



Faculty of science
Ain-Shams University

**Improvement and Purification of Bacteriocin
Production as an antimicrobial agent from *Lactobacillus
acidophilus* and *Lactobacillus plantarum***

Thesis

Submitted for the Ph.D. degree in microbiology

By

Hayam Abdelnabi Sayed
(Master Microbiology, 2011)

Supervisors

Prof. Hala M. Abu-Shady
Professor of bacteriology
Microbiology department
Faculty of science
Ain-shams University.

Prof. Kamal M. A. Khalil
Professor of genetic
Gen. and cyto. Dep.,
Gen. eng. and Biot. Div.
National research center

Dr. Arwa Hassan Soliman
Lecturer of microbiology
Microbiology department
Faculty of science
Ain-shams University.

Dr. Wafaa Farouk Mohamed
Fellow of microbiology
Ain-shams University
Special hospital

**Department of Microbiology
Faculty of science
Ain shams University
2016**

Approval sheet

Improvement and Purification of Bacteriocin Production as an antimicrobial agent from *Lactobacillus acidophilus* and *Lactobacillus plantarum*

Degree: Ph.D in Microbiology

Name of student: Hayam Abdelnabi Sayed

This thesis for Ph. D. Degree has been approved by:

- (1)
- (2)
- (3)
- (4)

Date of examination: / / 2016

ACKNOWLEDGEMENT

First and foremost, I feel always indebted to Allah, the most beneficent and merciful. I can do nothing without Him

I would like to express my deep gratitude and thanks to my dear supervisors for their help, encouragement, continuous advice and their expert supervision to bring this thesis to more than satisfactory finish. They were always patient, perfect in work organization and the best advisors.

Great thanks to Microbiology Department and all my Colleagues in for their assistance and support.

Deep thanks to my whole family especially my dear mother and dear father for everything they have done for me. AT last Very special and great thanks to my small family, my dear husband for his patience and encouragement; and the apple of my eyes my sweet children for their love.

CONTENTS

Subject	Pages
Abstract	1
Aim of work	3
Introduction	4
Literature Review	7
1-Lactic acid bacteria	7
2-Probiotics	8
2.1. Mode of action of probiotics	8
2.1.1. Competition for adhesion sites	8
2.1.2. Production of inhibitory compounds	9
2.1.2. a. Organic acids	9
2.1.2. b. Hydrogen peroxide	9
2.1.2. c. Bacteriocins	10
2.1.2. d. Other inhibitory compounds	10
3. Bacteriocins	
3.1. Historical account on bacteriocin	11
3.2. Definition of bacteriocin	11
3.3. Naming of bacteriocins	12
3.4. Physical and Chemical Properties and Classification of Bacteriocins Produced by LAB	12
3.4.1. Lantibiotics (Class I)	13
3.4.2. Class II: the Non-Lantibiotics	14
3.4.3. Class III: Bacteriocins	14
3.5. Biosynthesis of LAB Bacteriocins	17
3.6. Immunity of LAB Bacteriocins	17
3.7. Factors affecting the bacteriocin production	18
3.7.1. Microbial strain	18
3.7.2. Media	19
3.7.3. Effect of fermentation conditions	20
3.8. Bacteriocin detection and measurements	20
3.9. Purification of bacteriocins	21

