

ENTEROBACTERIAL REPETITIVE INTERGENIC CONSENSUS-PCR
(ERIC-PCR) ANALYSIS AMONG RAW VEGETABLES ISOLATES OF

Campylobacter jejuni

TUAN SURAYA BT TUAN SOH

DISSERTATION SUBMITTED IN FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF MASTER OF
BIOTECHNOLOGY

INSTITUTE OF BIOLOGICAL SCIENCES
FACULTY OF SCIENCE
UNIVERSITY OF MALAYA

2010

ACKNOWLEDGEMENT

Praise to Allah for the strength, wisdom and for the unconditional love that I got that, I am able to pursue and complete my Degree of Master of Biotechnology. Without that, I would never have the perseverance to make it until the end.

First of all, I would like to express my utmost appreciation to my supervisor, Dr Noraida bt Ismail and to my co-supervisor, Dr. Sahilah bt Abdul Mutalib, for their patience, kindness, guidance and useful advice given throughout this thesis project. Her wisdom and encouragement has inspired us to work harder, to make this thesis a special, successful and memorable one.

A million thanks to Prof. Dr. Yaakob Che Man, who is the Director of Halal Products Research Institute, Universiti Putra Malaysia, for the facilities and helps in making this, project a successful one

My thanks also go to my coursemates, Aishah, Kamila, Hani, Kak Gee, Teesilia and Kak Rafezah who worked together in the Food Biotechnology laboratory for the happy moment that we shared in the lab. I also would like to express my deepest gratitude to my husband, Mohd Syauki Hassan and my son, Nik Mohd Syamil for their support and guidance. Without your endless love, support and encouragement, I could never have finished this thesis. Thank you for always being there for me.

Finally, I would like to dedicate the thesis to my family; to my beloved Abah, Mama, Kak Su, Auk, Adik, Abg Mide, Ida and Marhamah (my loving sisters and brothers) and my dearest colleagues in SK Alor Lintah, Jerteh, Terengganu, especially for PPKIBP members, my deepest appreciation of love and thanks goes to all of you. I can never express my gratefulness for everything that you have brought into my life. I love you all and this I can do for you.

ABSTRACT

A total of 20 (n=20) raw vegetable isolates of *Campylobacter jejuni* were examined for the presence of virulence genes and genetic diversity using enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR) analysis. All *C. jejuni* isolates were obtained from the laboratory of Food Science and Biotechnology, University Putra Malaysia, Serdang, Selangor. When specific PCR amplification were determined, all raw vegetables isolates of *Campylobacter jejuni* contained *cadF* and *ceuE* genes. Whereas, 12 (12/20) isolates were positive *cdtB* and 6 (6/20) were positive *cdtC*. None of the *C. jejuni* isolates was *cdtA* positive. Using ERIC-PCR analysis, all 20 isolates of *C. jejuni* were subtyped and produced 18 ERIC-PCR profiles namely E1 to E18. Dendogram performed from cluster analysis showed the 20 isolates were group into 2 major clusters (cluster I and II) which may suggest the high level of local geographical genetic variation. From this study, the detection of virulence gene among *Campylobacter jejuni* strains isolated from raw vegetables is an evidence that raised concern on treatment of campylobacteriosis.

ABSTRAK

Sejumlah dua puluh ($n=20$) isolate *Campylobacter jejuni* yang diasingkan daripada sayur-sayuran mentah telah dikaji untuk mengesan gen virulen dan kepelbagaiannya genetik menggunakan analisis Enterobacterial Repetitive Intergenic Consensus-Polymerase Chain Reaction (ERIC-PCR). Kesemua isolat telah diperolehi dari Makmal Sains Makanan & Bioteknologi, Universiti Putra Malaysia, Serdang, Selangor. Kesemua isolat *Campylobacter jejuni* yang diasingkan daripada sayur-sayuran mentah mengkodkan gen cadF dan ceuE apabila amplifikasi PCR ditentukan. Sementara itu, 12 isolat (12/20) positif gen cdtB dan 6 isolat (6/20) positif gen cdtC. Namun demikian, tiada isolat *Campylobacter jejuni* yang positif bagi gen cdtA. Menggunakan analisis ERIC-PCR, kesemua 20 isolat *C. jejuni* disubtaipkan dan menghasilkan 18 profil ERIC-PCR yang dinamakan E1 hingga E18. Paparan dendogram daripada analisis kelompok menunjukkan kesemua 20 isolat dikumpulkan kepada 2 kelompok utama (kelompok I dan kelompok II) yang mana mencadangkan paras tinggi variasi genetik di geografi tempatan. Pengesan gen virulen di antara isolat *C. jejuni* yang diasingkan daripada sayuran mentah adalah bukti daripada kajian ini untuk meningkatkan kesedaran untuk merawat campylobacteriosis.

CONTENTS

	Page
ACKNOWLEDGEMENT	i
ABSTRACT	iii
ABSTRAK	iv
CONTENTS	v
LIST OF TABLES	viii
LIST OF FIGURES	ix
ABBREVIATIONS & SYMBOLS	x
CHAPTER 1	INTRODUCTION & OBJECTIVES
1.1	Introduction
1.2	Objectives
CHAPTER 2	LITERATURE REVIEW
2.1	<i>Campylobacter</i>
2.2	Campylobacteriosis
2.3	Pathogenicity of <i>Campylobacter</i>
2.4	Sources and Transmission of <i>Campylobacter</i>
2.5	Epidemiology of <i>Campylobacter</i>

2.6	Isolation of <i>Campylobacter</i>	12
2.6.1	Pre-enrichment media	12
2.6.2	Enrichment media	13
2.6.3	Selective plating media	13
2.7	Identification of <i>Campylobacter</i>	14
2.7.1	Culture Base Methods	14
2.7.2	Non culture Methods	15
2.8	Epidemiological typing systems	16
2.9	Molecular Typing	16
2.10	Polymerase Chain Reaction	17

CHAPTER 3 MATERIALS AND METHODS

3.1	<i>Campylobacter jejuni</i> strains	19
3.2	Strain maintenance	20
3.3	Sterilization technique	20
3.4	Solution	
3.4.1	Tris-borate EDTA (TBE) buffer (10X concentration)	20
3.5	Medium	
3.5.1	Nutrient Agar	21
3.5.2	cAMP agar	21
3.6	DNA extraction	21
3.7	PCR Detection using <i>cdtA</i> gene	22

3.8	PCR Detection using <i>cdtB</i> gene	22
3.9	PCR Detection using <i>cdtC</i> gene	23
3.10	Detection of <i>cadF</i> and <i>ceuE</i> gene	24
3.11	Enterobacterial Repetitive Intergenic Consensus (ERIC)-PCR Amplification	25
3.12	Data Analysis	26

CHAPTER 4 RESULTS

4.1	Detection of <i>cdtA</i> gene	27
4.2	Detection of <i>cdtB</i> gene	28
4.3	Detection of <i>cdtC</i> gene	29
4.4	Detection of <i>cadF</i> and <i>ceuE</i> genes by multiplex PCR	30
4.5	Enterobacterial Repetitive Intergenic Sequence	32
4.6	Unweighted Pair Group Method with Arithmetic mean (UPGMA)	35

CHAPTER 5 DISCUSSION

5.1	Discussion	37
-----	------------	----

CHAPTER 6 CONCLUSION

6.1	Conclusion	40
-----	------------	----

REFERENCES

APPENDICES

A: GENERAL MEDIA AND SOLUTIONS

B: SOLUTION FOR PCR

LIST OF TABLES

Table No.		Page
2.1	Epidemiologic studies of laboratory-confirmed cases of sporadic campylobacteriosis	11
3.1	Type of samples for the prevalences and numbers of <i>Campylobacter</i> spp. and their location	19
4.1	Virulence genes detected in raw vegetables isolates of <i>Campylobacter jejuni</i>	31

LIST OF FIGURES

Figure No.		Page
4.1	Detection of <i>Campylobacter jejuni</i> isolates using <i>cdtA</i> virulence gene among vegetable isolates of <i>Campylobacter jejuni</i> electrophoresed on 1.0% agarose gel	27
4.2	Detection of <i>Campylobacter jejuni</i> isolates using <i>cdtB</i> virulence gene among vegetable isolates of <i>Campylobacter jejuni</i> electrophoresed on 1.0% agarose gel	28
4.3	Detection of <i>Campylobacter jejuni</i> isolates using <i>cdtC</i> virulence gene among vegetable isolates of <i>Campylobacter jejuni</i> electrophoresed on 1.0% agarose gel	29
4.4	Multiplex detection of <i>cadF</i> and <i>ceuE</i> virulence gene among vegetable isolates of <i>Campylobacter jejuni</i> electrophoresed on 1.0% agarose gel	30
4.5	ERIC-PCR fingerprinting (E1 to E8) of <i>Campylobacter jejuni</i> isolates electrophoresed on 1.0% agarose gel	32
4.6	ERIC-PCR fingerprinting (E9 to E16) of <i>Campylobacter jejuni</i> isolates electrophoresed on 1.0% agarose gel	33
4.7	ERIC-PCR fingerprinting (E17 to E20) of <i>Campylobacter jejuni</i> isolates electrophoresed on 1.0% agarose gel	34
4.8	Dendogram generated from the ERIC-PCR profiles of the <i>Campylobacter jejuni</i> isolates from raw vegetables	36

ABBREVIATIONS & SYMBOLS

bp	Base pair
°C	Degree celcius/Centigrade
DNA	Deoxyribonucleic acid
dNTPs	Dinucleotide(s) triphosphate
ds	Double stranded
<i>et al.</i>	Consensus and associates
g	Gram(s)
>	Greater than
H	Hour
Kb	Kilo base pair
µg	Microgram(s)
µl	Microliter(s)
µm	Micrometer(s)
M	Molar
min	Minute
mg	Milligram(s)
mM	Millimole(s)
ml	Milliliter(s)
%	Per cent
Pmol	Per mole
rpm	Round per minute
UV	Ultra violet