PRODUCTION OF L-ASPARAGINASE THROUGH BIODEGRADATION OF CHICKEN BONE WASTES

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We hereby declare that we have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Master of Science in Biotechnology

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Thesis submitted in fulfillment of the requirements
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Master of Science (Biotechnology)

Faculty of Industrial Sciences and Technology

UNIVERSITI MALAYSIA PAHANG

JANUARY 2017

DEDICATION

Dedicated to my beloved family

ACKNOWLEDGEMENT

Writing this thesis has had a big impact on me. Praise Almighty God for making it able for me to achieve a master degree. I would like to reflect on the people who have supported and guided me so much throughout this research. Firstly, I would like to express the deepest appreciation to my main supervisor, Dr Essam Makky. This thesis would not have been possible without his endless support and persistent help.

Besides that, I would like to sincerely express my gratitude to my co-supervisor, Dr. Nina Suhaity binti Azmi. I want to thank you for your excellent cooperation and for all of the opportunities I was given to conduct my research. In addition to that, I would like to thank to Dr Kafi, Mr Hairul and Faculty of Industrial Sciences and Technology, UMP's lab assistants who provide me with valuable advices and guidance.

Apart from that, my research journey will not be exciting if without a group of special lab mates. Special thanks to Tahira Batool, Muna Jalal, Afifah and Hajar. They have been a great lab as well as group member of mine. Next, there are my friends. We were not only able to support each other by deliberating over our problems and findings, but also happily by talking about things other than just our papers.

I am grateful to all of my friends for their sacrifice, patience and understanding that were inevitable to make this research possible. Special thanks should be given to everyone who involved directly as well as indirectly. I would like to acknowledge their comments and suggestions, which was crucial for the successful completion of this study.

Finally, I acknowledge the sincerity of my family, who consistently encouraged me to pursue on my higher studies. Besides that, I would like to thank my parents for their wise counsel and sympathetic ear. You are always there for me. I cannot find the appropriate words that could properly describe my appreciation for their faith and devotion in my ability to attain my goals.

Thank you very much everybody!

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LIST OF SYMBOLS

%	Percentage
° C	Degree Celsius
:	Ratio
*	Highest value
↓	Lower than Control
2nd	Second
±	Plus-minus sign
Km	Michaelis constant
/	per
µ	Micro

LIST OF ABBREVIATIONS

ALL	Acute lymphoblastic leukemia
A. niger	*Aspergillus niger*
ATCC	American Type Culture Collection
BSA	Bovine serum albumin
cm	Centimetre
C. albicans	*Candida albicans*
CB	Chicken bone
CCB	Cooked chicken bone
CFF	Cell free-filtrate
CMC	Carboxymethyl cellulose
DEAE- Cellulose	Diethylaminoethyl cellulose
E. coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic
FDA	Food and Drug Administration
g	Gram
h	Hour

IU	International unit
kDa	kiloDalton
L	Litre
mA	milliampere
min	Minute
mg	Milligram
ml	Millilitre
mM	Millimolar
ms	millisecond
M	Molar
MTCC	Microbial Type Culture Collection
N	Normality
NA	Nutrient agar
NB	Nutrient broth
ND	Not detected
nm	Nanometre
PDA	Potato dextrose agar

PEG	Polyethylene glycol
pH	Potential hydrogen
PS	Phosphate solution
RCB	Raw chicken bone
rpm	Revolutions per minute
S. cerevisae	*Sarccharomyces cerevisae*
SD	Standard deviation
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEM	Scanning electron microscopy
sp.	Species
TEM	Transmission electron microscopy
T. reesei	*Trichorderma Reesei*
UMP	Universiti Malaysia Pahang
viz	*Videlicet*
v/v	volume per volume
w/v	weight per volume
WHO	World Health Organization

XRD	X-ray powder diffraction
XPS	X-ray photoelectron spectroscopy

LIST OF CHEMICAL FORMULAS

$CuSO_4.5H_2O$	Copper (II) Sulfate Pentahydrate
HCl	Hydrochloric acid
KCl	Potassium chloride
KH_2PO_4	Potassium dihydrogen phosphate
$KNaC_4H_4O_6.4H_2O$	Potassium sodium tartrate
$MgCl_2$	Magnesium chloride
$MgSO_4$	Magnesium sulfate
$NaCl$	Sodium chloride
Na_2CO_3	Sodium carbonate
NaH_2PO_4	Monobasic sodium phosphate
Na_2HPO_4	Dibasic sodium phosphate
NH_4Cl	Ammonium chloride
$(NH_4)_2SO_4$	Ammonium sulfate
$NaNO_3$	Sodium nitrate
$NaOH$	Sodium hydroxide

