Computational characterization and structure prediction of chitinase gene of *beauveria bassiana* using proteomic tools

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Chitinases occur in a wide range of organisms including bacteria, fungi, plants, insects, and animals. Chitinase has been widely used as one of the mycobiocontrol agent as it degrades chitin which is a chain homopolymer of N- acetylglucosamine (GlcNAc) connected by β-1,4 glucosidic linkages. Beauveria bassiana is an insect pathogenic fungus successfully used as an insect pest control agent worldwide. In this paper, 21 different chitinase, endochitinase and chit proteins / gene retrieved from Swiss-Prot database are analysed and characterized. Various Bioinformatics and molecular modeling approach were adopted to explore properties and structure of chitinase gene in Entomopathogenic fungi. Primary structure analysis predicted the physico-chemical properties such as pI, EC, AI, GRAVY and instability index and provides data about these proteins and their properties. Subcellular localization were predicted by MultiLoc software. Prediction of motifs, patterns, disulfide bridges and secondary structure were performed for functional characterization. Three dimensional structures for chitinase like proteins are not available as yet at PDB. Therefore, homology models for were developed. The modelling of the three dimensional structure of these proteins shows that models generated by Modeller were more acceptable in comparison to that by Geno3D and Swiss Model.

Keywords: Entomopathogenic fungi, Mycobiocontrol, Modeller, Homology.

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Introduction

Chitinases are cuticle-degrading enzymes [1, 2, 3] and have been reported as pathogenicity determinants in fungi [4]. Chitinase gene has been widely studied because of its characteristic feature of degrading chitin which is a chain homopolymer of N-acetylglucosamine connected by α 1, 4 glucosidic linkages [5]. They have been classified into two groups, endo- and exo-chitinases. Overproduction of endo-chitinases in fungi such as *Beauveria bassiana*, has been correlated with increased in their virulence [6].

Beauveria bassiana is an entomopathogenic fungi that parasitizes insects leading to their permanent disability or mortality. Entomopathogems were among the first organisms to be used as mycoinsecticide biocontrol agents [7, 8]. These fungi are reported to produces cuticle degrading proteins such as chitinases, proteases, and lipases [4, 9, 6]. Investigations on secretions of *Beauveria bassiana* reported production of multiple

chitinases that possess different functions [1, 2, 3]. Difference in the functions of these chitinases suggests difference in their structure, substrate specificity or catalytic mechanism. However, these differences in specificities and catalytic mechanism need to be proven and the correlation between structure and functions is to be deciphered.

Recent years have seen enormous increase in sequence data. Soft computing tools are being used to derive huge amount of information from these sequences that includes comparative analysis and properties prediction of putative proteins [10, 11]. The physicochemical and the structural properties of the proteins are well predicted with the help of several online computational packages [12]. These in silico approaches are a viable solution for decreasing time and cost involved in *in vitro* and *in vivo* studies [13].

In the present study, we characterized of a novel chitinase gene isolated from *Beauveria bassiana* by our group and used several bioinformatic tools to predict physic-chemical properties and compared it with characterized chitinases from other organisms.

Materials and method

The schematic flow-chart for the prediction of 3-D structure of protein through protein sequence is given below (figure 1).

1. Sequence Retrieval and analysis

Novel chitinase sequence (GenBank number: KF559204; NCBI Protein accession number: AHA93892.1) identified and sequenced by our group was translated to amino acid sequence with the help of online server DNA translation tool on Expasy (http://web.expasy.org/cgi-bin/translate/dna_aa). The translated sequence was subjected for BLASTP [14] for homology similarity. The protein sequences of maximum similarity with our protein (Table 1) were downloaded from SWISSPROT (http://www.expasy.ch), a public domain protein database [15] for further analysis. The proteins were analyzed for their subcellular location by online tool MultiLoc (http://abi.inf.uni-tuebingen.de/Services/MultiLoc), which is an extension of TargetLoc. The physicochemical analysis were calculated by ProtParam tool [16] (http://web.expasy.org/protparam/), which includes theoretical isoelectric point [12], molecular weight, total number of positive and negative residues, extinction coefficient [17], instability index [18], aliphatic index [19], and grand average hydropathy (GRAVY) [20] listed in Table 1.

