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URIDINE 5′-MONOPHOSPHATE (UMP) METABOLISING ENZYMES URACIL PHOSPHORIBOSYLTRANSFERASE AND OROTIDINE-5′-PHOSPHATE DECARBOXYLASE/UMP SYNTHASE IN CAMELS AND *Trypanosoma evansi*

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ABSTRACT

In this study, the metabolic pathways and the enzymes involved in uridine 5′-monophosphate (UMP) were investigated in camel and the blood parasite *Trypanosoma evansi* (*T. evansi*). The pyrimidine pathway of *T. evansi* was found to be devoid of uridine kinase and uridine 5–nucleotidase. Since this can affect the de novo synthesis using uracil, salvage enzymes as uracil phosphoribosyltransferase (UPRTase) could be important for the parasite life, given that the similarity rate is not more than 32% between the camel and *T. evansi* UPRTase. The source of UMP in *T. evansi* could be from uracil by the action of UPRTase. In addition, the bifunctional orotidine 5′-phosphate decarboxylase (OMPdecase)/UMP synthase shares also in UMP homeostasis. Owing to the diverse sources of UMP in *T. evansi*, the enzymes involved in UMP metabolism are underscored for drug discovery. However, supported by the lack of uridine kinase, further studies are recommended to estimate the impact of UPRTase inhibition on *T. evansi* growth.

Key words: Camel, OPRTase, pyrimidine, *Trypanosoma evansi*, UMP, UPRTase

Bioinformatics has largely enforced drug discovery process by rapid identification of a unique target for the life of pathogens. The recent announcement of camel genome (Jirimutu *et al,* 2012) had led it feasible to compare the target genes in camels and its corresponding in the pathogens. Previously, bioinformatics were used as tools in identification and characterisation of drug targets (Kandeel *et al,* 2019a; Kandeel *et al,* 2019b).

The pyrimidine pathway is essential for every organism for the synthesis of pyrimidine nucleotides. In this work, the metabolic pathways of UMP was investigated in the camel as well as in the blood parasite *T. evansi*. The KEGG maps (Kanehisa *et al,* 2007; Kanehisa *et al,* 2016) were used to map the enzymes involved in UMP binding.

At first, the standard metabolic paths are mapped followed by bioinformatics tools to find the most probable enzymes involved in these paths in camel and *T. evansi*. The comparison involved sequence similarity rate, the motif and domain content and phylogenetic mapping. These values were used in the context of comparison with human, camel species and various prokaryotic and eukaryotic enzymes.

Materials and Methods

Retrieval of genomic data

Collection of genomic data was carried out by extracting the information from the gene database (Hertz-Fowler *et al,* 2004), Kinetoplastom genome resources, protein and genome databases and the Arabian camel genome project information obtained from these sources included protein and nucleotide sequence information, gene annotation, paralogs and orthologs information, metabolic functions, signal peptides, transmembrane domains, predicted protein export domains, protein expression profiles, isoelectric point (pI), the number of predicted transmembrane helices, any predicted sequence motifs, and the E value of the closest PDB sequence homolog.

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Searching homologues

Protein sequence homologues was searched using the NCBI BLAST (Basic Local Alignment Search Tool) (Madden 2013) or PSI-BLAST (Position-Specific Iterated-BLAST) servers searching against the non redundant (nr) database, with filtering of low complexity regions.

Multiple sequence alignment program for proteins and construction of phylogenetic tree. It was carried out as per methods described previously (Sievers and Higgins, 2014). Multiple sequence alignment program (ClustalW2) was used to calculate the best match of the selected sequences. The resultant alignment was used to generate a phylogenic tree, which is visualised by Dendroscope phylogenic tree viewer (Huson *et al,* 2007) or CLC genomics workbench (Sequencing, 2011).

Putative domains— It was searched by the domain prediction program available at (http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml) (Marchler-Bauer *et al,* 2005). The genomic and molecular information was obtained from Kyoto Encyclopedia of Genes and Genomes (http://www.genome.jp/kegg/).

Proteomic and genomic tools

ExPASy Proteomics tools (http://us.expasy.org/tools/) (Gasteiger *et al,* 2003) and tools available at the website of the European Bioinformatics Institute (http://www.ebi.ac.uk/Tools/) (Labarga *et al,* 2007) was also used for analysis of nucleotide and gene sequences. Protein sequences of target genes was analysed for data such as pI, extinction coefficient and MW for the tagged protein sequence by PROTParam. PROSITE was used to search for patterns and profiles in the protein sequences of the target genes. The protein domains, families and functional sites was searched at http://prosite.expasy.org/.

