

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENT	iii
ABSTRACT	iv
LIST OF TABLES	xi
LIST OF FIGURES	xvi
ABBREVIATIONS AND SYMBOLS	xviii
CHAPTERS	
I. INTRODUCTION	1
II. LITERATURE REVIEWS	3
1. Lactic acid bacteria	3
2. Ecology of lactobacilli	13
3. Probiotics	13
4. Antimicrobial activity by lactobacilli against pathogens	15
5. Antimicrobial mechanism of bacteriocins	20
6. Antimicrobial activity of bacteriocins against microbial pathogens	22
7. Oral microbial ecology	25
8. Oral pathogens	28
9. <i>Porphyromonas gingivalis</i>	33
III. OBJECTIVES	46
IV. MATERIALS AND METHODS	47
1. Lactic acid bacteria	47
1.1 Collection of the microorganisms	47
1.2 Selection of antimicrobial lactic acid bacteria	47
1.3 Identification of potent antimicrobial lactic acid bacteria	48
1.4 Determination of casein utilization	49
1.5 Determination of antimicrobial susceptibility	49
1.6 Analysis of growth curve and generation time	50

TABLE OF CONTENTS (continued)

	Page
2. Characterization of cell-free supernatants	50
2.1 Preparation of cell-free supernatants	50
2.2 Determination of pH sensitivity	51
2.3 Determination of heat sensitivity	51
2.4 Determination of enzyme sensitivity	52
2.5 Extraction of crude bacteriocin by ammonium sulphate precipitation	52
2.6 Total protein assay	53
2.7 One-dimension polyacrylamide gel electrophoresis	53
2.8 Two-dimension polyacrylamide gel electrophoresis	54
3. Partial purification of crude bacteriocins	55
3.1 Anion exchange column chromatography	55
3.2 Determination of the antimicrobial activity	56
3.3 Effect of the solvent towards the antimicrobial activity	56
3.4 Determination of bacteriocin unit	57
3.5 Determination of minimum inhibitory concentration (MIC)	58
3.6 Time-killing assay	58
V. RESULTS	60
1. Lactic acid bacteria	60
1.1 Selection of antimicrobial lactic acid bacteria	60
1.2 Identification of potent antimicrobial lactic acid bacteria	60
1.3 Determination of casein utilization	61
1.4 Determination of antimicrobial susceptibility	62
1.5 Analysis of growth curve and generation time	63
2. Characterization of cell-free supernatants	65
2.1 Determination of pH sensitivity	65
2.2 Determination of heat sensitivity	65

TABLE OF CONTENTS (continued)

	Page
2.3 Determination of enzyme sensitivity	68
2.4 Extraction of crude bacteriocin by ammonium sulphate precipitation	68
2.5 Total protein assay of crude bacteriocin	76
2.6 One-dimension polyacrylamide gel electrophoresis	76
2.7 Two-dimension polyacrylamide gel electrophoresis	77
3. Partial purification of crude bacteriocins	80
3.1 Anion exchange column chromatography	80
3.2 Determination the antimicrobial activity	88
3.3 Effect of the solvent towards the antimicrobial activity	88
3.4 Determination of bacteriocin unit	90
3.5 Determination of minimum inhibitory concentration (MIC)	92
3.6 Time-killing assay	92
VI. DISCUSSION	93
VII. SUMMARY	105
VIII. REFERENCES	106
IX. APPENDICES	136
Appendix A Chemical and media preparations	137
Appendix B Lactic acid bacteria	147
Appendix C Characterization of cell-free supernatants	162
Appendix D Partial purification of crude bacteriocins	178
X. CURRICULUM VITAE	203

