

**Characterization of a temperature-responsive two component regulatory system from the Antarctic archaeon, *Methanococcoides burtonii***

**Najnin T, Siddiqui KS, Taha, Elkaid N, Kornfeld G, Curmi PMG and Cavicchioli R**

**Supporting Information**

**Figure S1. Multiple sequence alignment of LtrK with SKs from *Bacteria* and closely related methanogens.**

**Figures S2. Purity of LtrK and LtrR protein preparations.**

**Figure S3. Effect of temperature on LtrK phosphotransfer activity.**

**Table S1. Organization of HTH domain in RRs from *Archaea*.**

**Table S2. Temperature optimum values for proteins from psychrophiles.**

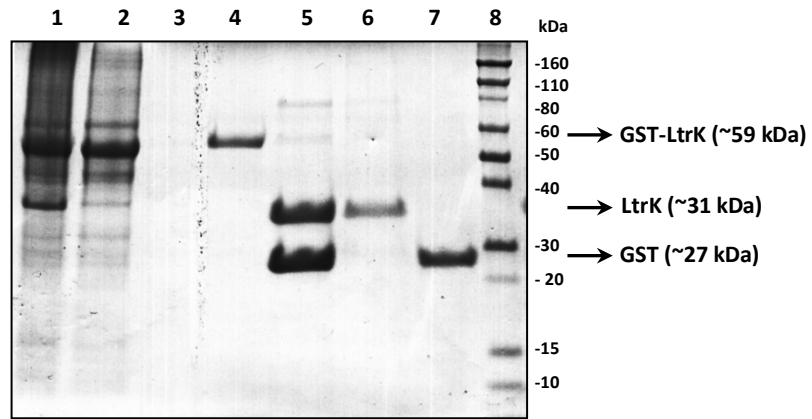
**References**

**Figure S1. Multiple sequence alignment of LtrK with SKs from *Bacteria* and closely related methanogens.** Multiple sequence alignments were performed using ClustalW. The conserved histidine residue H367 (labelled arrow) and the three non-conserved histidine residues, H367, H443, H448 and H502 are highlighted in the LtrK sequence in bold and yellow highlight. Amino acids identical in all sequences (\*); amino acids with strong structural similarity (:); amino acids with weak structural similarity (.). A. Multiple sequence alignment of the cytoplasmic domain of LtrK with bacterial SKs that have crystal structures available for their cytoplasmic domain and share at least 35% sequence identity with LtrK. *Thermotoga maritima* HK853 (PDB: 3DGE<sup>1</sup>); *Streptococcus mutans* VicK (PDB: 4I5s<sup>2</sup>); *Bacillus subtilis* WalK (PDB: 3SL2<sup>3</sup>); N, G1, F, G2 and G3 blocks (red boxes). B. Alignment of full-length LtrK sequence with sequences from closely related methanogens: *Methanohalophilus mahii*, *Methanolobus tindarius*, *Methanolobus psychrophilus* and *Methanococcoides methylutens*. C. Alignment of full-length LtrK sequence with the most similar sequence available (*M. methylutens*; 75% identity), showing the lack of sequence conservation for H2 (H443), H3 (H448) and H4 (H502).

