

UNIVERSITI TEKNOLOGI MARA

**CHARACTERIZATION OF FUR
REGULATORY REGION OF LOCAL
Pasteurella multocida 6:B ISOLATE**

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of the requirements for the degree of
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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the result of my own work, unless otherwise indicated or knowledge as referenced work. This thesis has not been submitted to any other academic institutions or non-academic institutions for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

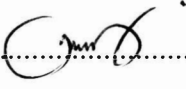
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ABSTRACT

Haemorrhagic Septicaemia (HS) is a major disease of cattle and buffaloes characterized by an acute, highly fatal septicaemia with high morbidity and mortality. In Asia, HS is caused by *Pasteurella multocida* serotype B:2 or 6:B. The production of iron regulated outer membrane proteins (IROMPs) is regulated by Fur protein through repression of the expression of the respective genes when complexed with iron. In this study, outer membrane proteins (OMPs) and iron-regulated outer membrane proteins (IROMPs) were prepared by extraction with 1.5 % sarcosyl from a *Pasteurella multocida* 6:B local isolate cultured on BHI medium and on a medium supplemented with different concentration of 2,2'-dipyridyl. Outer membrane proteins from the isolate were characterized by using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) technique. The approximate size of each OMPs detected were determined from log of molecular weight and Rf value. A total of 12 polypeptides ranging from 19.2 to 117.4 kDa were observed, including 33 and 37kDa major OMPs of *Pasteurella multocida* and two other (117.4 and 91kDa) prominent protein bands. In another study, several primers were designed for amplification around the *fur* gene. Sequence analysis from the amplified DNA revealed an additional sequence of 718bp upstream from *fur* gene which was unique to the local isolate of *Pasteurella multocida* 6:B as compared to *Pasteurella multocida* (PM70). BLAST analysis showed that the sequence possess significant identity towards partial sequence of *Pasteurella multocida* P934 region 2 capsule biosynthesis gene cluster. The potential of this additional sequence as a specific marker for rapid identification of HS diagnosis in Malaysian outbreak should be considered. A study on the function of this gene should also be carried out to give a better understanding of the specific serotypes that causes HS outbreak in Malaysia.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND AND PROBLEM STATEMENT

Haemorrhagic septicaemia (HS) is a contagious disease caused by *Pasteurella multocida* Asian type (B:2 = 6:B) mainly of cattle and buffalo in South East Asia including Malaysia. The disease continues to be the major cause of mortality of cattle and buffaloes in Malaysia. Two occurrences of HS in Malaysia had been reported in Kuala Terengganu (2000) and Rantau Panjang (2001) in which the disease was confirmed to be caused by *Pasteurella multocida* serotype B (Jamal, Chua, Frederick, Mahmood and Salmah, 2005). Khadak (2006) reported out of 11 states surveyed in Peninsular Malaysia, HS was identified as endemic in Terengganu, Kelantan and Perak. It was also reported in 2003 almost 204 buffaloes and four cattle died because of HS in Batang Padang, Perak and total 1489 death was recorded from January 1993 to December 2003.

A study conducted by Veterinary Research Institute (VRI) in the development of an improved haemorrhagic septicaemia vaccine shown that the whole cell vaccine prepared under iron limitation condition conferred a superior protection in mice and elicited high antibody titre in vaccinated cattle. VRI also found that outer membrane protein can afford up to 100% protection to immunized mice. Although the currently used oil-adjuvant vaccine produced in VRI is responsible for reducing the incidence of the disease but it is still unpopular among the farmers or veterinarian due to its high viscosity and post vaccination shock (Mohamed, 2000).

The OMPs of Gram negative bacteria have a role in disease processes as its acts at an interface between the host and pathogen (Lin, Huang, and Zhang, 2002). According to Boyce, Cullen and Nguye, (2006), the major proteins ompA and ompH and a limited number of minor OMPs were investigated as virulence factors or immunogens. This could be due to the presence of iron-regulated outer membrane proteins (IROMPs) that were expressed under iron-restricted condition. The production of IROMPs is regulated by Fur protein by repressing the expression of the respective genes when complexes with iron. The IROMPs of *Pasteurella multocida* have been recognized as an important immunodominant antigen and are thought to be responsible for cross-protective immunity hence can serve as vaccine candidates against haemorrhagic septicaemia (Confer, Suckow, Montelongo, Dao, Miloscio,