



**UNIVERSITI PUTRA MALAYSIA**

**PURIFICATION AND CHARACTERISATION OF THE 33-  
KILODALTON OUTER MEMBRANE PROTEIN OF  
*PASTEURELLA MULTOCIDA* TYPE 6: B**

**ZAMIRAH HJ. ZAINAL ABIDIN**

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**MASTER OF VETERINARY SCIENCE  
UNIVERSITI PUTRA MALAYSIA**

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6:B**

**By**

**ZAMIRAH HJ. ZAINAL ABIDIN**

**Thesis Submitted to the School of Graduate Studies, Universiti  
Putra Malaysia in Fulfilment of the Requirements for the Degree of  
Master of Veterinary Science**

**JULY 2002**



**DEDICATION**

ESPECIALLY DEDICATED TO....

**MAK AND ABAH,  
WHO NEVER STOP BELIEVING IN ME**

**ABANG, KAK MUNI AND SIRHAN,  
THANKS FOR EVERYTHING**



Abstract of thesis presented to the Senate of Universiti Putra Malaysia  
in fulfilment of the requirement for the degree  
of Master of Veterinary Science

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By

**ZAMIRAH HJ. ZAINAL ABIDIN**

**July 2002**

**Chairman : Associate Professor Dr. Hj. Abdul Aziz Saharee**

**Faculty : Veterinary Medicine**

The 33-kiloDalton (kDa) outer membrane protein (OMP) of *Pasteurella multocida* 6:B strain C82 was purified from the crude OMP extract, and its characteristics were investigated. The crude OMP extract was prepared from *P. multocida* 6:B grown in iron-restricted condition, using Sarkosyl extraction method. The purification was carried out by means of modified cylindrical preparative SDS-PAGE, and the purity of the 33kDa OMP was evaluated by SDS-PAGE gels. The protein was present as a single band when re-run on SDS-PAGE gels



stained with Coomassie brilliant blue R-250 and silver staining. However, judged from the 2-keto-3-deoxyoctonic acid (KDO) assay and also Western blotting results, it was observed that the purified 33kDa OMP was not completely devoid of lipopolysaccharide (LPS) and other contaminating proteins, which leads to conclusion that this particular protein was not purified to homogeneity,

Comparisons in terms of efficiency of purification and yield from the crude extract between modified cylindrical preparative SDS-PAGE and diethylaminoethyl-ion exchange chromatography (DEAE-IEX) was investigated. It was found out that the former was more superior, being less tedious to be carried out and had lower LPS contamination.

Protection studies showed that the 33kDa OMP afforded 20% protection level in mice. The ELISA antibody titres did not correspond to protective immunity, which means that the antibody produced was not protective against live challenge with *P. multocida* 6:B. This leads to conclusion that 33kDa OMP is not protective. This finding could be attributed to the harsh denaturation process during purification of the 33kDa OMP, rendering it being devoid of its protective capacity.



N-terminus amino acid sequencing and composition analysis of the 33kDa OMP revealed that it was similar to *P. multocida* major OMP, protein H, which was previously characterised as a porin. However, it was doubtful to conclude 33kDa OMP as porin, since it was not protective, and this warrants for more detailed analyses to verify the function(s) of this protein.

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

**PENULINAN DAN PENCIRIAN PROTIN SELAPUT LUAR  
33-KILODALTON *PASTEURELLA MULTOCIDA* TAIP 6:B**

Oleh

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Protin selaput luar (OMP) 33-kiloDalton (kDa) *Pasteurella multocida* 6:B strain C82 telah dituliskan dari ekstrak kasar OMP, dan cirri-cirinya telah dikaji. Ekstrak kasar OMP disediakan dari *P. multocida* 6:B yang dikulturkan di dalam persekitaran ferum terhad, menggunakan kaedah pengekstrakan Sarkosyl. Penulenan dibuat dengan menggunakan sodium dodesil sulfat-jel elektroforesis poliakrilamid (SDS-PAGE) silinder terubahsuai, dan ketulenan OMP 33kDa ditentukan dengan jel SDS-PAGE. Didapati OMP 33kDa hadir sebagai jalur tunggal apabila diperlihatkan oleh jel yang diwarnakan





dengan Coomassie brilliant blue R-250 dan pewarna perak. Walau bagaimanapun, berdasarkan kepada pemerhatian ke atas asai asid 2-keto-3 deoxyoctonic (KDO) dan pemblottan Western, didapati ia tidak sepenuhnya bebas daripada lipopolisakarida (LPS) dan pencemaran protin yang lain-lain, membawa kepada kesimpulan bahawa protin ini tidak ditulenkan ke homogeniti.

