



UNIVERSITI PUTRA MALAYSIA

**ADHESION PROPERTIES OF *BIFIDOBACTERIUM*
PSEUDOCATENULATUM G4 TO HT-29 EPITHELIUM CELL LINE**

ALI KAHTAN SULIMAN

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TO HT-29 EPITHELIUM CELL LINE**

BY

ALI KAHTAN SULIMAN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfillment of the Requirement for the Degree of Master of Science**

July 2009



Abstract of thesis presented to the Senate of University Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

**ADHESION AND ADHESION INHIBITION PROPERTIES OF
BIFIDOBACTERIUM PSEUDOCATENULATUM G4 TO HT-29 EPITHELIUM CELL
LINE**

BY

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July 2009

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Bifidobacterium pseudocatenulatum G4 has been recently identified as a safe probiotic for incorporation into functional food for human consumption. Preliminary investigations showed that the probiotic candidate *B. pseudocatenulatum* G4 strain possesses the required criteria for a being successful probiotic microorganism. Further enhancement of these criteria was undertaken by studying the adherence and inhibition properties potential of this probiotic candidate. Human colon carcinoma epithelium cell line HT-29 was used to evaluate the adherence of *B. pseudocatenulatum* G4, in simulated environmental factors of the colon, namely pH, calcium ions, and cholic acid. The effect of this strain on enhancing intestinal tract resistance to pathogenic *Escherichia coli* and *Clostridium* infections was examined.



Three different assays were used in order to differentiate between the competition, exclusion, and displacement of the pathogens by *B. pseudocatenulatum* G4.

The adherence ability of *B. pseudocatenulatum* G4 to HT-29 cell line was investigated as *in vitro* model. In addition the morphology observation of the organism was done by using scanning electron microscopy (SEM). The effect of human colon environmental factors on the adhesion quality was studied. The results showed that the highest adhesion was in the ascending and transverse acidic regions of the colon. Calcium was shown to increase, while cholic acid was shown to decrease the adhesion of *B. pseudocatenulatum* G4 to HT-29. The inhibitory effect of *B. pseudocatenulatum* G4 on the adherence of *Escherichia coli* O157:H7, *Clostridium scindens* and *Clostridium hiranonis* was demonstrated. A decrease in the number of adhering pathogens was observed.

Abstrak tesis yang dikemukakan kepada senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah master sains

**PERLEKATAN DAN KEBOLEHAN MERENCAT PERLEKATAN
BIFIDOBACTERIUM PSEUDOCATANULATUM G4 PADA PERMUKAAN
SEL EPITHELIUM HT-29**

Oleh

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Bifidobacterium pseudocatanulatum G4 telah dikenalpasti sebagai probiotik yang selamat untuk tambah ke dalam makanan berfungsi untuk kegunaan manusia. Kajian awal menunjukkan strain *B. pseudocatanulatum* G4 mempunyai kriteria yang diperlukan untuk bertindak sebagai mikroorganisma probiotik yang berjaya. Kriteria selanjutnya telah diselidik melalui kajian potensi kebolehan melekat and merencat perlekatan. Lapisan sel epithelium karsinoma kolon manusia HT-29 telah digunakan untuk menilai kebolehan melekat strain *B. pseudocatanulatum* G4 di dalam persekitaran kolon simulasi. Faktor-faktor persekitaran kolon yang disimulasi adalah pH, ion kalsium and asid kolik. Kesan strain ini dalam meningkatkan kerentangan saluran usus terhadap jangkitan *Esherichia coli* and *Clostridium* telah kaji. Tiga



analisis yang berlainan telah digunakan untuk mengenalpasti samada terdapat persaingan, pengusiran dan pengambilalihan antara patogen dan *B. pseudocatanulatum* G4. Kebolehan melekat *B. pseudocatanulatum* G4 kepada lapisan sel HT-29 telah diperhatikan menggunakan model *in vitro*. Kesan factor-factor persekitaran dalam kolon manusia terhadap kualiti kebolehan melekat juga telah dikaji. Selain itu, morfologi organisma telah diperhatikan menggunakan mikroskop pengimbas elektron. Keputusan kajian menunjukkan kebolehan melekat paling tinggi *B. pseudocatanulatum* G4 adalah pada bahagian kolon menaik dan melintang. Kehadiran kalsium dapat meningkatkan kebolehan melekat *B. pseudocatanulatum* G4 pada HT-29 tetapi asid kolik mempunyai kesan yang sebaliknya. Kesan kerencatan oleh *B. pseudocatanulatum* G4 terhadap kebolehan melekat *E. coli* O157:H7, *Clostridium scindens* and *Clostridium hiranonis* telah dipamerkan. Pengurangan bilangan patogen yang melekat telah diperhatikan.