3.10. Range of activity of bacteriocins	22
3.11. Mode of Action	23
3.12. Bacteriocins and Antibiotics	24
4. Some Applications of lactic acid bacteria (probiotics and bacteriocins)	25
4.1. Probiotics and health care	26
4.2. Bacteriocins and plants	27
4.3. Application of probiotics in food industry	28
4.4. Application of probiotics in aquaculture	29
Materials and methods	
1. Bacterial strains and growth media	30
2. Bacterial strains activation, Bacteriocin bioassay and activity range determination	30
3. Optimization of different growth conditions for the maximum bacteriocin production by <i>Lactobacillus acidophilus</i> and <i>Lactobacillus plantarum</i>	32
3.1. Effect of the growth medium initial pH on the bacteriocin production	32
3.2. Effect of the growth temperature on the production of the bacteriocin	32
3.3. Effect of Inoculum size	33
3.4. a. Effect of the carbon- source on the bacteriocin Production	33
3.4.b. Effect of different concentrations of the best carbon-source on bacteriocin production	33
3.5. Effect of nitrogen- source on the bacteriocin Production	33
3.6. Effect of the tween amount on the bacteriocin Production	34
3.7. Effect of the amount of different minerals (K_2HPO_4 , $MgSO_4$, $MnSO_4$, Tri-ammonium citrate and sodium acetate) on the bacteriocin production	34

3.8. Effect of the best conditions together	34
4. Mutation technique and mutant selection	35
5. Studies on crude bacteriocin	
5.1. Effect of temperature on the activity of the crude bacteriocin	36
5.2. Effect of pH on the activity of the crude bacteriocin	36
5.3. Effect of enzymes on crud bacteriocin	36
6-Bacteriocin purification	37
Microbiological media	39
Results	46
Discussion	96
English summary	109
References	122
Arabic summary	

LIST OF FIGURES

Fig. No.	Title	Page
Fig.(1):	showing the possible modes of action of bacteriocins	24
Fig. (2):	Effect of different fermentation broth amounts from (A) <i>Lactobacillus acidophilus</i> ATCC 4356 and (B) <i>Lactobacillus plantarum</i> ATCC 8014 against MRSA, (one of the selected tester organisms for this study).	48
Fig. (3):	Effect of different fermentation broth amounts from (1) the wild <i>Lactbacillus acidophilus</i> ATCC 4356 and (2) the best selected mutant (AM-UV5-12) on MRSA for best mutant selection from <i>Lactobacillus acidophilus</i> ATCC 4356	67
Fig. (4):	Effect of different fermentation broth amounts from (1) the wild strain <i>Lactobacillus plantarum</i> ATCC 8014 and (2) its best selected mutant (PM-UV3-4) with MRSA	70
Fig. (5):	Effect of autoclaving on activity of crude bacteriocins produced by (A) <i>Lactobacillus acidophilus</i> ATCC 4356, (B) its selected mutant (AM-UV5-12) , (C) <i>Lactobacillus plantarum</i> ATCC 8014 and (D) its mutant (PM-UV3-4); against (1) MRSA and (2) <i>Pseudomonas sp.</i> as testers.	89

LIST OF GRAPHS

Graph No.	Title	Page
Graph (1):	Effect of growth medium initial pH on bacteriocin production by (A) <i>Lactobacillus acidophilus</i> ATCC 4356 and (B) <i>Lactobacillus plantarum</i> ATCC 8014 against MRSA and <i>Pseudomonas sp.</i> as testers.	50
Graph (2)	Effect of growth temperature on bacteriocin production by (A) <i>Lactobacillus acidophilus</i> ATCC 4356 and (B) <i>Lactobacillus plantarum</i> ATCC 8014 against MRSA and <i>Pseudomonas sp.</i> as testers.	51
Graph (3):	Effect of different concentrations of best selected carbon- source (galactose) for <i>Lactobacillus acidophilus</i> ATCC 4356 compared with glucose against MRSA and <i>Pseudomonas sp.</i> as testers.	55
Graph (4):	Effect of different concentrations of best carbon-source (maltose) compared with glucose for <i>Lactobacillus plantarum</i> ATCC 8014 against MRSA and <i>Pseudomonas sp.</i> as testers.	56
Graph (5):	Effect of different tween concentrations on bacteriocin production by (A) <i>Lactobacillus acidophilus</i> ATCC 4356 and (B) <i>Lactobacillus plantarum</i> ATCC 8014 against MRSA and <i>Pseudomonas sp.</i> as testers.	60

