Determining the factors that promote high thermostable cellulase production of the fungus *Thermoascus aurantiacus*

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The filamentous thermophilic fungus *Thermoascus aurantiacus* secretes cellulases and hemicellulases with high thermotolerance and enzymatic activity, making this organism an intriguing host for low cost plant biomass conversion to renewable fuels and other products. Our goal is to use systems biology to generate a fundamental understanding of CAZy gene regulation in this fungus to design improved genetic- and bioprocess engineering strategies for up-scaling enzyme production in *T. aurantiacus* and lastly, perform saccharification studies by focusing on the *T. aurantiacus* LPMO to efficiently drive plant biomass deconstruction. We optimized a minimal growth medium for cultivation of this fungus and a novel fed-batch system to survey potential enzyme secretion inducers at 250 ml shake flask scale. Xylose, arabinose and cellobiose were identified as potent inducers for cellulases while only the first two sugars induced xylanases. With the low-feed fed batch set-up, a first-time RNA-Seq experiment was performed with fungal cultures grown under these conditions with the goal to study genome wide gene expression patterns when grown under these conditions compared to no carbon and high glucose medium. We also established an *Agrobacterium tumefaciens* mediated transformation (ATMT) system for *T. aurantiacus* ascospores with the hph resistance marker. The protocol conditions were optimized for temperature, co-incubation time and membranes, pH, acetosyringone concentration and agar media for spore production. A ku70 deletion stain was also generated to perform more efficient gene deletions. We overexpressed the xylanase and cellulase regulator xlnR which increased xylanase activity of respective isolates up to 500% compared to wild type in cellulose medium. Lastly, saccharification with *T. aurantiacus* enzymes on Avicel and acid pre-treated corn stover revealed increased glucose release respectively, presumably due to priming of LPMO activity.

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