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Biochemical Studies on Recombinant Interferon Gamma Produced in E. coli

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APPROVAL SHEET

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Declaration

I declare that this thesis has been composed by myself and that work of which it is a record has been done by myself. It has not been submitted for a degree at this or any other university.

Hend Okasha Ahmed Ali

TO SOULS OF MY FATHER, MY UNCLE,

AND MY DEAR GRAND MOTHER

TO MY GREAT MOTHER

TO WHOM I OWED MY DEEPEST GRATITUDE

MY SISTER

MY FRIENDS

MY DEAR HUSBAND

MY LOVELY DAUGHTER

"MARIAM"

&

MY SWEET HEART SON

"YASEEN"

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ABSTRACT

Production of therapeutic proteins in prokaryotic system, *Escherichia coli* (*E.coli*); has been recognized as an effective stage for production of recombinant human interferon gamma (rhIFN-γ). Modification of rhIFN-γ expression is a line that can be positively employed for increasing the yield of production through optimization of induction conditions in shaker flasks to be applied onto batch culture of recombinant *E. coli.* Hence, in this research induction conditions for the over-production of rhIFN-γ including type of media, pH, type and amount of inducer were optimized. The factors considered for optimized conditions of the recombinant *E.coli* were the use of growth medium LB, neutral pH7 and the inducer (lactose) at final concentration 2mM. These factors found to be useful in batch process development. The cell density was reached to 7gm/L wet cell weight after 12h of batch fermentation.

Commonly, the recombinant proteins were produced in *E. coli* as insoluble aggregates called inclusion bodies (IBs). A method for purification and refolding of rhIFN-γ from IBs has been designated. It includes solubilization of IBs in 8M guanidinium hydrochloride; refolding of rhIFN-γ by rapid dilution method; and protein purification by Hitrap Q XL strong anion chromatography. The rhIFN-γ obtained has been characterized by immunogenicity against the hIFN-γ antibodies. The specific activity of purified rhIFN-γ was 1.87 x 10^7 IU/mg compared to standard rhIFN-γ via new MxA reporter gene assay which depends on identifying MxA mRNA level using real time PCR. The rhIFN-γ increases expression of the MxA gene product in direct relation to the dose of rhIFN-γ in IU.

LIST OF ABBREVIATIONS

a.a	Amino acid
Ab	Antibody
AC	Affinity chromatography
Amp	Ampicillin
APCs	Antigen presenting cells
Arg	Arginine
Asp	Aspartic acid
BCGF	B-cell growth factor
BCIP	5-bromo-4-chloro-3-indolyl phosphate
bp	Base pair
BSA	Bovine serum albumin
cDNA	Complementary deoxyribonucleic acid
CGD	Chronic granulomatous disease
Cham	Chloramphenicol
CPE	Cytopathic effect
CPE	Cytopathic effect inhibition assay
Ct	Cycle threshold

DF	Dilution factor
DNA	Deoxribonucleic acid
dNTP	Deoxy nucleotide tri-phosphate
DTT	Dithiothritol
E.coli	*Escherichia coli*
EDTA	Ethyline diamine tetraacetate
EIA	enzyme immunoassay
FBSA	Fetal bovine serum albumin
FDA	Food and drug administration
FPLC	Fast protein liquid chromatography
GdmCl	Guanidinium hydrochloride
Gln	Glutamine
Glu	Glutamic acid
Gly	Glycine
GuSCN	Guanidinium thiocyanate
h	Hour
HIC	Hydrophobic interaction chromatography
HIV	Human immunodeficiency virus
IBs	Inclusion bodies
IEC	Ion exchange chromatography
IFN-α	Interferon alpha
IFN-β	Interferon beta

IFN-τ	Interferon tau
IFN-ω	Interferon omega
IgG	Immunoglobulin G
IL	Interleukin
IPTG	Isopropylthio -D-galactoside
ISG	Interferon stimulated gene
IU	International unit
JAK	Janus kinase
kDa	Kilo-dalton
lac	Lactose utilization operon
LB medium	Luria-Bertani medium
Leu	Leucine
Lys	Lysine
mAU	Milli absorbance unit
MCS	Multiple cloning site
MHC	Major histocompatibility complex
min	minute
MMLV-RT	moloney murine leukemia virus reverse transcriptase
mRNA	Messenger Ribonucleic Acid
mSc/cm	Milli Siemens per centimeter

Mwt	Molecular weight
MWCO	Molecular weight cutoff
MxA	Myxovirus resistance protein A
NBT	Nito blue tetrazoliium
NK	Natural killers
OAS1	2'-5'-oligoadenylate synthetase1
OD	Optical density
PAGE	Polyacrylamide gel electrophoresis
PBMCs	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PGK	3-phosphoglycerate kinase promoter
pI	Protein isoelectic point
PKR	Protein kinase RNA activated
PMN	Polymorphonuclear leukocytes
PMSF	Phenyl methyl sulfoxide
pSS	Primary Sjögren's syndrome
PTM	Post transitional modification
qPCR	Quantitative polymerase chain reaction
RBS	Ribosomal binding site
rhIFN-γ	Recombinant human interferon gamma
RNA	Ribonucleic acid

RNase L	Ribonuclease L
RPC	Reversed phase chromatography
rpm	Round per minute
RPMI	Roswell park Memorial institute
SB	Super broth
SDS	Sodium dodecyl sulfate
SEA	Staphylococcus enterotoxin A
SEB	Staphylococcus enterotoxin B
SEC	Size exclusion chromatography
SOB	Super optimal both
STAT	signal transducer and activator of transcription
TB	Terrific broth
TEMED	N, N, N`, N` –tetra-methylenediamine
Th	T helper cell
TNF	Tumor necrosis factor
tRNA	Transfer Ribonucleic Acid
TY	Tryptone yeast extract media
vvm	Vessel volume per minute
WCW	Wet cell weight
WHO	world health organization

LIST OF FIGURES