Perbandingan dalam erti kata kecekapan penulenan dan penghasilan penulenan OMP 33kDa dari ekstrak kasar di antara SDS-PAGE silinder terubahsuai dan diethylaminoethyl-kromatografi pertukaran ion (DEAE-IEX) telah dikaji. Didapati bahawa cara SDS-PAGE silinder terubahsuai adalah lebih baik, di mana ia kurang rumit untuk dijalankan dan juga lebih rendah kandungan pencemaran LPS.

Kajian perlindungan yang dijalankan mendapati bahawa OMP 33kDa memberikan 20% aras perlindungan pada mencit apabila dicabar dengan *P. multocida* 6:B hidup. Titer antibodi ELISA tidak berpadanan dengan imuniti berpelindung, memberi makna bahawa antibodi yang terhasil tidak dapat melindungi dari jangkitan *P. multocida* 6:B. Ini membawa kesimpulan bahawa OMP 33kDa adalah tidak berpelindung. Pemerhatian ini mungkin disebabkan oleh proses penyhaslian yang memudaratkan ketika penulenan OMP 33kDa,



membuatkan ia kehilangan keupayaan untuk melindungi dari jangkitan.

Penjujukan N-terminus asid amino dan analisis komposisi OMP 33kDa menunjukkan bahawa ia hampir sama dengan OMP major *P. multocida*, protin H, di mana sebelumnya ia telah dicirikan sebagai protin liang. Walaubagaimanapun, adalah diragui untuk menyimpulkan OMP 33kDa sebagai protin liang, kerana ianya tidak berpelindung, dan dengan ini disarankan agar lebih banyak kajian terperinci dijalankan untuk mengenalpasti fungsi protin ini.

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I certify that an Examination Committee met on 4<sup>th</sup> of July 2002 to conduct the final examination of Zamirah Hj. Zainal Abidin on her Master of Veterinary Science thesis entitled “Purification and Characterisation of the 33-kiloDalton Outer Membrane Protein of *Pasteurella multocida* Type 6:B” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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## DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



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**ZAMIRAH HJ. ZAINAL ABIDIN**

Date: 9 July 2007

## TABLE OF CONTENTS

	<b>Page</b>
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	vi
ACKNOWLEDGEMENTS	ix
APPROVAL SHEETS	xi
DECLARATION FORM	xiii
LIST OF TABLES	xviii
LIST OF FIGURES	xix
LIST OF PLATES	xx
LIST OF ABBREVIATIONS	xxii
CHAPTER	
1 INTRODUCTION	1
1.1 General Introduction	1
1.2 Hypothesis of the Study	5
1.3 Objectives of the Study	5
2 LITERATURE REVIEW	7
2.1 Introduction to <i>Pasteurella</i> Species	7
2.2 Serological Typing of <i>P. multocida</i>	9
2.2.1 Serum Protection Typing Characteristics	9
2.2.2 Capsule Serotyping	10
2.2.3 Somatic Typing	10
2.3 Haemorrhagic Septicaemia	11
2.4 Types of HS Vaccines	12
2.5 Experimental Vaccines	15
2.6 Gram-negative Bacteria Cell Wall	16
2.6.1 Peptidoglycan	17
2.6.2 Outer Membrane	18
2.6.3 Lipoprotein	19
2.6.4 Lipopolysaccharide (LPS)	20
2.7 Immunogens of <i>Pasteurella</i>	21
2.7.1 Capsular Polysaccharides	21
2.7.2 Lipopolysaccharides (LPS)	23
2.7.3 Outer Membrane Proteins (OMP)	25





