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Improving the Ionic Liquid Tolerance of a Mesophilic Bacteria *Agrobacterium Tumefaciens* β-glucosidase and its Mutants, in the Presence of the Enzyme Catalyzed Reaction Product Glucose

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Ligcellulosic biomass is most abundant biomass in the world. Enzymatic hydrolysis of this biomass becomes hard due to its insoluble in water. Pretreating biomass with ionic liquids (IL) increases enzyme accessibility and cellulose is recovered through precipitation with an anti-solvent. An industrially feasible pretreatment and hydrolysis process requires robust cellulases that are stable and active in the presence of either small amounts of ILs co-precipitated with recovered cellulose or for saccharifications in the presence of IL. β-glucosidase's (BG) hydrolyse cellobiose into two molecules of glucose (Glc) and is the last step of biomass hydrolysis. These enzymes are prone not only to product inhibition by glucose but also to inactivation by ILs. With increasing interest in IL based pretreatment methods, the focus has been towards a search for Glc tolerant BG as well as IL tolerance. We identified a putative BG belonging to the GH1 family, H0HC94, encoded in *Agrobacterium tumefaciens* 5A, and cloned and overexpressed the protein in *Escherichia coli.* H0HC94 exhibited high enzymatic activity with β-glycosidic substrates (248 µmol/min/mg on pNPGlc and 262 µmol/min/mg on cellobiose) and tolerant to higher Glc concentrations (apparent $K_{i,Glc}$= 686 mM).. Further evidence of Glc-based stabilization came from the increase in melting temperature of H0HC94, with increasing Glc concentrations. We have also generated mutants showing greater tolerance towards glucose. The half-life of H0HC94 increased between 2.5 fold to 10 fold in the presence of 100 mM Glc and different [C2mim] based ionic liquids.