Expression and Characterization of Chimeric CBHI in Yeasts and the Effects of CBHI Fusion to EGII

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Expression of an Endoglucanase-Cellbiohydrolase Fusion Protein in S. cerevisiae, Y. lipolytica, and L. starkeyi

The low secretion levels of cellbiohydrolase I (CBHI) in yeasts is one of the key barriers preventing yeast from directly degrading and utilizing lignocellulose. To overcome this barrier, we have explored the approach of genetically linking an easily secreted protein to CBHI, with CBHI being the last to be folded. The Trichoderma reesei eg2 (7E6GII) gene was selected as the leading gene due to its previously demonstrated outstanding secretion in yeast. To comprehensively characterize the effects of this fusion protein, we tested this hypothesis in three industrially relevant yeasts: Saccharomyces cerevisiae, Yarrowia lipolytica, and Lipomyces starkeyi.

Our initial assays with the L. starkeyi secretome expressing differing 7E6GII domains fused to a chimeric Talaromyces emersonii – T. reesei CBHI (7E6GII-7E7CBHI) showed that the complete 7E6GII enzyme, including the glycoside hydrolase (GH) 5 domain is required for increased expression level of the fusion protein when linked to CBHI (Figure 1). We found that this new construct (7E6GII-7E7CBHI, Fusion 3) had an increased secretion level of at least three-fold in L. starkeyi compared to the expression level of the chimeric Te7TCBHI (results not shown) and significantly improved secretome level activity against Avicel (Figure 2). However, activity was observed for the pNP-lactose substrate (results not shown).

The same benefits do not extend to S. cerevisiae and possibly other organisms to increase secretion levels and specific activity of not only CBP-biofuels relevant pursuits, but more broadly in the context of general secretion of enzymes from yeast.

Table I. Yield of purified TeTrCBHI and HPLC data. Higher numbers for the RI/UV area ratio indicate higher glucan content

Conclusions

We tested the hypothesis that 7E6GII-7E7CBHI fusion construct in S. cerevisiae was poor and only minimal activity was observed on the substrate pNP-cellulose substrate and no activity was observed for the pNP-lactose substrate (results not shown).

Characterization of Chimeric CBHI Expressed in Y. lipolytica, L. starkeyi and S. cerevisiae and the Effects of Glycosylation

Yeasts are known to have problems with properly folding all the expressed CBHI protein. To understand the properties of chimeric Te7TCBHI expressed in yeasts we compared the yields of purified active protein. Table I shows the yield of purified Te7TCBHI from Y. lipolytica, L. starkeyi, and S. cerevisiae. L. starkeyi has the lowest level of active purified Te7TCBHI at 0.08 mg/L followed by Y. lipolytica with a yield of 1.09 mg/L – a more than 10-fold increase. S. cerevisiae has a production level of at least 3-fold higher than Y. lipolytica but with the final recovery of three distinct active Te7TCBHI isomers.

To assess the purity and extent of glycosylation we did SDS-PAGE with Coomassie blue, Western blot and glycosylation staining (Figure 1). All chimeric CBHI samples had multiple bands in Western blot and glycosylation staining indicating degradation and/or variable glycosylation. To thoroughly understand what was happening we analyzed all samples with HPLC size exclusion chromatography and compared with 7E6GII (Table I).

The chimeric Te7TCBHI purified from L. starkeyi and Y. lipomyces, only converted 70% of the available PCS cellulose compared to 80% for 7E6GII purified from its native host while peak 1 from S. cerevisiae only converted 60% (Figure 3). Despite taking the best fraction for S. cerevisiae expressed Te7TCBHI it still clearly underperforms compared to L. starkeyi and Y. lipomyces expressed chimeric Te7TCBHI. YCI7 is still more active by reaching a conversion extend of over 80% in 100 hours compared to around 70% for the other two.

Table I. Yield of purified TeTrCBHI and HPLC data. Higher numbers for the RI/UV area ratio indicate higher glucan content

Conclusions

Saccharomyces hyper glycosylates Te7TCBHI

Some activity loss but the best yield of active enzyme

S. cerevisiae has the best yield but also the lowest extent of conversion with purified protein

L. starkeyi expressed chimeric Te7TCBHI have similar extents of conversion but Yarrowia produces over ten times more purified and active chimeric Te7TCBHI

L. starkeyi is an inferior CBP candidate compared to Yarrowia and Saccharomyces

Both Yarrowia and Saccharomyces would likely perform well as CBP microorganisms

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