UNIVERSITI PUTRA MALAYSIA

CHARACTERISATION OF LACTIC ACID BACTERIA AND BIFIDOBACTERIA AND THEIR POTENTIAL APPLICATION AS A PROBIOTIC AGAINST INFANT DIARRHEA

K.M. FORMUZUL HAQUE

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By

K.M. FORMUZUL HAQUE

Thesis Submitted in Fulfilment of the Requirements for the Degree of Doctor of Philosophy in the Faculty of Medicine and Health Sciences Universiti Putra Malaysia

November 2000

IN MEMORY OF MY FATHER

LATE QUAZI ERFANUL HAQUE

&

MY MOTHER, LATE FUARA BEGAM

AND MY LATE GRAND PARENTS

Abstract of thesis presented to the Senate of the Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy.

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Chairperson: Rokiah Mohd Yusof, Ph.D.

Faculty: Medicine and Health Sciences

The major health promoting probiotic bacteria found in the human gut are of the genera *Bifidobacterium* and *Lactobacillus*. The main objectives of the present studies were to isolate, characterise and identify the suitable probiotic strains of *Lactobacillus* (LAB) and Bifidobacteria (BB) from faeces of breast fed infants which could be used as effective probiotic for the control of infant diarrheal diseases.

From the isolation studies, the *Lactobacillus* was found to be Gram-positive, non-motile, short rods and catalase, nitrate, oxidase negative. The Bifidobacteria were Gram-negative, curved with characteristics of Y and V shapes. The high performance of liquid chromatography (HPLC) showed that almost all strains of BB produced more or less or equal amount of acetic and lactic acids. Based on the carbohydrate fermentation profile using API-CH-50 kits, out of 21 *Lactobacillus,* 16 species belong to the *L. casei*, four to *L. brevis,* one species to *L. plantarum* and one sub species *casei.* Twenty of Bifidobacteria were *B. infantis* species, where 13 belong to subspecies *infantis*, 7 were *lacentis*

The antagonistic activities of LAB and BB were tested against ETEC *E.coli* 0157:H7 and *Salmonella typhimurium* S-285 using double layered assay, results showed that strains LAB-3, 11, 21 and Bifi-11, 19, 20 produced wider inhibition zone compared to others. From bile tolerance studies, LAB-3, 11, 21 and Bifi-11,

19, 20 strains showed better bile tolerance compared to the other six LAB and BB strains. At pH 1.0 to 2.0, Bifi-19 and 20 survived better than the other strains.

Bifi-19 and Bifi-20 strains showed the highest inhibition against *E. coli* at 0.4% concentration in rice porridge compared to other BB and LAB strains. Out of the seventeen antibiotics tested, 6 LAB and BB strains were resistant to ceftriazone, cloxacillin, clindamycin, cefuroxime, cefuxime and tetracycline. Three LAB strains were moderately susceptible to cefuroxime, ceifixime and tetracycline, whereas, 3 BB strains were susceptible to ceftriazone.

Adhesion studies showed that LAB colonised better on the rats stomach whereas BB in the colon. There was a significant effect ($P < 0.01$) on the bacterial populations of LAB (8.18) and BB (8.09) log cfu/g, against *E. coli* 5.59 and 5.09 log cfu/g count respectively in the rats faeces after 15 days of feeding probiotic diets.

Mice were induced with diarrheal diseases by ingesting ETEC *E coli* 0157:H7 (WHO) at 10^8 concentration. After 24 hours of feeding probiotic diets to diarrheal mice, diarrhea had stopped. The pH in mice stomach, intestine, colon and ceacum varied significantly ($P < 0.01$) after feeding probiotic diets consisted of LAB or BB. The lower pH value for LAB probiotic diet was in mice stomach, while for BB diets, in the colon.

It could be postulated that the probiotic strains isolated from breast fed infants faeces belong to the *L. casei* and *B infantis* species, had a strong antagonistic activity against pathogens, tolerance towards bile acid, survived at low pH (1-2), resistant against antibiotics, inhibited *E. coli* in rice porridge, adhered on rats epithelial surface and overall could control diarrheal diseases in mice. Based on these probiotic characteristics, the strains LAB-3, 11 and Bifi-19, 20 were the best probiotic organisms compared to LAB-21 and Bifi-11.

Abstrak tesis yang dikemakanan kepada Senat Universiti Putra Malaysia
sebagi memenuhi keperluan untuk Ijazah Doktor Falsafah

PENCIRIAN BAKTERIA ASID LAKTIK DAN BIFIDOBAKTERIA DAN POTENSINYA SEBAGAI PROBIOTIK TERHADAP DIAREA BAYI

Oleh

K.M. FORMUZUL HAQUE

November, 2000

Pengerusi : Rokiah Mohd Yusof, Ph. D.

