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RESEARCH ARTICLE



# Cloning, expression and biochemical characterization of xanthine and adenine phosphoribosyltransferases from *thermus thermophilus* HB8

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**ABSTRACT**

Purine phosphoribosyltransferases, purine PRTs, are essential enzymes in the purine salvage pathway of living organisms. They are involved in the formation of C-N glycosidic bonds in purine nucleosides-5′-monophosphate (NMPs) through the transfer of the 5-phosphoribosyl group from 5-phospho-α-D-ribosyl-1-pyrophosphate (PRPP) to purine nucleobases in the presence of $Mg^{2+}$. Herein, we report a simple and thermostable process for the one-pot, one-step synthesis of some purine NMPs using xanthine phosphoribosyltransferase, XPRT or adenine phosphoribosyltransferase, APRT2, from *Thermus thermophilus* HB8. In this sense, the cloning, expression and purification of *Tt*XPRT and *Tt*APRT2 is described for the first time. Both genes, *xprt* and *aprt*2 were expressed as his-tagged enzymes in *E. coli* BL21(DE3) and purified by a heat-shock treatment, followed by Ni-affinity chromatography and a final, polishing gel-filtration chromatography. Biochemical characterization revealed *Tt*XPRT as a tetramer and *Tt*APRT2 as a dimer. In addition, both enzymes displayed a strong temperature dependence (relative activity >75% in a temperature range from 70 to 90 °C), but they also showed very different behaviour under the influence of pH. While *Tt*XPRT is active in a pH range from 5 to 7, *Tt*APRT2 has a high dependence of alkaline conditions, showing highest activity values in a pH range from 8 to 10. Finally, substrate specificity studies were performed in order to explore their potential as industrial biocatalyst for NMPs synthesis.

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## Introduction

Nucleic acid derivatives, NADs (nucleosides, nucleotides and nucleobases) are essential metabolites in numerous biochemical processes. In this way, they have been widely used for the treatment of several serious human illnesses, such as cancer or viral diseases among others (Galmarini et al. 2002; de Clercq 2005; Parker 2009).

Chemical synthesis of NMPs is routinely performed by 5′-phosphorylation of the precursor nucleoside. It often requires multiple protective group manipulations to control the regio- and stereochemistry of ribose phosphorylation and the use of chemical reagents, such as phosphoryl chloride ($POCl_3$) or phosphorus pentoxide ($P_2O_5$) and organic solvents which are expensive and environmentally harmful (Asano 2002; Li et al. 2015). Due to this, chemical synthesis of NMPs usually provide poor or moderate yields, low product purity and is also associated with harsh reaction conditions and waste disposal issues (Asano 2002; Serra et al. 2014). These drawbacks increase final production costs and lead to a prohibitively expensive price of the NADs, impeding their biological trials and studies, as well as limiting their wide therapeutic application. Due to the economic and social relevance of this kind of drugs, the availability of efficient, stereoselective and sustainable processes should be very relevant for the industry.

In this frame, the enzymatic synthesis of nucleosides-5′-monophospate (NMPs) is an attractive field since biotransformations shows many advantages, such as one-pot reactions under mild conditions, high stereo- and regioselectivity, and an environmentally friendly technology. The use of enzymes as industrial catalysts (white biotechnology) can thus provide novel and straightforward synthetic schemes and suitable and efficient methodologies for developing sustainable industrial processes.

Purine phosphoribosyltransferases belong to the phosphoribosyltransferases family (PRTs). They catalyse

