



**UNIVERSITI PUTRA MALAYSIA**

**ISOLATION AND CHARACTERIZATION OF BACTERIOCIN  
PRODUCING LACTIC ACID BACTERIA**

**SAHAR ABBASI LIASI**

**FBSB 2009 9**



**ISOLATION AND CHARACTERIZATION OF BACTERIOCIN-  
PRODUCING LACTIC ACID BACTERIA**

**By**

**SAHAR ABBASI LIASI**

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

**April 2009**



***Dedicated to my parents***  
For their endless supports



**Abstract of Thesis Presented to the Senate of Universiti Putra Malaysia in  
Fulfillment of the Requirement for the Degree of Master of Science**

**ISOLATION AND CHARACTERIZATION OF BACTERIOCIN-  
PRODUCING LACTIC ACID BACTERIA**

By

**SAHAR ABBASI LIASI**

**April 2009**

**Chairman: Professor Arbakariya Ariff, PhD**

**Faculty: Biotechnology and Biomolecular Sciences**

Lactic acid bacteria (LAB) in fermented foods produce a large variety of compounds which give these products their characteristic flavor and color apart from improving its safety and quality. LAB with potential applications in food industry have been isolated from a local fermented food (budu) and characterized based on their morphological and biochemical characteristics. Gram-staining, catalase and gas production tests were performed for identification while API 50 CHL (BioMérieux) was used for the determination of species. Twelve isolates were identified as genus *Lactobacillus* (5 isolates of *Lb. paracasei*, 2 isolates of *Lb. plantarum*, one isolate of *Lb. casei* and 4 isolates of *Lb. sp*). The highest population was *Lb. paracasei* (41.67%). All isolates showed gram-positive, catalase negative and some positive results for homo-fermentative characteristics .

Antibiotic sensitivity test of these twelve isolates of LAB to 24 different types of antibiotics was conducted using the disc diffusion method. Inhibition zone diameter was measured and calculated from the means of five determinations and expressed in



terms of resistance or susceptibility. All LAB isolates from this product were resistant to colestin sulphate, streptomycin, amikacin, norfloxacin, nalidixic acid, mecillinam, sulphamethoxazole/ trimethoprim, and kanamycin but susceptible to erythromycin, ceftriaxone, chlomphenicol, tetracycline, ampicillin and nitrofurantion.

The four isolates (*Lb. casei* LA17, *Lb. plantarum* LA22, *Lb. paracasei* LA07 and *Lb. sp.* LA19) were evaluated for their ability in producing bacteriocins. The inhibitory spectra of the isolates when evaluated against a range of gram-positive and gram-negative test microorganisms showed that antimicrobial substance from these isolates inhibit the growth of indicator microorganisms, such as *Bacillus cereus*, *Lactococcus lactis*, *Staphylococcus aureus*, *Salmonella enterica*, *Listeria monocytogenes* and *Escherichia coli*. Complete inactivation of antimicrobial activity from *Lb. paracasei* LA07 was observed after treatment of cell-free supernatant with proteinase K confirming its proteinaceous nature. Treatment with  $\alpha$ -amylase inactivated the antimicrobial activity suggesting that bacteriocin could be glycosylated. Stability of the bacteriocins in the present of catalase enzyme ruled out the possibility of antagonistic activity of bacteriocins due to hydrogen peroxide or organic acids. Lipase caused only a slight reduction of bacteriocin activity, indicating that besides the proteinaceous subunit, some lipid components may also involve in antibacterial activity. The partially purified bacteriocin produced by *Lb. paracasei* LA07 has molecular weight of 10 kDa, based on SDS-PAGE analysis. The antibacterial activity of cell free supernatant was significantly increased by the addition SDS, Triton x-100, Tween 80 and Tween 20. On the other hand, the antibacterial activity was lost with the addition of EDTA.

The influence of pH, temperature, and media composition on growth of *Lb. paracasei* LA07 and bacteriocin production was also conducted in shake flask culture. The highest bacteriocin production was obtained in cultivation using MRS, which was about 51%, 4%, 63 % and 22% higher than those obtained in BHI, M17 NB and TSB, respectively. Production of bacteriocin was also influenced by the cultivation temperature and the highest production was obtained at 30°C. Optimal pH for growth of *Lb. paracasei* LA07 and bacteriocin production was achieved at an initial pH of 8.5. Addition of (5 g/l) yeast extract and meat extract to MRS medium increased further the bacteriocin by about 8% and 5%. Supplementation with glucose at concentrations up to 10 g/l as the sole carbon source, gradually enhanced bacteriocin production, while higher concentrations (20 g/l) did not further increase bacteriocin production. Growth and bacteriocin production were not significantly affected by the agitation rate in either in anaerobic or in microaeration conditions.

