

**LACTIC ACID BACTERIA AS A PROBIOTIC  
FOR IMPROVING HUMAN HEALTH**

**By**

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**B. Sc. Agric. Sci., (Microbiology), Fac. Agric., Ain Shams Univ., 2004.**

**THESIS**

**Submitted in Partial Fulfillment of the  
Requirements for the Degree of**

**MASTER OF SCIENCE**

**In**

**Agriculture Sciences  
(Agriculture Microbiology)**

**Department of Agriculture Microbiology  
Faculty of Agriculture  
Cairo University  
EGYPT**

**2019**

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### **ABSTRACT**

Probiotics are live microbial food supplements that can be considered a functional food. They benefit the health of a host animal by maintaining their intestinal microbial balance. Colon cancer is the fourth most common cause of cancer-related mortality in the world. The aim of this study was to investigate the anti-proliferative effects of the cell-free filtrate of 12 isolates of lactic acid bacteria depending on different inhibitory effect.

The twelve isolates were selective from 150 isolates isolated from dairy product. The isolates selected were tested on human HCT116 cell line (colon cancer). The filtrates of LAB isolates were found to inhibit the growth of colon cancer cells in a dose-dependent manner as detected by the neutral red uptake assay for the estimation of cell viability/cytotoxicity protocol. The isolates gave different inhibitory effect. The isolate No.8 gave full inhibition of colon cancer viability and defined by API test as *Lactobacillus fermentum*.

**Key word:** Colon cancer, Lactic acid bacteria, *Lactobacillus*, Probiotic bacteria.

## DIDICATION

*In the name of Allah, the most gracious, the Most Merciful, all praise is to Allah, the lord of the world; prayers and peace be upon Prophet Mohammed His servant and messenger.*

*I dedicate this work to my dear parents, my dear brother, sister and my dear friends for all support they lovely offered during my post-graduate studies and all my life. I will always appreciate all they have done for me.*

## **ACKNOWLEDGEMENT**

*First of all, Alhamdulillah, all praise is to Allah, the Lord of the world. HE the Almighty gave me strength to complete this work and made me lucky to be supervised by kind and helpful supervisors. I wish to express my sincere thanks, deepest gratitude and appreciation to **Dr. Mohammed Zakaria Sedik**, Professor of Agricultural Microbiology and **Dr. Sayed Abd-Elkader** Professor of Agricultural Chemistry, Faculty of Agriculture Cairo University and **Dr. Hoaida El-Shazly** Professor of Food Science, Agricultural Research Center. Grateful appreciation is also extended to all staff members of the Agricultural Microbiology Department, Faculty of Agriculture, Cairo University.*

*Special deep appreciation is given to my Father, my mother, my brother and my sister. My sincere thanks are given to my friends for their help.*

## LIST OF ABBREVIATION

<b>ACF</b>	Aberrant crypt foci
<b>AFLP</b>	Amplified fragment length polymorphism
<b>API</b>	Application program interface
<b>ARDRA</b>	Amplified ribosomal DNA restriction analysis
<b>ATCC</b>	American type culture collection
<b>°C</b>	Degree Celsius
<b>C fatty acids</b>	Cyclopropane ring fatty acid
<b>cfu</b>	Colony forming unite
<b>CHL</b>	Carbo- hydrates
<b>CLAs</b>	Conjugated linoleic acids
<b>cm</b>	Centimeter
<b>CRC</b>	Colorectal cancer
<b>DGGE</b>	Denaturing gradient gel electrophoresis
<b>DMEA</b>	Dulbecco's Modified Eagle Medium
<b>DNA</b>	Deoxyribonucleic acid
<b>FAO</b>	Food and Agriculture Organization
<b>GI</b>	Gasterotenital
<b>GST</b>	Glutathione-S-transferase
<b>H</b>	Hour
<b>H1N1</b>	Hemagglutinin Type 1 and Neuraminidase Type 1 (influenza strain; aka swine flu)
<b>HCT</b>	Human clororectal carcinoma cel line
<b>HIV</b>	Human immunodeficiency virus
<b>HT-29</b>	Human colorectal adenocarcinoma cell line
<b>INF</b>	Interferon
<b>IQ</b>	Quinoline
<b>LAB</b>	Lactic acid bacteria
<b>LcS</b>	<i>Lactobacillus casei Shirota</i>
<b>LPS</b>	Lipopolysaccharide
<b>Lys</b>	Lysine
<b>M</b>	Moderately susceptibility
<b>MCF7</b>	Breast Carcinoma Cell Line
<b>MeIQ</b>	2-amino-3,4-dimethylimidazo [4,5-f] quinolone
<b>µg</b>	Microgram
<b>MHIQ</b>	2-amino-3-methyl-3H-imidazo [4,5-f] quinolone
<b>µl</b>	Microliter
<b>ml</b>	Milliliter
<b>µm</b>	Micrometer
<b>MNNG</b>	N-methyl-N9- nitro-N-nitrosoguanidine
<b>MRS</b>	De Man, Rogosa and Sharpe
<b>NADPH</b>	Nicotinamide adenine dinucleotide phosphate
<b>NF-KB</b>	Nuclear factor kappa-light-chain-enhancer of activated B cells

