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URIDINE 5-TRIPHOSPHATE (UTP) METABOLISING ENZYMES NUCLEOSIDE DIPHOSPHATE KINASE AND CYTIDINE TRIPHOSPHATE (CTP) SYNTHASE IN CAMELS AND *Trypanosoma evansi*

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ABSTRACT

In this work, the pyrimidine metabolic pathway Uridine 5'- Triphosphate (UTP) paths were investigated. In this context, two UTP enzymes were investigated by bioinformatics tools, nucleoside diphosphate kinase (NDK) and cytidine triphosphate (CTP) synthase. The dromedary, Bactrian and feral camels NDK showed high similarity > 97.7% to the human enzyme. The camel NDK was phylogenetically distant from eukaryotic NDK with the closest relation to prokaryotic NDK. *Trypanosoma evansi* NDK was phylogenetically distant from protozoal NDK and devoid of the histone H3 domain, which was found in eukaryotic NDK. Two isoforms of CTP synthase were retrieved from camel genome with medium homology per cent. These are replaced by one isoform in *T. evansi*. In terms of drug targets, both NDK and CTP synthase showed conserved and phylogenetically and motif distinctions. This enrolls the two targets as a choice for drug development against *T. evansi*.

Key words: Camel, CTP synthase, genome, NDK nucleotide, *Trypanosoma evansi*, UTP

Uridine 5'- Triphosphate (UTP) production process is proposed by three production routes, i.e. KEGG maps (Kanehisa *et al,* 2007; Kanehisa *et al,* 2016; Ogata *et al,* 1998). UTP can be synthesised from uridine-5'-diphosphate (UDP), cytidine-5'-triphosphate (CTP) and P(1),P(4)-bis(uridin-5'-yl) tetraphosphate (UppppU) by the actions of nucleoside-diphosphate kinase, deoxycytidine triphosphate deaminase and Bis (5'-nucleosyl)-tetraphosphatase enzymes, respectively. In the catabolic pathway, the phosphorylation of UTP by thymidine-triphosphatase and/or ATP-diphosphatase or by nucleoside-diphosphate kinase can yield UDP. Whereas the reduction of UTP by ribonucleoside-triphosphate reductase and/or ribonucleoside-triphosphate reductase (thioredoxin) can yield deoxyuridine-5'-triphosphate. In addition, pyrophosphates can be removed from UTP by the action of nucleotide diphosphatase to produce uridine-5'-monophosphate (UMP). Cytidine triphosphate synthetase uses UTP as a substrate to produce cytidine-5'-triphosphate (CTP).

In this study, the enzymes involved in UTP metabolic pathways in camels were investigated. Comparisons were made between dromedary camel and human, wild camel, and eukaryotic UTP metabolising enzymes. The comparisons included homology rate, conserved domains composition, functional motifs and signatures, phylogenetic relationships and genetic composition.

Materials and Methods

The enzymes involved in metabolic pathway of UTP are given in Table 1 and those expected enzymes involved in metabolic pathway of UTP in camel and *Trypanosoma evansi* are given in Table 2 and 3, respectively.

The proposed metabolic pathway of uridine triphosphate is given in Fig 1 and those for camel and *Trypanosoma evansi* are given in Fig 2 and 3, respectively.

Retrieval of genomic data

Collection of genomic data was carried out by extracting the information from the gene

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Table 1. Enzymes involved in metabolic pathways of UTP.

ID (E.C. number)	Definition (Enzyme name)
2.7.4.6	Nucleoside-diphosphate kinase
3.6.1.39	Thymidine-triphosphatase, thymidine triphosphate nucleotidohydrolase
3.6.1.5	Nucleoside triphosphate phosphohydrolases (nucleoside monophosphoate-forming) or ATP- diphosphatase, apyrase, ectonucleoside triphosphate diphosphohydrolase
3.6.1.9	Nucleotide diphosphatase
6.3.4.2	Cytidine triphosphate synthetase
3.5.4.13	Deoxycytidine triphosphate deaminase
3.6.1.17	Bis(5′-nucleosyl)-tetraphosphatase (asymmetrical)
1.17.4.2	Ribonucleoside - triphosphate reductase (thioredoxin)
1.1.98.6	Ribonucleoside- triphosphate reductase

