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Heavy metal tolerance of *Mesorhizobium delmotii* thymidylate kinase

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ABSTRACT

Metal ions play an important role in many metabolic processes in all living organisms. At low concentrations, heavy metals such as Fe²⁺, Cu²⁺ and Zn²⁺ are essential cofactors for many enzymes. However, at high concentrations they are toxic. *Mesorhizobium* species belong to the class α -proteobacteria and have high tolerance to soil acidity, salinity, temperature extremes, and metal-rich conditions. To identify factors responsible for this tolerance we have studied the effects of metal ions on *Mesorhizobium delmotii* thymidylate kinase (MdTMPK), an essential enzyme in the synthesis of dTTP, thus being vital for cell growth. We show that Mg²⁺ and Mn²⁺ are the divalent metal ions required for catalysis and that Mn²⁺ gives the highest catalytic efficiency. MdTMPK activity in the presence of Mg²⁺ was strongly inhibited by the co-presence of Zn²⁺, Ni²⁺ and Co²⁺. However, the addition of Cs⁺ caused >2-fold enhanced MdTMPK activity. For TMPK from *Bacillus anthracis* and humans, the effects of Mg²⁺ and Mn²⁺ were similar, whereas the effects of other divalent metal ions were different, and no stimulatory effect of Cs⁺ was observed. Together, our results demonstrate that MdTMPK and BaTMPK function well in the presence of high concentrations of heavy metal ions, introducing a potential contribution of these enzymes to the heavy metal tolerance of *Mesorhizobium delmotii* and *Bacillus anthracis*

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1. Introduction

Metal ions play vital role in many metabolic processes of all living organisms. At low concentrations, heavy metals such as Fe²⁺, Cu²⁺ and Zn²⁺ are essential cofactors for a large number of key enzymes in different metabolic pathways. However, at high concentrations these ions are toxic. Extensive mining generates mining spoils that are extremely rich in toxic heavy metals such as lead, mercury, nickel, copper, chromium, zinc,

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cadmium, antimony and arsenic. These heavy metals contaminate the surrounding soil and water and pose a threat to the ecosystem and the health of living organisms. For example, cadmium causes serious diseases in humans such as bone or kidney disease and cancer [1]. Covering mining waste pile with vegetation and removal of heavy metal contamination from industry effluents can thus reduce the release of toxic heavy metals to the environment [2]. Certain plants and microorganisms have evolved sophisticated mechanisms to deal with heavy metals, which allows them to grow in soil contaminated by heavy metals [3]. For example, *Mesorhizobium metallidurans* (*M. metallidurans*), a nitrogen-fixing bacteria, grows well in the presence of 1.5 mM zinc and 0.05 mM cadmium [4] and *Anthyllis vulneraria*, a leguminous species, can grow in heavy metal-contaminated soil. However, mining spoils are poor in nutrients, and therefore nitrogen-fixing bacteria, such as *M. metallidurans* and *Mesorhizobium delmotii* (*M. delmotii*), play an important role in assisting this type of plant growth [5, 6].

Bacillus species are gram-positive bacteria and are commonly found in soil and the gastrointestinal tracts of ruminants. Most strains of *Bacillus* are not pathogenic. However, *Bacillus anthracis* (*B. anthracis*) can cause deadly disease to animals and occasionally to humans. Nonpathogenic *Bacillus* strains, such as *Bacillus subtilis* (*B. subtilis*), and *Bacillus cereus*, have been used to de-contaminate industrial waste water and heavy metal polluted soil [7, 8]. Therefore, these organisms have great value in agriculture and environmental protection.

Thymidylate kinase (TMPK) is a key enzyme for the synthesis of deoxythymidine triphosphate (dTTP), an essential building block of DNA. Mg^{2+} or Mn^{2+} are the divalent metal ions known to be required for TMPK activity [9]. However, very little is known regarding the effects of heavy metals on TMPK enzymes. Here, we investigated the effects of heavy metal ions on TMPK from *M. delmotii*, *B. anthracis* and humans in order to clarify if these enzymes can be involved in why *Mesorhizobium* and *Bacillus* species can grow in metal-contaminated area.

2. Materials and methods

2.1. Materials

[3H -methyl]-thymidine ([3H]-dThd) was purchased from Perkin Elmer. [3H]-dTTP was synthesized using [3H]-dThd as starting material in a reaction catalyzed by thymidine kinase 1 [10]. Cadmium chloride, Cesium chloride, Zinc sulfate, Nickel sulfate, Cobalt chloride, Manganese dichloride, Magnesium chloride and Iron sulfate were from Sigma Aldrich. Lithium chloride was from Fluka.

