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HOMOLOGY MODELING AND STRUCTURAL STUDIES OF CELL WALL BINDING PROTEIN CHITINASE FROM NEUROSPORA CRASSA

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ABSTRACT

Back ground: Proteins serve a variety of functions within the cells. Some proteins are involved in structural support and movement others in enzymatic activity, and still others in interaction with the outside world. Some of the bioinformatics tools are very helpful to know the structure and functions of the proteins within the cell. **Methods:** The work reported here contains, the stereo chemical quality of the protein model was checked by using *in silico* analysis with PROCHECK and QMEAN servers. **Results:** In this paper the protein with 8 metal binding sites shows highest metal binding probability for the metal namely calcium in sites 1,4,5,6 & 7 with the metal probability of **0.398702, 0.627722,0.691595 0.627722 , & 0.516149 ;** magnesium in sites 1,2 & 8 with the probability of **0.351869,0.571190 & 0.571190** and zinc in sites 3 & 7 with the probability of **0.643216 & 0.316166** were shown. **Conclusion:** The result of the study may be a guiding point for further investigations on GH18_ Chitinase protein and metal binding sites.

KEYWORDS: cell wall proteins, chitinase, metal binding sites, homology modeling, *Neurospora crassa.*

INTRODUCTION

The overall architecture of the cell wall is raised by the interaction of different cell wall components.[1] Changes in osmotic pressure and environmental stresses were balanced by the dynamic functioning of the fungal cell wall.[2] Several lung diseases including allergic asthma, allergic bronchopulmonary aspergilllosis (ABPA), aspergilloma**,** and invasive aspergillosis (IA) were known to be treated by the etiological agents extracted from *Aspergillus sps*. Over 90% of cases of *Aspergillus*-related diseases are caused by *A.* fumigates.[3] Filamentous fungi have many different chitinases belonging to GH family 18. B-glucan is the main structural scaffold of the important cell wall component in fungi. Therefore, fungal cell wall degradation and remodeling was performed by the action of chitinases which decompose exogenous chitin in fungi.[4] Growth, metabolism and differentiation in yeast and fungi were tightly regulated by Ca^{2+}. If acts as a second messenger for transducing intracellular signals responsible for cellular functions.[5] In the present study, the model filamentous fungus, *Neurospora crassa* was selected for the isolation of cell wall proteins and their characterization.

METHODS

2D gel analysis of cell wall proteins

2D PAGE technique have been utilized for the proteomic examination of cell wall proteins.

BLAST P, multiple sequence alignment and phylogenetic tree construction

The amino acid sequence obtained by MALDI-TOF/MS analysis of chitinase isolated from the cell walls of *Neurosporca crassa*. The protein sequences are scanned by using the BLAST P algorithm we can obtain the homologous protein sequences from the available protein sequences of various organisms. Template search with Blast and HHBlits has been performed against the SWISS-MODEL template library. The target sequence was searched with BLAST[6] against the primary amino acid sequence contained in the SMTL. Overall 254 templates were found. A total of 18 templates were found. An initial HHblits profile has been built using the procedure outlined in Remmert, et al,[7] followed by 1iteration of HHblits against NR20. The obtained profile has then been searched against all profiles of the SMTL. A total of 236 templates were found (Table 1). For each identified template, the template's quality has been predicted from features of the target-template alignment. The templates with the highest quality have then been selected for model building. Models are built based on the target-template alignment using ProModIII. Coordinates which are conserved between the target and the template are copied from the template to the model. Insertions and deletions are remodeled using a fragment library. Side chains are then rebuilt. Finally, the geometry of the resulting model is regularized by using a force field. In case loop modelling with ProMod3 fails, an alternative model is built with PROMOD-II[8] or

MODELLER.[9] Phylogenetic tree was then constructed using phylogeny.fr (http://www.phylogeny.fr/) to determine the evolutionary relationships[10,11,12]

Secondary structure prediction

Secondary structure of GH18_ Chitinase family protein was predicted using SOPMA (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopma.html) tool in Expasy.

Homology modeling

The sequence of *Neurospora crassa* was downloaded from the universal protein resource (Uniprot KB) (http://www.uniprot.org/)[13] (entry ID: V5IPZ1). The suitable template for homology modeling was identified through searching *Neurospora crassa* on PDB using the BLAST P algorithm.[14] The 3D structure of *Neurospora crassa* was downloaded from PDB (PDB ID: 4tx6.1.A) as the template structure.

Model validation

The quality of the homology model was validated by assessing the stereo chemical quality of the model using Ramachandran plot obtained from the RAMPAGE (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php) server.[15] Verify 3D[16] and ERRAT[17] were used to assess the amino acid environment from the UCLA-DOE server (http://www.doe-mbi.ucla.edu/services).

Model Quality Estimation

The global and per-residue model quality has been assessed using the QMEAN scoring function.[18] For improved performance, weights of the individual QMEAN terms have been trained specifically for SWISS-MODEL.

Determination of Metal binding sites

In the majority of the metalloproteins, the residues involved in metal binding come close together in the tertiary structure to form the binding site, but are dispersed along the amino acid sequence. In this paper the protein shows highest metal binding probability for metal namely calcium, magnesium and zinc**.**