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

ID (E.C. number)	Definition (Enzyme name)
2.7.4.6	Nucleoside-diphosphate kinase
6.3.4.2	Cytidine triphosphate synthetase

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

ID (E.C. number)	Definition (Enzyme name)
2.7.4.6	Nucleoside-diphosphate kinase
6.3.4.2	Cytidine triphosphate synthetase

database (http://www.genedb.org) (Hertz-Fowler *et al,* 2004), protein and genome databases (http://www.ncbi.nlm.nih.gov), Kinetoplastom genome resources (http://tritrypdb.org/tritrypdb/) and the Arabian camel genome project (http://www.camel.kacst.edu.sa). 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.

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 (http://blast.ncbi.nlm.nih.gov/Blast.cgi) searching against the non redundant (nr) database, with filtering of low complexity regions.

Multiple sequence alignment program for proteins and construction of phylogenetic tree was carried out using the tools available at website (https://www.ebi.ac.uk/Tools/msa/clustalo/) (Sievers and Higgins, 2014). By the multiple sequence alignment programme (ClustalW2) the best matching among the chosen sequences was calculated. The phylogenic tree was generated from the resultant alignment, then visualised by Dendroscope phylogenic tree viewer (Huson *et al,* 2007) or CLC genomics workbench (Sequencing, 2011).

Putative domains was searched by the domain prediction programme 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) were also used for analysis of nucleotide and gene sequences. Several protein parameters including the molecular weight, atomic composition, amino acid composition, theoretical pI, estimated half-life, extinction coefficient, grand average of hydropathicity and aliphatic index was searched at (http://us.expasy.org/tools/protparam.html). Protein sequences of target genes was analysed for data such as pI, extinction coefficient and MW for the tagged protein sequence by PROTParam. PROSITE (http://www.expasy.org/proteomics/families__patterns_and_profiles) 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

Nucleoside diphosphate kinase (NDK)

Nucleoside-diphosphate kinase (NDPK; 2.7.4.6, or nucleoside 5′-diphosphate kinase; nucleoside diphosphokinase;) is a transferase enzyme catalyse the transfer of phosphates donor to acceptor nucleotide diphosphate. NDK maintains cellular homeostasis by controlling the cellular levels of nucleotides triphosphates, which are essential for many cellular activities. Moreover, NDPK is a highly conserved, multifunctional enzyme catalyses using ATP as the phosphate donor to form phosphorylated

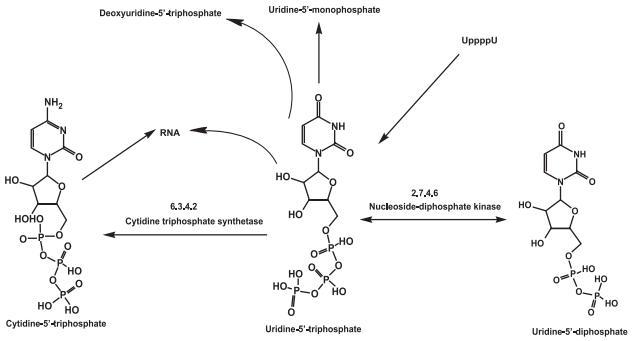


Fig 1. The proposed metabolic pathways of uridine triphosphate (UTP).

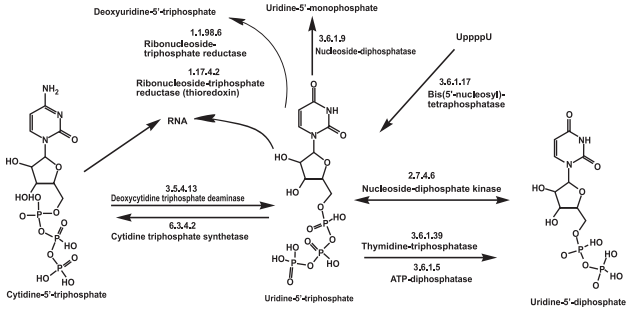


Fig 2. The proposed metabolic pathways of uridine triphosphate (UTP) in camels.

