# TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENT	iii
ABSTRACT (ENGLISH)	v
ABSTRACT (THAI)	viii
LIST OF TABLES	xvi
LIST OF FIGURES	xviii
CHAPTER 1 INTRODUCTION	1
CHAPTER 2 LITERATURE REVIEW	
2.1 Definition of probiotics	4
2.2 The benefit rule of probiotics	4
2.3 Microorganisms used as probiotics	5
2.4 Lactic acid bacteria (LAB) as probiotics	6
2.4.1 Lactic acid bacteria	6
2.4.2 Antimicrobial compounds produced by lactic acid bacteria	9
2.4.2.1 Organic acids	9
2.4.2.2 Hydrogen peroxide	10
2.4.2.3 Carbon dioxide	11
2.4.2.4 Aroma components	11
2.4.2.5 Fatty acids	12
2.4.2.6 Bacteriocins	13
2.5 Prebiotics and their effects	15

	Page
2.6 Safety of lactic acid bacteria	16
2.7 Exploitation of probiotic lactic acid bacteria	21
2.8 Researches for improving probiotic products	22
2.9 Tablets	25
2.10 Polymers in colon-specific drug delivery	27
2.10.1 Hydroxypropyl methylcellulose (HPMC)	28
2.10.2 Hydroxypropylmethylcellulose phthalate (HPMCP)	28
2.10.3 Pectins	29
2.10.4 Inulin	30
2.10.5 Alginates	32
2.11 Banana	32
CHAPTER 3 MATERIALS AND METHODS	
3.1 Materials	
3.1.1 Media and chemical reagents	34
3.1.2 Kits	36
3.1.3 Primers	37
3.1.4 Equipments	37
3.1.5 Animals Chiang Mai Univers	38
5.1.7 Samples	38
3.1.8 Bacterial indicators	38
3.2 Methods	
3.2.1 Bacterial strains and Isolation	39
3.2.2 Screening of acid and bile tolerant isolates	40

1	Page
3.2.3 Determination of acid resistant	40
3.2.4 Determination of bile resistant	41
3.2.5 Determination of antibacterial activity	41
3.2.6 Determination of cell surface hydrophobicity	42
3.2.7 Effect of inulin on <i>Lactobacillus</i> growth	42
3.2.8 Identification of bacterial isolates by biochemical reactions	43
3.2.9 Identification of bacterial isolates by molecular techniques	44
3.2.10 Antibiotic susceptibility test	46
3.2.11 Haemolytic activity	47
3.2.12 Safety study of viable lactobacilli	47
3.2.13 Effect of cryoprotectants	48
3.2.14 Tableting	
3.2.14.1 Bacterial strains and preparation of powder culture	49
3.2.14.2 Test of bacterial viability in tablets exposed to a test medium	50
3.2.14.3 Tablet evaluation	51
3.2.14.4 Effect of compression force on lactobacilli powder	51
3.2.14.5 Effect of excipients	
1) Tablets containing hydroxypropylmethyl cellulose phthalate (HPMCP)	51
2) Tablets containing inulin and banana powder	58
3) Tablets containing hydroxypropylmethyl cellulose phthalate	60
(HPMCP) and banana powder	
3.2.14.6 Stability test of probiotic tablets	60
CHAPTER 4 RESULTS AND DISCUSSION	62

	Page
4.1 Isolation of lactic acid bacteria (LAB)	62
4.2 Screening for acid and bile tolerant	63
4.3 Effect of pH	64
4.4 Effect of bile concentration	64
4.5 Detection of antibacterial activity	65
4.6 Cell surface hydrophobicity	66
4.7 Effect of inulin on selected Lactobacillus strains growth	69
4.8 Identification of bacterial isolates	70
4.9 Antibiotic susceptibility	79
4.10 Haemolytic activity	80
4.11 Safety of viable lactobacilli	82
4.12 Effect of cryoprotectants	85
4.13 Tableting	
4.13.1 Effect of compression force on probiotic powder	88
4.13.2 Effect of hydroxypropylmethyl-cellulosephthalate	89
(HPMCP 55)	
4.13.3 Effect of inulin and banana powder	100
4.13.4 Tablets containing HPMCP 55, banana powder, and sodium alginate	101
4.14 Stability of probiotic tablets	e <sub>107</sub>
CHAPTER 5 CONCLUSIONS	110
REFERENCES	112

#### Page

### APPENDICES

APPENDIX A Culture media	142
APPENDIX B Buffer	143
APPENDIX C Identification results of selected strains	144
CURRICULUM VITAE	153

**ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่** Copyright<sup>©</sup> by Chiang Mai University All rights reserved

# LIST OF TABLES

Table		Page
1	Advantages and disadvantages of direct compression	27
2	Formulations of tablet containing HPMCP and sodium alginate,	53
	apple-pectin, and Metolose <sup>®</sup>	
3	Formulations of tablet containing inulin and banana powder	59
4 %	Formulations of tablet containing HPMCP and banana powder	61
5	Number of strains obtained after purification and characterization	62
6	Number of viable strains after acid and bile tolerant screening test	63
7	Survival of selected acid- and bile-resistant strains in buffer	67
	pH 2, 2.5 and 3, and MRS containing bile salt at 0.3%,	
	0.5% and 1.0% of concentration.	
8	Antimicrobial activity of the selected strains	68
9	Hydrophobicity of the selected strains in hexadecane	69
10	Identification of selected strains using API 50CHL, Biolog system	76
	and PCR method (species specific primer)	
11	Physiological and biochemical characteristics of the selected strains	77
12	Sequencing of 16S rRNA of selected strains	77
13	Antibiotic susceptibility of the selected strains	81
14	Haemolytic activities of lactobacilli strains	82
15	Effect of viable lactobacilli on growth performance of Swiss albino mice	84

Table	Page
16 Survival rate of <i>Lacobacillus</i> spp. strains after lyophilization	87
17 The hardness, survival of <i>L.fermentum</i> 2311 and disintegration	94
of tablets containing HPMCP and sodium alginate,	
apple-pectin, or Metolose®	
18 The hardness, survival of <i>L.acidophilus</i> 72-4 and disintegration	102
of formulation tablets containing inulin and banana powder	
19 The hardness, survival of <i>L.fermentum</i> 2311 and disintegration of	103
formulation tablets containing inulin and banana powder	
20 The hardness, survival of <i>L.fermentum</i> 2311 and disintegration	105
of tablets containing HPMCP, banana, and sodium alginate.	
21. Proportion between $NaH_2PO_4$ and $Na_2HPO_4$	143

**ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่** Copyright<sup>©</sup> by Chiang Mai University All rights reserved

# LIST OF FIGURES

Figu	re	Page
1	Structure of hydroxypropyl methylcellulose phthalate (HPMCP)	29
2	Structure of pectin	30
3	Structure of inulin	31
4	Structure of alginate	32
5	The inhibitory effects of LAB isolates on Staphylococcus aureus	66
	TISTR 029, Escherichia coli TISTR 780, and Salnonella typhi	
	DMST 5784	
6	Comparison of maximum growth achived in 24 hour by nine	70
	selected strains on MRS broth containing glucose, or inulin, Raftiline,	
	bananal (Klouy Hom or Gros Michael or Musa AAA group),	
	banana2 (Klouy Nam Wa or Pisang Awak or Musa ABB group)	
7	The identification of LAB isolate by Biolog AN Plate.	71
8	Genus-specific PCR of selected isolates	72
9 V	Gel electrophoresis of PCR products from multiplex PCR assays	73
10	Gel electrophoresis of PCR product from Lactobacillus fermentum	74
	species specific PCR with primers Lferm3 and Lferm4	
11	Gel electrophoresis of PCR product from Lactobacillus plantarum	75
	species specific PCR with primers Lpla2 and Lpla3	

Figure		Page
12	Phylogenetic tree showing the positions of strains FTL 2311,	78
	FP 3007, FP 3010 and some Lactobacillus species based	
	on 16S rRNA	
13	Effect of compression force on viability of LAB cell powder	88
	(L. acidophilus La-5, L. acidophilus 72-4, and L. fermentum 2311)	
14	Survival of <i>L. fermentum</i> 2311in tablets compressed with	89
	various pressures.	
15	Effect of the amount of lactobacilli lyophilizate on survival rate	90
	of lactobacilli after exposure to 0.04 N HCl for 2 h	
16	Effect of <i>L. fermentum</i> FTL 2311 concentration in tablets on their cell	91
	viability (n=3)	
17	Survival of <i>L.fermentum</i> 2311in formulation no. 29 under	100
	gastric condition (0-2 h) in 0.04N HCl and further intestinal	
	fluid (2-7 h) at pH 6.8.	
18	tability of probiotic tablet containing HPMCP 55 and	108
SO	dium alginate with 171 mg of tablet weight	
19	Stability of selected formulations of <i>L.fermentum</i> 2311	109