Fakulti : Perubatan dan Sains Kesihatan

Bakteria probiotik penting dalam mempromosi kesihatan manusia yang terdapat dalam gastrousus terdiri daripada genera Bifidobacteria dan *Lactobacillus*. Objektif utama kajian ini adalah untuk mengasing, menciri dan mengenalpasti probiotik yang sesuai dari strain *Lactobacillus* (LAB) dan Bifidobacteria (BB) yang terdapat dalam feses bayi menyusu susu ibu, yang memberi kesan probiotik dalam mengawal penyakit diarea bayi.

Daripada kajian pengasingan, *Lactobacillus* adalah Gram-positif, tidak motil, rod pendek dan negatif terhadap katalase, nitrat dan oksidase. Bifidobacteria bercirikan rod Gram-positif, berbentuk Y dan V. Kaedah kromatografi cecair berprestasi tinggi (HPLC) menunjukkan bahawa semua strain BB menghasilkan lebih kurang sama banyak asid asetik dan laktik. Berdasarkan profil fermentasi karbohidrat menggunakan kit API-CH-50, daripada 21 spesies *Lactobacillus,* 16 spesies termasuk dalam kumpulan *L. casei*, 4 *L. brevis* dan satu *L. plantarum* dan satu sub-spesies *casei*. Terdapat 20 spesies Bifidobacteria adalah *B.infantis*, 13 termasuk dalam sub-spesies *infantis* dan 7 *lacentis.*

Kajian aktiviti antagonistik LAB dan BB keatas ETEC *E. coli* 0157:H7 dan *Salmonella typhimurium* S-285 menggunakan kaedah esei dua lapis menunjukkan strain LAB-3, 11, 21 dan Bifi-11, 19 dan 20 mengeluarkan zon perencatan yang lebih

luas berbanding dengan spesies lain. Daripada kajian toleransi hempedu, LAB-3, 11, 21 dan Bifi-11, 19, 20 menunjukkan lebih toleransi terhadap hempedu berbanding dengan enam strain LAB dan BB yang lain. Pada pH 1.0 hingga 2.0, strain Bifi-19 dan 20 tahan hidup lebih baik daripada strain lain.

Bifi-19 dan 20 telah menunjukkan perencatan terbaik terhadap *E. coli* pada kepekatan 0.4% dalam bubur nasi, berbanding dengan strain BB dan LAB yang lain. Rintangan LAB dan BB keatas 17 jenis antibiotik telah dikaji. Didapati 6 strain LAB dan BB resisten terhadap ceftriazone, cloxacillin, clindamycin, cefuroxime dan tetracycline. Cefuroxime, cefuxime dan tetracycline memberi kesan sederhana kepada tiga strain LAB, dan ceftriazone pula memberi kesan terhadap 3 strain BB.

Kajian pelekatan menunjukkan LAB telah membiak dengan baik dalam perut tikus dan BB dalam kolon. Selepas tikus diberi makan diet probiotik yang mengandungi LAB atau BB selama 15 hari, didapati bilangan LAB dan BB yang tertinggi dalam najis iaitu 8.18 dan 8.09 log cfu/g, yang mana telah merencat secara signifikan ($P < 0.01$) pertumbuhan *E. coli* ke 5.59 dan 5.09 log cfu/g masing-masing dalam feses tikus.

Mancit telah diarus mendapat diarea melalui pemberian makanan mengandungi ETEC *E. coli* 0157:H7 (WHO) pada kepekatan log 10^8. Selepas 24 jam pemberian diet probiotik kepada mancit berdiarea, yang mengandungi LAB dan BB berasingan, diarea berhenti. Nilai pH dalam perut, usus, kolon dan sekum mancit berbeza secara signifikan ($P<0.01$). Nilai pH terendah bagi LAB terdapat dalam perut mancit, sementara BB dalam kolon.

Sebagai rumusan, didapati strain probiotik yang telah diasingkan daripada feses bayi, terdiri daripada spesies *L. casei* dan *B infantis* yang telah menunjukkan aktiviti antagonistik yang kuat terhadap patogen, toleransi terhadap asid hempedu, ketahanan hidup pada pH rendah (pH 1.0-2.0), resisten terhadap antibiotik, perencat terhadap pertumbuhan *E. coli* dalam bubur nasi, pelekatan yang baik pada permukaan sel epitelia tikus dan berupaya mengawal penyakit diarea dalam mancit. Berdasarkan ciri-ciri probiotik yang dikaji, strain LAB-3, 11 dan Bifi-19, 20, adalah oragnisma probiotik yang terbaik berbanding dengan strain LAB-21 dan Bifi-11.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES