XYLANASES OF *MELANOCARPUS ALBOMYCES* IIS 68 BIOSYNTHESIS, PURIFICATION AND CHARACTERIZATION

BY

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CERTIFICATE

This is to certify that the thesis titled '**Xylanases of Melanocarpus albomyces IIS-68 - Biosynthesis, Purification and Characterization**', being submitted by **Mr. Vibhor Saraswat** to the Indian Institute of Technology, New Delhi, for the award of the degree of 'Doctor of Philosophy', is a record of the bonafide research work carried out by him, has been prepared under my supervision in conformity with rules and regulations of the 'Indian Institute of Technology, Delhi'. The research report and results presented in the thesis have not been submitted for any degree or diploma in any other University or Institute.

V.S. Bisaria

Prof. V.S. Bisaria

XYLANASES OF *MELANOCARPUS ALBOMYCES* IIS 68 - BIOSYNTHESIS, PURIFICATION AND CHARACTERIZATION

ABSTRACT

Xylan is the major component of monocotyledon hemicelluloses of plant cell walls in which ß-1,4-linked D-xylose residues constitute the backbone of xylan. After cellulose, xylan is the most abundant renewable polysaccharide in nature, accounting for 20 to 30% of the dry weight of woody tissue. In order to maximize the hydrolysis of lignocellulosic residues by cellulase enzymes, synergistic action of xylanase is also needed. For effective degadation of xylan a combined action of xylanase, ß-xylosidase, and xylan debranching enzymes is required. Xylanase is produced by a wide range of bacteria and fungi. Melanocarpus ***albomyces*** IIS 68, isolated from compost and soil, is a good producer of xylanase. The thermophilic fungus, M. ***albomyces*** was used to achieve the following objectives of the present investigation: Selection of best carbon source for production of xylanase and ß-xylosidase; induction of xylanase, ß-xylosidase and xylan debranching enzymes by various carbon sources; purification and characterization of xylanase isoenzymes; to study the effect of various carbon sources in terms of synthesis of specific xylanase isoenzymes; and to assess the potential of xylanases as prebleaching agents in pulp and paper industry.

In order to select the best carbon source for the production of xylanase and ß-xylosidase by M. ***albomyces, the*** fungus was grown on various carbon sources and wheat straw was found to be the best carbon source for xylanase (172 IU/ml) and xylose for ß-xylosidase (0.84 IU/ml) production. A maximum of seven extracellular xylanase isoenzymes were detected in the wheat straw grown-culture of M. ***albomyces. The*** xylan debranching enzymes namely, acetyl esterase , α -L-arabinofuranosidase and a-D-

glucuronidase, were also found to be inducible. The best carbon sources for the biosynthesis of acetyl esterase, α -L-arabinofuranosidase and a -D-glucuronidase, were wheat straw, arabinose and birch wood xylan, respectively. All the seven xylanase isoenzymes were purified using Sephadex G-75, DEAE-Sepharose, Sephadex G-50 and Sephacryl S-100 column chromatographic procedures. The molecular weights of purified isoenzymes namely, Ia, Ib, Ic, IIa, IIb, IIc and IId, were determined by to be 22,900; 20,650; 18,600; 31,300; 25,400; 38,500 and 34,300, respectively. The isoelectric points of Ia, Ib, Ic, IIa and IIb were 6.15, 6.25, 6.40, 3.70 and 4.35, respectively. All isoenzymes were also characterized with respect to pH and temperature optima, thermal stability and substrate specificity. The ranges of K, and Vmax of xylanase isoenzymes were 0.4 to 6.9 mg/ml and 16.39 to 198 a μmol/min.mg respectively, for the substrate on which the purified isoenzyme had the maximum activity. The highest value of turnover number (83.82 s^{-1}) and specificity constant (133.62 ml/mg s) were obtained for the isoenzymes IIb and Ic, respectively. The synergistic action of the xylanase isoenzymes were confirmed by the increased xylan saccharification as compared to the results obtained when xylanase isoenzymes were used individually. Moreover, ß-xylosidase and xylan debranching enzymes brought about enhanced xylan degradation when acted in combination with xylanases. The major product of xylan hydrolysis was xylose in case of Ib and IIa, whereas xylooligosaccharides (larger than xylobiose) were the major products in case of Ia, Ic, IIb, IIc and IId. The arabinose releasing property was detected in Ia, Ib, Ic and IIa. The xylanase system of M. ***albomyces*** was found to be effective in prebleaching of bamboo pulp. An enzymatically pretreated pulp, 'which had been subjected to chemical treatment, was found to have less kappa number, more pulp brightness and more pulp viscosity, .as compared to the chemically treated pulp.

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