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3D Structure Prediction and Validation of Novel Exo-beta-1, 3-Glucanase from *Psychroflexus torquis* ATCC700755/ACAM623

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ABSTRACT

Exo-beta-1,3-glucanase (EC 3.2.1.58) successively hydrolyse O-glycosidic linkage of beta-1,3-linked glucans from their nonreducing terminus to subsequently yield glucose. In this paper, we present the 3D structure prediction and validation of novel bacterial exo-beta-1, 3-glucanase of GH family 17 from Psychroflexus torquis ATCC700755/ACAM623-a psychrophilic bacteria. This is the first attempt to model this enzyme with a GH family 17 fold in bacteria. All known exo-beta-1, 3-glucanase of GH family 17 are of plant and fungal origin. Validation of the stereochemistry of the predicted model using the Rampage sever for Ramachandran plot indicates 97.1% of residues located in favoured and allowed regions while the remaining are in outlier region. This model also obtained an ERRAT score of 93.17% which is well above the 50% critical value for a good model. As a way of further evaluation, we generated profile files of the model and template by the 'asses_dope' command of MODELLER 9.15 programme. These profile files were used in gnuplot 4.6 ploting software to produce a comparative plot of Discrete Optimized Protein Energy (DOPE) per residue of the template and model. This model obtained an overall DOPE value better than the template.

Key words: *Psychroflexus torques, O-glycosidic, Exo-beta-1,3-glucanase*

INTRODUCTION

The increasing use of psychrotolerant enzymes in many industrial and biotechnological applications due to their ability to make processes run at lower temperature is evident[1]. This implies an efficient use in energy consequently, lowering production costs. Psychrotolerant enzymes are applied in environmental biotechnology, production of biofuels, fine chemical synthesis and in food and feed, detergent, pharmaceutical and medical industries[2].

In nature, cellulose, hemicelluloses and lignin are the major components of plant cell walls. Cellulose, the most common and abundant component of all plant matter comprising on about 35% - 50% [3] is a product of polymerization of saccharide monomers by O-glycosidic bonds. Since agricultural plant residues abound the use of beta-glucanases in conversion of waste to wealth is worthwhile.

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O-Glycosyl hydrolases (EC:3.2.1.) are a widespread group of enzymes that hydrolyse the glycosidic bond between two or more carbohydrates, or between a carbohydrate and a non-carbohydrate moiety. The Carbohydrate Active Enzyme (CaZy) database indicates that GH family 17 contains endo and exo-beta-1, 3-glucanases among others. Exo-beta-1,3-glucanase (EC 3.2.1.58) successively hydrolyse O-glycosidic linkage of beta-1,3-linked glucans from their nonreducing terminus to subsequently yield glucose[4].

Glycosyl hydrolase (GH) family 17 members currently known are from fungi and plants only (http://www.ebi.ac.uk/interpro/entry/IPR000490). All the 15 PDB accessions of the GH family 17 are of plant and fungal origin among which there is no exo-beta-1, 3-glucanase reported. (CaZy). Bacterial exo-beta-1, 3-glucanase have been reported in *Streptomyces* sp. SirexAA-E belonging to GH family 55 [5] and also in S. sioyaensis belonging to GH family 16 [6].

In this paper, we present the 3D structure prediction, validation and analysis of novel bacterial exo-beta-1, 3-glucanase of GH family 17 from *Psychroflexus torquis* ATCC700755/ACAM623-a psychrophilic bacteria. To the best of our knowledge, this is the first attempt to model this enzyme in bacteria with a GH family 17 fold. More so, it is the first report on modelling this enzyme from psychrophilic bacteria.

MATERIALS AND METHODS

Sequence Retrieval, Analysis and 3D structure prediction

The amino acid sequence for *Psychroflexus torquis* ATCC700755/ACAM623 was obtained from UniProt with accession number K4HIN2 (http://www.uniprot.org/). A UniProt-BLASTp was run and four exo-beta-1,3-glucanases from various species (Table 1.) were chosen for subsequent alignment. A multiple sequence alignment was performed using Multalin software 5.4.1 (http://multalin.toulouse.inra.fr/multalin/) at default settings to identify conserved regions which are potential active site location in study protein[7,8]. 3D structural prediction was performed using RAPTORX server[9].