Results and Discussion

Uridine-5′-monophosphate (UMP) production process was proposed by five production routes 1) phosphorylation of uridine 5′-triphosphate by the action of nucleotide diphosphatase, 2) the phosphorylation of uridine-5′-diphosphate by the actions of nucleoside diphosphate phosphatase and/or ATP-diphosphatase or by the action of UMP/CMP kinase and/or UMP kinase 3) from uridine by the kinase activity of uridine kinase 4) from uracil by the action of uracil phosphoribosyl transferase 5) the decarboxylation of orotidine-5′-phosphate by the action of orotidine-5′-phosphate decarboxylase as shown in Fig 1. In the catabolic direction, uridine is produced from UMP by 5′-nucleotidase. The enzymes involved in UMP pathways are shown in Tables 1-3.

Table 1. Enzymes involved in metabolic pathways of UMP.

ID (E.C. number)	Definition (Enzyme name)
3.6.1.8	ATP diphosphatase
2.7.1.48	Uridine kinase
3.1.3.5	Uridine 5′-nucleotidase
2.4.2.9	Uracil phosphoribosyltransferase
4.1.1.23	Orotidine-5′-phosphate decarboxylase
3.6.1.5	Nucleoside triphosphate phosphohydrolases (nucleoside monophosphoate-forming) or ATP- diphosphatase, apyrase, ectonucleoside triphosphate diphosphohydrolase
3.6.1.6	Nucleoside diphosphate phosphatase
2.7.4.22	UMP kinase
2.7.4.14	UMP/CMP kinase
3.6.1.9	Nucleotide diphosphatase

Table 2. The expected enzymes involved in metabolic pathways of UMP in camels.

ID (E.C. number)	Definition (Enzyme name)
2.4.2.9	Uracil phosphoribosyltransferase
3.6.1.5	nucleoside triphosphate phosphohydrolases (Nucleoside monophosphoate-forming) or ATP- diphosphatase, apyrase, ectonucleoside triphosphate diphosphohydrolase
2.7.4.14	UMP/CMP kinase

Table 3. The expected enzymes involved in metabolic pathways of UMP in *Trypanosoma evansi*

ID (E.C. number)	Definition (Enzyme name)
2.4.2.9	Uracil phosphoribosyltransferase
4.1.1.23	Orotidine-5′-phosphate decarboxylase
3.6.1.5	Nucleoside triphosphate phosphohydrolases (nucleoside monophosphoate-forming) or ATP- diphosphatase, apyrase, ectonucleoside triphosphate diphosphohydrolase
2.7.4.14	UMP/CMP kinase

After bioinformatics investigation, *T. evansi* was found to be devoid of uridine kinase and uridine 5′-nucleotidase (Fig 1-3). Therefore, uridine salvage is brought by uracil phosphoribosyltransferase.

Uracil phosphoribosyltransferase

Uracil phosphoribosyltransferase (EC 2.4.2.9; UMP pyrophosphorylase; UPRTase). Uridine nucleotides could be formed by energy-

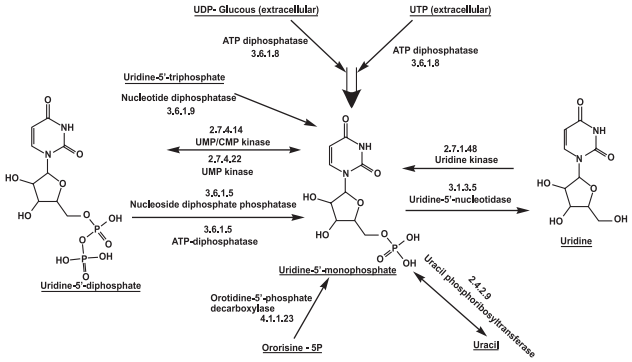

Fig 1. The proposed metabolic pathways of Uridine 5 monophosphate (UMP).

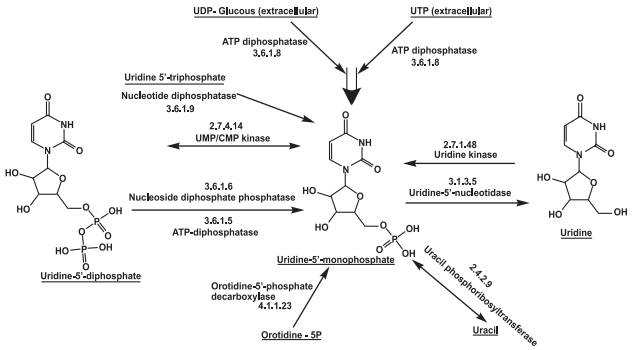

Fig 2. The proposed metabolic pathways of Uridine 5 monophosphate (UMP) in camels.

