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HOMOLOGY MODELING AND *INSILICO* ANALYSIS OF PROTEINS OF *BOMBYX MORI* - "A GREEN APPROACH"

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ABSTRACT

Sericin is a natural polymer produced by Silkworm also known as *Bombyx mori*. The Silk worm wounds around itself a fiber, cocoon which keeps together two fibroin filaments. The fibrion forms central core and sericin forms envelop and is water soluble. In this present study, 10 proteins of Sericin were analysed using bioinformatics tools. Structural prediction and functional characterization of proteins of Sericin were done using Expasy Protparam server, 3D structure was done using SWISS MODEL. Plants of different family showing identity 30% and above were selected and its sequences retrieved, aligned using Clustal Omega. Phylogenetic tree was constructed for the aligned sequence. Structure prediction showed that α – helix, random coil, β – turn and extended strand predominates. Transmembrane region was found in NADPH cytochrome P450 reductase, cytochrome c oxidase subunit 1**,** nicotonic acetylcholine receptor proteins. NADPH cytochrome P450 reductase possesses the abilty to reduce metals and hence Sericin was predicted for its ability to synthesis nanoparticles. Phylogenetic analysis of NADPH cytochrome P450 reductase of sericin reveals that the *Bombycidae* families are closely related. Thus, sericin is a potential biocompatible material for its biomedical applications.

KEYWORDS: *Bombyx mori,* NADPH cytochrome P450 reductase, sericin, Phylogenetic analysis.

INTRODUCTION

Silk protein filament has two protein fractions, fibroin, a fibrous component and glue-like sericin that holds the fibroin components together and often discarded as waste product in silk industry.[1] In the textile industry, the cocoon is processed and sericin is largely removed in a process called degumming. The fibroin is converted into raw silk and used in the production of many types of yarns and silk fabrics. For a long time, sericin has been disregarded in the field of sericulture. It is estimated that it 400,000 tons of dry cocoons worldwide, producing 50,000 tons of sericin. sericin is mostly discarded in wastewater. This generates a high chemical and biological oxygen demand as well as contamination of water. The removal and use of sericin could have a strong economic, social, and environmental impact, especially in countries where sericulture is practiced, such as China, India, and Brazil.[2] The silk fiber protein is synthesized by silk gland cells and stored in the lumen of the silk glands. Subsequently, it is converted into silk fibers. When the silkworms secrete the liquid silk during the spinning, it passes through the anterior gland and expelled out through the spinneret opening. Sericin is insoluble in cold water, however, it is easily hydrolyzed, where by the long protein molecules break down to smaller fractions, which are easily dispersed or solubilised in hot water.[3]

Chemically, sericin and fibroin two together have amino acids but in disparate balance. Fibroin is well off in glycine and alanine acids where as sericin is well heeled in serine and aspartic acids. Sericin protein consists of 18 amino acids, including some essential amino acids. Serine is the main part of human skin natural moisture factor (NMF). The content of serine in sericin is approximately 33.43%. Thus, sericin is an excellent moisturizing agent. Silkworm is the only source generating sericin, hence sericin is attained from cocoons, silk fabric, and silk waste or from the degumming liquor of silk industry. The structure and molecular weight affects functional properties of sericin. The chemical structure and molecular weight of sericin mainly depends on two factors: method of separation of sericin and fibroin and method of recovering sericin from degumming liquor.[4,5]

Silk sericin is highly hydrophilic with strong polar side chains such as hydroxyl, carboxyl, and amino groups. Recently, Silk sericin has been widely used in biomaterial applications due to its biocompatibility, biodegradability and anti-oxidative and bioactive activities.[6]

