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Structural prediction and comparative docking studies of psychrophilic β- Galactosidase with lactose, ONPG and PNPG against its counter parts of mesophilic and thermophilic enzymes

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Abstract:
Enzymes from psychrophiles catalyze the reactions at low temperatures with higher specific activity. Among all the psychrophilic enzymes produced, cold active β-galactosidase from marine psychrophiles revalorizes a new arena in numerous areas at industrial level. The hydrolysis of lactose in to glucose and galactose by cold active β-galactosidase offers a new promising approach in removal of lactose from milk to overcome the problem of lactose intolerance. Herein we propose, a 3D structure of cold active β-galactosidase enzyme sourced from Pseudoalteromonas haloplanktis by using Modeler 9v8 and best model was developed having 88% of favourable region in ramachandran plot. Modelling was followed by docking studies with the help of Auto dock 4.0 against the three substrates lactose, ONPG and PNPG. In addition, comparative docking studies were also performed for the 3D model of psychrophilic β-galactosidase with mesophilic and thermophilic enzymes. Docking studies revealed that binding affinity of enzyme towards the three different substrates is more for psychrophilic enzyme when compared with mesophilic and thermophilic enzymes. It indicates that the enzyme has high specific activity at low temperature when compared with mesophilic and thermophilic enzymes.

Keywords: Psychrozymes, β-galactosidase, Ramachandran plot, Homology modeling, Docking.

Background:
Enzymes from Extremophiles can be used extensively at industrial scale due to their defying nature at various robust processing conditions. However, most of the enzymes used in industrial processes are sourced from mesophiles and show many advantages, but their usage in bioprocess has been gradually decreasing due to their less stability at the extremes of temperature, pH and ionic strength **[1]**. In this context, much attention has been given to cold active enzymes as the efforts and cost cutting measures for downstream processing are less compared with other enzymes **[2]**. It was reported that Psychrophiles have the ability to degrade a wide range of polymeric substances and produce various enzymes like amylases, cellulases, pectinases, β-Galactosidases, oxidases, proteases and lipases etc. **[3]**. Cold adaptive enzymes catalyze the reactions at low temperatures with higher specific activity. They possess unique features when compared to thermophilic and mesophilic enzymes to cope up with the cold environment that leads the researchers to explore these enzymes for industrial applications. The enzyme β-Galactosidase (EC No. 3.2.1.23) hydrolyzes β (1→4) linkage in disaccharide lactose and liberates glucose and galactose as products. It has been reported that 75% of adults show decrease in lactase activity during adulthood which causes lactose intolerance **[4]**. Therefore, in present days the diary industries are mainly focusing on the production of lactose free products at a cheaper cost. This enzyme has potential application in dairy industry in removing lactose from milk and milk products to enhance the digestibility as well as sweetness of milk. Its high specific activity at low temperature is the main advantage and also it can be used while the food is in transportation or in storage. The present study is mainly focused on constructing a new 3D model of cold active β-Galactosidase enzyme from psychrophile by computational approach which can be used in comparing the relative affinities of enzyme towards different substrates (lactose, ONPG and PNPG) by comparative docking studies against to its counter parts of mesophilic and thermophilic enzymes.

Materials and Methodology:
Retrieval of Sequences and Multiple sequence alignment:
Amino acid sequence in the FASTA format for psychrophilic, (Accession No. AJ131635.1_1) was retrieved from NCBI database, where as for mesophilic and thermophilic enzymes the sequences were available in Protein Data Bank (PDB) with PDB IDs 1f49 and 1kwk, respectively. Multiple sequence alignment was carried out for all the three sequences using clustalW.