2. Structure and Functional Annotation

Identification of transmembrane region of proteins was done with SOSUI server (http://harrier.nagahama-i-bio.ac.jp/sosui/cgi-bin/adv_sosui.cgi). Table 2 represents the transmembrane region identified for these chitnase proteins. Disulphide linkages are important in functional characterization and bonds are predicted by the tool CYS_REC (http://sunI.softberry.com/berry.phtml?topic), which helps to identify the positions and total number of cysteines, and predicts the most probable "SS" bond pattern of pairs in the protein sequences which are listed in Table 2. Table 3 represents the output of Prosite [21], a database of protein families and domains, which was recorded in terms of the length of amino residues of protein with specific profiles and patterns.

3. Secondary structure prediction

The self-optimized, neural network [10] based alignment tool SOPMA was used for prediction of secondary structural features [22]. This method calculates the content of α-helix, β-sheets, turns, random coils and extended strands listed in Table 4.

4. Homology Modelling and Evaluation

An attempt was made to model the target protein. The three homology modelling programs Geno3D [23], Swissmodel [24], and Modeller [25] were used for modeling of the

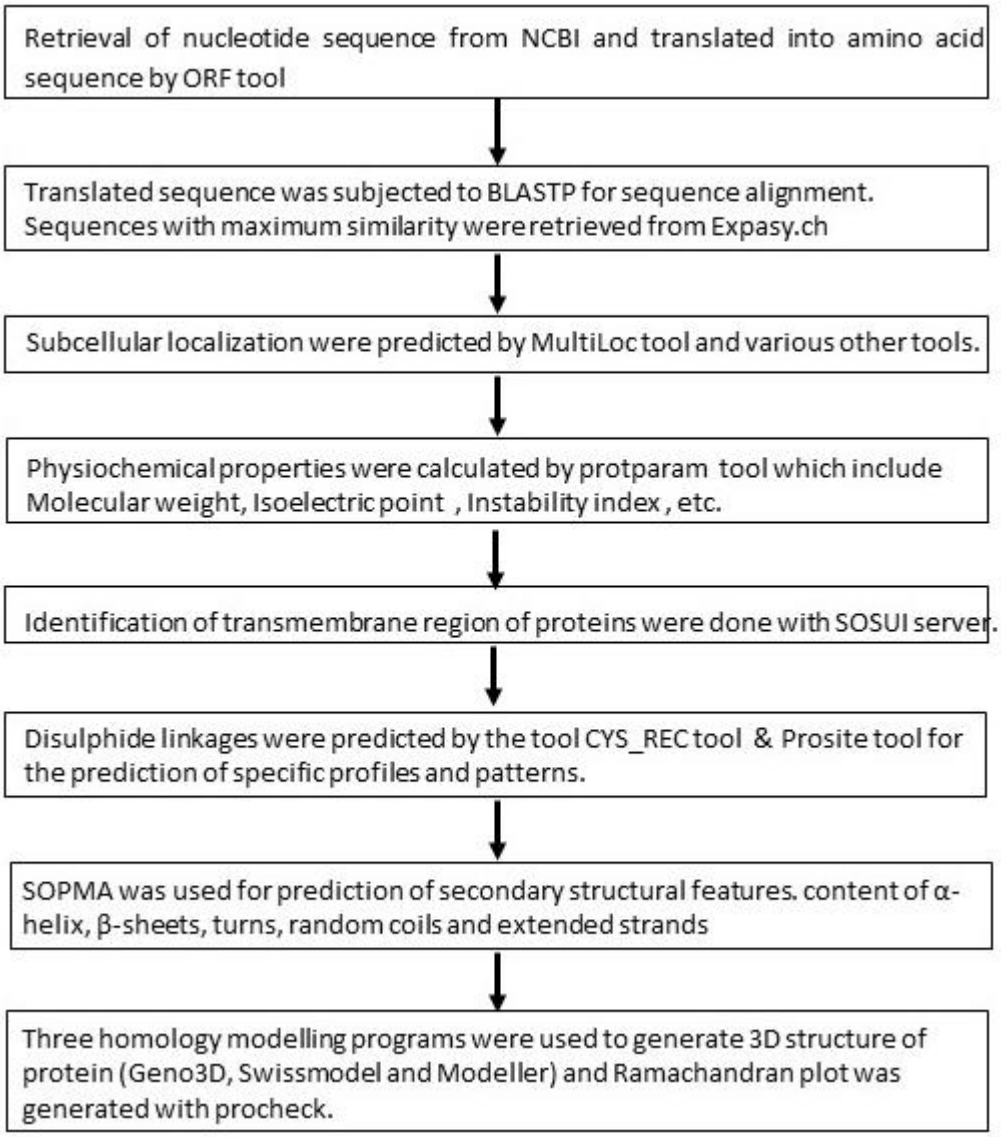

Figure 1. Flow-chart for the prediction of 3-D structure of protein through protein sequence.

three dimensional structure of the protein. The constructed model was evaluated in terms of quality and validation by Ramachandran plot (http://mordred.bioc.cam.ac.uk) to study the overall stereochemical property of protein [26] and Z score by using Qmean [27]. Structural analysis was performed and figures representations were generated with Pymol software.

Results and Discussions

BLAST analysis revealed 85% similarity with 18 sequences listed in Table 1 and further sequences of these proteins were retrieved from the SWISSPROT (http://www.expasy.ch/) and used for further analysis. The subjected protein with accession number AHA93892.1 (283 AA) of Chitinase protein of *Beauveria bassiana* showed maximum similarity with accession number G3JH30 (349 AA) (92 %)