2.2. Expression and purification of recombinant TMPKs

The *M. delmotii* TMPK (MdTMPK) sequence was retrieved from the GenBank (with access number: SJM35146) and the DNA was cloned into the pET-14b vector, which was transformed into an *E. coli* (DE3) pLysS strain. The expression of MdTMPK was induced by the addition of 0.1 mM IPTG to the culture and incubated overnight at 30°C. Cells were collected by centrifugation at $4800 \times g$ for 20 min at 4°C. The cell pellets were resuspended in buffer (10 mM Tris/HCl, pH 7.5, 200 mM NaCl, 4 mM MgCl₂, and 0.35 µg/ml DNase) and lysed by using a cell disruptor (Constant systems). The lysate was centrifuged at $15,000 \times g$ for 30 min at 4°C, and the supernatant was used to purify the recombinant MdTMPK using Ni²⁺-Sepharose column chromatography as described previously [11]. *B. anthracis* and human TMPK were expressed and purified under similar conditions.

2.3. TMPK activity assay

TMPK activity was determined by using a radiochemical method with [³H]-dTMP as substrate [12]. The reaction mixtures, containing 50 mM Tris/HCl, pH 7.5, 2 µM [³H]-dTMP, 1 mM ATP, variable concentrations of different metal ions, 5 mM DTT (dithiothreitol) and purified TMPK protein, were incubated at 37°C for 30 min. Aliquots of the reaction mixtures were spotted onto DEAE filter paper (DEAE filtermat, PerkinElmer) and dried. The filters were washed 3 times with 50 mM ammonium formate and then sorted into scintillation vials. The product was eluted with 0.5 ml solution of 0.1 M HCl and 0.2 M KCl and counted after the addition of scintillation liquid (Optiphase Hisafe 3, Perkin Elmer). In addition, aliquots of the reaction products were analyzed by thin layer chromatography developed in 0.2 M NaH₂PO₄. Non-radioactive dTMP and dTDP were used as standards, essentially as described [13].

2.4. Steady-state kinetics

Kinetic parameters for dTMP, ATP, dUMP, Mg²⁺ and Mn²⁺ were determined by using a coupled spectrophotometric method essentially as described [14]. Briefly, the reaction mixture contained 10 mM Tris/HCl pH 7.6, 5 mM DTT, 0.5 mM phosphoenolpyruvate, 0.1 mM NADH, 4 units/mL pyruvate kinase, 4 units/mL lactate dehydrogenase, various concentrations of substrates (ATP, dTMP, or dUMP) and metal ions (Mg²⁺ or Mn²⁺). The reaction was initiated by addition of MdTMPK and the decrease of NADH concentration was monitored at 340 nm for 2 min using a

spectrophotometer at room temperature (21 °C). Kinetic parameters were calculated using Michaelis-Menten equation from at least three independent experiments and given as mean \pm S.D.

The effects of Zn²⁺, Cd²⁺ and Cs⁺ were studied using radiochemical method with [³H]-dTMP as fixed substrate, and variable Mg²⁺ and other metal ions (Zn²⁺, Cd²⁺ and Cs⁺) concentrations. All assays were repeated at least three times and the results are given as mean \pm S.D.

2.5. Genome analysis

TMPK sequences were extracted from www.ncbi.nlm.nih.gov/. Multiple sequence alignments were performed by the method Muscle in MEGA7 [15]. Phylogenetic reconstruction was inferred by using the Maximum likelihood method based on the Jones-Taylor-Thornton (JTT) matrix-based model [16]. The tree with the lightest log likelihood (-3149.8070) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. 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 a 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.

2.6. Statistical analysis

All data was analyzed by using the GraphPad Prism and Excel 2010. Statistical comparisons were performed using two-tailed T-test. All data was derived from at least three independent measurements and are presented as the mean \pm S.D.

3. Results

3.1. Sequence and phylogenetic analyses of TMPKs

Mesorhizobium is a gram-negative, nitrogen-fixing soil bacterium belonging to the family *Rhizobiaceae* that also includes *Rhizobium*, *Allorhizobium*, *Sinorhizobium*. These organisms have high tolerance to soil acidity, salinity, temperature extremes, and metalcolous conditions [5, 17]. *M. delmotii* TMPK (MdTMPK) was identified in the *M. delmotii* genome by sequence homology search using human TMPK (HuTMPK) as query sequence, although the MdTMPK sequence shows only 21% sequence identity to HuTMPK. Using TMPK sequences identified in the genomes of *Mesorhizobium* and *Rhizobium* species and two representatives of gram-negative (*E. coli* and *Vibrio Cholerae* (*V. cholera*)), and gram-positive (*B.*

anthracis and *B. subtilis*) bacteria, a phylogenetic tree was built (Figure 1). As seen in Figure 1, there are distinct clusters of TMPK from gram-negative bacteria, which are diverged from TMPKs of gram-positive bacteria. Alignment of the TMPK sequences showed that the sequence identity among *Mesorhizobium* species is high. For example, between *M. delmotii* and *M. metallidurans*, the sequence identity is 98%, and among *Rhizobium* species the degree of sequence identity is also high (>90%). However, between *Mesorhizobium* and *Rhizobium* species the degree of sequence identity is much lower (< 60%). In contrast, a comparison of TMPKs from *Mesorhizobium* species to TMPKs from *Bacillus* species such as *B. anthracis* and to TMPK from *E. coli*, showed that the degree of sequence identity is only ~ 21%. However, important functional motifs, such as the ATP-binding p-loop, are conserved and the sequence diversity is mainly present at the C-terminal sequence.

