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Understanding the role of non-conserved residues of β-glucosidases in increasing glucose tolerance

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The abundant plant biomass on earth has the potential to become a sustainable source of transport fuels. The economics of enzymatic hydrolysis of biomass into ethanol largely depends upon the performance of cellulase cocktail used for saccharification of the biomass. β-glucosidase (EC 3.2.1.21) is a vital component of cellulase and catalyses the hydrolysis of β-1,4 linkages of disaccharides or glucose substituted molecules into glucose. Most β-glucosidase (BG) is sensitive to their end product glucose, which is a limiting step in saccharification. Very little is known about the mechanism of glucose inhibition or the difference in glucose tolerance among BG's. Elucidation of the mechanism of glucose tolerance is thus crucial towards engineering high glucose tolerance and in turn efficient saccharification.

To understand the mechanism of glucose tolerance and identification of key residues responsible for glucose tolerance we have characterised two thermotolerant BG's, B8CYA8 from *Halothermothrix orenii* and O08324 from *Thermococcus sp*. Non- conserved residues based on multiple sequence alignment amongst BGs along and a rational design based strategy in the active site tunnel of the enzyme was used to design mutations. Biochemical characterisation of the mutants suggests that residues at entrance of the tunnel (gatekeeper) and inside the tunnel regulate the effect of glucose on catalysis. Mutants also show high tolerance toward ionic liquids and enhanced thermal stability. In this poster, we will present data that suggests that reducing steric hindrance at the entrance of the tunnel and the presence of hydrophobic residues in the active site tunnel of BGs leads to higher glucose tolerance. Our findings will be helpful in engineering active glucose sensitive BGs into glucose tolerant variants.