



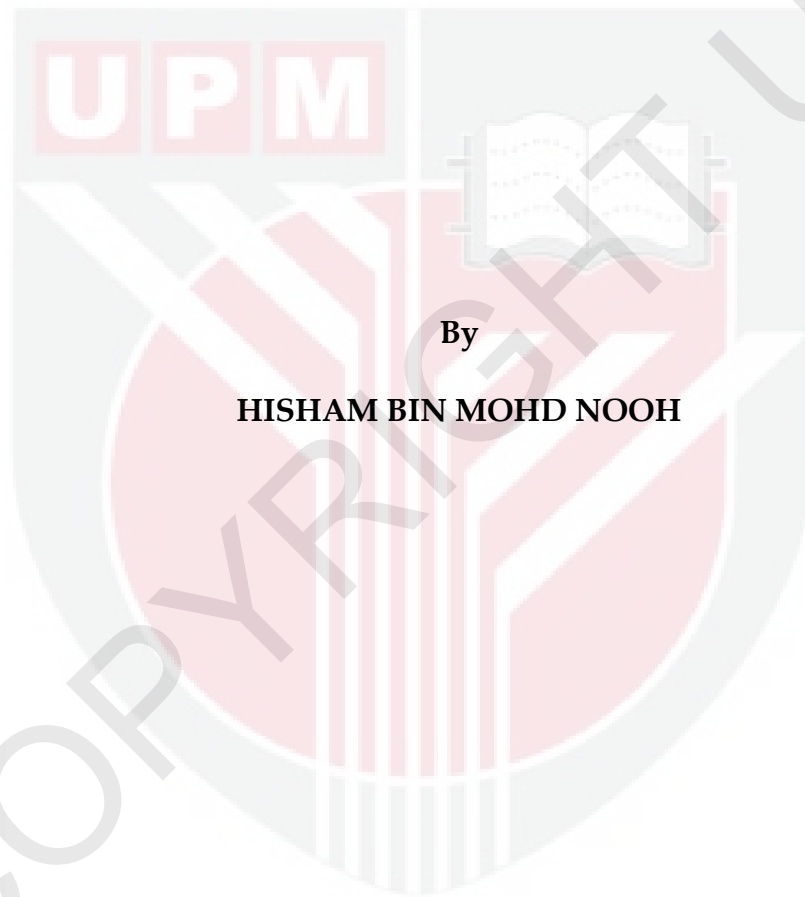
UNIVERSITI PUTRA MALAYSIA

**CLONING AND EXPRESSION OF *STAPHYLOCOCCUS EPIDERMIDIS*
AT2 LIPASE GENE IN *YARROWIA LIPOLYTICA***

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EPIDERMIDIS AT2 LIPASE GENE IN *YARROWIA LIPOLYTICA***



By

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CLONING AND EXPRESSION OF *Staphylococcus epidermidis* AT2 LIPASE GENE IN *Yarrowia lipolytica*

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October 2011

Chairman: Professor Raja Noor Zaliha Raja Abd Rahman, D. Eng.

Faculty : Biotechnology and Biomolecular Sciences

Proteolytic degradation and the production of protein that accumulates as misfolded form always occur in bacterial expression system. In view of that, *Yarrowia lipolytica* is chosen as a host to express heterologous protein. The gene encoding sequence of *Staphylococcus epidermidis* AT2 lipase (1.2kb) was cloned into *Y. lipolytica* expression vector (pYLEX1) and placed under the regulation of the strong hybrid promoter (hp4d) carrying four tandem copies of an upstream activator sequence (UAS1B) from *pXPR2* and a minimal *pLEU2* fragment. Previously, primers were designed on the basis of *S. epidermidis* lipase precursor (*geh1*) gene (AF053006). PCR (Polymerase chain reaction) was used to amplify the gene and cloned into pJET 1.2/blunt-end vector (Fermentas) transformed

into *E. coli* DH5 α competent cell. After the gene was propagated in *E. coli*, the gene were purified and ligated into pYLEX1 vector (Yeastern Biotech). The recombinant plasmid was extracted and linearized before it was transformed into *Y. lipolytica* host strain Po1g. The recombinant *Y. lipolytica* was grown on YNB selection medium. Five positive transformants harboured the expected size of AT2 lipase gene were obtained and one of the transformants showed the highest expression. The expression of AT2 lipase enzyme was optimized at of 28 $^{\circ}$ C with the agitation speed of 200 rpm in optimized YNB medium. Process of breaking the cells or sonication profile was optimized at 7.5 min and the highest activity obtained was 14 U/mL. The crude proteins were electrophoresed on 12% (w/v) of SDS-PAGE and estimated protein band of 43 kDa was detected when stained with Coomassie brilliant blue. The expressed enzyme retained 100% of its activity after 30 min incubation (37 $^{\circ}$ C) in n-hexane, p-xylene and dimethyl sulfoxide.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