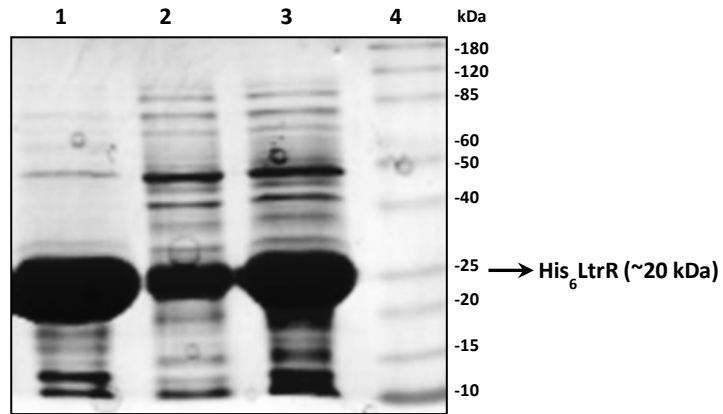


**Figure S2. Purity of LtrK and LtrR protein preparations.** The fractions from each purification step were analysed on a SDS-polyacrylamide gel (12%) with Coomassie brilliant blue staining. A. SDS-PAGE of different fractions during LtrK purification. Lanes: 1, soluble fraction of sonicated lysate; 2 & 3, flowthrough fractions; 4, eluted GST-LtrK by 20 mM glutathione; 5, GST tag cleaved LtrK; 6, Purified LtrK after cleavage reaction; 7, Eluted GST tag obtained after cleavage; 8, protein molecular weight standard. B. SDS-PAGE of different fractions during LtrR purification. Lanes: 1, eluted 6×His LtrR with 100 mM imidazole; 2, flowthrough fraction of 20 mM imidazole wash; 3, soluble fraction of sonicated lysate; 4, protein molecular weight standard.

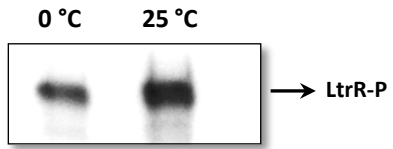
A)



B)



**Figure S3. Effect of temperature on LtrK phosphotransfer activity.** GST-LtrK (25  $\mu$ g) bound to glutathione beads was phosphorylated in presence of [ $\gamma$ - $^{32}$ P]-ATP at room temperature for 30 min, free [ $\gamma$ - $^{32}$ P]-ATP washed off and one column was placed on ice (0  $^{\circ}$ C) and the other was placed at room temperature (25  $^{\circ}$ C) for 10 min. Ice cold LtrR (100  $\mu$ g) was passed through the cold column and an equivalent amount of LtrR was warmed to room temperature for 5 min and then added to the column kept at room temperature. LtrR-P was collected in the flowthrough and 10  $\mu$ l samples were added to 3  $\mu$ l of 100 mM EDTA containing sample buffer and analysed by gel-phosphorimaging.



**Table S1. Organization of HTH domain in RRs from *Archaea*.**

<i>Archaea</i>	N-terminal	C-terminal
<b>Halophilic</b>		
<i>Halonotius</i> sp. J07HN		J07HN4v2_01675, J07HN4v2_00780, J07HN4v2_02997, J07HN6v2_01307
<i>Halorubrum aidingense</i>		C461_00607
<i>Halorubrum lacusprofundi</i>		Hlac_3274
<i>Halobiforma lacisalsi</i>		C445_12891
<i>Halopiger djelfamassiliensis</i>		Ga0036365_12412, Ga0036365_12651, Ga0036367_103616
<i>Haloterrigena turkmenica</i>		Htur_0128, Htur_0348, Htur_0709
<i>Haloquadratum walsbyi</i>		Hqrw_3103
<i>Haloferax volcanii</i>		HVO_1357, HVO_B0272
<i>Haloarcula marismortui</i>	rrnB0301	pNG7159, pNG7223
<i>Halopiger xanaduensis</i>		Halxa_3599
<i>Halovivax ruber</i>		Halru_0454
<i>Halobacterium</i> sp. DL1	HalDL1_2148, HalDL1_1858	HalDL1_2066
<i>Halostagnicola larsenii</i>		Halla_0473
<i>Halalkalicoccus jeotgali</i>	HacjB3_0162	HacjB3_04505, HacjB3_00310, HacjB3_18828
<i>Halorhabdus utahensis</i>		Huta_1740, Huta_0317
<i>Halohasta litchfieldiae</i> tADL		halTADL_2543
<i>Natrinema pallidum</i>		C487_17780
<i>Natrinema pellirubrum</i>		C488_09871
<i>Natrinema versiforme</i>		C489_01256
<i>Natronolimnobius innermongolicus</i>		C493_05780
<i>Natronorubrum tibetense</i>		C496_05962
<i>Natrialba hulunbeirensis</i>		C483_14902
<i>Natrialba magadii</i>	Nmag_2044	Nmag_1012, Nmag_2147, Nmag_2692, C500_06936
<i>Natrialba taiwanensis</i>		C484_14808
<i>Natrialba aegyptia</i>		C480_05466
<i>Natrialba chahannaoensis</i>		C482_19154
<i>Natronobacterium gregoryi</i>		Natgr_1657
<i>Natronococcus amylolyticus</i>		C491_04490
<i>Natronomonas pharaonis</i> Gabara		NP0654A
<b>Psychrophilic &amp; mesophilic methanogens</b>		
<i>Methanococcoides methylutens</i>	Ga0070849_114114	
<i>Methanohalophilus mahii</i>	Mmah_0822	
<i>Methanolobus psychrophilus</i>	Mpsy_1252	
<i>Methanosarcina barkeri</i>	Mbar_A2896	
<i>Methanosarcina mazei</i>	MM2351	
<i>Methanococcoides burtonii</i>	Mbur_0695 (LtrK)	



