**Poster Presentation**

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*Structure based improvement of the glucose tolerance of a β-glucosidase*

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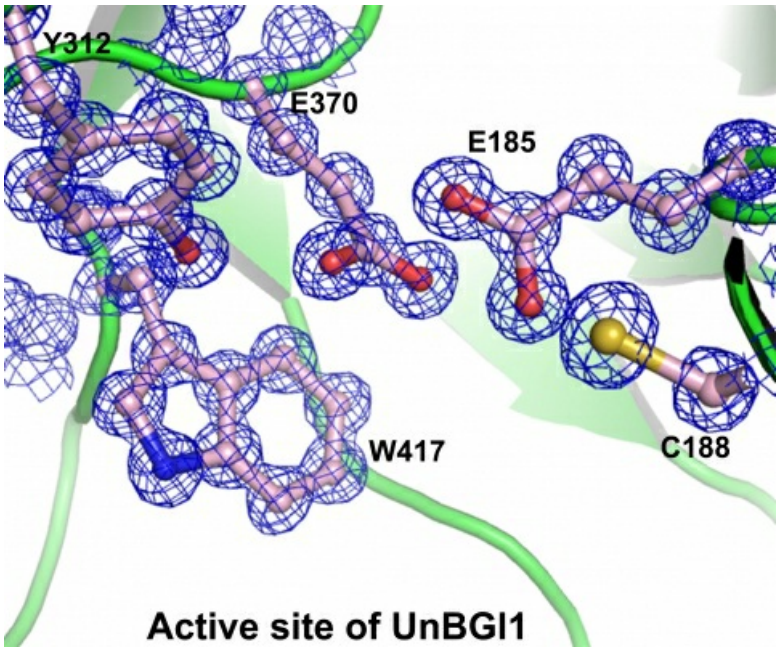
Cellulases, a family of enzymes those are responsible for hydrolyzing cellulose or hemicellulose to produce glucose, have lots of industrial importance in cellulosic biofuel production. β-glucosidase is the rate limiting cellulase which produces glucose from cellobiose. Feedback inhibition by glucose is one of the reasons for low catalytic efficiency of this enzyme and slower enzymatic conversion rate of cellulose to glucose. Therefore, engineering of β-glucosidase is essential to produce enzymes with high catalytic efficiency and higher tolerance to product inhibition. Recently, a β-glucosidase (UnBGl1) from soil metagenome has been isolated. We have cloned this enzyme (UnBGl1) with a C-terminal His-tag. The wild type recombinant enzyme was expressed and purified to homogeneity. The glucose tolerance level of the purified enzyme was measured to be 0.9 M. The enzyme showed highest optimal activity at pH 6.0 at 55 °C. The stability of the enzyme over the time was also monitored. The enzyme retains almost 35 % of the activity after 24 hours at 55 °C. The enzyme was crystallized and atomic resolution (0.95 Å) structure of its Tris bound form has been determined using the diffraction data collected at PX-BL21 beamline, Indus 2 at Raja Ramanna Centre for Advanced Technology, India. Very high resolution crystal structures of active UnBGl1 have also been determined as apo-enzyme as well as complexed with cellobiose and glucose. The cellobiose bound form of this enzyme is the first structure of an active β-glucosidase with its substrate in the active site. The structural analysis indicates the mode of cellobiose binding in the active site. The cellobiose bound structure has revealed that the substrate binding pocket contains two catalytic glutamate residues (E185 and E370) and the substrate is anchored to the enzyme via several polar interactions. The glucose bound structure represents a state of the enzyme in which glucose is covalently bound to the active site. These structures of UnBGl1 provide a detailed understanding about the reaction mechanism of this enzyme. Our results also indicate that the active site is located at the closed end of a tunnel that opens at the surface of the enzyme. This tunnel might be involved in supply of cellobiose to the active site and removal of product glucose. Based on the structural analysis a cysteine residue (C188) was identified to stabilize a glucose molecule adjacent to the catalytic center. The C188V mutant of UnBGl1 was created and the purified mutant enzyme showed 55 % higher glucose tolerance as compared to the wild type. The crystal structure of C188V mutant has been determined at 1.1 Å resolution and it serves as a tool for further improvement of glucose tolerance of this enzyme to enhance its performance in industrial biofuel production process.

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