BIOPROCESS STUDIES ON RECOMBINANT *E. coli* EXPRESSING PROTEINS WITH GroEL-GroES ASSISTED FOLDING

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by

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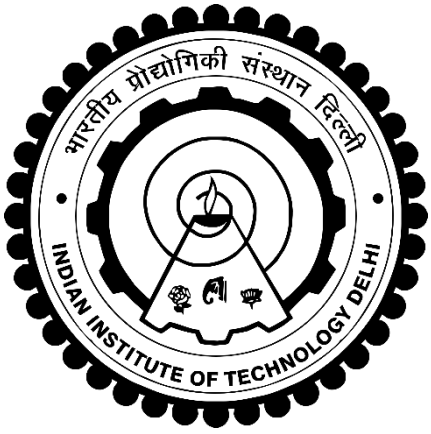

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CERTIFICATE

This is to certify that the thesis titled **"Bioprocess Studies on Recombinant *E. coli* Expressing Proteins with GroEL-GroES Assisted Folding**" submitted by **Ms. Gayathri. R** (2007BEZ8175) for the award of **Doctor of Philosophy** in **Biochemical Engineering and Biotechnology** is a record bonafide work carried out by her under our guidance and supervision at the **Department of Biochemical Engineering and Biotechnology**. The work presented in this thesis has not been submitted elsewhere either in part or full, for the award of any other degree or diploma.

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ABSTRACT

The recombinant protein production is an essential tool for many biotechnological applications including large-scale production of proteins, which are of therapeutic and industrial significance and also for the investigation of the structural and functional aspects of the proteins. The enteric bacterium, *Escherichia coli*, is the most popular prokaryotic expression system, known for its rapid and high yields of heterologous protein production. The major bottleneck in using this host system for recombinant protein production is the frequent formation of inclusion bodies, which are devoid of functionality. The chaperonins GroEL/ES assist in the folding of these recombinant proteins and hence improve their solubility and activity. The physiological expression of chaperonins in the host cells are quite low and hence the need arises to co-express the molecular chaperones concomitantly with the recombinant proteins.

The process variables such as temperature, inducer concentration, media components, presence of co-expressed chaperones and physiological stresses like osmotic and heat stress, are shown to affect the folding of recombinant proteins in the complex cellular environment. Hence, we have developed a bench-scale screening platform to enhance the yield of functional recombinant proteins.

The kinetic studies were carried out on the recombinant *E. coli* during the transient state continuous culture to understand the advantages the cells possess during GroEL/ES chaperone co-expression. It is found that the co-expression of GroEL/ES alleviates the stress produced in the recombinant cells during the overexpression of proteins. The cells expressing the chaperones GroEL/ES show enhancement in both the specific growth rate of the induced cells and the aconitase productivity.

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Abbreviations

Arab	Arabinose
APS	Ammonium per sulphate
BA	Benzyl alcohol
Bet	Betaine
bp	basepair
BSA	Bovine serum albumin
D	Dilution rate (h^{-1})
dAco/dt	Rate of change in aconitase concentration (mg/h)
DCW	Dry cell weight (g)
DO	Dissolved oxygen
DTT	Dithiothrietol
F	Media Feed rate (L/h)
h	hour
HPLC	High pressure liquid chromatography
IPTG	Isopropyl β-D- thiogalactoside
KCl	Potassium chloride
kDa	kilodaltons
K-Glu	Potassium glutamate
LA	Luria Agar
LB	Luria Broth
LBS	Luria broth augmented with 0.5M Sodium Chloride
max	maximum
mg	milligram
min	minute

mL	millilitre
MM	Minimal media
NaCl	Sodium Chloride
OD_{600}	Optical density at 600nm
PAGE	Polyacrylamide gel electrophoresis
pETAco	Plasmid pET29a containing yeast mitochondrial aconitase gene
pGro7	Plasmid carrying GroEL and GroES gene
pQE60Aco	Plasmid pQE60 carrying aconitase gene
PMSF	Phenylmethylsuphonyl fluoride
q_{Aco}	Aconitase Productivity (mg/g DCW/h)
rpm	revolution per minute
RPP	Recombinant protein production
S_f	Substrate concentration in the feed (g/L)
S	Substrate concentration from the reactor exit (g/L)
SDS	Sodium dodecyl sulphate
Sorb	Sorbitol
TB	Terrific Broth media
V	Volume of the reactor (L)
VVM	Volumetric flow rate of air (L/min) per volume of reactor
μ_i	Post-induction specific growth rate of induced cells (h^{-1})
μ_p	Pre-induction Specific growth rate of cells (h^{-1})
TEMED	N ,N ,N', N'- Tetramethylethylenediamine
X	Biomass concentration (mg/mL)
$Y_{p/x}$	recombinant protein yield (mg/ g DCW)
YT	Yeast extract – Tryptone media