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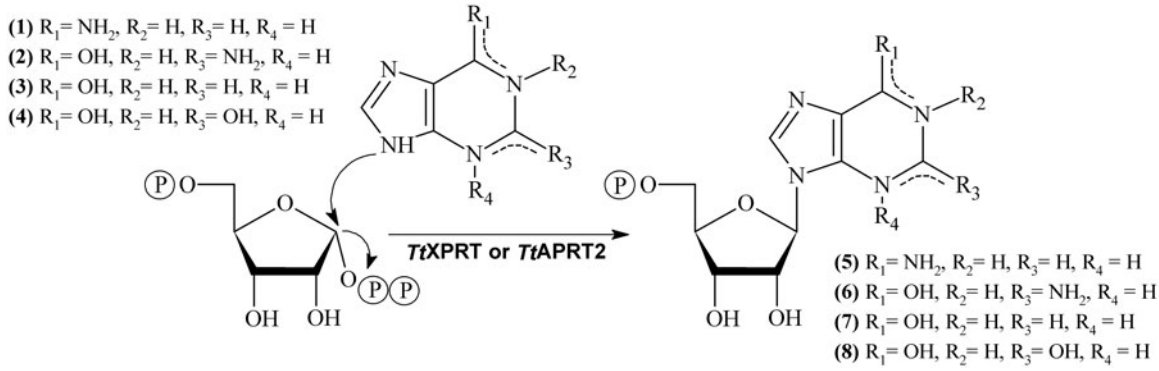


**Figure 1.** Enzymatic synthesis of purine nucleosides-5′-monophosphate (NMPs) catalyzed by *Tt*XPRT and *Tt*APRT2.

the reversible transfer of the 5-phosphoribosyl group from 5-phospho-α-D-ribosyl-1-pyrophosphate (PRPP) to N9 in 6-amino or 6-oxopurines, such as adenine **(1)**, hypoxanthine **(2)**, guanine **(3)** and xanthine **(4)**, in the presence of $Mg^{2+}$ to synthesize adenosine-5′-monophosphate, AMP **(5)**, inosine-5′-monophosphate, IMP **(6)**, guanosine-5′-monophosphate, GMP **(7)** or xanthosine-5′-monophosphate, 5′-XMP **(8)** (el Kouni 2003; Craig and Eakin 2000) (Figure 1). These kind of enzymes are essential in the salvage pathways for purine nucleotide synthesis of all organisms. Due to this, the use of PRTs as therapeutic targets has been extensively reported (el Kouni 2003). In contrast, only a few examples about their use as catalysts in NMP synthesis has been described (Scism et al. 2007, 2010; Valino et al. 2015; Iglesias et al. 2015; Esipov et al 2016; Hansen et al 2014).

According to their substrate specificity, purine PRTs are classified into two general classes: 6-aminopurine PRTs (APRT), strictly specific for 6-aminopurines, such as adenine and 6-aminopurine derivatives (Craig and Eakin 2000; Iglesias et al. 2015; Esipov et al. 2016), and 6-oxopurine PRTs (HPRT, GPRT, XPRT, HGPRT, XGPRT or HGXPRT) that can recognize different kinds of 6-oxopurines, such as hypoxanthine, guanine, xanthine and other 6-oxopurine analogs (el Kouni 2003; Craig and Eakin 2000; Scism et al. 2007; Scism & Bachmann 2010; Iglesias et al. 2015; Valino et al. 2015).

Nowadays, it is very common use of enzymes from thermophilic organisms in industrial bioprocesses. The use of thermozymes offers the possibility to use high-temperature reactions, resulting in a higher solubilization of substrates, a diminution of medium viscosity and an increase in substrate diffusion coefficients, leading to higher overall reaction rates.

The present work aims to explore the potential of xanthine phosphoribosyltransferase (XPRT, EC 2.4.2.22) and adenine phosphoribosyltransferase (APRT, EC 2.4.2.7) from *Thermus thermophilus* HB8 as biocatalysts for the synthesis of NMPs. In this sense, we report for the first time the cloning of the *xprt* and *aprt*2 genes from *T. thermophilus* (HB8), the expression in *Escherichia coli*, purification of the recombinant proteins (*Tt*XPRT and *Tt*APRT2), as well as their biochemical characterization. In addition, detailed comments about oligomeric state, substrate specificity and architecture of active site were included.

## Materials and methods

### Materials

Cell culture medium reagents were from Difco (St. Louis, MO). Trimethyl ammonium acetate buffer and 5-phospho-α-D-ribosyl-1-pyrophosphate were purchased from Sigma-Aldrich (Madrid, Spain). All other reagents and organic solvents were purchased to Scharlab (Barcelona, Spain) and Symta (Madrid, Spain). All natural and non-natural purine bases used in this work were provided by Carbosynth Ltd. (Compton, United Kingdom).

