

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

BY

VIBHOR SARASWAT
**Department of Biochemical Engineering
and Biotechnology**

*THESIS SUBMITTED
FOR THE AWARD OF THE DEGREE OF
DOCTOR OF PHILOSOPHY*



**INDIAN INSTITUTE OF TECHNOLOGY, DELHI
HAUZ KHAS, NEW DELHI - 110016, INDIA
MARCH 1997**

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.



Prof. V.S. Bisaria

XYLANASES OF *MELANOCARPUS ALBOMYCES*
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ABSTRACT

Xylan is the major component of monocotyledon hemicelluloses of plant cell walls in which β -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 degradation of xylan a combined action of xylanase, β -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 β -xylosidase; induction of xylanase, β -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 β -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 β -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 α -D-

glucuronidase, were also found to be inducible. The best carbon sources for the biosynthesis of acetyl esterase, α -L-arabinofuranosidase and α -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_m and V_{max} of xylanase isoenzymes were 0.4 to 6.9 mg/ml and 16.39 to 198 μ mol/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, β -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.

ACKNOWLEDGEMENT

I am indebted to my Ph.D. Supervisor, Prof. **V.S. Bisaria**, for his invaluable guidance and encouragement in my work. His inspiration made me achieve the objectives of the project. At the time of problems he was always there to solve them at the earliest.

I am thankful to the faculty members of the department for their interest and suggestions regarding my project.

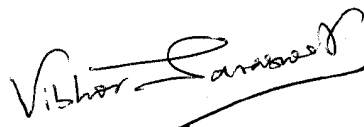
I thank the members of supporting staff of the department, especially those associated with Biochemical Research Lab for their constant help. I am thankful to **Mr. C.P. Kalra, Mr. Subhash Anand and Mrs. Sunita Varma** for their help in various official matters,

I express my gratitude to **Dr. Manjula Pandey** for her intelligent suggestions all through my work and her involvement in the typing, proof reading and thesis compilation which led to timely submission of this thesis.

I am thankful to **Mr. Neeraj Chauhan** for patiently bearing with me, especially in the last six months,

I acknowledge the help of all my friends whose company made the period of my -stay at IITD, a memorable one.

Last, but not the least, I am grateful to my parents for their constant encouragement and support.


(VIBHOR SARASWAT)

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