UNIVERSITI PUTRA MALAYSIA

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

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FPV 2002 7

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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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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

ZAMIRAH HJ. ZAINAL ABIDIN

Julai 2002

Pengerusi : Profesor Madya Dr. Hj. Abdul Aziz Saharee

Fakulti : Perubatan Veterinar

Protin selaput luar (OMP) 33-kiloDalton (kDa) *Pasteurella multocida* 6:B strain C82 telah ditulenkan 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 penyahaslian 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 sebgai 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 4th 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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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Master of Veterinary Science. Members of the Supervisory Committee are as follows:

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Date: 12 SEP 2002

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.

ZAMIRAH HJ. ZAINAL ABIDIN

Date: 9 [illegible] 200[illegible]

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ABBREVIATIONS

µg	Microgram
µl	Microlitre
µm	Micrometre
^0C	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_2SO_4	Sulphuric acid
HCl	Hydrochloric acid
HIO_4	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