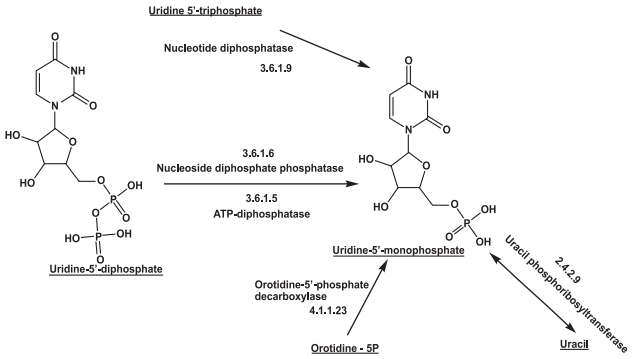

Fig 3. The proposed metabolic pathways of Uridine 5 monophosphate (UMP) in *Trypanosoma evansi*.

using pyrimidine de novo biosynthesis or by the energy-saving retrieving of nucleosides resultant of nucleotide catabolism. The UPRTs is energy-saving pentosyltransferases enzyme involved in the metabolism of uracil and related compounds that catalysing reversible salvage of pyrimidines by catalysing the formation of uridine monophosphate from uracil and 5-phospho-alpha-D-ribose 1-diphosphate (Mainguet *et al,* 2009). The UPRTase activity in *Giardia intestinalis* was remarkably increased by GTP and dGTP with no effect on the Michaelis constants. The GTP exhibited similar effect for UPRTase from other prokaryotes as *E. coli* but not from other eukaryotes (Dai *et al,* 1995). The protozoan parasite *Leishmania donovani* uracil phosphoribosyltransferase (LdUPRT), is an enzyme not found in mammalian cells, is inhibited by uracil and 4-thiouracil, nonetheless 5-fluorouracil toxicity emerges through another mechanism (Soysa *et al,* 2013).

There was high similarity rate (89%) between the camel and human UPRTase (Fig 4). Meanwhile, there was little difference of only 2-3 amino acids replacement between the three camel species, dromedary, Bactrian and feral camels (Fig 5).

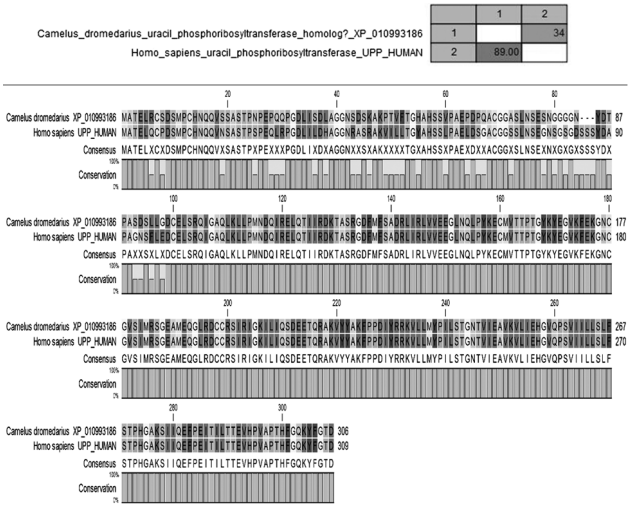

Fig 4. Multiple sequence alignment of dromedary camel and human uracil phosphoribosyltransferase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

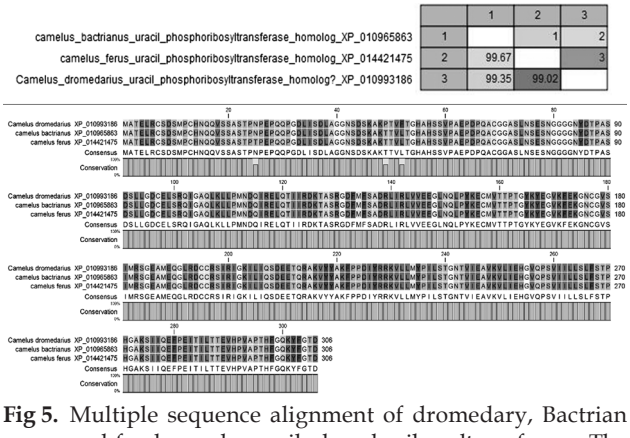

Fig 5. Multiple sequence alignment of dromedary, Bactrian and feral camels uracil phosphoribosyltransferase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences

In comparison of camel UPRTase with other vertebrate enzymes, the differences were less than 39 amino acid replacement with high similarity rate of 90-99%. There was great and obvious difference between the camel and prokaryotic and protozoal enzymes and differences were up to 227. This indicates large amount of amino acid differences between camel and lower organisms UPRTases. Interestingly, direct comparison of camel and *T. evansi* (Fig 6) revealed high rate of differences with similarity not exceeding 30.12% and 225 differences. Additionally, the camel UPRTase had 305 amino acids as compared to 240 amino acids in *T. evansi*.

The phylogenetic analysis of UPRTase in different prokaryotic and eukaryotic UPRTases revealed distant relation between the camel and *T. evansi*. The *T. evansi* UPRTase was highly related to the bacterial enzyme and very distant from the camel enzyme (Fig 7).

Domains and motifs search of UPRTase from camel and *T. evansi* revealed the presence of one domain of PRTase-1 superfamily including uracil (Tables 4 and 5).

Table 4. The motif and domain content of camel and *T. evansi* UPRTase.

Trypanosoma		
Pfam	**Position (Independent E-value)**	**Description**
Trypanosoma		
UPRTase	29..238(1.6e-68)	PF14681, Uracil phosphoribosyltransferase
Pribosyltran	139..178(4.5e-06)	PF00156, Phosphoribosyl transferase domain
Camel		
UPRTase	113..291(1.1e-53)	PF14681, Uracil phosphoribosyltransferase
Pribosyltran	223..275(0.0003)	PF00156, Phosphoribosyl transferase domain

Orotidine-5′-phosphate decarboxylase (UMP synthase) uridine 5′-monophosphate synthase

Orotidine-5′-phosphate decarboxylase (orotidine-5′-monophosphate (OMP) decarboxylase; 4.1.1.23). The OMP decarboxylase (OMPdecase) is a carboxy-lyase enzyme from higher eukaryotes and is equivalent with orotate phosphoribosyltransferase (EC 2.2.2.10). Moreover, OMPdcase is one of most competent enzymes that catalyses the decarboxylation of orotidine-5′- monophosphate (OMP) to uridine-5′-monophosphate (UMP)(Kotra and Pai, 2008). Furthermore, OMP-DC has studied as a drug target.

Table 5. The motif and domain content of camel and *T. evansi* OMPdecase.

Trypanosoma		
Pfam	**Position (Independent E-value)**	**Description**
OMPdecase	17..245(1.5e-34)	PF00215, Orotidine 5′-phosphate decarboxylase / HUMPS family
Pribosyltran	294..410(1.4e-17)	PF00156, Phosphoribosyl transferase domain
PRTase_2	363..404(0.00099)	PF15609, Phosphoribosyl transferase
UPRTase	365..413(0.00096)	PF14681, Uracil phosphoribosyltransferase
PUA	360..399(0.15)	PF014 PUA domain
Camel		
OMPdecase	252..466(1.5e-73)	PF00215, Orotidine 5′-phosphate decarboxylase / HUMPS family
Pribosyltran	44..162(1.3e-13)	PF00156, Phosphoribosyl transferase domain

Figure legends

In which a potent ligands of OMPdcase with diverse structures were examined for structural interactions with the active site of OMPdcase as trials to design novel inhibitors of OMPdcase. These ligands involve of pyrazole or pyrimidine nucleotides comprising the mononucleotide derivatives of pyrazofurin, barbiturate ribonucleoside, and 5-cyanouridine, in addition to, 1,4-dihydropyridine-based non-nucleoside inhibitors such as nifedipine and nimodipine. Binding of these ligands to OMPdecase active site displaying diverse interactions paving the way to design novel inhibitors against OMPdecase (Meza-Avina *et al*, 2010).

