Development of *Saccharomyces cerevisiae* mutant over-expressing *Trichoderma reesei* cellobiohydrolase II.

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Developing a *Saccharomyces cerevisiae* mutant strain over expressing cellobiohydrolase enzyme has always been a drawback owing to the lack of efficient screening procedure. In our previous study, we found that the fusion of cellulose binding domain (CBD2) from *Trichoderma reesei* cellobiohydrolase II to a β-glucosidase (BGL1) enzyme from *Saccharomycopsis fibuligera* significantly hindered the latters expression and secretion in *S. cerevisiae*, suggesting that the possible low expression of cellulases in *S. cerevisiae* could be attributed to the presence of CBD in these enzymes. As a most ideal situation, in our present study, we used this chimeric enzyme (*CBGL1*) as a reporter enzyme for screening mutagenized yeast strains for increased ability to produce CBD associated enzymes like cellulases. The haploid *Saccharomyces* strain W303-1A, transformed with a yeast integration vector pCBGL1 containing the *CBGL1* gene under the PGK1 promoter and *MFα-S1* secretion signal, produced β-glucosidase activity of 65 U L^{-1}. On the average 75% of the total activity was secreted into the culture medium. The transformed W303-CBGL1 strain was mutagenized and the mutant WM91-CBGL1 was isolated exhibiting up to 200 U L^{-1} of activity, a 3-fold increase than the parent strain. The ability of the mutant strain to grow on cellobiose as sole carbon source was significantly improved in comparison to its parent strain. Subsequently, the mutant yeast strain transformed with vector pCBH2 containing the *T. reesei CBH2* gene produced cellobiohydrolase activity up to 2-fold more than the parent strain. The cellobiohydrolase enzyme from both the parent and the mutant strain were purified, and characterized for physical and hydrolytic properties.