Table 1. Sequence Retrieval and analysis.

Protein sequences considered for the study				Primary structure prediction analysis using Protparam						
Accessions No.	**Species**	**Proteins**	**Length**	**M.wt**	**pI**	**II**	**AI**	**-R**	**+R**	**GRAVY**
AHA93892.1	*Beauveria bassiana*	Chitinase	283	29783.9	5.12	26.57	85.23	22	18	0.043
G3JH30	*Cordeyceps militaris*	Endochitinase	349	36617.9	6.81	28.16	86.22	22	22	0.136
K9JFD0	*Beauveria bassiana*	Chitinase	348	36794.9	5.94	30.14	84.17	26	23	-0.027
E5LEW9	*Beauveria bassiana*	Chit1	348	36784.9	5.94	29.59	84.45	36	23	-0.024
Q8J1Y3	*Beauveria bassiana*	Chit1	348	47644.5	8.59	55.98	59.32	25	23	-0.937
F6MIV5	*Beauveria bassiana*	Chit1	348	36794.9	5.94	30.14	84.45	26	23	-0.026
D1MGZ8	*Beauveria bassiana*	Endochitinase	348	36730.7	5.94	28.69	82.76	26	23	-0.037
E9DX57	*Metarhizium acridum*	Chitinase	345	36580.9	6.41	31.32	88.29	23	22	0.083
D6N0Z5	*Trichoderma longibrachiatum*	Chitinase	322	33754.4	4.6	30.81	87.64	22	14	0.17
A2VEC4	*Hypocrea jecorina*	Chitinase	342	33754.4	4.6	30.81	87.64	22	14	0.17
D6N0Z4	*Trichoderma ghanense*	Chitinase	323	33828.4	4.6	31.46	86.78	22	14	0.168
D6N0Z3	*Trichoderma citrinoviride*	Chitinase	322	33751.3	4.35	29.46	89.16	25	13	0.182
C9WJD1	*Metarhizium anisopliae*	Chi4	282	29669.7	5.3	33.25	86.21	22	18	0.058
Q8NJQ4	*Trichoderma inhamatum*	Chitinase	337	35538.2	4.55	23.88	85.13	24	15	0.103
Q8NJQ5	*Trichoderma harzianum*	Chitinase	337	35478.1	4.55	25.08	85.16	24	15	0.104
E9F7R6	*Metarhizium anisopliae*	Chitinase	345	36500.6	5.61	32.29	85.71	25	22	0.035
A2SW11	*Bionectria ochroleuca*	Endochitinase	348	36547.5	5.49	26.39	86.38	22	18	0.103
D6N0Z1	*Trichoderma croceum*	Chitinase	322	33791.6	4.87	25.54	88.79	18	13	0.212

endochitinase protein of *Cordeyceps militaris* and least similarity (85%) with accession number D6N0Z1 (322 AA) chitinase protein of *Trichoderma croceum*. The identified protein showed similarities with other chitinases identified from *Beauveria*, which suggested multiple isoforms of chitinase gene and large group of chitinases available in this fungus.