LIST OF TABLES

Table	Page
1 The habitats of genus <i>Lactobacillus</i>	9
2 Taxonomy of lactobacilli which based on the phenotypic subdivision	11
3 The examples of bacteriocins	17
4 The antimicrobial studies of <i>Lactobacillus</i> bacteriocins against the gastrointestinal pathogens	23
5 The antimicrobial studies of <i>Lactobacillus</i> bacteriocins against the vaginal pathogens	24
6 The antimicrobial studies of <i>Lactobacillus</i> against the oral pathogens	25
7 Oral human microorganisms	26
8 Proinflammatory cytokine induction by <i>P. gingivalis</i> and its cellular constituents	40
9 <i>P. gingivalis</i> proteinases; substrate specificity and genes	43
10 Identification of potent antimicrobial lactic acid bacteria by API [®] software database	60
11 Identification of potent antimicrobial lactic acid bacteria by BBL [™] software database	61
12 Antibiogram patterns of the selected lactobacilli by using agar-disc diffusion technique	62
13 The generation time of the potent antimicrobial lactobacilli	65
14 Total protein concentration of each pooled fraction of the partial purified bacteriocin by using BCA protein assay kit	85
15 Bacteriocin purifications of <i>L. paracasei</i> subsp. <i>paracasei</i> strain B85/4, B282 and B63/8	91
16 The killing times of ampicillin, B85/4 cell-free supernatant, partial purified bacteriocin at MIC level and partial purified bacteriocin at 2-fold MIC level	92

LIST OF TABLES (continued)

Table	Page
B1 The antimicrobial activity of the potent antimicrobial lactic acid bacteria against various tested bacteria	148
B2 Biochemical identification table of some lactobacilli according to API 50 CHL V5.0 biochemical identification software	149
B3 Biochemical reactions of 3 potent antimicrobial producing lactic acid bacteria compared with 4 standard strains of lactobacilli according to API 50 CHL kit	151
B4 Biochemical reactions of 3 potent antimicrobial lactic acid bacteria compared with 4 standard strains of lactobacilli according to BBL Crystal ID kit	153
B5 The zone diameters interpretive standard of <i>S. aureus</i> ATCC 25923 provided by Oxoid®	154
B6 The zone diameters in millimeter of the potent antimicrobial lactobacilli to antimicrobial agents	155
B7 The calculations of growth curve of <i>L. paracasei</i> subsp. <i>paracasei</i> B85/4 according to their OD ₆₀₀ and viable counts	156
B8 The calculations of growth curve of <i>L. paracasei</i> subsp. <i>paracasei</i> B282 according to their OD ₆₀₀ and viable counts	158
B9 The calculations of growth curve of <i>L. paracasei</i> subsp. <i>paracasei</i> B63/8 according to their OD ₆₀₀ and viable counts	160
C1 The effect of pH on the antimicrobial activity of the potent antimicrobial producing lactobacilli B85/4 isolate and MRS broth against <i>P. gingivalis</i> W50	163
C2 The effect of pH on the antimicrobial activity of the potent antimicrobial producing lactobacilli B282 isolate and MRS broth against <i>P. gingivalis</i> W50	165

LIST OF TABLES (continued)

Table	Page
C3 The effect of pH on the antimicrobial activity of the potent antimicrobial producing lactobacilli B63/8 isolate and MRS broth against <i>P. gingivalis</i> W50	167
C4 The effect of heat on the antimicrobial activity of the potent antimicrobial producing lactobacilli B85/4 isolate and MRS broth against <i>P. gingivalis</i> W50	169
C5 The effect of heat on the antimicrobial activity of the potent antimicrobial producing lactobacilli B282 isolate and MRS broth against <i>P. gingivalis</i> W50	170
C6 The effect of heat on the antimicrobial activity of the potent antimicrobial producing lactobacilli B63/8 isolate and MRS broth against <i>P. gingivalis</i> W50	171
C7 The effect of proteolytic enzyme on the antimicrobial activity of the potent antimicrobial producing lactobacilli 85/4 isolate and MRS broth against <i>P. gingivalis</i> W50	172
C8 The effect of proteolytic enzyme on the antimicrobial activity of the potent antimicrobial producing lactobacilli B282 isolate and MRS broth against <i>P. gingivalis</i> W50	173
C9 The effect of proteolytic enzyme on the antimicrobial activity of the potent antimicrobial producing lactobacilli B63/8 isolate and MRS broth against <i>P. gingivalis</i> W50	174
C10 Total protein concentration and protein increasing folds of 3 isolates of the potent antimicrobial producing lactobacillus	175
C11 The absorbance at 590 nm of standard BSA at 590 nm	176
C12 The absorbance at 590 nm and total protein calculation of each crude bacteriocin and MRS broth	177

LIST OF TABLES (continued)