### Enzyme cloning, expression and purification

The encoding *ttha0535* (*xprt*) and *ttha1614* (*aprt*2) genes, which codifies xanthine phosphoribosyltransferase, *Tt*XPRT (NCBI Reference Sequence: YP_143801.1XPRT), and adenine phosphoribosyltransferase, *Tt*APRT2 (NCBI Reference Sequence: YP_144880.1) from *T. thermophilus* HB8, were ordered and purchased from Genscript (Piscataway, NJ). The coding sequences appeared as *Nde*I-*Eco*RI fragments subcloned into two different expression vectors pET28b(+). The resultant recombinant vectors, pET28b*Tt*XPRT and pET28b*Tt*APRT2, provided two different N-terminal His6-tagged fusion proteins with a thrombin cleavage site between the tag and the enzyme. Both enzymes were expressed in *E. coli* BL21(DE3) grown in LB medium at 37 °C supplemented with kanamycin 50 μg/mL. Protein overexpression was induced by adding 0.5 mM β-D-1-thiogalactopyranoside and the cells

further grown for 4 h. These cells were harvested via centrifugation at 3500 × *g*, and the resulting pellets were resuspended in 20 mM Tris-HCl buffer, pH 8, containing 100 mM NaCl. Crude extracts were prepared by ultrasonic cell treatment using a digital sonifier. The lysates were centrifuged at 17,500×*g* for 20 min at 4 °C. After this, lysates were then treated at 70 °C for 20 min. Insoluble material was removed by centrifugation 17,500×*g* for 20 min at 4 °C and the supernatant was filtered through a 0.22-μm filter (Millipore). Cleared lysates were loaded onto a 5-mL HisTrap FF column (GE Healthcare), pre-equilibrated in a binding buffer (20 mM Tris-HCl buffer, pH 8.0, with 100 mM NaCl and 20 mM imidazole). Bound proteins were eluted using a linear gradient of imidazole (from 10 to 500 mM). Fractions containing *Tt*XPRT or *Tt*APRT2 were identified by SDS-PAGE, pooled, concentrated and loaded onto a HiLoad 16/60 Superdex 200 prep grade column (GE Healthcare) pre-equilibrated in 20 mM Tris-HCl buffer, pH 8.0. Fractions with the protein of interest identified by SDS-PAGE were pooled and the protein was concentrated and stored at 4 °C until its use. Electrophoresis was carried out on 15% polyacrylamide slab gel with 25 mM Tris-HCl buffer, pH 8.6, 0.1% SDS. Protein concentration was determined spectrophotometrically by UV absorption at 280 nm using $\varepsilon_{280} = 31{,}970\ M^{-1}cm^{-1}$ for *Tt*XPRT and $\varepsilon_{280} = 4470\ M^{-1}cm^{-1}$ for *Tt*APRT2 (Gill and Von Hippel 1989).

### Analytical ultracentrifugation analysis

Sedimentation velocity for *Tt*UPRT were carried out in 20 mM Tris-HCl buffer, pH 8.0 at 20 °C and 50,000×*g* in an Optima XL-I analytical ultracentrifuge (Beckman-Coulter Inc., Madrid, Spain), equipped with UV-VIS absorbance and Raleigh interference detection systems, using an An-60Ti rotor and standard (12-mm optical path) double-sector centre pieces of Epon-charcoal. Sedimentation profiles were recorded at 292 nm. Sedimentation coefficient distributions were calculated by least-squares boundary modelling of sedimentation velocity using the continuous distribution *c(s)* Lamm equation model as implemented by SEDFIT 14.7 g.

Baseline offsets were measured afterwards at 200,000×*g*. The apparent sedimentation coefficient of distribution, *c*(*s*), and sedimentation coefficient *s* were calculated from the sedimentation velocity data using the program SEDFIT (Brown and Schuck 2006). The experimental sedimentation coefficients were corrected to standard conditions (water, 20 °C and infinite dilution) using the software SEDNTERP (Holde 1985) in order to get the corresponding standard *s* values ($s_{20,w}$).