3.2. Steady-state kinetic analysis of *M. delmotii* TMPK (MdTMPK)

TMPK is known to phosphorylate dTMP and dUMP using ATP or other nucleoside triphosphates as phosphate donors, and it is known that a divalent metal ion is required for catalysis. The kinetic

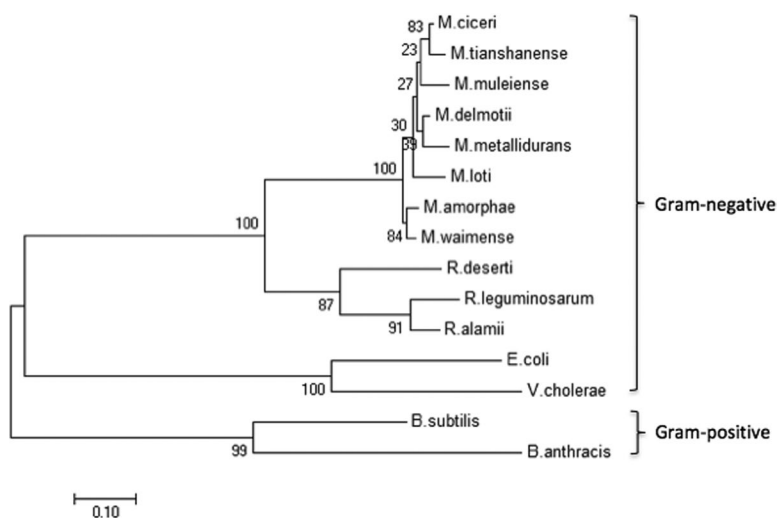


Figure 1. Phylogenetic analysis of TMPK sequences. The phylogenetic tree was constructed by using the MEGA7 software using the Maximum likelihood method based on the Jones-Taylor-Thornton matrix-based model as described in the Materials and Methods section. The numbers at the nodes are bootstrap values. TMPK Sequences with accession number are: *M. Ciceri* (WP_166487953.1); *M. tianshanense* (WP_145716400.1); *M. muleiense* (SDI51051.1); *M. delmotii* (SJM35146.1); *M. metallidurans* (WP_008875775.1); *M. loti* (BAV45273.1); *M. amorphae* (OWK18273.1); *M. waimense* (WP_120015323.1); *Rhizobium deserti* (*R. deserti*) (WP_133314036.1); *R. leguminosarum* (WP_116405974.1); *R. alamii* (WP_037102407.1); *E. coli* (BAA35905.1); *V. cholerae* (KFE22709.1); *B. subtilis* (SPY 13780.1) and *B. anthracis* (AJG86461.1).

Table 1. Kinetic parameters of MdTMPK.

Substrate	K_M (mM)	k_{cat} (s^{-1})	k_{cat}/K_M ($(M^{-1}s^{-1})$)
dTMP	0.065 ± 0.008	3.9 ± 0.03	5.95×10^4
ATP	0.18 ± 0.04	0.4 ± 0.03	2.09×10^3
dUMP	36.3 ± 2.99	0.003 ± 0.004	8.27×10^0
Mg^{2+}	3.5 ± 0.18	2.2 ± 0.02	6.26×10^2
Mn^{2+}	2.2 ± 0.9	3.6 ± 0.5	1.64×10^3

All assays were performed with one variable substrate concentrations and fixed other co-substrate concentrations, which were in excess. Kinetic parameters were calculated using Michaelis-Menten equation from at least three independent experiments and given as mean \pm SE.

parameters of MdTMPK were determined with either dTMP, dUMP or ATP as substrates, in the presence of excess of the other co-substrates. As shown in Table 1, The K_M values for dTMP and ATP were in the micromolar range while the K_M value for dUMP was in millimolar scale, which indicated MdTMPK is specific for dTMP, similar to other known TMPKs.

3.3. The effects of divalent and monovalent metal ions on TMPK activity

To assess the metal ion dependence for TMPK activity, we tested the effects of Mg^{2+} , Mn^{2+} and a number of other metal ions on the activity of MdTMPK, BaTMPK and HuTMPK. As shown in Figure 2 (left panel), only Mg^{2+} and Mn^{2+} could act as efficient cofactors for catalysis, with other metal ions having minimal or no effect on the activity of either of the three enzymes. Furthermore, steady-state kinetics analyses with Mg^{2+} and Mn^{2+} showed that Mn^{2+} is better fitted as cofactor for MdTMPK (Table 1).

Next, we evaluated whether the co-presence of Fe^{2+} , Zn^{2+} , Li^+ , Ni^{2+} , Co^{2+} , Mn^{2+} , Cd^{2+} or Cs^+ together with Mg^{2+} (3 mM) influenced the enzymatic activity of the respective TMPKs. This analysis revealed that the co-presence of Zn^{2+} , Ni^{2+} , Co^{2+} , and Cd^{2+} (> 0.5 mM) strongly inhibited MdTMPK activity at 3 mM Mg^{2+} . Unexpectedly, the presence of Cs^+ caused ~ 2 -fold increase in MdTMPK activity with 3 mM Mg^{2+} , as compared with the activity in the presence of 3 mM Mg^{2+} only (Figure 2 right panel).

