ENGINEERING OF β-GLUCOSIDASE I OF *Pichia etchellsii* FOR ENHANCED ORGANIC SOLVENT AND THERMAL TOLERANCE AND ITS USE IN ARYL-OLIGOSACCHARIDE SYNTHESIS

JYOTI BATRA

Department of Biochemical Engineering and Biotechnology

INDIAN INSTITUTE OF TECHNOLOGY

OCTOBER 2014

ENGINEERING OF β-GLUCOSIDASE I OF *Pichia etchellsii* FOR ENHANCED ORGANIC SOLVENT AND THERMAL TOLERANCE AND ITS USE IN ARYL-OLIGOSACCHARIDE SYNTHESIS

by

JYOTI BATRA

Department of Biochemical Engineering and Biotechnology

Submitted

In fulfillment of requirement of degree of

DOCTOR OF PHILOSOPHY

to the

INDIAN INSTITUTE OF TECHNOLOGY

OCTOBER 2014

.....Dedicated to my
Dear Parents

CERTIFICATE

This is to certify that the thesis entitled **"Engineering of β-glucosidase I of *Pichia etchellsii* for enhanced organic solvent and thermal tolerance and its use in aryl-oligosaccharide synthesis"** being submitted by **Ms. Jyoti Batra** 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, which has been prepared under my supervision in conformity with the rules and regulations of the 'Indian Institute of Technology Delhi'. The research reports and the results presented in this thesis have not been submitted for any degree or diploma in any other University or Institute.

Prof. Saroj Mishra

(Department of Biochemical Engineering

and Biotechnology)

Indian Institute of Technology Delhi

ACKNOWLEDGEMENT

An endeavor cannot be completed successfully single-handedly. A few people work together for it. And it is the time to acknowledge those intellectuals in successful completion of my project work.

First and foremost, I bow down to almighty for showering His blessing on me for successful completion of this phase of my life and for all blessings He has conferred upon me.

I am extremely thankful to my supervisor Prof. Saroj Mishra for her valuable guidance on different aspects of the thesis. She always gave patient hearing to my work and her wide knowledge helped formulating this thesis in a good way. Her faith in me and the liberty that she gave me has helped in bringing out the best in me. It is indeed a profound privilege to work under her distinguished guidance.

I sincerely express deep gratitude to the members of my 'Scientific Research Committee', Prof. Sunil Nath, Prof. Prashant Mishra and Dr. S.K. Khare for guiding me through all these years and critically reviewing the progress of this research work to make necessary improvements.

I would like to gratefully acknowledge the support of my seniors Md. Asif Shah, Richa Baranwal and Meenu Chhabra for helpful discussions and for their help rendered in carrying out the experiments. I would also like to specially thank my friends Younis Rather, Prabha Arya, Gayatri, Rani Verma, Sarvesh Singh, Nisha, Varsha and Hema for their scintillating cooperation and mental support during the exhaustive work period which was of great importance to me. I am extremely thankful to my best friend Sunaina for her unconditional support and timely help. I am also thankful to Saumya Jain, B. Arun, B. Gopi, Shweta, Beri, Humayra, Anshul, Arpita and Nitu for making me feel very friendly in lab hours and immense help by them whenever needed.

Special thanks to my parents who have put in their efforts and prayers for me to attain success in life. I am falling short of words to express my feelings towards them. Their blessings and encouragements were beyond comparison. I am greatly thankful to my brother and sister for their motivation.

Jyoti Batra

ABSTRACT

Biotransformation in organic solvents has numerous advantages over reactions carried out in aqueous medium. Glycosyl hydrolases have been used as enzymes of choice for synthesis of glycosidic bonds but these enzymes exhibit low stability in organic solvents. In the present study, β-glucosidase I (BGLI) from thermo-tolerant yeast *Pichia etchellsii* was taken as the candidate enzyme for generating an organic solvent tolerant variant. The objective was to understand reaction chemistry of such a system. *BGL1* gene coding for BGLI enzyme was subjected to random mutagenesis using error prone PCR. Mutant gene library was transformed in to *E. coli* and 500 colonies were screened for recombinant plasmids. Plasmid DNA isolated from positive bacterial transformants was pooled together and electroporated into *Pichia pastoris* GS115 host cells. Out of a large number (200) of yeast transformants, 21 transformants were selected for functional expression on MUG plates. These transformants were further screened for organic solvent tolerant β-glucosidase activity in presence of acetonitrile, DMF, DMSO, methanol and 2-propanol. The wild type enzyme (WT-BGLI) was taken as reference for all these studies. Enzyme variant expressed by one transformant (namely, EP14) exhibited 1.7-10 fold higher stability in presence of DMF, 2-propanol and methanol than that of the wild type enzyme. Mutations imparting stability were identified by sequencing the entire length of *bgl1* amplified from the genomic DNA of *Pichia pastoris*. Six substitutions were observed, three of which were silent and the other three (G414D, Y722H, N789D) led to changes in amino acid sequence. Interactions among mutated amino acids and neighbouring residues favored formation of new salt bridges as well as new hydrogen bonds. The organic solvent tolerant variant (OT-BGLI) was also found to possess higher thermal stability. Half-life values of OT-BGLI were 5.02 h, 2.45 h and WT-BGLI were 2.22 h, 2.1 h at 40 °C and 45 °C respectively. At 50 °C and 55 °C, the half-

life values for OT-BGLI were 1.52 h and 0.14 h while that for the WT-BGLI were 0.43 h and 0.04 h respectively. Tm of OT-BGLI (62.4 °C) was higher than that of WT-BGLI (61 °C) as determined from thermal denaturation curve obtained from CD spectroscopy. No major changes in secondary and tertiary structures were observed between OT-BGLI and WT-BGLI. Kinetic parameters (Km and Vmax) were also nearly similar for both the enzymes.

Autocondensation reactions were favored by OT-BGLI in presence of 2-propanol (10%, v/v) with aryl glycosides which acted as an acceptor and a donor for the glycosyl moiety. OT-BGLI catalyzed synthesis of aryl disaccharides in regioselective manner in aqueous-organic medium. Also, the rates of reactions catalyzed by OT-BGLI were higher than that of WT-BGLI. Products were purified and characterized by ESI-MS and NMR. The study thus demonstrates immense potential of organic solvent tolerant β-glucosidase in carrying out reactions in aqueous-organic medium for synthesis of oligosaccharides.