<b>NK</b>	Natural killer cell
<b>Nm</b>	Nanometer
<b>Ornlys</b>	Ornithine
<b>PBS</b>	Phosphate buffer solution
<b>PCR</b>	Polymerase chain reaction
<b>PFGE</b>	Plasmid profiling and pulsed field gel electrophoresis
<b>pH</b>	Hydrogen ion concentration
<b>PhMIP</b>	5-phenyl-2-amino-1-methylimidazo [4,5-f] pyridine
<b>PPAR</b>	Peroxisome proliferator-activated receptors
<b>R</b>	Resistant
<b>RAPD</b>	Randomly amplified polymorphic DNA
<b>REA</b>	Restriction enzyme assay
<b>REP-PCR</b>	Repeated sequence extragenic palindromic PCR
<b>RNA</b>	Ribonucleic acid
<b>ROS</b>	Reactive oxygen species
<b>Rpm</b>	Round per minute
<b>S</b>	Susceptible
<b>S fatty acids</b>	Straight-chain saturated
<b>SCFAs</b>	Short chain fatty acids
<b>SL</b>	Selective <i>lactobacillus</i> agar
<b>SSCP</b>	Single strand conformation polymorphism
<b>TLRs</b>	Toll-like receptors
<b>TNF</b>	Tumor Necrosis Factor
<b>U fatty acids</b>	Monosaturated
<b>WHO</b>	World Health Organization

# CONTENTS

	Page
<b>INTRODUCTION</b> .....	1
<b>REVIEW OF LITERATURE</b> .....	3
Probiotic definition.....	3
<b>1. Probiotic bacteria</b> .....	4
<b>2. Probiotic for human health</b> .....	5
<b>3. Probiotic strains</b> .....	6
a. <i>Lactobacillus</i> .....	6
b. Isolation of probiotic lactobacilli.....	7
c. Identify probiotic species of the genus <i>Lactobacillus</i> by.... API50 CHL.....	8
d. Molecular methods for identification of lactobacilli.....	10
<b>4. Colorectal cancer (CRC)</b> .....	11
a. Probiotics in controlling cancer.....	12
<b>5. Lactic acid bacteria and cancer: Mechanistic perspective mechanisms by which lactic acid bacteria may be inhibiting colon cancer</b> .....	15
a. Alteration of the metabolic activities of intestinal microflora.....	16
b. Alteration of physico-chemical conditions in the colon.....	17
c. Binding and degrading potential carcinogens.....	18
d. Quantitative and/or qualitative alterations in the intestinal microflora.....	20
e. Production of antitumourigenic or antimutagenic compounds.....	20
f. Enhancing the host's immune response.....	21
g. Effects on the physiology of the host.....	23
<b>6. Probiotics in colorectal cancer (CRC) with emphasis on mechanisms of action and current perspectives</b> .....	24
a. Effects on the gut epithelial barrier.....	24
b. Effects on the gut physico-chemical conditions.....	26
c. Effect of probiotics on metabolic and carcinogenic.....	27
1. Activity of bacterial enzymes in CRC.....	27

	Page
2. Inhibition of harmful enzymatic activity.....	28
3. Removal of carcinogenic products by probiotics carcinogenic compounds.....	29
4. Binding of carcinogens.....	30
5. Inactivation of carcinogens.....	30
6. Anti-carcinogenic and antioxidant metabolites.....	31
7. Probiotics favorably modulate the host immune response to reduce CRC risk.....	32
<b>MATERIAL AND METHODS.....</b>	<b>35</b>
<b>1. MATERIAL.....</b>	<b>36</b>
<b>a. Sampling.....</b>	<b>36</b>
<b>b. Microorganisms.....</b>	<b>36</b>
1. Lactic acid bacteria .....	36
2. Pathogenic microorganisms .....	36
<b>c. Media used.....</b>	<b>36</b>
1. Rogosa agar (SL agar) .....	36
2. MRS agar .....	37
3. The API 50 CHL Medium .....	38
4. Nutrient agar medium .....	38
5. Baird-Parker agar medium .....	38
6. MacConkey broth medium .....	39
7. Simmons citrate agar medium .....	39
8. Glucose broth medium .....	40
9. Trypticase nitrate broth (Indol nitrate medium) .....	40
10. Bile esculin agar medium .....	40
11. Arginine hydrolase broth medium .....	41
12. Mueller Hinton Agar medium (MHA) .....	41
13. Material for Neutral red uptake assay for the estimation of cell viability/cytotoxicity protocol .....	41
<b>2. Methods.....</b>	<b>42</b>
<b>a. Microbiological determinations.....</b>	<b>42</b>
<b>1. Sample preparation.....</b>	<b>42</b>
<b>2. Identification of selected lactobacilli.....</b>	<b>43</b>
<b>3. Microscopic examination.....</b>	<b>43</b>
<b>4. Physiological characterization of isolates.....</b>	<b>43</b>
<b>5. Biochemical characterization of isolates.....</b>	<b>44</b>
<b>6. Assessment of antimicrobial activity of isolates...</b>	<b>45</b>
<b>7. Anticarcinogenic activity of isolates.....</b>	<b>46</b>