We further studied the effects of Zn^{2+} , Cd^{2+} and Cs^+ on MdTMPK with varied Mg^{2+} concentrations. At fixed Mg concentrations and variable Zn^{2+} and Cd^{2+} concentrations, the Dixon plots showed that Zn^{2+} and Cd^{2+} were potent noncompetitive inhibitors toward Mg^{2+} (Figure 3A). The K_i values for Zn^{2+} and Cd^{2+} were calculated from replots of the slope of Dixon plots versus Mg^{2+} concentration; they were 18.0 μ M and 147.1 μ M, respectively. In contrast, the presence of Cs^+ resulted in enhancement of MdTMPK activity, in a concentration dependent manner at ≤ 3 mM Mg^{2+} . At higher Mg^{2+} concentration, no stimulatory effect was observed (Figure 3B).

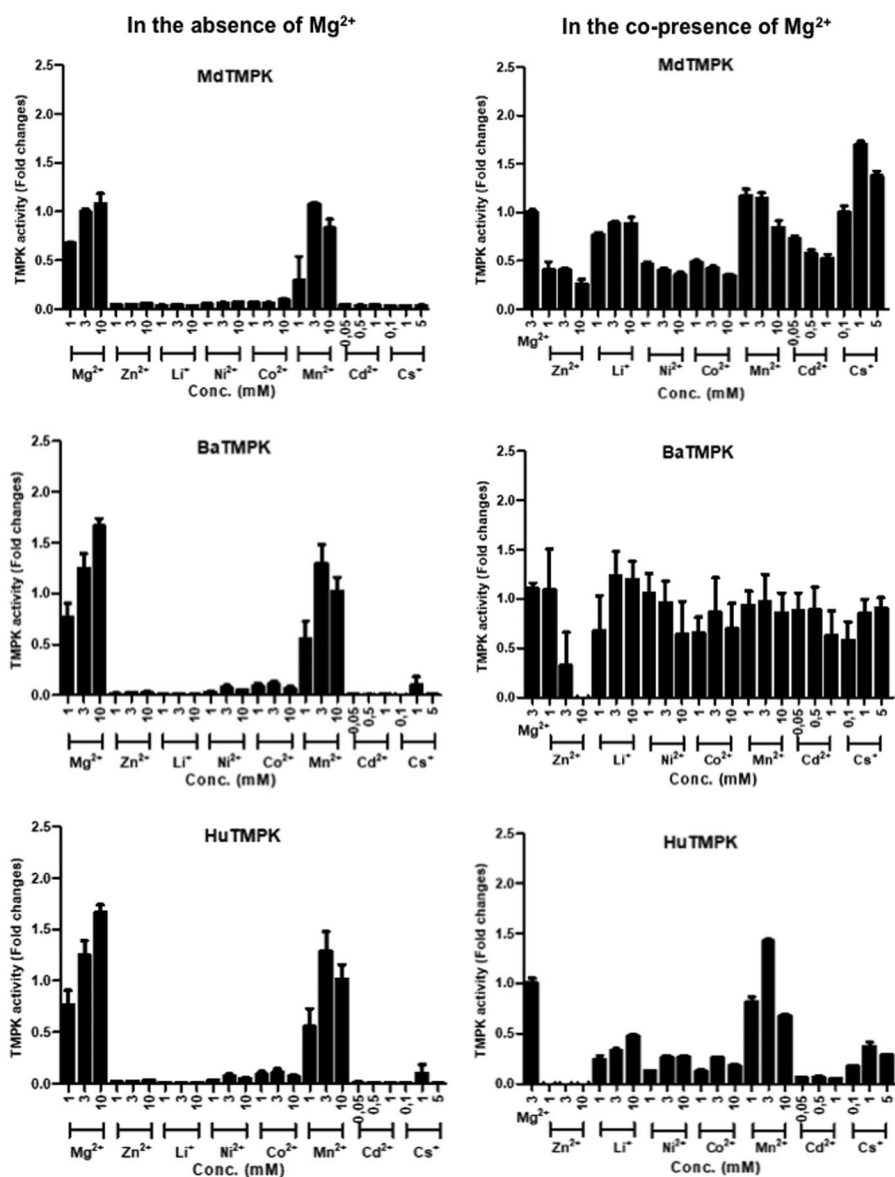


Figure 2 Effects of metal ions on TMPK activity. Left panel: TMPK activity in the presence of various metal ions; Right panel: TMPK activity in the presence of different metal ions with the co-presence of 3 mM Mg²⁺. TMPK activity was measured by using [³H]-dTMP as substrate and shown as fold changes as compared with that with 3 mM Mg²⁺ (set to 1.0). All data were derived from at least three independent measurements and shown as mean ± S.D.

For BaTMPK, an inhibitory effect of the co-presence of Zn²⁺ together with 3 mM Mg²⁺ was observed at ≥3 mM of Zn²⁺. However, the other tested metal ions produced no significant inhibition at concentrations up to 10 mM (Figure 2 right panel).

