

DETECTION OF TRAILED CYTOBANE IN OCEANS INFECTED BY
Rickettsia rickettsii Rickettsiae 3:2 USING POLYMERASE
CHAIN REACTION (PCR) TECHNIQUE

HARVY GHAFT

FAKULTI SAINS DAN TEKNOLOGI
UNIVERSITI MAJLISIA MELAKKA
2007

Ch 16.16.2

1100051132

Perpustakaan Sultanah Nur Zahirah (UMT)
Universiti Malaysia Terengganu



LP 19 FST 2 2007



1100051132

Detection of fimbrial gene in organs infected by *Pasteurella multocida* B:2 using polymerase chain reaction (PCR) technique / Harny Chapi.

**PERPUSTAKAAN
UNIVERSITI MALAYSIA TERENGGANU (UMT)
21030 KUALA TERENGGANU**

100051132

Lihat sebelah

HAK MILIK
PERPUSTAKAAN UMT

DETECTION OF FIMBRIAL GENE IN ORGANS INFECTED BY
Pasteurella multocida B: 2 USING POLYMERASE
CHAIN REACTION (PCR) TECHNIQUE

By

Harny Chapi

Research Report submitted in partial fulfillment of
the requirements for the degree of
Bachelor of Science (Biological Sciences)

Department of Biological Sciences
Faculty of Science and Technology
UNIVERSITI MALAYSIA TERENGGANU
2007

1100051132

This project should be cited as:

Harny, C. 2007. Detection of fimbrial gene in organs infected by *Pasteurella multocida* B: 2 using Polymerase Chain Reaction (PCR) technique. Undergraduate thesis, Bachelor of Science in Biological Sciences, Faculty of Science and Technology, Universiti Malaysia Terengganu , Terengganu. 40p

No part of this project report may be produced by any mechanical, photographic, or electronic process, or in the form of phonographic recording, nor may it be stored in a retrieval system, transmitted, or otherwise copied for public or private use, without written permission from the author and the supervisor(s) of the project.



JABATAN SAINS BIOLOGI
FAKULTI SAINS DAN TEKNOLOGI
UNIVERSITI MALAYSIA TERENGGANU

UNIVERSITI MALAYSIA TERENGGANU

PENGAKUAN DAN PENGESAHAN LAPORAN
PROJEK PENYELIDIKAN I DAN II
RESEARCH REPORT VERIFICATION

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: DETECTION OF FIMBRIAL GENE IN ORGANS INFECTED BY *Pasteurella multocida* B: 2 USING POLYMERASE CHAIN REACTION (PCR) TECHNIQUE oleh Harny Chapi, no. matrik: UK10785 telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan memperolehi Ijazah Sarjana Muda Sains (Sains Biologi), Fakulti Sains dan Teknologi, Universiti Malaysia Terengganu.

Disahkan oleh: /Verified by:

Penyelia Utama/Main Supervisor

Nama: Assoc. Prof Dr. Mohd Effendy Abd Wahid

Cop Rasmi:

Tarikh: April 20, 2007

PROF MADYA DR. MOHD. EFFENDY ABD WAHID
Pengarah
Institut Bioteknologi Marin
Universiti Malaysia Terengganu
21030 Kuala Terengganu, Terengganu.

Ketua Jabatan Sains Biologi/Head, Department of Biological Sciences

Nama: Dr. Aziz Ahmad

Cop Rasmi:

DR. AZIZ BIN AHMAD
Ketua
Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Universiti Malaysia Terengganu
21030 Kuala Terengganu

Tarikh: 6/5/2007

ACKNOWLEDGEMENTS

Thanks to God, this project was finally successfully done. And I want to take this opportunity to express my deepest thanks to all that support and help me from the beginning until the end of this project. First of all, I want to give a million thanks to my supervisor Assoc. Prof. Dr. Mohd Effendy Abd. Wahid who has gives me lots of idea, guidance, advice and knowledge within this study. Thanks for the time that you have spent for guiding and supervise me.

Lot's of thank also to Vijay, who always guide and teach me a lot of new thing especially in molecular part. Thanks for your continuous guidance and encouragement. Not forget to the science officers and laboratory assistants at FST and INOS, thank you for your willingness to help me especially in my laboratory work.

Finally, I would like to give thanks to my friends and family for always beside me and encourage me all the time. Without you all, I will unable to finish this project successfully. Thanks, for always being there for me.

TABLE OF CONTENTS

TITLE	PAGE
ACKNOWLEDGEMENTS	ii
LIST OF TABLES	v
LIST OF FIGURES	vi
LIST OF ABBREVIATIONS	vii
LIST OF APPENDICES	viii
ABSTRACT	ix
ABSTRAK	x
CHAPTER 1 INTRODUCTION	1
1.1 Objective of the study.	3
CHAPTER 2 LITERATURE REVIEW	
2.1 <i>Pasteurella multocida</i> .	4
2.2 Virulence factors	5
2.3 Fimbriae	6
2.3.1 <i>Pasteurella multocida</i>	6
2.3.2 <i>Eschericia coli</i>	7
2.4 Polymerase Chain Reaction (PCR).	7
CHAPTER 3 MATERIALS AND METHODS	
3.1 Animals	9
3.2 <i>Pasteurella multocida</i> B: 2 cultures	9
3.3 Identification test	9
3.3.1 Primary isolation	9
3.3.2 Staining	10
3.3.3 Biochemical test	10
3.3.4 Growth on McConkey	11
3.3.5 Commercial Identification Kit	12
3.4 Bacteria preparation	12
3.5 Experiment design	12
3.5.1 Bacteria isolation	12
3.5.2 Tissue organs	13
3.6 DNA extraction	13

TITLE	PAGE
3.7 Purification and quantification	14
3.7.1 Electrophoresis	14
3.7.2 Bio photometer	14
3.8 Optimization of PCR component	14
3.9 Detection of fimbriae gene by Polymerase Chain Reaction (PCR).	15
4.0 Identify the presence of <i>P. multocida</i> B: 2 in exposed tissue organs.	15
CHAPTER 4 RESULT	
4.1 Identification test	17
4.1.1 Primary isolation	17
4.1.2 Staining	17
4.1.3 Biochemical test	17
4.1.4 Growth on McConkey	18
4.1.5 Commercial Identification Kit	18
4.2 Purification and quantification of extracted DNA	18
4.2.1 Electrophoresis	19
4.2.2 Bio photometer	19
4.3 Detection of fimbriae gene by Polymerase Chain Reaction (PCR).	19
4.4 Identify the presence of <i>P. multocida</i> B: 2 in infected tissue organs	19
CHAPTER 5 DISCUSSION	26
CHAPTER 6 CONCLUSION AND RECOMMENDATION	30
REFERENCES	31
APPENDICES	37
CURRICULUM VITAE	40

LIST OF TABLES

TABLES	PAGE
3.1 The final volume of PCR component used for PCR reaction.	15
3.2 Fimbriae gene primers sequence.	16
3.3 Amplification conditions for PCR reaction.	16

LIST OF FIGURES

FIGURES		PAGE
4.1	Genomic DNA extracted from <i>P. multocida</i> B: 2 and <i>E. coli</i>	20
4.2a	Extracted genomic DNA from unexposed tissue organs	21
4.2b	Extracted genomic DNA from exposed tissue organs	21
4.3a	PCR products generated by amplification of <i>P. multocida</i> B: 2 genomic DNA using 3 sets of fimbriae primers	22
4.3b	PCR products generated by amplification of <i>E. coli</i> genomic DNA using 3 sets of fimbriae primers	22
4.4a	PCR product (fimbriae gene) generated by amplification of <i>Pasteurella multocida</i> B: 2 genomic DNA isolated from exposed tissue organs using Pair 1; EZ 1(F) and EZ 2 (R) primer	23
4.4b	PCR product (fimbriae gene) generated by amplification of <i>Pasteurella multocida</i> B: 2 genomic DNA isolated from unexposed tissue organs using Pair 1; EZ 1(F) and EZ 2 (R) primer	23
4.5a	PCR product (fimbriae gene) generated by amplification of <i>Pasteurella multocida</i> B: 2 genomic DNA isolated from exposed tissue organs using Pair 2; EZ 3(F) and EZ 4 (R) primer	24
4.5b	PCR product (fimbriae gene) generated by amplification of <i>Pasteurella multocida</i> B: 2 genomic DNA isolated from unexposed tissue organs using Pair 2; EZ 3(F) and EZ 4 (R) primer	24
4.6a	PCR product (fimbriae gene) generated by amplification of <i>Pasteurella multocida</i> B: 2 genomic DNA isolated from exposed tissue organs using Pair 3; EZ 5(F) and EZ 6 (R) primer	25
4.6b	PCR product (fimbriae gene) generated by amplification of <i>Pasteurella multocida</i> B: 2 genomic DNA isolated from unexposed tissue organs using Pair 3; EZ 5(F) and EZ 6 (R) primer	25