Table	Page
D1 The elution profiles of B85/4's crude bacteriocin by using anion exchange column chromatography	179
D2 The elution profiles of B282's crude bacteriocin by using anion exchange column chromatography	181
D3 The elution profiles of B63/8's crude bacteriocin by using anion exchange column chromatography	183
D4 The elution profiles of MRS's crude proteins by using anion exchange column chromatography	185
D5 The inhibition zone of each purified fractions which obtained from an anion exchange column chromatography	187
D6 Total protein concentration of each step of the purification of bacteriocin	188
D7 The inhibition zone demonstrated by the partially purified bacteriocin of B85/4 in each solvent	189
D8 The inhibition zone demonstrated by the partially purified bacteriocin of B282 in each solvent	190
D9 The inhibition zone demonstrated by the partially purified bacteriocin of 63/8 in each solvent	191
D10 Bacteriocin unit of each sample of B85/4 against <i>P. gingivalis</i> W50	192
D11 Bacteriocin unit of each sample of B282 against <i>P. gingivalis</i> W50	194
D12 Bacteriocin unit of each sample of B63/8 against <i>P. gingivalis</i> W50	196
D13 The <i>P. gingivalis</i> W50 growth curve	198
D14 The time killing assay against <i>P. gingivalis</i> W50 at the MIC of ampicillin	199
D15 The time killing assay against <i>P. gingivalis</i> W50 at the MIC of B85/4's supernatant	200

LIST OF TABLES (continued)

Table		Page
D16	The time killing assay against <i>P. gingivalis</i> W50 at the MIC of B85/4's partially purified bacteriocin	201
D17	The time killing assay against <i>P. gingivalis</i> W50 at 2-folds MIC of B85/4's partial purified bacteriocin	202



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

LIST OF FIGURES

Figure	Page
1 Homolactic fermentation scheme of lactic acid bacteria	4
2 Heterolactic fermentation scheme of lactic acid bacteria	5
3 Hypothesized relationship between the addition of species during microbial succession leading to the development of gingival inflammation	33
4 The casein utilization of B85/4, B282, B63/8, <i>L. plantarum</i> TISTR 541 and <i>L. casei</i> TISTR 390	61
5 The MIC of clindamycin after tested towards B282 isolate	63
6 The growth curves of the potent antimicrobial lactobacilli, B85/4, B282 and B63/8	64
7 The effect of pH toward the antimicrobial activity of the tested lactobacilli, B85/4, B282 and B63/8	66
8 The effect of heat toward the antimicrobial activity of the tested lactobacilli, B85/4, B282 and B63/8	67
9 The effect of enzyme toward the antimicrobial activity of the tested lactobacilli, B85/4, B282 and B63/8	69
10 The affected proteins of B85/4 and B282 after characterized	70
11 The affected proteins of B63/8 and MRS broth after characterized	71
12 Crude bacteriocins of B85/4 extracted by ammonium sulfate precipitation	72
13 Crude bacteriocins of B282 extracted by ammonium sulfate precipitation	73
14 Crude bacteriocins of B63/8 extracted by ammonium sulfate precipitation	74
15 Crude protein of MRS broth extracted by ammonium sulfate precipitation	75
16 The protein patterns of each crude bacteriocins	76

LIST OF FIGURES (continued)

Figure	Page
17 The proteins pattern of B85/4's crude bacteriocins observing by 2D-PAGE	77
18 The proteins pattern of B282's crude bacteriocins observing by 2D-PAGE	78
19 The proteins pattern of B63/8's crude bacteriocins observing by 2D-PAGE	79
20 The purification chromatograms of the B85/4's bacteriocins according to an anion exchange column chromatography	81
21 The purification chromatograms of the B282's bacteriocins according to an anion exchange column chromatography	82
22 The purification chromatograms of the B63/8's bacteriocins according to an anion exchange column chromatography	83
23 The purification chromatograms of the MRS's crude proteins according to an anion exchange column chromatography	84
24 The protein patterns of the partially purified bacteriocins extracted from B85/4 and B282	86
25 The protein patterns of the partially purified bacteriocins extracted from B63/8 and MRS	87
26 The percentage of residual activity of unbound fraction and pooled fractions 23-27 of B85/4, B282 and B63/8 in each solvent	89
C1 The standard curve of BSA from the BCA protein assay	176
D1 Bacteriocin curve of each sample of B85/4 against <i>P. gingivalis</i> W50	193
D2 Bacteriocin curve of each sample of B282 against <i>P. gingivalis</i> W50	195
D3 Bacteriocin curve of each sample of B63/8 against <i>P. gingivalis</i> W50	197