For HuTMPK, the co-presence of Zn²⁺ completely inactivated the enzyme at 3 mM Mg²⁺. Other metal ions, i.e., Li⁺, Ni²⁺, Co²⁺, Cd²⁺ and Cs⁺ also

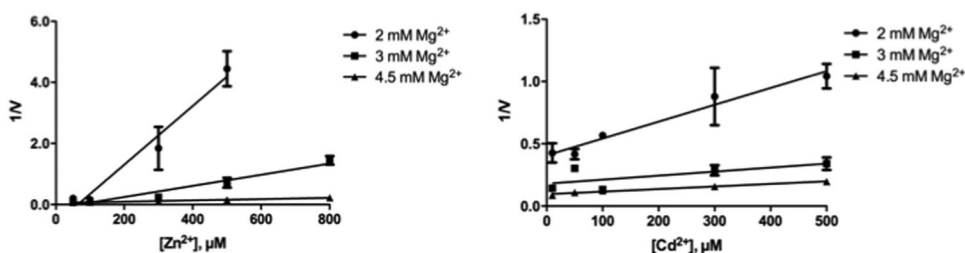
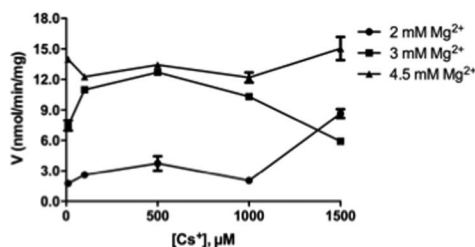
A**B**

Figure 3. Effects of Zn^{2+} , Cd^{2+} and Cs^+ on MdTMPK activity. Dixon plots of Zn^{2+} and Cd^{2+} (A) and Cs^+ (B) concentrations versus Mg^{2+} concentrations. MdTMPK activity was measured by using [3H]-dTMP (2 μM) and ATP (1 mM) as substrates at fixed Mg^{2+} concentration (2, 3, or 4.5 mM), and variable concentrations of Zn^{2+} , Cd^{2+} and Cs^+ . Initial velocity (V) is in nmol/min/mg. All data were derived from at least three independent measurements and shown as mean \pm S.D.

strongly inhibited HuTMPK activity at Mg^{2+} (3 mM) (Figure 2 right panel). Thus, HuTMPK is sensitive to the presence of monovalent and divalent metal ions other than Mg^{2+} and Mn^{2+} , whereas MdTMPK and BaTMPK are resistance to most metal ions.

4. Discussion

Many divalent metal ions participate in biological processes through binding to specific proteins, as exemplified by Mg^{2+} and Mn^{2+} in TMPK catalysis. However, except for Mg^{2+} and Mn^{2+} , little is known regarding the effects of other mono- and divalent metal ions on TMPK function. In this study we investigated the effects of a number of mono- and divalent metal ions on *M. delmotii*, *B. anthracis* and human TMPK. Our results revealed that the effects of monovalent metal ions (Li^+ , Cs^+) and divalent metal ions (Cd^{2+} , Zn^{2+} , Ni^{2+} , Co^{2+}) on TMPK activity differed considerably between MdTMPK, BaTMPK and HuTMPK. Intriguingly, Cs^+ had in fact

a stimulatory effect on MdTMPK, but not on BaTMPK or HuTMPK. These results suggest that these differential effects may be a reason for the high tolerance of *M. delmotii* and *B. anthracis* to heavy metals.

In human TMPK, Mg²⁺ is located in the dTMP-binding site (Figure 5A). Mg²⁺ has a high affinity for water and prefers oxygen-containing ligands/residues because of favorable electrostatic interaction energy [18]. As shown in Figure 5B, all six bonds from Mg²⁺ are formed directly to oxygen (PDB code: 1E2F) [19].

Earlier studies have shown that, among divalent metal ions, Zn²⁺ has higher binding affinity than other divalent metal ions such as Mg²⁺, Mn²⁺, Fe²⁺, Co²⁺, Ni²⁺ or Cu²⁺, which have a binding affinity order of Mg²⁺ < Mn²⁺ < Fe²⁺ < Co²⁺ < Ni²⁺ < Cu²⁺, according to Irving-Williams series [20]. Our kinetic study of the effect of Zn²⁺ and Cd²⁺ on MdTMPK showed that these two metal ions indeed bind stronger than Mg²⁺, but at a different site, resulting in noncompetitive inhibition.

The regulatory effects of monovalent metal ions on TMPKs have not been studied earlier but studies with ribokinases have shown that monovalent cations such as K⁺ and Cs⁺ have stimulatory effects on *E. coli* ribokinase activity [21]. In contrast, these monovalent metal ions have inhibitory effects on *E. coli* phosphofructokinase-2 [22]. Structural studies have revealed that the allosteric effects of the monovalent cations are due to their binding to a conserved monovalent cation-binding site at the C-terminal part of the proteins. This leads to conformational changes of the ATP-Mg²⁺ binding pocket, resulting in enzyme activation or inhibition [21, 23]. Alignment of the C-terminal sequences of MdTMPK, BaTMPK and HuTMPK with ribokinases from *E. coli* and *Vibrio cholerae* (*V. cholerae*) shows that the residues that bind Cs⁺ in the *E. coli* ribokinase [21] are conserved in *V. cholerae* ribokinase and MdTMPK, but are not present in BaTMPK or HuTMPK (Figure 4). This suggests that, in MdTMPK, Cs⁺ may bind to the C-terminal of the protein, thereby inducing conformational changes resulting in a stimulatory effect on MdTMPK activity. Accordingly, Cs⁺ had no effect on BaTMPK and HuTMPK activity since they lack the corresponding residues that can bind Cs⁺.

TMPK is an essential enzyme for cell growth, as demonstrated in *E. coli* [24]. In order to survive in heavy metal-contaminated soil, an active TMPK is vital. Thus, a TMPK that tolerates heavy metals is a pre-requisite for organism growth. The ability of MdTMPK to tolerate heavy metals, together with the stimulatory effect of monovalent metal ions (Cs⁺) on MdTMPK activity, suggests that, in a heavy metal-contaminated area, *M. delmotii* can efficiently synthesize dTTP, which ensures adequate dTTP supply for DNA replication and cell growth. Similarly, the heavy metal-resistance of *B. anthracis* TMPK may, at least partly, explain why *B. anthracis* can propagate in heavy metal-contaminated area.

MdTMPK	-VVDASADPDAVEN VVTATVFAALETMP-----VHRK--QVSPA	224
HuTMPK	-MVDASKSIEAVHEELRVLSEDAIRTEKPLGELWK	212
BaTMPK	-LVNADQPMK----LIEEVIQVIEDKLL	208
EcRK	-AVDTIAAGDTF---NGALITAL--LEEKPLPEAIRFAHAAAAIAVTRKGAQPSVWPREEIDAFDRQR	261
VcRK	-ATDTTAAGDTF---NGALVTGL--LQEMPLESAIKFAHAAAAISVTRFGAQTSSIPTRAEVEAFLAEHS	306
	*** *****	

Figure 4. Alignment of C-terminal sequences. MdTMPK (accession number SJM35146.1), HuTMPK (accession number AAA21719), BaTMPK (accession number AJG86461), *E. coli* ribokinase (EcRK) (accession number WP_136750898.1), and *V. cholerae* ribokinase (VcRK) (accession number AKB06975). The C-terminal regions that involved in Cs^+ binding in EcRK sequence are marked with *. Residues in EcRK that are directly involved in Cs^+ binding are in red, and the corresponding conserved residues in VcRK and MdTMPK are also in red.

Since the TMPK sequences from nitrogen-fixing bacteria such as *Mesorhizobium* and *Rhizobium* species are similar to MdTMPK sequence, it is plausible that their TMPKs have similar properties as MdTMPK. TMPKs from *Bacillus* subspecies have also high sequence homology to *B. anthracis* and, therefore, TMPK of other *Bacillus* species may also be resistant to the presence of heavy metals. The results presented here concerning MdTMPK and BaTMPK may thus help to explain, at least in part, their ability to adapt to an environment contaminated with heavy metals.

5. Conclusion

Symbiotic nodule-forming bacteria such as *M. delmotii* and *M. metallaridans* are of great agricultural and ecological importance. These bacteria species promote the growth and uptake of heavy metals in specific plants from contaminated soil [25]. The nonpathogenic *Bacillus* species can absorb and accumulate heavy metals from industrial waste effluents and contaminated soil [7]. Therefore, these microorganisms may play an important role in soil bioremediation. Our findings show that TMPK from *M. delmotii*, *B. anthracis* and humans can use Mg^{2+} and Mn^{2+} as cofactors for

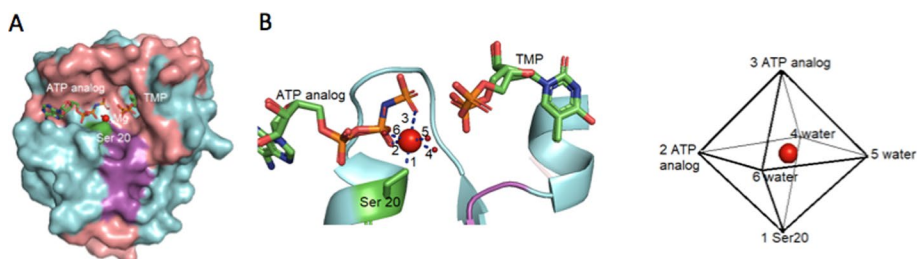


Figure 5. Human TMPK structure. (A) HuTMPK (PDB code: 1E2F) in complex with dTMP, ATP analog and Mg^{2+} . Mg^{2+} binding site is located on the surface, adjacent to dTMP. (B) In human TMPK Mg^{2+} forms 6 bonds and is in an octahedral geometry with its binding partner, one bond to Ser20 of the protein, two bonds to ATP analog and three bonds with water. All the bond distances are 2.1 Å.

catalysis. Intriguingly, MdTMP and BaTMPK were able to tolerate heavy metals at relatively high concentrations, with MdTMPK in fact being stimulated by Cs⁺. Altogether, we may propose that the ability of MdTMP and BaTMPK to tolerate heavy metals at relatively high concentrations, at least partly, may explain why *M. delmotii* and *B. anthracis* can grow in heavy metal-contaminated soil.