### Enzyme activity assay

The standard activity assay was performed by incubating 10–50 μL of free extracts or 1–5 μg of pure enzymes with a 40 μL solution containing 10 mM PRPP, 10 mM adenine (*Tt*APRT) or xanthine (*Tt*XPRT), 12 mM $MgCl_2$ in 12 mM Tris-HCl buffer pH 8. The reaction mixture was incubated at 60 °C for 10 min (300 rpm). Enzyme was inactivated by the addition of 40 μL of cold methanol in icebath and heating for 5 min at 100 °C. After centrifugation at 9000×*g* for 5 min, samples were half-diluted with water and the NMP production was analysed by HPLC to quantitatively measure the reaction products as described below in the analytical methods. All determinations were carried out by triplicate and the maximum error was below 5%. In such conditions, one international activity unit (IU) was defined as the amount of enzyme producing 1 μmol/min of the corresponding NMP under the assay conditions.

### Influence of pH and T on enzyme activity

The optimum pH of both enzymes was initially determined according to the standard activity assay described earlier, using sodium citrate (pH 4–6), sodium phosphate (pH 6–8) and sodium borate (pH 8–10) as reaction buffers (50 mM). The optimum temperature of enzyme activity was determined using standard assay in a temperature range from 20 to 90 °C.

### Storage stability

*Tt*XPRT and *Tt*APRT2 were stored at 4 °C in 20 mM Tris-HCl buffer, pH 7.0 for 100 days. Periodically, samples were taken, and enzymatic activity was evaluated. Storage stability was defined as the relative activity between the first and the successive reactions.

### Substrate specificity

Enzymatic synthesis of NMPs using different natural nucleobases (adenine, hypoxanthine, guanine and xanthine) was performed under standard assay conditions. The reaction mixtures were incubated in different reaction buffers (12 mM sodium citrate pH 6 or 12 mM sodium borate pH 8.5) at 60 °C and 300 rpm orbital shaking at different reaction times (5–15 min). After this, the reaction mixture was processed as previously described above and the NMP production was analysed by HPLC.

### Analytical methods

The production of NMPs was quantitatively measured by HPLC (Agilent 1100 series). Reactions were analysed with an ACE EXCEL 5 μm CN-ES 250 × 4.6 mm equilibrated with 100% trimethyl ammonium acetate at a flow rate of 0.8 mL/min. Retention times for the reference natural compounds (hereafter abbreviated according to the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature) were as follows: adenine (Ade), 10.2 min; adenosine-5′-monophosphate (AMP), 5.50 min; guanine (Gua), 4.8 min; guanosine-5′-monophosphate (GMP), 2.8 min; hypoxanthine (Hyp), 4.5 min; inosine-5′-monophosphate (IMP), 2.5 min; xanthine (Xan), 4.4 min; xanthosine-5′-monophosphate (XMP), 2.5 min. In order to confirm the reaction products, commercial AMP, GMP, IMP and XMP were used as HPLC controls.

## Results and discussion

### Bioinformatic analysis of xanthine and adenine phosphoribosyltransferases encoding genes of T. Thermophilus *HB8*

The genomic information of *T. thermophilus* HB8 has been analysed and published online (Genbank No. AP008226). Several genes that potentially encode purine phosphoribosyltransferases were annotated throughout the genome. Among these, two ORFs, *ttha0535* (*xprt*) and *ttha1614* (*aprt2*), which are annotated as putative xanthine phosphoribosyltransferase (*Tt*XPRT) and a putative adenine phosphoribosyltransferase (*Tt*APRT), were subjected to bioinformatic analysis to identify their physical and chemical properties and their possible active site architecture. Since other putative *aprt* gene (*ttha1613*) was detected in *T. thermophilus* HB8 genome, we renamed *ttha1613* as *aprt1*.

BLAST analysis of amino acid sequences (http://blast.ncbi.nlm.nih.gov/Blast.cgi) reveals that *xprt* and *aprt2* encodes putative xanthine and adenine phosphoribosyltransferases. Multiple sequence alignment of predicted both enzymes with amino acid sequences of xanthine and adenine phosphoribosyltransferases from other thermophilic and mesophilic bacteria and protozoan characterized so far, reveals that both enzymes display a conserved 13-residue "fingerprint" region, PRPP binding domain of PRTs type I, typically composed by four hydrophobic residues followed by two acidic residues (which interact with the vicinal hydroxyl groups of the ribose phosphate moiety), two hydrophobic residues, and four small residues (Figure 2). All these characteristics suggest that these genes could code for putative intracellular phosphoribosyltransferases.