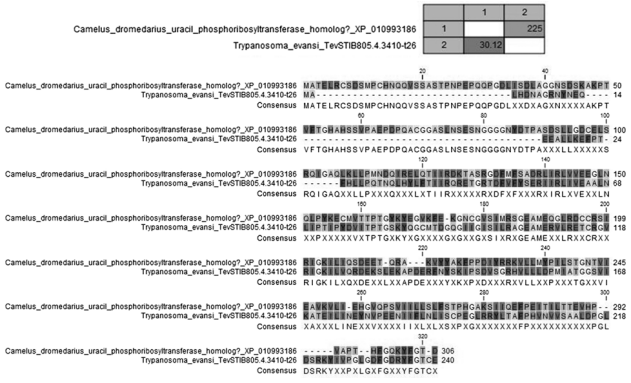

Fig 6. Pairwise sequence alignment of camel and *T. evansi* uracil phosphoribosyltransferase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences

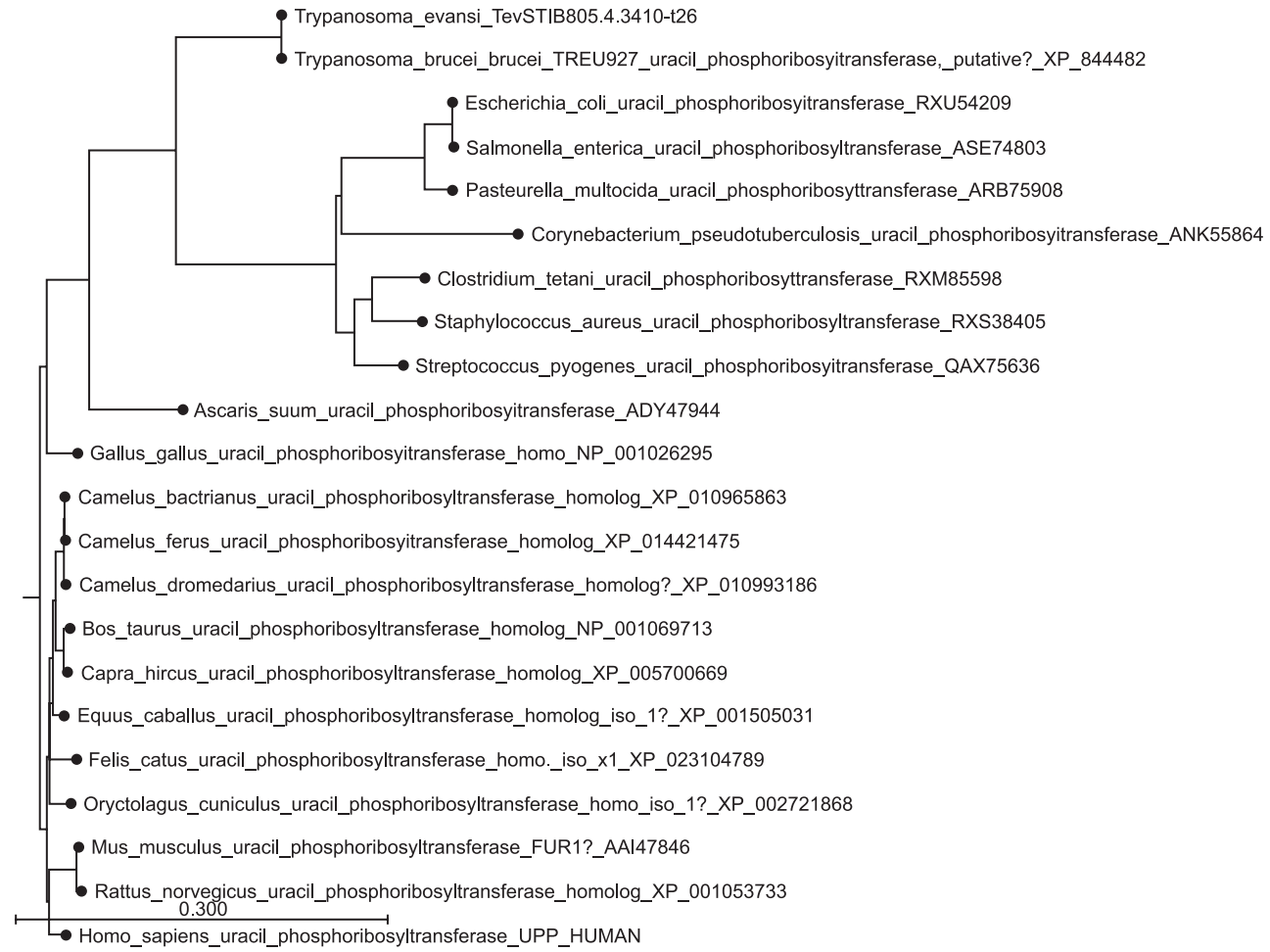

Fig 7. Phylogram of camel and Trypanosoma uracil phosphoribosyltransferase in relation to a set of eukaryotic organisms.

