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OVEREXPRESSION, PURIFICATION AND CHARACTERIZATION OF *Aspergillus niger* BE TA-GLUCOSIDASE IN *Pichia pastoris*

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ABSTRACT

This study describes the expression of ?-glucosidase (BglA) from *Aspergillus niger* in *Pichia pastoris* , a methylotrophic yeast strain, under the regulation of an alcohol oxidase promoter. The h eterologous expression of BglA was optimized in a shake flask.
Optimal conditions were achieved using an initial cell density (OD $_{600}$) of 4-5 and an inducer concentration of 2.5% methanol for 72 hours. A recombinant protein with a molecular weight of ~116 kDa was produced.
This recombinant BglA has optimal activity at 60°C in sodium acetate buffer at pH 4. T his enzyme is stable between pH 3.0-6.0 and retained more than 50% of its maximum activity at pH 6.0 after incubation at 60°C for 30 min. However, it lost almost 80% of its maximal activity at pH 7.0 under the same conditions. A thermostability assay of this enzyme revealed that BglA is relatively stable up to 60°C. This enzyme retained 50% of its original activity at 60°C but was completely inactive after incubation at 70°C for 30 min.
BglA showed highest activity and specificity towards the synthetic substrate *p* -nitrophenol-?-D-glucopyranoside with a specific activity of 347.62 U mg $^{-1}$ and a specificity constant of 466.19 mL mg $^{-1}$

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. BglA had a specific activity of 6.2 U mg
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and a specificity constant of 6.01 mL mg
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for cellobiose.

Key words: *Aspergillus niger*, ?-glucosidase, *Pichia pastoris*, heterologous expression