INHIBITORY ACTIVITIES OF A PROBIOTIC BACTERIUM
(BIFIDOBACTERIUM PSEUDOCATANULATUM) ON A COMMON
DIARRHEAGRONIC PATHOGEN (SALMONELLA ENTERICA) IN HUMAN

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FSMB 2003 9
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By
ANIS SHOBIRIN MEOR HUSSIN

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

May 2003
Special Dedication
To my husband Azmi Alias for his patience, endurance and support throughout this project and also to my son Muhammad Aniq and my daughter Aliah Batrisyia
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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Chairman: Associate Professor Dr. Mohd Yazid Abdul Manap

Faculty: Food Science and Biotechnology

Sixteen strains of Salmonella were isolated from clinically diagnosed diarrhea patients. They were tested against a range of antimicrobial agents, and typed by serological test and RAPD fingerprinting. All the strains have the similar pattern of antimicrobial susceptibility. The serological test has typed them into 3 serovars but the RAPD fingerprinting has classed them into 2 major clusters. Three strains of bifidobacteria were analyzed for their survival rate in human stomach condition. It showed that the ability of bifidobacteria to survive was strains dependant. Bifidobacterium pseudocatunulatum F117 and Bifidobacterium infantis can survive at pH value of human stomach after exposure for 90 minutes but not Bifidobacterium pseudocatunulatum G48. The survival of bifidobacteria was higher in the pH after meal compared to the pH before meal (fasted state). The dose effect study demonstrated, that the initial concentration of bifidobacteria would affect the duration of inhibitory activity
against Salmonella. Lower initial concentration exhibit greater inhibitory activity. The inhibition of Salmonella was due to the production of acetate and lactate by bifidobacteria and the effectiveness was higher at low pH. Acetate and lactate production was excessive when the initial concentration of bifidobacteria was low due to the high growth rates, metabolism, and competition of energy sources.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan Ijazah Master Sains

AKTIVITI PERENCATAN BAKTERIA PROBIOTIK (BIFIDOBACTERIUM PSEUDOCATANULATUM) TERHADAP PATOGEN PENYEBAB DIAREA (SALMONELLA ENTERICA) PADA MANUSIA

Oleh

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Pengerusi: Profesor Madya Dr. Mohd Yazid Abdul Manap

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Enam belas strain Salmonella telah dipencilkan daripada pesakit diarea yang telah didiagnosa secara klinikal. Ujian terhadap beberapa agen antibiotik telah dilakukan terhadap pencilan tersebut dan ia telah dikelaskan melalui ujian serologi dan capjari RAPD. Kesemua strain tersebut mempunyai corak ketahanan yang sama terhadap semua antibiotik yang digunakan. Ujian serologi telah mengkelaskannya kepada 3 jenis serovar manakala capjari RAPD kepada 2 kluster utama. Tiga strain bifidobacteria telah melalui ujian keupayaan untuk hidup pada keadaan dalam perut manusia. Ujian tersebut menunjukan keupayaan bifidobacteria untuk hidup dalam perut manusia adalah bergantung kepada jenis strain. Bifidobacterium pseudocatatanulatum F117 dan Bifidobacterium infantis ATCC 27920 masih mampu hidup pada nilai pH dalam
ACKNOWLEDGEMENTS

All praise to Allah S.W.T, who has showered me with patience and bless to complete my Master thesis. Alhamdulillah.

I would like to extend my deepest appreciation to the Chairman of the Supervisory Committee, Associate Prof. Dr. Mohd Yazid Abd Manap, for his guidance, invaluable advice, continuous supervision and support throughout the course of my study. I am also thankful to Associate Prof. Dr. Arbakariya Ariff and Prof. Abd Manaf Mohd Ali, my co-supervisors, for their help, constructive criticism and guidance, which have greatly benefited me.

I also forward my special thanks to the staff of KK7 Ward, Pediatric Institute, Kuala Lumpur Hospital for helping me to get sample, Mr. Halim for allowing and teaching me to use HPLC equipment, and also to Mr. Rosli Aslim for their kindness to lend me the bioreactor apparatus.

I would also like to express my sincere thanks to my friends, Shuhaimi Mustafa, Lim Long Chang, Shanti Mugundan, Wendy Yap Keng Wai and others for their help and kind friendship.
My sincere thank is also extended to staff in Faculty of Food Science and
Biotechnology, UPM and to Ministry of Science and Technology of Malaysia for
providing the financial support through IRPA fund.

Finally, my deepest gratitude and appreciation is dedicated to my family
and in particular, to my husband, Azmi Alias, for being very supportive and
patient, and to my adorable kids, Muhammad Aniq and Aliah Batrisya, for being
good throughout the duration of my study.
I certify that an Examination Committee met on 4 July 2003 to conduct the final examination of Anis Shobirin Meor Hussin on her Master of Science thesis entitled "Inhibitory Activities of a Probiotic Bacterium (Bifidobacterium pseudocatanulatum) on a Common Diarrheagenic Pathogen (Salmonella enterica) in Human" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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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 institution.