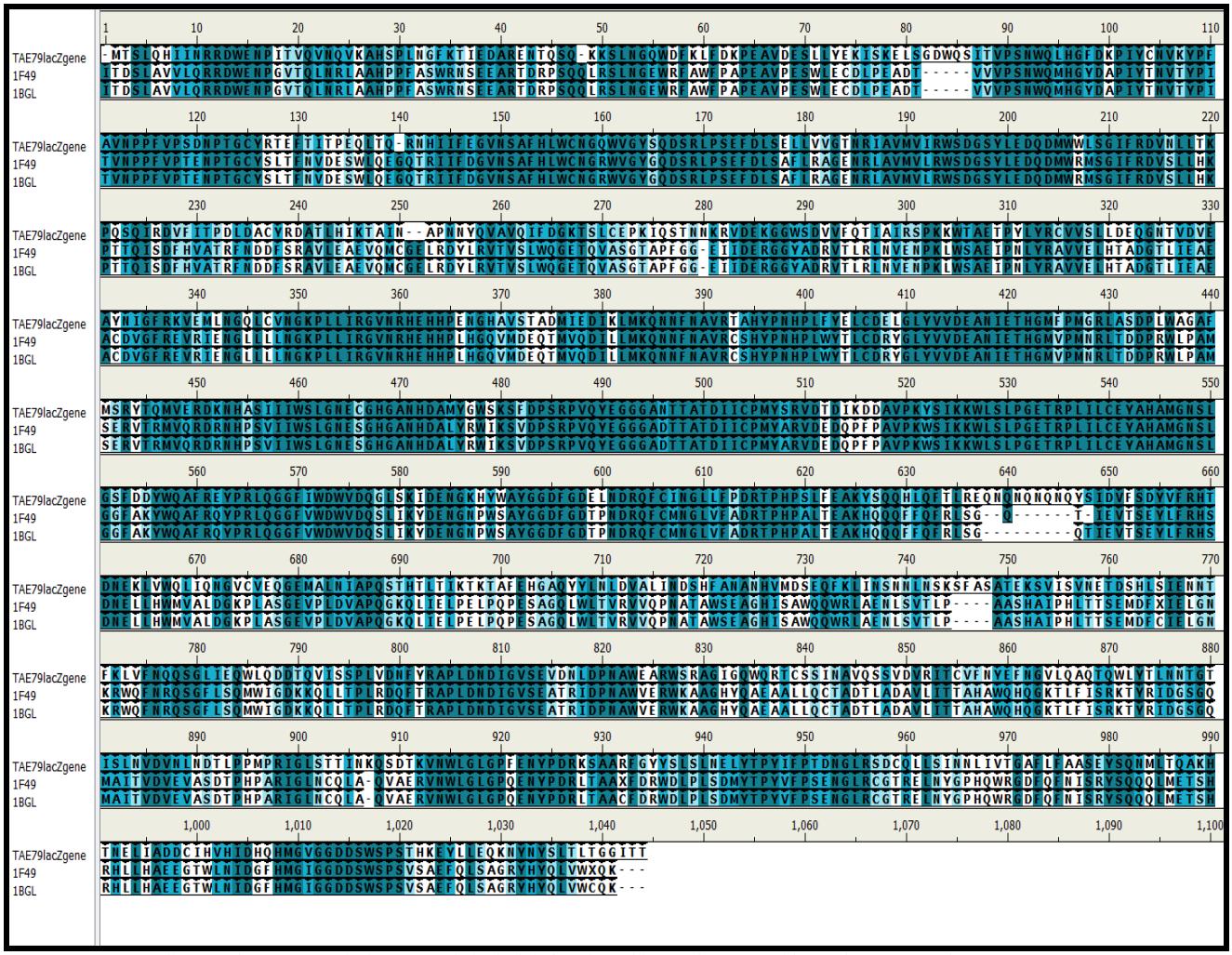

Figure 1: Sequence alignment between Pseudoalteromonas haloplanktis β -galactosidase and enzyme with templates 1F49 and 1BGL.

Homology Modeling:
Psychrophilic amino acid sequence was searched against PDB using BLAST-P **[5, 6, and 7]** to identify the potential template structure for molecular modelling. MODELLER 9v8 program was used for predicting the 3D model of the enzyme **[8]** and was subjected to PROCHECK **[9, 10]** and ProSA (Protein Structure Analysis) to determine the stereo chemical quality of the structure.

