Evaluation of Lactic Acid Bacteria Isolated from the Honey Bee *Apis mellifera* L. for the Control of the American Foulbrood Disease

A Thesis
Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science
(Entomology)

By

Fatma Mahmoud El-Sayed Mohammed
(B.Sc. Entomology)

Supervisors

Prof. Dr. Akila Mohammed El-Shafei
Professor of Entomology
Entomology department - Faculty of Science - Ain Shams University

Prof. Dr. Ahmed Saad Abou Zeid
Professor of Entomology
Entomology department - Faculty of Science - Ain Shams University

Dr. Shireen Ahmed Mahmoud Ma'moun
Lecturer of Entomology
Entomology department - Faculty of Science - Ain Shams University

Department of Entomology
Faculty of Science
Ain Shams University

2017

Biography

Name:	Fatma Mahmoud El-Sayed Mohammed.
Degree awarded:	B.Sc. (Entomology).
Department:	Entomology.
Faculty:	Science.
University:	Ain Shams University.
Date of Graduation:	July 2013.
Date of Appointment:	April 2014.
Occupation:	Demonstrator, Department of Entomology, Faculty of Science, Ain Shams University.
Date of registration For the M.Sc. Degree:	April 2015.

Evaluation of lactic acid bacteria isolated from the honey bee *Apis mellifera* L. for the control of the American foulbrood disease

Board of Supervision:

Prof. Dr. Akila Mohammed El-Shafei
Professor of Entomology
Faculty of Science
Ain Shams University

Prof. Dr. Ahmed Saad Abou Zeid
Professor of Entomology
Faculty of Science
Ain Shams University

Dr. Shireen Ahmed Mahmoud Ma'moun
Lecturer of Entomology
Faculty of Science
Ain Shams University

Approval Sheet

Evaluation of lactic acid bacteria isolated from the honey bee *Apis mellifera* L. for the control of the American foulbrood disease

Approved By:

Prof. Dr. …… .

Prof. Dr. …… .

Prof. Dr. …… .

Prof. Dr. …… .

(Committee in charge)
Date: / /

ACKNOWLEDGEMENTS

First of all, thanks to "ALLAH" to whom I relate any success in achieving any work in my life.

My deepest appreciation and respect with sincere thanks go to ***Prof. Dr. Akila El Shafei,*** *Professor of Entomology for suggesting the problem, her excellent ideas, energetic guidance, constructive discussion and close supervision.*

I am also particularly indebted to ***Prof. Dr. Ahmed Saad Abou Zeid,*** *Professor of Entomology for his generous assistance and guidance.*

My deepest appreciation and respect with sincere thanks go to ***Dr. Shireen Ahmed Mahmoud Ma'moun,*** *lecturer of Entomology for suggesting the problem, her excellent ideas, energetic guidance, constructive discussion and close supervision.*

Finally, I wish to express my deep thanks to ***Dr. Rasha Mohamed Ahmed Farag,*** *Researcher in Apiculture Research Department, Plant Protection Institute for her help.*

Special thanks go to all members of the Entomology Department, Faculty of Science, Ain Shams University for their encouragement and help during my work.

Finally, ***ALLAH*** *was the only one who made this work possible.*

Abstract

The purpose of this study is to investigate a new applicable, reliable, promising and highly effective treatment for American Foulbrood (AFB) disease in honey bee colonies. Some of the honey bee gut microbial diversity in worker bees using 560 amplicon assays of the 16S rRNA gene were investigated. The presence of nine novel anaerobic lactic acid bacterial (LAB) flora were reported within honey bee gut. Four of the LABs are carefully related to four different strains of *Lactobacillus plantarum* species. Two are closely identical to two different strains of *Lactobacillus kunkeei* species. One is closely related to a strain of *Lactobacillus pentosus* species. The last two are matching two different strains of *Lactobacillus sp.*. A strong inhibitory effect of the honey bee stomach LAB flora on AFB bacterial pathogen, *Paenibacillus larvae larvae* (*P. l. larvae*) growth *in vitro* were demonstrated. The individual LAB phylotypes showed different inhibition zones ranges from 0.4 to 1.8 cm. Artificial infection was accompanied by the administration of a mixture of five of the most effective endogenous LABs, previously tested for their inhibitory effect on agar plates *in vitro*. It was observed that the honey bee endogenous LAB inhibited *P. l. larvae* in an *in vivo* system. LAB mixture added to the larval food in honey bee colonies significantly reduced the number of infected larvae when pooled data from all experiments were analyzed ($P \simeq 0.000$, $P < 0.001$). Confidence intervals analysis on the effect of the time LAB was added to the colony didn’t show significant different from adding LAB to the food on first or second day post infection and throughout the feeding period. Both *in vitro* and *in vivo* studies demonstrated that the LAB microbiota in *Apis mellifera* inhibit one important honey bee pathogen, the bacterial brood pathogen

P. l. larvae that is cause of the brood disease AFB. The results point to new avenues for the prophylactic or therapeutic treatment of honey bee diseases.

Keywords:

Honey bee, *Apis mellifera*, American Foulbrood, Lactic Acid Bacteria, LAB identification, Probiotic treatment and AFB control.

Contents

List of Tables

List of Figures