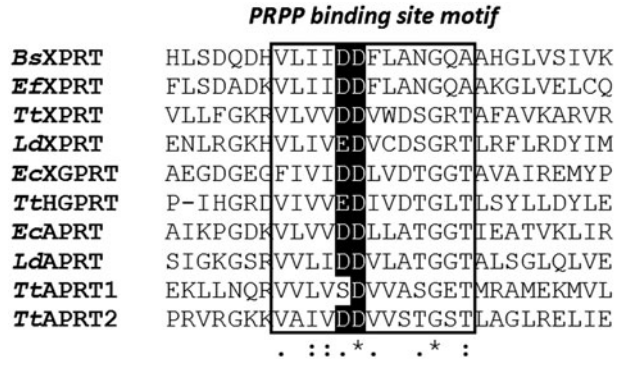


**Figure 2.** Multiple sequence alignment of purine phosphoribosyltransferases from *Leishmania donovani* (*Ld*XPRT), *Thermus thermophilus* (*Tt*APRT1, *Tt*APRT2 and *Tt*XPRT), *Escherichia coli* (*Ec*XGPRT), *Mycobacterium tuberculosis* (*Mt*UPRT), *Escherichia coli* (*Ec*UPRT) and *Bacillus subtilis* (*Bs*XPRT). Sequences were aligned by CLUSTALΩ. Conserved PRPP motif are encircled. *Asterisk* (*), indicates single, fully conserved residues; *colon* (:), conservation of strong groups; *period* (.), conservation of weak groups.

*Tt*XPRT was predicted to contain 154 amino acid residues and have a relative molecular mass of 17.10 kDa (Protparam, http://web.expasy.org/protparam/). Moreover, putative *Tt*APRT2 was predicted to contain 180 amino acids and a relative molecular mass of 19.15 kDa.

### Production and purification of recombinant enzymes

The corresponding *xprt* and *aprt2* genes, encoding *Tt*XPRT and *Tt*APRT2 were cloned, and overexpressed in *E. coli* BL21(DE3) as described in the experimental section. The recombinant N-terminal His6-tagged proteins were purified by two chromatographic steps. SDS-PAGE analysis of purified enzymes shows only one protein band with an apparent molecular mass of 19 kDa for *Tt*XPRT and 21 kDa for*Tt*APRT2.

These results agree with those from analytical ultracentrifugation analysis. Under the conditions assayed, sedimentation velocity experiments revealed *Tt*XPRT as a group of four species with experimental sedimentation coefficients of 3.41 S ($s_{20,w}$ = 3.43 S), 5.84 S ($s_{20,w}$ = 5.89 S), 13.48 S ($s_{20,w}$=13.60 S) and 16.12 S ($s_{20,w}$ = 16.26) compatible with the theoretical behaviour of different oligomeric forms. The major specie found in solution corresponds to a tetrameric state ($M_w$ = 76.08 kDa) and is compatible with a single-species monomeric model of 19.02 kDa, similar to the molecular mass calculated from the amino acid sequence of His6-tagged protein (18.90 kDa). Different

oligomeric states are described for XPRTs. While XPRT from *Streptococcus faecalis* and *Bacillus subtilis* seems to be a homodimer of 42.00 kDa (Miller et al. 1974; Arent et al. 2006), XGPRT from *E. coli* has been reported as a homotetramer (Vos et al. 1997) after the determination of its native molecular mass by Superose-gel filtration chromatography.

On the other hand, experimental data revealed *Tt*APRT2 as a single species with an experimental sedimentation coefficient of 3.06 S ($s_{20,w}$=3.09) compatible with a dimer ($M_w$=41.66 kDa). This value is compatible with a single-species monomeric model of 20.83 kDa, similar to the molecular mass calculated from the amino acid sequence of His6-tagged protein (20.95 kDa). This result agrees with other reported bacterial, archaea, fungal, protozoan and eukaryotic APRTs, such as APRT from *E. coli* (Hochstadt-Ozer and Stadtman 1971), *Sulfolobus solfataricus* (Hansen et al. 2014), *Saccharomyces cerevisiae* (Alfonzo et al. 1997), *Leishmania donovani* (Tuttle and Krenitsky 1980) or human APRT (Holden et al. 1979). In this sense, it seems that the oligomeric state of APRTS is highly conserved among different living organisms.

