Molecular and Biochemical Analysis of *Setaria digitata* Novel Parasitic Nematode Specific Protein

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ABATRACT

Setaria digitata is an ivory colored thread like animal parasitic nematode, which naturally resides in the peritoneum cavity of ungulates such as cattle and buffaloes. *S. digitata* share morphological, histological and drug responses similarities with *Wucheraria bancrofti* and *Brugia malayi*. Hence, it is used as a model organism to study *W. bancrofti* and *B. malayi* when specimens were not available. *S. digitata* novel protein (SDNP), is a single copy gene. It was found to be ubiquitously expressed in all the life stages of the nematode. It also shows a significant sequence resemblance with novel parasite specific genes of *W. bancrofti, Loa, Brugia malayi* and *Onchocerca volvulus*.

In this study, the bioinformatics analysis, co-immunoprecipitation, RNA interference *in-vitro* (culture medium) with adults and *in-vivo* (inside the mosquito, *Culex quinquefasciatus*) with microfilariae were carried out to investigate the function of the SDNP.

According to the bioinformatics analysis, the SDNP is a positively charged, basic protein with a better *in-vivo* half-life and thermostable tertiary structure. Due to the hydrophilic nature of the protein, it can be predicted that the protein is most likely to be in the cytosol than it to be in the membrane. The phylogenetic analysis of the protein showed that all of the taxonomic units in its cluster are animal parasitic nematodes and surprisingly this cluster is phylogentically related to a cluster having human parasites. To predict the three dimensional structure of the protein, CABS fold server was selected. The 3D structure obtained from the CABS fold server was refined using the Mod Refiner server and the model validation was carried out using ProSA and RAMPAGE tools. The outcomes of these analyses suggested that, a stable 3D structure had been predicted.

The adult female *S. digitata* worm was subjected to RNAi by microinjecting the *in-vivo* synthesized *SDNP* siRNA and phenotypic and cellular changes associated with the interference were analyzed. The qPCR analysis of the *SDNP* transcript levels revealed a significant reduction of *SDNP* mRNA following siRNA microinjection into *S. digitata* adult worms. Similarly, immunohistochemical staining indicated a reduction of SDNP expression. Furthermore, worms treated with siRNA showed a significant reduction of microfilariae release together with embryonic lethality by arresting an early developmental stage compared to non-treated worms. A distinct motility reduction was also observed in treated worms compared to non-treated counterparts showing the amenability of *S. digitata* to the siRNA induced RNAi for the first time. The presence of interdomain linkers of muscle-specific twitchin kinase and calcium-dependent protein kinase

isoform CDPK1, together with the results of foregoing and previous studies suggested that *SDNP* is most likely a protein involved in muscle movement, growth, and development of the nematode.

In this study *S. digitata* larval stages were cultured in its intermediate host, *Culex quinquefasciatus*, and the RNAi trigger (*SDNP* siRNA) was directly injected into the mosquito. The red color fluorescence detected following RNAi injection to the thorax of *C. quinquefasciatus* indicated the uptake of dsRNA by *S. digitata* larvae. The reduction of *SDNP* transcripts in siRNA treated larvae compared to non-treated larvae, as determined by qPCR, indicated that the siRNA pathway is operational in *S. digitata* larvae. The observation of motility reductions and deformities during the development indicated the association of SDNP in larvae locomotion and development processes, respectively. The irregularities in the migration of larvae in mosquitoes and elevated survival rates of mosquitoes compared to their untreated counterparts indicated reduced parasitism of *S. digitata* larvae in mosquitoes upon targeted down regulation of SDNP by siRNA treatment. Hence, it can be concluded that SDNP plays vital roles in muscle contraction, locomotion, development processes, larval development and parasitism of *S. digitata*. Its ubiquitous presence in parasitic nematodes and its absence in their hosts provide a tantalizing prospect of the possibility of targeting *SDNP* for future development of anthelmintic drugs. The susceptibility of the larval stages of *S. digitata* for RNAi in *Culex quinquefasciatus* was also demonstrated for the first time in this study.

Since proteins interact with its partners to exert its biological function, to understand such process if any that SDNP performs in *S. digitata*, the Co-immunoprecipitation assay was undertaken. In this study the expression and purification of the recombinant SDNP, purification of anti SDNP polyclonal rabbit antibody and co-immunoprecipitation were performed. SDS-PAGE analysis of the resulting co-immunoprecipitated proteins indicated two possible interacting partners with SDNP and the characterization of these protein partners will facilitate further detailing the biological function of SDNP.