Disclosure statement

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References

- [1] Tchouwou, P. B.; Yedjou, C. G.; Patlolla, A. K.; Sutton, D. J. Heavy Metal Toxicity and the Environment. *Exp. Suppl.* **2012**, *101*, 133–164.
- [2] Mohamad, R.; Maynaud, G.; Le Quere, A.; Vidal, C.; Klonowska, A.; Yashiro, E.; Cleyet-Marel, J.-C.; Brunel, B. Ancient Heavy Metal Contamination in Soils as a Driver of Tolerant *Anthyllis Vulneraria* Rhizobial Communities. *Appl. Environ. Microbiol.* **2017**, *83*, e01735–16. DOI: [10.1128/AEM.01735-16](https://doi.org/10.1128/AEM.01735-16).
- [3] Alirzayeva, E.; Neumann, G.; Horst, W.; Allahverdiyeva, Y.; Specht, A.; Alizade, V. Multiple Mechanisms of Heavy Metal Tolerance Are Differentially Expressed in Ecotypes of *Artemisia Fragens*. *Environ. Pollut.* **2017**, *220*, 1024–1035. DOI: [10.1016/j.envpol.2016.11.041](https://doi.org/10.1016/j.envpol.2016.11.041).
- [4] Maynaud, G.; Brunel, B.; Yashiro, E.; Mergeay, M.; Cleyet-Marel, J.-C.; Le Quéré, A. CadA of *Mesorhizobium metallidurans* Isolated from A Zinc-Rich Mining Soil is a P(IB-2)-type ATPase Involved in Cadmium and Zinc Resistance. *Res. Microbiol.* **2014**, *165*, 175–189. DOI: [10.1016/j.resmic.2014.02.001](https://doi.org/10.1016/j.resmic.2014.02.001).
- [5] Laranjo, M.; Alexandre, A.; Oliveira, S. Legume Growth-Promoting *Rhizobia*: An Overview on the *Mesorhizobium* Genus. *Microbiol. Res.* **2014**, *169*, 2–17. DOI: [10.1016/j.micres.2013.09.012](https://doi.org/10.1016/j.micres.2013.09.012).
- [6] Mohamad, R.; Willems, A.; Le Quéré, A.; Maynaud, G.; Pervent, M.; Bonabaud, M.; Dubois, E.; Cleyet-Marel, J.-C.; Brunel, B. *Mesorhizobium Delmotii* and *Mesorhizobium Prunaredense* Are Two New Species Containing Rhizobial Strains within the Symbiovar *Anthyllidis*. *Syst. Appl. Microbiol.* **2017**, *40*, 135–143. DOI: [10.1016/j.syapm.2017.01.004](https://doi.org/10.1016/j.syapm.2017.01.004).
- [7] Alotaibi, B. S.; Khan, M.; Shamim, S. Unraveling the Underlying Heavy Metal Detoxification Mechanisms of *Bacillus Species*. *Microorganisma* **2021**, *9*, 1628. DOI: [10.3390/microorganisms9081628](https://doi.org/10.3390/microorganisms9081628).

