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Phylogenetic Studies on tRNA Dependent Amidotransferase from Plasmodium Falciparum

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Abstract

Malaria remains the major concern in most of the developing countries. It is caused by the protozoan, Plasmodium, which is a member of apicomplexan. This family is characterized by the presence of plastid like structure known as apicoplast. Proteins and metabolic pathways of plastid are more like bacterial one. Protein tRNA-dependent amidotransferase (PfAdT) is one of the many proteins encoded by nuclear genome and targeted towards apicoplast. In this study, we have performed the phylogenetic analysis of tRNA-dependent amidotransferase with respect to other species from all the three domains of life. Results showed the closeness of PfAdT to the archaeal and human mitochondrial homolog. We hope that this observation would be valuable in defining evolutionary history of parasite and also in designing new chemotherapy against malaria parasite.

Keywords: Amidotransferase, alternate aminoacylation pathway, plasmodium, phylogeny.

Introduction

Protein synthesis is a process where mRNA is decoded into protein sequence. Amino acid gets linked with cognate tRNA molecules with the help of aminoacyl-tRNA synthetases (aaRSs)[1]. All the 20 amino acids are charged to their cognate tRNA with respective aaRS[2-3]. On the other hand few bacteria, chloroplast and most of the archaea does not code for all the 20 amino aminoacyl-tRNA synthetases. Charging of glutamine amino acid to its tRNA is done through alternate pathway of aminoacylation[4]. First, non-discriminating glutamyl-tRNA synthetase charge glutamate onto tRNAGln and then enzyme called tRNA-dependent amidotransferase converts glutamate into glutamine[5-6]. This secondary pathway is also found in the plastid of malaria parasite[7-11]. Apart from having 20 canonical aaRSs, malaria parasite also code for additional tRNA synthetases which are targeted towards apicoplast which also include tRNA-dependent amidotransferase (PfAdT) [12]. Most of the proteins require for protein synthesis in apicoplast are encoded by nucleus and transported to apicoplast[13]. It has been already shown that apicoplast is indispensible, probably because of pathways operating inside plastids are essential for parasite survival[14]. Indirect aminoacylation is one of those pathways important in plastid biology. Therefore, in this study, we have performed phylogenetic analysis on tRNA-dependent amidotransferase of malaria parasite. Multiple sequence alignment was performed with various other sequence homologs from all three domains of life and further phylogenetic tree was constructed using neighbour-joining method. Results show that PfAdT clustered with human mitochondrial and archaeal counterparts. We think this study will definitely shed light on evolutionary relationship of malaria parasite and also emphasis on prokaryotic nature of apicoplast proteins which can be targeted as potential drug targets.

Materials and Methods

The sequence of tRNA-dependent amidotransferase (PfAdT) was extracted from PlasmoDB with accession number of PFD0780w. The protein sequence of PfAdT was pasted in NCBI Blast column to run against referenced sequences of various proteomes. Total of 67 sequences of tRNA-dependent amidotransferase from various phyla of living organisms were selected for sequence alignment[15-16]. ClustalW online server was used for generating multiple sequence alignment of above selected sequences. The output file of the multiple sequence alignment was further submitted to MEGA5 program for generation of phylogenetic tree. Test neighbour-joining method[17] was used for making phylogenetic tree. The pair-wise distances were calculated for each 67 sequences. Images were created with MEGA5 program.

Results and Discussion

Multiple sequence alignment of tRNA-dependent amidotransferase from various organisms including Plasmodium falciparum was performed using ClustalW. Interestingly, we found that P. falciparum AdT has a unique extension at N-terminal of protein which was absent in all other sequence homolog (figure1A). Insertion of small sequence or motif in protein coding gene is a common phenomenon in malaria proteins which leads to the increase in the size of the protein. Although, significance of this N-terminal extension is not yet known but could be involved in making protein-protein interactions. Figure 1B showing the alignment of 67 sequences of AdT from various organisms with different colour coding

based on the degree of conservation of amino acid residue at that particular position. In addition, evolutionary tree of selected AdT sequences was constructed using MEGA5 program. Various methods of phylogenetic analysis were employed including maximum-likelihood, neighbour-joining. The constructed tree was further analyzed for computing pair wise distances using MEGA5 utility tools. The P. falciparum AdT sequence was tagged with red triangle in the constructed tree (figure 2). Phylogenetic tree revealed that PfAdT was clubbed with archaea bacterial and human mitochondrial AdT sequences. These results clearly support the hypothesis of secondary endosymbiosis in which apicoplast is the part of cynobacteria which was engulfed by eukaryotic cell during evolutionary time. Hence, PfAdT is evolutionary closer to the prokaryotic counterparts which makes it suitable target for the development of anti-malarials.

