UNIVERSITI PUTRA MALAYSIA

ISOLATION AND CHARACTERIZATION OF *SALMONELLA* SPECIES FROM STREET FOOD AND CLINICAL SAMPLES

TUNUNG ROBIN

FSTM 2007 1

ISOLATION AND CHARACTERIZATION OF *SALMONELLA* SPECIES FROM STREET FOOD AND CLINICAL SAMPLES

By

TUNUNG ROBIN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

May 2007

Dedicated to my beloved family and dear friends for their loving support

To God Almighty for the strength and wisdom

Fear of the Lord is the beginning of knowledge,

Fools despise wisdom and instruction

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

ISOLATION AND CHARACTERIZATION OF *SALMONELLA* SPECIES FROM STREET FOOD AND CLINICAL SAMPLES

By

TUNUNG ROBIN

May 2007

Chairman: **Associate Professor Fatimah Abu Bakar, PhD**

Faculty: **Food Science and Technology**

The aims of this study were to isolate and characterize *Salmonella* spp. from food sampled randomly from street vendors in Selangor, Malacca, Kuala Lumpur and Negeri Sembilan, and from clinical samples in a hospital in Klang, Selangor (collected from January to September 2004). A total of 24 *Salmonella* strains, belonging to seven different serotypes, were isolated from 129 different street-vended foods and drinks and 12 rectal swabs from hospital patients suspected with salmonellosis. The most frequently encountered serotype from street foods was *Salmonella* Biafra (66.7%) while the most frequently encountered serotype from clinical samples was *Salmonella* Typhi (66.7%). To the best of our knowledge, this is the first reported occurence of *Salmonella* Biafra in street foods in Malaysia. Other serotypes isolated from street foods were *Salmonella* Braenderup (25%) and *Salmonella* Weltevreden (8.3%), and from clinical samples were *Salmonella* Typhimurium (17%), *Salmonella* Paratyphi A (0.08%) and *Salmonella* Paratyphi B (0.08%). All isolates were tested for their resistance to the following 14 antibiotics: streptomycin, trimethoprim, sulphamethoxazole, tetracycline, cefuroxime,

ciprofloxacin, ampicillin, chloramphenicol, gentamicin, rifampin, penicillin, nalidixic acid, norfloxacin and erythromycin. All strains (100%) were found resistant to the antibiotic rifampin. None of the strains were however resistant to cefuroxime. A dendrogram was generated for antibiotic resistances of the isolates, and six clusters were defined, with similarity levels ranging from 18.8% to 100%. Generally, street foods and clinical isolates tend to cluster apart. A dendrogram to cluster the antibiotics was also generated, and they could be grouped according to their classes based on mode of inhibition: cell wall synthesis, protein synthesis, or nucleic acid synthesis. The most related compounds were chloramphenicol and tetracycline (46.6% similarity), which is acceptable as they share the same mode of inhibition. Ampicillin was grouped in the same cluster at 13.5% similarity although of different inhibition mode, possibly due to cross-resistance. Out of the 24 isolates screened for the presence of plasmid, 15 harbored plasmids. The plasmid sizes ranged from 3.0 to 38.5 MDa. Randomly amplified polymorphic DNA (RAPD) and enterobacterial repetitive intergenic consensus (ERIC) analysis were performed for the molecular genetic typing of the strains. RAPD fingerprinting with the primers OPAR3 and OPAR8 produced a combination of 21 fingerprint patterns, while ERIC fingerprinting generated 19 patterns. Cluster analysis of *S.* Biafra showed that similar serotypes were found in different food samples collected at different locations. The likely source of this result was cross contamination during food handling. Cluster analysis of *S.* Typhi showed that all the serotypes were different, even though the location of sampling was the same. The results indicate multiple source of *S.* Typhi infection of the patients. From this study, RAPD was found to be more discriminative than ERIC.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PEMENCILAN DAN PENCIRIAN SPESIS *SALMONELLA* DARI SAMPEL MAKANAN GERAI DAN KLINIKAL