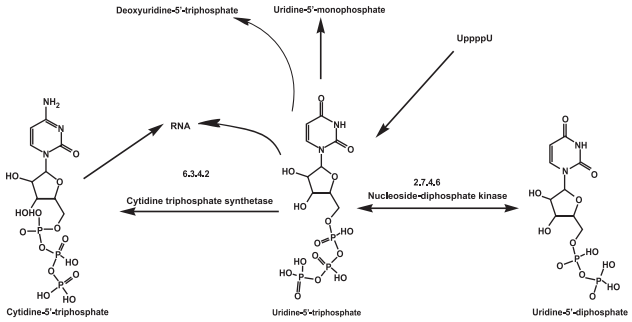
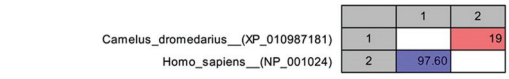


Fig 3. The proposed metabolic pathways of uridine triphosphate (UTP) in *Trypanosoma evansi*.



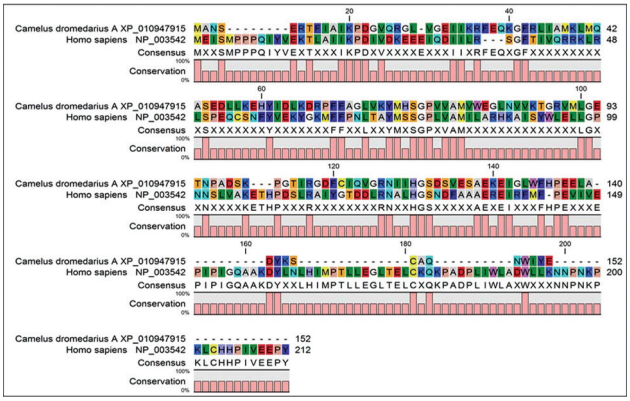


Fig 4. Multiple sequence alignment of dromedary camel and human nucleoside-diphosphate kinase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per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

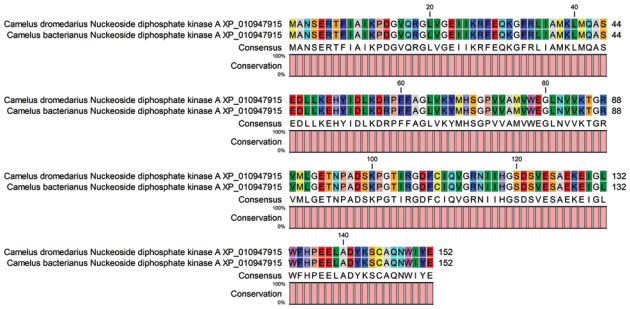


Fig 5. Multiple sequence alignment of dromedary and bactrian nucleoside-diphosphate kinase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

histidine residue with high energy intermediate form (Yu *et al*, 2017). Additionally, the enzyme is nonspecific and can recognise a wide range of nucleotides and ribonucleotides (Kandeel and Kitade, 2010).

Recently, additional functions of NDPK has been appreciated. For instance, NDK was able to transphosphorylate other proteins, resembling reminiscent of bacterial two-component systems. Additional newly discovered features were the DNA-binding of NDK. The genome of the parasitic protozoon *Trypanosoma brucei* contains a single gene for NDK (Hunger-Glaser *et al*, 2000). Its sequence was conserved with high similarity with other species. NDK. Procyclic and bloodstream forms were highly expressing NDK. Immunofluorescence and immuno-electron microscopy prove that trypanosomal NDK is located in the nucleus. LY266500, a strong inhibitor of histidine phosphorylation was unable to stop the NDK activity. On the other hand, (Pereira *et al*, 2014) revealed that the immunofluorescence microscopy NDK from *Trypanosoma cruzi* enzyme NDK overexpressing parasites has a cytosolic distribution with higher sequestration at the nucleus. The *Plasmodium falciparum* NDK is characterised by broad substrate range binding all nucleotides with almost similar high efficiency (Kandeel *et al*, 2009; Kandeel and Kitade, 2010).

