PURIFICATION AND CHARACTERIZATION OF β-GLUCOSIDASES FROM A THERMOTOLERANT YEAST *PICHIA ETCHELLSII*

by

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CERTIFICATE

This is to certify that the thesis entitled **"Purification and characterization of β-glucosidases from a thermotolerant yeast *Pichia etchellsii*"** being submitted by **Ms. ANU WALLECHA** to the Indian Institute of Technology, Delhi, for the award of the degree of '**DOCTOR OF PHILOSOPHY**', is a record of the bonafide research work carried out by her, prepared under my supervision in conformity with the rules and regulations of the 'Indian Institute of Technology', Delhi. The research report and the results presented in the thesis have not been submitted to any other University or Institute for the award of any other degree or diploma.

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ABSTRACT

The thermotolerant yeast *Pichia etchellsii* produced two inducible, cell wall bound β-glucosidases viz. BGLI and BGLII. The highest level of enzyme production occurred in the presence of 10 mM cellobiose as an inducer. The enzymes BGLI and BGLII were separated and purified from *P. etchellsii* by ammonium sulfate precipitation, ion-exchange and hydroxyapatite chromatography with an overall recovery of 17 % and 7.5 % respectively. The molecular weight of BGLI and BGLII were 186,000 Da (dimer of 102,000 Da) and 340,000 Da (tetramer of 90,000 Da) respectively.

The two enzymes were biochemically and kinetically characterized. BGLI and BGLII exhibited a similar pH and temperature optimum i.e., 6.0 and 50°C. Both the enzymes were stable in the pH range of 3.0-9.0. The half-life of BGLI and BGLII was above 7 h at 35 and 40° C. BGLI and BGLII differed in their preference of hydrolysis of different substrates. The hydrolysis of various substrates performed by BGLI were aryl-glucosides ≥ alkyl-glucosides ≫ disaccharides and for BGLII were disaccharides > aryl-glucosides ≥ alkyl-glucosides. BGLI was most active on salicin whereas BGLII displayed maximum activity on cellobiose. The K_M and V_{max} of BGLI and BGLII on pNPG were 0.33 mM, 55 μmol min^{-1} mg of $protein^{-1}$ (BGLI) and 0.33 mM, 25 μmol min^{-1} mg of $protein^{-1}$ (BGLII). The kinetic specificity of BGLI and BGLII were also determined for other substrates and is discussed. Both the enzymes were competitively inhibited by glucose and gluconolactone. The value of K_I of BGLI and BGLII for glucose and gluconolactone were 12, 0.12 mM (BGLI) and 25, 0.038 mM (BGLII). The metal ions were not found to increase the activity of BGLI and BGLII but Cu^{2+}, Fe^{2+} and Hg^{2+} ions were inhibitory. Activity of BGLI and BGLII was stimulated in the presence of 4 %, v/v 1-propanol (10 % stimulation of BGLI), 4 %,

v/v ethanol (10 % stimulation of BGLII). The purified enzymes displayed glycosyl transferase activity and the synthesis of different alkyl-glucosides was performed with purified BGLI and BGLII.

The residues essential for catalytic activity of BGLI and BGLII were determined by modification of BGLI and BGLII using chemicals specific for modification of Asp/Glu, His, Cys and Trp residues. Asp/Glu residues were found to be essential for catalysis of BGLI and BGLII as modification of these residues was protected by pNPG. His residues were also found to be essential for catalysis of BGLII. Other residues like Cys and Trp were found to be structurally important, as their modification caused complete loss in the activity of BGLI and BGLII and was not protected by any of the substrates or competitive inhibitor.

This work describes the isolation, purification, characterization and identification of likely amino acids involved in the action of β-glucosidases from a thermotolerant yeast *P. etchellsii*.

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