3D Model Validation

For assessing the validity of the predicted 3D model, the Rampage server was used[10]. This tool calculates the phi (Φ) and psi (Ψ) angles thus generate a Ramachandran plot for the model. The model was also submitted to VERIFY-3D and ERRAT servers to further validate its structure.

Analysis of the Model

Superimposition of the template and model was performed using PyMOL 1.7.4.5 visualisation software to facilitate observation of structural similarities and differences of the structures. Secondary structure alignment of template and model was run using ENDscript server[11] to enable analysis of secondary structure-profile energy relations. To facilitate the energy relations analysis hitherto mentioned, a comparative plot of Discrete Optimized Protein Energy (DOPE) per residue of template and model was made using gnuplot 4.6 software and profile files generated by the 'asses dope' command of MODELLER 9.15 software[12].

Table1. UniProt-BLASTp result for exo-beta-1,3-glucanases

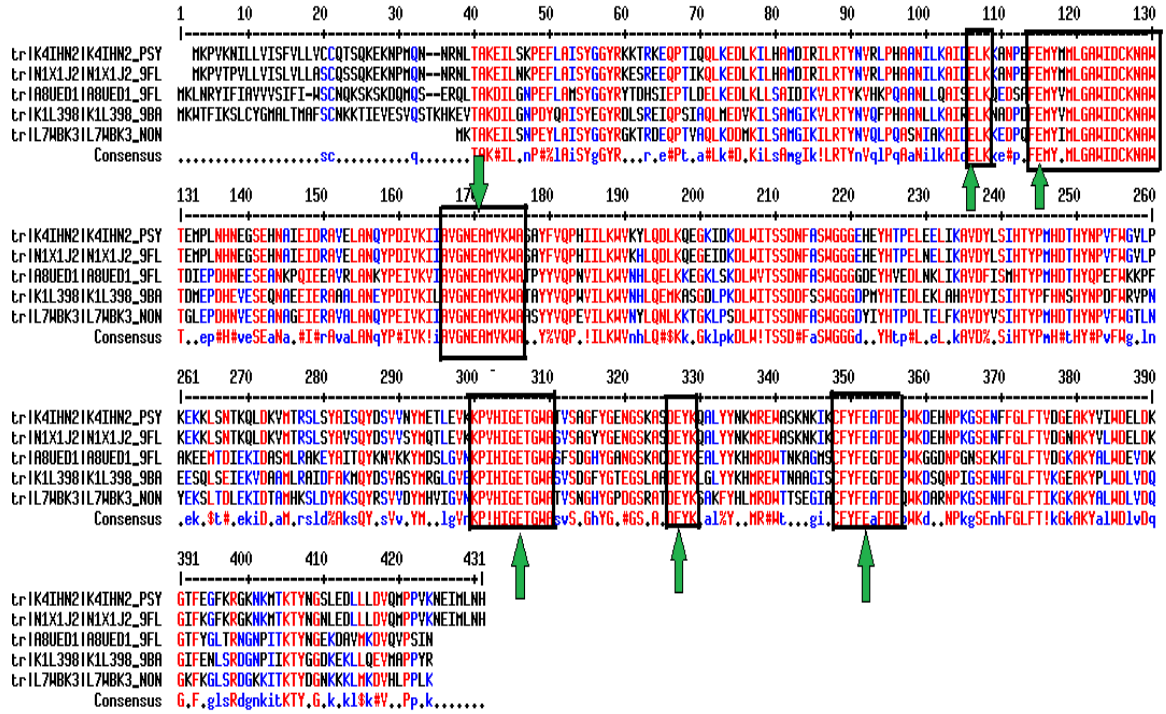
Accession	Enzyme	Organism	Identity (%)	E-value
K4IHN2	Exo-beta-1,3-glucanases	*Psychroflexus torquis (strain ATCC 700755 / ACAM 623)*	100	0.0
N1X1J2	Exo-beta-1,3-glucanases	*Psychroflexus gondwanensis ACAM 44*	94.1	0.0
L7WBK3	Exo-beta-1,3-glucanases	*Nonlabens dokdonensis (strain DSM 17205 / KCTC 12402 / DSW-6)*	68.6	0.0
A8UED1	Exo-beta-1,3-glucanases	*Flavobacteriales bacterium ALC-1*	60.0	0.0
K1L398	Exo-beta-1,3-glucanases	*Cecembia lonarensis LW9*	58.7	8.2e-180