Camel and human NDKs showed 97.6% homology rates with about 19 residues differences

Fig 6. Multiple sequence alignment of dromedary camel and other eukaryotes nucleoside-diphosphate kinase. 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 4). The camel NDK showed shorter protein length of 152 residues, compared to 212 residues in the human enzyme. This was mostly due to the absence of 41 residues at the C-terminus of the enzyme.

Bactrian and feral camels showed 98.35 and 97.85% homology with the Arabian camel with 17 and 28 residues differences, respectively (Fig 5). Additionally, they also shared a short amino acids length of 152 residues. Fig 6 shows multiple comparisons of NDK from camel compared with higher eukaryotes including bovine, caprine, equine, swine, feline, mouse, rat, rabbit, chicken and human NDKs.

BLAST search of NDK using the *T. brucei* sequence against *T. evansi* database revealed two enzymes with little bit low homology rate (Fig 7). While 132-153 amino acid proteins were retrieved. The differences were 88-130 amino acid to constitute low homology rates ranging between 22.16 and 37.59%. This low rate might indicate variations in structures and functional features of NDKs in protozoa.

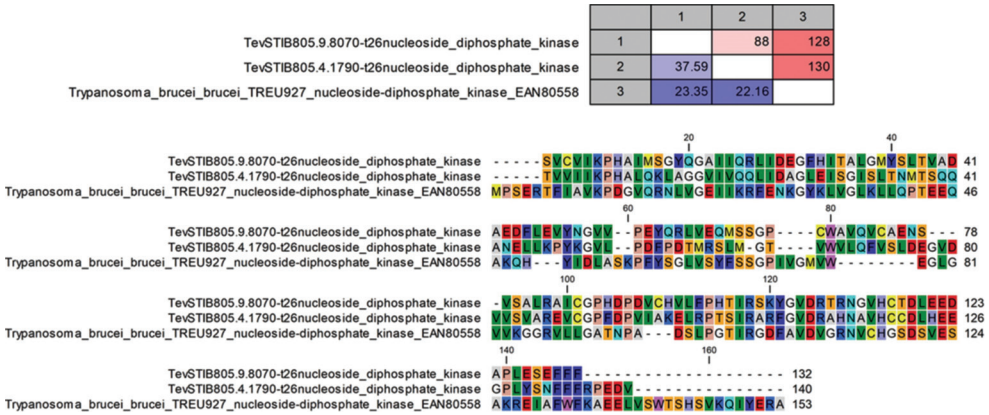


Fig 7. Multiple sequence alignment of *Trypanosoma brucei* and *T. evansi* nucleoside-diphosphate kinase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

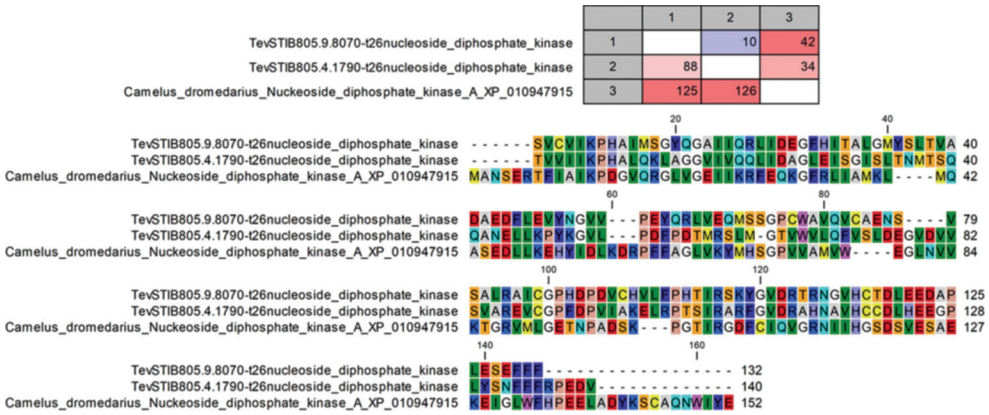


Fig 8. Multiple sequence alignment of dromedary camel and *T. evansi* nucleoside-diphosphate kinase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

In comparing camel and *T. evansi* NDKs, identity per cent was 42 and 43 (Fig 8) with about 126 and 126 differences from the two *T. evansi* enzymes.