ABBREVIATIONS AND SYMBOLS

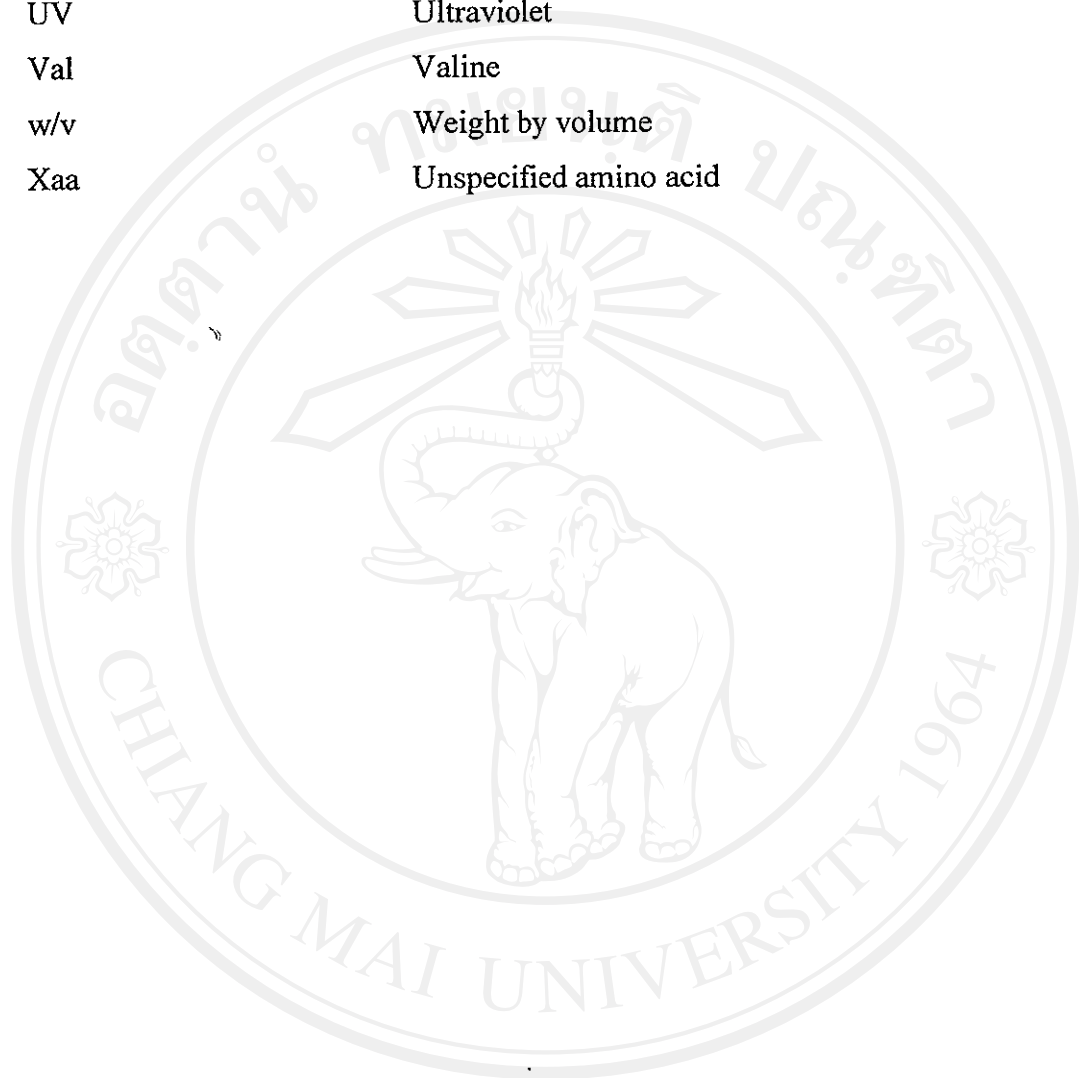
%	Percentage
%C	Crosslinker, acrylamide monomer ratio of the monomer solution
%T	Acrylamide monomer concentration
α	Alpha
β	Beta
κ	Kappa
$^{\circ}\text{C}$	Degree Celsius
2D-PAGE	Two-dimensional polyacrylamide gel electrophoresis
μA	Microampere
μg	Microgram
μl	Microlitre
μm	Micrometre
aa	Amino acid
ADP	Adenosine 5'-diphosphate
ANR	Anaerobe
APF	Aggregation-promoting factor
AR	Analytical
Arg	Arginine
Asn	Asparagine
ATCC	American Type Culture Collection
ATP	Adenosine 5'-triphosphate
BCA	Bicinchronic acid
B_f	Final number bacteria
B_i	Initial number bacteria
BSA	Bovine serum albumin
BU	Bacteriocin unit
C	Cytosine
CFU	Colony forming unit

