MICROBIAL PRODUCTION OF TANNASE AND ITS APPLICATION IN HYDROLYSIS OF TANNINS AND SYNTHESIS OF GALLIC ACID ESTERS

by

MALINI SETH

DEPARTMENT OF BIOCHEMICAL ENGINEERING AND BIOTECHNOLOGY

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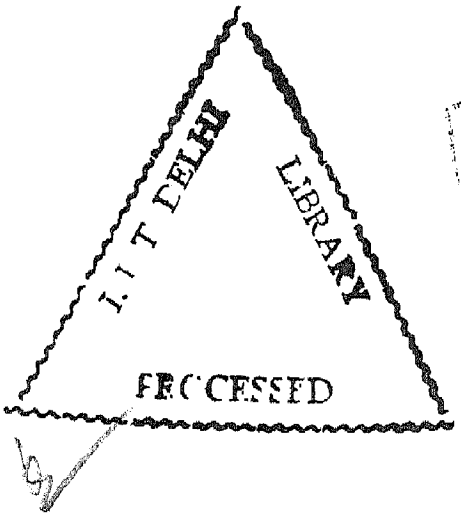

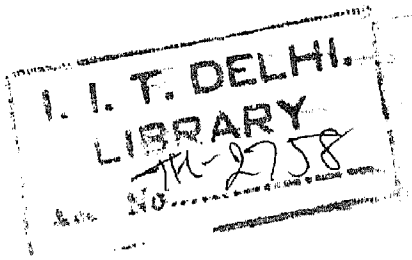

Microbial biotechnology

CERTIFICATE

This is to certify that the thesis entitled " **Microbial Production of Tannase and its Application in Hydrolysis of Tannins and Synthesis of Gallic acid Esters**', being submitted to the Indian Institute of Technology, Delhi, for the award of Degree of Doctor of Philosophy, has been prepared under my supervision and guidance in conformity with the rules and regulations of Indian Institute of Technology, Delhi. The Research report and results presented in the thesis have not been submitted to any other University or Institute for the award of any other degree or diploma.

Subhash Chand, Professor
Dept. of Biochemical Engineering and Biotechnology,
Indian Institute of Technology, Delhi
Hauz Khaz, New Delhi-110016

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Malini Seth

MALINI SETH

ABSTRACT

Tannin acyl hydrolase is an industrially important enzyme produced by a number of fungi. *Aspergillus awamori* was selected as the potential producer of intracellular tannase. Using Plackett-Burman's 2-factorial design, the tannic acid concentration, agitation speed and pH during the fermentation were identified as important process parameters affecting the cell growth and enzyme synthesis. These parameters were optimized in a laboratory bioreactor by Response Surface Methodology using Box and Benkehn's experimental design for enzyme production and gallic acid accumulation. Under optimum conditions for enzyme synthesis, the fermentation run lasted 60 hours with an initial tannic acid concentration of 35.0 g l^{-1}, yielding biomass concentration of 7.13 g l^{-1} containing 771 IU of intracellular tannase per gram dry cell weight and 19 g l^{-1} of gallic acid. However, maximum gallic acid accumulation (40.3 g l^{-1}) was obtained in 24 hours with an initial substrate concentration of 45 g l^{-1}. Intracellular tannase was isolated by disintegrating the cells. Different strategies based on ultrafiltration; ammonium sulphate fractionation and sorption were studied to get the purified enzyme sample for immobilization to DEAE-cellulose. The enzyme sample obtained after the above strategy had the specific activity of 53.6 IU mg^{-1} protein with purification factor of 134 and 68 % yield.

The purified enzyme sample was also used to study the mode of action of tannase on gallotannins. Tannase contains both the depsidase and esterase activities which hydrolyze depside and ester bonds of gallotannins respectively. Assuming the two reactions to follow Michaelis and Menten's kinetics and their stepwise action on the tannins, a kinetic rate expression has been developed. A reasonable fit between the experimental and theoretical release profiles confirmed the proposed hypothesis.

The immobilized enzyme, whole cells and the permeabilized mycelia were used to carry out the hydrolysis of gallotannins to gallic acid. The permeabilized cells were used for the hydrolysis of gallotannins in a stirred tank reactor in batch/continuous mode. The immobilized enzyme was also used to synthesize gallic acid esters as the permeabilized mycelia showed a very low esterification rate. The effect of nucleophilic donor, temperature and water activity was determined on the esterification rate. The immobilized enzyme reactor for the production of propyl gallate was operated in a batch mode for a series of 10 runs exhibiting a reasonably good operational stability.

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