1. Primary structure prediction analysis

The physiochemical properties showed that the molecular weight of AHA93892.1 (29783.9 Da)

Table 2. Structure and Functional Annotation.

Accessions no	Species	Gene names	Transmembrane regions identified by SOSUI server			Disulphide (SS) bond pattern of pairs predicted, by CYS_REC
			Transmembrane region	Length	Type of protein	
AHA93892.1	*Beauveria bassiana*	Chitinase	No Transmembrane region	---	Soluble	Cys29-Cys251 Cys33-Cys270
Q8NJQ4	*Trichoderma inhamatum*	Chitinase	TRLLDASFLLLPVI VSTLFGTAS	23	Transmembrane	Cys 29- Cys 247
Q8NJQ5	*Trichoderma harzianum*	Chitinase	TRLLDASFLLLPVI VSTLFGTAS	23	Transmembrane	Cys 29- Cys 247
G3JH30	*Cordeyceps militaris*	Endochitinase	-	-	-	Cys 29-Cys 251 Cys 33- Cys 270
Q8J1Y3	*Beauveria bassiana*	Chit1	-	-	-	Cys 408- Cys 424
A2SW11	*Bionectria ochroleuca*	Endochitinase	-	-	-	Cys 32- Cys 250
D6N0Z1	*Trichoderma croceum*	Chitinase	-	-	-	Cys 30- Cys 267

Table 3. Functional characterization of proteins of chitinase at Prosite.

Gene name	Accession numbers	Motif identified	Profile	Position in the protein	Description
Chitinase	AHA93892.1	Chitinase	Chitinase_18	140 – 148	Chitinases are enzymes that catalyze the hydrolysis of the β-1,4-N-acetyl-D-glucosamine linkages in chitin polymers. From the view point of sequence similarity chitinases belong to either family 18 or 19 in the classification of glycosyl hydrolases. Chitinases of family 18 (also known as classes III or V) groups a variety of proteins. Catalysis of the hydrolysis of (1->4)-beta linkages of N-acetyl-D-glucosamine (glcnac) polymers of chitin and chitodextrins
	K9JFD0	Chitinase	Chitinase_18	155 – 163	
	E9DX57	Chitinase	Chitinase_18	155 – 163	
	D6N0Z5	Chitinase		153 - 161:	
	A2VEC4	Chitinase	Chitinase_18	153 – 161	
	D6N0Z4	Chitinase	Chitinase_18	153 – 161	
	D6N0Z3	Chitinase	Chitinase_18	153 – 161	
	Q8NJQ4	Chitinase	Chitinase_18	152 – 160	
	Q8NJQ5	Chitinase	Chitinase_18	152 – 160	
	E9F7R6	Chitinase	Chitinase_18	153 – 163	
	D6N0Z1	Chitinase	Chitinase_18	153 – 161	
Endochitinase	G3JH30	Endochitinase	Chitinase_18	156 – 164	
	D1MGZ8	Endochitinase	Chitinase_18	155 – 163	
	A2SW11	Endochitinase	Chitinase_18	155 – 163	
Chi4	C9WJD1	Putative chitinase	Chitinase_18	104 – 112	
Chit1	E5LEW9	Chitinase	Chitinase_18	155 – 163	
	Q8J1Y3	Chitinase	Chitinase_18	155 – 163	
	F6MIV5	Chitinase	Chitinase_18	155 – 163	

Table 4. Calculated secondary structure elements by SOPMA.