From this study, it can be concluded that the locally fermented fish product, budu contained LAB where the highest population was *Lb. paracasei*. The antimicrobial activity produced by the LAB isolated in this research could act as a potential barrier to inhibit the growth of spoilage bacteria and food-borne pathogens.

**Abstrak Tesis Untuk Dikemukakan Kepada Senat Universiti Putra Malaysia  
Sebagai Memenuhi Keperluan Untuk Ijazah Master Sains**

**PEMENCILAN DAN PENCIRIAN BAKTERIA ASID LAKTIK -PENGHASIL  
BAKTERIOSIN**

Oleh

**SAHAR ABBASI LIASI**

**April 2009**

**Pengerusi : Professor Arbakariya Ariff, PhD**

**Fakulti : Bioteknologi dan Sains Biomolekul**

Bakteria asid laktik (BAL) dalam makanan tertapai menghasilkan pelbagai sebatian yang memberikan produk tersebut ciri-ciri rasa dan warna selain meningkatkan kualiti dan keselamatannya. BAL yang berpotensi diaplikasikan dalam industri makanan telah dipencilkan dari makanan tertapai tempatan (budu) dan dicirikan berdasarkan morfologi dan biokimianya. Perwarna gram, ujian katalase dan penghasilan gas telah dijalankan untuk pengenalpastian bakteria tersebut sementara API 50 CHL (BioMerieux) telah digunakan untuk penentuan spesis. Dua belas isolat telah dipencilkan dan dikenalpasti sebagai genus *Lactobacillus* (5 isolat *Lb. paracasei*, 2 isolat *Lb. plantarum*, one isolat *Lb. casei* and 4 isolat *Lb. sp.*). Populasi tertinggi yang diperolehi ialah *Lb. paracasei* (41.67%). Kesemua isolat yang dipencil telah menunjukkan ciri-ciri gram-positif, katalase-negatif dan hasil yang positif bagi ciri-ciri homofermentatif.

Ujian sensitiviti antibiotik bagi dua belas isolat BAL terhadap 24 jenis antibiotik berbeza telah dijalankan menggunakan kaedah keserapan cakera. Garis pusat zon

perencanaan telah diukur dan dikira dari purata lima penentuan dan dinyatakan dalam sebutan rintang atau penerimaan. Semua isolat BAL yang dipencil dari produk ini adalah rintang kepada sulfat kolestin, streptomisin, amikasin, norfloksasin, asid nalidiksik, mesilinam, sulphametoksazol/trimetoprim dan kanmisin tetapi penerimaan kepada eritromisin, seftriakson, kloramfinikol, tetrasiklin, ampicilin dan nitrofurantion.

Empat isolat tersebut (*Lb. casei* LA17, *Lb. plantarum* LA22, *Lb. paracasei* LA07 dan *Lb. sp.* LA19) telah dinilai keupayaannya dalam menghasilkan bakteriosin. Spektra perencanaan yang dinilai terhadap satu julat ujian mikroorganisma gram-positif dan gram-negatif menunjukkan bakteriosin dari isolat tersebut dapat merencat pertumbuhan mikroorganisma penunjuk seperti *Bacillus cereus*, *Lactococcus lactis*, *Staphylococcus aureus*, *Salmonella enterica*, *Listeria monocytogenes* dan *Escherichia coli*. Penyahaktifan lengkap aktiviti antimikrobia *Lb. paracasei* LA07 diperoleh selepas rawatan supernatant bebas-sel dengan proteinase K yang mengesahkan ianya berbentuk protein. Rawatan dengan  $\alpha$ -amilase tidak menyahaktif aktiviti antimikrobia yang menunjukkan bakteria mungkin terglisosilat. Kestabilan bakteriosin dalam kehadiran enzim katalase menolak kemungkinan aktiviti penentangan bakteriosin disebabkan oleh hydrogen peroksida atau asid organik. Lipase menyebabkan hanya sedikit pengurangan aktiviti bakteriosin yang menunjukkan, di samping subunit berprotein, terdapat sedikit komponen lipid yang mungkin terlibat dalam aktiviti antibakteria. Bakteriosin separa taleh yang dihasilkan oleh *Lb. paracasi* LA07 mempunyai 10 kDa berat molekul, berdasarkan analisis SDS-PAGE. Aktiviti antibakteria bagi supernatant bebas sel telah ditingkatkan oleh