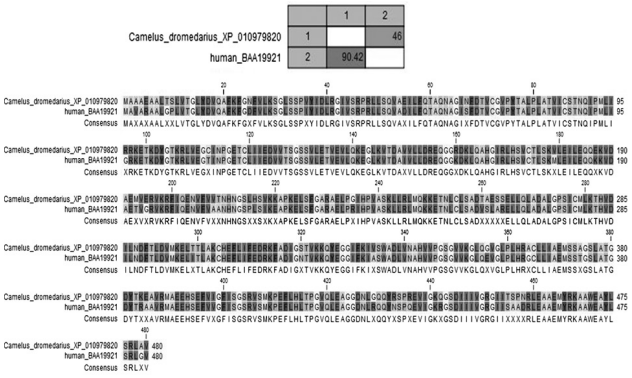

Fig 8. Pairwise sequence alignment of human and camel orotidine-5′-phosphate decarboxylase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

Both camel and human OMPdecase retained high similarity of 90.42% (Fig 8) with prefect complementarity of dromedary and Bactrian camels (Fig 9). The camel and *T. evansi* shared a common feature of OMPdecase length more than 450 amino acids. However, the similarity rate was as low as 18.15% (Fig 10). There was similar high similarity between the camel and other vertebrate OMPdecase with less than 67 differences (Fig 11), which extend to more than 450 differences with bacterial or protozoal enzyme.

The phylogenetic analysis of OMPdecase was having a similar pattern of UPRTase by showing distant relation between the camel and *T. evansi*. The *T. evansi* UPRTase was highly related to the bacterial enzyme and very distant from the camel enzyme (Fig 12).

The enzyme is monofunctional (about 233 amino acid) in prokaryotes, while in eukaryotes including camel and TEV the protein is about 450 amino acid and composed of two domains or bifunctional protein for UMP synthesis. Domains and motifs search of OMPdecase from camel and *T. evansi* revealed the presence of two predicted domains in camel and 5 domains in *T. evansi* (Table 5). In camel,

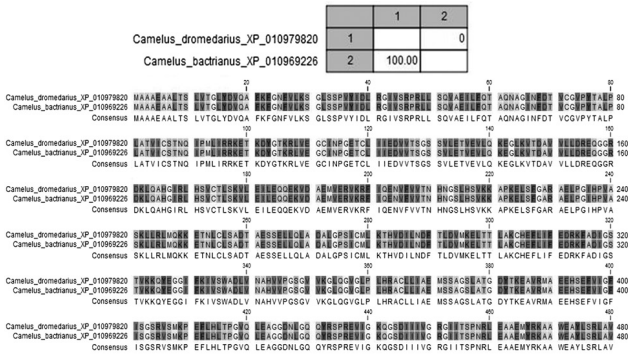

Fig 9. Pairwise sequence alignment of dromedary and Bactrian camels orotidine-5′-phosphate decarboxylase (UMP synthase). The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

the two domains include the OMPdecase and a PRTase domain. In *T. evansi*, PRTase-2 and UPRTase and confirmed with high E-values.

A recent report suggested that a null strain of UMP synthase in *T. brucei* affected the lethality of blood infection in mice (Ong *et al,* 2013). However, this was bypassed after longer culture time due to conversion of orotate to uridine by the host enzymes (Ong *et al,* 2013).

UMP-CMP kinase (UMP/CMP kinase)

A bifunctional enzyme present in eukaryotes and catalyses the phosphorylation of CMP and UMP. Prokaryyotes has two different monofunctional enzymes EC 2.7.4.25, which act as CMP kinase and EC 2.7.4.22, which act as UMP kinase.

UMP kinase

Uridylate kinase; UMPK, 2.7.4.22) has restricted kinase activity for UMP only and specific for prokaryotes. It is replaced by the bifunctional UMP-CMP kinase.

Nucleoside triphosphate phosphohydrolases

EC no. 3.6.1.5 present in vertebrates including camel and some protozoa. The enzyme was previously found by experimental procedures (Weiss *et al,* 2015), yet it was not found in the gene sequence databases after searching by accession number or name of enzyme.