Proteins/ Secondary structure	Alpha helix	310 helix	Pi helix	Beta bridge	Extended strand	Beta turn	Bend region	Random coil	Ambiguous states	Other states
AHA93892.1	22.89%	0.00%	0.00%	0.00%	18.66%	8.45%	0.00%	50.00%	0.00%	0.00%
G3JH30	31.23%	0.00%	0.00%	0.00%	15.47%	6.30%	0.00%	46.99%	0.00%	0.00%
K9JFD0	26.72%	0.00%	0.00%	0.00%	19.54%	8.33%	0.00%	45.40%	0.00%	0.00%
E5LEW9	29.31%	0.00%	0.00%	0.00%	19.25%	6.32%	0.00%	45.11%	0.00%	0.00%
Q8J1Y3	22.54%	0.00%	0.00%	0.00%	3.99%	2.82%	0.00%	70.66%	0.00%	0.00%
F6MIV5	27.87%	0.00%	0.00%	0.00%	18.68%	6.61%	0.00%	46.84%	0.00%	0.00%
D1MGZ8	27.01%	0.00%	0.00%	0.00%	17.82%	5.75%	0.00%	49.43%	0.00%	0.00%
E9DX57	27.25%	0.00%	0.00%	0.00%	19.13%	6.67%	0.00%	46.96%	0.00%	0.00%
D6N0Z5	29.81%	0.00%	0.00%	0.00%	19.57%	9.32%	0.00%	41.30%	0.00%	0.00%
A2VEC4	26.53%	0.00%	0.00%	0.00%	19.24%	5.25%	0.00%	48.98%	0.00%	0.00%
D6N0Z4	27.24%	0.00%	0.00%	0.00%	17.96%	6.50%	0.00%	48.30%	0.00%	0.00%
D6N0Z3	31.37%	0.00%	0.00%	0.00%	16.15%	7.45%	0.00%	45.03%	0.00%	0.00%
C9WJD1	27.66%	0.00%	0.00%	0.00%	19.15%	11.35%	0.00%	41.84%	0.00%	0.00%
Q8NJQ4	32.34%	0.00%	0.00%	0.00%	18.40%	6.23%	0.00%	43.03%	0.00%	0.00%
Q8NJQ5	24.33%	0.00%	0.00%	0.00%	19.29%	5.93%	0.00%	50.45%	0.00%	0.00%
E9F7R6	28.77%	0.00%	0.00%	0.00%	19.65%	5.96%	0.00%	45.61%	0.00%	0.00%
A2SW11	30.17%	0.00%	0.00%	0.00%	19.25%	7.18%	0.00%	43.39%	0.00%	0.00%
D6N0Z1	27.64%	0.00%	0.00%	0.00%	18.32%	9.32%	0.00%	44.72%	0.00%	0.00%

is nearly equal to C9WJD1 (29669.7 Da). The physiochemical properties showed that molecular weight is highest in Q8J1Y3 (47644.5 Da) and lowest in C9WJD1 (29669.7 Da). Isolelectric point (pI) plays an important role in the stability of proteins and is used to study the net charge on the surface of proteins. The computed pI value of all proteins are < 7 with pH value of 4.6 to 5.96, i.e. the pH value of AHA93892.1 is 5.12, which is similar and close to other proteins, indicating proteins are acidic in nature except Q8J1Y3 with pH of 8.59. The ph range suggested that for chitinase activity, an ionized acidic group and protonated basic group is required for the regulation of chitinase enzyme activity [28, 29]. Instability index helps in the stability of proteins and instabilty index less than 40 considered to be best, as protein is stable at this values [18]. The instability index (II) revealed that all proteins are stable except the Q8J1Y3. The instability value ranges from 22.92 to 55.98. It was concluded that the positive value of Instability index leads to the thermostability of proteins [30]. Aliphatic index infers the stability of protein at wide range of temperature and is defined as the relative volume occupied by aliphatic side chains (alanine, valine, isoleucine, and leucine). AHA93892.1 showed the aliphatic index value of 85.23 which predicted it as thermostable protein like all other listed proteins.

The Grand Average hydropathy (GRAVY) value tells us whether protein is hydrophilic or hydrophobic in nature. The positive value of AHA93892.1 as 0.043 indicated that protein is hydrophobic except accession numbers K9JFD0, D1MGZ8, E5LEW9, Q8J1Y3, and F6MIV5, which is accepted as hydrophilic in nature. The hydrophobic nature of protein was proved as evidence for the stability and secondary structure of proteins and helps in determining the protein folding properties [31].

