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DEVELOPMENT OF OPTIMAL
FERMENTATION EXPRESSION
SYSTEMS FOR RECOMBINANT
PROTEINS

A thesis submitted in partial fulfilment
of the requirements for the degree of

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in
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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. *Eg95* 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 (ϕ FSH)) and develop parameters for the expression of those proteins in a small scale stirred tank bioreactor.

Production of *Eg95* 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 *Eg95* 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 *Eg95* 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 1×10^6 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	G-CSF granulocyte colony stimulating factor
<i>bla</i> ampicillin resistance gene	GST , glutathione-s-transferase
<i>bsd</i> blasticidin resistance gene	HCl hydrochloride
BCA bicinchoninic acid	HIC hydrophobic interaction chromatography
BMG buffered minimal glycerol medium	His₆ polyhistidine epitope tag
BMM buffered minimal methanol medium	HRP horse radish peroxidase
BMGY buffered complex glycerol medium	IgG Immunoglobulin G
BMMY buffered complex methanol medium	IMAC immobilised metal chelating resin
CARE continuous absorption recycle extraction	INF interferon
CDW cell dry weight	IPTG isopropyl β -D-thiogalactopyranoside
DO dissolved oxygen	LB Luria-Bertani broth
DTT dithiothreitol	LPM litres per minute
EDTA ethylene diamine tetra-acetic acid	MBP maltose binding protein
ELISA enzyme-linked immunosorbent assay	MGY minimal glycerol medium
F(t) flow rate of feed at time t (h^{-1})	MM minimal methanol medium
GFP green fluorescence protein	MOI multiplicity of infection

NaOH sodium hydroxide	SEC size exclusion chromatography
NMW nominal molecular weight	S(t) substrate concentration at time t (g/L)
OD optical density	TB Terrific Broth
PID proportional integral derivative	TBS tris buffered solution
PTM <i>P.pastoris</i> trace metal solution	TMB 3, 3', 5, 5'-tetramethylbenzidine
RBS ribosome binding site	TRX thioredoxin gene
REC reverse phase chromatography	TSB tryptic soy broth
RO reverse osmosis	UF ultrafiltration
SB superbroth	V(t) reactor volume at time t (L)
SD Shine-Dalgarno site	WCW wet cell weight (g/L)
SDS-PAGE sodium dodecyl sulphate polyacrylamide gel electrophoresis	X(0) cell concentration at time t
Sf <i>Spodoptera frugiperda</i>	X(t) cell concentration at time t (g CDW/L)
S_F substrate concentration in feed (g/L)	YEPD yeast extract peptone dextrose medium
Y_{x/s} cell titre of substrate (g CDW/g)	
μ specific growth rate (h ⁻¹)	

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