RESULTS AND DISCUSSION

2D gel analysis of cell wall proteins

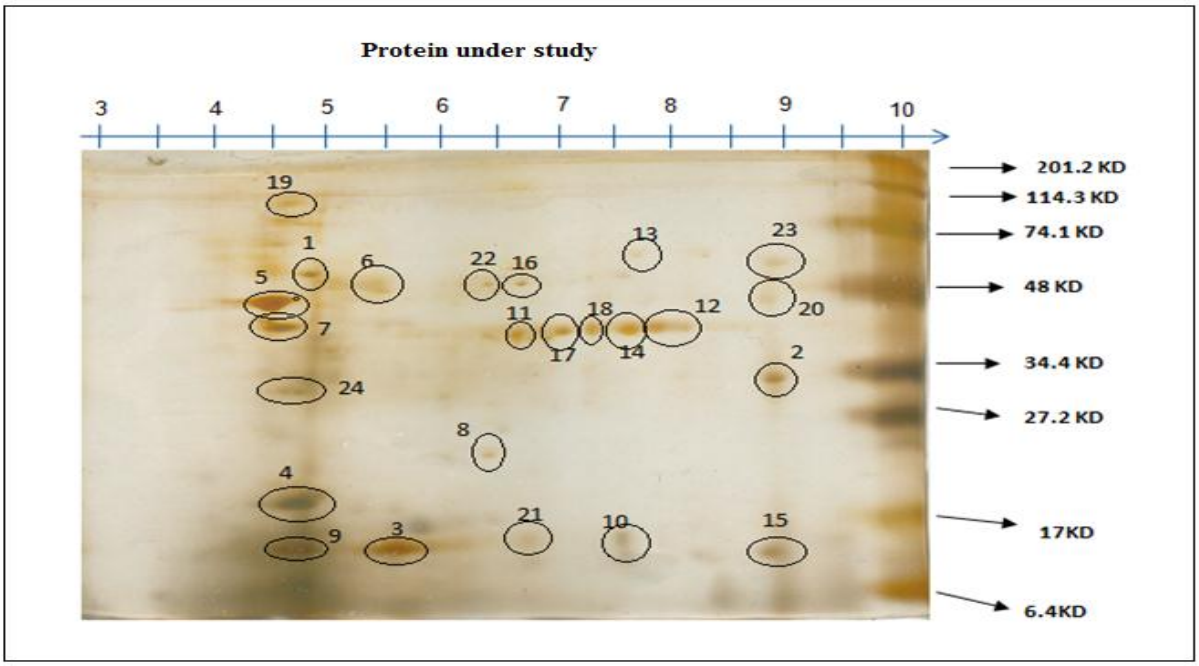

Fig.1: 2D gel analysis showing protein under study

Scanning of protein sequence databases using BLAST P with the sequence obtained by MALDI-TOF/MS analysis of the purified lipase revealed that the protein is a hypothetical protein from *Neurospora crassa* with an entry ID: V5IPZ1 was shown in (Fig. 2). A phylogram constructed based on multiple sequence alignment using phylogeny.fr revealed that GH18_ Chitinase was closely related to a conserved hypothetical protein from *Neurospora tetrasperma* was shown in (Fig.3).

```
>tr|V5IPZ1|V5IPZ1_NEUCR Chitinase-1 OS=Neurospora crassa
(strain ATCC 24698 / 74-OR23-1A / CBS 708.71 / DSM 1257 / FGSC
987) GN=chit-1 PE=4 SV=1
MHKSLVAAAVLAATARAHTLTEAKVNVYWGQRGDARLRDHCDQANFDYVTIGAHCDATYY
TNGTTSGHMNGKCSVVASDIKHCQEKGKKVLLSLDGVEHMGSRFSLSSEAKAEEFASFLW
GAFGPYDAKWTGPRPFDFAGHRVSVDGFNLDGELKLNGAGEGAYAAMAKKLRELYNGNAE
LLLTAAPGCSLDDVKMKAIFDNAQFDALFIQFYNNPSCEAASASGFNYLQWEKAIAAGMS
KEAKLFIGLAGASDAAGSGYIEPLEAAALINTYKTRSSFGGAMVLDAFRGQTLANGMTFL
DVINTVVSSAEAVDLSSEFCEDESALPKVPSVTEGSAGGFFTVADPSAITSGPVVLPSGG
SSVLPTGGSSEDEVCEDENVIPKVPSVTDGAHEVNPTIVDTSIITSVPVALPSGDRSEII
GGSPATEDDVCEGEIMTEDPLRSGVVPSGVMPTGALPSGVLPSGDRSDIIGAIPSGSIPL
GSAPSGLVPSGSIPLGSAPSGLVPSGSIPLGSAPSGLVPSGAVPSGSIPLGSVPSGDRSD
IIGAIPSGSIPLGSVPSGVVPSGDRSEIIGSPADEDDVCEGEDPVRSGLVPSGAVPTGDR
SDIIGAIPSGSIPLGSAPSGLVPSGAVPSGDRSEIIGAIPSGSIPLGSVPSGAVPSGDRS
EIIGAIPSGSIPLGSAPSGAVPSGLVPSGIVPSGAVPSGDRSEIIGSPADEDDYCEGEIT
PEDPVRSGLVPSGIVPTGAVPTGLVPSGGVIGSTVPGGVIPSGVAPGGILPSGVVSSVGS
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GLPSGAIASGVIPSGVIPSGIVPSGVVSSIGSKVTGPIDGDNVALPSVGLPSGDLPSGVV
PSGVVPSGVLSSVGSKITGPINGHNVAVPSAGVPSGVVPSGILSNVGSKVTGPIDGNNVV
PTGILPSGSVPSGVVSSVVSMVTADPINGDKIADPSAVTAPAEWTTSTIYATTTSTITSC
APEVTDCPAKIGQVTTVTVPIGVTVCPVTATETAARAPTGIFTSVPAESIPAGFTTSTVY
STTTSTIASCAPEMTDCAGKIGQVTTVIVPVGVTVCPVTATETAATETAARAPTGIMTSV
PVESIPAGYTTSTVYSTATSTIASCAPEMTDCAGKIGQVTTIIVPVGVTVCPITEAFPPA
TSVPAAPAAVPTGAASVPAVSAVPTGGAGVPAVSAPAVPTGGAGVPAVSTPVVPSGVAGV
PAVSAPAVPSGGADVPAVSAPAVPSGGADVPAVPSNGAPGAGVNKVATSSVVSVSSMPFT
TITVAKPAASAPGAPGAPGAGVPAESAAVPAGGAPAVPSAVNAPVVPSGAVPTGTGVSVA
PSSAASTYSMPAPPAQTEPVTGSEPSEVPVTAGAGRNVVAMGVPALVAALVLAL
```

Fig. 2: Search for the *Neurospora crassa* sequence in UNIPORT KB revealed that the sequence is GH18_ Chitinase protein from *Neurospora tetrasperma.*

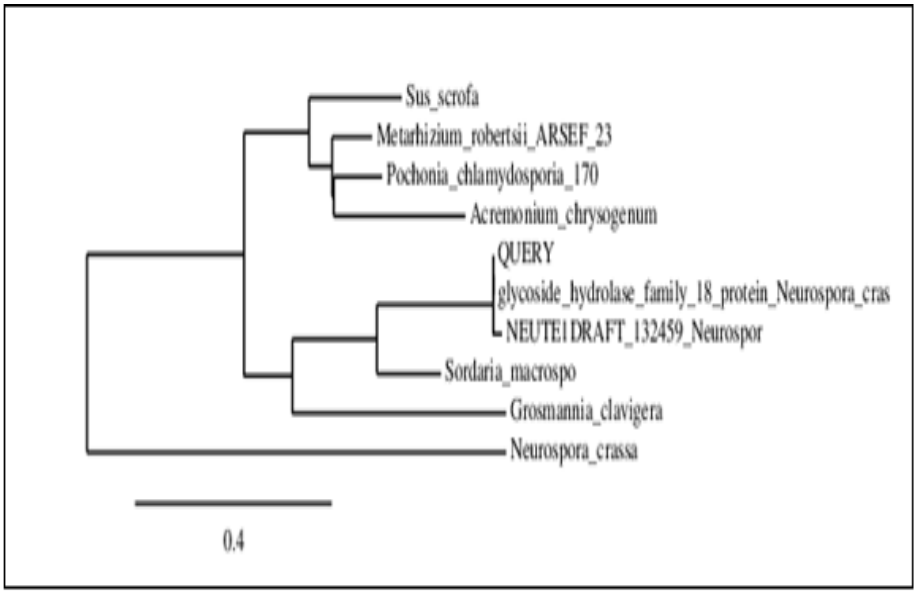
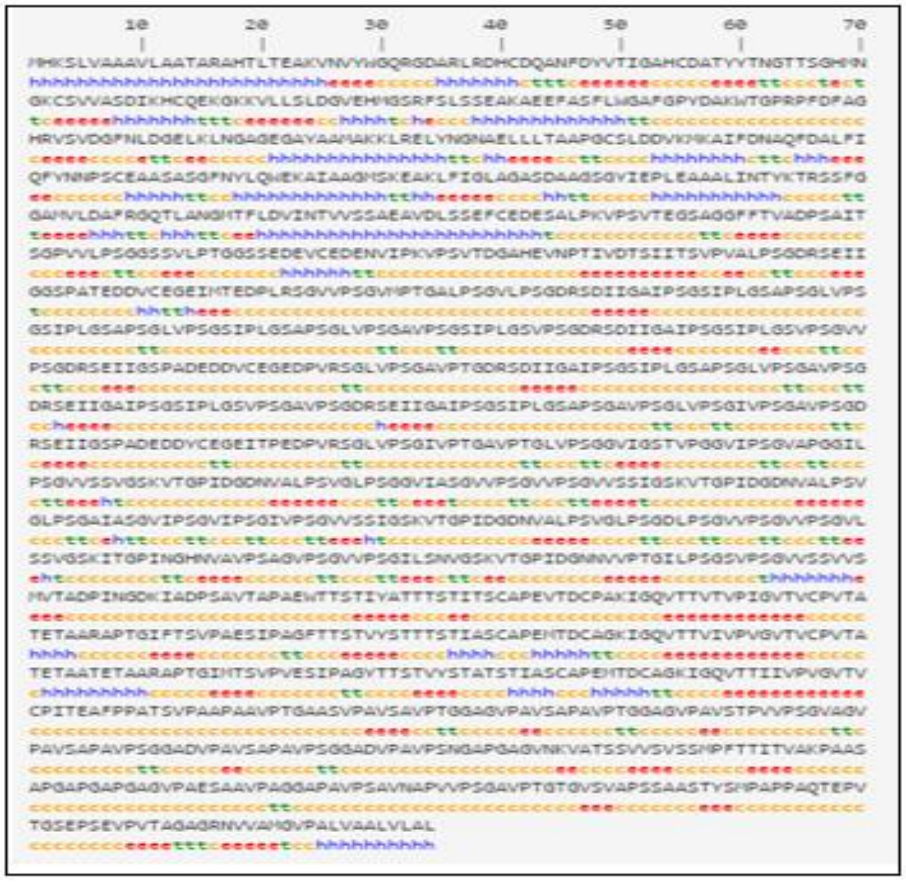


Fig. 3: Phylogenetic tree of *Neurospora crassa*, A phylogenetic tree of *Neurospora crassa*, constructed using phylogeny.fr showing that *Neurospora crassa* is closely related to Sordaria macrospo.

