UNIVERSITI PUTRA MALAYSIA

PLASMID CHARACTERIZATION OF *Enterobacter sp.* FROM HUMAN BREAST MILK IN MALAYSIA

FATIN ILLANI ABD SAMAD

FBSB 2015 145

PLASMID CHARACTERIZATION OF *Enterobacter sp.* FROM HUMAN BREAST MILK IN MALAYSIA

By

FATIN ILLANI BT ABD SAMAD

Thesis Submitted to the Department of Cell and Molecular Biology,

Faculty of Biotechnology and Biomolecular Science,

Universiti Putra Malaysia,

In Fulfilment of the Requirement for the Degree of

Cell and Molecular Biology

June 2015

Abstract of thesis presented to the Department of Cell and Molecular Biology in fulfilment of the requirement for the degree of Cell and Molecular Biology

PLASMID CHARACTERIZATION OF *Enterobacter sp.* FROM HUMAN BREAST MILK IN MALAYSIA

By

FATIN ILLANI BT ABD SAMAD

June 2015

Chair: Dr. Nurulfiza Mat Isa, PhD

Faculty: Biotechnology and Biomolecular Sciences.

Enterobacter sp. species are nosocomial pathogens that could cause variety of infections, such as pneumonia, urinary tract infections or surgical wound infections. This ability in infecting human can cause by present of antibiotic-resistant plasmids which resistant toward antibiotics and this become a challenging clinical problem. The aim of this study is to isolate and characterize the plasmid extracted from *Enterobacter cloacae from* breast milk in Malaysian women. Extraction method of modified alkaline lysis and using Promega PureYield™ Plasmid Miniprep System were used to obtain high concentration of plasmids. The product size was expected to be ~11kbp and in this experiment, highly purified and concentrated plasmids were difficult to obtain thus, several modifications in plasmid extraction a required. PCR reaction was run using M13 universal primers and send for sequencing for the fragments which being amplify. This project was also being carried out to design a more versatile plasmid DNA which might aid in cloning system.

Abstrak tesis yang dikemukakan kepada Jabatan Biologi Sel dan Molekul sebagai memenuhi keperluan untuk ijazah Biologi Sel dan Molekul

PENCIRIAN *Enterobacter sp*. PLASMID DARI SUSU BADAN MANUSIA DI MALAYSIA

Oleh

FATIN ILLANI BT ABD SAMAD

Jun 2015

Pengerusi : Dr. Nurulfiza Mat Isa, PhD

Fakulti: Bioteknologi dan Sains Biomolekul

Enterobacter sp. ialah nosokomial patogen yang mampu mengundang pelbagai jenis jangkitan seperti pneumonia, jangkitan saluran kencing atau jangkitan pada luka pembedahan. Kemampuan bakteria ini dalam menjangkiti tubuh badan manusia boleh disebabkan oleh ketahanan antibodi plasmid yang mampu bertahan walau dengan kehadiran antibodi dan ini menjadi masalah dalam dunia perubatan. Tujuan utama projek ini adalah untuk mengekstrak dan mengalpasti ciri-ciri plasmid dari *Enterobacter cloacae* yang dijumpai dalam kandungan susu badan wanita di Malaysia. Kaedah pengekstrakan plasmid melalui lisis alkali yang telah di olah dan pengunaan Promega PureYield™ Plasmid Miniprep Sistem telah diaplikasikan demi memperoleh kepekatan *plasmid* DNA yang tinggi. Dianggarkan saiz plasmid adalah ~11 kbp dan ianya agak sukar untuk memperoleh plasmid yang kurang tercemar lalu menyebabkan beberapa langkah pengekstrakan yang biasa dilakukan perlu diolah. PCR reaksi telah dilakukan dengan menggunakan M13 primer universal

dan dihantar untuk jujukan bagi ruas DNA yang telah diperbanyakkan. Selain itu, projek ini juga bertujuan untuk mencipta vektor yang serba guna yang mungkin mampu meningkatkan keupayaan sistem pengklonan yang sedia ada pada masa kini.

APPROVAL

This thesis was submitted to the Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences and has been accepted as fulfilment of the requirement for the degree of Cell and Molecular Biology. The member of the Supervisory Committee was as follows:

Name: Prof. Madya Dr. Janna Ong Abdullah, PhD

Title: Professor Madya

Faculty of Biotechnology and Biomolecular Science

Universiti Putra Malaysia

……

(Prof. Madya Dr. Janna Ong Abdullah)

Head of Department of Cell and Molecular Biology

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

Date :

DECLARATION

Declaration by undergraduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by The Department of Cell and Molecular Biology
- written permission must be obtained from supervisor before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials.
- There is no plagiarism or data falsification/fabrication in the thesis.
- The thesis has undergone plagiarism detection software (TURNITIN)

Signature: …….. Date: ……..

Name and Matric No.: Fatin Illani Bt Abd Samad (161047)

Declaration by Supervisor

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision

Supervisor,

……

Dr. Nurulfiza Bt Mat Isa,PhD

ACKNOWLEDGEMENTS

First of all, Alhamdulillah, I would like to thank Allah for all of His guidance, opportunity, and strength that were given to me along the process to finish my final year project. Indeed, there were much challenges and difficulties while doing this project, however with the help of Him especially, and others, I managed to accomplish this study successfully.

I would like to take this opportunity to thank my supervisor, Dr. Nurulfiza Mat Isa for her invaluable knowledge, guidance and supports that have been given to me in this study. My gratitude also goes to all postgraduate and undergraduate students at Molecular Biology Lab, Faculty of Biotechnology and Biomolecular Sciences for their help and cooperation. Also my sincere thanks go to my family for their advices, encouragement, love and understanding in all my life throughout these years. Last but not least, a million thanks to all my friends especially my course mates, for their help and warm friendship. Thanks to all, and only The Almighty Allah will repay all your kindness.

TABLE OF CONTENTS

CONTENTS	PAGE
TITLE	**i**
ABSTRACT	**ii**
ABSTRAK	**iii**
APPROVAL	**v**
DECLARATION	**vi**
ACKNOWLEDGEMENT	**viii**
LIST OF ABBREVIATION	**xi**
LIST OF FIGURES	**xii**
CHAPTER	
1. INTRODUCTION	1
2. LITERATURE REVIEW	3
2.1 *Enterobacter cloacae*	3
2.2 Breast milk	
2.3 Bacterial content in breast milk	6
2.4 Characterization of plasmid	7
2.4.1 Agarose Gel Electrophoresis	7
2.4.2 Plasmid DNA sequencing using universal primers	8
2.4.3 Identification of Plasmid DNA using 16s primers	8
2.4.4 Restriction Enzyme Mapping Plasmid	9
2.4.5 DNA manipulation and analysis	10
2.4.6 Plasmid copy number	10
2.4.7 Bioinformatic Analysis	11

3. MATERIALS AND METHODS 12

3.1 Bacterial Culture 12

3.2 Genomic DNA extraction 12

3.3 Plasmid extraction using kit 13

3.4 Plasmid DNA extractions using alkaline lysis 14

3.5 Agarose gel electrophoresis 16

3.6 Gel Purification 17

3.7 PCR amplification 18

3.8 PCR Purification 19

4. RESULTS AND DISCUSSION 21

4.1 Genomic extraction 21

4.2 PCR amplification using specific primers 23

4.3 Plasmid extraction using alkaline lysis 25

4.4 Plasmid extraction using kit 27

4.5 Gel purification 30

4.6 Amplification of plasmid DNA with M13 primer 32

4.7 Sequencing analysis 33

5. CONCLUSION AND RECOMMENDATION 35

REFERENCES 36

APPENDICES 40

LIST OF ABBREVIATIONS

DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
RE	Restriction enzyme
PCR	Polymerase chain reaction
LB	Luria Bertani media
TE / TAE Buffer	Tris-CI EDTA / Tris-acetate EDTA
$MgCl_2$	Magnesium chloride
bp	Base pairs
kb	Kilo base
v/v	Volume per volume
w/v	Weight per volume
µM/ mM/ M	Micro molar/ Millimolar/ Molar
µl/ ml	Microliter/ Milliliter
rpm	Rotation per minute
g	Relative centrifugal force (RCF)