## Biochemical characterization

Effect of temperature and pH on the activity of *Tt*XPRT and *Tt*APRT2 were studied to select optimal conditions for their use as biocatalysts in NMPs synthesis. Experimental results revealed that the enzymatic activity of *Tt*XPRT and *Tt*APRT2 presents a strong dependence with temperature, as can be expected for proteins from hyperthermophilic organisms, but they also show very different behaviour under the influence of pH.

On one hand, the temperature profile revealed that *Tt*XPRT is active (more than 75%) at high temperature values (more than 75% of retained activity in a temperature range 60–90 °C) showing maximum activity at 80 °C (Figure 3(A)). In addition, recombinant *Tt*XPRT also displayed high activity (more than 75%) in a narrow pH range (5–7), with a maximum activity value at pH 6. (Figure 3(B)). As it is expected from thermophilic enzymes, *Tt*XPRT is very active at high temperatures; unfortunately, there is not any described other strict xanthine phosphoribosyltransferase from thermophiles to compare these data. The most similar enzymes are HGXPRTs from thermoacidophile archaeon *Sulfolobus solfataricus* and hyperthermophilic archaeon *Pyrococcus horikoshii*, which are active at high temperatures (Hansen et al. 2014; de Souza et al. 2008). Regarding the effect of pH on enzyme activity, all described specific XPRTs work reasonably better in neutral-alkaline conditions, such as XPRTs from *Streptococcus faecalis* (Miller et al. 1974) and *E. coli* (Vos et al. 1997). Moreover, other non-specific 6-oxo-purines, such as HGXPRT from *Toxoplasma gondi*i (Naguib et al. 1995), *Bacillus subtilis* (Arent et al. 2006) and *Sulfolobus solfataricus* (Hansen et al. 2014) display a similar pattern. In this sense, *Tt*XPRT differs slightly from other XPRTs.

On the other hand, *Tt*APRT2 displayed maximum activity at 80 °C, with more than 80% of retained activity in a temperature interval from 70 to 90 °C (Figure 3(C)), but contrary to *Tt*XPRT, it could be observed that *Tt*APRT2 showed high activity at high pH values (pH range 8–10) (Figure 3(D)). In this case, *Tt*APRT2 displays a similar susceptibility to alkaline conditions than other mesophilic or thermophilic APRTS, such as APRTS from *Plasmodium falciparum* (Queen et al. 1989), *Leishmania donovani* (Tuttle and Krenitsky 1980) or *Sulfolobus solfataricus* (Jensen et al. 2015). These results are very interesting due to the fact that both proteins, *Tt*XPRT and *Tt*APRT2, display optimal activity values at high temperatures, but *Tt*APRT2 also displays a non-usual tolerance in alkaline conditions that is not expected. Since purine bases usually display poor solubility in aqueous medium (Fernández-Lucas et al. 2012), *Tt*APRT2 could be an interesting biocatalyst for the industrial synthesis of purine NMPs using low soluble bases.

The storage of *Tt*XPRT and *Tt*APRT2 at 4 °C was analysed to ensure biocatalyst stability. Both enzymes exhibited more than 85% of retained activity over more than 70 days (data not shown). Unfortunately, *Tt*XPRT and *Tt*APRT2 suffered a high decrease in activity (20–30%) when they were stored at −20 °C or −80 °C.

## Substrate specificity

Taking into account all previous results, substrate specificity studies were carried out under optimal conditions (*Tt*XPRT, pH 6 and 70 °C; *Tt*APRT2, pH 8.5 and 70 °C). Table 1 summarizes the specific activities of *Tt*XPRT and *Tt*APRT2 over adenine, guanine, hypoxanthine and xanthine. It can be observed that *Tt*XPRT recognizes xanthine as an acceptor for the synthesis of XMP; consequently, *Tt*XPRT has been confirmed as a xanthine phosphoribosyltransferase. Furthermore, *Tt*XPRT could also slightly recognize hypoxanthine and guanine as substrates. However, the remaining activity is negligible; so *Tt*XPRT should not be re-classified as a HGXPRT. Due to the lack of literature on any specific thermophilic XPRT so far, *Tt*XPRT is reported as the first hyperthermophilic XPRT.

