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

ANIS SHOBIRIN MEOR HUSSIN

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

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

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May 2003

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 pseudocatanulatum* F117 and *Bifidobacterium infantis* can survive at pH value of human stomach after exposure for 90 minutes but not *Bifidobacterium pseudocatanulatum* 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

ANIS SHOBIRIN MEOR HUSSIN

Mei 2003

Pengerusi: Profesor Madya Dr. Mohd Yazid Abdul Manap

Fakulti: Sains Makanan dan Bioteknologi

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 pseudocatanulatum* F117 dan *Bifidobacterium infantis* ATCC 27920 masih mampu hidup pada nilai pH dalam

perut manusia setelah 90 minit didedahkan pada keadaan tersebut tetapi tidak bagi *Bifidobacterium pseudocatanulatum* G48. Kemampuan untuk hidup bifidobacteria adalah lebih tinggi pada pH dalam perut selepas makan berbanding sebelum makan. Kajian kesan dos mempamerkan kepekatan permulaan bifidobacteria akan memberi kesan kepada jangkamasa aktiviti perencatan terhadap Salmonella. Kepekatan permulaan yang rendah mempamerkan aktiviti perencatan yang lebih cepat. Perencatan Salmonella adalah disebabkan oleh penghasilan asid asetik dan asid laktik oleh bifidobacteria. Dan keberkesanannya adalah lebih baik pada pH rendah. Penghasilan asid asetik dan asid laktik adalah lebih tinggi apabila kepekatan permulaan bifidobacteria adalah rendah. Ini adalah disebabkan oleh kadar pertumbuhan dan metabolisma yang tinggi, dan pertandingan untuk sumber tenaga.

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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
μ_m	:	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 (Saveedra *et al.,* 1994; Hilton *et al.,* 1996; Shornikova e*t 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