- [8] Ayangbenro, A. S.; Babalola, O. O. Genomic Analysis of *Bacillus cereus* nwuab01 and Its Heavy Metal Removal from Polluted Soil. *Sci. Rep.* **2020**, *10*, 19660.
- [9] Lee, L.-S.; Cheng, Y.-C. Human Thymidylate Kinase. Purification, Characterization, and Kinetic Behavior of the Thymidylate Kinase Derived from Chronic Myelocytic Leukemia. *J. Biol. Chem.* **1977**, *252*, 5686–5691.
- [10] Carnrot, C.; Wehelie, R.; Eriksson, S.; Bölske, G.; Wang, L. Molecular Characterization of Thymidine Kinase from *Ureaplasma urealyticum*: Nucleoside Analogues as Potent Inhibitors of *Mycoplasma* Growth. *Mol. Microbiol.* **2003**, *50*, 771–780. DOI: [10.1046/j.1365-2958.2003.03717.x](https://doi.org/10.1046/j.1365-2958.2003.03717.x).
- [11] Carnrot, C.; Wang, L.; Topalis, D.; Eriksson, S. Mechanisms of Substrate Selectivity for *Bacillus anthracis* Thymidylate Kinase. *Protein Sci.* **2008**, *17*, 1486–1493. DOI: [10.1110/ps.034199.107](https://doi.org/10.1110/ps.034199.107).
- [12] Hu Frisk, J. M.; Eriksson, S.; Pejler, G.; Wang, L. Identification of a Novel Thymidylate Kinase Activity. *Nucleosides Nucleotides Nucleic Acids*. **2020**, *39*, 1359–1368. DOI: [10.1080/15257770.2020.1755043](https://doi.org/10.1080/15257770.2020.1755043).
- [13] Wang, L. The Role of *Ureaplasma* Nucleoside Monophosphate Kinases in the Synthesis of Nucleoside Triphosphates. *FEBS J.* **2007**, *274*, 1983–1990. DOI: [10.1111/j.1742-4658.2007.05742.x](https://doi.org/10.1111/j.1742-4658.2007.05742.x).
- [14] Schelling, P.; Folkers, G.; Scapozza, L. A Spectrophotometric Assay for Quantitative Determination of K_{cat} of *Herpes Simplex* Virus Type 1 Thymidine Kinase Substrates. *Anal. Biochem.* **2001**, *295*, 82–87. DOI: [10.1006/abio.2001.5191](https://doi.org/10.1006/abio.2001.5191).
- [15] Kumar, S.; Stecher, G.; Tamura, K. Mega7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol. Biol. Evol.* **2016**, *33*, 1870–1874. DOI: [10.1093/molbev/msw054](https://doi.org/10.1093/molbev/msw054).
- [16] Jones, D. T.; Taylor, W. R.; Thornton, J. M. The Rapid Generation of Mutation Data Matrices from Protein Sequences. *Comput. Appl. Biosci.* **1992**, *8*, 275–282. DOI: [10.1093/bioinformatics/8.3.275](https://doi.org/10.1093/bioinformatics/8.3.275).
- [17] Laranjo, M.; Oliveira, S. Tolerance of *Mesorhizobium* Type Strains to Different Environmental Stresses. *Antonie Van Leeuwenhoek* **2011**, *99*, 651–662. DOI: [10.1007/s10482-010-9539-9](https://doi.org/10.1007/s10482-010-9539-9).
- [18] Dudev, T.; Nikolova, V. Determinants of Fe^{2+} over M^{2+} ($\text{M}=\text{Mg}, \text{Mn}, \text{Zn}$) Selectivity in Non-Heme Iron Proteins. *Inorg. Chem.* **2016**, *55*, 12644–12650. DOI: [10.1021/acs.inorgchem.6b01822](https://doi.org/10.1021/acs.inorgchem.6b01822).
- [19] Ostermann, N.; Schlichting, I.; Brundiers, R.; Konrad, M.; Reinstein, J.; Veit, T.; Goody, R. S.; Lavie, A. Insights into the Phosphoryltransfer Mechanism of Human Thymidylate Kinase Gained from Crystal Structures of Enzyme Complexes along the Reaction Coordinate. *Structure* **2000**, *8*, 629–642. DOI: [10.1016/S0969-2126\(00\)00149-0](https://doi.org/10.1016/S0969-2126(00)00149-0).
- [20] Dudev, T.; Lim, C. Competition among Metal Ions for Protein Binding Sites: Determinants of Metal Ion Selectivity in Proteins. *Chem. Rev.* **2014**, *114*, 538–556. DOI: [10.1021/cr4004665](https://doi.org/10.1021/cr4004665).
- [21] Andersson, E.; Mowbray, S. Activation of Ribokinase by Monovalent Cations. *J. Mol. Biol.* **2002**, *315*, 409–419. DOI: [10.1006/jmbi.2001.5248](https://doi.org/10.1006/jmbi.2001.5248).
- [22] Baez, M.; Cabrera, R.; Pereira, H. M.; Blanco, A.; Villalobos, P.; Ramirez-Sarmiento, C. A.; Caniuguir, A.; Guixé, V.; Garratt, R. C.; Babul, J. A Ribokinase Family Conserved Monovalent Cation Binding Site Enhances the MgATP-Induced Inhibition in *E. coli* Phosphofructokinase-2. *Biophys. J.* **2013**, *105*, 185–193. DOI: [10.1016/j.bpj.2013.05.028](https://doi.org/10.1016/j.bpj.2013.05.028).

- [23] Li, J.; Wang, C.; Wu, Y.; Wu, M.; Wang, L.; Wang, Y.; Zang, J. Crystal Structure of Sa239 Reveals the Structural Basis for the Activation of Ribokinase by Monovalent Cations. *J. Struct. Biol.* **2012**, *177*, 578–582. DOI: [10.1016/j.jsb.2011.12.010](https://doi.org/10.1016/j.jsb.2011.12.010).
- [24] Chaperon, D.-N. Construction and Complementation of in-Frame Deletions of the Essential *Escherichia coli* Thymidylate Kinase Gene. *Appl. Environ. Microbiol.* **2006**, *72*, 1288–1294. DOI: [10.1128/AEM.72.2.1288-1294.2006](https://doi.org/10.1128/AEM.72.2.1288-1294.2006).
- [25] Sobariu, D. L.; Ionela, D.; Fertu, T.; Diaconu, M.; Pavel, L. V.; Hlihor, R.-M.; Dragoi, E. N.; Curteanu, S.; Lenz, M.; Corvini, P.; F-X; Gavrilescu, M. *Rhizobacteria* and Plant Symbiosis in Heavy Metal Uptake and Its Implications for Soil Bioremediation. *N Biotechnol.* **2017**, *39*, 125–134. DOI: [10.1016/j.nbt.2016.09.002](https://doi.org/10.1016/j.nbt.2016.09.002).