penambahan SDS, Triton x-100, Tween 80 dan Tween 20. Selain itu, aktiviti antibakteria hilang dengan penambahan EDTA.

Kajian ke atas pengaruh pH, suhu dan komposisi media kepada pertumbuhan *Lb. paracasei* LA07 dan penghasilan bakteriosin juga telah dijalankan didalam kelalang bergoncang. Penghasilan bakteriosin tertinggi diperolehi daripada pengkulturan menggunakan MRS yang mana lebih kurang 51%, 4%, 63% dan lebih tinggi berbanding BHI, M17 NB dan TSB. Masing-masing. Penghasilan bakteriosin adalah juga dipengaruhi oleh suhu kultur dan penghasilan tertinggi diperolehi pada suhu 30°C. pH optimal untuk pertumbuhan *Lb. paracasei* LA07 dan penghasilan bakteriosin diperolehi pada pH permulaan 8.5. Penambahan ekstrak yis dan ekstrak daging (5 g/l) ke dalam medium MRS meningkatkan lagi penghasilan bakteriosin sebanyak 8% dan 5%. Penambahan glukosa pada kepekatan sehingga 10 g/l sebagai sumber karbon tunggal meningkatkan penghasilan bakteriosin; sebaliknya kepekatan yang lebih tinggi (20 g/l) tidak meningkatkan penghasilan bakteriosin seterusnya. Adalah jelas tiada perbezaan yang ketara samada dalam pertumbuhan bakteria atau penghasilan bakteriosin dalam keadaan aerobik atau anaerobik.

Kesimpulan daripada kajian ini menunjukkan bahawa budu mengandungi BAL dengan populasi *Lb. paracasei* yang tertinggi. Aktiviti antimikrob bakteriosin yang dihasilkan oleh BAL dalam kajian ini boleh bertindak sebagai penghalang untuk merencat pertumbuhan bakteria perosak dan patogen bawaan makanan.

## ACKNOWLEDGMENTS

I would like to express my sincere appreciation and gratitude to my supervisor **Prof. Dr. Arbakariya Ariff** under whose guidance and supervision had provided me the opportunity and conducive environment to complete this study. His invaluable constructive criticism and continuous support had built in me the confidence to undertake the laboratory investigations with patience and optimism throughout the course my studies.

Special thanks go to the members of my supervisory committee **Dr. Rosfarizan Mohamad**, **Associate Professor Dr. Shuhaimi Mustafa** and **Associate Professor Dr. Hassan Hj. Daud** for their suggestions and useful deliberative discussions to make this study more comprehensive and meaningful.

I would also like to thank **Prof. Dr. Tengku Azmi Ibrahim** for his invaluable advice and guidance on the electron microscopic aspect of this investigation. I am equally indebted to **Dr. Zunita Zakaria** and **Dr. Goh Yong Meng** for their useful advice when dealing with specific problems related to bacteriology and statistics.

Thanks are due to **Prof. Dr. Raha Abdul Rahim** for extending me the use of facilities of her laboratory. I would like to thank all the staff in the Department of Biotechnology, Faculty of Biotechnology and Biomolecular Sciences for their numerous help and support during the course my work. I am equally grateful to the staff of the Microscopy Unit, Institute of Bioscience, especially to **Mr. Ho Oi Kuan** for their kind assistance in the preparation of samples for electron microscopy.



I am immensely and forever grateful to those individuals who have, in one way or another provided me with the courage and resilience to pave the way towards the successful completion of this study.

Last but not least, I wish to thank my family for their continuous moral support and encouragement. By remaining close to me through constant contact and being there when I need them, they made me feel as if I am at home with them even though they are far away from me.