3	ISOLATION AND PURIFICATION OF THE 33KDA OUTER MEMBRANE PROTEIN (OMP) OF <i>Pasteurella multocida</i> 6:B	29
3.1	Introduction	29
3.2	Materials and Methods	32
3.2.1	Chemicals and Reagents	32
3.2.2	Bacteria	
3.2.3	Bacteria Cultures in Brain Heart Infusion Broth	33
3.2.4	Preparation of Crude OMP Extract	33
3.2.5	Purification of 33kDa Protein From Crude OMP by Modified Cylindrical Preparative SDS-PAGE	34
3.2.6	Determination of Protein Concentration	35
3.2.7	Determination of 2-keto-3-deoxyoctulosonic Acid	36
3.2.8	Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)	38
3.2.9	SDS-PAGE of The Crude OMP	39
3.2.10	Raising of Antiserum in Mice	39
3.2.11	Coomassie Brilliant Blue R-250 Staining and Destaining	39
3.2.12	Bio-Rad Silver Staining for Proteins	40
3.2.13	Protein Profile Visualisation	40
3.2.14	Western Blotting Technique	41
3.2.15	Preparation of 33kDa OMP	42
3.2.16	Solubilisation of Crude OMP by Zwittergent	43
3.2.17	DEAE-Ion Exchange Chromatography (DEAE-IEX)	43
3.3	Results	47
3.3.1	Protein Profile of <i>P. multocida</i> 6:B crude OMP	47
3.3.2	Purification of Crude OMP by Modified Cylindrical Preparative SDS-PAGE	47
3.3.3	Purity of 33kDa After Excision and Electroelution	48
3.3.4	Purification of 33kDa OMP by DEAE-IEX	53
3.3.4.1	Elution Profile of Solubilised Crude OMP on DEAE-Sephadex Column	53
3.3.4.2	Purity of the Eluted Fractions	55
3.3.4.3	Comparative Yield of 33kDa OMP by Modified Cylindrical Preparative SDS-PAGE and DEAE-IEX	57



3.4	Discussion	58
3.4.1	Conclusion	63
4	PROTECTIVE CAPACITY OF 33KDA OUTER MEMBRANE PROTEIN OF <i>Pasteurella multocida</i> 6:B	64
4.1	Introduction	64
4.2	Materials and Methods	66
4.2.1	Preparation of 33kDa OMP	66
4.2.2	Enumeration of Bacteria	65
4.2.3	Experimental Animals	66
4.2.4	Active Protection Study	67
4.2.4.1	Estimation of Minimum Moribund Dose (MMD)	67
4.2.4.2	Immunisation of Mice With 33kDa OMP and Challenge With Live <i>P.</i> <i>multocida</i> 6:B	67
4.2.4.3	Enzyme-linked Immunosorbent Assay (ELISA)	67
4.2.4.4	Calculation of ELISA Titres	70
4.2.5	Passive Mouse Protection Test (PMPT)	71
4.2.6	Complement-mediated Bactericidal Assay	71
4.2.7	Statistical Analysis	72
4.3	Results	74
4.3.1	Estimation of Minimum Moribund Dose (MMD)	74
4.3.2	Active Protection Study	75
4.3.3	Passive Protection Mouse Test (PMPT)	76
4.3.4	Complement-mediated Serum Bactericidal Assay	76
4.4	Discussion	76
4.4.1	Conclusion	79
5	AMINO ACID SEQUENCING AND COMPOSITION ANALYSIS OF THE 33KDA OUTER MEMBRANE PROTEIN OF <i>Pasteurella</i> <i>multocida</i> 6:B	80
5.1	Introduction	80
5.2	Materials and Methods	81
5.2.1	Amino Acid Sequencing and Composition Analysis	81



5.3	Results	82
5.3.1	Sequence Determination and Similarity Search	82
5.3.2	Amino Acid Composition Analysis	83
5.4	Discussion	84
5.4.1	Conclusion	86
6	GENERAL DISCUSSION AND CONCLUSION	88
	REFERENCES	93
	APPENDICES	106
	VITA	118



## LIST OF TABLES

Table	Page
1.1 Livestock census figures for cattle and buffaloes, For year 1999 (source: Department of Veterinary Services).	1
3.1 The eluted peaks and fractions representing them	53
3.2 Comparisons of 33kDa yield between modified cylindrical preparative SDS-PAGE (MCPS) and DEAE-IEX.	57
4.1 Estimation of the minimum moribund dose of <i>P. multocida</i> 6:B strain C82	74
4.2 Active protection study of the purified 33kDa OMP.	75
5.1 Comparison of the amino acid composition of 33kDa OMP of <i>P. multocida</i> 6:B with protein of <i>P. multocida</i> serotype D:2. (Source of amino acid composition of protein H: Chevalier <i>et al.</i> , 1993).	84



## LIST OF FIGURES

Figure	Page
2.2 Schematic drawing of molecular organisation of the typical cell wall of gram-negative bacteria. (AA)-OMP,(PP)-pore protein (matrix protein), (BP)-nutrient-binding protein, (PPS)-periplasmic space, (LPS)-lipopolysaccharide, (LP)-Lipoprotein, (PG) Peptidoglycan (CM)-cytoplasmic membrane. (Source: Jawetz <i>et al.</i> , 1987)	17
3.1 Summary of materials and methods	45
3.2 Schematic diagram of the Prep Cell set-up	46
3.3 Elution profile of zwittergent-solubilised crude OMP of <i>P. multocida</i> 6:B on a DEAE-Sephadex column by ion-exchange chromatography.	54
4.1 Summary of materials and methods	73



## LIST OF PLATES

Plate	Page
3.1 Modified preparative SDS-PAGE on cylindrical gel, performed on Prep Cell column (Model 491, Bio-Rad Laboratories) using 12% sodium dodecyl sulphate-cylindrical polyacrylamide gel.	46
3.2 The electrophoretic profile of <i>P. multocida</i> 6:B crude OMP upon Coomassie Brilliant Blue R-250 staining. Lane 1; Kaleidoscope Prestained MW Standard (Bio-Rad Laboratories). Lane 2; Crude OMP of <i>P. multocida</i> 6:B.	49
3.3 Cylindrical gel of modified preparative SDS-PAGE stained with Coomassie Brilliant Blue R-250. Arrow indicates the thickest band corresponding to the MW of 33kDa of crude OMP profile in Plate 4.2.	49
3.4 Electrophoretic profile on SDS-PAGE of crude and eluted purified outer membrane protein upon staining with Coomassie Brilliant Blue R-250. Lane 1; Kaleidoscope Prestained MW Standard (Bio-Rad Laboratories). Lane 2; Crude OMP of <i>P. multocida</i> 6:B. Lane 3; Eluted protein fraction appearing as a single band.	50
3.5 Electrophoretic profile on SDS-PAGE of crude and eluted purified outer membrane protein upon staining with Bio-Rad silver stain. Lane 1; High Range MW Standard (Bio-Rad Laboratories). Lane 2; Crude OMP of <i>P. multocida</i> 6:B. Lane 3; Eluted protein fraction appearing as a single band.	51



- 3.6 Western blotting of crude and eluted purified outer membrane protein. Lane 1; Kaleidoscope Prestained MW Standard (Bio-Rad Laboratories). Lane 2; Crude OMP of *P. multocida* 6:B. Lane 3; Eluted protein fraction reacted specifically to the primary antibody at 33kDa, appearing as a single band. 52
- 3.7 Electrophoretic profile of P2 fractions, stained with Coomassie Brilliant Blue R-250. Lane 1; (Kaleidoscope Prestained MW Standard, Bio-Rad Laboratories). Lane 2; (Crude OMP of *P. multocida* 6:B). Lane 3-6; (Fraction 110-113). 56
- 3.8 Electrophoretic profile of P2 fractions, stained with Bio-Rad silver staining. Lane 1; (Kaleidoscope Prestained MW Standard, Bio-Rad Laboratories). Lane 2; (Crude OMP of *P. multocida* 6:B). Lane 3-6; (Fraction 110-113). 56



## ABBREVIATIONS

µg	Microgram
µl	Microlitre
µm	Micrometre
°C	Degree Celcius
APV	Alum precipitated vaccine
BCIP	Bromochloroindoyl phosphate
BHI	Brain heart infusion
BSA	Bovine serum albumin
CFU	Colony forming unit
DIP	α, α - dipyridyl
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DVS	Department of Veterinary Services
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
g	Gram
GDP	Gross domestic product
H <sub>2</sub> SO <sub>4</sub>	Sulphuric acid
HCl	Hydrochloric acid
HIO <sub>4</sub>	Periodic acid
HS	Haemorrhagic septicaemia
i/p	Intraperitoneally
IgG	Immunoglobulin G
kDa	KiloDalton
KDO	2-keto-3-deoxyoctonic acid
km	Kilometre
L	Litre
LPS	Lipopolysaccharide
M	Molar
ml	Millilitre
mm	Millimetre
mM	Millimolar
MMD	Minimum moribund dose
MW	Molecular weight
N	Normal





NBT	Nitrobluetetrazolium
ng	Nanogram
nm	Nanometre
OAV	Oil adjuvant vaccine
OD	Optical density
OMP	Outer membrane protein
PBS	Phosphate buffered saline
PVDF	Polyvinylidene difluoride
RPM	Revolution per minute
RT	Room temperature
SBA	Sheep blood agar
USA	United States of America

