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Heterogeneous expression and functional characterization of β-glucosidase gene from *Aspergillus niger* BE-2 and construction of its hyper-expression system in *Trichoderma orientalis* EU7-22 by (cbh1) promoter optimization

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The cDNA gene (AnBgL1), encoding GH3 family β-glucosidase (EC3.2.1.21) from *Aspergillus niger* BE-2 (abbreviated to AnBgL1) was amplified and inserted into the yeast expression pPIC9K vector at the site of Bln I (Avr II) and Not1. The recombinant expression vector designated as pPIC9K-AnBgL1 was transformed into *Pichia pastoris* GS115. The transformants were screened on a MD plate which inoculated on geneticin G418-containing YPD plates. The transformants expressed the high β-glucosidase activity of 22.6 U/ml. SDS-PAGE assay demonstrated that the AnBgL1 was extracellularly expressed with an apparent M.W. of 90.0 kDa. The purified AnBgL1 displayed the maximum activity at pH 6.0 and 60° C. It was highly stable at a broad pH range of 4.0-7.5, and at a temperature of 60° C. The K_m and V_{max}, towards p-NPG at pH 5.5 and 60° C were 1.45 mg/ml and 2,365 U/mg, respectively. The AnBgL1 displays high similarity to the β-glucosidases of *A. niger* (FN430671) and *A. niger* (DQ655704), the members of the GH3 family. The β-glucosidase gene (Bgl1) from *A. niger* was cloned and recombined with cbh1 optimized promoter (pcbh1) and terminator trpC. The expression cassette was ligated to the binary vector to form pUR5750-Bgl1 and then transferred into the host strain EU7-22 via *Agrobacterium tumefaciens* mediated transformation (ATMT) using hygromycin B resistance gene as the screening marker. Bgl-1 transformants was screened. The enzyme activities of filter paper (FPA) and β-glucosidase (BG) of transformants increased by 8.5% and 15.2% under induction condition, respectively compared with the host strain EU7-22. The results showed that the cbh1 promoter (pcbh1) has successfully driven the over-expression of Bgl1 gene in *T. orientalis* under glucose repression condition.

Biography

Nasir Ali has completed his PhD from Xiamen University. He is born in 1987 at KP province Pakistan. He has completed his Master's degree from University of Peshawar. He has published more than 5 papers in reputed journals and has been serving as an Editorial Board Member of repute.

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