

DETECTION OF FIBRINOLYTIC GENE IN LIVE AND LIVE-  
ATTENUATED *Proteusella mitsucida* B:2 USING PCR

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DETECTION OF FIMBRIAL GENE IN LIVE AND LIVE-ATTENUATED  
*Pasteurella multocida* B:2 USING PCR

By

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PROJEK PENYELIDIKAN I DAN II**

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Detection of Fimbrial Gene In Live and Live Attenuated *Pasteurella multocida* B:2 Using PCR  
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## TABLE OF CONTENTS

TITLE	PAGE
<b>ACKNOWLEDGEMENTS</b>	ii
<b>LIST OF TABLES</b>	vi
<b>LIST OF FIGURES</b>	vii
<b>LIST OF ABBREVIATIONS</b>	viii
<b>LIST OF APPENDICES</b>	x
<b>ABSTRACT</b>	xi
<b>ABSTRAK</b>	xii
<b>CHAPTER 1 INTRODUCTION</b>	1
1.1 Objectives	3
<b>CHAPTER 2 LITERATURE REVIEW</b>	
2.1 <i>Pasteurella multocida</i>	4
2.2 Haemorrhagic septicaemia	5
2.3 Role of fimbriae	7
2.3.1 Type 4 fimbriae	8
2.4 Live-attenuated bacteria	9
2.5 Polymerase Chain Reaction	10
2.5.1 Advantages of PCR	12

## **CHAPTER 3    METHODOLOGY**

3.1	Bacterial isolation and maintenance	14
3.2	Staining and cell morphology	14
3.2.1	Gram staining technique	15
3.2.2	Giemsa staining technique	15
3.3	Biochemical characterization	16
3.3.1	Catalase test	16
3.3.2	Oxidase test	16
3.3.3	Indole test	17
3.3.4	Hydrogen sulphide production	17
3.3.5	Motility test	17
3.4	Attenuation of <i>P. multocida</i>	18
3.5	DNA extraction	18
3.6	Purity and quantity of genomic DNA	19
3.7	Screening of fimbrial subunit primers	20
3.8	Optimization of final concentration of Taq polymerase	22
3.9	PCR amplification and cycling conditions	22

## **CHAPTER 4    RESULTS**

4.1	Bacterial isolation and maintenance	24
4.2	Staining and cell morphology	24
4.3	Biochemical characterization	25
4.3.1	Catalase test	25
4.3.2	Oxidase test	25



4.3.3	Indole test	26
4.3.4	Hydrogen sulphide production	26
4.3.5.	Motility test	26
4.4	DNA extraction	27
4.5	Purity and quantity of genomic DNA	27
4.6	Optimization of final concentration of Taq polymerase	28
4.7	Screening of fimbrial subunit primers	32
4.8	Amplification of fimbrial gene following PCR technique	32
<b>CHAPTER 5 DISCUSSION</b>		<b>35</b>
<b>CHAPTER 6 CONCLUSION AND RECOMMENDATION</b>		
6.1	Conclusion	41
6.2	Recommendation	42
<b>REFERENCES</b>		<b>43</b>
<b>APPENDICES</b>		<b>47</b>
<b>CURRICULUM VITAE</b>		<b>49</b>

## LIST OF TABLES

TABLES		PAGE
3.1	Sequences of fimbrial subunit primers and annealing temperature	21
3.2	The final concentration and volume of PCR mixture	23
3.3	Thermal cycling conditions	23
4.1	Purity and quantity of DNA of live and attenuated <i>P. multocida</i> B:2	30

## LIST OF FIGURES

FIGURES	PAGE
4.1 Agarose gel electrophoresis of extracted genomic DNA from live and attenuated <i>P. multocida</i> B:2	29
4.2 Effect of different concentration of Taq DNA polymerase on PCR product by the primer EZ1 and EZ2	31
4.3 Screening for fimbrial subunit primers using live (7%) <i>P. multocida</i> B:2	33
4.4 Agarose gel electrophoresis showing putative DNA bands obtained from the PCR reactions	34