Bioinformatics is the field of science in which biology, computer science and information technology merges to

form a single discipline. It focuses more on hypothesis testing and discovery in the biological domain. The task used in bioinformatics ranges from the creation and maintenance of database of biological information to the analysis of sequence information. The wide range of application of bioinformatics include molecular medicine, gene therapy, drug development, waste cleanup, forensic analysis of microbes, evolutionary studies, comparative studies . Structural Bioinformatics is one of the key research areas in the field of Computational Biology. Structural Bioinformatics concerns the analysis and prediction of three-dimensional (3-D) structures of biological macromolecules such as Proteins1, RNA and DNA. This structural information corresponds to 3-D macromolecular structures obtained through different experimental methods such as protein crystallography (X-ray diffraction), electron microscopy or nuclear magnetic resonance (NMR). This information allows one to study folds and local motifs in proteins, molecular folding, and evolution and structure/function relationships. One of the main research problems in structural bioinformatics is the prediction of three-dimensional protein structures. Proteins are long sequences formed out of 20 different amino acid residues that in physiological conditions adopt a unique 3-D structure.[7] The Ramachandran plot is the display of phi, psi angle pairs of polypeptide chain in a given protein structure in an easily comprehensible way.[8]

However there remains still a huge scope for use of modern scientific methods - genomics, proteomics and bioinformatics in the Sericin. Bioinformatics shall facilitate analysis and integration of information from these related fields to enable the identification of genes and gene products and elucidate the functional relationships between genotype and observed phenotype. This research report provides a state-of-the-art overview of bioinformatics study of *Bombyx mori* with emphasis on the current progress and future directions, which shall provide tools and resources necessary to understand and promote advances in this important field.

MATERIALS AND METHODS

Sequence Retrieval: The FASTA sequences of the proteins were retrieved from Genbank database hosted by the NCBI (http://www.ncbi.nlm.nih.gov).[10]

Primary Structure Prediction

For Physio-chemical characterization, theoretical Isoelectric Point (pI), molecular weight, total number of positive and negative residues, extinction coefficient, instability index, aliphatic index and grand average of hydropathy (GRAVY) were computed using the Expasy Protparm server.[11] **(**http://us.expasy.org/tools/protparam.html).

Secondary Structure Prediction

SOPMA (Self Optimized Prediction Method with Alignment) was used for the secondary structure prediction and the corresponding alpha helix, extended strand, beta turn and random coil of the proteins were predicted.

Functional Characterization: SOSUI and TMHMM v.2.0 tools were used to characterize whether the protein is soluble or transmembrane in nature. InterPro is an integrated resource for protein families, domains and functional sites. InterPro incorporates the major protein signature databases into a single resource. These include: PROSITE, which uses regular expressions and profiles, PRINTS, which uses Position Specific Scoring Matrix-based (PSSM-based) fingerprints, ProDom, which uses automatic sequence clustering, and Pfam, SMART, TIGRFAMs, PIRSF, SUPERFAMILY, Gene3D and PANTHER, all of which use hidden Markov models (HMMs). Superfamily and molecular function were predicted by Interpro protein sequencing and classification.[12] (http://www.ebi.ac.uk/interpro/).

Sequence Alignment: Sequence alignment of was performed using pair wise sequence alignment tool (NCBI- BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and multiple sequence alignment was done using the EBI-CLUSTAL OMEGA (http://www.ebi.ac.uk/Tools/msa/clustalo/) tool. Clustal Omega also has powerful features for adding sequences to and exploiting information in existing alignments, making use of the vast amount of recomputed information in public databases like Pfam.[13] The emphasis of this work was to find the regions of sequence similarity, which in other words allows us to yield functional and evolutionary relationships among the proteins considered in this study.

Phylogenetic Analysis: The phylogentic analysis of ten proteins was performed to determine the number of proteins that share common structural and functional features. As an input to Clustal Omega all sequences in fasta formats were supplied with default options. The output was analyzed for sequences that are aligned for the complete length, scores, alignment, conserved residues, substitutes and semi conserved substituted residue patterns. The phylogenetic tree was constructed based on the bootstrap Neighbour Joining (NJ) method.[14] The stability of the internal nodes was assessed by bootstrap analysis with 1000 replicates.