LIST OF GRAPHS

Graph No.	Title	Page
Graph (6)	Effect of different minerals concentrations on bacteriocin production from by <i>Lactobacillus plantarum</i> ATCC 8014 against MRSA and <i>Pseudomonas sp.</i> as testers	62
Graph (7):	Effect of different minerals concentrations on bacteriocin production by <i>Lactobacillus acidophilus</i> ATCC 4356 against MRSA and <i>Pseudomonas sp.</i> as testers.	63
Graph (8):	Effect of best growth conditions together on bacteriocin production by (A) <i>Lactobacillus acidophilus</i> ATCC 4356 and (B) <i>Lactobacillus plantarum</i> ATCC 8014 against MRSA and <i>Pseudomonas sp.</i> as tester bacteria.	65
Graph (9):	Effect of growth medium initial pH on bacteriocin production from (A) the mutant (AM-UV5-12) and (B) the mutants (PM-UV3-4) against MRSA and <i>Pseudomonas sp.</i> as testers.	71
Graph (10)	Effect of growth Temperature on bacteriocin production by the mutants (A) (AM-UV5-12) and (B) (PM-UV3-4) against MRSA and <i>Pseudomonas sp.</i> as testers.	72
Graph (11):	Effect of different C-sources on bacteriocin production by selected mutant (AM-UV5-12) against MRSA and <i>Pseudomonas sp.</i> as tester bacteria.	74
Graph (12)	Effect of different carbon- sources on bacteriocin produced by the selected mutant (PM-UV3-4) against MRSA and <i>Pseudomonas sp.</i> as testers.	75

LIST OF GRAPHS

Graph No.	Title	Page
Graph (13):	Effect of different tween concentrations on bacteriocin production by (A) the mutant (AM-UV5-12) and (B) the mutant (PM-UV3-4) against MRSA and <i>Pseudomonas sp.</i> as testers.	81
Graph (14):	Effect of different minerals concentrations on bacteriocin production by the selected mutant (AM-UV5-12) against MRSA and <i>Pseudomonas sp.</i> as testers.	83
Graph (15):	Effect of different minerals concentrations on bacteriocin production by the mutant (PM-UV3-4) against MRSA and <i>Pseudomonas sp.</i> as testers.	84
Graph (16)	Effect of best growth conditions together on bacteriocin production by (A) the mutant (AM-UV5-12) and (A) the mutant (PM-UV3-4) against MRSA and <i>Pseudomonas sp.</i> as testers.	85
Graph (17):	Effect of different heat treatments on the crud bacteriocin activity produced by the parent strain <i>Lactobacillus acidophilus</i> ATCC 4356 and its mutant (AM-UV5-12) and the parent strain <i>Lactobacillus plantarum</i> ATCC 8014 and its mutant (PM-UV3-4) against MRSA and <i>Pseudomonas sp.</i> as testers.	88
Graph (18):	Effect of autoclaving on the crud bacteriocin produced by (A) the parent strain <i>Lactobacillus acidophilus</i> ATCC 4356 and its mutant (AM-UV5-12) (B) the parent strain <i>Lactobacillus plantarum</i> ATCC 8014 and its mutant (PM-UV3-4) against MRSA and <i>Pseudomonas sp.</i> as testers.	89

LIST OF GRAPHS

Graph No.	Title	Page
Graph (19):	Effect of pH on activity of crude bacterocins produced by (A) <i>Lactobacillus acidophilus</i> ATCC 4356 and its selected mutant (AM-UV5-12) and (B) <i>Lactobacillus plantarum</i> ATCC 8014 and its selected mutant (PM-UV3-4) against (1) MRSA and (2) <i>Pseudomonas sp.</i> as testers.	92