Conclusion

Construction of phylogenetic tree tells you about the evolutionary closeness of given sequence among available sequences chosen for studies. In the analysis of PfAdT in terms of its phylogenetic relationship with other species provided the direct evidence of prokaryotic nature of apicoplast proteins. Hence, we conclude that this study will not only pave the way for drug development but also helps in the understanding of evolution of present day malaria parasite.

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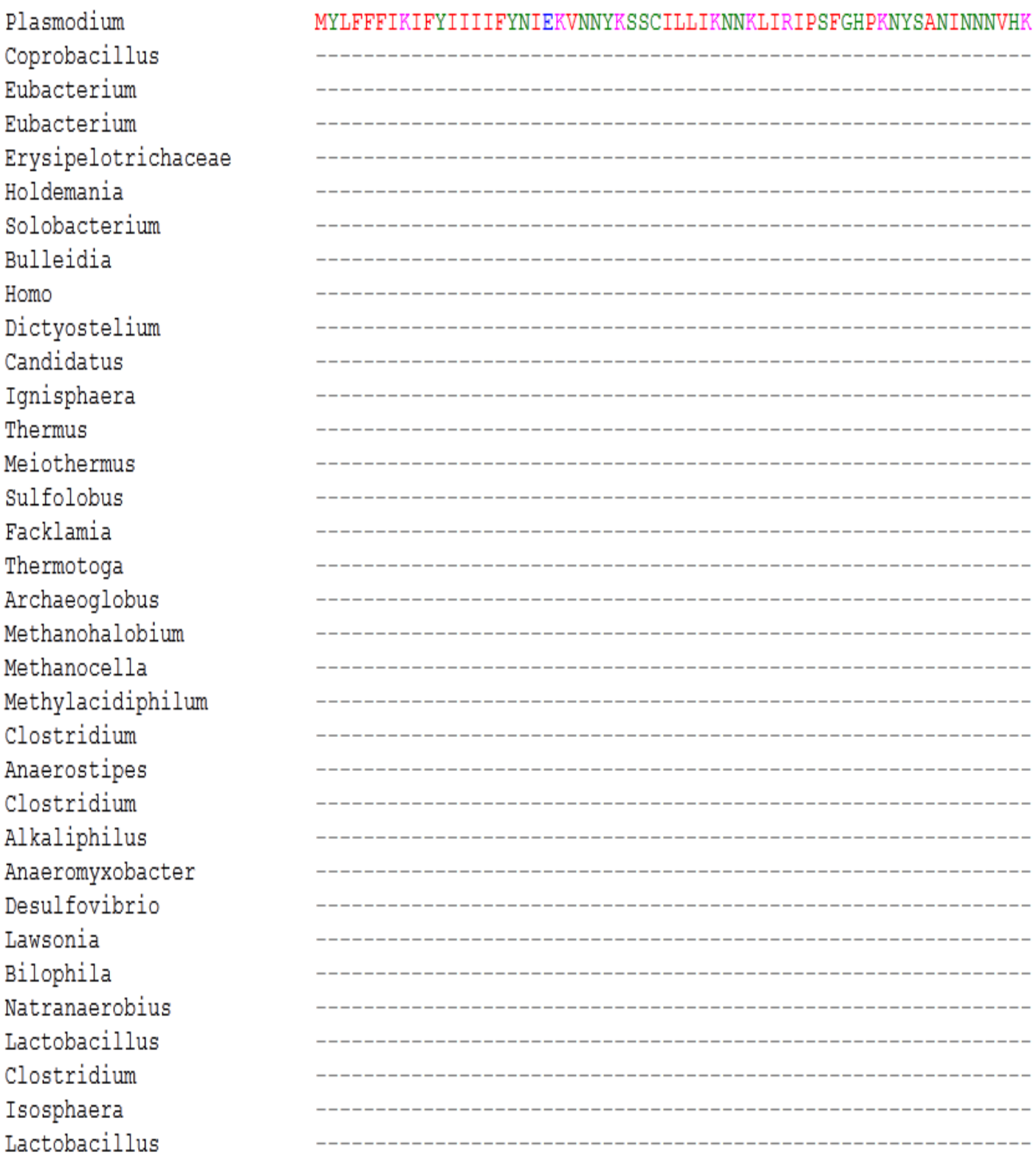
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Coprobacillus
Eubacterium
Eubacterium
Erysipelotrichaceae
Holdemania
Solobacterium
Bulleidia
Homo
Dictyostelium
Candidatus
Ignisphaera
Thermus
Meiothermus
Sulfolobus
Facklamia
Thermotoga
Archaeoglobus
Methanohalobium
Methanocella
Methylacidiphilum
Clostridium
Anaerostipes
Clostridium
Alkaliphilus
Anaeromyxobacter
Desulfovibrio
Lawsonia
Bilophila
Natranaerobius
Lactobacillus
Clostridium
Isosphaera
Lactobacillus