**Table S2. Temperature optimum values for proteins from psychrophiles.**

<b>Organism</b>	<b>Protein</b>	<b>T<sub>opt</sub></b>	<b>Reference No.</b>
<b><i>Bacteria</i></b>			
Antarctic bacterium	Citrate synthase	31 °C	4
Antarctic <i>Pseudomonas</i> sp.	Phosphoglycerate Kinase	35 °C	5
Antarctic <i>Pseudomonas syringae</i>	RNA polymerase	37 °C	6
Antarctic seawater bacterium	Alkaline phosphatase	25 °C	7
Antarctic seawater bacterium	DNA ligase	16 °C	8
Arctic sea shore sediment bacterium	Esterase	30 °C	9
<i>Bacillus globisporus</i>	Adenylate kinase	35 °C	10
<i>Bacillus</i> TA41	Subtilisin	40 °C	11
<i>Carnobacterium piscicola</i> BA	β-galactosidase	30 °C	12
<i>Chlamys islandica</i>	Chlamysin	22 °C	13
<i>Colwellia psychrerythraea</i>	Aminopeptidase	39 °C	14
<i>Colwellia psychrerythraea</i>	Phenylalanine hydroxylase	25 °C	15
<i>Colwellia maris</i>	Malate synthase	45 °C	16
<i>Colwellia maris</i>	Isocitrate lyase	20 °C	16
Cold sea sediment sample bacterium	Lipase	25 °C	17
<i>Cytophaga</i> sp.	Valine dehydrogenase	20 °C	18
<i>Desulfotalea psychrophila</i>	Isocitrate dehydrogenase	45 °C	19
Marine psychrophile strain PA-43	Serine protease	58 °C	20
<i>Micrococcus</i> sp.	Pollulanase (extracellular)	50 °C	21
<i>Moraxella</i> sp.	Alcohol dehydrogenase	25 °C	22
<i>Pseudoalteromonas haloplanktis</i>	α-amylase	28 °C	23
<i>P. haloplanktis</i>	Cellulase	40 °C	24
<i>P. haloplanktis</i>	Xylanase	35 °C	25
<i>P. haloplanktis</i>	Pectate lyase (extracellular)	30 °C	26
<i>P. haloplanktis</i>	Aspartate amino transferase	64 °C	27
<i>Pseudomonas</i> strain DY-A	Serine Alkaline protease	40 °C	28
<i>Sphingomonas paucimobilis</i> (Antarctic marine bacterium)	Extracellular metalloprotease	25 °C	29
<b><i>Archaea</i></b>			
<i>Cenarchaeum symbiosum</i>	DNA polymerase	40 °C	30
<i>Methanococcoides burtonii</i>	EF2	34 °C	31
<b><i>Eucarya</i></b>			
Antarctic fish	Lactate dehydrogenase	50 °C	32
Fish liver	Imidase	55 °C	33
Psychrophilic green alga	Argininosuccinate lyase	37 °C	34
<i>Sclerotinia borealis</i>	Polygalacturonase (extracellular)	45 °C	35

\*When data were available for multiple enzymes of the same type (e.g. α-amylases from different psychrophiles), data were included for the enzyme with the lowest T<sub>opt</sub>.

