GENETIC CONSTRUCTION OF NEW STRAINS OF LACTIC ACID BACTERIA

BY

RASHA GOMMA SAID SALIM

B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2001
M.Sc. Agric. Sci. (Genetics), Fac. Agric., Cairo Univ., 2009

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APPROVAL SHEET

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APPROVAL COMMITTEE

Dr. SAMIR ABDEL AZIZ IBRAHIM.....
Professor of Genetics, Fac. Agric., Ain Shams University

Dr. HAGGAG SALAH ZAIN MOSTAFA.....
Associate Professor of Genetics, Fac. Agric., Cairo University

Dr. NIVIEN ABDEL RAHMAN ABOSEREH.....
Researcher Professor of Genetics, National Research Center

Dr. ABDELHADI ABDALLAH ABDELHADI.....
Associate Professor of Genetics, Fac. Agric., Cairo University

Date: / / 2016

SUPERVISION SHEET

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SUPERVISION COMMITTEE

Dr. AHAMED NAGIB El-Sayed Sharaf (late)
Professor of Genetics, Fac. Agric., Cairo University

Dr. ABDELHADI ABDALLAH ABDELHADI
Associate Professor of Genetics, Fac. Agric., Cairo University

Dr. NAGWA IBRAHIM ABDELFATTAH ELARABI
Associate Professor of Genetics, Fac. Agric., Cairo University

Dr. NIVIEN ABDEL RAHMAN ABOSEREH
Researcher Professor of Genetics, National Research Center

Name of Candidate Rasha Gomaa Said Salim **Degree**: Ph.D.
Title of Thesis: Genetic Construction of New Strains of Lactic Acid Bacteria
Supervisors: Late Prof. Dr. Ahmed Nagib El-Sayed Sharaf
Dr. Abdelhadi Abdallah Abdelhadi
Dr. Nagwa Ibrahim Abdelfattah Elarabi
Prof. Dr. Nivien Abdel Rahman Abosereh
Department: Genetics **Approval**: / / 2016

ABSTRACT

Ten local isolates of lactic acid bacteria (LAB) able to produce bacteriocin were purified from traditional dairy products and then identified morphologically. These isolates were tested for activity of bacteriocin production against a wide range of food spoilage pathogens and termed as I_1 to I_{10}. Three different isolates I_1, I_2 and I_3 were selected as the best bacterial isolates that gave a high activity of the bacteriocin production. The morphological, biochemical and molecular identifications based on (*16s rRNA* gene). It characterized I_1 as *E. faecium*, I_2 as *E. faecium* and I_3 as *Pediococcus pentosaceus*. The sequences of these isolates were deposited in GenBank database under accession numbers LC063691, LC063692 and LC063861. The genetic improvement was done *via* conjugation as one important method to obtain strains have high bacteriocin production and activity compared with parents. Genetic improvement of selected bacterial isolates were carried out using two different techniques of conjugation; filter and broth mating techniques. The transconjugtion frequencies of the filter mating technique (4.6×10^{-5}) was higher than the broth mating technique (2.4×10^{-5}). The genetic variability among the transcojugants lines were tested using RAPD analysis and showed 13.76% polymorphism percentages for donor, recipient and tranconjugants lines The genes encoding proteins involved in bacteriocin production were isolated and sequenced from *E . faecium* AH2 (*entA, entI, entF, entR , orfA2 , orfA3*), *Pediococcus pentoceseus* AH1(*PapA, PedB*) and *nis A* from *Lactococcus lactis sub lactis.* All sequences of genes were deposited in the GenBank database under accession numbers: LC064146, LC064147, LC064148, LC064149, LC064150, LC064151, LC101300, LC101489 and LC101789.

Keywords: Bacteriocin, Lactic acid bacteria, *16S rRNA* gene, RAPD PCR Conjugation, Bacteriocin genes , Cloning.

DEDICATION

I dedicate this work to Prof.Dr. Ahmed Nagib El-Sayed Sharaf, Prof.Dr. Nivien Abdelrahman Abosereh, my mother the most kind person on the earth, my late father for all the support they lovely offered along the period of my post graduation., whom my heart felt thanks; to my brothers and sisters, my husband Mahdy and my sons Mohamed, Mahmoud, mostafa and my daughter Nivien for their patience, my frinds, and to every one want that world to be abetter place to live.

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LIST OF ABBREVIATIONS

LAB	**Lactic acid bacteria**
ddH_2O	Double-distilled water
X-Gal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside
IPTG	isopropyl- β -D-thiogalactopyranoside
No	number
ORF	Open reading frame
rpm	Round per minute
API	Analytical Profile Index
LB	Luria-Beratni
KAA	Kanamycin aesculin azide
MRS	DeMan, Rosa and sharpa
MDR	Multi drug resistance
GRAS	Generally recognized as safe

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