INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

Name of the Student : **KATLA SRIKANTH**

Roll Number : **126106006**

Programme of Study : **Ph.D.**

Thesis Title:
Cloning, Expression, Process Analytical Technology (PAT) Enabled Monitoring and Control of Glycoengineered *Pichia pastoris* Cultivation for Human Interferon α2b Production

Name of Thesis Supervisor(s) : **Dr. Senthilkumar Sivaprakasam**

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Key words for description of Thesis Work : **Interferon alpha 2b, *Pichia pastoris*, Process Analytical Technology, Glycosylation**

SHORT ABSTRACT

Recombinant huIFNα2b has been expressed on various platforms, which includes bacteria, yeast, insect cell lines, mammalian cell lines and plants. Expression of recombinant huIFNα2b in *E. coli* yielded high huIFNα2b titer but the expressed protein forms inclusion bodies and loses the activity during renaturation process. Also, the expressed protein lacks post-translational modifications and exhibited very less plasma life. The plasma life of huIFNα2b expressed by *E. coli* increased to certain extent through pegylation but could affect the patient's quality life on long-term use. To achieve the post translational modifications of the recombinant huIFNα2b insect cell lines and mammalian cell lines were considered as viable platform. But, the expressed huIFNα2b yield is low and the protein is partially glycosylated. Mammalian cell lines were used to express rightly processed glycosylated huIFNα2b but it is limited by low yield, high cost, challenges of scale-up, and the problems associated with purification. Few literature reports are available for the expression of recombinant huIFNα2b in *P. pastoris* but failed to report the extent of post translational modifications of the expressed protein and the maximum protein yield reported was 600 mg/L. The present thesis work is aimed to address the gaps pertaining to reported literature. In this work a glycoengineered *P. pastoris* clone expressing homogenous human-like N-glycosylated huIFNα2b was achieved using N-Glycoengineering approach. Medium optimization and Process Analytical Technology (PAT) based real-time monitoring and control studies (Dielectric Spectroscopy) resulted in enhanced expression of recombinant huIFNα2b (1.48 g/L). Finally an optimum mixed feed strategy (Methanol + sorbitol) was deduced using Biocalorimetry as PAT tool for real-time monitoring. This strategy resulted in reduced biomass heat yield coefficient ($Y_{Q/X}$), Oxycalorific value ($Y_{Q/O2}$) and enhanced expression. The purified glycosylated huIFNα2b of the present thesis work was biologically active exhibiting antiviral and antiproliferative properties and also with increased pharmacokinetic property (plasma half-life)