LIST OF TABLES

Table No.	Title	Page
Table (1):	Comprehensive classification system for bacteriocin from Cotter <i>et al.</i> , 2013.	16
Table (2)	Bacterial strains used in this study.	31
Table (3):	Antibacterial activity of (A) <i>Lactobacillus acidophilus</i> ATCC 4356 and (B) <i>Lactobacillus plantarum</i> ATCC 8014 using eight different tester strains.	48
Tables (4):	Effect of inoculum size on bacteriocin production by (A) <i>Lactobacillus acidophilus</i> ATCC 4356 and (B) <i>Lactobacillus plantarum</i> ATCC 8014 against MRSA and <i>Pseudomonas sp.</i> as testers.	52
Tables (5):	Effect of different carbon- sources on bacteriocin production from <i>Lactobacillus acidophilus</i> ATCC 4356 against MRSA and <i>Pseudomonas sp.</i> as testers.	53
Tables (6):	Effect of different carbon-sources on bacteriocin produced by <i>Lactobacillus plantarum</i> ATCC 8014 against MRSA and <i>Pseudomonas sp.</i> as testers.	54
Table (7):	Effect of different N-sources on bacteriocin production from <i>Lactobacillus acidophilus</i> ATCC 4356 against MRSA and <i>Pseudomonas sp.</i> as testers.	58

LIST OF TABLES

Table	Title	Page
Table (8):	Effect of different nitrogen-sources on bacteriocin production from <i>Lactobacillus plantarum</i> ATCC 8014 against MRSA and <i>Pseudomonas sp.</i> as testers	59
Table (9)	Inhibition zones diameters of selected mutants isolates after UV- treatment of <i>Lactobacillus acidophilus</i> ATCC 4356.	67
Table (10)	Inhibition zones diameters of selected mutants isolates after UV- treatment <i>Lactobacillus plantarum</i> ATCC 8014.	70
Tables (11)	Effect of inoculum size on bacteriocin production by (A) the mutant (AM-UV5-12) and (B) the mutant (PM-UV3-4) against MRSA and <i>Pseudomonas sp.</i> as testers.	73
Tables (12)	Effect of different concentrations of best carbon- source (maltose); on bacteriocin production by the mutant (AM-UV5-12) compared with glucose against MRSA and <i>Pseudomonas sp.</i> as testers.	76
Tables (13):	Effect of different concentrations of best C-source (sucrose) on bacteriocin production by the mutant (PM-UV3-4) compared with glucose against MRSA and <i>Pseudomonas sp.</i> as testers.	77
Table (14):	Effect of different N-sources on bacteriocin production by the mutant (AM-UV5-12) against MRSA and <i>Pseudomonas sp.</i> as testers..	79

LIST OF TABLES

Table No	Title	Page
Table (15):	Effect of different nitrogen- sources on bacteriocin production by the selected mutant (PM-UV3-4) against MRSA and <i>Pseudomonas sp.</i> as testers	80
Table (16):	Effect of catalase, protease k and trypsin enzymes on the activity of the crude bacteriocins produced by (A) <i>Lactobacillus acidophilus</i> ATCC 4356 and its selected mutant (AM-UV5-12) and (B) <i>Lactobacillus plantarum</i> ATCC 8014 and its selected mutant (PM-UV3-4) against (1) MRSA and (2) <i>Pseudomonas sp.</i> as testers.	93

ABBREVIATIONS

ABC-transporter system	ATP-Binding Cassette transporters
ATCC	American Type Culture Collection
AMPs	Antimicrobial peptides
c.f.s.	Cell free supernatant
Da., K. Da.	Dalton, Kilo Dalton (units of protein measurement)
ELISA	Enzyme Linked Immune-Sorbent Assay
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration Organization
GIT	Gastro Intestinal Tract
GRAS	Generally Recognized as Safe.
G⁻	Gram negative
G⁺	Gram positive
LAB	Lactic Acid Bacteria
MRSA	Methicillin Resistant <i>Staphylococcus aureus</i>
MRS	de Man Rogosa and Sharpe (name of the medium inventor)
pH	Hydrogen ion activation.
PMF	The Proton Motive Force
SOS function	Save our selves
WHO	World Health Organization