RESULTS AND DISCUSSION

Analysis of primary sequence

UniProt-BLASTp was run for the complete exo-beta-1,3-glucanase sequence of *Pschroflexus torquis* obtained from UniProt and this returned many hits with varying percentage identities among which 4 other exo-beta-1,3-glucanases from other organisms were chosen for alignment with the query protein. (Accessions in Table 1.). The multiple sequence alignment identified the conserved regions as shown in Figure.1, which shows green arrows pointing to possible active site residues. Location of these active site residues have been confirmed to be in agreement with conserved domain database (CDD). The mechanism of catalysis in glycosyl hydrolase (GH) family 17 involve the use of two glutamic acid (GLU/E) residues with one acting as a proton donor and the other, a nucleophile[8].

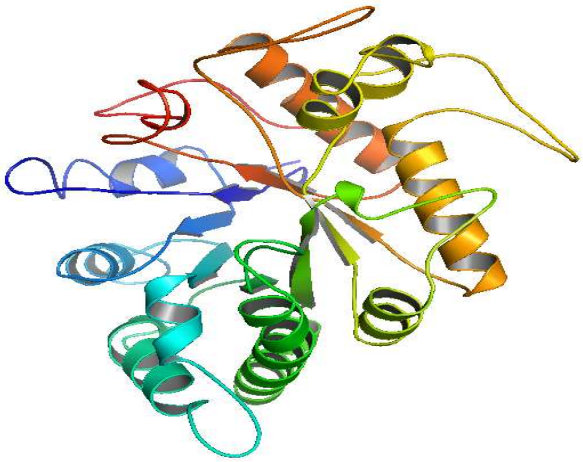
Fig. 1: Output of multiple sequence alignment of four exo-beta-1,3-glucanases with the novel enzyme using Multalin 5



Model Prediction

The 3D model predicted by RAPTORX contains a central TIM barrel (Fig. 2).

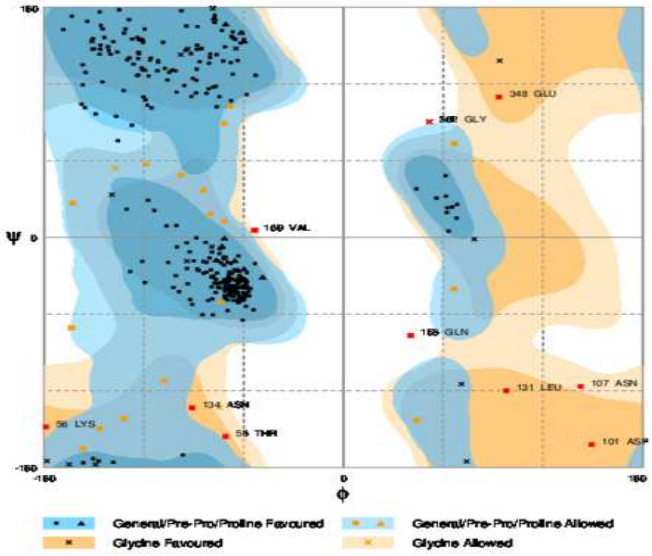
Fig. 2: 3D Model of exo-1,3-beta glucanase from psychroflexus torquis using RaptorX



3D Model Validation

The Rampage server was used to validate the stereo chemical quality of the model. This tool calculates the phi (Φ) and psi (Ψ) angles thus generate a Ramachandran plot for the model. The Ramachandran plot (Figure 3.) indicated the model has 91.5% of residues located in favoured region while 5.6% and 2.9% are located in allowed and outlier region respectively. In general, a score (97.1%) close to 100% implies good stereo-chemical quality of the model.