## References

1. Marina, A., Waldburger, C. D. & Hendrickson, W. A. Structure of the entire cytoplasmic portion of a sensor histidine-kinase protein. *EMBO J.* **24**, 4247-4259 (2005).
2. Wang, C. *et al.* Mechanistic insights revealed by the crystal structure of a histidine kinase with signal transducer and sensor domains. *PLoS Biol.* **11**, e1001493 (2013).
3. Celikel, R., Veldore, V. H., Mathews, I., Devine, K. M. & Varughese, K. I. ATP forms a stable complex with the essential histidine kinase WalK (YycG) domain. *Acta Crystallogr. D Biol. Crystallogr.* **68**, 839-845 (2012).
4. Russell, R. J., Gerike, U., Danson, M. J., Hough, D. W. & Taylor, G. L. Structural adaptations of the cold-active citrate synthase from an Antarctic bacterium. *Structure* **6**, 351-361 (1998).
5. Bentahir, M. *et al.* Structural, kinetic, and calorimetric characterization of the cold-active phosphoglycerate kinase from the Antarctic *Pseudomonas* sp. TACH18. *J. Biol. Chem.* **275**, 11147-11153 (2000).
6. Uma, S., Jadhav, R., Kumar, G. S., Shivaji, S. & Ray, M. A RNA polymerase with transcriptional activity at 0 C from the Antarctic bacterium *Pseudomonas syringae*. *FEBS Lett.* **453**, 313-317 (1999).
7. Kobori, H., Sullivan, C. W. & Shizuya, H. Heat-labile alkaline phosphatase from Antarctic bacteria: rapid 5' end-labeling of nucleic acids. *Proc. Natl. Acad. Sci. USA* **81**, 6691-6695 (1984).
8. Georlette, D. *et al.* Structural and functional adaptations to extreme temperatures in psychrophilic, mesophilic, and thermophilic DNA ligases. *J. Biol. Chem.* **278**, 37015-37023 (2003).
9. Jeon, J. H. *et al.* Cloning and characterization of a new cold-active lipase from a deep-sea sediment metagenome. *Appl. Microbiol. Biotechnol.* **81**, 865-874 (2009).
10. Bae, E. & Phillips, G. N. Structures and analysis of highly homologous psychrophilic, mesophilic, and thermophilic adenylate kinases. *J. Biol. Chem.* **279**, 28202-28208 (2004).
11. Davail, S., Feller, G., Narinx, E. & Gerday, C. Cold adaptation of proteins. Purification, characterization, and sequence of the heat-labile subtilisin from the antarctic psychrophile *Bacillus* TA41. *J. Biol. Chem.* **269**, 17448-17453 (1994).

12. Coombs, J. M. & Brenchley, J. E. Biochemical and phylogenetic analyses of a cold-active  $\beta$ -galactosidase from the lactic acid bacterium *Carnobacterium piscicola* BA. *Appl. Environ. Microbiol.* **65**, 5443-5450 (1999).
13. Nilsen, I. W. *et al.* Protein purification and gene isolation of chlamysin, a cold-active lysozyme-like enzyme with antibacterial activity. *FEBS Lett.* **464**, 153-158 (1999).
14. Huston, A. L., Haeggström, J. Z. & Feller, G. Cold adaptation of enzymes: Structural, kinetic and microcalorimetric characterizations of an aminopeptidase from the Arctic psychrophile *Colwellia psychrerythraea* and of human leukotriene A 4 hydrolase. *Biochim. Biophys. Acta.* **1784**, 1865-1872 (2008).
15. Leiros, H.-K. S. *et al.* Structure of phenylalanine hydroxylase from *Colwellia psychrerythraea* 34H, a monomeric cold active enzyme with local flexibility around the active site and high overall stability. *J. Biol. Chem.* **282**, 21973-21986 (2007).
16. Watanabe, S., Takada, Y. & Fukunaga, N. Purification and characterization of a cold-adapted isocitrate lyase and a malate synthase from *Colwellia maris*, a psychrophilic bacterium. *Biosci. Biotechnol. Biochem.* **65**, 1095-1103 (2001).
17. Jeon, J. H., Kim, J.-T., Kang, S. G., Lee, J.-H. & Kim, S.-J. Characterization and its potential application of two esterases derived from the arctic sediment metagenome. *Mar. Biotechnol.* **11**, 307-316 (2009).
18. Oikawa, T., Yamanaka, K., Kazuoka, T., Kanzawa, N. & Soda, K. Psychrophilic valine dehydrogenase of the antarctic psychrophile, *Cytophaga* sp. KUC-1. *Eur. J. Biochem.* **268**, 4375-4383 (2001).
19. Fedøy, A.-E., Yang, N., Martinez, A., Leiros, H.-K. S. & Steen, I. H. Structural and functional properties of isocitrate dehydrogenase from the psychrophilic bacterium *Desulfotalea psychrophila* reveal a cold-active enzyme with an unusual high thermal stability. *J. Mol. Biol.* **372**, 130-149 (2007).
20. Irwin, J. A., Alfredsson, G. A., Lanzetti, A. J., Gudmundsson, H. M. & Engel, P. C. Purification and characterisation of a serine peptidase from the marine psychrophile strain PA-43. *FEMS Microbiol. Lett.* **201**, 285-290 (2001).
21. Kimura, T. & Horikoshi, K. Characterization of pullulan-hydrolysing enzyme from an alkalopsychrotrophic *Micrococcus* sp. *Appl. Microbiol. Biotechnol.* **34**, 52-56 (1990).
22. Tsigos, I., Velonia, K., Smonou, I. & Bouriotis, V. Purification and characterization of an alcohol dehydrogenase from the Antarctic psychrophile *Moraxella* sp. TAE123. *Eur. J. Biochem.* **254**, 356-362 (1998).