(A)

(B)

Figure-1

A and B showing the multiple sequence alignment of PfAdT along with other sequence homolog from 67 different species from all the three domains of life

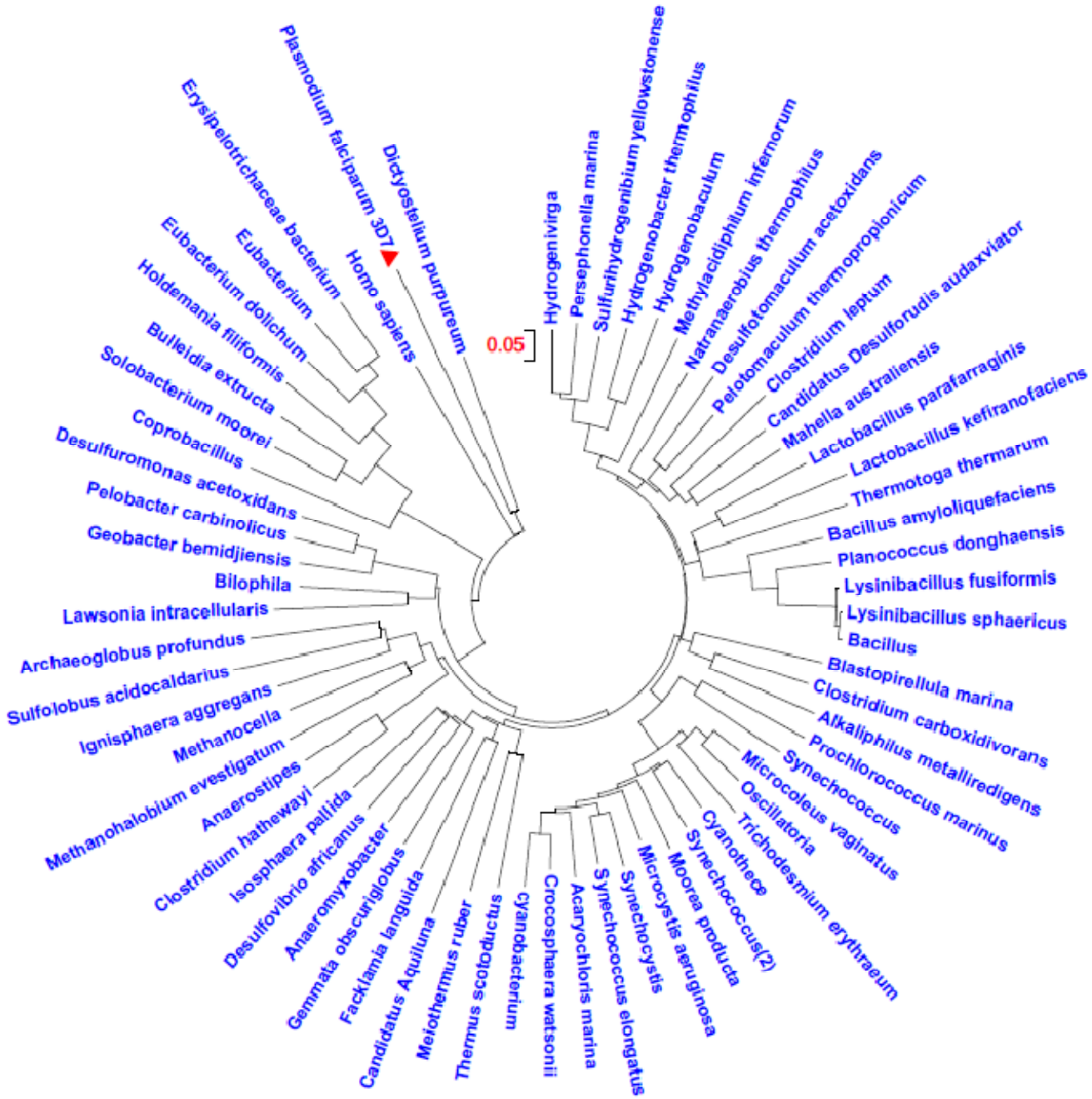

Figure-2
Showing the phylogenetic tree constructed with PfAdT along with other sequence homolog from 67 different species from all the three domains of life, using neighbour-joining method