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ISOLATION AND COMPUTATIONAL CHARACTERIZATION OF GLUTATHIONE PEROXIDASE GENE FROM *AZOLLA PINNATA*

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ABSTRACT: *Azolla pinnata* has been recently referred to as the super organism due to its emense potential in carbon dioxide sequestration, role in biofertilizer industry as well as as food and fodder. Apart from these, different economic uses of the organism, several studies have indicated towards the effectivity of these organisms as bioindicators to different metal pollution of water bodies. The capacity of azolla to withstand harsh environments is attributed to its strong oxidative stress evasion mechanism, in which glutathione peroxidases play a major role. In this work we isolate and sequence the glutathione peroxidase gene from *Azolla pinnata* and characterize it at the phylogenetic and structure levels using computational tools.

KEYWORDS: Azolla, super organism, Phylogenetic Analyses, Structural Studies, Interaction Studies.

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1.INTRODUCTION

Azolla is a worldwide heterosporous floating or semi-aquatic pteridophyte, having scale like leaves, bilobed which overlaps and which covers a rhizomatous stem. It is generally found floating on water surfaces, having roots appearing like pendulums[1]. It grows rapidly and enables the sequestration of greenhouse gases such as carbon dioxide which increases the biomass of the organism[2]. *Azolla pinnata* as a wide distribution. It has been reported from parts of Africa, India, China and Japan, Malaysia, Philippines, the New Guinea mainland and Australia[3]. Azolla was found in fossils of eocene sediments (~48.5 Ma) in the central arctic ocean near north pole[4]. Ganguli et.al [5]have reported that azolla has the capacity of lead accumulation and sequestration[6]. Glutathione peroxidase represents one of the most ubiquitously occurring oxidative stress enzymes of the plant kingdom. Over the years several reports have accumulated regarding the phylogenetic implications of glutathione peroxidases[7]. We have recently reported the sequencing and analyses of a glutathione peroxidase from *Salvinia molesta* Mitch.

2. MATERIALS AND METHODS

Data collection, isolation and sequencing followed by submission to Genbank was done according to the protocol described in Rahaman et.al. 2016. [8]

Flowchart of Work of Computational Analysis:

1. Nucleotide sequence (EF620782.1) was retrieved from NCBI data warehouse and it was used to find homologues using Basic Local Alignment Search Tool (BLAST)[9] of the NCBI.
2. Sequences of best 25 homologues were retrieved.
3. Homologues and our query sequence were aligned using MEGA 6.0[10] tool following MUSCLE[11] algorithm.
4. The aligned sequence was further utilized for Phylogenetic analysis and molecular clock analysis[12].
5. Further real time tree divergence rates and probable ancestors were computed.
6. ORF for the protein sequence was predicted using ORF Finder[13].
7. The amino acid sequence obtained was used for homology modeling using Modeller 9.12[14].
8. The model obtained was simulated using AMBER ff93[15] and CHARMM force field[16].
9. Simulated protein was further validated using PROCHECK[17] and QMEANS[18] server.
10. Structural analyses of protein was carried out.
11. Protein was docked with Glutathione and Hydrogen peroxide separately.
12. Interactomics analysis was performed on the docked complexes.

3. RESULTS AND DISCUSSION

PCR amplification was successful and the product was sent for capillary sequencing which was converted to FASTA format and submitted in GENBANK with the id EF620782.1

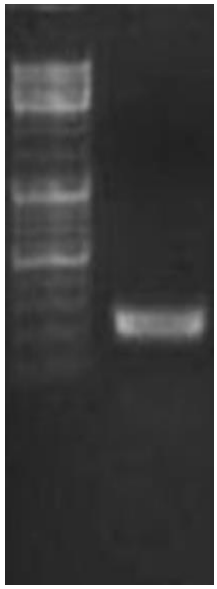

Fig1: PCR amplicon corresponding to the *Azolla pinnata* glutathione peroxidase gene

The sequence was then subjected to BLAST analyses and the best 25 homologues were detected which were then used for phylogenetic analyses. Upon Phylogenetic it was revealed that query sequence formed a clade with another OTU (gi|189162400|dbj|AP009589.1| *Lotus japonicus* genomic DNA chromosome 4 clone). The divergence rate computed HTU (Hypothetical Taxonomic Unit) for query was 0.66 which is much higher than remaining HTU and had probably three changes in nucleotide (GTA) at HTU having divergence rate of 0.66 while rest of the HTUs have variation of only one nucleotide. This implicates the variation in divergence rate of query from remaining other OTUs (Operational Taxonomic Unit) only exception being its sister OTU.