(ANIS SHOBIRIN MEOR HUSSIN)

Date: 8 Sept 2003
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LISTS OF ABBREVIATIONS

CFU : Colony Forming Unit

g : gram

h : hour

h⁻¹ : per hour

L : Liter

mg : milligram

min : minute

ml : milliliter

mm : millimeter

mM : milliMolar

N : Normality

rpm : revolution per minute

v/v : volume/volume

μ : Specific Growth Rate

μg : microgram

μm : Maximum Specific Growth Rate

μm : micrometer
CHAPTER 1

INTRODUCTION

Infectious diarrhea is a worldwide public health problem. In many developing countries, diarrheal diseases remained a leading cause of illness and death among infants and children (Snyder and Merson, 1982; Ho et al., 1988). In more developed nations, nosocomially acquired diarrheal disease can significantly lengthened hospital stays and increase both direct and indirect medical costs (Saavedra, 2000). The most serious aspect of this disease is fluid loss with resultant dehydration and electrolyte disturbances. In most cases, replacing lost fluid to prevent dehydration is the only treatment necessary. The use of oral rehydration solutions (ORS) is the main treatment, but it does not shorten the recovery of diarrhea.

Bacterial pathogens probably cause less than 20 % of cases of acute childhood diarrhea (Moffet, 1989). This incidence rises in warm climates, particularly where sanitary conditions are poor. Globally, Salmonella, Shigella and Campylobacter remain major contributors to diarrheal diseases (Stutman, 1994; Liesenfeld et al., 1993). Some strains of Escherichia coli, a normal inhabitant of the distal bowel, are pathogenic, causing sporadic cases of acute enteritis, epidemic diarrhea (particularly in young infants) and traveler’s diarrhea. Severe diarrhea in children has now been attributed to enteroadherent
strains of *E. coli*. In Malaysia, investigation for common diarrheal bacteria has not much been documented.

Numerous probiotic agents have been studied in the management of infectious diarrheal diseases (Saavedra *et al.*, 1994; Hilton *et al.*, 1996; Shornikova *et al.*, 1997). Preliminary experimental and clinical findings show that probiotics are emerging as an important, new therapy for preventing and treating infectious diarrhea (Saavedra, 2000). Ingestion of probiotics can exert a positive influence on the health or physiology of the host. It was believed that it could influence intestinal physiology either directly or indirectly through modulation of the endogenous ecosystem or immune system.

Bifidobacteria, a probiotic, comprised a major group in the human and animal intestinal flora along with bacteroides and eubacteria. They are thought to exert some of the protective effect against acute diarrhea diseases. Bifidobacteria are Gram positive, non-acid fast, non-spore forming and non-motile organism. These organisms have been isolated from the faeces of breast-fed infant, adult human intestine, vagina and mouth as well as in the alimentary tract of various kinds of animal (Rasic and Kurman, 1983; Yazid *et al.*, 1999). In the large intestine, bifidobacteria produce acetic and lactic acids and is thought to inhibit the proliferation of putrefactive bacteria such as escherichiae, clostridia and eubacteria.
In order to use bifidobacteria as an alternative to manage acute diarrhea, it is necessary to establish the strain that can survive in the acidic conditions of the stomach. And also, the dose of the bifidobacteria that is able to reduce the growth of the causative microorganism should also be established. Considering these reasons, the objectives of the present study are:

a) To isolate and characterize the major diarrheagenic pathogens from stool of acute diarrhea patients below 3 years of age

b) To study the dose-effect of *Bifidobacterium* spp. against the *Salmonella* spp. in simulated human colon environment
CHAPTER 2

LITERATURE REVIEW

2.1 Etiology of *Bifidobacteria* spp.

Bifidobacteria were first isolated from the faeces of breast-fed infants by Tissier (1990), who used the name *Bacillus bifidus communis*. Today, this genus, which belongs to the *Actinomycetaceae* group, includes 30 species including nine species found in human (Ishibashi *et al.*, 1997). *Bifidobacterium* are generally characterized as Gram-positive, non-sporeforming rods with bifurcating pleomorphic cellular morphology, non-motile and catalase negative (Rasic and Kurman, 1983). Bifidobacteria are anaerobic microorganisms but some species can tolerate oxygen only in the presence of carbon dioxide (Scardovi, 1986). The colonies may be smooth, convex to pulvinate, entire edges, cream to white, glistening and soft consistency depending on nutritional condition and strain characteristics. Most human strains of bifidobacteria grow at an optimum temperature of 37-38 °C (Rasic, 1983).

2.2 Inhibitory Activity of Bifidobacteria

Bifidobacteria are known as probiotic organisms because of the potential beneficial roles of bifidobacteria in the intestinal tract of humans (Hughes and
Hoover, 1991). These bacteria were shown to play a significant role in controlling the acidity of the large intestinal tract and capable of hydrolyzing indigestible complex carbohydrate such as lactulose, into acetic and lactic acids. These acids are responsible for maintaining the intestinal microbial balance by inhibiting the growth of potential pathogens (Rasic, 1983).

In vitro study by Araya-Kojima et al. (1995) shows that the inhibitory effects of bifidobacteria were due to the decreased of pH resulting from the synthesis of lactic and acetic acids. It may also compete with a pathogen for luminal nutrients that are rates limiting substrates or occupy adhesion receptors and inhibit attachment to the mucosa (Bernet et al., 1994). There may be indirect effects that result from enhancement of host responses such as activation of microphages or stimulation of secretary antibody (Kaila et al., 1992). These possible mechanisms would be dependent on the ability of the probiotic to survive and colonize the gut.

2.3 Probiotics and Acute Diarrhea

Probiotic, have long been suggested to have a role in the management of diarrheal diseases. The best-established benefit of using probiotic agents has been in the management of acute pediatric diarrheal disease. Several large and well-controlled studies showed a significant decrease in the duration of diarrhea in children who received Lactobacillus GG, either as a supplement or in