Fig. 3: Ramachandran plot of the Model

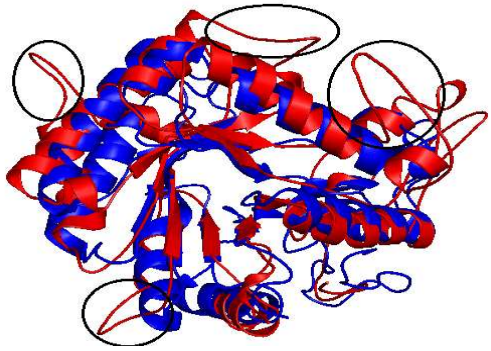


The result of analysis by VERIFY-3D revealed that 73.3% of the residues had an average 3D-1D score above 0.2 and the remaining did not attain this score. For 65% ≤ VERIFY-3D<80, the model quality is scored 'warning' while ≥80% is 'pass' (http://services.mbi.ucla.edu/Verify_3D/). The overall quality of the model was also assessed by the ERRAT programme for nonbonded atomic interaction by comparing the statistics of highly refined structures. The ERRAT score of a good model is above 50% whereas higher scores indicate a better quality[13]. The model obtained an ERRAT score of 93.17% which is acceptable. Considering that the model structure has a sequence identity less than 30% to the template, the overall scores obtained for the model using different evaluation tools are considered reasonable.

Analysis of the model

The 3D model of K4IHN2 sequence shown in Figure 2 has similar structural features to those of other members belonging to GH family 17. This structure consists of alternating α-helices and β-sheets in its conserved domain which houses the catalytic residues. The 3D model structure is a TIM-barrel structure in which the stability of the structure is due to the 8 central β-sheets that form the central barrel.

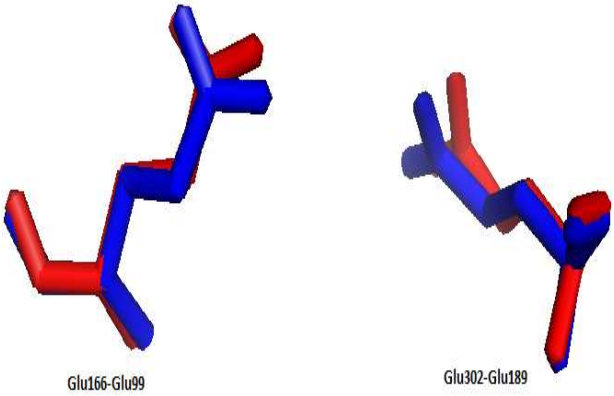
Fig. 4: Superposition of template (blue) and model (red)



The superimposition of the Model with its best template (4wtp returned by RAPTORX server) is represented in Figure 4. This superimposition reveals similarities in the template and model structures which are not completely detailed here.

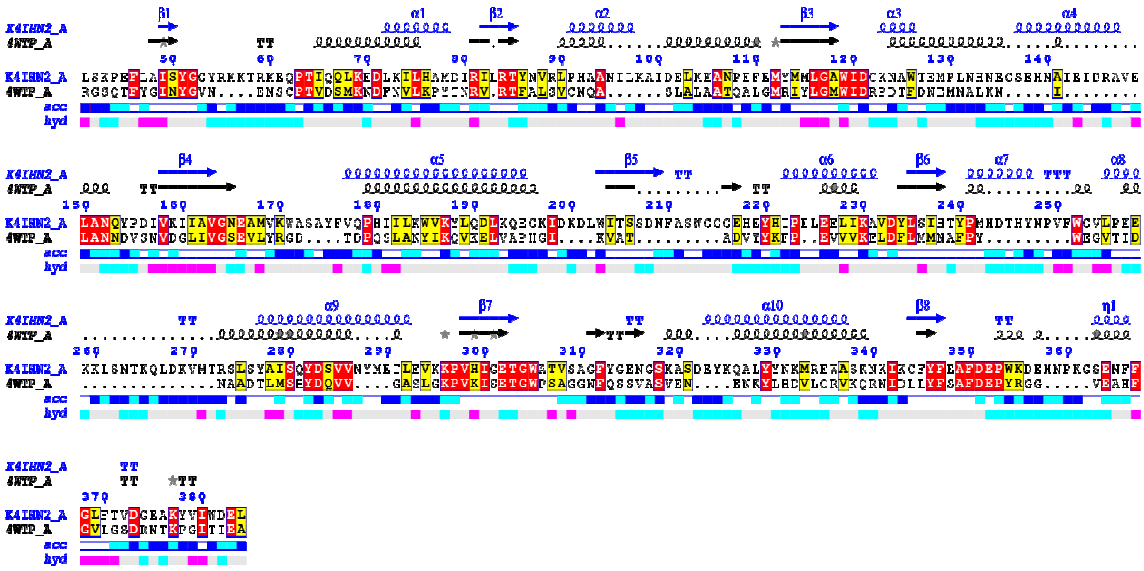
The superimposition reveals the catalytic residues of the model. Figure 5 shows that the GLUs166 and 302 of the model align to GLUs 99 and 189 respectively. GLUs 99 and 189 are the catalytic residues of the template. This indicates to the active site of the model.