**PENGLONAN DAN PENGEKSPRESAN GEN AT2 LIPASE
Staphylococcus epidermidis DALAM *Yarrowia lipolytica***

Oleh

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Degradasi proteolitik dan pengeluaran protein yang tersalah bentuk seperti tersalah-lipatan selalu berlaku dalam sistem ekspresan bakteria. Sehubungan itu, *Yarrowia lipolytica* dipilih sebagai hos untuk mengekspreskan protein heterologous. Jujukan gen pengekodan *Staphylococcus epidermidis* AT2 lipase (1.2kb) diklonkan pada *Y. lipolytica* vektor (pYLEX1) dan diletakkan di bawah pengaruh promoter hibrid (hp4d) membawa empat salinan sejajar urutan (UAS1B) pXPR2 dan serpihan minimum pLEU2. Sebelum ini, primer telah direka berdasarkan *S. epidermidis* lipase (geh1) gen (AF053006). PCR (Tindak Balas Berantai Polymerase) digunakan untuk mengandakan gen dan diklon pada pJET 1.2/blunt-end vektor (Fermentas) yang dimasukkan di dalam *E. coli*

DH5 α sel kompeten. Selepas gen itu digandakan dalam *E. coli*, gen yang telah diasingkan dan diligat pada pYLEX1 vektor (Yeastern Biotech). Plasmid rekombinan diekstrak dan diluruskan sebelum ia dimasukkan kedalam hos *Y. lipolytica* strain Po1g. *Y. lipolytica* rekombinan telah disaring pada medium pemilihan YNB. Lima transformants positif menunjukkan saiz yang dijangka pada AT2 lipase gen telah diperolehi dan salah satu yang transformants menunjukkan ekspresan yang paling tinggi. Ekspresan enzim AT2 lipase dioptimumkan pada suhu 28 °C dengan kelajuan perkocakan 200 rpm dalam medium YNB dioptimumkan. Proses pemecahan sel-sel atau profil sonikasi dioptimumkan pada kadar 7.5 min dan aktiviti tertinggi yang diperolehi adalah 14.2 U / mL. Protein mentah dielektrophorisiskan di dalam 12% (w / v) SDS-PAGE dan jalur protein yang dianggarkan pada 43 kDa, dikesan apabila direndam dengan Coomassie Brilliant Blue. Enzim tersebut telah mengekalkan 100% aktiviti selepas 30 min diinkubasi (37 °C) dalam n-heksana, p-xylene dan dimetil sulfoxide.

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I certify that an Examination Committee has met on **date of viva voce** to conduct the final examination of **name of student** on his (or her) **degree** thesis entitled " **CLONING AND EXPRESSION OF *Staphylococcus epidermidis* AT2 LIPASE GENE IN *Yarrowia lipolytica***" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the degree of Master of Science.

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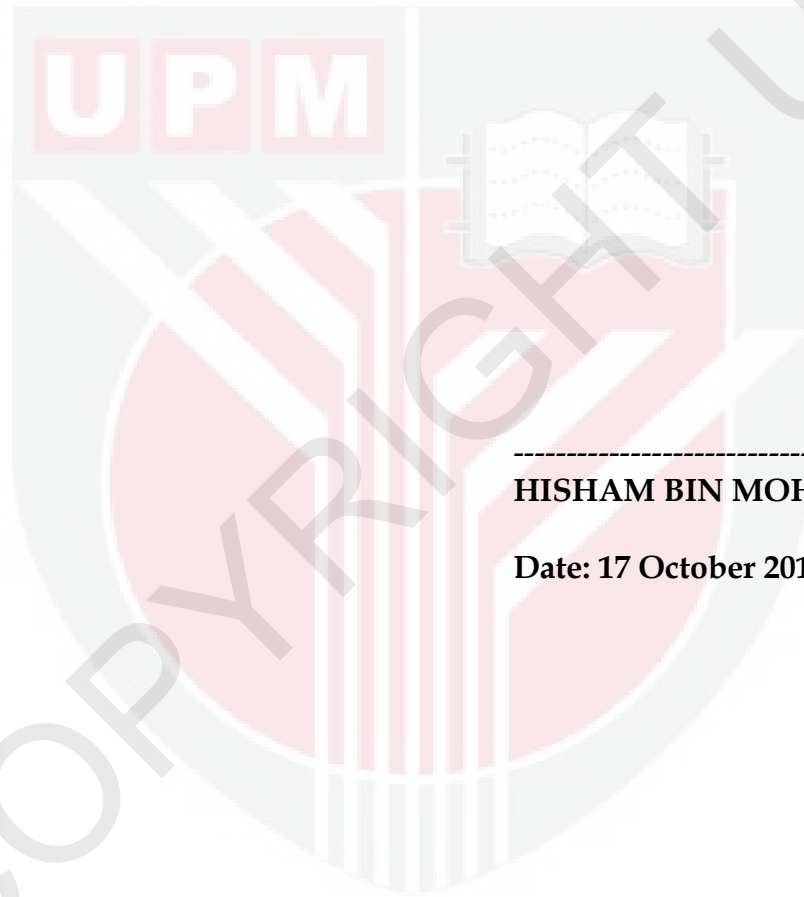
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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.