8. Identification by API Kits.....	48
9. Molecular method.....	51
10. Analysis of growth and viability under varied pH.....	53
11. Antibiotics susceptibility testing.....	54
<b>RESULT AND DISCUSSION.....</b>	<b>57</b>
1. Isolation of lactic acid bacteria isolated from dairy product.....	57
2. Identification and characterization of isolates.....	58
3. Physiological Characterization.....	60
4. Biochemical characterization of isolates.....	63
5. Assessment of antimicrobial activity of lactobacilli isolates..	65
6. Anticarcinogenic activity of pure isolates.....	68
7. Classification of some bacterial isolates.....	73
8. Molecular identification.....	75
9. Effect of pH on <i>L. fermentum</i> (Ls8) growth and viability.....	77
10. Susceptibility of <i>L. fermentum</i> (Ls8) to antibiotic.....	78
<b>SUMMARY.....</b>	<b>81</b>
<b>REFERENCES.....</b>	<b>85</b>
<b>ARABIC SUMMARY.....</b>	

## LIST OF TABLES

No.	Title	Page
1.	The sequences of oligonucleotide primers used for PCR	52
2.	Amplification program used in PCR reaction .....	53
3.	The standards zones of inhibition for specific antibiotics	55
4.	Colonial morphology of the twelve selected isolates .....	58
5.	Cell morphology of the pure isolated.....	59
6.	Physiological characterization of the pure isolates .....	62
7.	The biochemical characterization of the pure isolates ...	64
8.	Assessment of antimicrobial activity of LAB isolates against pathogenic bacterial strains by inhibition zone in mm .....	65
9.	Viability percent of each isolates .....	68
10.	Classification of isolate No.8 by API 50 CHL test .....	74
11.	Susceptibility of <i>L.fermentum</i> LS 8 to antibiotics .....	80

## LIST OF FIGURES

<b>No.</b>	<b>Title</b>	<b>Page</b>
1.	Steps of research diagram. ....	35
2.	Assessment of antimicrobial activity of isolates against pathogenic bacteria .....	67
3.	The viability percent of twelve pure isolates against HCT 116 cell line.....	68
4.	Anticarcinogenic activity of isolates .....	72
5.	API test for the isolate Ls 8.....	73
6.	Agarose gel electrophoresis of PCR products .....	75

## INTRODUCTION

Probiotics 'live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance. Probiotics usually refer to highly selected lactic acid bacteria (LAB), e.g. *Lactobacillus* sp., *Bifidobacterium* sp. and *Streptococcus* sp. with defined gut survival properties and associated biological activities and which can be ingested in fermented milk products or as a supplement. The list of healthful effects attributed to probiotic bacteria is extensive and includes: alleviation of lactose intolerance symptoms, serum cholesterol reduction, anticancer effects, alleviation of constipation and relief of vaginitis. While, prebiotics (non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, that have the potential to improve host health and symbiotic (combinations of pro- and prebiotics), (Salminen *et al.*, 1998).

Lactobacilli are important for the maintenance of the intestinal microbial ecosystem. Colonization of the gut with lactobacilli starts within the first week of life. The presence of this group of bacteria in the gut is considered to have several potential benefits such as growth enhancement of farm animals, protection from pathogens, alleviation of lactose intolerance, relief of constipation, anti-cholesterolaemic effect and immunostimulation (Ehlers and Kaufmann, 2010).

Lactobacilli exert their protective or therapeutic effect through production of antimicrobial compounds (Dodd and Gasson, 1994), reduction of gut pH by stimulating the lactic acid producing microflora (Langhendries *et al.*,1995), competition for binding of receptor sites

that pathogens occupy (Kailasapathy and Chin, 2000), stimulation of immunomodulatory cells and competition with pathogens for available nutrients (Rolfe, 2000).

The precise mechanisms by which LAB may inhibit colon cancer are presently unknown. However, mechanisms may include: alteration of the metabolic activities of intestinal microflora, alteration of physico-chemical conditions in the colon, binding and degrading potential carcinogens, quantitative and/or qualitative alterations in the intestinal microflora incriminated in producing putative carcinogen (s) and promoters (*e.g.* bile acid-metabolising bacteria), production of antitumourigenic or antimutagenic compounds, enhancing the host's immune response and effects on physiology of the host (Le Leu *et al.*, 2010).

Many foreign compounds are detoxified by glucuronide formation in the liver before entering the intestine via the bile. The bacterial enzyme  $\beta$ -glucuronidase has the ability to hydrolyze many glucuronides due to its wide substrate specificity, and thus may liberate carcinogenic glycines in the intestinal lumen. Several other bacterial enzymes have also been suggested to be implicated in the carcinogenic process, releasing carcinogens in the intestinal tract.

So that, the present study concerned with isolation, purification and identification of lactic acid bacteria which improving human health by antagonistic activities against pathogenic microorganisms (*Staphylococcus aureus*, *Escherichia coli* and *Bacillus cereus*) as well as studying the effect of these isolates on colon cancer (HCT116 cell line).