Secondary structure of the target protein was predicted by using SOPMA tool in Expasy **(Fig.4).** The results indicate that GH18_ Chitinase has 14.71%, α-helix thus making it stable for homology modeling[19].



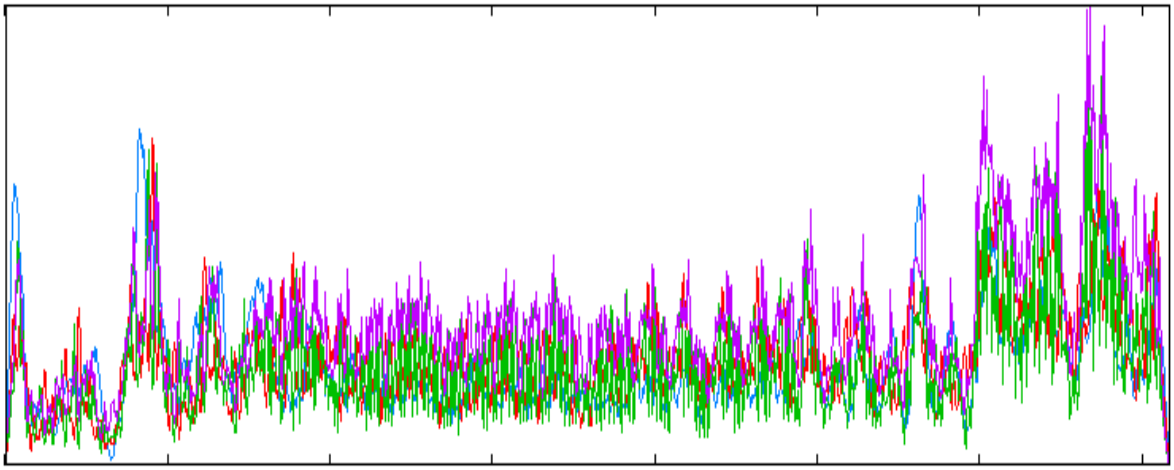


Fig. 4: (a-b) secondary structure of Neurospora crassa. (a) Sequence length: 1434; Alpha helix(Hh): 211 is 14.71%, Extended strand (Ee) :264 is 18.41%, Beta turn (Tt) : 145 is 10.11%, Random coil (Cc): 814 is 56.76% and (b) Distribution of Secondary structure elements of *Neurospora crassa.* Blue line-Alpha Helix, Red-Extended strand, Green-beta turn, Orange-random coil.

The first step in homology modeling involves identification of a suitable template. This was met by performing a BLAST P search against known protein structures deposited in PDB. The studies of Rost[20] and Yang and Honig[21] demonstrated that 3D structures will be similar if the sequence identity between target and template proteins is higher than 25%. Generally, a target which shares a sequence similarity of 30% or more to an experimentally solved protein structure (template) can only be employed for homology modeling. The crystal structure of Chitinase Chi A1 (4tx6.1.A) with a sequence identity of 36.23% to the target sequence was selected based on BLAST P search against PDB database **(Table 1).** Overall 254 templates were found **(Table 1).** The sequence alignment between the template (4tx6.1.A) and the target was shown in **(Fig.5).**

Table 1: Blast P. search against PDB and Target-Template alignment, (a) BLAST results of target sequence of GH18_ Chitinase against PDB for the identification of template for homology modeling.

Template	Seq Identity	Oligo-state	Found by	Method	Resolution	Seq Similarity	Coverage	Description
4tx6.1.A	36.23	monomer	HHblits	X-ray	1.90Å	0.38	0.19	Class III chitinase ChiA1
2xtk.1.A	36.36	monomer	HHblits	X-ray	2.00Å	0.38	0.19	CLASS III CHITINASE CHIA1
2xuc.3.A	36.36	monomer	HHblits	X-ray	2.30Å	0.38	0.19	CHITINASE
2xvn.1.A	36.36	monomer	HHblits	X-ray	2.35Å	0.38	0.19	ASPERGILLUS FUMIGATUS CHITINASE A1
2xvn.3.A	36.36	monomer	HHblits	X-ray	2.35Å	0.38	0.19	ASPERGILLUS FUMIGATUS CHITINASE A1
2uy4.1.A	39.39	monomer	BLAST	X-ray	1.75Å	0.40	0.18	ENDOCHITINASE
2uy4.1.A	36.57	monomer	HHblits	X-ray	1.75Å	0.38	0.19	ENDOCHITINASE
2xvn.1.A	41.43	monomer	BLAST	X-ray	2.35Å	0.41	0.18	ASPERGILLUS FUMIGATUS CHITINASE A1
2xvn.3.A	41.43	monomer	BLAST	X-ray	2.35Å	0.41	0.18	ASPERGILLUS FUMIGATUS CHITINASE A1
4tx6.1.A	41.43	monomer	BLAST	X-ray	1.90Å	0.41	0.18	Class III chitinase ChiA1
2xtk.1.A	41.43	monomer	BLAST	X-ray	2.00Å	0.41	0.18	CLASS III CHITINASE CHIA1
2xuc.3.A	41.43	monomer	BLAST	X-ray	2.30Å	0.41	0.18	CHITINASE
4toq.1.A	33.72	monomer	HHblits	X-ray	1.60Å	0.37	0.18	Class III chitinase
1llo.1.A	29.92	monomer	HHblits	X-ray	1.85Å	0.36	0.18	HEVAMINE
2gsj.1.A	31.03	monomer	HHblits	X-ray	1.73Å	0.36	0.18	protein PPL-2
1cnv.1.A	25.46	monomer	HHblits	X-ray	1.65Å	0.33	0.19	CONCANAVALIN B
1kqz.1.A	29.17	monomer	HHblits	X-ray	1.92Å	0.35	0.18	Hevamine A
1kr1.1.A	29.55	monomer	HHblits	X-ray	2.00Å	0.35	0.18	Hevamine A
1kr0.1.A	29.17	monomer	HHblits	X-ray	1.92Å	0.35	0.18	Hevamine A
3mu7.1.A	31.30	monomer	HHblits	X-ray	1.29Å	0.35	0.18	xylanase and alpha-amylase inhibitor protein
3hu7.1.A	31.80	monomer	HHblits	X-ray	2.00Å	0.35	0.18	Haementhin
3o9n.1.A	31.54	monomer	HHblits	X-ray	2.40Å	0.35	0.18	Haementhin
4toq.1.A	37.40	monomer	BLAST	X-ray	1.60Å	0.39	0.17	Class III chitinase
3hu7.1.A	38.17	monomer	BLAST	X-ray	2.00Å	0.39	0.17	Haementhin
3mu7.1.A	37.86	monomer	BLAST	X-ray	1.29Å	0.39	0.17	xylanase and alpha-amylase inhibitor protein
3o9n.1.A	37.76	monomer	BLAST	X-ray	2.40Å	0.39	0.17	Haementhin
1llo.1.A	37.24	monomer	BLAST	X-ray	1.85Å	0.40	0.17	HEVAMINE
1te1.1.A	25.29	hetero-oligomer	HHblits	X-ray	2.50Å	0.33	0.18	xylanase inhibitor protein I