Phylogenetic comparison (Fig 9) reveals a distant relation of camel NDK from human and most vertebrates with a closer relation to prokaryotic and protozoal enzymes. In comparison, the *T. evansi* enzymes were highly distant from the tested set of organisms. This might indicate interesting features of NDKs in both camel and *T. evansi* in comparison with other orthologous organisms.

Domains and motifs search revealed an eminent difference between camel and Trypanosoma NDKs. Camel was similar to human NDK showing two potential domains, including NDK domain and the histone H3 methylation domain (DPY30). The former domain is common with the protozoal enzymes, while the latter is unique to camel NDK.

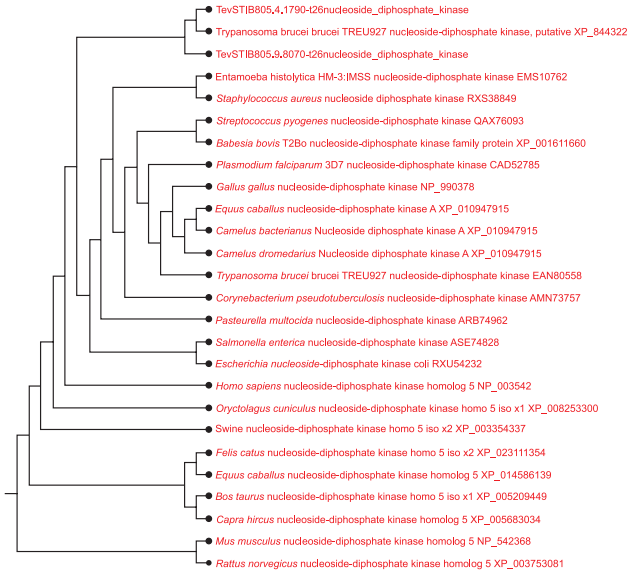


Fig 9. Cladogram of camel and *T. evansi* bifunctional nucleoside-diphosphate kinase in relation to a set of prokaryotic and eukaryotic organisms.

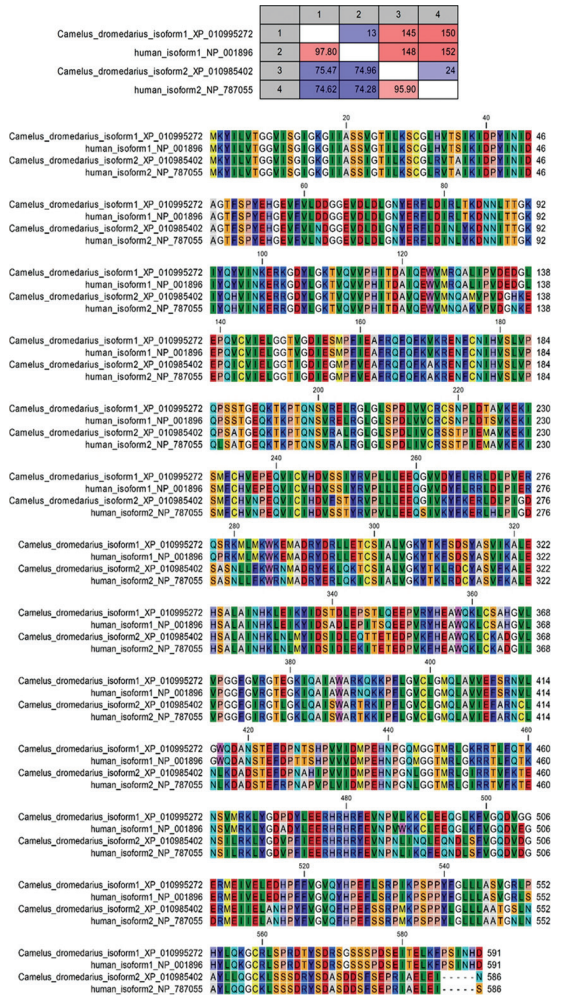


Fig 10. Multiple sequence alignment of dromedary camel and human Cytidine triphosphate synthetase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