Figure 2: Screenshot of Phylogenetic tree displaying Real time tree divergence and probable ancestors at different HTUs and OTUs.

The molecular clock analysis revealed divergence rate of evolution to be

	lnL	Parameters	(+G) (+I)		
With Clock	-26604.463	29	n/a	n/a	
Without Clock	-3770.479	52	n/a	n/a	

Table 1: Molecular Clock Analyses which was performed for parameter based comparison between Maximum Likelihood value for tree topology both without and with molecular clock constraint based upon Tamura-Nei model(1993).

Protein Modelling and Simulation:

Amino sequence was obtained using ORF Finder and the protein was modeled by following comparative modeling approach using Modeller 9.12. Model which was generated was then simulated using CHARRM force field and AMBER ff3. The simulated protein was further validated through QMEANS and PROCHECK server.

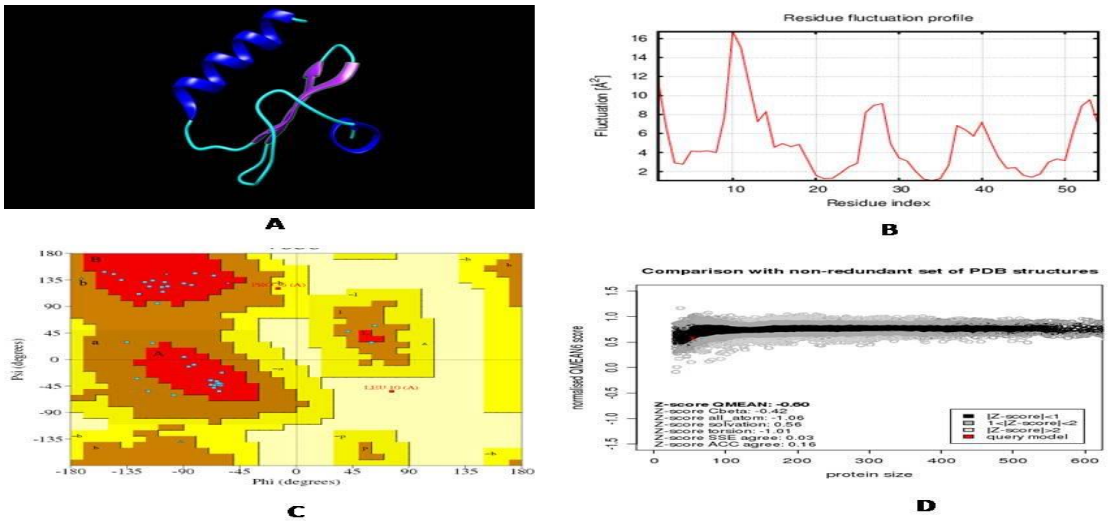

Figure 3: A represents modeled protein post simulation, B represents residue fluctuation profile during simulation, C represents Ramachandran Plot for Validation of Simulated protein and D represents QMEANS score of simulated protein for validation.

Structural Analysis:

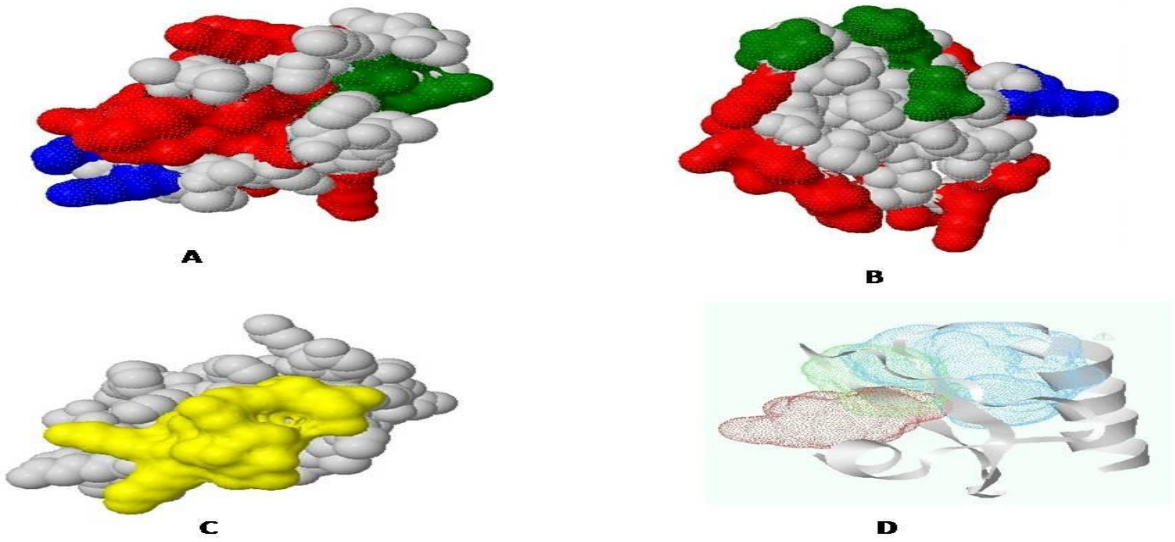

Figure 4: A represents cavities, B represents Protrusions, C represents Flat regions and D represents Pocket and Subpockets of protein using 3DSURFER [19]

Interactomics:

Post validation protein was docked with Glutathione and Hydrogen Peroxide separately using Autodock[20]. The docked complex was analyzed for presence of interacting bonds using LIGPLOT[21].

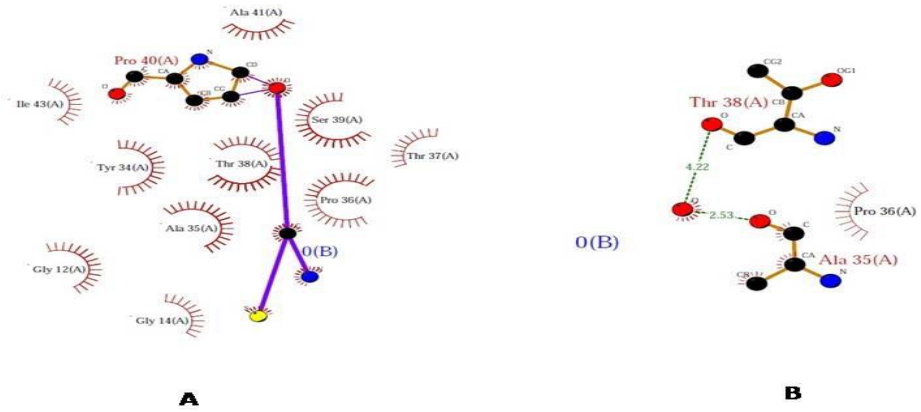

Figure 5: A shows the interaction between Modeled Protein and Glutathione while B represents the interaction between modeled protein and hydrogen peroxide.

DISCUSSIONS

Comparative modeling and simulation revealed the presence of a robust domain topology was revealed by comparative modeling and molecular dynamics simulation and features such as cavity, protrusion and flat were identified within the structure using Zernike descriptors and Delaunay Trangulation scores.

Variations in the rate of divergence were observed by interpretation of molecular clock and phylogenetic tree. The null hypothesis of assumption of equal evolutionary rate throughout the maximum likelihood tree was rejected at a 5% significance level (P = 0). Interactomics revealed that Ala 314, Ala 315, Ala 316, Ala 317, Ala 318, Pro 322, Pro 323, Thr 335 and Thr 338 were identified to be common residues which took part in VanderWaal's Interaction for both of studies. For Glutathione peroxidases and hydrogen peroxide complex Ala 317 was found to be taking part in formation of two hydrogen bonds while Thr 338 was involved in formation of one hydrogen bond. No hydrogen bond was identified during analyses of Glutathione peroxidases and glutathione complex.

4. CONCLUSION

Structural and Phylogenetic analyses revealed that the isolated gene was truly a glutathione peroxidase since it shared sister clades with known glutathione peroxidase goes and interacted stably with glutathione and hydrogen peroxide - its two most important substrates

CONFLICT OF INTEREST

The authors have no conflict of interest.

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