Conclusions

Investigation of UMP metabolising enzymes in camel and *T. evansi* revealed the absence of uridine conversion to UMP due to the lack of

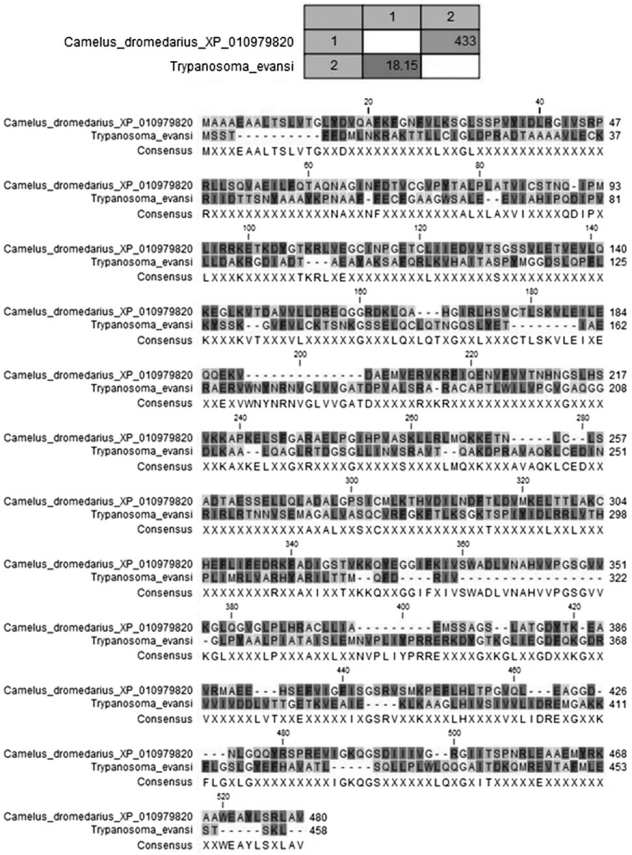

Fig 10. Pairwise alignment of dromedary camel and *T. evansi* orotidine-5′-phosphate decarboxylase (UMP synthase). The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

uridine kinase. In this context, two other enzymes are important, including UPRTase and OMPdecase. Both enzymes showed high sequence variability and distant evolutionary pattern. Inhibition of UPRTase might be of a value due to the absence of uridine kinase. However, the abundance of UMP synthetic pathways, the adaptation of parasite or compensatory mechanisms leading to UMP production and the high mw of the enzymes might affect the lab identification of these enzymes. These factors underscores using the UMP pathway as a drug target. However, more details on UPRTase inhibition in *T. evansi* is required.

Acknowledgements

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		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Camelus_dromedarius_XP_010979820	1		0	0	2	1	1	0	92	92	255	235	238	241	273	273	305	310	306
Camelus_bactrianus_XP_010969226	2	0		0	2	1	1	0	92	92	255	235	238	241	273	273	305	310	306
human_BAA19921	3	46	46		2	1	1	0	92	92	255	235	238	241	273	273	305	310	306
Felis_catus_XP_003991769	4	48	48	49		3	3	2	94	94	257	237	240	243	275	275	307	312	308
Mouse_NP_033497	5	67	67	57	73		0	1	93	93	254	234	237	242	272	272	304	309	305
Rattus_norvegicus_AAH98033	6	64	64	51	68	16		1	93	93	254	234	237	242	272	272	304	309	305
Bos_taurus_AAI12873	7	67	67	77	70	87	85		92	92	255	235	238	241	273	273	305	310	306
Trypanosoma_brucei_brucei_TREU927_orotidine-5-phosphate_decarboxylase,_putative_(XP_845051)	8	428	428	431	432	431	428	432		0	257	245	280	271	285	285	315	314	312
Trypanosoma_evansi	9	428	428	431	432	431	428	432	0		257	245	280	271	285	285	315	314	312
Babesia_bovis_T2Bo_orotidine-5'-phosphate_decarboxylase_(XP_001611096)	10	457	457	462	459	461	461	463	471	471		24	247	246	190	190	158	159	161
Plasmodium_falciparum_3D7_orotidine-5'-phosphate_decarboxylase_(XP_001347509)	11	461	461	460	464	459	458	459	485	485	225		255	262	208	208	176	181	183
Clostridium_tetani_orotidine-5'-phosphate_decarboxylase_(RYU99202)	12	457	457	460	459	456	457	457	482	482	383	396		39	197	197	225	228	234
Corynebacterium_pseudotuberculosis_orotidine-5'-phosphate_decarboxylase_(RKT29909)	13	453	453	453	454	452	453	450	472	472	378	394	220		210	210	236	233	239
Escherichia_coli_orotidine-5'-phosphate_decarboxylase_(QAY44275)	14	450	450	452	453	453	453	448	483	483	347	366	354	351		0	32	39	39
Salmonella_enterica_orotidine-5'-phosphate_decarboxylase_(QBA00760)	15	456	456	456	459	459	459	454	481	481	345	364	357	354	34		32	39	39
Pasteurella_multocida_orotidine-5'-phosphate_decarboxylase_(ATN16523)	16	454	454	453	456	454	455	455	479	479	314	330	354	356	119	118		9	9
Staphylococcus_aureus_orotidine-5'-phosphate_decarboxylase_(RYV48081)	17	463	463	464	463	462	463	462	479	479	313	333	357	355	175	171	135		6
Streptococcus_pyogenes_orotidine-5'-phosphate_decarboxylase_(QBB63177)	18	466	466	463	465	463	464	464	484	484	308	332	357	352	169	169	139	104	