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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 been previously or concurrently submitted for any other degree at UPM or other institution.

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TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	v
ACKNOWLEDGEMENTS	vi
APPROVAL	vii
DECLARATION	ix
LIST OF TABLE	xii
LIST OF FIGURE	xiv
LIST OF ABBREVIATION	xvii
CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	5
2.1 Definition of Probiotic	5
2.1.1 Benefit of Probiotic	8
2.2 Probiotic Characteristics	9
2.2.1 Survive of Probiotic with Present of Bile and Low pH	11
2.2.2 Adhesion to Human Intestinal Cell	15
2.2.3 Competition between Probiotic and Pathogen Bacteria	20
2.2.4 The Relationship between Probiotic Bacteria and Immune System	23
2.3 Relationship between Intestinal Flora and Intestinal Cells: Adhesion and Colonization	25
2.4 Bifidobacteria Discovery and History	27
2.4.1 Bifidobacteria	29
3 MATERIAL AND METHODS	32
3.1 Bacterial Strains and Culture Conditions	32
3.2 Growth Phases of Bifidobacterial Strains	33
3.3 Cell Culture Preparation	34
3.3.1 HT-29 Epithelium Cell Line	34
3.3.2 Tissue Culture 6 –Well Plates with cover slips preparation	37
3.4 In vitro Study of the Effect of Environmental Factors on the Adherence of Bifidobacterial Strains in Human Colon	39
3.4.1 Evaluation the Effect of pH on Bifidobacterial Strains Adhesion Quality	39
3.4.2 The Effect of Calcium Ions on Adherence of Bifidobacterial Strains Quantity	41
3.4.3 The Ability of Bifidobacterial Strains with Acquired	42



	Resistance to Cholic Acid to Adhere to Epithelium Cells	
3.4.4	Analysis Fixation Techniques	43
3.5	Observing of Morphology by Using Scan Electron Microscopy (SEM)	44
3.5.1	Tissue Culture Cell Preparation	44
3.5.2	Specimen Preparation for SEM	44
3.6	The Interaction between Bifidobacterial Strains with Pathogens	45
3.6.1	Tissue Culture 6 –Well Plate Preparation	45
3.6.2	Bacterial Strain and Specimen Preparation	45
3.6.3	Competition Assays	46
3.6.4	Exclusion Assays	46
3.6.5	Displacement Assays	47
3.6.6	Microbiology Analysis	47
4	RESULTS AND DISCUSSION	48
4.1	Growth Phases of Bifidobacterial Strains	48
4.2	Environment Factors Effect on the Adherence of Bifidobacterial Strains on HT-29 Human epithelium cell line – <i>in vitro</i> Study	51
4.2.1	Evaluation the Effect of pH on Bifidobacterial Strains Adhesion Quality	51
4.2.2	The Effect of Calcium Ions on Adherence of Bifidobacterial Strains Quantity	61
4.2.3	The Ability of Bifidobacterial Strains with Acquired Resistance to Cholic Acid to Adhere on HT-29 Epithelium Cells Line	80
4.3	Observing of Morphology by Using Scan Electron Microscopy (SEM)	92
4.4	Relationship between Bifidobacterial Strains and Pathogens	97
5	GENERAL CONCLUSION	104
	REFERENCES	107

LIST OF TABLES

Table	Page
2.1 <i>Lactobacilli</i> and <i>Bifidobacterium</i> used as Human probiotics	7
4.1 Comparison of ANOVA Result between Different pH Levels	56
4.2 Comparison of ANOVA Result between Different Times	56
4.3 ANOVA Full Factorials Design Comparing the Effect of Different pH Levels and Different Times on <i>B. longum</i> BB536 and <i>B. pseudocatenulatum</i> adhesion Quality	57
4.4 Comparing of <i>T</i> -test Results between Adhesion before and after Adding Calcium Ions to DMEM with pH Level 5.6	68
4.5 Comparing of <i>T</i> -test Results between Adhesion before and after Adding Calcium Ions to DMEM with pH Level 5.7	68
4.6 Comparing of <i>T</i> -test Results between Adhesion before and after Adding Calcium Ions to DMEM with pH Level 6.6	69
4.7 Comparing of <i>T</i> -test Results between Adhesion before and after Adding Calcium Ions to DMEM with pH Level 6.8	69
4.8 Factorial Design Adhesion of <i>B. longum</i> BB536 and <i>B. pseudocatenulatum</i> G4 in pH Level 5.6 with 30, 60 and 120 min	76
4.9 Factorial Design Adhesion of <i>B. longum</i> BB536 and <i>B. pseudocatenulatum</i> G4 in pH Level 5.7 with 30, 60 and 120 min	76
4.10 Factorial Design Adhesion of <i>B. longum</i> BB536 and <i>B. pseudocatenulatum</i> G4 in pH Level 6.6 with 30, 60 and 120 min	77
4.11 Factorial Design Adhesion of <i>B. longum</i> BB536 and <i>B. pseudocatenulatum</i> G4 in pH Level 6.8 with 30, 60 and 120 min.	77
4.12 Comparing of <i>T</i> -test Results between Adhesion before and after Adding Cholic Acid to Buffer with pH Level 5.6	86
4.13 Comparing Of <i>T</i> -test Results between Adhesion before and after Adding Cholic Acid to Buffer with pH Level 6.6	86
4.14 Factorial Design Adhesion of <i>B. longum</i> BB536 and <i>B. pseudocatenulatum</i> G4 in pH Level 5.6 with 30, 60 and 120 min	91