LIST OF ABBREVIATIONS

bp	base pair
CFU	colony forming unit
CO ₂	carbon dioxide
DNA	deoxyribonucleic acid
dNTP	deoxynucleoside triphosphate
ddH ₂ O	deionise distil water
dH ₂ O	distil water
H ₂ O ₂	hydrogen peroxide
H ₂ S	hydrogen sulphide
kDa	kilo Dalton
LPS	lipopolysaccharide
mer	Oligomer
mg	milligram
MgCl ₂	Magnesium chloride
mL	mililiter
OMP	outer membrane protein
OD	optical density
PCR	Polymerase Chain Reaction
rpm	rotation per minute
SDS	Sodium Dodcyl Sulphate
TE	Tris- EDTA
TAE	Tris-Acetate EDTA
TSI	Triple Sugar Ion
U	Unit(s)
V	Volt(s)
µg	microgram
µM	micromolar
µL	microliter
°C	Degree Celsius

LIST OF APPENDICES

APPENDIX		PAGE
A1	50X TAE Buffer (Tris-Acetate-EDTA)	37
A2	Phosphate buffer pH7.2	37
A3	Phosphate Buffer Saline (PBS)	37
B1	1.2% Agarose gel	38
B2	5% Blood agar	38
B3	Nutrient agar	38
C1	Giemsa	39
C2	Peptone water	39
C3	Brain Heart Infusion (BHI) broth	39

ABSTRACT

Rapid detection of *Pasteurella multocida* B: 2 are important due to the facts that these bacteria can cause serious outbreak that bring mortality to the affected animals. This study was conducted to detect the presence of fimbrial gene in rat's organs that infected with *P. multocida* B: 2 by using PCR technique. This technique was used to determine the ability of this technique to detect the presence of *P. multocida* B: 2 in the tissue organs. Three sets of fimbriae gene primers were used, Pair 1; EZ1(F) and EZ2(R), Pair 2; EZ3(F) and EZ4(R) and Pair 3; EZ5(F) and EZ6(R). This study has successfully identified the presence of fimbrial gene in the infected tissue organs; lung, liver and kidney which suggested that there was a presence of *P. multocida* B: 2 in the tissue organs. Results indicated that all 3 set of primers have generated fimbriae gene of approximately 400-500bp. The finding of this study has indicated that within 7 days of exposure, the bacteria has spread and colonize the tissue organs and it was suspected that the bacteria have spread through the blood stream of the infected animals. These studies have demonstrated that PCR technique using a fimbriae gene primers can be used as a diagnostic tool in detecting and identifying the presence of *P. multocida* B: 2 in the infected tissue organs.

**PENGESANAN GEN FIMBRIAL DALAM ORGAN YANG DIJANGKIT
OLEH *Pasteurella multocida* B: 2 MENGGUNAKAN TEKNIK
TINDAK BALAS RANTAI POLIMERASE (PCR)**

ABSTRAK

Pengesan *Pasteurella multocida* B: 2 yang cepat adalah penting berdasarkan kepada fakta bahawa bakteria ini boleh menyebabkan wabak yang serius dan membawa kematian kepada haiwan yang dijangkiti. Kajian ini telah dijalankan untuk mengenalpasti kehadiran gen fimbria dalam organ tikus yang dijangkiti oleh *Pasteurella multocida* B: 2 menggunakan teknik PCR. Teknik ini digunakan untuk mengesan kebolehan teknik ini untuk mengesan kehadiran *P. multocida* B: 2 dalam tisu organ tersebut. Tiga set primer gen fimbriae digunakan, P1; EZ1 (F) dan EZ2(R), P2; EZ3 (F) dan EZ4(R) dan P3; EZ5 (F) dan EZ6(R). Kajian ini telah berjaya mengenalpasti kehadiran gen fimbria dalam tisu organ yang dijangkiti; peparu, hati dan ginjal yang menunjukkan kehadiran *P. multocida* B: 2 dalam organ tersebut. Keputusan menunjukkan kesemua 3 set primer telah menghasilkan gen fimbria iaitu kira-kira 400-500bp. Hasil penemuan kajian ini telah menunjukkan bahawa dalam tempoh 7 hari selepas didedahkan, bakteria tersebut telah tersebar dan menghuni tisu organ, dan disyaki kemungkinan bakteria tersebut tersebar melalui saluran darah haiwan yang telah dijangkiti. Kajian ini telah menunjukkan bahawa teknik PCR menggunakan primer gen fimbriae boleh digunakan sebagai alat diagnostik untuk mengesan dan mengenalpasti kehadiran *P. multocida* B: 2 dalam tisu organ yang dijangkiti.