



UNIVERSITI PUTRA MALAYSIA

**EFFECTS OF MUSA SAPIENTUM (BANANA) AND IPOMEA BATATAS  
(SWEET POTATO) EXTRACTS ON THE GROWTH OF  
LACTOBACILLUS, E. COLI AND S. THYHIMURIUM IN MONOCULTURE  
AND CO-CULTURE**

NOR ZAWANI CHE JAAFAR

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## PENGESAHAN

Dengan ini adalah disahkan bahawa projek yang bertajuk “EFFECTS OF *MUSA SAPIENTUM* (BANANA) AND *IPOMEA BATATAS* (SWEET POTATO) EXTRACTS ON THE GROWTH OF *LACTOBACILLUS*, *E. COLI* AND *S. THYHIMURIUM* IN MONOCULTURE AND CO-CULTURE” telah disiapkan serta dikemukakan kepada Jabatan Mikrobiologi oleh NOR ZAWANI BINTI CHE JAAFAR (162897) sebagai syarat untuk kursus BMY 4999 projek.

Disahkan oleh:

.....  
Prof. Madya Dr. Sieo Chin Chin

Penyelia

Jabatan Mikrobiologi

Fakulti Bioteknologi dan Sains Biomolekul

Universiti Putra Malaysia

Tarikh: .....

.....  
Prof. Madya Dr. Muhajir Hamid

Ketua

Jabatan Mikrobiologi

Fakulti Bioteknologi dan Sains Biomolekul

Universiti Putra Malaysia

Tarikh: .....

## ABSTRACT

Prebiotics are substrates that are able to promote the growth of beneficial bacteria in the intestine such as lactic acid bacteria. Commercial prebiotics are costly and to explore the possibility of obtaining prebiotic-like compound from local resources, the water and methanolic extracts of *Musa sapientum* (banana) and *Ipomea batatas* (sweet potato) peels were tested on the growth of beneficial bacteria. In this study, the growth of *Lactobacillus reuteri* C1, *Lactobacillus salivarius* I 24, *Lactobacillus brevis* I 25 and *Lactobacillus gallinarum* I 26 in the extracts were tested. The effects of the extracts on the growth of non beneficial bacteria such as *Escherichia coli* and *Salmonella thyphimurium* was also tested in mono and co-culture with *Lactobacillus*. The growth of the bacteria was compared against growth in the modified basal de Man Rogosa Sharpe (MRS) supplemented with 1% glucose for *Lactobacillus* and nutrient broth (NB) supplemented with 1% glucose for *E. coli* and *S. thyphimurium*. The results showed that the *Lactobacillus* have higher growth in the methanolic extracts. *Lactobacillus reuteri* C1 and *L. gallinarum* I26 achieved 8.48 log CFU/ml and 7.65 log CFU/ml, respectively after 24 hour incubation in methanolic extract of *Musa sapientum*. *Lactobacillus sallivarius* I24 achieved 7.68 log CFU/ml after 24 hour growth in methanolic extract of *Ipomea batatas*. *Lactobacillus brevis* I25 could grow equally well in methanolic extract of *Musa sapientum* and *Ipomea batatas*. Although the growth of *E. coli* and *S. thyphimurium* achieved 9.72 log CFU/ml and 10.00 log CFU/ml in *Ipomea batatas* water extraction and *Ipomea batatas* methanolic extraction, the growth of these bacteria was reduced when grown in co-culture with selected *Lactobacillus* strains. The growth of *E. coli* and *S. thyphimurium* was reduced by 3 to 5 log CFU/ml in water and methanolic extracts of *Musa sapientum*. Similarly the growth of *E. coli* and *S. thyphimurium* was reduced by 3 to 4 log CFU/ml in water and methanolic extracts of *Ipomea batatas*.

