INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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SHORT ABSTRACT

IFN-γ is a dimerized, pleotropic cytokine and plays an important role in stimulating immune reactions. It is also critical for innate and adaptive immunity against viral and intracellular bacterial infections. With its already approved FDA status for the treatment of two diseases and excellent indications and positive results in the treatment of Tuberculosis and MDR-TB and other diseases, IFN-γ has the potential of becoming a lifesaver drug. IFN-γ as a drug is available in majority of developed/developing countries throughout the world; however it is not available in India. A cost effective method for the production of Recombinant Human IFN-γ can reduce the cost of treatment of chronic granulomatous disease and malignant osteopetrosis significantly which currently stands as $50000/year. In this study Recombinant Human IFN-γ was expressed in two separate expression systems a) *E. coli* GalG20 and b) *Kluyveromyces lactis* GG799. *E. coli* GalG20 strain is newly developed strain (originally MG1655 strain) developed by metabolic engineering tools where *endA, recA* and *pgi* genes of metabolic pathway have been knocked out to redirect the carbon flow into the pentose phosphate pathway and to promote nucleotide biosynthesis. Originally this strain was developed for enhanced plasmid biosynthesis for the purpose of plasmid DNA vaccine. We hypothesized that it could be a hyper protein producer strain by the virtue of its enhanced plasmid producing capabilities. For the purpose of expressing IFN-γ in GalG20 strain, this plasmid producing strain was engineered into a protein producing strain by λDE3 lysogenization technique. GalG20DE3 produced 230±20 mg/l of IFN-γ i.e. 1.5 fold more IFN-γ as compared to BL21DE3, a most commonly strain used for protein production, and 3.3 fold more as compared to its parent strain MG1655DE3. *Kluyveromyces lactis,* a "budding yeast" from phylum Ascomycota was developed as an alternative host to *Escherichia coli* GalG20DE3 for the production of IFN-γ. Five physical parameters (pH, agitation rate, incubation temperature and time, age of inoculum) for IFN-γ expression were optimized. After the optimization of physical parameters for IFN-γ expression a total increase of 2.2 fold in the expression level was observed. Production medium was optimized by assessing the tolerance level of carbon source by *K. lactis* cells and substrate inhibition was observed over and above 80 gms/litre of lactose. Response surface methodology and aritificial neural network was used for the optimization of complex growth medium. IFN-γ purification was performed using reverse miceller extraction. IFN-γ production in bioreactor yielded a total biomass of 25±3 g/l and a 6 fold increase in IFN-γ protein (1100±80 µg/l) under optimized conditions.