Tools: Primary tools for sequence comparison and assembly have grown in line with an expansion of the datasets that they analyze. Without basic local alignment search tool (BLAST) and related sequence comparison tools, much of the data coming from the many high-throughput sequencing laboratories would be nothing more than strings of letters. BLAST remains the fastest means by which to identify specific sequences in large datasets and enables the rapid annotation of novel sequences. Although BLAST is the standard tool for identifying sequence similarities in large datasets, there are several options for assembling sequence datasets, the

choice of which depends on hardware availability, dataset size, data format, structure and the genetic structure of the organism.

Sequence similarity search and assembly tools are the foundation of many software applications for analyzing plant genomic information. The ability to rapidly identify similarities to previously characterized sequences greatly enhances the sequence annotation process. This has led to the development of comparative sequence databases, whereas sequence assembly packages both reduce the high level of redundancy in datasets and enable variations in sequence to be identified.

RESULT AND DISSCUSSION

Silk worm, *Bombyx Mori* is gaining a much important space in the area of biomedical technology because of its two powerful protein members Sericin and Fibroin. Fibroin is widely used in textiles, industrial and medical applications. *In silico* study in medicine is thought to have the potential to speed the rate of discovery while reducing the need for expensive lab work and clinical trials. One way to achieve this is by producing and screening drug candidates more effectively.

Table 1: Primary Structure of Proteins of Bombyx Mori.

S. No	Accession number	Protein	Length
1	BAA95684.1	NADPH cytochrome P450 reductase	642
2	BAH97090.1	Glutathione peroxidase	637
3	AAF73765.1	Cytochrome C oxidase subunit 1	510
4	BAE96011.1	Glyceraldehyde 3 phosphate dehydrogenase	332
5	BAD38853.1	Catalase	507
6	AAY86076.1	Superoxide dismutase(Cu-Zn)	172
7	ACT64133.1	Alpha amylase	500
8	ABV45520.2	Nicotinic acetylcholine receptor	497
9	H9J6L6	DNA polymerase alpha subunit B	696
10	AAA29189.1	Lysozyme	139

The primary structure prediction was done with the help of protparam tool (Table 2). The parameters were computed using Expasy‘s protparam tool which revealed that the molecular weights for ten different proteins as 73121.90 (NADPH cytochrome P450 reductase), 73488.34 (Glutathione peroxidase), 56832.88 (Cytochrome C oxidase subunit 1), 35428.48 (Glyceraldehyde 3 phosphate dehydrogenase), 56920.19 (Catalase), 18285.36 (Superoxide dismutase(Cu-Zn)) and 56679.69 (Alpha amylase), 56199.04 (Nicotinic acetylcholine receptor), 77934.45 (DNA polymerase alpha subunit B), 15877.26 (Lysozyme). The pI of 5 proteins was less than 7 which indicated that they are acidic and remaining 4 proteins were greater than 7 which showed that it is basic in character. The proteins are found to be compact and stable at their pI.[15] Among the ten proteins eight proteins showed instability index lesser than 40, indicating that the protein are stable.

Aliphatic index of the proteins ranged between 71.97–109.76. The computed extinction coefficients help in the quantitative study of protein–protein and protein–ligand interactions in solution. The range of GRAVY (Grand Average of Hydropathicity) of *Bombyx mori* proteins was found to be -0.259 to -0.475 and 0.025 to 0.648. The lowest value of GRAVY indicates the possibility of better interaction with water.[16]

Table 2: Parameters Computed Using Expasy’s *Bombyx Mori.*

S. No	Accession number	Protein	Length	Mol.Wt	PI	-R	+R	EC	II	AI	Gravy
1	BAA95684.1	NADPH cytochrome P450 reductase	642	73121.90	5.62	94	77	76960	44.74	80.30	-0.499
2	BAH97090.1	Glutathione peroxidase	637	73488.34	6.85	69	67	112315	31.84	85.38	-0.314
3	AAF73765.1	Cytochrome C oxidase subunit 1	510	56832.88	5.87	24	15	106800	30.51	109.76	0.648
4	BAE96011.1	Glyceraldehyde 3 phosphate dehydrogenase	332	35428.48	7.70	34	35	33015	21.19	92.80	0.025
5	BAD38853.1	Catalase	507	56920.19	8.11	58	57	65000	30.84	71.97	-0.475
6	AAY86076.1	Superoxide dismutase (Cu-Zn)	172	18285.36	6.12	19	13	4595	22.71	84.42	-0.259

Mol. Wt – molecular weight(Daltons), pI – Isoelectric point, -R - Number of negative residues, +R – Number of Positive residues, EC – Extinction Coefficient at 280 nm, II – Instability Index, AI – Aliphatic Index, GRAVY – Grand Average Hydropathicity, * - No Trp, Tyr or Cys residue (should not be visible by UV spectrophotometry).

Table 3: Secondary Structure Results of Proteins of *Bombyx Mori.*

S No	Secondary Structure	BAA95684.1	BAH97090.1	AAF73765.1	BAE96011.1	BAD38853.1	AAY86076.1	ACT64133.1	ABV45520.2	H9J6L6	AAA29189.1
1	Alpha helix	41.34%	35.16%	43.73%	32.53%	28.40%	13.37%	28.80%	33.00%	34.34%	46.04%
2	Extended strand	15.57%	17.27%	19.80%	26.20%	15.58%	30.81%	19.20%	23.34%	15.09%	13.67%
3	Beta turn	4.51%	5.49%	6.86%	7.53%	5.33%	6.40%	5.60%	4.43%	3.74%	7.91%
4	Random coil	38.57%	42.07%	29.61%	33.73%	50.69%	49.42%	46.40%	39.24%	46.84%	32.37%

The secondary structure prediction of *Bombyx mori* proteins (Table-3) was analyzed by SOPMA which revealed that alpha helix, extended strand, beta turn and random coil, were more predominant. In most of the proteins alpha helix dominates which is followed by random coil, extended strand and beta turn. The secondary structure were predicted by using default parameters (Window width: 17, similarity threshold: 8 and number of states: 4). TMHMM v.2.0 and SOSUI predicted were three proteins are Transmembrane proteins and remaining seven were soluble proteins.

Table 4: Transmembrane Region Predicted by Sosui Server.

1. NADPH cytochrome P450 reductase

This amino acid sequence is of a MEMBRANE PROTEIN which have 1 transmembrane helices.

Table 4.1: Transmembrane region of NADPH cytochrome P450 reductase predicted by SOSUI server.

No.	N terminal	Transmembrane region	C terminal	type	length
1	19	AAAAGGSLFSTFDIIVLALLLGG	41	PRIMARY	23

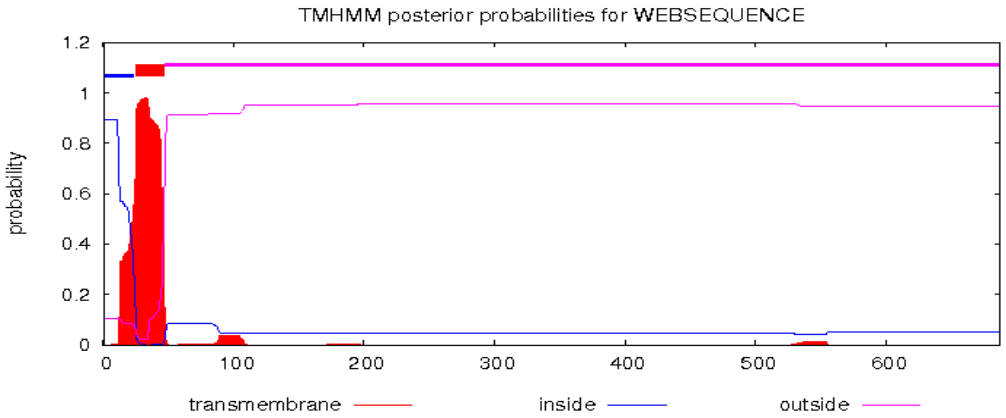

Fig. 1: TMHMM Result Showing Transmembrane Region of NADPH cytochrome P450 reductase.

2. Cytochrome C Oxidase Subunit 1

This amino acid sequence is of a MEMBRANE PROTEIN which have 6 transmembrane helices.

Table 4.2: Transmembrane Region of Cytochrome C Oxidase Subunit 1 Predicted By Sosui Server.

No.	N terminal	transmembrane region	C terminal	type	length
1	17	FIFGIWSGMIGTSLSLLIRAELG	39	SECONDARY	23
2	60	FIMIFFMVMPIMIGGFGNWLVPL	82	PRIMARY	23
3	94	MNNMSFWLLPPSLMLLISSSIVE	116	SECONDARY	23
4	142	LAIFSLHLAGISSIMGAINFITT	164	SECONDARY	23
5	182	VWAVGITAFLLLLSLPVLAGAIT	204	PRIMARY	23
6	237	HPEVYILILPGFGMISHIISQES			

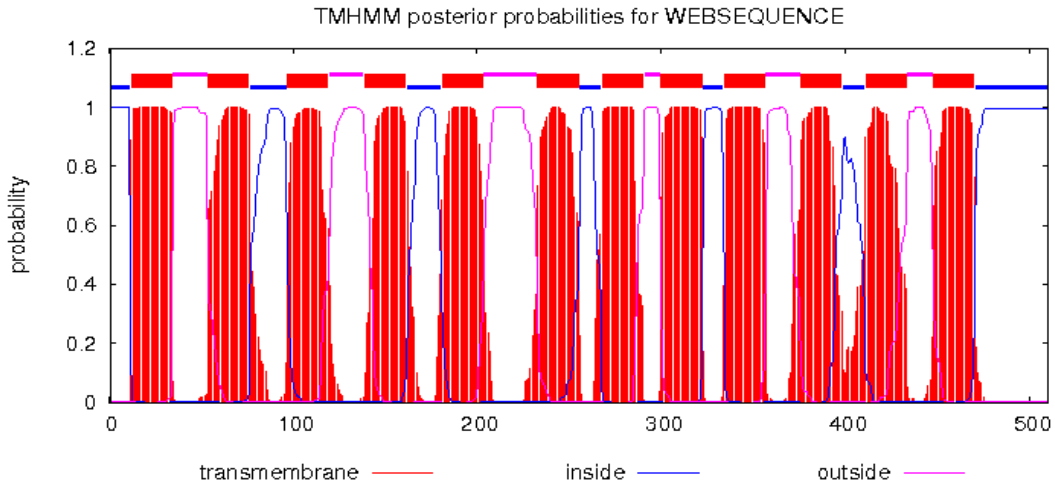

Fig. 2: TMHMM Result Showing Transmembrane Region of Cytochrome c oxidase subunit.

3. Nicotonic Acetylcholine Receptor

This amino acid sequence is of a MEMBRANE PROTEIN which have 6 transmembrane helix.

Table 4.3: Transmembrane Region of Nicotonic Acetylcholine Receptor Predicted By Sosui Server.

No.	N terminal	transmembrane region	C terminal	type	length
1	9	LLAAPAGLLLLLLGLLWPRGVCG	31	PRIMARY	23
2	242	TLYYFFNLIVPCVLIASMALLGF	264	SECONDARY	23
3	272	EKLSLGVTILLSLTVFLNMVAET	294	SECONDARY	23
4	306	GTYFNCIMFMVASSVVSTILILN	328	PRIMARY	23
5	338	EMSDWIRCVFLYWLPWILRMSRP	360	SECONDARY	23
6	468	VDRLCLIIFTLFTIIATLAVLLS			

