

Extracellular Secretion of Cellobiohydrolase 1 from *Trichoderma harzianum* in *Pichia pastoris* by Using Heterologous Expression and Revealing of Its Cellulolytic Enzyme Activity

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Trichoderma harzianum is a filamentous fungus that produces and secretes extracellular hydrolytic enzymes used in plant cell wall degradation. In this report, we identified a major unknown cellulase from *T. harzianum* and investigated characteristics of the cellulase using heterologous expression. One enzyme which had an exoglucanase activity was purified from *T. harzianum* through anion exchange chromatography. The purified enzyme revealed that it was cellobiohydrolase 1 (CBH1). Open reading frame of the *cbh1* gene was cloned in the expression vector pPICZαA and transformed into *Pichia pastoris* X-33. The CBH1 was expressed in BMMY medium and the specific activity was displayed against Avicel of 0.07 U/ml. Recombinant cellobiohydrolase1 (rCBH1) was purified from the culture by Ni-NTA affinity chromatography and the purity of the enzyme was checked by SDS-PAGE. Further studies were investigated for characterization of rCBH1 enzyme activity. Our results revealed the cellulolytic enzyme activity of rCBH1 and secreted rCBH1 relevant to biofuel production.