSM 3638	PF1702	PF1701	PF1703 PF1704	PF0291
<i>Pyrococcus furiosus</i> COM1	PFC_10095	PFC_10100	PFC_10085 PFC_10090	PFC_00520
<i>Pyrococcus horikoshii</i>				
<i>Pyrococcus abyssi</i>				
<i>Pyrococcus</i> sp. NA2				
<i>Pyrococcus yayanosii</i>				
<i>Pyrococcus</i> sp. ST04	Py04_1613	Py04_1614	Py04_1612	Py04_0178
<i>Pyrococcus kukulkanii</i>				
<i>Pyrococcus chitonophagus</i>	CHITON_1956	CHITON_1957	CHITON_1955	CHITON_0241
<i>Thermococcus kodakarensis</i>	TK0260	TK0261	TK0259	
<i>Thermococcus onnurineus</i>	TON_1141	TON_1140	TON_1142	
<i>Thermococcus gammatolerans</i>	TGAM_1590	TGAM_1591	TGAM_1589	
<i>Thermococcus sibiricus</i>				
<i>Thermococcus barophilus</i>	TERMP_00428	TERMP_00427	TERMP_00429	
<i>Thermococcus</i> sp. 4557				
<i>Thermococcus</i> sp. AM4				
<i>Thermococcus cleftensis</i>				
<i>Thermococcus litoralis</i>	OCC_05516	OCC_05521	OCC_14085 OCC_14090	
<i>Thermococcus paralvinellae</i>	TES1_0491	TES1_0490	TES1_0492	
<i>Thermococcus nautili</i>				
<i>Thermococcus eurythermalis</i>				
<i>Thermococcus guaymasensis</i>				
<i>Thermococcus</i> sp. 2319x1	ADU37_CDS10220	ADU37_CDS10210	ADU37_CDS10230	
<i>Thermococcus peptonophilus</i>				
<i>Thermococcus piezophilus</i>				
<i>Thermococcus gorgonarius</i>				
<i>Thermococcus celer</i>	A3L02_04745	A3L02_04740	A3L02_04750	
<i>Thermococcus barossii</i>				
<i>Thermococcus</i> sp. 5-4				
<i>Thermococcus siculi</i>				
<i>Thermococcus thioireducens</i>				
<i>Thermococcus profundus</i>				
<i>Thermococcus radiotolerans</i>				
<i>Thermococcus pacificus</i>				
<i>Thermococcus</i> sp. P6				
<i>Thermococcus indicus</i>				
<i>Thermococcus camini</i>				
<i>Thermococcus aciditolerans</i>				
<i>Thermococcus</i> sp. IOH2				
<i>Palaeococcus pacificus</i>				

The presence of homologs is indicated in black.



G1

G2

G3

G4

G5

G6

G7

G8

cysteine  
desulfurase

Fig. S1. Amino acid sequences of class I aminotransferases were collected from all Thermococcales genomes registered on the KEGG database (1-3). Homologs of cysteine desulfurase (TK1990) were added into the dataset as an outgroup. The sequences were aligned by using MUSCLE algorithm (4). The phylogenetic analysis was performed by using the Maximum Likelihood method and JTT matrix-based model (5). The tree with the highest log likelihood (-39302.18) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Bootstrap values above 50 are shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the JTT model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 291 amino acid sequences. All positions with less than 95% site coverage were eliminated, i.e., fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position (partial deletion option). There were a total of 279 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (6).

1. Kanehisa, M. and Goto, S.; KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Res.* 28, 27-30 (2000).
2. Kanehisa, M; Toward understanding the origin and evolution of cellular organisms. *Protein Sci.* 28, 1947-1951 (2019).
3. Kanehisa, M., Furumichi, M., Sato, Y., Ishiguro-Watanabe, M., and Tanabe, M.; KEGG: integrating viruses and cellular organisms. *Nucleic Acids Res.* 49, D545-D551 (2021).
4. Edgar, Robert C. (2004), MUSCLE: multiple sequence alignment with high accuracy and high throughput, *Nucleic Acids Research* 32(5), 1792-1797.
5. Jones D.T., Taylor W.R., and Thornton J.M. (1992). The rapid generation of mutation data matrices from protein sequences. *Computer Applications in the Biosciences* 8: 275-282.
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# Fig. S2

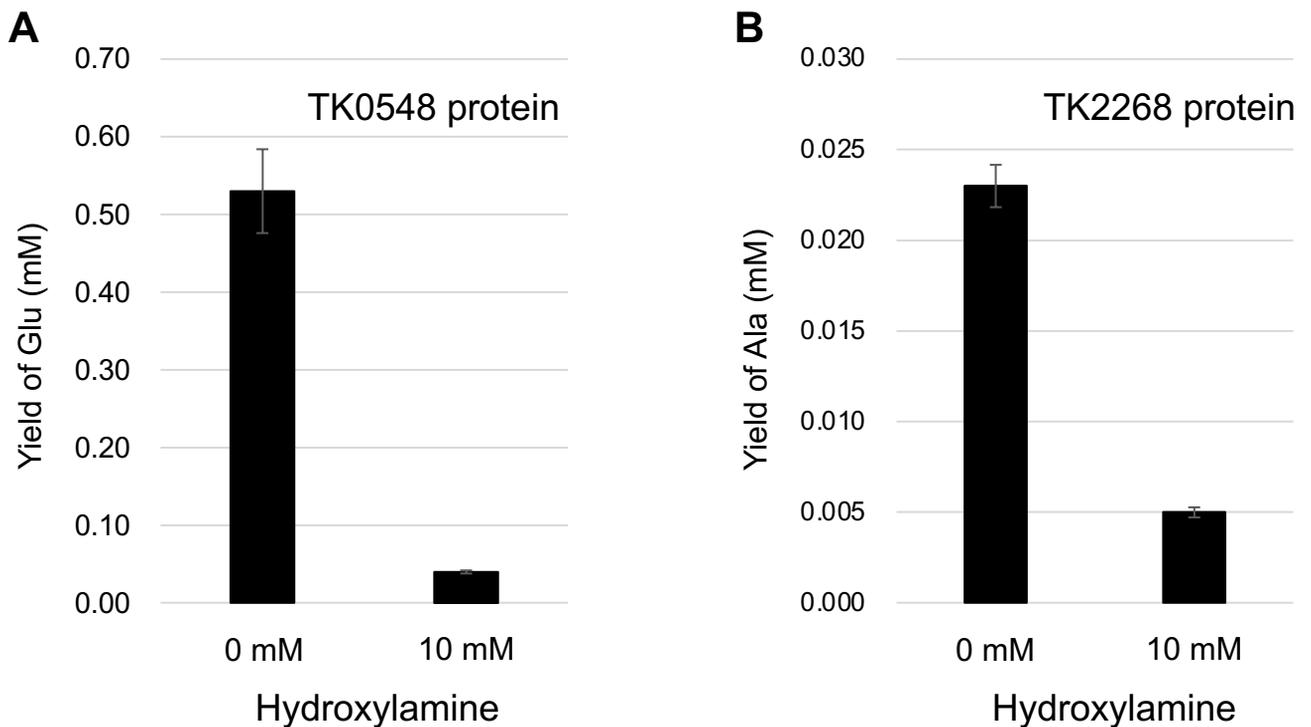


Fig. S2. PLP-dependency of the aminotransferase activity of the TK0548 protein (A) and TK2268 protein (B). Aminotransferase activity was measured in the absence or presence of 10 mM hydroxylamine without the addition of exogenous PLP. The TK0548 protein reaction was measured with 10 mM Phe and 10 mM 2-oxoglutarate, while the TK2268 protein reaction was measured with 10 mM Asp and 10 mM pyruvate. Reactions were carried out for 5 min at 80°C after treating the proteins with 10 mM hydroxylamine on ice for 1 h. The results are the means of three independent assays and error bars indicate standard deviations.