

**PURIFICATION AND CHARACTERIZATION
OF β -GLUCOSIDASES FROM A
THERMOTOLERANT YEAST
*PICHIA ETHELLSII***

by

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Submitted

in fulfillment of the requirements of the degree of Doctor of Philosophy

to the



INDIAN INSTITUTE OF TECHNOLOGY, DELHI

DECEMBER 2000

CERTIFICATE

This is to certify that the thesis entitled “**Purification and characterization of β -glucosidases from a thermotolerant yeast *Pichia etchellsii*”** being submitted by **Ms. ANU WALLECHA** to the Indian Institute of Technology, Delhi, for the award of the degree of ‘**DOCTOR OF PHILOSOPHY**’, is a record of the bonafide research work carried out by her, prepared under my supervision in conformity with the rules and regulations of the ‘Indian Institute of Technology’, Delhi. The research report and the results presented in the thesis have not been submitted to any other University or Institute for the award of any other degree or diploma.


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ACKNOWLEDGEMENT

I would like to thank my supervisor Dr. Saroj Mishra for her enormous contribution in allowing me to do independent research and her numerous discussions in which she sharpened and shaped my scientific ability and moulded my perceptions of “ the spirits of scientific approach and enquiry”. I shall never fail to remember her optimism, constant support and encouragement that stood by me in the dark hours of my laboratory. My warmest thanks are due to her unfailing helpful advice at every stage and having made task easier in all ways a gifted and devoted guide can.

I take this opportunity to express my deep gratitude to Prof. V. S. Bisaria, Prof. M. N. Gupta, Prof. R. Bhatnagar and Dr. Prashant Mishra for their generous advice and also for their sustained reasonableness. Thanks to head of the Deptt. Prof. G. P. Aggarwal and all the faculty members for their help and support.

I am grateful to Mr. V. K. Ghosh who contributed his help in numerous ways. I shall ever be obliged with his care in giving me ideas and making things available to me. I am also thankful to Mr. Sharma, Mr. Mukesh and Mr. Kishan for making me available with equipments and chemicals. A special thanks to Ramgopal and Meharchand for providing with washed glasswares in time.

It would have been impossible for me to complete my work without the resources of BTIS and Doc. Unit and the courtesies of Sapan, Roshni, Mrs. Mathur, Sunita and Neera madam.

My greatest debts of gratitude are to my friends Savita and Shipra for many years of support and generous assistance and for help throughout. My special thank to them for making my stay in hostel comfortable and for their undemanding help and unceasing support. I am thankful to Chitra and shall never cease to admire the way in which she stood beside me.

A special acknowledgement is due to Yukti for all her help and constructive criticism. I shall be never be able to reciprocate her timely help and unconditional support. I would like to make a special thanks to Shivani and Salony for their good humour, which acted as an enthusiasm to my work. My thanks are due to Manjula and Benu for their help at initial stages. I am also thankful to other number of friends and colleagues, Vandana, Malini, Preeti, Pranita and all others for their hospitality and invaluable advice. Special thanks to Shivendra for his generosity.

I'm deeply beholden to my parents for their painstaking care, warm hospitality and constant encouragement. My special thanks to my brother and sisters for their love and patiently putting up in my absence. I would like to extend thanks to my husband Gurpreet who stood by me through thick and thin and offered me support and encouragement in whatever way he could have. I am also thankful to my in laws for their support.

Anu Wallecha
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v/v ethanol (10 % stimulation of BGLII). The purified enzymes displayed glycosyl transferase activity and the synthesis of different alkyl-glucosides was performed with purified BGLI and BGLII.

The residues essential for catalytic activity of BGLI and BGLII were determined by modification of BGLI and BGLII using chemicals specific for modification of Asp/Glu, His, Cys and Trp residues. Asp/Glu residues were found to be essential for catalysis of BGLI and BGLII as modification of these residues was protected by pNPG. His residues were also found to be essential for catalysis of BGLII. Other residues like Cys and Trp were found to be structurally important, as their modification caused complete loss in the activity of BGLI and BGLII and was not protected by any of the substrates or competitive inhibitor.

This work describes the isolation, purification, characterization and identification of likely amino acids involved in the action of β -glucosidases from a thermotolerant yeast *P. etchellsii*.

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