Molecular cloning and production of type III Hsp40 protein co-chaperone PfZRF1 of human malaria parasite Plasmodium falciparum

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Background: Despite remarkable progress in combating malaria, this deadly disease still accounts for more than a half million deaths annually. In the light of growing incidences of drug resistance, an understanding of parasite biology is necessary for the development of new antimalarials. During life cycle in two different hosts Plasmodium falciparum experiences frequent thermal variations and physiological stress. Heat shock proteins (Hsps) are key players for its survival and making them attractive drug targets. Hence, present research emphasizes on in silico analysis, cloning and production of a type III Hsp40 protein, PfZRF1.

Methods & Materials: Orthologs of PfZRF1 were assigned by BLASTp and literature search. Domain architecture was drawn by SMART and Pfam. ClustalW and T-coffee were employed for multiple sequence alignment analysis. Phylogenetic tree was generated by NJ method using Phylip-3.695 for evolutionary relationship analysis. PfZRF1 and its domain constructs were amplified and cloned in pETM11 vector between NcoI, XhoI and Kpn1 restriction sites. His-tagged PfZRF1 and other domain constructs were expressed in E. coli B834.

Results: PfZRF1 orthologs were identified in 31 eukaryotes however found to be absent in prokaryotes. PfZRF1 is composed of a Hsp70-binding DnaJ domain and two DNA-binding SANT domains. An ubiquitinated histone H2A binding UBD domain was also identified based on human ortholog HsZRF1. As compared to HsZRF1, DnaJ domain has a 50 aas long parasite-specific insertion in loop region between helix II and III near Hsp70-binding HPD motif. Additionally, ribosome associating RAC_head domain was found less conserved with many insertions in PfZRF1. Phylogenetic analysis depicted PfZRF1 to be closer to unicellular eukaryotes. Full ORF (2820 bp) and several constructs covering different domains were amplified and cloned in pETM11. Different constructs were expressed after inducing with 0.5 mM IPTG at 16 °C in E. coli B834 cells for soluble expression.

Conclusion: This study presents preliminary characterization of a type III Hsp40 co-chaperone of the parasite. Further functional characterization of this putative multifunctional chaperone would undoubtedly provide an insight in parasite molecular biology for the development of novel antimalarials.

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Molecular evidence of Bothriocephalus acheilognathi (Cestoda: Bothriocephalidea) from India

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Background: Bothriocephalus acheilognathi, a tapeworm that infects a variety of fishes worldwide. This worm is native to East Asia and spread throughout the world due to aquaculture trade of ornamental fishes. Although, this worm has not been parasitize to any mammals, but recently, a study from Saint Laurent du Maroni (French Guiana) describes the first report of the egg isolation of B. acheilognathi from human stool, a case of accidental infection. However, this worm is also described from Northern part of India but currently there are no reliable data is available based on molecular studies in India. This study aimed to assess the molecular phylogenetic analyses of B. acheilognathi based on sequences of 18S and 28S ribosomal DNA and its distribution in India.

Methods & Materials: Specimens of Bothriocephalus were collected from Xiphophorus hellerii, a native of North and Central America. For morphology, worms were washed in saline and were fixed in 70% ethanol for further processing. For molecular study, a small fragment from the strobila was cut and stored in 95% ethanol until DNA extraction. Genomic DNA was extracted and ribosomal 18S and 28S were amplified and sequenced. The data were then