23. D'Amico, S., Gerday, C. & Feller, G. Temperature adaptation of proteins: engineering mesophilic-like activity and stability in a cold-adapted  $\alpha$ -amylase. *J. Mol. Biol.* **332**, 981-988 (2003).
24. Garsoux, G., Lamotte, J., Gerday, C. & Feller, G. Kinetic and structural optimization to catalysis at low temperatures in a psychrophilic cellulase from the Antarctic bacterium *Pseudoalteromonas haloplanktis*. *Biochem. J.* **384**, 247-253 (2004).
25. Collins, T., Meuwis, M.-A., Gerday, C. & Feller, G. Activity, stability and flexibility in glycosidases adapted to extreme thermal environments. *J. Mol. Biol.* **328**, 419-428 (2003).
26. Tuyen, H., Helmke, E. & Schweder, T. Cloning of two pectate lyase genes from the marine Antarctic bacterium *Pseudoalteromonas haloplanktis* strain ANT/505 and characterization of the enzymes. *Extremophiles* **5**, 35-44 (2001).
27. Birolo, L. *et al.* Aspartate aminotransferase from the Antarctic bacterium *Pseudoalteromonas haloplanktis* TAC 125. *J. Biochem.* **267**, 2790-2802 (2000).
28. Zeng, R., Zhang, R., Zhao, J. & Lin, N. Cold-active serine alkaline protease from the psychrophilic bacterium *Pseudomonas* strain DY-A: enzyme purification and characterization. *Extremophiles* **7**, 335-337 (2003).
29. Turkiewicz, M., Gromek, E., Kalinowska, H. & Zielińska, M. Biosynthesis and properties of an extracellular metalloprotease from the Antarctic marine bacterium *Sphingomonas paucimobilis*. *J. biotechnol.* **70**, 53-60 (1999).
30. Schleper, C., Swanson, R. V., Mathur, E. J. & DeLong, E. F. Characterization of a DNA polymerase from the uncultivated psychrophilic archaeon *Cenarchaeum symbiosum*. *J. Bacteriol.* **179**, 7803-7811 (1997).
31. Thomas, T. & Cavicchioli, R. Effect of temperature on stability and activity of elongation factor 2 proteins from Antarctic and thermophilic methanogens. *J. Bacteriol.* **182**, 1328-1332 (2000).
32. Coquelle, N., Fioravanti, E., Weik, M., Vellieux, F. & Madern, D. Activity, stability and structural studies of lactate dehydrogenases adapted to extreme thermal environments. *J. Mol. Biol.* **374**, 547-562 (2007).
33. Huang, C.-Y. & Yang, Y.-S. A novel cold-adapted imidase from fish *Oreochromis niloticus* that catalyzes hydrolysis of maleimide. *Biochem. Biophys. Res. Commun.* **312**, 467-472 (2003).

34. Loppes, R., Devos, N., Willem, S., Barthélemy, P. & Matagne, R. F. Effect of temperature on two enzymes from a psychrophilic chloromonas (*Chlorophyta*). *J. Phycol.* **32**, 276-278 (1996).
35. Takasawa, T. *et al.* Polygalacturonase isolated from the culture of the psychrophilic fungus *Sclerotinia borealis*. *Can. J. Microbiol.* **43**, 417-424 (1997).