4.15	Factorial Design Adhesion of <i>B. longum</i> BB536 and <i>B. pseudocatenulatum</i> G4 in pH Level 6.6 with 30, 60 and 120 min	91
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LIST OF FIGURES

Figure	Page
2.1	10
2.2	13
2.3	13
3.1	35
3.2	36
3.3	38
3.4	38
4.1	49
4.2	51
4.3	51
4.4	53
	53
4.5	53
4.6	54



4.7	30 min Adhesion of <i>B. longum</i> BB536 and <i>B. pseudocatenulatum</i> G4 with pH 6.8, 6.6, 5.7 and 5.6	55
4.8	15 min Adhesion of <i>B. longum</i> BB536 and <i>B. pseudocatenulatum</i> G4 with pH 6.8, 6.6, 5.7 and 5.6	55
4.9	Interactional between Times and Numbers of <i>Bifidobacterium</i> adhere to get the optimum pH for <i>B. longum</i> BB536 and <i>B. pseudocatenulatum</i> G4	58
4.10	(A) 30, (B) 60 and (C) 120min Adhesion of <i>B. longum</i> BB536 and <i>B. pseudocatenulatum</i> G4 with pH 5.6 and Ca ⁺²	62
4.11	(A) 30, (B) 60 and (C) 120min Adhesion of <i>B. longum</i> BB536 and <i>B. pseudocatenulatum</i> G4 with pH 5.7 and Ca ⁺²	63
4.12	(A) 30, (B) 60 and (C) 120min Adhesion of <i>B. longum</i> BB536 and <i>B. pseudocatenulatum</i> G4 with pH 6.6 and Ca ⁺²	64
4.13	(A) 30, (B) 60 and (C) 120min Adhesion of <i>B. longum</i> BB536 and <i>B. pseudocatenulatum</i> G4 with pH 6.8 and Ca ⁺²	65
4.14	Time Course Adhesion of <i>Bifidobacterium</i> with and without Calcium Ions in pH level 5.6	71
4.15	Time Course Adhesion of <i>Bifidobacterium</i> with and without Calcium Ions in pH level 5.7	72
4.16	Time Course Adhesion of <i>Bifidobacterium</i> with and without Calcium Ions in pH level 6.6	73
4.17	Time Course Adhesion of <i>Bifidobacterium</i> with and without Calcium Ions in pH level 6.8	74
4.18	(A) 30, (B) 60 and (C) 120min Adhesion of <i>B. longum</i> BB536 and <i>B. pseudocatenulatum</i> G4 with pH 5.6 with concentration 0.019/50 (v/w)	82
4.19	(A) 30, (B) 60 and (C) 120min Adhesion of <i>B. longum</i> BB536 and <i>B. pseudocatenulatum</i> G4 with pH 6.6 with concentration 0.0019/50 (v/w)	84
4.20	Time Course Adhesion of <i>Bifidobacterium</i> with and without Cholic Acid 0.94µmol/ml in pH level 5.6	88
4.21	Time Course Adhesion of <i>Bifidobacterium</i> with and without Cholic Acid 0.094µmol/ml in pH level 6.6	89

4.22	Observation by Scanning Electron Microscopy of The Adhesion of <i>B. Longum</i> BB536 and <i>B. Pseudocatenulatum</i> G4 onto Brush Border Of HT-29	93
4.23	Observation by Scanning Electron Microscopy of the Adhesion of <i>B. Longum</i> BB536 and <i>B. Pseudocatenulatum</i> G4 onto Brush Border of HT-29	94
4.24	Observation by Scanning Electron Microscopy of the Adhesion of <i>B. Longum</i> BB536 and <i>B. Pseudocatenulatum</i> G4 Closely Associated With Microvilli of HT-29	95
4.25	Competition between Probiotics Bacteria and Pathogens Bacteria onto Human Epithelium Cell Line. Results are shown as mean and Standard Deviation	98
4.26	Exclusion between Probiotics Bacteria and Pathogens Bacteria onto Human Epithelium Cell Line. Results are shown as mean and Standard Deviation	100
4.27	Displacement between Probiotics Bacteria and Pathogens Bacteria onto Human Epithelium Cell Line. Results are shown as mean and Standard Deviation	102



LEST OF ABBREVIATIONS

ATCC	American Type Culture Collection
CFU	Colony Forming Unit
CO ₂	Carbon dioxide
DMEM	Dulbecco's modified Eagle's minimal essential medium
DNA	Deoxyribonucleic acid
e.g.	Example gratia (for example)
<i>et at.</i>	Et cetera (and company)
FBS	Fetal Calf Serum
GIT	Gastrointestinal tract
H	Hour
H ⁺	Hydrogen ion
HCL	Hydrochloric acid
JCM	Japan Collocation of Microbiology
L	Liter
LAB	Lactic Acid Bacteria
Log	Logarithm
M	Molar
mL	Milliliter
mM	Millimolar
MRS	De Man Rogosa Sharpe Media
NaOH	Sodium Hydroxide
PBS	Phosphor Buffer Saline



RAPD	Randomly amplified polymorphic DNA
rpm	Revolution per minute
SEM	Scanning electron microscopy
S.D.	Stander Deviation
spp.	Species
v/v	Volume per volume
WHO	World Health Organization
w/v	Weight per volume



CHAPTER 1

INTRODUCTION

The normal microflora of the human body is extensive and diverse. The largest portion is found in the gastrointestinal tract (GIT), specifically the colon. Bifidobacteria represents one of the predominant groups of the GIT microflora in breast-fed children (Caglar *et al.*, 2005), and it has been the focus of researches on probiotic in recent years.

There are more than 10^{13} – 10^{14} total bacteria in the human GIT, most of them are anaerobes. Bifidobacteria was discovered in 1899 as naturally present in the colon microflora, including *Bifidobacterium bifidum* and *Bifidobacterium longum*. Tissier has isolated the genus *Bifidobacterium* from the stool of human beings and observed that it represents up to 25% of the cultivable faecal bacteria in adults and 80% of it in infants (Salminen *et al.*, 2004; Picard *et al.*, 2005).

In 1989, Fuller defined probiotic as "a live microbial feed supplement which beneficially affects on the host animal by improving its microbial balance." Probiotic survive gastric acidity, bile, and pancreatic secretions. They attach to the epithelial cells and colonize in the intestinal tract, where they inhibit the pathogenic bacteria



and stimulate the immune system (Saito, 2004; Del Piano *et al.*, 2006). It is probable that adhesion bacteria interact to a greater degree with the intestinal mucosa and therefore, with the host as in comparison to flora bacteria, many of which have little or no influence on the host (Del Piano *et al.*, 2006).

Many of *Bifidobacterium* human isolation species are probiotic as *B. longum*, *B. breve*, *B. lactis*, *B. bifidum*, and *B. pseudocatenulatum* (Ouwehand *et al.*, 2002; Shuhaimi *et al.*, 2004). Since few studies have been carried out on *Bifidobacterium pseudocatenulatum* G4, this research is a comprehensive study on this species. A special emphasis has been made on the species isolation by PCR- based and 16S rDNA partial sequences analysis methods (Shuhaimi *et al.*, 2002), a generation of genomic DNA fingerprints of *B. pseudocatenulatum* G4 is isolated by RAPD (Shuhaimi *et al.*, 2001).

The theoretical benefits of probiotic bifidobacteria are mediated by modulating the functionality of the intestinal microbial flora, gut barrier, and host immune system. Their therapeutic and prophylactic roles have been proposed and trailed in animals and human beings. Probiotic bifidobacteria adhesion properties, resistance to infectious diseases, and prevention of colon cancer have been assessed in current years (Salminen *et al.*, 2004). A number of bifidobacteria have now a long history of safe use in dairy products; *B. adolescentis*, *B. animalis*, *B. lactis*, *B. bifidum*, *B. breve*, and *B. longum* are generally regarded as safe status (Salminen *et al.*, 2004).

Probiotic strains adhesion to the intestinal surface and their following colonization in the human GIT have been suggested as an important requirement for probiotic action. Adhesive strains of probiotic bacteria probably persist longer in the intestinal tract and thus have better potential of showing metabolic and immunomodulatory effects than non adhesive strains. Adhesion provides an interaction with the mucosal surface, facilitating the contact with gut associated lymphoid tissue and mediating local and systematic immune effects. Adhesion may also provide means of competitive exclusion of pathogenic bacteria from the intestinal epithelium (Saarela *et al.*, 2000).

The bacterial surface properties determine their ability to adhere to intestinal mucus, enterocyte cells, and gut epithelial tissue (Saarela *et al.*, 2000). These surface properties also determine the microorganism's resistance to pH and bile, and its production of antimicrobial substances. Resistance to bile, however, is one of the criteria used to select probiotic strains that would potentially be capable of performing effectively in the GIT (Begley *et al.*, 2005). As the calcium receptor is expressed along the entire GIT, the present of the calcium ion in human colons may enhance the adhesion of probiotic (Larsen *et al.*, 2007).

Clearly avoiding pathogenic colonization and reducing the risk of potential pathogenic bacteria is beneficial to the host. The idea is that probiotic change the host's normal microflora from a potential harmful composition towards a beneficial one. Generally, this means reducing the growth of pathogens and increasing lactobacilli and/ or bifidobacteria (Ouweland *et al.*, 2002).

Further assessment of probiotic qualities, this study is focused on the adhesion and adhesion inhibition properties of *B. pseudocatenulatum* G4 with the following objectives:

1. Evaluating the effect of time, pH, calcium, and cholic acid on *B. pseudocatenulatum* G4 adhesion quality.
2. Determines the ability of *B. pseudocatenulatum* G4 to impair the adhesion of several pathogens.

CHAPTER 2

LITRETURE REVIEW

2.1 Definition of Probiotic

Recent researches regarding probiotics as beneficial bacteria, concentrate basically on *Lactobacillus* spp. and *Bifidobacterium* spp. Bifidobacteria represents one of the predominant groups in the gastrointestinal tract (GIT). However; the presence of bifidobacteria has decreased after vertebrate weaning and potentially pathogenic bacteria begin to predominate. Some bifidobacteria was documented today as probiotic, that is to say, bacteria which improve the properties of the intestinal flora and contribute to better health (Haschke *et al.*, 1998).

In the last century, microbiologists differentiated between microbiota in the GIT of healthy individuals from those found in diseased individual. The beneficial microorganisms found in the GIT are termed as probiotic. Probiotic, meaning "for life," are microorganisms that have proven to extract healthy promoting influences in human beings and animals (Parvez *et al.*, 2006). Probiotic by the general accepted definition is a live microbial feed supplement which beneficially affects the host animal by improving intestinal microbial balance (Ferna'ndez *et al.*, 2003). The term 'probiotic' was first used by Lilly and Stillwed in (1965) to describe substances secreted by one microorganism which stimulate the growth of another. A powerful evolution of this definition was coined by Parker in (1974), who proposed that



probiotic are "organisms and substances which contribute to intestinal microbial balance". Fuller; then modified the definition in (1989) to "a live microbial feed supplement which beneficially affects the host animal by improving its microbial balance". Kalantzopoulos (1997), defined probiotic as "fermented food containing specific live microorganisms or live microbial food or feed supplement, which beneficial affects the human or the host animal by improving its intestinal microbial balance". Salminen *et al.*, in (1998), defined probiotic as "food which contains live bacteria which are beneficial to health". While Marteau, (2002) defined them as preparations or components of microbial cells that have beneficial effects on the health.

The first probiotic species been introduced into research were *Lactobacillus acidophilus* by Hull *et al.*, (1984) and *Bifidobacterium bifidum* by Holcomb *et al.*, (1991). The idea was that bacteria in fermented products compete with microorganisms that were harmful to health. The bacterial population of the human GIT constitutes an enormously complex ecosystem. Most of the organisms are beneficial e.g. *Bifidobacterium* and *Lactobacillus* but some are harmful e.g. *Salmonella spp.*, *Helicobacter pylori*, *Escherichia coli*, and *Clostridium spp.* Most of the beneficial bacteria fall into the group of organisms known as lactic acid bacteria. These organisms are usually produced and consumed in the form of yoghurt, fermented milk or other fermented foods. Some of the positive impacts of lactic acid bacteria include consumption, improving intestinal tract health, strengthening the immune system, enhancing bioavailability of nutrients, and reducing the risk of certain cancers (Parvez *et al.*, 2006).