Fig 11. Multiple sequence alignment of Dromedary camel and *T. evansi* orotidine-5′-phosphate decarboxylase (UMP synthase) with some prokaryotic and eukaryotic enzymes. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

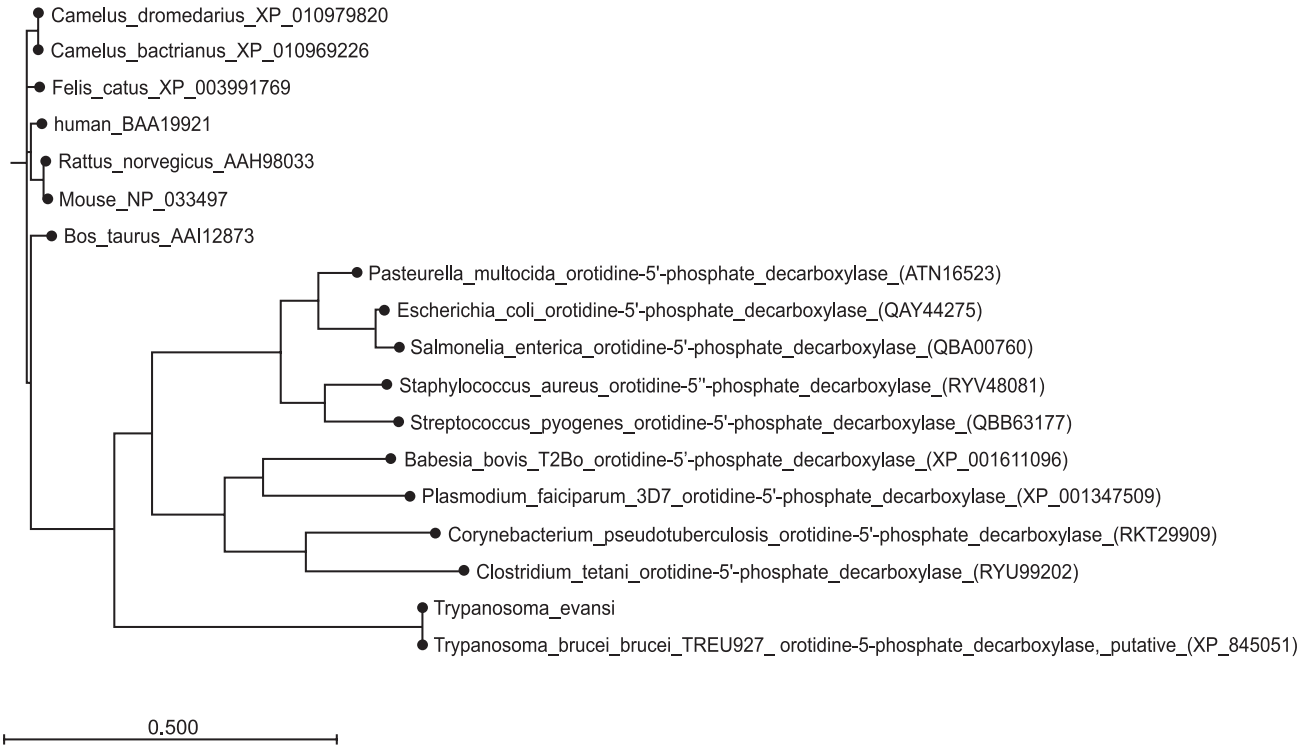

Fig 12. Phylogram of Trypanosoma orotidine-5′-phosphate decarboxylase in relation to a set of eukaryotic and prokaryote organisms.

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