I certify that an Examination Committee has met on 3 April 2009 to conduct the final examination of Sahar Abbasiasi on her thesis entitled “Isolation and Characterization of Bacteriocin-Producing Lactic Acid Bacteria “ in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Examination Committee were as follows:

**Mohd. Yunus Abd. shukur, PhD**

Associate Professor

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

(Chairman)

**Sieo Chin Chin, PhD**

Lecturer

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

(Internal Examiner)

**Nor ‘Aini Binti Abdul Rahman, PhD**

Lecturer

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

(Internal Examiner)

**Aidil Bin Abdul Hamid, PhD**

Associate Professor

Faculty of Science and Technology

Universiti Kebangsaan Malaysia

(External Examiner)

-----  
**BUJANG KIM HUAT, PhD**

Professor and Deputy Dean

School of Graduate Studies

Universiti Putra Malaysia

Date:



This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

**Arbakariya Ariff, PhD**

Professor

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

(Chairman)

**Rosfarizan Mohamad, PhD**

Associate Professor

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

(Member)

**Shuhaimi Mustafa, PhD**

Associate Professor

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

(Member)

**Hassan Hj Mohd Daud , PhD**

Associate Professor

Faculty of Veterinary Medicine

Universiti Putra Malaysia

(Member)

-----  
**HASANAH MOHD GHAZALI, PhD**

Professor and Dean

School of Graduate Studies

Universiti Putra Malaysia

Date: 9 July 2009



## **DECLARATION**

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any degree at Universiti Putra Malaysia or at any other institutions.

-----  
**SAHAR ABBASI LIASI**

Date: 17 June 2009

## TABLE OF CONTENTS

	<b>Page</b>
<b>ABSTRACT</b>	iii
<b>ABSTRAK</b>	vi
<b>ACKNOWLEDGMENTS</b>	ix
<b>APPROVAL</b>	xi
<b>DECLARATION</b>	xiii
<b>LIST OF TABLES</b>	xvii
<b>LIST OF FIGURES</b>	xix
<b>LIST OF ABBREVIATIONS</b>	xx
<b>CHAPTER</b>	
<b>1 INTRODUCTION</b>	<b>1</b>
<b>2 LITERATURE REVIEW</b>	<b>4</b>
2.1 Food Fermentation by Lactic Acid Bacteria	4
2.2 Microbiological and Biochemical Characteristics of Lactic Acid Bacteria	6
2.3 Identification of Lactic Acid Bacteria	10
2.4 Optimal Nutrition Conditions and Metabolism of Lactic Acid Bacteria	11
2.5 Metabolic Products of Lactic Acid Bacteria	13
2.5.1 Aroma Components	14
2.5.2 Hydrogen Peroxide and Carbon Dioxide	17
2.5.3 Reuterin	19
2.5.4 Organic Acids	19
2.5.5 Bacteriocins	21
2.6 Classification of Bacteriocins Produced by LAB	22
2.7 Antimicrobial Spectrum of Bacteriocins	23
2.8 Mechanism of Inhibitory Effect in Bacteriocins	24
2.9 Antibacteria vs Antibiotic	25
2.10 Factors Affecting to the Production of Bacteriocins	26
2.10.1 Growth Medium	27
2.10.2 pH and Fermentation Temperature	29
2.11 Bacteriocins from Different Species of Lactic Acid Bacteria	29
2.11.1 Bacteriocin from <i>Lactobacillus</i> spp.	30
2.11.2 Bacteriocin from <i>Leuconostoc</i> spp.	32
2.11.3 Bacteriocin from <i>Pediococcus</i> spp.	33
2.11.4 Bacteriocin from <i>Carnobacteria</i> spp.	33
2.11.5 Bacteriocin from <i>Lactococcus</i> spp.	34
2.11.6 Bacteriocin from Enterococci and Streptococci	35
2.12 Application of Bacteriocins in Industries	39
2.13 Concluding Remarks	39

<b>3</b>	<b>ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF THE BACTERIOCINS-PRODUCING LAB FROM BUDU</b>	41
3.1	Introduction	41
3.2	Materials and Methods	42
3.2.1	Flow of Experimental Design	42
3.2.2	Isolation of Lactic Acid Bacteria	43
3.2.3	Maintenance of Culture	43
3.2.4	Morphological, Physiological and Biochemical Examinations	43
3.3	Results	45
3.4	Discussion	53
3.5	Conclusions	55
<b>4</b>	<b>ASSESSMENT OF ANTIBIOTIC SENSITIVITY OF LACTIC ACID BACTERIA ISOLATES FROM BUDU</b>	56
4.1	Introduction	56
4.2	Materials and Methods	57
4.2.1	LAB Isolates	57
4.2.2	Antibiotic Susceptibility of Isolates	58
4.3	Results	59
4.4	Discussion	74
4.5	Conclusions	76
<b>5</b>	<b>CHARACTERIZATION OF THE BACTERIOCIN PRODUCED BY THE SELECTED LAB ISOLATE (<i>Lb . paracasei</i> strain LA07)</b>	77
5.1	Introduction	77
5.2	Materials and Methods	78
5.2.1	LAB Isolates	78
5.2.2	Assay for Antimicrobial Activity	78
5.2.3	Inhibitory spectrum	78
5.2.4	Effect of Enzymes and Detergents on Antimicrobial Activity	79
5.2.5	Partially Purified, Antibacterial Activity and Protein Concentration	79
5.3	Results	80
5.3.1	Assay for Antibacterial Activity	80
5.3.2	Inhibitory Spectrum	81
5.3.3	Effect of Enzymes and Detergents on Antimicrobial Activity	82
5.3.4	Partial Purification, Antibacterial Activity and Protein Concentration	83
5.4	Discussion	87
5.5	Conclusions	92
<b>6</b>	<b>INFLUENCE OF CULTURE CONDITIONS AND MEDIUM COMPOSITION ON THE GROWTH AND PRODUCTION OF BACTERIOCIN BY <i>Lactobacillus paracasei</i> LA07</b>	93
6.1	Introduction	93
6.2	Materials and Methods	94
6.2.1	Flow of Experimental Design	94
6.2.2	Bacterial Strain and Culture Conditions	95
6.2.3	Fermentation Medium	96
6.2.4	Inoculum Preparation	96



6.2.5 Analytical Procedure	96
6.2.6. Assay for Antibacterial Activity	96
6.2.7 Bacterial Growth, Bacteriocin Production and Determination of Bacteriocin Activity/ Titer	97
6.2.8 Effect of Growth Conditions on Bacteriocin Production	97
6.3 Results	99
6.3.1 Bacterial Growth, Bacteriocin Production and Determination of Bacteriocin Activity/ Titer	99
6.3.2 Growth of <i>Lb. paracasei</i> LA07 and Production of Bacteriocin in Different Incubation Temperature and Growth Media	100
6.3.3 Effect of Different Concentrations of Carbon and Nitrogen Source on Growth of <i>Lb. paracasei</i> LA07 and Production of Bacteriocin	101
6.3.3.1 Effect of Nitrogen Sources	102
6.3.3.2 Effect of Carbon Sources	104
6.3.4 Effect of Initial pH on Growth of <i>Lb. paracasi</i> LA07 and Production of Bacteriocin	104
6.3.5 Effect of Agitation Speed and Microaeration on Growth of <i>Lb. paracasei</i> LA07 and Production of Bacteriocin	105
6.4 Discussion	106
6.5 Conclusions	111
<b>7 CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK</b>	112
BIBLIOGRAPHY	115
BIODATA OF STUDENT	140
LIST OF PUBLICATION	141

## LISTS OF TABLES

Table	Page
2.1 Commercial significance of metabolic products of LAB (Holzapfel <i>et al.</i> , 1995)	5
2.2 Antimicrobial products of low molecular mass (Helaner, <i>et al.</i> , 1997)	13
2.3 Some of the bacteriocin producing microorganisms (Mojgani, 1997)	22
2.4 Classification of bacteriocins (De Vuyst & Vandamme, 1994; Cleveland <i>et al.</i> , 2001)	23
2.5 Bacteriocins vs. antibiotics (Cleveland <i>et al.</i> , 2001)	26
2.6 Bacteriocin produced by lactobacilli	31
2.7 Bacteriocin of <i>Leuconostoc</i> spp.	32
2.8 Bacteriocin of pediococci	33
2.9 Bacteriocin from carnobacteria	34
2.10 Bacteriocin from <i>Lactococcus</i> spp.	35
2.11 Bacteriocin from enteococci	37
2.12 Bacteriocin from streptococci	38
3.1 Morphological, culture and biochemical characteristics of isolates	47
3.2 The biochemical profile of isolates	51
3.3 Species identification results	52
4.1 Susceptibility of isolate LA02 to 24 different types of antibiotics	60
4.2 Susceptibility of isolate LA07 to 24 different types of antibiotics	61
4.3 Susceptibility of isolate LA08 to 24 different types of antibiotics	62
4.4 Susceptibility of isolate LA11 to 24 different types of antibiotics	63
4.5 Susceptibility of isolate LA14 to 24 different types of antibiotics	64
4.6 Susceptibility of isolate LA16 to 24 different types of antibiotics	65