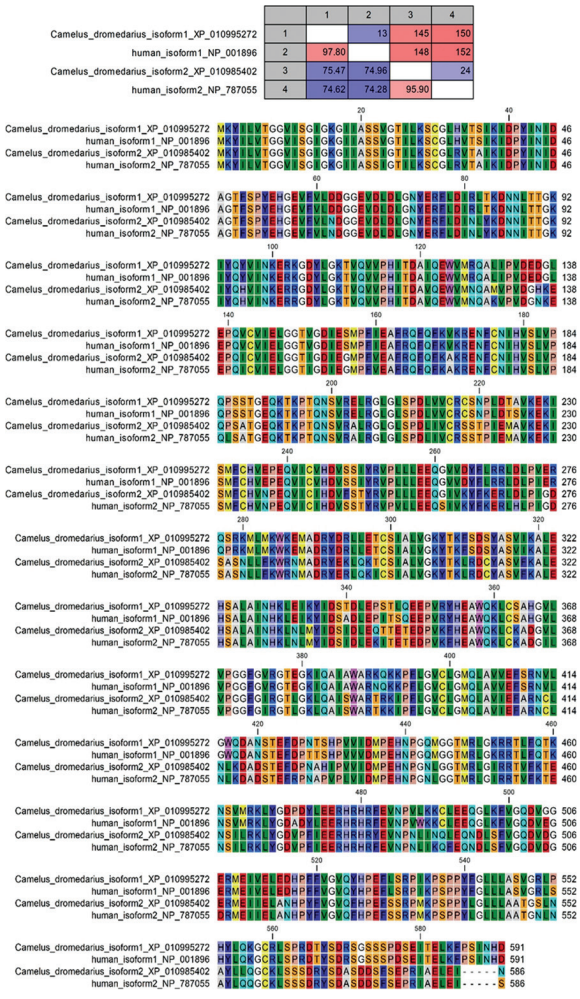


Fig 11. Multiple sequence alignment of dromedary and bacterian camels Cytidine triphosphate synthetase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

Cytidine triphosphate synthetase

Cytidine triphosphate synthetase (CTP synthase; 6.3.4.2) is a ligase enzyme build carbon-nitrogen linkages catalyses the synthesis of CTP from UTP. This synthetase enzyme also known as glutamine hydrolysing; UTP—ammonia ligase; uridine triphosphate aminase; cytidine 5′-triphosphate synthetase; UTP (uridine 5′-triphosphate): ammonia ligase (ADP-forming). *Trypanosoma brucei* is protozoon parasite causing is a fatal disease known as African sleeping sickness. The concentration of CTP is very tiny in *T. brucei* comparing to mammalian cells. Moreover, the limited power for *de novo* synthesis cytidine/cytosine and the lack of salvage pathways of Trypanosomes. Therefore, the parasite CTP synthetase (TbCTPS) is rendered a prospective target for the creating of antiprotozoal agents. (Fijolek *et al,* 2007; Steeves and Bearne, 2011). Furthermore, *T. brucei* CTP synthase is stopped by purine derivatives such as GTP, guanosine, caffeine, and uric acid. Additionally, the purine ring may perform a useful structure for the construction of inhibitors of trypanosomal CTP synthase. As well, the exploitation of CTP synthetase inhibitors alone or in concoction with proper nucleosides or bases might help in management of sleeping sickness. (Hofer *et al,* 2001).

Two isoforms of CTP synthase were retrieved from the camel database of proteins (Fig 10). Isoform 1 and isoform 2 showed 97.8 and 95.9% similarity to the human isoforms, while the variation between the two isoforms extends to include 26-26% differences.