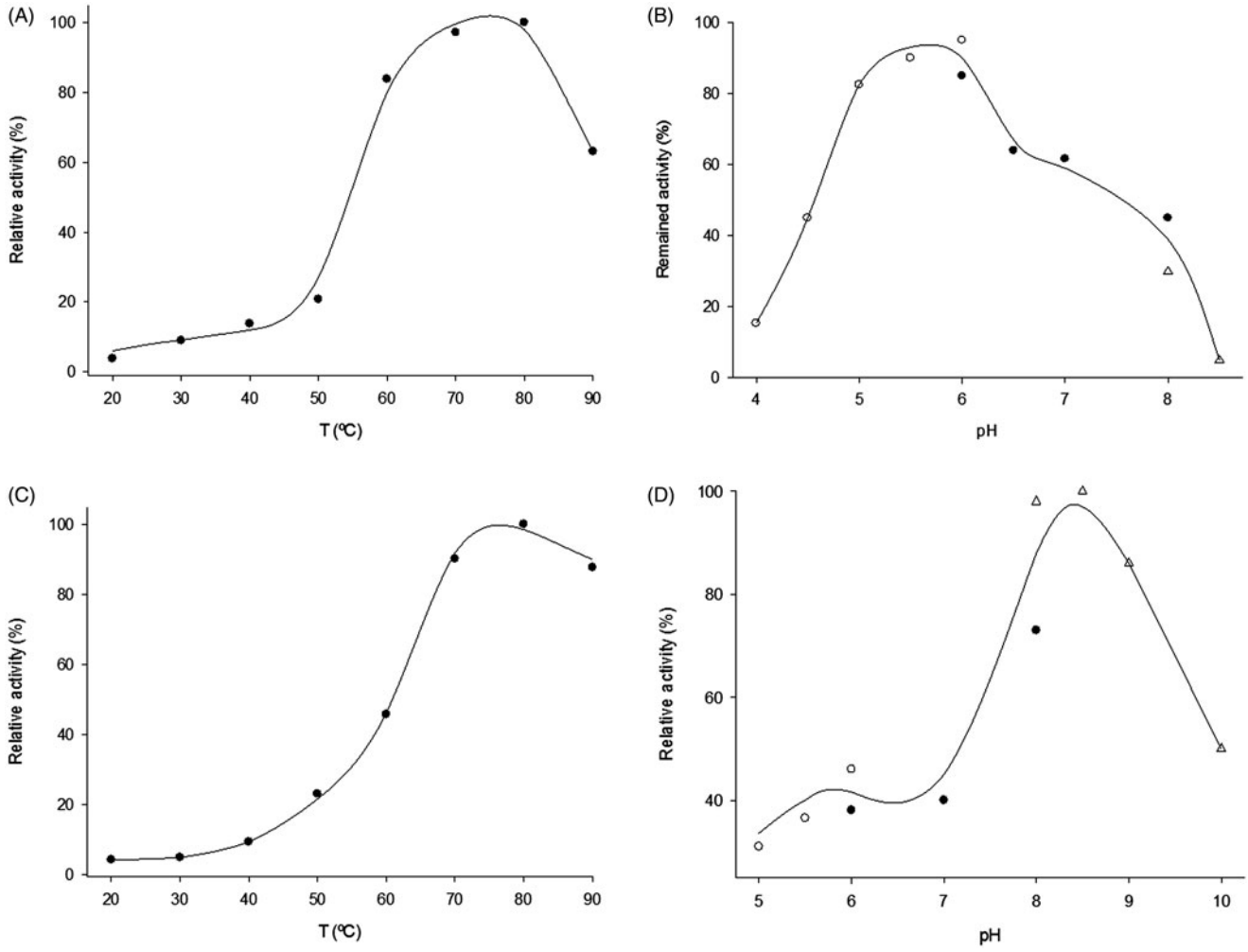


**Figure 3.** Temperature and pH dependence on enzymatic activity. (A) Effect of temperature on *Tt*XPRT activity (●). (B) Effect of pH on *Tt*XPRT activity, (○) sodium acetate 50 mM (pH 4–6), (●) sodium phosphate 50 mM (pH 6–8), (Δ) sodium borate 50 mM (pH 8–10). (C) Effect of temperature on *Tt*APRT2 activity (●). (D) Effect of pH on *Tt*APRT2 activity, (○) sodium acetate 50 mM (pH 4–6), (●) sodium phosphate 50 mM (pH 6–8), (Δ) sodium borate 50 mM (pH 8–10).

