# ENZYME CATALYZED TRANSGLYCOSYLATION FOR SYNTHESIS OF ALKYL-GLUCOSIDES AND OLIGOSACCHARDES USING CELL BOUND α-GLUCOSIDASE FROM *Microbacterium sp.*

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**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**

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## DEDICATION

*TO MY DEAR PARENTS AND MY LOVING*

*BROTHER SAURABH*

# CERTIFICATE

This is to certify that the thesis entitled **"Enzyme catalyzed transglycosylation for synthesis of alkyl-glucosides and oligosaccharides using cell-bound α-glucosidase from *Microbacterium sp.*"** being submitted by **Ms. Swati Ojha** 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 our supervision and guidance 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.

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# ABSTRACT

The use of whole cells displaying cell bound α-glucosidase activity for synthesis of alkyl-α-D-glucosides, –polyglucosides and isomaltooligosaccharides was demonstrated for a bacterial isolate. Alkyl-α-D-glucosides are used, in various pharmaceutical applications, as initiators in manufacture of rigid polyurethane foams, as surfactants, humectants and viscosity modifiers. Isomalto-oligosaccharides are multifunctional health molecules that act as prebiotics. These cause least flatulence, are of low glycemic index and non-cariogenic.

The morphological and biochemical tests indicated that the isolate belonged to the genus of *Microbacterium.* The molecular characterization by 16S rRNA gene sequencing showed that the sequence bore 99% similarity with *Microbacterium paraoxydans* strain 3131 and strain 76. G+C content of the isolate was determined to be 69±2 mol%. Further, physiological studies on growth in presence of sugars and salts indicated that the isolate was moderately salt-tolerant and able to grow in LB containing up to 4% NaCl. The isolate could also be grown on high concentration of sucrose (30%) and glucose (20%) after a brief period of adaptation to these sugars. To characterize the cell bound glycosyl hydrolase activity, different substrates with various linkages were tested and it was found that the cells were most active on α-1,4 linked substrates. Kinetic parameters of the cell bound α-glucosidase were compared with the purified α-glucosidase. The peptide mass fingerprinting of the purified α-glucosidase showed homology with the published α-glucosidase of family 13. The cell bound α-glucosidase activity was also determined in the presence of various organic solvents by taking solvent adapted cells and unadapted

cells. Almost 65% activity was retained in 5% DMSO. The solvent adapted cells gave higher residual hydrolytic activities as compared to the unadapted cells.

Transglycosylation reactions were carried out using maltose and hexanol as a glycosyl acceptor molecule in monophasic and bi-phasic reaction systems. Optimization of water activity, time profile, substrate concentration and enzyme units lead to nearly 25% conversion. Addition of co-solvents (DMSO, benzene, toluene) in the synthesis reaction mixture did not lead to any significant increase in yield. The yield increased drastically in the bi-phasic system where an increase in water content from 10-60% lead to 60% conversion with a total yield of 11 g/l. The synthesized hexyl glucoside was extracted and purified and the structure determined by NMR.

The whole cells were further evaluated for synthesis of oligosaccharides. Maltose was chosen for the synthesis of isomalto-oligosaccharides by transglycosylation. Time profile of the reaction, substrate concentration, enzyme units were optimized and about 23% conversion was obtained at 400 g/l maltose. Addition of salt such as LiCl increased the yield by nearly 15%. The degree of polymerization of the formed products was determined by ESI-MS and the structures elucidated by $^{1}H$ and $^{13}C$ NMR analysis. To improve the performance, the cells were immobilized in Ca-alginate beads and packed in a packed bed reactor. The bioreactor gave nearly 4 times higher productivity than the free cells.

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