## ABSTRAK

Prebiotik adalah substrat yang berkebolehan untuk menggalakkan pertumbuhan bakteria yg bermanfaat di dalam usus seperti bakteria asid laktik. Prebiotik komersial mempunyai harga yang mahal dan untuk meneroka kemungkinan mendapatkan bahan bercirikan prebiotik daripada sumber tempatan, ekstrak air dan methanol bagi kulit tumbuhan *Musa sapientum* (pisang) dan *Ipomea batatas* (kentang manis) diuji atas pertumbuhan bakteria baik. Dalam kajian ini, pertumbuhan *Laktobacillus reuteri* C1, *Laktobacillus salivarius* I 24, *Laktobacillus brevis* I 25 dan *Laktobacillus gallinarum* I 26 didalam ekstrak diuji. Kesan ekstrak kepada pertumbuhan bakteria tidak bermanfaat seperti *Escherichia coli* dan *Salmonella thyphimurium* juga diuji dalam ujian mono dan ko-kultur bersama *Lactobacillus*. Pertumbuhan bakteria dibandingkan dengan pertumbuhan di dalam basal de Man Rogosa Sharpe (MRS) yang diubahsuai dan dicampurkan dengan 1% glukos untuk *Lactobacillus* dan nutrient broth (NB) ditambahkan dengan 1% glukos untuk *E. coli* an *S. thyphimurium*. The results showed that the *Laktobacillus* have higher growth in the methanolic extracts. *Laktobacillus reuteri* C1 dan *L. gallinarum* I26 mencapai 8.48 log CFU/ml an 7.65 log CFU/ml, selepas 24 jam penyimpanan dalam ekstrak metanol *Musa sapientum*. *Laktobacillus sallivarius* I24 mencapai 7.68 log CFU/ml selepas 24 jam pertumbuhan dalam ekstrak metanol *Ipomea batatas*. *Laktobacillus brevis* I25 mempunyai pertumbuhan yang sama banyak dalam ekstrak methanol *Musa Sapientum* dan *Ipomea batatas*. Walaupun pertumbuhan *E. coli* dan *S. thyphimurium* mencapai 9.72 log CFU/ml and 10.00 log CFU/ml dalam ekstak air *Ipomea batatas* dan metanol *Ipomea batatas*, pertumbuhan bakteria ini mengalami penurunan apabila disatukan dengan ko-kultur bersama strain *Laktobacillus* yang terpilih. Pertumbuhan *E. coli* dan *S. thyphimurium* berkurangan sebanyak 3 ke 5 log CFU/ml dalam ekstrak air dan metanol *Musa sapientum*. Bersamaan dengan pertumbuhan *E. coli* dan *S. thyphimurium* yang berkurangan sebanyak 3 ke 4 log CFU/ml dan ekstrak air dan metanol *Ipomea batatas*.

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## TABLE OF CONTENTS

PENGESAHAN	i
ABSTRACT	ii
ABSTRAK	iii
ACKNOWLEDGEMENT	iv
LIST OF TABLES	vii
LIST OF FIGURES	viii
LIST OF ABBREVIATIONS	ix
CHAPTER 1	1
INTRODUCTION	1
1.1 Objectives :	3
CHAPTER 2	4
LITERATURE REVIEW	4
2.1 Probiotics	4
2.1.1 Properties of probiotics	5
2.1.2 The Genus <i>Lactobacillus</i>	6
2.2 Prebiotic	7
2.2.1 Properties of prebiotics	8
2.2.2 Source of prebiotics	9
2.3 Musa sapientum	11
2.4 Ipomea batatas	13
CHAPTER 3	15
MATERIALS AND METHODS	15
3.1 Bacterial strains and growth conditions	15
3.2 Basal growth media	15
3.3 Sample collection	17
3.4 Extraction methods	17
3.4.1 Hot water extraction	17
3.4.2 Methanolic extraction	17
3.5 Preparation of experimental extracts	18
3.6 Growth of bacteria on extracts	18
3.7 Growth of bacteria in co-culture in extracts	19

CHAPTER 4	20
RESULTS & DISCUSSION	20
4.1    Growth of bacteria in monoculture in different extracts	20
4.1.1    Growth of bacteria on extracts ( $OD_{620}$ )	20
4.1.2    pH Measurement	21
4.1.3    Viable Cell Counts	22
4.2    Growth of bacteria in co-culture in different extracts	25
4.2.1    pH measurement	25
4.2.2    Viable Cell Count	26
CHAPTER 5	30
CONCLUSION & RECOMMENDATIONS	30
REFERENCES	31



## LIST OF TABLES

	Caption	Page
1	Probiotic microorganisms studied and commercial isolation sources	3
2	Types and sources of prebiotics	11
3	Industrial productions of prebiotics	11
4	Components of carbohydrate-free MRS broth	16
5	Components of carbohydrate-free nutrient broth	16
6	The combination of co-culture	19
7	Growth on extracts in each extractions (log CFU/mL)	23

## LIST OF FIGURES

Figures	Caption	Page
1	The solid waste (banana peel) of <i>Musa sapientum</i>	12
2	<i>Ipomea batatas</i> (sweet potato)	14
3	The pH of bacteria culture in glucose (control) and different extracts.	21
4	The pH of isolates in co-culture of <i>Musa sapientum</i> (banana) water and methanolic extraction	25
5	The pH of isolates in the co-culture of <i>Ipomea batatas</i> (sweet potato) water and methanolic extraction	26
6	Bacterial growth in the co-culture of water and methanolic extraction of <i>Musa sapientum</i> (banana)	27
7	Bacterial growth in the co-culture of water and methanolic extraction of <i>Ipomea batatas</i> (sweet potato)	28