CHAPS	3-[(3-cholamidopropyl)-dimethyl-ammonia]-1-propane sulfonate
cm	Centimetre
CO ₂	Carbon dioxide
Cys	Cysteine
Da	Dalton
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
ESI-MS	Electrospray ionization mass spectrometry
<i>et al.</i>	et alii (and colleagues)
[Fe ³⁺ PPIX] ₂ O	Iron (III) porphyrin
<i>fimA</i>	Fimbrillin A
FPLC	Fast-Protein Liquid Chromatography
g	Gram
G	Guanine
Gly	Glycine
GM-CSF	Granulocyte macrophage colony-stimulating factor
GRAS	Generally regarded as safe
<i>hag</i>	Hemagglutinin gene
Hag	Hemagglutinin protein
<i>hem</i>	Hemin-regulated gene
HCl	Hydrochloric acid
HmuR	Hemoglobin-hemin receptor
HPLC	High-Performance Liquid Chromatography
hrs.	Hours
i.e.	id est (that is)
IEC	Ion exchange chromatography
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IL.	Illinois state
IL	Interleukin

IPG	Immobilized pH Gradients
K	Potassium
KC	Kuffer cells cytokine
kDa	Kilodalton
<i>kgp</i>	Lysine-specific cysteine proteinase gene
kVh	Kilovolt
l	Litre
LAB	Lactic acid bacteria
LPS	Lipopolysaccharide
Lys	Lysine
M	Molar
MCP	Macrophage chemoattractant protein
MD	Maryland
mg	Milligram
MIC	Minimal inhibitory concentration
MIRCEN	Microbiological Resources Centre
ml	Millilitre
MMP	Matrix metalloprotease
mMRS	Modified De Mann-Rogosa-Sharpe broth
mRNA	Messenger ribonucleic Acid
MW	Molecular weight
N	Normality
NaCl	Sodium Chloride
NAD	Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide Adenine Dinucleotide Phosphate
NaOH	Sodium hydroxide
NC.	North Carolina state
NF- κ B	Nuclear factor kappa beta
NK	Natural killer
mAu	Milliabsorbance unit
nm	Nanometre

No.	Number
NY.	New York state
ON.	Ontario state
OH.	Ohio state
p	Probability
P	Phosphate
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffer saline
PEP-PTS	Phosphoenolpyruvate phosphotransferase system
PG	Packing group
pH	power of Hydronium
pI	Isoelectric point
PMF	Proton motive force
PMN	Polymorphonuclear leukocytes
<i>prtC</i>	Protease collagenase gene
PS	Polysaccharide
<i>rgp</i>	Arginine-specific cysteine proteinase gene
Rgp	Arginine-specific cysteine proteinase
RP	Reverse phase
rpm	Revolution per minute
rRNA	Ribosomal ribonucleic acid
sec	Second
SDS	Sodium dodecyl sulfate
SOD	Superoxide dismutase
spp.	Species
TEMED	N,N,N',N'-tetra-methyl-ethylenediamine
Th	T-helper cell
TISTR	Thailand Institute of Scientific and Technological Research
<i>tla</i>	TonB-linked adhesion gene
TNF	Tumor necrosis factor

Tyr	Tyrosine
USA	The United States of America
UV	Ultraviolet
Val	Valine
w/v	Weight by volume
Xaa	Unspecified amino acid



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved