195g Purification and Characterization a Novel Thermostable Endoglucanase from a Mesophilic Fungus for Bio-Chemimechanical Pulp,Fusarium Oxysporum

Shuyan Liu, Xuemei Lu, Xinyuan Duan, and Peiji Gao

Cellulose is an abundant and renewable energy resource on earth. Microorganisms produce multiple enzymes to degrade cellulose, known as enzyme system. However,one of major drawbacks in industrial terms is that most cellulases apparently lack thermostability. Thermostable cellulolytic enzymes have been mainly isolated from thermophiles and hyperthermophiles.

In this paper, we found a typical mesophilic fungus F. oxysporum L19 is capable of producing multiple thermostable endoglucanases. They have the maximal activity at around 75°C, which are apparently much higher than the physiological growth optimum temperature of the organism. And the enzyme was stable over 60min incubated at 60°C. The strain was used for short time fermentation of wheat straw to produce bio-chemimechnical pulp.

The effect of temperature on the growth of F. oxysporum L19

The strain grew rapidly at 28°C, grew very slowly at 37°C; or 15°C; and did not grow above 45°C. This fact suggested that the strain was a typical mesophilic fungus.

Purification of thermostable endoglucanase

As a result of purification, at least four thermostable endoglucanases and several mesophilic endoglucanases were found in extracellular cellulases system of F. oxysporum L19. By DEAE-Sepharose FF, Bio-gel P100 and CM-Sepharose FF column chromatography, a thermostable endoglucanase was purified. The purified enzyme preparations were essentially homogeneous; showing a single band by SDS-PAGE with a mobility corresponding to an apparent molecular mass of 42.7 kDa and isoelectric focusing resulted in single band with pI value of 5.0.

Properties of the thermophilic endoglucanase

The thermal activity and thermostablity of the thermophicl endoglucanase have been examined following enzymatic activity as a function of temperature. It displayed the highest apparent activity towards carboxymethyl cellulose, sodium salt (CMC-Na) at 75°C and the enzyme was stable over 60min incubated at 60°C. Analysis of amino acid composition suggested it contains more proline residues. The N-terminal amino acid sequence of it is SYRVPAANGFPNPDASQEKQ. Extensive alignment results showed it was not homologous to any previously known endoglucanases from the same organism or other species including thermostable endoglucanases that have been published.

Properties of bio-chemimechanical puple treated with the fungus

After pretreatment with the fungus for only 5 days, the wheat straw fibres were obviously separated, resulting in the improvement of the chemicals immersion efficiency during pulping.

Conclusion

A thermostable extracellular endoglucanase was first purified from Fusarium oxysporum L19 which is a typical mesophilic fungus. The result of treating wheat straw fibres with the fungus proved that short time treatment of wheat straw with non white-rot fungi has a good applying outlook on bio-pulping. Further research focuses on the gene characterization of the novel thermophilic endoglucanase and

relationship between a series of thermophilic endoglucanases produced by the fungus and properties of biopulping pretreated by the fungus.