## LIST OF ABBREVIATIONS

\$	US Dollar
%	percent
€	Euro
®	Registered
µm	Micrometer
°C	Degree Celsius
h	hour
FAO	Food and Agriculture Organization
FOS	Fructooligosaccharide
GC	Guanine & cytosine
GI	Gastrointestinal tract
GOS	Galactooligosaccharide
IMO	Isomaltooligosaccharide
L <sup>-1</sup>	per 1 litre
NDCs	Non-digestible carbohydrate
OD	Optical density
SPFE	Sweet Potato Fibre Extract
USA	United States of America
v/v	Volume per volume
w/v	Weight per volume
WHO	World Health Organization

## CHAPTER 1

### INTRODUCTION

Consumers nowadays are more health conscious and prefer health promoting foods. Food products containing probiotics are gaining acceptance. Probiotics such as *Lactobacillus* spp which are also one of the bacterial members of the normal human intestinal flora (Tamime *et al.*, 2005) have been reported to show certain benefits to human health. They are reported to improve the intestinal microbial balance, resulting in the inhibition of bacterial pathogens, reduction of colon cancer, improvement of the immunity system and also helps in lowering serum cholesterol levels (Saarela *et al.*, 2002).

Probiotics are live microorganisms which administered in adequate amounts confer a health benefit to the host (FAO/WHO, 2003). Probiotics are being applied in dairy products, particularly in yogurts and the market value of this kind of products is on the rising trend. In order to achieve the health benefits claims, one of the most crucial requirement is to maintain a high number of probiotic organisms at the point of consumption and after consumption (Lourens Hattingh & Viljoen, 2001).

Growth of probiotics can be enhanced by prebiotic that needed during selective stimulation of the growth and/or activity of one or limited number of bacteria (Gibson & Roberfroid, 1995). Gibson *et al.*, (2004) defined prebiotic as a ‘non-digestible’ nutritional ingredients that benefits the host and help to stimulate the growth and activity of one or more beneficial bacteria such as lactobacilli and bifidobacteria in the colon (Rayes *et al.*, 2007). Thus, prebiotics are known as “food” of the probiotic bacteria (Denipote *et al.*, 2010 ; Park & Floch 2007). These prebiotic able to enhance and stimulate the growth of the probiotics in our gut.

Beneficial effects of prebiotics include improvement of mineral absorption, reduction in serum lipid levels and inhibition of gut pathogens (Cummings and Macfarlane, 2002; Marteau and Botron-Rualt, 2002).

The prebiotics isomaltooligosaccharides (IMO), galactooligosaccharides (GOS) and inulin are the example of non-digestible oligosaccharides which have been reported to dominate prebiotic effects (Desai *et al.*, 2004; Pennacchia *et al.*, 2006). Several studies suggested that the probiotics may not able to stand alone and are not competitive enough to allocate and adapt themselves in the gut system (Granato *et al.*, 2010). Thus, synbiotic as a combination of probiotic and prebiotic is reported to have competitive edge in synergistic effect to promote the growth of exiting beneficial microorganisms in the gut and improve the survival of newly added probiotic strains (Schrezenmeir & de Vrese, 2001).

According to Rycroft *et al.* (2001), the intake of prebiotics can either modulate significantly the colonic microbiota by increasing the number of specific probiotic bacteria, such as lactobacilli and bifidobacteria or decreasing the undesired pathogenic bacteria by imitating their attachment sites on the intestinal mucosa (Iji and Tivey, 1998). Prebiotics, which are available in the market are mostly for human consumptions and are costly for example in infant formula with probiotics, add to an additional cost of € 51 or about 55.62 US Dollar (Lenoir *et al.*, 2012). As reported by Cruz *et al* (2014), they are mostly imported products such as capsules, powdered milk formula and fermented milk manufactured in USA and Mexico as shown in Table 1.

**Table 1: Probiotic microorganisms studied and commercial isolation sources.**

Commercial product	Type of product	Manufacturer
Culturell®	Capsules	ConAgra Foods USA
NAN 1®	Powder milk formula	Nestle Mexico
Yakult®	Fermented milk	Yakult Mexico

(Sources : Cruz et al., 2014)

Malaysia is rich in flora and fauna and it is a good source of potential prebiotic like materials. Thus, in the present study, the ability of the extracts from two local materials which are *Musa sapientum* (cardaba banana) and *Ipomea batatas* (sweet potato) peels are examined to promote the growth of lactic acid bacteria but not non beneficial bacteria such as *Escherichia coli* and *Salmonella thyphimurium* was determined.

### **1.1 Objectives :**

- i. To investigate the effects of the water and methanolic extracts on the growth of selected lactic acid bacteria and non beneficial bacteria (*E.coli* and *S.thyphimurium*) in monoculture and co-culture.

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