STUDIES ON INTERFERON-α2b PRODUCTION USING *PICHIA PASTORIS*

By

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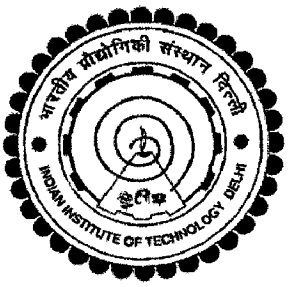
Submitted
in fulfillment of the requirement of the degree of

DOCTOR OF PHILOSOPHY

to the



INDIAN INSTITUTE OF TECHNOLOGY DELHI

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DEDICATION

to my Uncle, late Shri. G. K, Pradhan

CERTIFICATE

This is to certify that the thesis entitled "Studies on interferon-α2b production using *Pichia pastoris*" being submitted by Mr. Anand Ghosalkar 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 him, which has been prepared under our 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.

Dr. Aradhana Srivastava

Dr. Vikram Sahai

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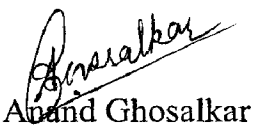

Anand Ghosalkar

Abstract

The objective of the thesis was to study the production of human interferon-α2b (IFN-α2b) in *Pichia pastoris*. Gene coding for IFN-α2b was cloned and expressed in *P. pastoris* under the control of alcohol oxidase promoter (*AOX1*) using three different secretion signals. Native secretion signal of IFN-α2b, *Saccharomyces cerevisiae* MF-α factor prepro sequence and a mutated α prepro sequence without the Glu-Ala (EAEA) repeats were used separately for directing the secretion of IFN-α2b into the culture medium of *P. pastoris*. The mutated α prepro sequence secreted a maximum of 200 mg/L IFN-α2b into the culture medium, with the same amino acid sequence as that of the native IFN-α2b secreted by human lymphocytes.

A chemically defined medium was optimized for maximum biomass production of recombinant *P. pastoris* in the fermentor cultures using glycerol as the sole carbon source. The response surface methodology was used for the optimization of nutritional requirements of salts, trace metals and vitamins for the growth of recombinant *P. pastoris*. Using the optimized medium biomass yield of 0.55 g dry cell weight/g of glycerol was achieved in a batch culture. In the chemically defined medium, recombinant IFN-α2b was produced by fed-batch fermentation of recombinant *P. pastoris* in three phases; batch phase, transition phase (glycerol fed-batch phase) and methanol fed-batch phase. Exponential feeding of glycerol was used in the transition phase and a step increasing feeding of methanol was used during the methanol fed-batch phase. Maximum IFN-α2b production of 363 mg/L was achieved by maintaining a specific growth rate of 0.08 h^{-1} during the transition phase.

CONTENTS