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Title: Engineering Thermostable *Trichoderma reesei* Cellulases

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Abstract: *Trichoderma reesei* cellulases are currently the gold standard for converting the cellulosic content of biomass to fermentable sugars for biofuels production. The ability of this fungus to produce and secrete large quantities of cellulases is paramount for the economical production of biofuels from cellulosic biomass. Lignocellulose hydrolysis using cellulases at high temperatures (65-70°C) has many potential advantages such as higher solid loadings due to reduced viscosity, lower risk of microbial contamination, greater compatibility with high temperature pretreatments, enhanced mass transfer and faster rates of hydrolysis. The instability of *Trichoderma reesei* cellulases at temperatures above 50°C combined with severely limited expression and production of existing thermophilic cellulases from non-fungal sources motivates the development of thermostable *Trichoderma reesei* cellulases that can hydrolyze lignocellulose efficiently at higher temperatures, preferably in the range of 65-70°C. A B-factor guided approach for improving thermostability was used to engineer a variant of *Trichoderma reesei* endoglucanase I (TrEGI) with greater than 3-fold improved activity at 60-65°C on insoluble lignocellulosic substrates as well as a ~4°C increase in T_m as compared to the native enzyme. The expression of engineered enzymes with varying degrees of glycosylation (by cell-free protein synthesis and yeast) shed light on the role of glycosylation on the activity and stability of TrEGI mutants.