STUDIES ON CELL GROWTH, PLASMID STABILITY AND PROTEIN EXPRESSION BY RECOMBINANT *ESCHERICHIA COLI*

by

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Submitted
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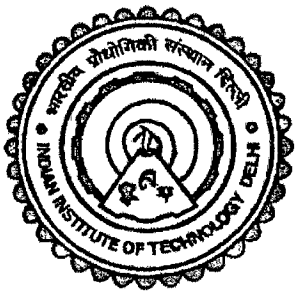

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Therapeutic Proteins

CERTIFICATE

This is to certify that the thesis entitled, "***Studies on Cell Growth, Plasmid Stability and Protein Expression by Recombinant Escherichia coli***" being submitted by **Mr. Ashwani Mathur** to the Indian Institute of Technology Delhi, for the award of the degree of **Doctor of Philosophy** in Biochemical Engineering and Biotechnology is a bonafide record of original research work carried out by him under my supervision in conformity with the rules and regulations of the institute.

The results presented in this thesis have not been submitted, in part or full, to any other university or institute for the award of any degree or diploma.

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(Ashwani Mathur)

ABSTRACT

Recombinant protein expression in *Escherichia coli* is always an issue of concern because of problems such as protein aggregation, lack of protein secretion and plasmid instability. Present study shows the effect of four different expression vectors (pET29(b), pET32(b), pET43(b) and pCS22) on expression, localization and secretion of a model recombinant protein (α-amylase). An analysis of vector characteristics on cell growth and protein expression was observed. The reduction in specific growth rate of recombinant bacteria as compared to untransformed host can be attributed to higher metabolic load. Metabolic load on the host cell was analyzed in terms of oxygen uptake rate (OUR) by recombinant hosts in both uninduced and induced conditions and was found to increase with increase in plasmid size. Effect of metabolic load on bacterial size was also compared and no significant difference in the size of hosts in both uninduced and induced conditions was observed.

Localization of the enzyme protein indicated its distribution as intracellular, periplasmic and extracellular fractions. Activity was analyzed using in-gel assay (zymogram) in different cellular fractions. Transmission electron microscopy (TEM) showed intracellular aggregation of recombinant protein expressed in chemically (IPTG) induced recombinant cells as inclusion bodies while no such aggregation was seen with host transformed with temperature inducible pCS22 vector. Total activity of α-amylase under uncontrolled and controlled dissolved oxygen conditions was highest in host transformed with pCS22 vector with values of 56.8 and 80.1 U/ml respectively. An analysis of plasmid instability by different recombinant system was studied and results were analyzed based on available mathematical models. It was observed that segregational instability (R) shown by recombinant host increased with increase in plasmid size and ranged between 1.4×10^{-3} per generation (pCS22-*amyE* ,6.6 kb) to

2.2 x 10^{-3} per generation (pET43(b)-*amyE*, 8.76 kb). Difference in specific growth rate (dμ) of plasmid free and plasmid bearing cells does not show marked variation with variation in plasmid size and the ratio of growth rates of plasmid free to plasmid bearing cells (α) was around unity showing the equal competence of plasmid free and plasmid bearing cells. Experimental results showed a correlation between vector size and segregational instability. Based on protein expression, folding and secretion studies, pCS22 vector was found to be a better vector compared to other chemical inducible vector and was used for further studies.

Cell immobilization was used as a strategy to improve plasmid stability. Though some improvement in stability of vector was observed but that was at the cost of reduced growth rate. However, complete stability was not attained. Use of *par* gene for improving plasmid instability showed 100% improvement. The presence of *par* gene in pCS22 vector increased the metabolic load on the cell as analyzed by OUR but it does not affect protein expression.

In order to improve extracellular secretion of recombinant protein, glycine was added to growing culture at the time of induction. Increase in extracellular enzyme activity was observed when the system was treated with 1% glycine after induction. Recombinant bacteria transformed with pCS22 and pCS22*par* showed approximately four-fold increase in extracellular α-amylase activity. However, no such significant effect in secretion was seen using other recombinant systems. It indicates the role of glycine in enhancing the excretion of periplasmic recombinant protein fraction, rather than intracellular recombinant protein fraction.

Keywords: *Escherichia coli,* Metabolic load, Protein secretion, Periplasmic fraction, Inclusion bodies, Signal sequence, *par* gene, Cell immobilization

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