2. Structure and Functional Annotation

In the prediction of transmembrane regions, the disulfide bonds are important characterstics of functional annotation. The SOSUI server distinguishes between membrane and soluble proteins from amino acid sequences and

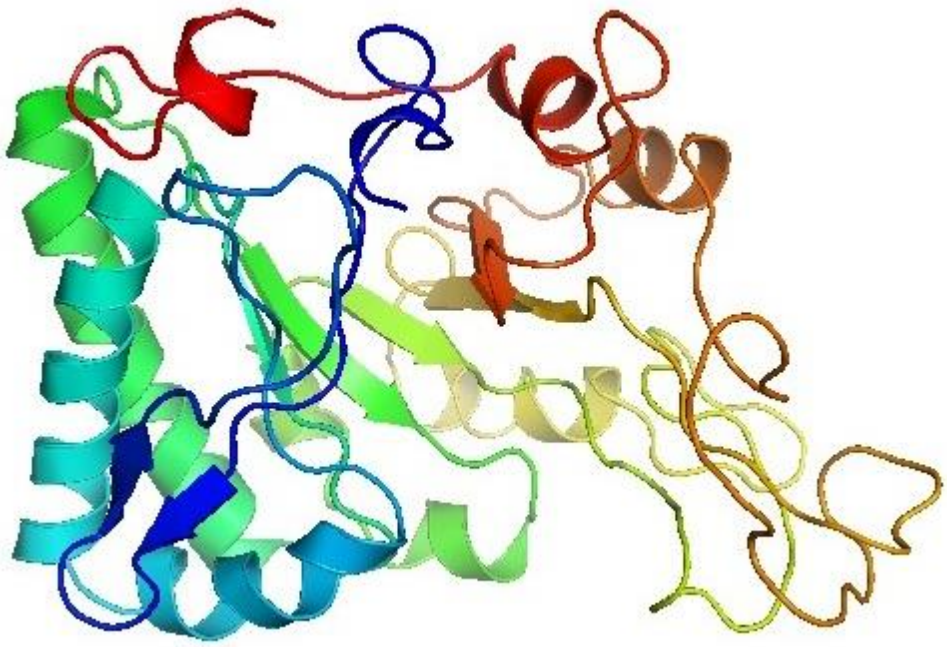

Figure 2. Modelled structure of Chitinase protein AHA93892.1.

predicts the transmembrane helices. The SOSUI server classified AHA93892.1 as soluble protein with water molecules and Q8NJQ4 and Q8NJQ5 as membrane proteins. The server identified one transmembrane region in both proteins. Cys_Rec tool helped in the prediction of disulphide linkages which plays an important role in determining the thermostability of proteins. Possible pairing and patterns with accessions numbers G3JH30, Q8NJQ4, Q8NJQ5, Q8J1Y3, A2SW11, D6N0Z1 contain disulphide linkages along with translated protein. It was calculated that there is no transmembrane region present in AHA93892.1 and no signal peptide was present which showed that protein is secretory in nature.

The domain analysis was conducted by Prosite database which helps in calculating motif length and its Profile. The motif of about 10 – 15 amino acids length belonging to chitinase 18 retrieved which is functionally important for the biological and structural studies. Chitinase enzyme plays an important role in cuticle degradation and the data supports that our protein plays an important role in catalyzing the linkages in chitin hydrolysis of the β-1, 4-N-acetyl-D-glucosamine polymers.

3. Secondary structure prediction

The SOPMA tool predicts the Secondary structure of proteins was predicted by SOPMA tool to classify whether a given amino acid lies in a helix, strand or coil. The default parameters were used for the prediction of secondary structure [32, 33]. The results revealed that random coils dominated among secondary structure elements followed by alpha helix, extended strand and beta turns for all sequences. In case of AHA93892.1, Random coil is 50.00% with alpha strand as 22.89% and it was clearly noticed that beta turns showing very less percentage of conformations below 10%, i.e. 8.45% with extended strand value of 18.66%. The studies of high random coil lead to intrinsic conformations with respect to correlating with low energy conformations and have significance with regard to protein structure prediction and design [34, 35, 36].

Table 5. Ramachandran plot calculation with Procheck program.