Molecular docking studies:
Preparation of protein structure:
Modeled psychrophilic enzyme along with PDB structures of mesophilic enzyme (1F49) and thermophilic enzymes (1kwk) were taken and hetero atoms in the form of water molecules, bound ligands were removed. With the help of Q-site finder, functional site amino acids were predicted for the enzyme.

Preparation of Ligand structures:
The 3D structures of all the three substrates namely lactose, ortho-Nitrophenyl-β-Galactoside (ONPG) and para nitro phenyl-β-D-Galactopyranoside (PNPG) were obtained from the Chemspider database **[11]**.

Molecular Docking Studies:
AutoDock 4.0 was used for docking simulation **[12, 13, and 14]** which employs the preparation of receptor by adding hydrogens and assigning Kollman charges, followed by conversion of PDB file to pdbqt. Ligands were assigned with Gasteiger charges and non polar hydrogens. Docking simulations were run using Lamarckian Genetic algorithm(LGA) which is known to be the most efficient and reliable method of Auto Dock. The grid points for Autogrid calculations were set to be 44 × 44 × 40 Å with the active site residues at the centre of the grid box. The docking parameters were set to a LGA calculation of 10,000 runs. The energy evaluations were set to 1,500,000 and 27,000 generations. Population size was set to 150 and the rate of gene mutation and the rate of gene crossover were set to 0.02 and 0.8 respectively. The obtained conformations were then summarized, collected and extracted by using Autodock Tool.

