EXECUTIVE SUMMARY
UGC-MAJOR REASEARCH PROJECT
F. 41-571/2012 (SR)

"Development of a cost-effective strategy for production of recombinant human tumor necrosis factor alpha (TNF alpha) in *Escherichia coli*"

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Executive Summary

Summary of the UGC-Major research project entitled "Development of a cost-effective strategy for production of recombinant human tumor necrosis factor alpha (TNF alpha) in *Escherichia coli*" sanctioned to Dr. Sanjay Kumar, Assistant Professor, Department of Microbiology, Maharshi Dayanand University, Rohtak-124001 by UGC New Delhi (F. No 41-571/2012 (SR))

SUMMARY

Tumor necrosis factor-alpha (TNF-α) is a cytokine which plays a major role in inflammation, immunity and cellular organization. The present study was undertaken to overproduce recombinant hTNF-α in *Escherichia coli* by optimization of paramount influencing factors related to the target gene, expression system, and culture conditions. The plasmid-based expression of a codon-optimized gene sequence under control of T7 promoter could yield a substantial amount (207 mg/g DCW) of insoluble rhTNF-α in *E. coli*. The optimization of media and other culture parameters increased the yield up to 1.26 g/L in the bioreactor. A series of chemostat cultures operated at 0.2 h^{-1}, 0.3 h^{-1} and 0.4 h^{-1} dilution rates revealed a positive correlation between pre-induction specific cell growth rate (μ) and protein formation kinetics (q_p) for $\mu \leq 0.4 h^{-1}$. A fed-batch cultivation, employing all the pre-optimized parameters, gave a volumetric yield of 5.5 g/L with 36 g dry cell weight and ~29 % of total cellular protein. The inclusion bodies of rhTNF-alpha were refolded and purified with 54.7 % overall yield. The bioprocess and downstream strategies employed in the study could yield a high amount (up to 3g/L) of purified soluble rhTNF-α.

FUTURE PERSPECTIVE

The production strategy developed in this study can give an overall yield of 3 g/L soluble rhTNF-α. As a working volume of 3.5 L was used for fed-batch culture, thus total 10.5 g purified protein can be produced form single run of fed-batch. This amount is sufficient for preparing nearly ten thousand vials (1 mg/vial) of rhTNF-α. The cost of a package of 4 vials (Beromun) in the present market is ~ 8.7 lakh rupees. Thus, a technology designed in this study can be used for producing TNF-α at industrial scale. Moreover, the technology can be further upscaled for increasing the overall productivity which would further cut down the cost of production.

CONTRIBUTION TO THE SOCIETY

TNF is a very important immunostimulant which is helpful in fighting against cancer. This drug is very expensive and there is a need for a cost-effective production strategy can help the drug to reach to more population. The significance of a cost-effective production strategy can be far more in developing countries like India. We have developed a strategy for achieving very high productivity in a Lab-scale bioreactor which would be helpful in reducing the production cost of the drug. The effort will be made towards technology transfer to a pharmaceutical company for manufacturing the drug.

PUBLICATIONS

1. Tapan Kumar Singha, Pooja Gulati, Aparajita Mohanty, Yogender Pal Khasa, Rajeev Kumar Kapoor, Sanjay Kumar. Efficient genetic approaches for improvement of plasmid-based expression of recombinant protein in *Escherichia coli*: A review. *Process Biochemistry*. 55 (2017) 17–31.

2. Tapan Kumar Singha, Pooja Gulati, Sanjay Kumar. Nonconventional induction strategies for production of recombinant human tumor necrosis factor-alpha in *Escherichia coli*. J App Biol Biotech. 2018; 6(1):23-27.

3. Tapan Kumar Singha, Vikas Kumar Dagar, Pooja Gulati, Sanjay Kumar. Kinetic studies and bioprocess optimization of recombinant human Tumor Necrosis Factor-alpha (rhTNF-α) production in *Escherichia coli.* (**Under Review**)

POSTER PRESENTATIONS

1. **Tapan Kumar Singha**, Vikas Dagar, Yogender Pal Khasa, Pooja Gulati, Sanjay Kumar* "Over-expression of recombinant human TNF-alpha in *Escherichia coli* by using codon optimized gene sequence with T7 promoter-based expression system" in the 54th AMI, Maharshi Dayanand University, Rohtak, Haryana, India, November 17-20, 2013.

2. **Tapan Kumar Singha**, Pooja Gulati and Sanjay Kumar*. ‘‘Optimization of recombinant human tumor necrosis factor-alpha (rhTNF-α) production in *Escherichia coli*’’ in the 56th AMI, JNU, New Delhi, India and ‘International Symposium on ‘Emerging Discoveries in Microbiology’ December 07-10, 2015.

3. **Tapan Kumar Singha**, Pooja Gulati and Sanjay Kumar*. ‘Kinetics of Recombinant Human Tumor Necrosis Factor-alpha (rhTNF-α) expression in *Escherichia coli* with different

induction strategies: a comparative approach' in the 103rd India Science Congress Association held at University of Mysore, Mysore, Karnataka. January 03-07, 2016.

4. **Tapan Kumar Singha**, Pooja Gulati and Sanjay Kumar*. 'Expression of Human recombinant Tumor necrosis factor-α (hTNF-α) in chemostat culture in *E. coli*' in India International Science Festival held at CSIR-NPL December 07-11, 2016.

ORAL PRESENTATION

1. Tapan Kumar Singha, Pooja Gulati and Sanjay Kumar*. 'Bioprocess Studies for production of recombinant Human Tumor Necrosis Factor-alpha (rhTNF-α) in *Escherichia coli*' in Bioepoch held at School of Biotechnology, Jawaharlal Nehru University, Delhi, March 23-24, 2017.