Fig. 5: Superimposed catalytic residues; K4IHN2-4wtp shown as sticks



However, Structural differences exist between the superimposed structures. The model has longer or insertions of loops (highlighted by cycles in Fig.4.) at four locations: ILE100-MET112 and THR127-GLU138 are loop insertions while ALA211-THR222 and GLU259-SER280 are longer loops. Other loops worthy of mention but not highlighted in Fig.4 are located at GLY51-ASP71, GLY303-GLU321 and PRO353-GLY362. These can be observed in the secondary structure alignment between template and model (Figure 6). The presence of these loops can be seen as an adaptation to psychrophilic life since loops confer more flexibility to proteins.

Fig. 6: Secondary structure alignment between model (K4IHN2_A) and template (4WTP_A) using ENDscript 2.0 server



The Energy profile plot in Figure 7 reveals location of high energy in the model. These regions correspond to the longer loops or insertions which are possible adaptations to psychrophilic life. However, the overall DOPE score (Table 2) of the model is reasonable and indicative of a good model.

Fig. 7: Comparative DOPE per residue plot of Template and model

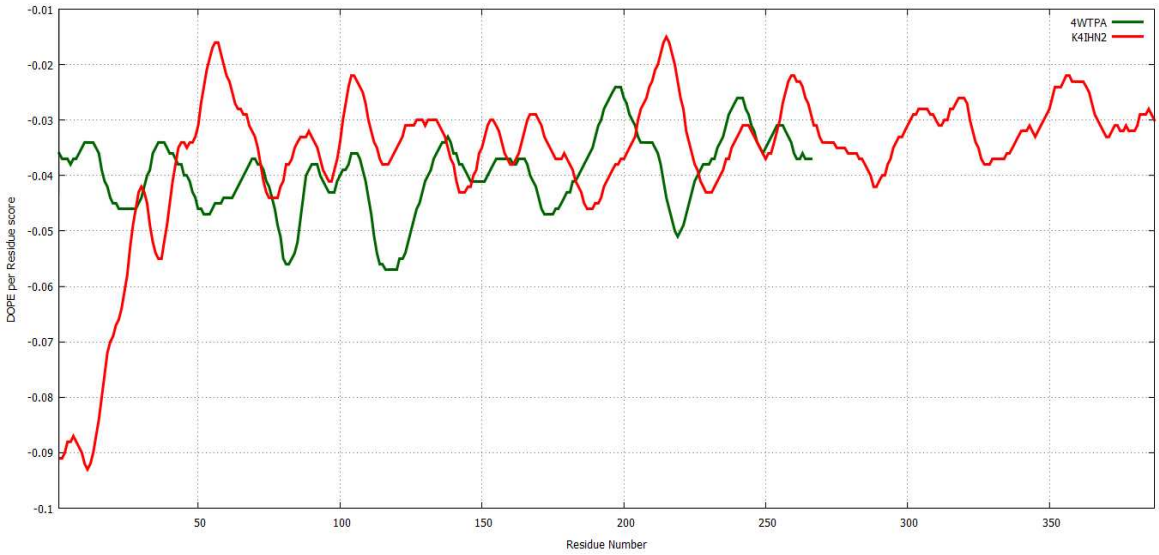


Table 2: Overall DOPE score of template and model

Structure	Overall DOPE score
4WTPA	-34224.464844
K4IHN2	-41547.304688

CONCLUSION

The prediction, validation and analysis of 3D model of this exo-beta-1, 3-glucanase in this paper reveals its characteristics as a cold adapted enzyme. Presence of longer loops as well as loops insertions instead of α-helices or β strands confer greater flexibility to this model enzyme and enhance the capability of the enzyme to be active in cold temperatures. This study also elucidates the first bacterial exo-beta-1, 3-glucanase with a GH family 17 fold.

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