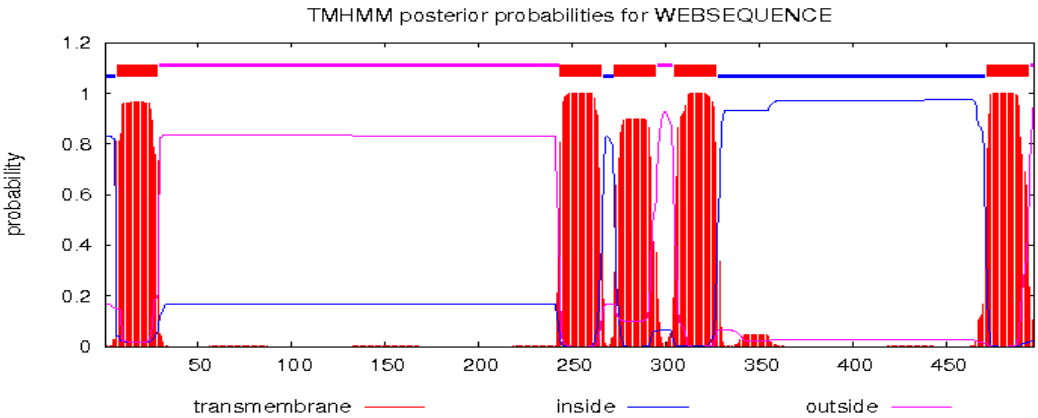

Fig. 3: TMHMM Result Showing Transmembrane Region of Nicotonic acetylcholine receptor.

Tertiary Structure of *Bombyx Mori*

1. NADPH cytochrome P450 reductase

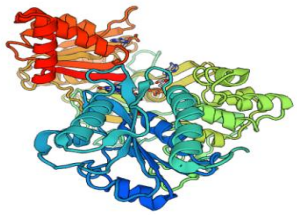

Fig. 4: Tertiary structure of NADPH cytochrome P450 reductase.

Table 5: Interpro Results of Proteins of *Bombyx Mori.*

S.NO	Accession Number	Super Family	Molecular Function
1	BAA95684.1	NADPH--cytochrome P450 reductase	Binds 1 FAD per monomer Binds 1 FMN per monomer.
2	BAH97090.1	Glutathione peroxidase	hydrolase activity Oxidoreductase glutathione peroxidase activity
3	AAF73765.1	Cytochrome c oxidase subunit 1	cytochrome-c oxidase activity heme binding iron ion binding
4	BAE96011.1	Glyceraldehyde-3-phosphate dehydrogenase	glyceraldehyde-3-phosphate dehydrogenase (NAD+) (phosphorylating) activity NAD binding NADP binding
5	BAD38853.1	Catalase	heme binding iron ion binding response to oxidative stress
6	AAY86076.1	Superoxide dismutase [Cu-Zn]	Binds 1 copper ion per subunit. Binds 1 zinc ion per subunit Destroys radicals
7	ACT64133.1	Alpha-amylase	releasing maltohexaose cation binding Glycosidase
8	ABV45520.2	Nicotinic acetylcholine receptor subunit alpha 7	acetylcholine-gated cation-selective channel activity transmembrane signaling receptor activity Ligand-gated ion channel
9	H9J6L6	DNA polymerase alpha subunit B	DNA binding DNA-directed DNA polymerase activity Golgi vesicle transport
10	AAA29189.1	Lysozyme	lysozyme activity Glycosidase Antibiotic, Antimicrobial.

Phylogenitic Analysis of *Bombyx Mori*

Phylogeny is the history of descent of a group of taxa such as species from their common ancestors including the order of branching and sometimes the times of divergence. In molecular phylogeny, the relationships among organisms or genes are studied by comparing homologues of DNA or protein sequences. Dissimilarities among the sequences indicate genetic divergence as a result of molecular evolution during the course of time.

The phylogentic analysis of NADPH cytochrome P450 reductase was performed to determine the number of proteins that share common structural and functional features. As an input to Clustal Omega all sequences in Fasta formats were supplied with default options. The study of evolutionary history of some organisms using tree-like diagrams is known as phylogenetic tree construction or phylogenetic analysis. Each time a branch divides into a smaller branch, it shows the emergence of a new group of organisms. The most popular distance-based methods which are being used for the comparison are the Un-weighted pair group method with arithmetic mean (UPGMA) and Neighbor joining (NJ).[17] NADPH-cytochrome P450 reductase is a 78 kDa flavoprotein bound to the cytoplasmic surface of the endoplasmic reticulum and the outer membrane of the nuclear envelope in eukaryotic cells.[18] Cytochrome P450 (P450) enzymes have been studied for more than 50 years. They have important roles in the metabolism of steroids, fat-soluble vitamins, carcinogens and drugs. NADPH–cytochrome P450 reductase can be used as checks on the integrity of a biological system.[19]

Table 6: Lists of Plant Species Showing Similarity of 60% and Above With the Nadph Cytochrome P450 Reductase.