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JANUARY 2017

ABSTRAK

L-asparaginase mendapat permintaan yang tinggi dari pelbagai industri seperti farmaseutikal, makanan mahupun biopenderia. Ini adalah disebabkan oleh keunikan enzim L-asparaginase dalam menghidrolisis asid amino L-asparagina kepada L-asid aspartik dan ammonia. Oleh yang demikian, enzim L-asparaginase dihasilkan secara besar-besaran melalui proses fermentasi mikrob. Kajian ini bertujuan untuk menghasilkan enzim L-asparaginase melalui proses fermentasi mikrob yang ditambah dengan sisa tulang ayam selaku substrat oleh penghasil enzim yang terbaik. Lantaran itu, kajian ini boleh dikategorikan kepada 3 bahagian di mana bahagian pertama merangkumi beberapa eksperimen dalam usaha mengenalpasti penghasil enzim serta substrat yang terbaik manakala bahagian kedua pula mengenalpasti impak proses parameter yang optimum dengan menggunakan pendekatan Satu-Faktor-Pada-Satu-Masa. Selain itu, enzim L-asparaginase juga diteruskan dengan proses penulenan serta pencirian. Enam mililiter (5 x 10^8 sel/ml) *Escherichia coli* ATCC 10536 merupakan saiz inokulum yang optimum apabila ia diinkubasikan pada suhu 40 °C dan pH 9 untuk 2 hari dalam setiap 50 ml media fermentasi. Tambahan pula, pertumbuhan maksimum *Escherichia coli* ATCC 10536 selaku penghasil enzim L-asparaginase apabila ia ditambah dengan 1.0 % w/v kanji, 0.2 % w/v ammonium klorida serta 1.0 % w/v sisa tulang ayam yang telah dimasak selaku sumber karbon, nitrogen serta substrat. Impak proses parameter yang dikaji mendapati bahawa *Escherichia coli* ATCC 10536 merupakan penghasil enzim L-asparaginase yang terbaik dan ia lebih mengemari kaldu nutrient sewaktu proses fermentasi. Enzim L-asparaginase yang terhasil juga diteruskan dengan proses penulenan termasuk pemendakan ammonium sulfat, dialisis serta selulosa DEAE kromatografi turus. Sebanyak 0.42 % enzim L-asparaginase diperoleh di akhir proses penulenan. Di samping itu, proses pencirian juga dilaksanakan pada enzim yang diperoleh selepas proses dialisis dan didapati bahawa 40 °C dan pH 8 merupakan suhu inkubasi dan pH yang optimum. Selain itu, Na^+ merupakan ion logam yang terbaik dalam usaha mengawal selia aktiviti enzim manakala EDTA pula ialah perencat enzim. Kesimpulannya, *Escherichia coli* ATCC 10536 merupakan penghasil enzim L-asparaginase yang terbaik apabila media fermentasi ditambah dengan sisa tulang ayam yang telah dimasak yang tinggi kandungan proteinnya.

ABSTRACT

L-asparaginase is highly demanded in pharmaceutical, food and biosensor industry due to its remarkable properties in hydrolysing L-asparagine into aspartic acid and ammonia. Owing to this significant property, L-asparaginase is widely produced through microbial fermentation. This research aims to produce L-asparaginase enzyme through microbial fermentation by the most potent isolate in the presence of chicken bone wastes as the substrate. Therefore, this research can be categorised into 3 parts where the first part was to perform several studies in determining the best enzyme producer and substrate while the second part was the effect of process parameters using One-Factor-at-a-Time (OFAT) method and the third part was the purification and characterisation study of L-asparaginase. Six millilitres (5 x 108 cells/ml) of *Escherichia coli* ATCC 10536 is the optimum inoculum size in 50 ml of pH 9 growth media at 40 °C for 2 days. In addition to that, *Escherichia coli* ATCC 10536 was fully enhanced when it was engaged with 1.0 % w/v of starch, 0.2 % w/v of ammonium chloride and 1 % w/v of cooked chicken bone (CCB) as the carbon source, nitrogen source and substrate respectively. The effect of process parameters studied using OFAT method revealed that *Escherichia coli* ATCC 10536 is the most potent L-asparaginase producer in this research and it preferred nutrient broth as the growth media in producing L-asparaginase. Furthermore, *Escherichia coli* ATCC 10536 L-asparaginase was purified by ammonium sulfate precipitation, dialysis and DEAE-cellulose chromatography. At the end of purification, 0.42 % yield of L-asparaginase was obtained. In addition, the dialysed ammonium sulfate fraction of L-asparaginase was partially characterised which resulted to 40 °C, pH 8, Na^+ and ethylenediaminetetraacetic acid (EDTA) being the optimum incubation temperature, pH, metal ion and inhibitor respectively. In conclusion, *Escherichia coli* ATCC 10536 is an excellent L-asparaginase producer when the fermentation is supplemented with high protein content of cooked chicken bone as substrate.

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