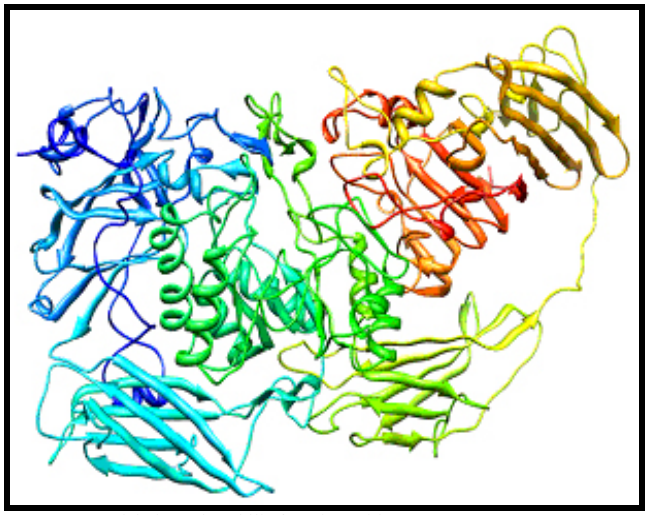

Figure 2: Modeled psychrophilic β -galactosidase enzyme

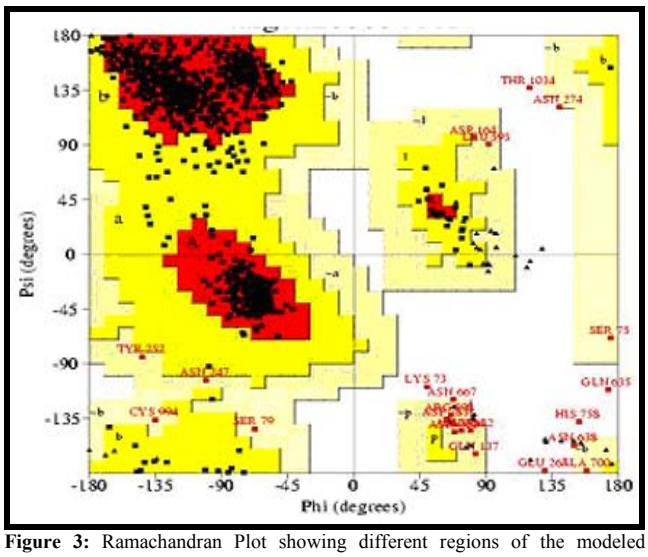

Figure 3: Ramachandran Plot showing different regions of the modeled enzyme

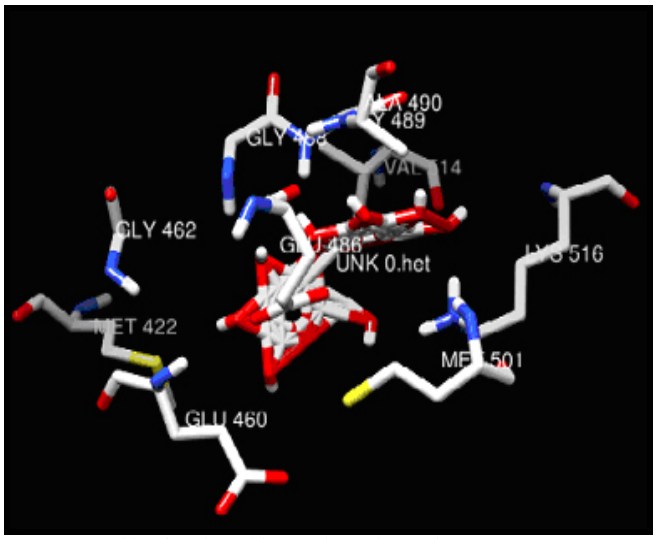

Figure 4: Docking β- galactosidase against substrate lactose

Results and Discussion:

Multiple sequence alignment:

The psychrophilic and mesophilic enzymes showed 50.2% identity and 65.1% similarity as when compare to psychrophilic and thermophilic enzymes which showed 11.0% identity and 17.4% similarity. From the above results it was clear that structurally, psychrophilic enzymes were more related to mesophilic enzymes when compared to thermophilic enzymes.

Homology modeling of β-Galactosidase enzyme:

Template structure for modeling the protein was searched through BLAST and among all the hits obtained, PDB ID:1F49 was selected, having 0.0 E-value Using this, a valid 3D structure was generated by the help of Modeler 9v8 **(Figure 1)**. The DOPE (Discrete optimized protein Energy) score values for all the five models generated were -113439.30469, -112441.01563, -113630.60938, -112431.86719 and -113359.10938. Among all, the model having lowest DOPE score (-113630.60938) was considered as the final model **(Figure 2)**. The final model was confirmed and used for further studies as the ProSA Z-score value of -9.6 and 88% of favored region was obtained through ramachandran plot **(Figure 3)**.

Validation of functional Site:

Among the binding pockets obtained through Q-site finder, the related active site amino acids were selected on the basis of available literature **[15]** and the residues in the active site were confirmed as Glu460, Glu536, Met501, Tyr502 and Arg387.

Molecular Docking Studies:

Modeled psychrophilic, mesophilic and thermophilic enzymes were docked against the three substrates viz., lactose **(Figure 4)**, ONPG and PNPG. It was observed that the modeled enzyme of Psychrophilic origin has significantly low binding energy values with all the three substrates (-20.18, -39.24 and -39.48 K.Cal/mole) when compared with mesophilic and thermophilic enzymes **(Table 1, see supplementary material)**. The reasonable low binding energy values of psychrophilic enzyme indicate that the ligands are in most favorable region of the protein and that the enzyme has more affinity towards all the three substrates when compared with enzymes from other origins.

Conclusion:

From molecular Docking studies it was observed that the modeled enzyme has good affinity towards substrates like lactose, ONPG and PNPG when compared with mesophilic and thermophilic counterparts. Low binding energy values with respect to psychrophilic enzymes indicates the high specificity of the enzyme towards substrates at low temperatures. Moreover, the valid and stable 3D model of β-galactosidase from P. haloplanktis structure will help in further in silico studies to evaluate the structural stability and provide better understanding of topological parameters of the enzyme and molecular basis of cold adaptation.

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Supplementary material:

Table 1: Binding Energies of β- Galactosidase from various sources against different substrates

Organism	Lactose	ONPG	PNPG
Psychrophile	-20.18 Kcal/mole	-39.24 Kcal/mole	-39.48 Kcal/mole
Mesophile	-16.32 Kcal/mole	-39.08 Kcal/mole	-38.78 Kcal/mole
Thermophile	-19.35 Kcal/mole	-23.49 Kcal/mole	-23.69 Kcal/mole