Servers	Modeller
Residues in the most favored Region	85.4%
Residues in additionally allowed region	12.7%
Residues in generously allowed region	1.4%
Residues in disallowed region	0.5%

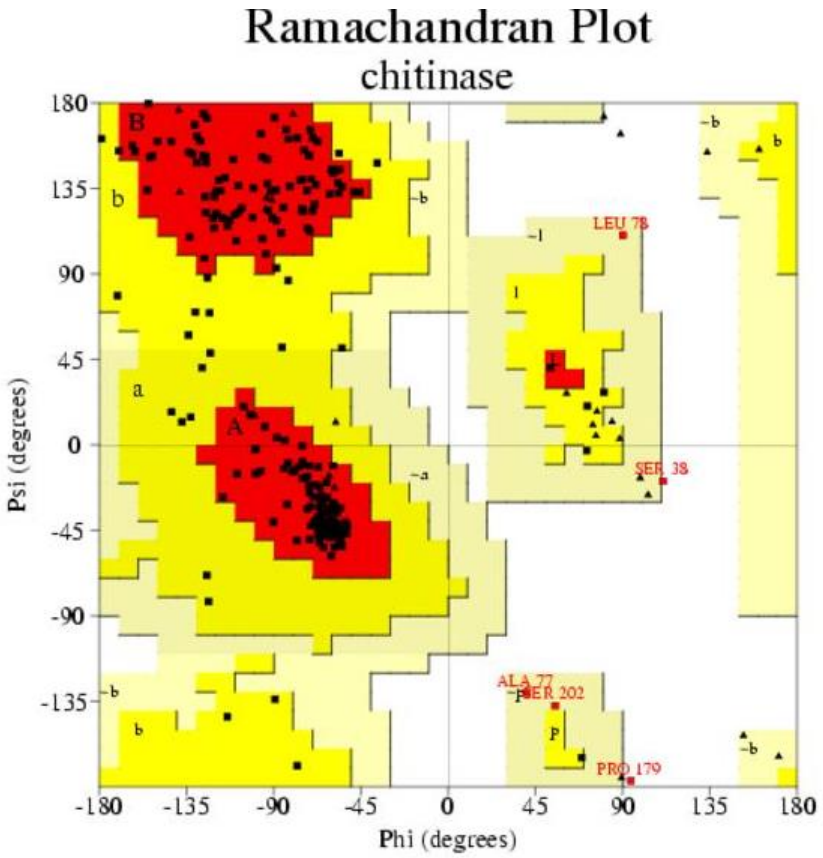

Figure 3. Ramachandran plot showing the phi -psi torsion angles for all residues in most stable Predicted 3-D conformation of chitinase protein AHA93892.1 (except those at the chain termini).

4. 3D Modelling and evaluation

Geno 3D, Swiss Model and Modeller were used to construct the 3D model of AHA93892.1 (figure 2). The modelled structure was helpful in studying the function of proteins and its active sites. PDB id 1w9v was selected as template with 52.1 identity with query sequence. The structure was validated by using Qmean server with its Z-score as -0.67, which means 67% similarity with template. The structure and quality of model was analyzed by Ramachandran plot (figure 3). RAMPAGE analysis showed that only 1.4% residues in outer region, 12.7% allowed regions and 85.4% in favored regions (table 5). The values indicated that the modelled structure is reliable and of good quality. The results obtained signified new perspectives and importance to

biological or computational assays and bioinformatics analyses to improve biological models [37]. The most important part of this research was to predict 3D structure of chitinase gene through an insilico analysis which is not yet available in PDB. This hypothetical structure can be used as model structure for other DNA or protein sequences and can be verified through NMR and X-ray Crystallography. Moreover, this pipeline is very easy for the new researchers to predict.

Conclusion

This study presents a comprehensive in silico assessment and structure prediction of protein AHA93892.1. In this study 18 sequences were selected to acquire an understanding of its physical and chemical properties along with its functional and structural levels. Primary structure analysis reveals that AHA93892.1 is acidic in nature. Protein is stable in nature as it have hydrophilic interactions and disulphide linkages. The 3D structure was more acceptable by modeller with Qmean score of -0.65 with 65% similarity. The model was verified by Ramachandran plot. The data calculated will be helpful in formulating their uses in industries and protein structure by NMR and X-ray crystallography. The derived properties and structure will provide insights into functional analysis of this protein enabling researchers to design *in vivo* assays.

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