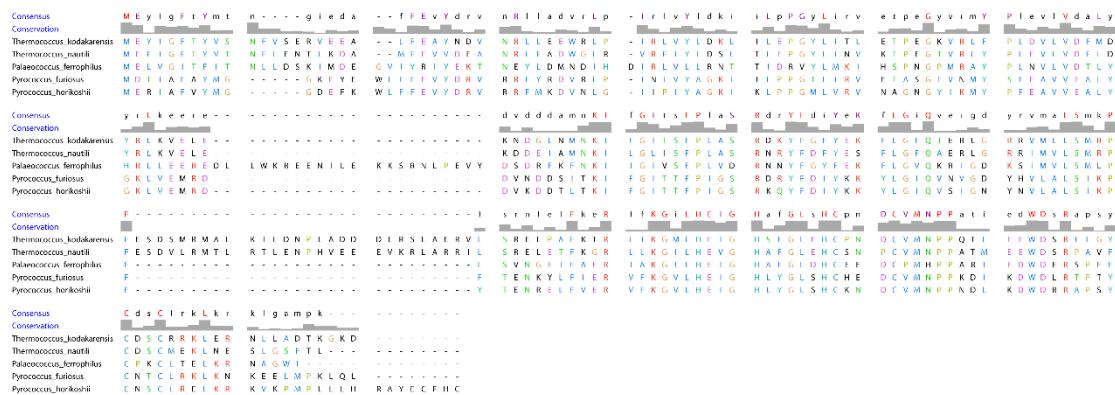


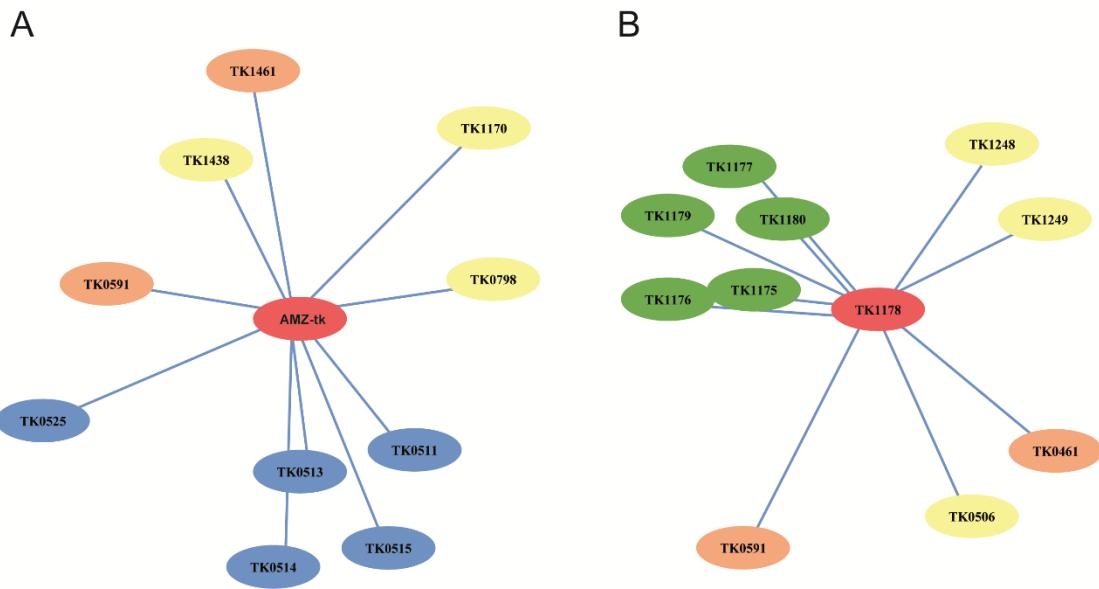
A zinc-dependent protease AMZ-tk from a thermophilic archaeon is a new member of the archaemetzincin protein family

Supplementary Fig. 1



Supplementary Fig. 1 Sequence analysis of AMZ-tk. Archaemetzincin sequences from *Thermococcus kodakarensis* (YP_182925.1), *Thermococcus nautili* (WP_042689681.1), *Pyrococcus furiosus* (WP_011012973.1), *Pyrococcus horikoshii* (WP_010885849.1), and *Palaeococcus ferrophilus* (WP_048151914.1) were aligned. The conservation level of each residue is indicated by the height of the bars above each residue.

Supplementary Fig. 2.



Supplementary Fig. 2. Predicted protein-protein interaction network of AMZ-tk

(A) and TK1178 (B). The graph was constructed in the STRING tools using standard parameters. The proteins that may be involved in detoxifying are shown in blue and the proteins that may regulate DNA metabolism are shown in green. The proteins that may interact with both AMZ-tk and TK1178 are shown in orange. These proteins are annotated as: TK0506, translation-associated GTPase; TK0511, pyruvate kinase; TK0514, camphor resistance protein CrcB; TK0525, superoxide reductase; TK0591, N-acetyltransferase; TK0798, DNA topoisomerase VI subunit A; TK1175, type Holliday junction resolvase; TK1177, deblocking aminopeptidase; TK1179, adenylate cyclase; TK1438, fibronectin-binding protein; TK1461, leucyl-tRNA synthetase; TK1770, cyclomaltodextrinase; and TK0513, TK0515, TK1180, TK1176, TK1248, and TK1249 are hypothetical proteins. Protein functions were predicted using the software listed in ‘Materials and Methods.’

Supplementary Table I. Archaemetzincin sequences from different organisms used for phylogenetic analysis.

Organism	Gene Bank Accession No.
<i>Aeropyrum pernix</i>	WP_010865524.1
<i>Archaeoglobus fulgidus</i>	WP_010877837.1
<i>Archaeoglobus veneficus-1</i>	YP_004342225.1
<i>Archaeoglobus veneficus-2</i>	WP_013682907.1
<i>Aspergillus flavus</i>	XP_002379891.1
<i>Aspergillus nidulans</i>	CBF83223.1
<i>Chitinophaga</i> sp.	WP_029464671.1
<i>Coniosporium apollinis</i>	XP_007781354.1
<i>Dictyoglomus thermophilum</i>	WP_012547438.1
<i>Flavobacterium chungangense</i>	WP_031453746.1
<i>Galerina marginata</i>	KDR81884.1
<i>Haloarcula hispanica</i>	YP_008877092.1
<i>Haloarcula marismortui</i>	YP_136662.2
<i>Halobacterium salinarum</i>	WP_010903951.1
<i>Halobacterium</i> sp.	AHG02721.1
<i>Haloquadratum walsbyi</i>	WP_021055743.1
<i>Halorubrum lacusprofundi</i>	YP_002565008.1
<i>Homo sapiens-1</i>	NP_597720.1
<i>Homo sapiens-2</i>	NP_001275985.1
<i>Lentisphaera araneosa</i>	WP_007280897.1
<i>Methanocaldococcus jannaschii</i>	WP_010869779.1
<i>Methanocaldococcus</i> sp.	WP_012979875.1
<i>Methanoculleus bourgensis</i>	YP_006545080.1
<i>Methanosaeta harundinacea</i>	WP_014586234.1
<i>Microscilla marina</i>	WP_002697137.1
<i>Mus musculus-1</i>	NP_775581.1
<i>Mus musculus-2</i>	NP_001239122.1
<i>Natrialba magadii</i>	YP_003479690.1
<i>Natronomonas moolapensis</i>	WP_015408079.1
<i>Ophiophagus hannah-1</i>	ETE59834.1
<i>Ophiophagus hannah-2</i>	ETE71868.1
<i>Penicillium expansum</i>	KGO44057.1
<i>Pyrobaculum aerophilum</i>	WP_011008336.1
<i>Pyrobaculum arsenaticum</i>	WP_011901510.1
<i>Pyrococcus furiosus-1</i>	WP_011013000.1
<i>Pyrococcus furiosus-2</i>	WP_011012973.1
<i>Pyrococcus yayanosii-1</i>	WP_013904868.1
<i>Pyrococcus yayanosii-2</i>	WP_013904896.1
<i>Pyrus bretschneideri</i>	XP_009350639.1
<i>Rattus norvegicus-1</i>	NP_001040557.1
<i>Rattus norvegicus-2</i>	NP_001014143.1
<i>Rhodopirellula</i> sp.	WP_009102142.1
<i>Sulfolobus acidocaldarius</i>	WP_011277458.1
<i>Sulfolobus islandicus</i>	WP_012711449.1
<i>Sulfolobus solfataricus</i>	WP_009991235.1
TK0512	YP_182925.1
TK1178	YP_183591
<i>Tolypothrix bouteillei</i>	WP_038111967.1
<i>Vulcanisaeta distributa</i>	YP_003901466.1

Supplementary Table II. Comparison of the biochemical properties of protease-Tk and other proteases.

	MW (kDa)	Optimal pH	Optimal temp	Specific activity (U/mg)	References
AMZ-tk	28	8.0-9.0	55 °C	78.37	This study
Protease-Vs	48	9.5-10.0	55 °C	467	Fukuda et al., 1998
Protease-Ts	35	8.5	75 °C	N.D.	Murao et al., 1991
Protease-Ss	42	7.0	70 °C	5.65	Colombo et al., 1992
Protease-Bs	48	6.8	80 °C	744	Aqel et al., 2012

The proteases are from *T. kodakarensis* KOD1 (AMZ-tk), *Vibrio* sp. NUF-BPP-1 (Protease-Vs), *Sulfolobus solfataricus* (Protease-Ss), *Bacillus* strain HUTBS62 (Protease-Bs), and *Thermomicrobium* sp. KN-22 (Protease-Ts). N.D.: not determined.

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