4.7	Susceptibility of isolate LA17 to 24 different types of antibiotics	66
4.8	Susceptibility of isolate LA18 to 24 different types of antibiotics	67
4.9	Susceptibility of isolate LA19 to 24 different types of antibiotics	68
4.10	Susceptibility of isolate LA20 to 24 different types of antibiotics	69
4.11	Susceptibility of isolate LA21 to 24 different types of antibiotics	70
4.12	Susceptibility of isolate LA22 to 24 different types of antibiotics	71
4.13	Susceptibility of isolates to 24 different types of antibiotics	73
5.1	Antimicrobial activity of isolates against closely related bacteria	81
5.2	Inhibitory spectrum of selected on gram-positive and gram-negative bacteria	81
5.3	Physico-chemical stability of cell-free culture supernatant from <i>Lb. paracasei</i> LA07 after incubation for 24 hours at 37°C	83
6.1	Effect of different temperatures and media on growth of <i>Lb. paracasei</i> LA07 and production of bacteriocin	101
6.2	Effect of tryptone as a nitrogen source on growth of <i>Lb. paracasei</i> LA07 and production of bacteriocin	102
6.3	Effect of yeast extract as a nitrogen source on growth of <i>Lb. paracasei</i> LA07 and production of bacteriocin	103
6.4	Effect of meat extract as a nitrogen source on growth of <i>Lb. paracasi</i> LA07 and production of bacteriocin	103
6.5	Effect of glucose as a carbon source on growth of <i>Lb. paracasei</i> LA07 and production of bacteriocin production	104
6.6	Effect of initial pH on growth of <i>Lb. paracasei</i> LA07 and production of bacteriocin	105
6.7	Effect of agitation speed and microaeration on growth of <i>Lb. paracasei</i> LA07 and production of bacteriocin	106

## LIST OF FIGURES

Figure		Page
2.1	Metabolic pathways in Lactic Acid Bacteria (Danone world newsletter, 1994)	12
2.2	General pathway by which acetoin and diacetyl are produced from citrate by group N lactococci and <i>Leuconostoc</i> spp.(Jay <i>et al.</i> , 2005)	14
2.3	Overview of the different metabolic pathway in LAB that could lead to acetaldehyde formation and Acetyl CoA (Chaves <i>et al.</i> , 2002)	16
3.1	Flow diagram of the experimental work	42
3.2	Percentage of species isolated from Budu	52
5.1	Antimicrobial activity of isolates against <i>L. monocytogenes</i> by agar well diffusion method	82
5.2	SDS-PAGE (15% gel) of the partially purified antimicrobial substance	84
5.3	SDS-PAGE (20% gel) of the partially purified antimicrobial substance	84
5.4	Antibacterial activity of the partially purified antimicrobial substance on MRS agar plate	85
5.5	Antibacterial activity of the partially purified antimicrobial substance on MRS agar plate	86
6.1	Flow diagram of the experimental work	95
6.2	Typical time course of bacteriocins fermentation by <i>Lb. paracasei</i> LA07 (Symbols represent: ( □ ) cell density; ( ○ ) bacteriocin concentration; (-.-) culture pH).	100

## LIST OF ABBREVIATIONS

Acetyl CoA	Acetyl Coenzyme A
AK	amikacin
AMP	ampicillin
APT	All purpose tween
ATCC	American Type Culture Collection
ATP	Adenosine Triphosphate
AU	Arbitrary Unit
B	bacitracin
BHI	Brain Heart Infusion
BSA	Bovine Serum Albumin
°C	Degree centigrade
C	chloramphenicol
CFU	Colony Forming Unit
CH <sub>3</sub> CHO	Acetaldehyde
OB	cloxacillin
CIP	ciprofloxacin
CO <sub>2</sub>	Carbon dioxide
CN	gentamycin
CRO	ceftriaxone
CT	colestin sulphate
CXM	cefuroxime sodium
Da	Dalton, Identically the atomic mass unit, used mainly for biochemical molecular weights