Oleh

TUNUNG ROBIN

Mei 2007

Pengerusi : Profesor Madya Fatimah Abu Bakar, PhD

Fakulti : Sains dan Teknologi Makanan

Matlamat kajian ini adalah untuk memencilkan dan mencirikan pencilan-pencilan spesis *Salmonella* dari sampel makanan gerai dan klinikal yang diperolehi dari lokasi rawak di Selangor, Melaka, Kuala Lumpur dan Negeri Sembilan, dan dari sampel klinikal di sebuah hospital di Klang, Selangor (dikumpulkan dari Januari hingga September 2004). Sejumlah 24 pencilan *Salmonella*, yang terdiri daripada tujuh serotip berbeza, dipencilkan daripada 129 makanan dan minuman daripada gerai yang berbeza dan 12 sampel klinikal (swab rektum). Serotip yang paling kerap dijumpai daripada makanan gerai adalah *Salmonella* Biafra (66.7%) dan daripada sampel klinikal adalah *Salmonella* Typhi (66.7%). Pada pengetahuan kami, ini merupakan laporan yang pertama tentang kehadiran *Salmonella* Biafra dalam makanan gerai di Malaysia. Serotip lain yang dipencilkan dari makanan gerai adalah *Salmonella* Braenderup (25%) dan *Salmonella* Weltevreden (8.3%), dan dari sampel klinikal adalah *Salmonella* Typhimurium (17%), *Salmonella* Paratyphi A (0.08%) dan *Salmonella* Paratyphi B (0.08%). Kerintangan pencilan-perncilan tersebut telah dinilai dengan menggunakan antibiotik berikut: streptomycin, trimethoprim,

sulfamethoxazole, tetracycline, cefuroxime, ciprofloxacin, ampicillin, chloramfenicol, gentamicin, rifampin, penicillin, nalidixic acid, norfloxacin dan erythromycin. Semua pencilan tersebut (100%) didapati rintang terhadap rifampin. Namun tiada pencilan yang rintang terhadap cefuroxime. Dendrogram kerintangan antibiotik pencilan-pencilan tersebut dihasilkan, dan enam kluster telah ditakrifkan, dengan tahap persamaan 18.8% ke 100%. Secara amnya, pencilan makanan gerai dan klinikal cenderung untuk mengkluster berasingan. Dendrogram untuk mengkluster antibiotik juga dihasilkan, dan antibiotik-antibiotik tersebut boleh dikumpulkan mengikut kelas berdasarkan mod inhibisi: inhibisi sintesis dinding sel, sintesis protein atau sintesis asid nukleik. Antibiotik yang paling berkait rapat adalah chloramphenicol dan tetracycline (persamaan 46.6%), yang mana ianya boleh diterima kerana berkongsi mod inhibisi yang sama. Ampicillin berkumpul di dalam kluster yang sama pada persamaan 13.5% walaupun mempunyai mod inhibisi yang berbeza, kemungkinan disebabkan kerintangan silang. Daripada 24 pencilan yang telah diuji untuk kehadiran plasmid, 15 pencilan mengandungi plasmid. Saiz plasmid berada dalam lingkungan 3.0 ke 38.5 MDa. Analisis amplifikasi polimorfik DNA rawak (RAPD) dan konsensus intergenik repetitif enterobakterial (ERIC) telah dilaksanakan untuk mengetip genetik molekular pencilan-pencilan tersebut. RAPD dengan OPAR3 dan OPAR8 menghasilkan kombinasi 21 pola, sementara ERIC menghasilkan 19 paten. Analisis kluster *S.* Biafra menunjukkan bahawa serotip yang sama dijumpai daripada sampel makanan gerai yang berbeza daripada lokasi yang berbeza. Kemungkinan ini adalah kerana kontaminasi silang semasa pengendalian makanan. Analisis *S.* Typhi menunjukkan bahawa serotip adalah berbeza walaupun lokasi sampel sama. Keputusan tersebut menunjukkan sumber jangkitan *S.* Typhi

yang berlainan pada pesakit-pesakit tersebut. Melalui kajian ini, RAPD adalah lebih diskriminatif berbanding ERIC.

ACKNOWLEDGEMENTS

Praise to the Lord God Almighty, Jesus Christ for the strength that He has given to me, for the wisdom that He had granted me, and for the unconditional love that He has shown me, that I am able to pursue and complete my Degree of Master of Science. Without You, I would never have the perseverance to make it until the end. Thank You.

I would like to dedicate my heartfelt thanks to Associate Professor Dr. Fatimah Abu Bakar as the chairman of my supervisory committee, for her continuous support and guidance throughout the years. Thank you for the trust that you have in me. Your love and kindness will remain in my heart always.

A million thanks to Professor Dr. Son Radu who has contributed tremendously of his time and expertise in this research and in my study. He has acted as a guardian and also as a friend to me throughout the journey. Thank you so much for your guidance and patience, and for always believing in me and seeing the potential more than the weaknesses. You will always be an inspirational role model.

My gratitude also goes to Dr. Pradeep K. Malakar as my co-supervisor, who has been giving me encouragement and advice throughout my research. You are a blessing! To Professor Dr. Rani Abdul Rahman (Veterinary Faculty), my deepest appreciation goes to you for the encouragement, advice, and support that you gave endlessly. Also

to Dr. Farinazleen Mohd. Ghazali, a million thanks especially to you for the encouragement and love that you have shown.

A special appreciation goes to Dr. Noorzaleha from the Ministry of Health Malaysia and Encik Subki and also members from the Public Health Malaysia for their assistance in this research.

Sincere thanks to Lesley Maurice, who is also a senior in the Microbiology Lab, for her tremendous help in my research. I owe you a lot! Thank you for the time and energy that you have spent to help me and to mentor my work. You have been a very good friend to me. Also to Jurin who has been guiding me continuously, thank you so much. I really appreciate all that you have done for me in my study.

A thousand heartfelt thanks to my dearest friends Cheah Yoke Kqueen, Yuli Haryani, Azura Liana, Marlina, Yousr, Patrick, Lawrence, Chandrika, Daniel, Henie, Tang Sui Yan, Chai Lay Ching, Usha, Salleh (Universiti Kebangsaan Malaysia), Elaine (Universiti Malaysia Sabah), and Sharon (UMS). All of you have contributed so much in my study and in my life, I wish you know how grateful I am to have all of you in my life. Your advices, encouragements, supports and guidance have brought me to where I am now. You are all the best!

To the wonderful undergraduates Lee Hai Yen (Universiti Tunku Abdul Rahman), Teh Kheng Pyn, Lim Shin Lan, Bong Siew Fui, Chan Kok Loon, Azizah, Sharifah Zuhaira, Dayu (Indonesia), Rini (Indonesia), Ratnasari (Indonesia), Lis (Indonesia),

Zulqifli (Indonesia), Ervina (Australia), Maria (Denmark) and Lise (Denmark), I really appreciate knowing all of you. Without you realizing, I learnt a lot from you guys. I thank you for the joy and fun that all of you have brought in my life. A heartfelt thanks also to the members of the National Food Safety Research Center, to the members of the Postgraduate Students Society (PERSIS), and to all postgraduates and the staffs of the Food Science and Technology Faculty for the support and assistance directly and indirectly throughout my study.

To my housemates Anja, Jennifer, Elvinah, Cecilia, Dayanie, Loreta, Claire, my dear friends Wagner, Helena, Verhda, Hasni, Zainal, Lawrence, Jhawn, Jill, Kah Wai, Sagau, Sarah, Elsie, Adrian, Dr. Vani, my church members, friends I have not mentioned here, millions and thousands of gratitude I dedicate to all of you. It is a blessing from heaven to have all of you in my life. Especially to Mervin, thank you so much for without your endless love, support, and encouragement, I could never have finished this thesis. Thank you for always being there for me.

To Amai, Amak (my beloved parents), Linda, Vivia, Grace, Mary, and Jayvier (my loving sisters and brother), and my dearest relatives, 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 is all I can do for you.

I certify that an Examination Committee has met on 29th May 2007 to conduct the final examination of Tunung Robin on her Master of Science thesis entitled "Isolation and Characterization of *Salmonella* species from Street Food and Clinical Samples" in accordance with Universiti Putra Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Jinap Selamat, PhD
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Saleha Abdul Aziz, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal Examiner)

Nor Amalina Emran, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Iddya Karunasagar, PhD
Professor
University of Agricultural Sciences
College of Fisheries Mangalore, India
(External Examiner)

_____

HASANAH MOHAMAD GHAZALI, PhD
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 16 August 2007

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

Fatimah Abu Bakar, PhD
Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Son Radu, PhD
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

Pradeep K. Malakar, PhD
Research Scientist
Institute of Food Research
Norwich, United Kingdom
(Member)

_____
AINI IDERIS, PhD
Professor/Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 13 September 2007

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

_____

TUNUNG ROBIN

Date: 10 August 2007

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AP-PCR	arbitrary primered polymerase chain reaction
BGA	brilliant green agar
BPW	buffered peptone water
BSA	Bismuth Sulphite Agar
CDC	Centers for Disease Control and Prevention
DNA	deoxyribonucleic acid
EC	European Commission
EDTA	ethylenediaminetetra-acetic acid
EMBA	eosin-methylene blue agar
ERIC	enterobacterial repetitive intergenic consensus
FAO	Food and Agriculture Organization
FDA	Food Drug Administration
FSRI	Food Safety Research Institute
GET	glucose-EDTA-Tris
H_2S	hydrogen sulphide
HEA	Hektoen Enteric agar
kb	kilo basepair
LB	Luria-Bertani
MA	MacConkey agar
MAR	multiple antibiotic resistance
MDa	mega Dalton
MOH	Ministry of Health

NCCLS	National Committee for Clinical Laboratory Standards
NST	New Straits times
PCR	polymerase chain reaction
RAPD	randomly amplified polymorphic DNA
rpm	revolution per minute
RV	Rappaport-Vassiliadis
SDS	sodium dodecyl sulphate
SSA	*Salmonella-Shigella* agar
TBE	Tris-Borate-EDTA
TSI	triple sugar iron
TS	The Star
UV	ultra violet
V	volts
WHO	World Health Organization
x g	unit gravity
XLD	xylose lysine deoxycholate

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

Food borne illness is a major international public health concern (Cardinale *et al.*, 2005) and was estimated to be the cause of 76 million illnesses, 325,000 hospitalizations, and 5000 deaths in the United States annually (Mead *et al.*, 1999; Badrie *et al.*, 2005). According to the World Health Organization (WHO, 2005), 90% of the annual deaths from food borne illnesses are among children particularly in developing countries. The WHO defines food borne illnesses as diseases, usually either infectious or toxic in nature, caused by agents that enter the body through the ingestion of food. Food borne illnesses remain widespread but their impact on public health has not been well documented, especially in developing countries (King *et al.*, 2000).

Many countries reported contamination of food to be the major factor contributing to food borne illness. Several countries have reported high bacterial counts in foods when microbiological studies were carried out on street-food vending (Kubheka *et al.*, 2000). According to WHO, street food refers to food and beverages prepared and sold by vendors in streets and other public places for immediate consumption or consumption at a later time without further processing or preparation (WHO, 2005). In developing countries, the street food trade has evolved as a result of rapid

urbanization and is known as a phenomenon that has great economical, socio-cultural, and sanitary importance (Lucca *et al.*, 2006). Street-food vendors play an important economic role as they provide a source of inexpensive, nutritious meals to a large number of office and construction workers, and to people in transit. The vendors themselves benefit from positive cash flow, as they are able to establish business with minimum capital and do not pay tax on their earning.

The usual congregation conditions for vendors (such as overcrowded areas, limited access to basic sanitary facilities and large amounts of garbage) provide harborage for insects and animal pests. In many incidences consumers are concerned about the price of food rather than its safety and hygiene. This has given rise to concerns regarding the sanitary standards of street vending operations. The main factor that currently compromises food quality is the hygienic-sanitary aspect (in which the sale of food in public places is highly controversial from a health standpoint). This practice represents a serious threat to consumer health and is a matter of great importance for public health (Lucca *et al.*, 2006). Nonetheless, food borne illnesses remain responsible for morbidity and mortality in the general population, particularly infants and children, the elderly and immunocompromised.

One of the most common causes of food borne illness worldwide is *Salmonella* infection or salmonellosis (Cardinale *et al.*, 2005). *Salmonella* is considered to be the cause of the largest number of outbreaks, cases, and fatalities that result from food borne infections among enteric pathogens. Salmonellosis is caused by the two species of *Salmonella*; *Salmonella* enterica and *Salmonella* bongori, and it is an

infectious disease of humans and animals. Investigations in developed countries have shown that *Salmonella* infections are serious in frequency and severity of symptoms (Cardinale *et al.,* 2005). Clinical symptoms of human salmonellosis include acute onset fever, abdominal pain, diarrhea, nausea and vomiting, while dehydration can become life threatening.

The main source of salmonellosis is usually contaminated food, especially poultry, poultry products, cattle and dairy products (Rychlik *et al.,* 2000). During the last decade, several salmonellosis outbreaks have been documented worldwide due to the consumption of contaminated food products or water with *Salmonella* (Llewellyn *et al.,* 1998; Harakeh *et al.,* 2004). In developing countries, street foods in particular have been reported to be contaminated with *Salmonella* and have been implicated in a few outbreaks of food borne diseases (Mankee *et al.,* 2003). *Salmonella* is one of the most frequently isolated bacteria from food, and *Salmonella* species have been isolated from a variety of foods such as milk, beef, pork, and chicken (Lim *et al.,* 2005). In Malaysia, the Ministry of Health Malaysia has reported that the food poisoning cases associated with *Salmonella* spp. were 2.3% (Shobirin *et. al.*, 2003).

The incidence of food borne infections caused by *Salmonella* species has increased dramatically during the past few years. Mrema *et al.* (2004) reported that in the United States, salmonellosis is estimated to affect 1.4 million people each year, and 95% of the cases are foodborne. Outbreaks of *Salmonella* food poisoning has become the most frequent health problem in Japan, as indicated by the number of cases reported and the number of patients affected. In Malaysia, an outbreak of