Table 1. Activity of *Tt*XPRT and *Tt*APRT2 over different natural nucleobases.

| Purine base | Specific activity *Tt*XPRT[a] (U/mg$_{enz}$) | Specific activity *Tt*APRT2[b] (U/mg$_{enz}$) |
|---|---|---|
| Adenine | n.d. | 0.32 |
| Guanine | 0.10 | n.d. |
| Hypoxanthine | 0.12 | n.d. |
| Xanthine | 1.70 | n.d. |

[a]Reaction conditions: 1.25 μg of enzyme in 40 μL reaction volume. Substrate concentrations were 10 mM PRPP, 10 mM uracil, 12 mM $MgCl_2$ in 12 mM sodium citrate buffer, pH 6. Reaction time: 10 min, $T = 70\,^{\circ}C$, 350 r.p.m.
[b]Reaction conditions: 5 μg of enzyme in 40 μL reaction volume. Substrate concentrations were 10 mM PRPP, 10 mM uracil, 12 mM $MgCl_2$ in 12 mM sodium borate, pH 8.5. Reaction time: 10 min, $T = 70\,^{\circ}C$, 350 r.p.m.
n.d.: not detected.

*Tt*APRT2 could use adenine as acceptor for NMP synthesis. In contrast, neither guanine nor hypoxanthine or xanthine, were recognized as substrates by *Tt*APRT2. As mentioned earlier, two different putative APRTs are annotated in *T. thermophilus* HB8 genome (Genbank No. AP008226), *Tt*APRT1 (YP_144879.1) and *Tt*APRT1 (NCBI Reference Sequence: YP_144880.1). Recently, Esipov et al. (2016) have described APRT from *T. thermophilus* HB27, which is identical to putative *Tt*APRT1 from *T. thermophilus* HB8. Since *Tt*APRT2 is also encoded in *T. thermophilus* HB27 genome, it leads to think that two different APRTS are present in *T. thermophilus*, the first one, *Tt*APRT1, displays an activity value of 8.8 μM $min^{-1}mg^{-1}$ (referred to AMP synthesis) (Esipov et al. 2016), while *Tt*APRT2 display an activity of 8000 μM$min^{-1}mg^{-1}$ (deduced from Table 1). In this sense, it seems that *Tt*APRT2 should play a more important role in purine scavenging in *T. thermophilus* HB8 than *Tt*APRT1. According to multiple sequence alignment of PRPP sequence motifs of different 6-amino and 6-oxopurines PRTs (Figure 2), these experimental results could be expected considering

that *Tt*APRT1 shows only one of two acidic residues, which interacts with the vicinal hydroxyl groups of the ribose phosphate moiety from PRPP. This probably leads to a less effective binding of PRPP and a subsequently decrease in catalytic activity.

## Conclusions

In this work, we describe for the first time the cloning, expression and biochemical characterization of xanthine phosphoribosyltransferase, *Tt*XPRT and adenine phosphoribosyltransferase2, *Tt*APRT2, from *T. thermophilus* HB8. Biochemical characterization revealed *Tt*XPRT as a homotetramer with significant activity in a broad range of temperature pH (from 60 to 90 °C). Additionally, *Tt*XPRT was also found to exhibit activity in a pH interval from 5.0 to 7.0. Moreover, experimental results revealed *Tt*APRT2 as a homodimer, with a similar temperature dependence to *Tt*XPRT (optimal activity in temperature range 70–90 °C), but it displays a very different behaviour under pH influence (from 7.0 to 9.0). The thermophilic origin of both enzymes involves several advantages, such as an easy purification method and tolerance to high temperatures, what makes these enzymes attractive biocatalysts for the industrial synthesis of nucleoside derivatives.

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## Disclosure statement

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