Template	Seq Identity	Oligo-state	Found by	Method	Resolution	Seq Similarity	Coverage	Description
1ta3.1.A	25.29	hetero-oligomer	HHblits	X-ray	1.70Å	0.33	0.18	xylanase inhibitor protein I
1kr1.1.A	35.98	monomer	BLAST	X-ray	2.00Å	0.39	0.17	Hevamine A
1kr0.1.A	35.56	monomer	BLAST	X-ray	1.92Å	0.39	0.17	Hevamine A
1kqz.1.A	35.56	monomer	BLAST	X-ray	1.92Å	0.39	0.17	Hevamine A
4tx8.1.A	21.13	monomer	HHblits	X-ray	2.17Å	0.31	0.18	Probable chitinase A
3n13.1.A	20.38	monomer	HHblits	X-ray	1.70Å	0.30	0.18	Chitinase A
3n17.1.A	20.15	monomer	HHblits	X-ray	1.20Å	0.30	0.18	Chitinase A
3n18.1.A	19.70	monomer	HHblits	X-ray	1.60Å	0.30	0.18	Chitinase A
3n15.1.A	20.31	monomer	HHblits	X-ray	1.94Å	0.30	0.18	Chitinase A
3ebv.1.A	21.40	monomer	HHblits	X-ray	1.50Å	0.32	0.18	Chinitase A
3n11.1.A	20.77	monomer	HHblits	X-ray	1.35Å	0.31	0.18	Chitinase A
5hbf.1.A	16.10	monomer	HHblits	X-ray	1.95Å	0.28	0.19	Chitotriosidase-1
5hbf.2.A	16.10	monomer	HHblits	X-ray	1.95Å	0.28	0.19	Chitotriosidase-1
3ian.1.A	17.56	monomer	HHblits	X-ray	1.75Å	0.30	0.18	Chitinase
4hmc.1.A	18.56	homo-dimer	HHblits	X-ray	2.10Å	0.29	0.18	Chitinase 60
4hme.1.A	18.56	homo-dimer	HHblits	X-ray	2.07Å	0.29	0.18	Chitinase 60
4b15.1.A	20.87	homo-dimer	HHblits	X-ray	1.49Å	0.32	0.18	CHITINASE LIKE LECTIN
4w5z.1.A	18.94	monomer	HHblits	X-ray	1.32Å	0.29	0.18	Chitinase 60
4mb3.1.A	18.32	monomer	HHblits	X-ray	1.55Å	0.29	0.18	Chitinase 60
2gsj.1.A	39.11	monomer	BLAST	X-ray	1.73Å	0.41	0.16	protein PPL-2
4axn.1.A	17.37	monomer	HHblits	X-ray	1.68Å	0.29	0.18	CHITINASE C1
4ac1.1.A	18.40	monomer	HHblits	X-ray	1.30Å	0.30	0.17	ENDO-N-ACETYL-BETA-D-GLUCOSAMINIDASE
2y8v.1.A	15.29	homo-dimer	HHblits	X-ray	1.99Å	0.28	0.18	CLASS III CHITINASE, PUTATIVE
4rl3.1.A	17.46	monomer	HHblits	X-ray	1.57Å	0.29	0.18	Chitinase A
3wij.1.A	15.56	monomer	HHblits	X-ray	1.30Å	0.27	0.18	Chitinase A
4mnj.1.A	15.56	monomer	HHblits	X-ray	1.58Å	0.27	0.18	Chitinase A
3fnd.1.A	14.40	monomer	HHblits	X-ray	1.90Å	0.28	0.17	Chitinase
3co4.1.A	14.40	monomer	HHblits	X-ray	1.92Å	0.28	0.17	Chitinase
4q6t.1.A	15.20	monomer	HHblits	X-ray	1.40Å	0.27	0.17	Glycosyl hydrolase, family 18

Template	Seq Identity	Oligo-state	Found by	Method	Resolution	Seq Similarity	Coverage	Description
1c91.1.A	15.06	monomer	HHblits	X-ray	2.10Å	0.28	0.17	ENDO-BETA-N-ACETYLGLUCOSAMINIDASE H
1eom.1.A	17.80	monomer	HHblits	X-ray	2.10Å	0.29	0.16	ENDO-BETA-N-ACETYLGLUCOSAMINIDASE F3
1c3f.1.A	15.13	monomer	HHblits	X-ray	2.10Å	0.28	0.17	ENDO-BETA-N-ACETYLGLUCOSAMINIDASE H
1c92.1.A	15.13	monomer	HHblits	X-ray	2.10Å	0.28	0.17	ENDO-BETA-N-ACETYLGLUCOSAMINIDASE H
1edt.1.A	16.46	homo-dimer	HHblits	X-ray	1.90Å	0.28	0.17	ENDO-BETA-N-ACETYLGLUCOSAMINIDASE H, ENDO H
1c93.1.A	16.95	monomer	HHblits	X-ray	2.10Å	0.28	0.16	ENDO-BETA-N-ACETYLGLUCOSAMINIDASE H
3w4r.1.A	15.04	monomer	HHblits	X-ray	1.70Å	0.27	0.16	Chitinase
1kfw.1.A	18.92	monomer	HHblits	X-ray	1.74Å	0.29	0.15	chitinase B
1wno.1.A	18.89	monomer	HHblits	X-ray	2.10Å	0.30	0.15	Chitinase
4cd8.1.A	15.63	monomer	HHblits	X-ray	1.47Å	0.27	0.16	ENDO-BETA-1,4-MANNANASE
1cnv.1.A	33.51	monomer	BLAST	X-ray	1.65Å	0.38	0.14	CONCANAVALIN B
2ebn.1.A	18.98	monomer	HHblits	X-ray	2.00Å	0.30	0.15	ENDO-BETA-N-ACETYLGLUCOSAMINIDASE F1
2a3c.1.A	20.09	monomer	HHblits	X-ray	2.07Å	0.30	0.15	Chitinase
5d8w.1.A	10.36	monomer	HHblits	X-ray	2.86Å	0.27	0.15	Endoglucanase
3g6m.1.A	18.57	monomer	HHblits	X-ray	1.65Å	0.30	0.15	Chitinase
1wb0.1.A	16.90	monomer	HHblits	X-ray	1.65Å	0.29	0.15	CHITOTRIOSIDASE 1
1d2k.1.A	18.10	monomer	HHblits	X-ray	2.20Å	0.29	0.15	CHITINASE 1
1ll6.1.A	17.06	monomer	HHblits	X-ray	2.80Å	0.29	0.15	CHITINASE 1
3qok.1.A	17.06	monomer	HHblits	X-ray	2.60Å	0.29	0.15	Putative chitinase II
3alf.1.A	17.13	monomer	HHblits	X-ray	1.20Å	0.27	0.15	Chitinase, class V
2dsk.1.A	12.04	homo-dimer	HHblits	X-ray	1.50Å	0.27	0.15	Chitinase
5fip.1.A	10.50	monomer	HHblits	X-ray	1.88Å	0.26	0.15	GH5 CELLULASE
4w5u.1.A	20.00	monomer	HHblits	X-ray	2.77Å	0.29	0.15	Chitinase
3qr3.1.A	8.72	monomer	HHblits	X-ray	2.05Å	0.26	0.15	Endoglucanase EG-II
3a4x.1.A	11.11	monomer	HHblits	X-ray	1.76Å	0.26	0.15	Chitinase
4u3a.1.A	9.01	monomer	HHblits	X-ray	2.42Å	0.24	0.15	Endoglucanase H
1owq.1.A	16.98	monomer	HHblits	X-ray	2.00Å	0.28	0.15	signal processing protein
1syt.1.A	16.59	monomer	HHblits	X-ray	2.60Å	0.28	0.15	BP40
1zl1.1.A	16.59	monomer	HHblits	X-ray	3.50Å	0.28	0.15	Chitinase-3 like protein 1