DNA	Deoxyribonucleic acid
E	erythromycin
<i>E</i>	<i>Enterococcus</i> spp.
EDTA	Ethylenediaminetetraacetic acid
EMP pathway	Embden-Meyerhof-Parnas pathway
F	nitrofurantion
g/l	Gram per liter
GAP	Glyceraldehyde phosphate
GRAS	Generally regarded as safe
H	Hour
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
HCl	Hydrochloride acid
HMM	High-molecular-mass
I	Intermediate
K	kanamycin
kDa	Kilo Dalton
LAB	Lactic Acid Bacteria
<i>L</i>	<i>Lactococcus</i> spp.
<i>Lb</i>	<i>Lactobacillus</i> spp.
<i>Lc</i>	<i>Leuconostoc</i> spp.
LDH	lactate dehydrogenase
LMM	low-molecular-mass
MEL	mecillinam
µg	Microgram, 10 <sup>-6</sup> of a gram
Min	Minutes

ml	Milliliter, one thousandth ( $10^{-3}$ ) of a liter
$\mu$ l	Microliter, one-millionth ( $10^{-6}$ ) of a liter
Mm	Milimeter, one thousandth ( $10^{-3}$ ) of a meter
MRS	De Man, Rogosa and Sharpe
MY	lincomycin
N	neomycin
NA	nalidixic acid
NAD (NADH)	Nicotinamide adenine dinucleotide, A coenzyme, $C_{21}H_{27}N_7O_{14}P_2$ , occurring in most living cells and utilized alternately with NADH as an oxidizing or reducing agent in various metabolic processes.
NaOH	Sodium Hydroxide
NB	Nutrient Broth
Nm	nanometer, $10^{-9}$ m
NOR	norfloxacin
NV	novobiocin
OD	Optical Density
P2	penicillin G
PAGE	Poly Acrylamide Gel Electrophoresis
pH	The Power of Hydrogen
$pK_a$	Acid dissociation constants are also known as the acidity constant or the acid-ionization constant. The term is also used for $pK_a$ , which is equal to minus the decimal logarithm of $K_a$ (cologarithm of $K_a$ )
ppm	The parts of a substance per million parts (by weight) of a solution; equal to 0.0001%
R	Resistant

rRNA	Ribosomal RNA (The RNA that is a permanent structural part of a ribosome, Ribosomal RNA accounts for nearly 80% of the RNA content of the bacterial cell).
RPM	Revolution per Minute
S	Susceptible
S	streptomycin
SDS	Sodium Dodecyl Sulphate
SXT	Sulphanethoxazole/Trimethoprim
TE	tetracycline
TSB	Tryptic Soy Broth
v/v	Volume per volume
VA	vancomycin
w/v	Weight per volume



## CHAPTER 1

### INTRODUCTION

Antagonistic substances are vital factors in microbial ecology. Among the many different substances known to play a role in bacterial interactions, bacteriocins are the most specific and efficient antagonist (Iqbal, 1998). Bacteriocins are antibacterial proteins or protein complexes produced by several gram-positive and gram-negative bacteria.

Although bacteriocins are produced by many gram-positive and gram-negative species, those produced by lactic acid bacteria (LAB) are of particular importance to the food industry (Nettles & Barefoot, 1993). These bacteria have generally been regarded as safe (GRAS status) and as the majority of bacteriocin-producing LAB are natural food isolates, therefore they are ideally suited to food applications. Thus, the production of bacteriocins by LAB is not only advantageous to the bacteria themselves but also to the food industry. When utilised as a tool to control the growth of undesirable bacteria it is directed towards making the food product more acceptable to consumers (Deegan *et al.*, 2006).

The demand by consumers for a decrease in the use of chemical additives in food has led to the use of natural antimicrobial substances secreted by food fermentation bacteria to inhibit the growth of undesirable microorganisms (Berry *et al.*, 1991; Schillinger *et al.*, 1991). The use of LAB as starter cultures in fermented food industry has become widespread. Among the beneficial properties of LAB are the production of organic acids, hydrogen peroxide, and bacteriocins which are