S.NO	Protein name	Family	Accession Number	Identity
1	Helicoverpa armigera	Noctuidae	ADK25060.1	89.2%
2	Aedes albopictus	Culicidae	JAC13190.1	69.7%
3	Plutella xylostella	Plutellidae	AIJ00849.1	84.1%
4	Drosophila virilis	Drosophilidae	EDW63490.1	68.5%
5	Fopius arisanus	Braconidae	JAG80572.1	65.1%
6	Rhopalosiphum padi	Aphididae	ANE10154.1	61.8%
7	Orchesella cincta	Entomobryidae	ODN02614.1	61.6%
8	Tabanus bromius	Tabanidae	JAI17457.1	68.4%
9	Tenebrio molitor	Tenebrionidae	AKZ17715.1	67.5%
10	Clunio marinus	Chironomidae	CRK973329.1	68.3%

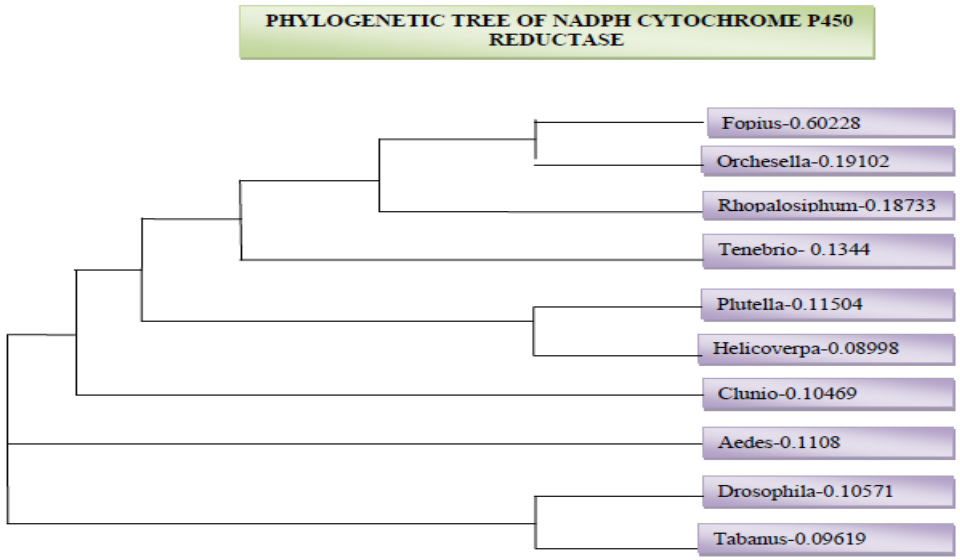

Fig. 6: Phylogenitic Tree of Nadph Cytochrome P450 Reductase Protein Containing Plants.

