STRUCTURAL AND FUNCTIONAL ANALYSIS OF β-GLUCOSIDASES FROM *PICHIA ETCHELLSII*

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By

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Submitted

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Dedicated to my dear parents

for their everlasting love and affection

CERTIFICATE

This is to certify that the thesis entitled **"Structural and functional analysis of β-glucosidases from *Pichia etchelssii*"** being submitted by **Mr. Mohammad Asif Shah** to the Department of Biochemical Engineering and Biotechnology, India Institute of Technology, Delhi, for the award of the degree of '**Doctor of Philosophy**' is a record of the bonafide research work carried out by him, prepared under our supervision in conformity with the rules and regulations of 'Indian Institute of Technology', Delhi. The research report and the results presented in the thesis have not been submitted to any other University or Institute for the award of any other degree or diploma.

Prof. Saroj Mishra **Dr. Tapan K. Chaudhuri** **Prof. Tej P. Singh**

(Deptt. of Biophysics, AIIMS)

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ABSTRACT

The era of structural genomics ushered in generating a wealth of information about the organization of the genomes. We came to understand how the code of life is written at the molecular level. However, to understand what those threads of life meant, a new era of functional genomics was born. The aims were to understand how the genome works. How the one dimensional information is transformed into a well defined three dimensional function. The understanding of the protein structure-function relationship is a key aspect of functional genomics. This work is an attempt to address the issue from a different perspective, the world of multi-domain proteins. Their structure and function relationship has been studied. The two proteins chosen for the study were β-glucosidases from thermo-tolerant yeast, *Pichia etchellsii.*

β-glucosidases are a wide group of enzymes present across all organismal levels ranging from prokaryota to eukaryota. The enzymes have hydrolyzing activity, cleaving a β-glucosidic bond between two glucose molecules or a glucose and an aglycone part. A remarkable feature of these enzymes is their broad substrate specificity generated from the same TIM-barrel fold. Although the sequence similarity is less among them, but the overall fold is similar consisting of eight β-sheets which are surrounded by eight α helices. The arrangement is different in family 1 and family 3 members of glycosylhydrolases. Family three members contain an additional α/β sandwich domain. It is formed from the organization of six β-sheets and six α-helices. Since the general organization is similar, they are expected to show similar structure-function relationship. In this study, it was observed that the two β-glucosidaes, BGL I and BGL II, show some interesting features when their structure-function relation is probed. BGL I seemed to be more rigid in structure and more stable than BGL II by about 1 kcal when unfolded by GdnHCl. In case of alkaline and thermal unfolding transitions, BGL I showed late transition to unfolded states than BGL II, again indicative of more rigid structure. Since both the proteins belong to the category of multi-domain proteins, their refolding yields were low, owing to misfolding or aggregation of the proteins. Osmolytes have been proved to be helpful in preventing the aggregation of large proteins, but even in their presence, low refolding yields were observed in these proteins.

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