Template	Seq Identity	Oligo-state	Found by	Method	Resolution	Seq Similarity	Coverage	Description
4s06.1.A	17.06	monomer	HHblits	X-ray	1.49Å	0.28	0.15	Chitinase-3-like protein 1
4u5i.1.A	9.55	monomer	HHblits	X-ray	2.50Å	0.24	0.15	Endoglucanase H
4u5i.2.A	9.55	monomer	HHblits	X-ray	2.50Å	0.24	0.15	Endoglucanase H
4lgx.1.A	16.19	monomer	HHblits	X-ray	1.49Å	0.28	0.15	Glycoside hydrolase family 18
3alg.1.A	16.90	monomer	HHblits	X-ray	1.80Å	0.27	0.15	Chitinase, class V
3aqu.1.A	15.42	monomer	HHblits	X-ray	2.01Å	0.26	0.15	At4g19810
2pi6.1.A	16.11	monomer	HHblits	X-ray	1.65Å	0.27	0.15	Chitinase-3-like protein 1
3a4w.1.A	11.27	monomer	HHblits	X-ray	1.80Å	0.27	0.15	Chitinase
4s19.1.A	16.67	monomer	HHblits	X-ray	1.65Å	0.27	0.15	Chitinase-3-like protein 1
1ll7.1.A	17.24	monomer	HHblits	X-ray	2.00Å	0.29	0.14	CHITINASE 1
1nar.1.A	12.32	monomer	HHblits	X-ray	1.80Å	0.26	0.15	NARBONIN
4uri.1.A	19.80	monomer	HHblits	X-ray	1.85Å	0.29	0.14	CHITINASE-RELATED AGGLUTININ
4mnm.1.A	17.07	monomer	HHblits	X-ray	1.80Å	0.27	0.14	Chitinase A
2ybu.1.A	13.30	monomer	HHblits	X-ray	2.25Å	0.28	0.14	ACIDIC MAMMALIAN CHITINASE
3wh9.1.A	8.61	monomer	HHblits	X-ray	1.57Å	0.25	0.15	Endo-beta-1,4-mannanase
3cz8.1.A	17.17	homo-dimer	HHblits	X-ray	2.20Å	0.29	0.14	Putative sporulation-specific glycosylase ydhD
3sim.1.A	15.66	monomer	HHblits	X-ray	2.10Å	0.28	0.14	Protein, Family 18 Chitinase
4wiw.1.A	14.43	monomer	HHblits	X-ray	2.64Å	0.26	0.14	Glycoside hydrolase family 18
1c8x.1.A	18.18	monomer	HHblits	X-ray	2.00Å	0.30	0.13	ENDO-BETA-N-ACETYLGLUCOSAMINIDASE H
4w84.1.A	12.82	monomer	HHblits	X-ray	1.79Å	0.26	0.14	Xyloglucan-specific endo-beta-1,4-glucanase
1c90.1.A	15.34	monomer	HHblits	X-ray	2.10Å	0.28	0.13	ENDO-BETA-N-ACETYLGLUCOSAMINIDASE H
1g0c.1.A	17.28	monomer	HHblits	X-ray	1.90Å	0.27	0.13	ENDOGLUCANASE
4w85.1.A	12.37	monomer	HHblits	X-ray	1.92Å	0.26	0.14	Xyloglucan-specific endo-beta-1,4-glucanase
4w7u.1.A	10.47	monomer	HHblits	X-ray	1.48Å	0.26	0.13	Cellulase
1c8y.1.A	17.39	monomer	HHblits	X-ray	2.00Å	0.28	0.13	ENDO-BETA-N-ACETYLGLUCOSAMINIDASE H
1qno.1.A	12.70	monomer	HHblits	X-ray	2.00Å	0.26	0.13	ENDO-1,4-B-D-MANNANASE
3civ.1.A	16.67	monomer	HHblits	X-ray	1.90Å	0.27	0.13	Endo-beta-1,4-mannanase
1h1n.1.A	12.90	monomer	HHblits	X-ray	1.12Å	0.27	0.13	ENDO TYPE CELLULASE ENGI
1gzj.1.A	12.30	monomer	HHblits	X-ray	1.62Å	0.26	0.13	ENDO TYPE CELLULASE ENGI

Template	Seq Identity	Oligo-state	Found by	Method	Resolution	Seq Similarity	Coverage	Description
3wfl.1.A	11.17	monomer	HHblits	X-ray	1.60Å	0.26	0.13	beta-mannanase
4jkk.1.A	9.84	homo-tetramer	HHblits	X-ray	2.59Å	0.24	0.13	Beta-glucuronidase
4jkl.1.A	9.84	homo-tetramer	HHblits	X-ray	2.29Å	0.24	0.13	Beta-glucuronidase
4ee9.1.A	12.04	monomer	HHblits	X-ray	1.38Å	0.24	0.13	Endoglucanase
4xzw.1.A	12.02	monomer	HHblits	X-ray	1.50Å	0.27	0.13	endo-glucanase chimera C10
3wsu.1.A	18.23	monomer	HHblits	X-ray	1.60Å	0.28	0.13	Beta-mannanase
5jh8.1.A	14.21	monomer	HHblits	X-ray	1.02Å	0.27	0.13	Probable chitinase
4y7e.1.A	17.78	monomer	HHblits	X-ray	1.50Å	0.28	0.13	Endoglucanase
4pmy.1.A	9.68	monomer	HHblits	X-ray	1.60Å	0.25	0.13	Xylanase
4xzb.1.A	13.81	monomer	HHblits	X-ray	1.62Å	0.27	0.13	CelA
2whj.1.A	13.33	monomer	HHblits	X-ray	1.78Å	0.27	0.13	BETA-MANNANASE
2whl.1.A	12.92	monomer	HHblits	X-ray	1.40Å	0.27	0.12	BETA-MANNANASE
4nzc.1.A	17.50	monomer	HHblits	X-ray	1.45Å	0.28	0.11	Glycoside hydrolase family 18
2wly.1.A	17.31	monomer	HHblits	X-ray	2.40Å	0.30	0.11	CHITINASE A
3wkz.1.A	15.82	monomer	HHblits	X-ray	2.00Å	0.29	0.11	Chitinase
1ffq.1.A	18.06	monomer	HHblits	X-ray	1.90Å	0.30	0.11	CHITINASE A
1ctn.1.A	18.06	homo-dimer	HHblits	X-ray	2.30Å	0.30	0.11	CHITINASE A
1ehn.1.A	17.42	monomer	HHblits	X-ray	1.90Å	0.30	0.11	CHITINASE A
3wqw.1.A	17.09	monomer	HHblits	X-ray	2.00Å	0.28	0.11	Chitinase
3fy1.1.A	16.67	monomer	HHblits	X-ray	1.70Å	0.29	0.11	Acidic mammalian chitinase
3fxy.1.A	16.67	monomer	HHblits	X-ray	2.00Å	0.29	0.11	Acidic mammalian chitinase
3fxy.2.A	16.67	monomer	HHblits	X-ray	2.00Å	0.29	0.11	Acidic mammalian chitinase
2wk2.1.A	17.42	monomer	HHblits	X-ray	2.05Å	0.30	0.11	CHITINASE A
1k9t.1.A	18.06	homo-dimer	HHblits	X-ray	1.80Å	0.30	0.11	CHITINASE A
1ffr.1.A	17.42	monomer	HHblits	X-ray	1.80Å	0.30	0.11	CHITINASE A
1eib.1.A	17.42	monomer	HHblits	X-ray	1.80Å	0.30	0.11	CHITINASE A
1vf8.1.A	16.03	monomer	HHblits	X-ray	1.31Å	0.29	0.11	secretory protein
3wl0.1.A	16.46	monomer	HHblits	X-ray	2.20Å	0.28	0.11	Chitinase
1x6l.1.A	18.83	monomer	HHblits	X-ray	1.90Å	0.30	0.11	Chitinase A

Template	Seq Identity	Oligo-state	Found by	Method	Resolution	Seq Similarity	Coverage	Description
1nh6.1.A	17.53	monomer	HHblits	X-ray	2.05Å	0.30	0.11	chitinase A
1hkk.1.A	16.03	monomer	HHblits	X-ray	1.85Å	0.28	0.11	CHITOTRIOSIDASE-1
3oa5.1.A	15.92	monomer	HHblits	X-ray	1.74Å	0.28	0.11	Chi1
4p8u.1.A	16.67	monomer	HHblits	X-ray	2.40Å	0.28	0.11	Chitinase-3-like protein 2
1hjv.1.A	17.31	homo-tetramer	HHblits	X-ray	2.75Å	0.28	0.11	CHITINASE-3 LIKE PROTEIN 1
1hjx.1.D	17.31	homo-tetramer	HHblits	X-ray	1.85Å	0.28	0.11	CHITINASE-3 LIKE PROTEIN 1
1hjw.2.A	17.31	monomer	HHblits	X-ray	2.30Å	0.28	0.11	CHITINASE-3 LIKE PROTEIN 1
1zb5.1.A	16.67	monomer	HHblits	X-ray	2.45Å	0.28	0.11	signal processing protein
1ljy.1.A	17.31	monomer	HHblits	X-ray	2.90Å	0.28	0.11	MGP-40
4ay1.1.A	16.67	monomer	HHblits	X-ray	1.95Å	0.28	0.11	CHITINASE-3-LIKE PROTEIN 2
2esc.1.A	16.67	monomer	HHblits	X-ray	2.10Å	0.28	0.11	Chitinase-3-like protein 1
2aos.1.A	18.06	monomer	HHblits	X-ray	2.90Å	0.28	0.11	Signaling protein from goat, SPG-40
4ptm.1.A	18.18	monomer	HHblits	X-ray	1.70Å	0.29	0.11	Glycoside hydrolase family 18
4a5q.1.A	15.38	monomer	HHblits	EM	17.00Å	0.28	0.11	CHI1
2qf8.1.A	17.53	monomer	HHblits	X-ray	2.80Å	0.28	0.11	Chitinase-3-like protein 1
1tfv.1.A	17.53	monomer	HHblits	X-ray	2.90Å	0.28	0.11	mammary gland protein 40
4nsb.1.A	17.53	monomer	HHblits	X-ray	3.05Å	0.28	0.11	Chitinase-3-like protein 1
3bxw.1.A	16.11	homo-dimer	HHblits	X-ray	2.70Å	0.28	0.10	Chitinase domain-containing protein 1
4s3k.1.A	14.57	monomer	HHblits	X-ray	1.70Å	0.27	0.11	Spore germination protein YaaH
3zmr.1.A	14.97	monomer	HHblits	X-ray	1.43Å	0.28	0.10	CELLULASE (GLYCOSYL HYDROLASE FAMILY 5)
3zmr.2.A	14.97	monomer	HHblits	X-ray	1.43Å	0.28	0.10	CELLULASE (GLYCOSYL HYDROLASE FAMILY 5)
4s3j.1.A	12.67	monomer	HHblits	X-ray	1.60Å	0.26	0.10	Cortical-lytic enzyme
4s3j.2.A	12.67	monomer	HHblits	X-ray	1.60Å	0.26	0.10	Cortical-lytic enzyme
4dws.1.A	14.86	monomer	HHblits	X-ray	1.80Å	0.27	0.10	Chi2
4nuy.1.A	13.19	monomer	HHblits	X-ray	2.61Å	0.27	0.10	Endo-beta-N-acetylglucosaminidase F2
4nuz.1.A	12.50	monomer	HHblits	X-ray	1.91Å	0.27	0.10	Endo-beta-N-acetylglucosaminidase F2
3ndy.1.A	12.98	hetero-oligomer	HHblits	X-ray	2.10Å	0.27	0.09	Endoglucanase D
4lyp.1.A	9.02	monomer	HHblits	X-ray	1.28Å	0.25	0.09	Exo-beta-1,4-mannosidase
1itx.1.A	24.59	monomer	HHblits	X-ray	1.10Å	0.31	0.09	Glycosyl Hydrolase

Template	Seq Identity	Oligo-state	Found by	Method	Resolution	Seq Similarity	Coverage	Description
1cec.1.A	13.28	monomer	HHblits	X-ray	2.15Å	0.28	0.09	ENDOGLUCANASE CELC
3wd0.1.A	19.01	monomer	HHblits	X-ray	1.70Å	0.30	0.08	Chitinase B
1goi.1.A	19.01	homo-dimer	HHblits	X-ray	1.45Å	0.30	0.08	CHITINASE B
1goi.1.B	19.01	homo-dimer	HHblits	X-ray	1.45Å	0.30	0.08	CHITINASE B
1ceo.1.A	12.80	monomer	HHblits	X-ray	1.90Å	0.27	0.09	CELLULASE CELC
1h0i.1.A	18.18	monomer	HHblits	X-ray	2.00Å	0.30	0.08	CHITINASE B
1h0i.2.A	18.18	monomer	HHblits	X-ray	2.00Å	0.30	0.08	CHITINASE B
1gpf.1.A	18.18	homo-dimer	HHblits	X-ray	1.85Å	0.30	0.08	CHITINASE B
1gpf.1.B	18.18	homo-dimer	HHblits	X-ray	1.85Å	0.30	0.08	CHITINASE B
1te1.1.A	39.62	hetero-oligomer	BLAST	X-ray	2.50Å	0.40	0.07	xylanase inhibitor protein I
1ta3.1.A	39.62	hetero-oligomer	BLAST	X-ray	1.70Å	0.40	0.07	xylanase inhibitor protein I
3w0k.1.A	14.52	monomer	HHblits	X-ray	1.60Å	0.28	0.09	Bifunctional endomannanase/endoglucanase
1e6z.1.A	18.18	monomer	HHblits	X-ray	1.99Å	0.30	0.08	CHITINASE B
1e6z.2.A	18.18	monomer	HHblits	X-ray	1.99Å	0.30	0.08	CHITINASE B
4nf7.1.A	14.29	monomer	HHblits	X-ray	2.11Å	0.27	0.09	Endo-1,4-beta-glucanase Cel5C
1e6n.1.A	17.36	monomer	HHblits	X-ray	2.25Å	0.30	0.08	CHITINASE B
1e6n.2.A	17.36	monomer	HHblits	X-ray	2.25Å	0.30	0.08	CHITINASE B
1ogg.1.A	17.36	homo-dimer	HHblits	X-ray	1.97Å	0.30	0.08	CHITINASE B
1ogg.1.B	17.36	homo-dimer	HHblits	X-ray	1.97Å	0.30	0.08	CHITINASE B
1ogb.1.B	17.36	homo-dimer	HHblits	X-ray	1.85Å	0.30	0.08	CHITINASE B
1ur9.1.A	17.36	homo-dimer	HHblits	X-ray	1.80Å	0.30	0.08	CHITINASE B
1ur9.1.B	17.36	homo-dimer	HHblits	X-ray	1.80Å	0.30	0.08	CHITINASE B
3arq.1.A	20.17	monomer	HHblits	X-ray	1.50Å	0.30	0.08	Chitinase A
3b8s.1.A	20.17	monomer	HHblits	X-ray	2.00Å	0.30	0.08	Chitinase A
3b8s.2.A	20.17	monomer	HHblits	X-ray	2.00Å	0.30	0.08	Chitinase A
3arr.1.A	20.17	monomer	HHblits	X-ray	1.65Å	0.30	0.08	Chitinase A
3ars.1.A	20.17	monomer	HHblits	X-ray	2.45Å	0.30	0.08	Chitinase A
1jnd.1.A	16.39	monomer	HHblits	X-ray	1.30Å	0.28	0.09	Imaginal disc growth factor-2
4wjx.1.A	17.36	monomer	HHblits	X-ray	1.00Å	0.29	0.08	Chitotriosidase-1

Template	Seq Identity	Oligo-state	Found by	Method	Resolution	Seq Similarity	Coverage	Description
3b9e.1.A	18.33	monomer	HHblits	X-ray	1.70Å	0.30	0.08	Chitinase A
1lq0.1.A	17.36	monomer	HHblits	X-ray	2.20Å	0.29	0.08	CHITOTRIOSIDASE
1hkj.1.A	17.36	monomer	HHblits	X-ray	2.60Å	0.29	0.08	CHITOTRIOSIDASE
1guv.1.A	17.36	monomer	HHblits	X-ray	2.35Å	0.29	0.08	CHITOTRIOSIDASE
4zn2.1.A	14.40	monomer	HHblits	X-ray	2.00Å	0.26	0.09	PslG
3aof.1.A	13.11	monomer	HHblits	X-ray	1.29Å	0.27	0.09	Endoglucanase
3aof.2.A	13.11	monomer	HHblits	X-ray	1.29Å	0.27	0.09	Endoglucanase
3mmw.1.A	14.05	monomer	HHblits	X-ray	1.85Å	0.28	0.08	Endoglucanase
3amc.1.A	14.05	monomer	HHblits	X-ray	1.40Å	0.28	0.08	Endoglucanase
3azr.1.A	13.22	monomer	HHblits	X-ray	1.71Å	0.27	0.08	Endoglucanase
3azt.3.A	13.22	monomer	HHblits	X-ray	1.80Å	0.27	0.08	Endoglucanase
3poh.1.A	20.51	monomer	HHblits	X-ray	1.55Å	0.30	0.08	Endo-beta-N-acetylglucosaminidase F1
3rjy.1.A	11.57	monomer	HHblits	X-ray	2.20Å	0.27	0.08	Endoglucanase FnCel5A
3amg.1.A	12.40	monomer	HHblits	X-ray	2.40Å	0.27	0.08	Endoglucanase
3amg.2.A	12.40	monomer	HHblits	X-ray	2.40Å	0.27	0.08	Endoglucanase
3nco.1.A	11.57	monomer	HHblits	X-ray	1.50Å	0.27	0.08	Endoglucanase FnCel5A
4ff5.1.A	22.32	monomer	HHblits	X-ray	1.86Å	0.30	0.08	Glycosyl hydrolase 25
1h4p.1.A	13.59	monomer	HHblits	X-ray	1.75Å	0.26	0.07	GLUCAN 1,3-BETA-GLUCOSIDASE I/II
2jep.1.A	13.00	monomer	HHblits	X-ray	1.40Å	0.28	0.07	XYLOGLUCANASE
2jeq.1.A	13.00	monomer	HHblits	X-ray	1.94Å	0.28	0.07	XYLOGLUCANASE
1edg.1.A	15.00	monomer	HHblits	X-ray	1.60Å	0.27	0.07	ENDOGLUCANASE A
4x0v.1.A	9.80	monomer	HHblits	X-ray	2.80Å	0.25	0.07	Beta-1,3-1,4-glucanase
4x0v.3.A	9.80	monomer	HHblits	X-ray	2.80Å	0.25	0.07	Beta-1,3-1,4-glucanase
3pz9.1.A	15.63	monomer	HHblits	X-ray	1.42Å	0.28	0.07	Mannan endo-1,4-beta-mannosidase. Glycosyl Hydrolase family 5
4lx4.1.A	17.05	monomer	HHblits	X-ray	1.56Å	0.30	0.06	Endoglucanase(Endo-1,4-beta-glucanase)protein
3ayr.1.A	19.32	monomer	HHblits	X-ray	2.00Å	0.29	0.06	Endoglucanase
5g56.1.A	11.24	monomer	HHblits	X-ray	2.64Å	0.28	0.06	CARBOHYDRATE BINDING FAMILY 6
4im4.1.A	13.48	monomer	HHblits	X-ray	2.42Å	0.26	0.06	Endoglucanase E
5byw.1.A	11.76	monomer	HHblits	X-ray	2.60Å	0.26	0.06	Endoglucanase H

Template	Seq Identity	Oligo-state	Found by	Method	Resolution	Seq Similarity	Coverage	Description
3fhq.1.A	19.48	homo-dimer	HHblits	X-ray	2.45Å	0.28	0.05	Endo-beta-N-acetylglucosaminidase
2vtf.1.A	18.18	monomer	HHblits	X-ray	1.79Å	0.28	0.05	ENDO-BETA-N-ACETYLGLUCOSAMINIDASE
3fha.1.A	18.42	homo-dimer	HHblits	X-ray	2.00Å	0.27	0.05	Endo-beta-N-acetylglucosaminidase
4oou.1.A	10.00	monomer	HHblits	X-ray	2.36Å	0.25	0.05	Beta-1,4-mannanase
4qp0.1.A	8.47	monomer	HHblits	X-ray	2.30Å	0.25	0.04	Endo-beta-mannanase
1hjq.1.A	12.07	monomer	HHblits	X-ray	2.55Å	0.26	0.04	BETA-1,4-GALACTANASE
1edt.1.A	9.26	homo-dimer	HHblits	X-ray	1.90Å	0.27	0.04	ENDO-BETA-N-ACETYLGLUCOSAMINIDASE H, ENDO H
5eyu.1.A	12.50	homo-tetramer	HHblits	X-ray	1.72Å	0.25	0.03	Betaine aldehyde dehydrogenase
5ez4.1.A	12.50	homo-tetramer	HHblits	X-ray	2.11Å	0.25	0.03	Betaine aldehyde dehydrogenase
4mpb.1.A	12.50	homo-dimer	HHblits	X-ray	1.70Å	0.25	0.03	Betaine aldehyde dehydrogenase
4mpb.2.A	12.50	homo-dimer	HHblits	X-ray	1.70Å	0.25	0.03	Betaine aldehyde dehydrogenase
3efv.1.A	17.78	homo-tetramer	HHblits	X-ray	1.90Å	0.27	0.03	Putative succinate-semialdehyde dehydrogenase
2imp.1.A	13.04	homo-tetramer	HHblits	X-ray	2.10Å	0.25	0.03	Lactaldehyde dehydrogenase
4m29.1.A	15.38	monomer	HHblits	X-ray	2.10Å	0.26	0.02	Beta-xylosidase
4ekj.1.A	15.38	monomer	HHblits	X-ray	2.50Å	0.26	0.02	Beta-xylosidase
4tuf.1.A	20.00	monomer	HHblits	X-ray	2.70Å	0.28	0.02	Major extracellular endoglucanase
3ziz.1.A	13.04	monomer	HHblits	X-ray	1.40Å	0.30	0.02	GH5 ENDO-BETA-1,4-MANNANASE
1uhv.1.A	8.33	homo-tetramer	HHblits	X-ray	2.10Å	0.26	0.02	Beta-xylosidase
3m07.1.A	17.39	monomer	HHblits	X-ray	1.40Å	0.27	0.02	Putative alpha amylase
4awe.1.A	18.18	monomer	HHblits	X-ray	1.40Å	0.30	0.02	ENDO-BETA-D-1,4-MANNANASE
2gft.1.A	18.18	monomer	HHblits	X-ray	2.30Å	0.28	0.02	Glycosyl Hydrolase Family 53
1ur0.1.A	18.18	monomer	HHblits	X-ray	2.50Å	0.28	0.02	GALACTANASE
1vbu.1.A	11.11	monomer	HHblits	X-ray	1.80Å	0.28	0.01	endo-1,4-beta-xylanase B

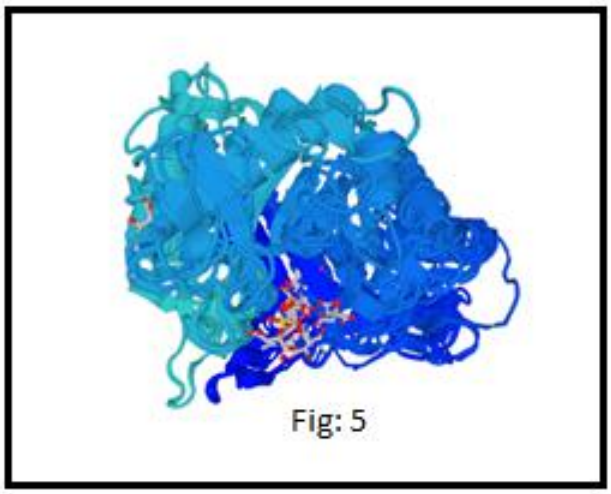


Fig. 5: Alignment between target (GH18_Chitinase) and template Chitinase Chi A1 (4tx6.1.A) Overall 254 templates were found.

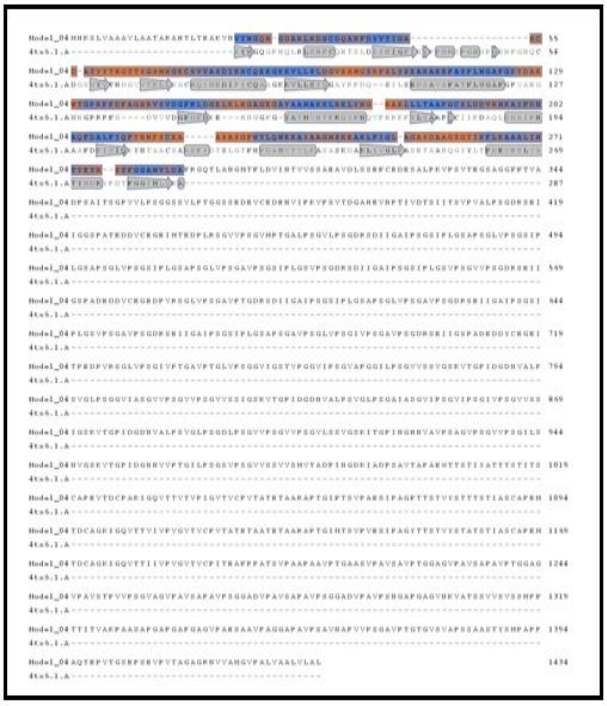


Fig. 6: Target-Template Alignment showed overall 254 templates out of them the selected template Chitinase Chi A1 (4tx6.1.A) were used to build this model.

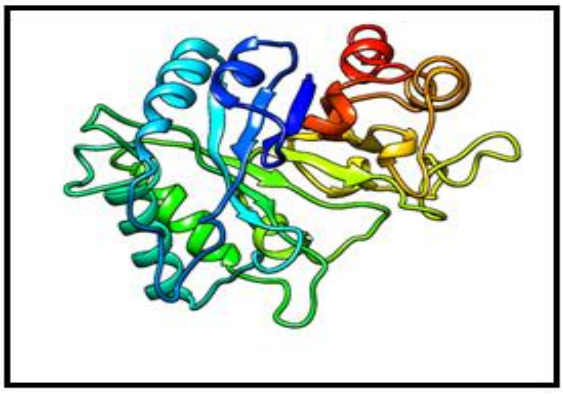


Fig. 7: Modeled protein image of protein obtained from Target-template alignment.

