Isolation of efficient cellulase producing Aspergillus unguis UCSC324 and determination of the kinetic properties of its crude cellulose

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Abstract: Bioengineering of cellulolytic enzymes with enhanced catalytic efficiency and thermostability is important in the commercialization processes. This study describes the isolation of efficient cellulase producing fungi and determination of the kinetic properties of the crude cellulase. Among the fungal strains isolated from cow dung, hot rice water, water used in autoclave and decaying coconut wood, the strains growing on decaying coconut wood was selected for this study because of the higher amount of cellulase production measured by the rate of zone of clearance on the Carboxymethylcellulose (CMC)sodium salt agar plates by Congo red test. The three isolated fungal strains isolated from coconut wood were identified and confirmed as Aspergillus niger FL17, Aspergillus oryzae CBS108.24 and Aspergillus unguis UCSC324 based on the morphological studies and molecular analysis done by amplifying the ITS5.8SrDNA sequence, PCR amplification and multiple sequence alignment. Since there had been no reports recorded about the production of cellulase from Aspergillus unguis UCSC324, kinetic properties of the cellulase from this fungal strain were studied. Fermentation medium contained (gL-1) 2.0g cellulose; 3.0g carboxymethyl cellulose; 0.3g ammonium sulphate and 100mL of distilled water was used at an optimal conditions of temperature 20±1ºC, pH7.0 for 5 days at 100rpm.Crude cellulase showed zero order kinetics for 5 minutes. When the activity of cellulase was measured at different temperatures ranging from 20°C to 75°Cat pH 7.0, the optimum temperature for the enzyme activity was 50°C. When the pH of the media was changed from 2.0 to 8.0, while temperature was kept at 50°C with 1g/100mL cellulose substrate, highest cellulase activity was observed at pH 5.0. Michaelis constant and the Vmax of the cellulase enzyme to soluble cellulose by Lineweaver-Burk Plot were4.545×10-2 moldm-3and 26.66 mgml-2mins-1respectively at pH 5.0 and 50°C. The crude enzyme was stable for at least 90 minutes at pH 5.0 and at 50°C. Since the cellulase enzyme from Aspergillus unguis was active in moderately acidic pH and showed better stability at 50°C, it could be a good candidate for the cellulase dependent industrial applications.

Keywords: Aspergillus unguis, Cellulase, Decaying coconut wood, Kinetic properties.