MS06-2-10 Structural and biochemical studies on a GH5 cellulase from Aspergillus oryzae with beta-glucosidase activity
#MS06-2-10

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Abstract
Saccharification of cellulose to yield glucose holds particular commercial interest in the biofuel industry. This glucose can be fermented and distilled to produce fuel grade ethanol. The hydrolysis of cellulose is carried out by cellulase, an ensemble of enzymes that includes exoglucanases, endoglucanases and β-glucosidases, which act synergistically on cellulose. The endoglucanases act initially, followed by exoglucanases to produce cellobiose which is hydrolysed to glucose by β-glucosidases[1]. The reaction involving β-glucosidases is the rate determining step in the entire process of cellulose saccharification. β-glucosidases are inhibited by accumulation of the product glucose in the reaction chamber[2]. Apart from glucose, these enzymes are also inhibited by conditions of high temperature and acidic pH. As a result of these factors, thermotolerant β-glucosidases which have higher tolerance towards glucose and can function in lower pH, are considered important for the industrial bioethanol production processes. In this study, we report a GH5 cellulase of *Aspergillus oryzae* (AoBgl) which has β-glucosidase activity. The gene coding for aobgl has been cloned and over-expressed in *Escherichia coli* BL21 (DE3). The protein was purified using Ni-NTA affinity chromatography, followed by anion exchange and size exclusion chromatography. The purified protein was crystallized and structures of both the apo as well as cellobiose-bound forms of the enzyme was solved at high resolutions (1.65 Å for apo form and 1.73 Å for cellobiose bound complex). AoBgl has an overall TIM barrel like structural fold that is similar to β-glucosidases of GH1 family, this structural feature might be responsible in conferring β (1-4) glycosidic linkage hydrolysing features to this GH5 enzyme. On substrate binding, a change in conformation in a loop region has been observed. This loop region involves residues present in the +2 subsite of substrate binding (Figure 1) . Biochemical studies on AoBgl revealed that the optimum temperature of its activity is 55 ˚C and optimum pH of activity was 5.5 (Figure 2 A, B). Apart from these features, the enzyme had a very high glucose tolerance of 1.35 M (Figure 2 C). These biochemical properties indicate that AoBgl is highly suitable candidate for industrial bioethanol production processes. Guided by these structural and biochemical characteristics, further superior variants of AoBgl can be generated for use in biofuel industry.

References
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2. Sawant, S., Birhade, S., Anil, A., Gilbert, H., & Lali, A. (2016). Two-way dynamics in βglucosidase catalysis. Journal of Molecular Catalysis B: Enzymatic, 133, 161-166.

Figure 1: Structural changes on cellobiose binding

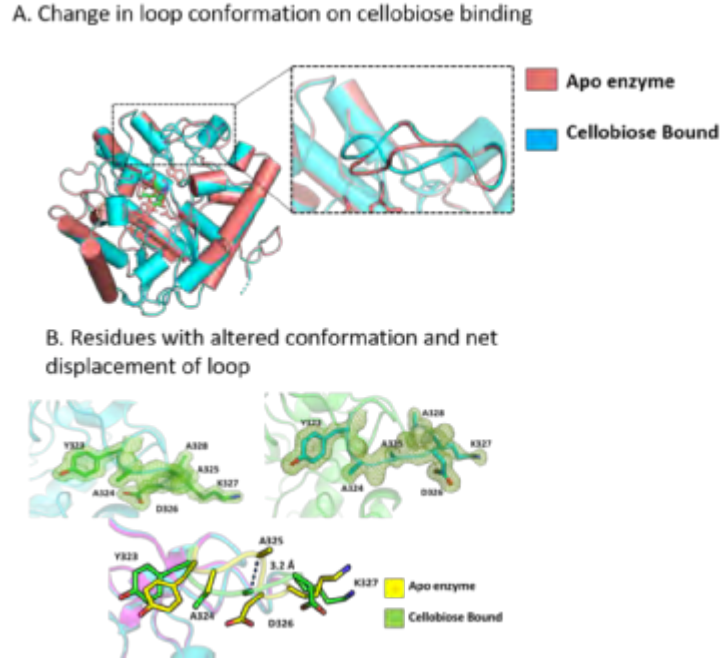

Figure 2: Biochemical characterization of AoBgl