The stereo chemical quality of the 3D model was validated by Ramachandran plot using RAMPAGE server. **Fig.8** and **Table 2** shows that around 13.0% residues were present in the allowed regions, 85.9% residues in the favored region and only 1.1% residues were present in the outlier region indicating that the quality of the model was good.

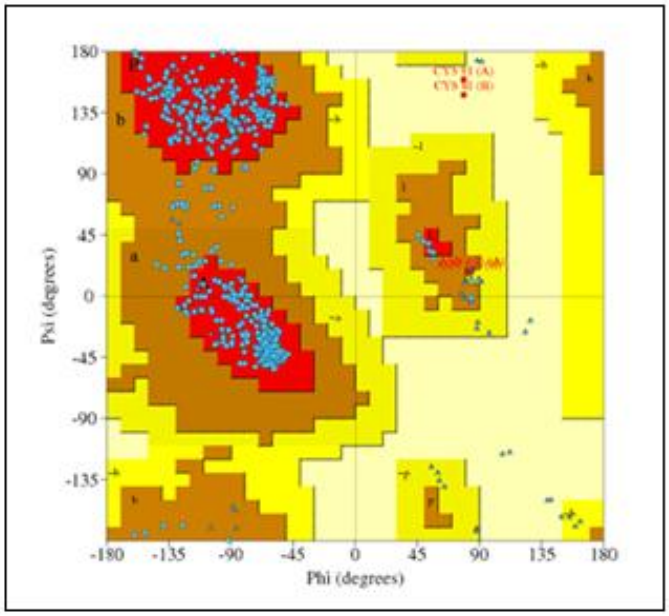


Fig. 8: Ramachandran plot statistics for modeled protein

Table 2: Ramachandran plot statistics GH18 Chitinase homology model using RAMPAGE server.

Amino acid residues and regions (%)	Percentage
Residues in most favored regions [A,B,L]	89.2 %
Residues in the allowed [a, b, l, p]	10.1 %
Residues in the outlier regions	0.4 %

The quality of estimated model is based on the QMEAN scoring function were normalized with respect to the number of interactions[18]. The QMEAN score of the model was 0.58 and the Z-score was -2.83, which was very close to the value of 0 and this shows the fine quality of the model[22, 23] because the estimated reliability of the model was expected to be in between 0 and 1 and this could be inferred from the density plot for QMEAN scores of the reference set **(Fig.9A).** A comparison between normalized QMEAN score (0.40) and protein size in non-redundant set of PDB structures in the plot revealed different set of Z-values for different parameters such as C-beta interactions (0.18), interactions between all atoms (-0.47), solvation (-0.65), torsion (-2.79) **(Fig.9B).**

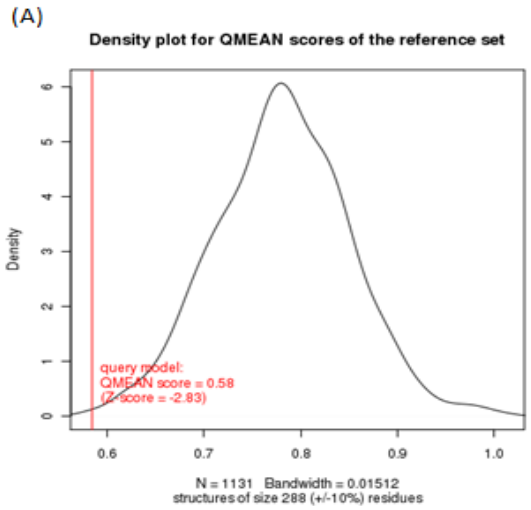
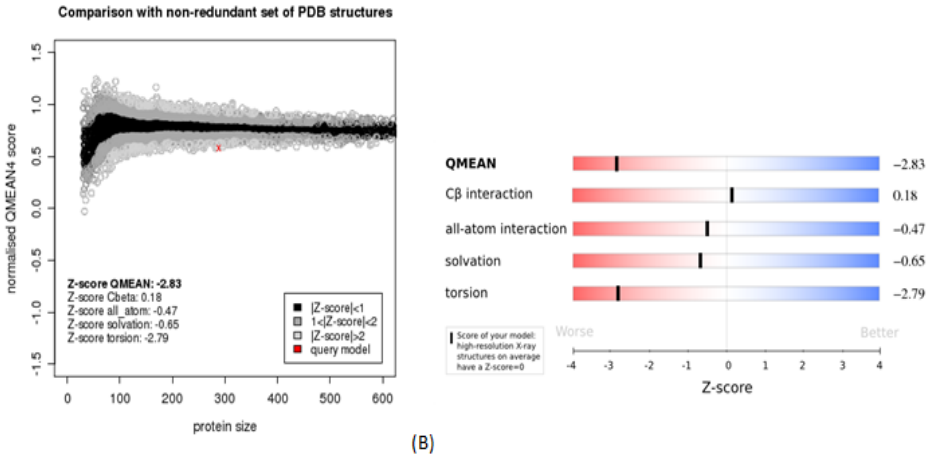


Fig. 9: (A) The density plot for QMEAN showing the value of Z-score and QMEAN score (B) plot showing the QMEAN value as well as Z-score.



(B)

Metalloproteins are proteins capable of binding one or more metal ions, which may be required for their biological function, for regulation of their activities or for structural purposes. Metal-binding properties remain difficult to predict as well as to investigate experimentally at the whole-proteome level. Consequently, the current knowledge about metalloproteins is only partial. In this paper the protein with 8 metal binding sites shows highest metal binding probability for the metal namely calcium in sites 1, 4, 5, 6 & 7 with the metal probability of 0.398702 , 0.627722 , 0.691595, 0.627722 , & 0.516149 ; magnesium in sites 1, 2 & 8 with the probability of 0.351869 , 0.571190 & 0.571190 and zinc in sites 3 & 7 with the probability of 0.643216 & 0.316166 were showed in (Table 3).

Table 3: Metal binding site prediction of chitinase proteins using CHED server.

Protein	Metal Binding Sites	Metal binding probability								Metal Binding Pockets	
		CA	CO	CU	FE	MG	MN	NI	ZN	Amino acids	Position
Cell wall protein Chitinase from N.crassa	Site-1	**0.398702**	0.013877	0.016652	0.052384	**0.351869**	0.039895	0.007979	0.118644	S*	107
										A	112
										A	159
										A*	163
										M	167
	Site-2	0.127722	0.016751	0.020101	0.063233	**0.571190**	0.048157	0.009631	0.143216	R	172
										G*	177
										N*	178
										A	179
	Site-3	0.127722	0.016751	0.020101	0.063233	0.071190	0.048157	0.009631	**0.643216**	D*	34
										R	36
										R	38
										D*	39
										T	292
	Site-4	**0.627722**	0.016751	0.020101	0.063233	0.071190	0.048157	0.009631	0.143216	A*	267
										N*	271
										T*	272
										T	275
	Site-5	**0.691595**	0.013877	0.016652	0.052384	0.058975	0.039895	0.007979	0.118644	V*	306
										G	238
										K	241
										E*	242
										A	243
										K	244
										S*	277
										S*	278
	Site-6	**0.627722**	0.016751	0.020101	0.063233	0.071190	0.048157	0.009631	0.143216	S*	66
										G*	67
										H*	68
										N	70
										F	115

	Site-7	**0.516149**	0.012262	0.014715	0.046290	0.052114	0.035254	0.007051	**0.316166**	A	128
										H*	141
										V	143
										S	144
										N*	178
										A*	179
										E*	180
										L	181
	Site-8	0.127722	0.016751	0.020101	0.063233	**0.571190**	0.048157	0.009631	0.143216	V	302
										I	303
										N*	304
										T*	305
										V	307

Significant statement: In this paper the authors have reported the first successful solution to the challenging problem of predicting protein metal binding geometry from sequence alone. Learning with structured outputs is a fairly difficult task and in spite of the fact that several methodologies have been proposed, no single general approach can effectively solve every possible application problem.

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