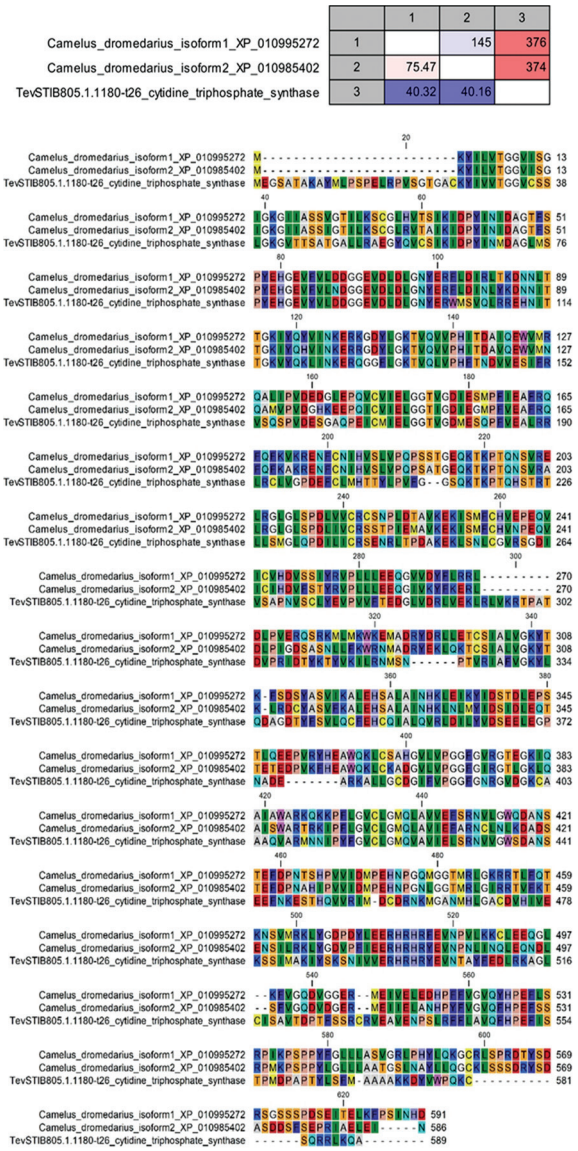


Fig 12. Multiple sequence alignment of *T. evansi* and dromedary camel cytidine triphosphate synthetases. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

Similarly, comparison of dromedary and Bactrian camels revealed about 100% similarity within the same isoform and about 25% differences across the isoforms (Fig 11).

BLAST search using *T. brucei* against *T. evansi* retrieved one protein with more than 95% similarity. Furthermore, BLAST search using the two camel isoforms retrieved the same hit protein. The retrieved *T. evansi* CTP synthase showed about 40% similarity with both camel isoforms comprising average of 375 amino acids differences.

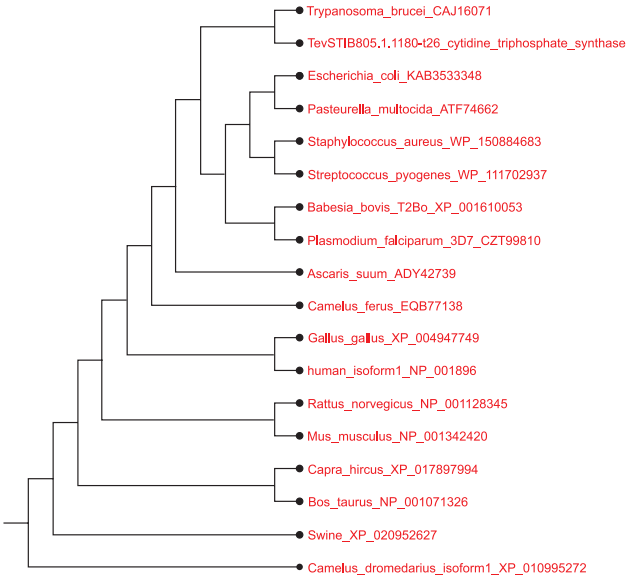


Fig 13. Cladogram of camel and *T. evansi* CTP synthetase in relation to a set of prokaryotic and eukaryotic organisms.

Phylogenetic comparison (Fig 13) reveals a close relation of camel CTP synthase from human and most vertebrates with a closer relation to prokaryotic and protozoal enzymes. In comparison, the *T. evansi* enzyme was highly distant from the tested set of organisms. This might indicate interesting features of evolutionary differences between the camel and Trypanosoma CTP synthase.

Domains and motifs search revealed almost constant and conserved domain and motifs features in both of camel and Trypanosoma. The domain content includes CTP synthase and glutamine amino transferase.

Acknowledgements

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