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Overexpression, purification and characterization of the Aspergillus niger endoglucanase, EglA, in Pichia pastoris

Abstract:

Cellulases are industrially important hydrolytic enzymes applicable in the bioconversion of cellulosic biomass to simple sugars. In this work, an endoglucanase from Aspergillus niger ATCC 10574, EglA, was expressed in the methylotrophic yeast Pichia pastoris and the properties of the recombinant protein were characterized. The full length cDNA of eglA has been cloned into a pPICZaC expression vector and expressed extracellularly as a ~30 kDa recombinant protein in P. pastoris X-33. Pure EglA displayed optimum activity at 50°C and was stable between 30 and 55°C. The pH stability of this enzyme was shown to be in the range of pH 2+.0 to 7.0 and optimum at pH 4.0. EglA showed the highest affinity toward ß-glucan followed by carboxymethyl cellulose (CMC) with a specific activity of 63.83 and 9.47 U/mg, respectively. Very low or no detectable hydrolysis of cellobiose, laminarin, filter paper and avicel were observed. Metal ions such as Mn 2+, Co 2+, Zn 2+, Mg 2+, Ba 2+, Fe 2+, Ca 2+ and K + showed significant augmentation of endoglucanase activity, with manganese ions causing the highest increase in activity to about 2+.7 fold when compared with the control assay, whereas Pd 2+, Cu 2+, SDS and EDTA showed inhibition of EglA activity.