DEVELOPMENT OF OPTIMAL FERMENTATION EXPRESSION SYSTEMS FOR RECOMBINANT PROTEINS

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This is to certify that the research carried out in the Masterate Thesis entitled Development of Optimal Fermentation Expression Systems for Recombinant Proteins in the Institute of Technology and Engineering at Massey University, New Zealand:

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ABSTRACT

DEVELOPMENT OF OPTIMAL FERMENTATION EXPRESSION SYSTEMS FOR RECOMBINANT PROTEINS

by Daniel Manderson

This research set out to maximise the titre of four recombinant protein products (i.e. *Eg*95 vaccine antigen against *Echinococcus granulosus*; a aspartyl protease inhibitor homologue, *Aspin*; a secreted cytokine granulocyte colony stimulating factor (G-CSF); a secreted gonadotropin ovine follicle stimulating factor (*o*FSH)) and develop parameters for the expression of those proteins in a small scale stirred tank bioreactor.

Production of *Eg*95 as inclusion bodies in *E. coli* was influenced by the medium, feeding strategy, induction timing and dissolved oxygen concentration. Expression was greatest using the medium Terrific Broth. Higher *Eg*95 titres were favoured using exponential feeding, a low dissolved oxygen concentration and with cells induced in mid-exponential growth. A maximum titre of 1.73 g/L of *Eg*95 was produced in a fed-batch fermentation controlled at 37°C, pH 7.0 and 30% dissolved oxygen. Induction with 0.1 mM of IPTG added four hours after inoculation, was optimal. The maximum titre attained, was a 360% improvement on fermentations prior to this research.

Aspin was used to investigate the culture conditions for maximizing the production of soluble protein in *E.coli*. Soluble *Aspin* production was favoured at low expression rates. A volumetric titre of 0.220 g/L of soluble *Aspin* was attained in batch fermentation by inducing with 2 g/L of L-arabinose, with the temperature reduced from 37°C to 23°C and by maintaining a low dissolved oxygen (DO) concentration. This yield was relatively high compared to previous reports [1-3].

G-CSF production in the yeast *Pichia pastoris* was influenced by the medium, pH and methanol-to-cell ratio. A maximum titre of 0.028g/L of G-CSF was produced in shaker flasks of enhanced yeast extract Hy-Soy dextrose medium (YEHD), maintained at 200 rpm, 30°C, pH 6.0 and with 1% (v/v) methanol fed per day. Cells were resuspended to an optical density of 8 prior to induction. No improvement in G-CSF was achieved in the fermenter, likely due to an inhibition by toxic materials. The optimised shaker flask yield was consistent with previous reports [4-6].

Production of *o*FSH in insect cells was influenced by the cell density at inoculation and rate of agitation. 0.001g/L of *o*FSH was produced in shaker flasks inoculated at a density of 1 x 10^6 cells/mL, cultured at 27°C and agitated at 140rpm. This represented an improvement over previous yields [7].

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LIST OF ABBREVIATIONS AND SYMBOLS

AOX alcohol oxidase

bla ampicillin resistance gene

bsd blasticidin resistance gene

BCA bicinchoninic acid

BMG buffered minimal glycerol medium

BMM buffered minimal methanol medium

BMGY buffered complex glycerol medium

BMMY buffered complex methanol medium

CARE continuous absorption recycle extraction

CDW cell dry weight

DO dissolved oxygen

DTT dithiothreitol

EDTA ethylene diamine tetra-acetic acid

ELISA enzyme-linked immunosorbent assay

F(t) flow rate of feed at time t (h^{-1})

GFP green fluorescence protein

G-CSF granulocyte colony stimulating factor

GST. glutathione-s-transferase

HCl hydrochloride

HIC hydrophobic interaction chromatography

His$_6$ polyhistidine epitope tag

HRP horse radish peroxidase

IgG Immunoglobulin G

IMAC immobilised metal chelating resin

INF interferon

IPTG isopropyl β-D-thiogalactopyranoside

LB Luria-Bertani broth

LPM litres per minute

MBP maltose binding protein

MGY minimal glycerol medium

MM minimal methanol medium

MOI multiplicity of infection

NaOH sodium hydroxide

NMW nominal molecular weight

OD optical density

PID proportional integral derivative

PTM *P.pastoris* trace metal solution

RBS ribosome binding site

REC reverse phase chromatography

RO reverse osmosis

SB superbroth

SD Shine-Dalgarno site

SDS-PAGE sodium dodecyl sulphate polyacrylamide gel electrophoresis

Sf *Spodoptera frugiperda*

S_F substrate concentration in feed (g/L)

$Y_{x/s}$ cell titre of substrate (g CDW/g)

μ specific growth rate (h^{-1})

SEC size exclusion chromatography

S(t) substrate concentration at time t (g/L)

TB Terrific Broth

TBS tris buffered solution

TMB 3, 3', 5, 5'-tetramethyl-benzidine

TRX thioredoxin gene

TSB tryptic soy broth

UF ultrafiltration

V(t) reactor volume at time t (L)

WCW wet cell weight (g/L)

X(0) cell concentration at time t

X(t) cell concentration at time t (g CDW/L)

YEPD yeast extract peptone dextrose medium

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