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Date: 17 October 2011

TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	iv
ACKNOWLEDGEMENTS	vi
APPROVAL	vii
DECLARATION	ix
LIST OF TABLES	xiii
LIST OF FIGURES	xiv
LIST OF ABBREVIATIONS	xvi
CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	5
2.1 Lipase	5
2.1.2 Sources of lipases	6
2.1.3 Applications of microbial lipases in industries	7
2.2 <i>Staphylococcal</i> lipase	11
2.2.1 <i>Staphylococcus epidermidis</i> AT2	12
2.2.2 Advantages of organic solvent tolerant lipase	13
2.3 Comparison between <i>E. coli</i> and yeast expression system	14
2.4 Yeast expression system platform	16
2.4.1 <i>Saccharomyces cerevisiae</i>	16
2.4.2 <i>Pichia pastoris</i>	17
2.4.3 <i>Hansenula polymorpha</i>	18
2.4.4 <i>Arxula adeninivorans</i>	18
2.4.5 <i>Yarrowia lipolytica</i>	19
2.5 <i>Yarrowia</i> expression system	19
3 METHODOLOGY	
3.1 Materials	23
3.2 AT2 lipase gene	
3.2.1 <i>Staphylococcus epidermidis</i> AT2	26
3.2.2 Extraction and quantitation of genomic DNA	26
3.2.3 Amplification of mature AT2 lipase gene	28
3.3 Cloning AT2 lipase gene into <i>Escherichia coli</i> host	
3.3.1 Preparation of AT2 lipase gene and pYLEX1 vector	30

3.3.2	Heat-shock transformation of <i>Escherichia coli</i>	31
3.3.3	Analysis of recombinant plasmids	31
3.4	Sequencing and N-glycosylation potential site prediction	32
3.5	Cloning AT2 lipase gene into <i>Yarrowia lipolytica</i>	
3.5.1	Transformation AT2 lipase gene into <i>Y. lipolytica</i>	34
3.5.2	Preparation of yeast competent cells	34
3.5.3	One step transformation	35
3.5.4	Direct PCR analysis of the <i>Yarrowia</i> transformants	35
3.6	Protein expression in <i>Yarrowia lipolytica</i>	36
3.6.1	Standard procedure	37
3.7	Optimization Studies of AT2 Lipase Expression in Shake Flask	
3.7.1	Effect of media on AT2 lipase expression	38
3.7.2	Effect of medium component on AT2 lipase production	39
3.7.3	Effect of temperature on AT2 lipase expression	40
3.7.4	Effect of agitation rate on AT2 lipase expression	40
3.7.5	Effect of sonication profiles on AT2 lipase expression	40
3.8	Effect of AT2 lipase in <i>Y. lipolytica</i> on organic solvent	41
3.9	Quantification of lipase expression	
3.9.1	Assay of lipase activity	41
3.9.2	Protein concentration determination	43
3.10	Gel Analysis	
3.10.1	SDS PAGE	44
3.10.2	Native PAGE	44
3.10.3	Zymogram of AT2 lipase	45
3.11	Statistical analysis	46

4 RESULT AND DISCUSSION

4.1	Cloning AT2 lipase gene into <i>E. coli</i>	
4.1.1	Genomic DNA extraction	47
4.1.2	Construction of recombinant plasmid	52
4.1.3	Sequencing Result	53
4.2	N-glycosylation site prediction of recombinant AT2 lipase	56
4.3	Cloning of AT2 lipase in <i>Yarrowia lipolytica</i>	
4.3.1	Selection of recombinant <i>Y. lipolytica</i>	60
4.3.2	Direct PCR screening	61

4.4	Intracellular expression of AT2 lipase	63
4.5	Optimization studies of AT2 lipase expression in shake flask	65
4.5.1	Effect of media on AT2 lipase expression	66
4.5.2	Effect of medium component on AT2 lipase production	68
4.5.3	Effect of temperature on AT2 lipase expression	71
4.5.4	Effect of agitation rate on AT2 lipase expression	73
4.5.5	Effect of sonication profiles on AT2 lipase production	77
4.5.6	Time course study of AT2 lipase expression	79
4.6	Gel Analysis of AT2 lipase	
4.6.1	SDS PAGE	81
4.6.2	Native PAGE	83
4.6.3	Zymogram of AT2 lipase	85
4.7	Stability of AT2 Lipase in <i>Y. lipolytica</i> on Various Organic Solvents	87
5	CONCLUSION AND RECOMMENDATIONS	
5.1	Conclusion	91
5.2	Recommendations	92
	REFERENCES	93
	APPENDICES	
7.1	Appendix A	100
7.2	Appendix B	100
7.3	Appendix C	101
7.4	Appendix D	101
7.5	Appendix E	102
	BIODATA OF STUDENT	116