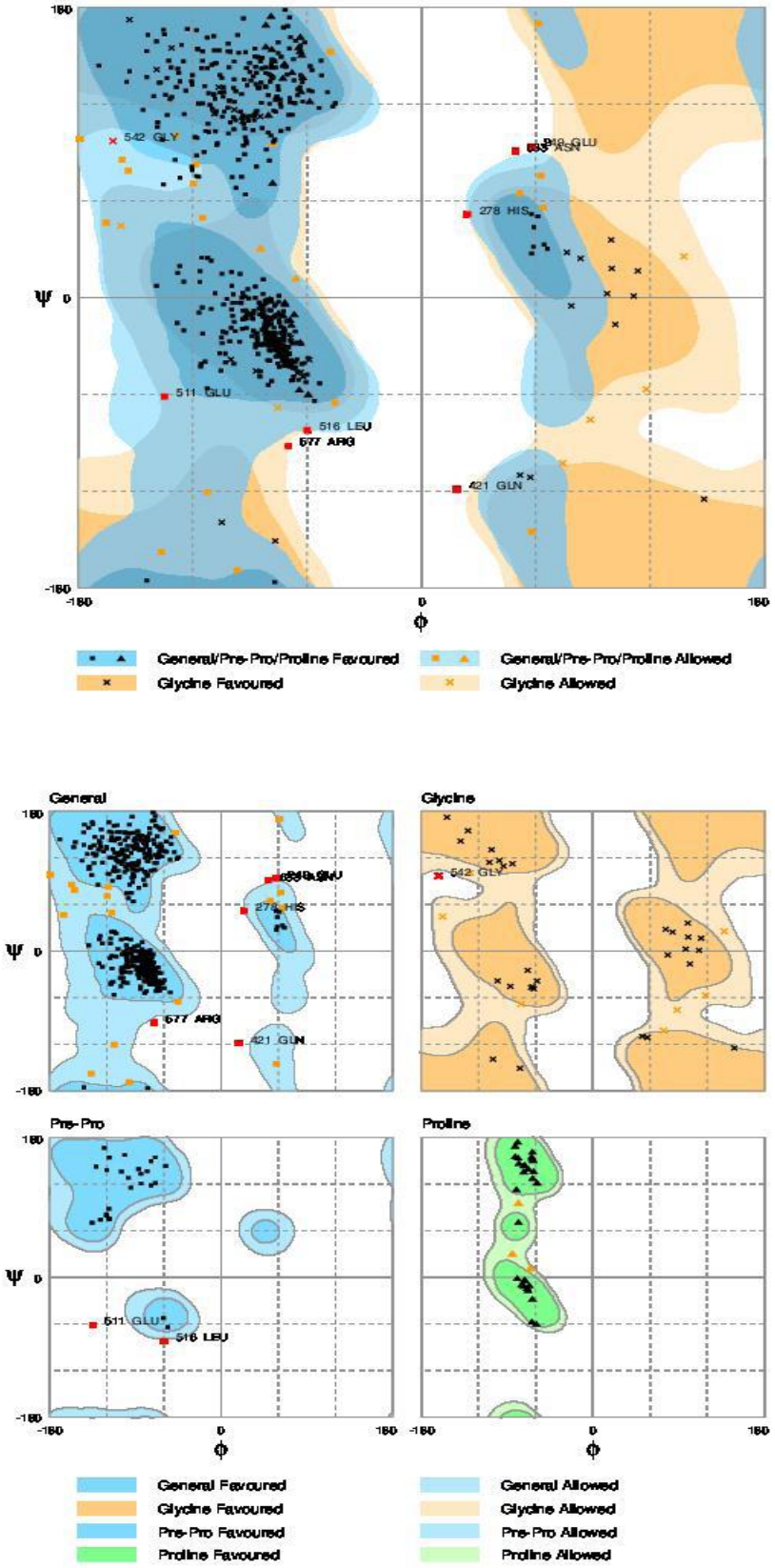

Fig. 7: Ramachandran Plot for Nadph Cytochrome P450 Reductase.

Number of residues in favoured region (~98.0% expected): 578 (94.3%)
Number of residues in allowed region (~2.0% expected): 27 (4.4%)
Number of residues in outlier region: 8(1.3%)

CONCLUSION

The use of *in silico* methods for drug discovery in natural products has increased during the previous decade. Computational studies of its key biosynthetic enzymes may provide valuable insights into the mechanism of action of the enzymes aiding in the ultimate aim of improving quality and quantity of the reaction products. Deep sequencing data analysis is a growing field. The overflow of available bioinformatics tools for each of the optional analysis steps represents a challenge for the researcher aiming to evaluate and interpret deep sequencing data. The field is rapidly evolving both in sequencing platform technology and in computational tools. The development of high throughput technologies has not only increased the amount of data, but also the types of data available, opening new prospects for investigations. However, even the application of currently available chemo- and bioinformatics resources and approaches provides valuable information for discovery of novel applications of environmental and industrial wastes beyond their traditional use. *Bombyx mori* was selected. Expasy's Prot Param tool predicted the physio-chemical characters of the proteins. Further analyses are required for drug target identification.

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