## LIST OF ABBREVIATIONS

$\mu\text{M}$	micromolar
ATP	adenosine triphosphate
bp	base pair
dATP	deoxyadenosine triphosphate
dCTP	deoxycytidine triphosphate
dGTP	deoxyguanosine triphosphate
dH <sub>2</sub> O	distilled water
DNA	deoxyribonucleic acid
dNTPs	deoxynucleoside triphosphates
dTTP	deoxythymidine triphosphate
EDTA	ethylenediaminetetraacetate
H <sub>2</sub> S	hydrogen sulphide
HS	Haemorrhagic septicaemia
Kb	kilo base
kDa	kilo Dalton
LPS	Lipopolysaccharide
mer	oligomer
MgCl <sub>2</sub>	magnesium chloride
NaCl	sodium chloride
ng	nanogram

<i>P. multocida</i>	<i>Pasteurella multocida</i>
PCR	polymerase chain reaction
rpm	rotation per minute
TAE	Tris-Acetate EDTA
U	Unit(s)
V	Volt(s)

## LIST OF APPENDICES

APPENDIX	PAGE
A.1 Blood agar	47
A.2 Peptone water	47
B.1 50x TAE Buffer for PCR reaction	48
B.2 10x PCR Buffer for PCR reaction	48

## ABSTRACT

Gram-negative bacteria use various adhesion strategies to colonize host tissue and type 4 fimbriae are one of them. Their presence has been associated with pathogenesis of several bacterial species. The type 4 fimbriae have been identified on the surfaces of *Pasteurella multocida* serotype B:2, the causative agent of Haemorrhagic Septicaemia (HS) in Asia. These structures have been shown to mediate colonization of mucosal surfaces. The present study was conducted to detect the fimbrial gene in live and attenuated *P. multocida* strain M16 (serotype B:2) following the PCR amplification. The results showed the existence of fimbrial gene in both live *P. multocida* strain M16 and also the attenuated strains derived from the live strain after passaging the latter in decreasing concentration of blood supplement from 7% to 0% of blood in the agar from for a period of two months. The results demonstrated that the gene is an important survival factor of the organism as it still remains in the genome of the bacteria even after multiple passages in suppressed environment that have been reduced from 7% of blood supplement to 0% of total absent of blood in the growth medium. The results also verified that the type 4 fimbriae are one of the important virulence determinants in the *P. multocida* type B:2 that is crucial for host invasion upon their prime role to first colonize the mucosal site of infection. The findings from this study are important to initiate further research concerning the role of fimbrial protein in the development of disease and vaccine.

**PENGENALPASTIAN GEN FIMBRIA MELALUI TEKNIK PCR DALAM *P. multocida*  
B:2 YANG HIDUP DAN YANG DILEMAHKAN**

**ABSTRAK**

Bakteria Gram-negatif menggunakan pelbagai strategi pelekatan untuk mengkoloni tisu hos dan fimbria kelas 4 merupakan salah satu daripada strategi tersebut. Fimbria kelas 4 ini wujud pada lapisan permukaan *Pasteurella multocida* B:2 yang merupakan agen penyebab penyakit Haemorrhagic Septicaemia (HS) di Asia. Struktur fimbria ini dapat mengkoloni permukaan mukosa dan mempercepatkan infeksi bakteria terhadap hos. Kajian ini telah dijalankan untuk mengenalpasti gen fimbria melalui teknik PCR dalam *P. multocida* B:2 yang hidup dan yang telah dilemahkan berikutan pengsubkulturan yang berulang selama dua bulan di dalam media agar yang mengandungi kepekatan darah yang semakin berkurang iaitu dari 7% hingga 0%. Keputusan kajian ini menunjukkan bahawa gen fimbria adalah satu faktor kemandirian yang penting pada *P. multocida* B:2 kerana gen ini kekal di dalam genom dan tidak hilang walaupun dilemahkan dalam keadaan yang tertekan, yakni kekurangan nutrien darah dalam media. Hasil kajian ini juga memutuskan bahawa gen fimbria merupakan faktor virulen yang penting dalam *P. multocida* B:2 di mana ia menyerang tisu hos sebaik sahaja bakteria mengkoloni permukaan mukosa. Keputusan daripada kajian ini dapat digunakan untuk menginisiasi kajian lanjutan mengenai peranan protein fimbria dalam jangkitan penyakit dan penemuan vaksin.