TABLE OF CONTENTS

TABLE OF CONTENTS	i
LIST OF TABLES	ii
LIST OF FIGURES	iv
LIST OF SYMBOLS AND ABBREVIATIONS	V
INTRODUCTION	1
OBJECTIVES	4
LITERATURE REVIEW	5
MATERIALS AND METHODS	35
RESULTS	48
DISCUSSION	85
CONCLUSION AND RECOMMENDATION	93
LITERATURE CITED	94
APPENDIX	110

LIST OF TABLES

Table		Page
1	Origins and associations of LAB	6
2	Classification of bacteriocins from Gram-positive bacteria	12
3	Examples of lantibiotics produced by LAB	13
4	LAB producing class IIa bacteriocins and their origins	15
5	Summary of known nonlantibiotic two peptide bacteriocins (Class IIb)	16
6	Class IIc bacteriocins	17
7	Class III bacteriocins produced by LAB	18
8	Class IV bacteriocins produced by LAB	18
9	Methods of testing bacteria for production of bacteriocins	20
10	Bacteriocins and antibiotics	25
11	Bacteriocins as food preservatives: example of suggested applications	33
12	List of indicator strains and their growth conditions	36
13	Primers used for PCR amplification of bacteriocin gene	45
14	Average number of LAB isolated from different part of chicken intestine	48
15	Antibacterial activities of K4 and K7 against indicator strains	50
16	Effect of enzymes on the antibacterial activities produced by K4	
	and K7 against <i>Lb. sakei</i> subsp. <i>sakei</i> JCM 1157^{T}	51
17	Effect of pH and heat on the antibacterial activities produced by K4	
	and K7 against <i>Lb. sakei</i> subsp. <i>sakei</i> JCM 1157 ^T	52
18	Effect of pH on growth and bacteriocin production of K4	57
19	Effect of NaCl on growth and bacteriocin production of K4	57
20	Effect of pH on growth and bacteriocin production of K7	58
21	Effect of NaCl on growth and bacteriocin production of K7	58
22	Characteristics of K4 and K7	59
23	Carbohydrate fermentation patterns of K4 and K7	60
24	Antibacterial activities of the amberlite eluates of Lb. salivarius K4	67
25	Antibacterial activities of SP-Sepharose fractions of Lb. salivarius K4	68
26	Purification of bacteriocin FK15 produced by Lb. salivarius K4	76
27	Bacteriocin activity of Salvicin K against indicator strains	81

ii

LIST OF TABLES (Continued)

Table		Page
28	Effect of proteolytic enzyme, pH and heat on bacteriocin, Salvicin K,	
	activities against <i>Lb. sakei</i> subsp. <i>sakei</i> JCM 1157 ^T	82

Appendix Table

1	Profile of growth and bacteriocin production of K4 at 30°C	113
2	Profile of growth and bacteriocin production of K4 at 37°C	113
3	Profile of growth and bacteriocin production of K4 at 42°C	114
4	Profile of growth and bacteriocin production of K7 at 30°C	114
5	Profile of growth and bacteriocin production of K7 at 37°C	115
6	Profile of growth and bacteriocin production of K7 at 42°C	115
7	Mode of action of partially purified bacteriocins produced by	
	Lb. salivarius K4	116
8	Mode of action of Salvicin K produced by Lb. salivarius K4	116

iii

LIST OF FIGURES

Figure	P	Page
1	Biosynthesis of Class IIa bacteriocins	27
2	General models for mechanism of pore formation by bacteriocins	29
3	Schematic representation for bacteriocin and membrane interactions	31
4	Illustration of primer set designed from data base of ORF3 and abp 118 β	45
5	Profile of growth of K4 and bacteriocin production at various temperatures	54
6	Profile of growth of K7 and bacteriocin production at various temperatures	55
7	Alignment of the 16S rRNA gene sequence of K4 (99% similarity with	
	Lb. salivarius, accession No. AF 420311)	63
8	Alignment of the 16S rRNA gene sequence of K7 (98% similarity with	
	Lb. salivarius, accession No. AY 389803)	65
9	Antibacterial activities of the SP-Sepharose in stepwise of NaCl; AU/ml	68
10	HPLC-1 chromatogram of the purification of fraction from SP-Sepharose	69
11	Mass spectrums (MALDI-TOF MS) of HPLC-1-fraction at 18 min	70
12	Mass spectrums (MALDI-TOF MS) of HPLC-1-fraction at 19 min	70
13	Chromatogram at 220 nm from RP-HPLC-2 of bacteriocin FK12	71
14	Molecular weight of purified bacteriocin fraction FK 12	72
15	Mass spectrums (MALDI-TOF MS) of HPLC-1-fraction at 20 min	73
16	Mass spectrums (MALDI-TOF MS) of HPLC-1-fraction at 21 min	73
17	Chromatogram at 220 nm from RPHPLC-2 of bacteriocin FK15	74
18	Molecular weight of purified bacteriocin fraction FK15	75
19	Partial N-terminal amino acid sequences of bacteriocins FK12 and FK15	77
20	Nucleotide sequence of the structural gene of FK12 and FK15	79
21	Alignment of amino acid sequences deduced from DNA sequences	
	compare to amino acid sequences in GenBank database (Underline	
	sequence showed the different)	80
22	Mode of action of partially purified bacteriocin produced by K4	83
23	Mode of action of bacteriocin Salvicin K	84

LIST OF SYMBOLS AND ABBREVIATIONS

ATCC	=	American Type Culture Collection
CFU	=	Colony Forming Unit
AU	=	Arbitary Unit
bp	=	base pair
°C	=	Celsius
Da	=	Dalton
DNA	=	Deoxyribose Nucleic Acid
ESI MS	=	Electrospray-ionization (ESI) Mass Spectrometer (MS)
GRAS	=	Generally Recognized As Safe
HPLC	=	High Performance Liquid Chromatography
JCM ^T	=	Japan Collection of Microorganisms (T = type strain)
Kb	=	Kilobase
kDa	=	kilodalton
LAB	=	Lactic Acid Bacteria
LB	=	Luria-Bertani
Μ	=	Molar
MRS	=	De Man, Rogosa and Sharpe media
MW	=	Molecular Weight
NaCl	=	Sodium Chloride
NCBI	=	National Center for Biotechnology Information
OD	=	Optical Density
PCR	=	Polymerase Chain Reaction
rDNA	=	Ribosomal deoxyribonucleic acid
rRNA	=	Ribosomal ribonucleic acid
Subsp.	=	Subspecies
TFA	=	Triflouro Acetic Acid
v/v	=	volume /volume

vi