LIST OF FIGURES

FIGURE	TITLE	PAGE
Figure 1	Genomic extraction	22
Figure 2	PCR amplification using specific primers	24
Figure 3	Plasmid Extraction using alkaline lysis method	26
Figure 4	Plasmid Extraction using kit	29
Figure 5	Gel Purification	31
Figure 6	Amplification of plasmid DNA with M13 primers	32
Figure 7	Chromatogram result using M13F_29	34
Figure 8	Chromatogram result using M13R_24	34
Figure 9	Chromatogram result using M13F_41	34
Figure 10	Chromatogram result using M13R_27	34

CHAPTER 1

INTRODUCTION

Enterobacter *spp.* is in the family *Enterobacteriaceae* and can be classified as facultative anaerobic Gram-negative bacilli. They produce acid upon glucose fermentation with an optimal growth temperature of 30 °C. This species can lead to various infections, including pneumonia, sepsis and intestinal infection. *Enterobacter cloacae* infections have the highest mortality rate compared to other *Enterobacter* strains. This bacterium can be easily found on human skin, within our foods, urine and can also be detected within infected breast milk. Thus, children with long breastfeeding might expose toward a higher level of risk for associated developmental ill effects. The pathogenicity of the plasmid can be due to the presence of the resistance plasmid families in *Enterobacteriaceae*. Plasmids are also known as extrachromosomal that did not carry genes involve in the bacteria growth. Plasmid consist few elements like Origin of Replication (ORI) that allow the plasmid to replicate independently without depending on the genome replication mechanism. To create an exact copy, plasmid will recruit or use the replicative machinery in their replication process. Another element would be the multiple cloning sites (MCS) or known as the polylinkers are the region where unique restriction enzyme presents. This makes the plasmid act as the vector and vehicle for genes of interest for cloning purposes. Selectable marker elements are the antibiotics resistance gene that

involve for the maintenance of the plasmid within the host bacteria. Selectable markers are useful for the cells for surviving under selective environment allowing plasmid to stay within the cells at very long time. Example of the resistant gene is amp^r gene resistant toward ampicillin, cam^r gene allow the bacteria to survive in the present of Chloramphenicol antibiotics.

Identifying and classification of the plasmid in homogeneous group on their phylogenetic relatedness are crucial to analyze and obtain information of their pathogenicity characteristics. The standard procedure in isolating and characterizing the plasmid involve in gel electrophoresis method to determine the size of the plasmid. After the sequencing by Sanger Sequencing method, the information can be analyses through Bioinformatics tools for instant BLAST and Pairwise Alignment analysis to identify the phylogeneticity for the plasmid . Understanding on the properties of the plasmid within the host cell allows us to understand more on the causative disease of *Enterobacter cloacae* thus, reduce the clinical challenge in disease diagnose. Other than that, new versatile plasmid system can be develop and may offers several advantages over previously cloning system.

OBJECTIVE

To characterized the plasmid from *Enterobacter sp.*

REFERENCES

Alavi, M. R. ((2011)). An Enterobacter plasmid as a new genetic background for the transposon Tn1331. Infection and drug resistance. *4*, 209.

Athas, G. (1999, November 8). *The Scientist*. Retrieved January 2015, from Pure and Simple: http://www.the-scientist.com/?articles.view/articleNo/19641/title/Pure-and-Simple/

Fernándeza L, Langaa S, Martína V, Maldonadoa A, Jiméneza E., Martínd R., Rodríguez J.M. (2013, March). The human milk microbiota: Origin and potential roles in health and disease. *Pharmacological Research, 69*(1), 1-10.

Gao X, M. R. (2012). Temporal changes in milk proteomes reveal developing milk functions. *11(7)*, 3897–3907.

Geis A., H. A. (2002, December). Sequence analysis and Characterization of Plasmid from Streptococcus thermophilus. *PLASMID*, 53-69.

Han J., L. A. (2012, December). DNA Sequence Analysis of Plasmids from Multidrug Resistant Salmonella enterica Serotype Heidelberg Isolates. *7*(12).

Han J., Lynne A. M., David D. E., Tang H., Xu J., Rajesh Nayak, Pravin Kaldhone, Logue C. M., Foley S. L. (2012, December 10). DNA Sequence Analysis of

Plasmids from Multidrug ResistantSalmonella enterica Serotype Heidelberg Isolates. *7*(12).

Hegyi G., Kardos J., Kovács M., Csizmadia A. M., Nyitray L., Pál G., Radnai L., Reményi A., Venekei I. (2013). *BiochemistryAnalysis of plasmid DNA by gel electrophoresis.*

Hoffmann H. & Roggenkamp A. (2003 , September). Population Genetics of the Nomenspecies Enterobacter cloacae. *69*(9), 5306–5318.

Hui L. C., Jen H. L., Hsin H. W. (2013). Clinical analysis of Enterobacter bacteremia. *Microbiology, Immunology and Infection*, 381-386.

Introduction to Practical Biochemistry. (2013). Retrieved March 12, 2015, from ELTE TTK ONLINE: http://elte.prompt.hu/sites/default/files/tananyagok/practical_biochemistry/ch10s06.html

Jalilsood T., Baradaran A., Ling F. H., Mustafa S., Yusof K., Abdul rahim A. (2014). Characterization of pR18, a novel rolling-circle replication plasmid from Lactobacillus plantarum. *Plasmid, 73* , 1-9.

Jorgensen T.S., Xu Z., Hansen M. A., Sorensen S. J., Hansen L.H. (2014). Hundreds of Circular Novel Plasmid and DNA Elements Identified in a Rat Cecum Metamobilome.

Kneebone G. M., K. R. (1985, April). Fatty Acids Composition of Breast Milk From Three Racial Groups From Penang, Malaysia. *The American Journal of Clinical Nutrition*, pp765-769.

L., B. O. (2013 , February). Human Milk Composition: Nutrients and Bioactive Factors. *60*(1), 49–74.

L., B. O. (2013, February). Human Milk Composition: Nutrients and Bioactive Factors. *NIH Public Access, 60(1)*, 49–74.

Lyons, D. R. (2015, June 3 Wednesday). *THE UNIVERSITY OF MICHIGAN*. Retrieved April 2015, from DNA Sequencing Core: http://seqcore.brcf.med.umich.edu/doc/dnaseq/trouble/pcrprimers.html

MEYERS J. A., SANCHEZ D., ELWELL L. P., FALKOW S. (1976). Simple Agarose Gel Electrophoretic Method for the. *JOURNAL OF BACTERIOLOGY, 127*, 1529-1537.

Mohammad R.A., Antonic V., Ravizee A., Weina P. J., Izadjoo M., Stojadinovic A. ((2011)). An Enterobacter Plasmid as a New Genetic Background for the Transposon Tn1331. 4: 209–213.

P., B. (2010). The mucosal immune system and its integration with the mammary glands. The Journal of pediatrics.

P., B. (2010). The mucosal immune system and its integration with the mammary glands. The Journal of pediatrics. *156(2 Suppl)*, :S8–15.

R. Cabrera-Rubio, M. C. (2012). The Human Milk Microbiome Changes Over Lactation and is Shaped by Maternal Weight and Mode of Delivery. *American Journal of Clinical Nutrition, 96(3),* 544.

Raetz, C. R. (2002). Lipopolysaccharide endotoxins. Annual Review of Biochemistry. 71, 635-700.

Sasa H., Marko J., Strancar A., Peterka M., Hodzic D., Miklavcic D. (2013). Comparison of Alkaline Lysis with Electroextraction abd Optimization of Electric Pluses to Extract Plasmid from Escherichia coli.

Strand T.A., S. P. (2012). Risk factors for extended duration of acute diarrhea in young children. *7(5).*

Strand T.A., Sharma P.R., Gjessing H.K., Ulak M., Chandyo R.K., Adhikari R.K., Sommerfelt H. (2012). Risk factors for extended duration of acute diarrhea in young children. *5*(7).