



Early Diagnosis of the American Foulbrood Disease of the Honeybee (*Apis mellifera* Linnaeus) in Egypt

**A Thesis Submitted for the
Requirements for the Degree of Philosophy of
Doctor (Entomology)**

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DEDICATION

**This work is dedicated to the late eminent Prof.
Dr. Ahmad Hassan Kaschef, whose contributions
in the field of biological research work are never
forgettable**

بسم الله الرحمن الرحيم

وأوحى ربك إلى النحل أن أتخذي من الجبال بيوتا

ومن الشجر ومما يعرشون ^{٦٨} ثم كلي من كل

الثمار فاسلكي سبل ربك ذللا يخرج من بطونها

شراب مختلف ألوانه فيه شفاء للناس إن في ذلك

لآية لقوم يتفكرون ^{٦٩}

صدق الله العظيم

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Abstract

The aim of this thesis is to investigate some practical aspects of American Foulbrood (AFB) diagnosis and control. Article I investigates the possibility to use composite sampling for diagnosis of AFB in apiaries through composite sampling of adult bees and honey. A reliable procedure for early detection of *Paenibacillus larvae* subsp. *larvae* (*P. l. larvae*), the causal agent of American Foulbrood disease (AFB) of honeybees (*Apis mellifera* L.) based on the polymerase chain reaction (PCR) and subspecies – specific KAT primers. A PCR amplicon of the expected size 550 bp only found in *P. l. larvae* strains was used for positive AFB. This PCR assay provides a specific detection for *P. l. larvae* from week 1 post infection even if there is no clinical symptoms appeared in a colony. The technique can be directly used to detect presence or absence of *P. l. larvae* spores in honeybee samples and contaminated honeys.

In article II we report the presence of the probiotic lactic acid bacterium (LAB) in the gut of the honeybee *Apis mellifera*. Partial 16S rRNA gene sequences of the bacterial flora found in the gut of the honeybee workers, revealed the presence of 7 novel LAB flora. Five of the anaerobic LABs are closely related to 5 different strains of the lactic acid bacteria *Lactobacillus kunkeei* species, One is closely related to a strain of *Lactobacillus plantarum* species and the last one is identical to a strain of species *Fructobacillus fructosus*. We evaluated the antagonistic effects of newly identified lactic acid bacteria (LAB) in the genera *Lactobacillus* and *Fructobacillus*, originating from the honeybee gut, on the honeybee pathogen, *Paenibacillus larvae*. We used inhibition assays on agar plates to investigate the effects of honeybee LAB on *P. larvae* growth in vitro. The individual LAB phylotypes showed

different inhibition properties against *P. larvae* growth on agar plates, whereas a combination of all seven LAB phylotypes resulted in a total inhibition (no visible growth) of *P. larvae*. The results demonstrate that honeybee specific LAB possess beneficial properties for honeybee health. Enhancing growth of LAB or applying LAB to honeybee colonies should be further investigated.

Keywords:

Honeybee, American Foulbrood, Molecular Diagnosis, AFB control, Lactic Acid Bacteria, LAB identification and Probiotic control.

CONTENTS

Subject	Pages
ACKNOWLEDGMENTS	
Abstract	
List of Tables.....	i
List of Figures.....	ii
List of Abbreviations.....	vii
I. Introduction.....	1
II. Literature Review.....	6
1. Study organism: the Honeybee and Beekeeping hives.....	6
1. A. Honeybees' caste structure.....	6
1. B. Beekeeping hives.....	12
2. American Foulbrood disease; diagnosis and treatment.....	13
2. A. Diagnosis of American Foulbrood disease.....	13
2. B. Treatments and prophylaxis of AFB disease.....	24
III. Materials and Methods.....	35
1. Source and rearing of honeybees	35
2. Isolation and preparation of the, <i>Paenibacillus</i> <i>larvae larvae</i> , AFB bacterial pathogen.....	35

Subject	Pages
2.1. Source of the bacterial pathogen.....	35
2.2. Isolation and Cultivation of the bacterial pathogen <i>P. l. larvae</i>	36
2.3. Identification and characterization of the bacterial pathogen.....	37
2.3.1. Morphological tests.....	38
2.3.2. Microscopical tests.....	38
2.3.3. Biochemical tests.....	38
3. Field experiment and samples collection.....	39
3.1. Detection of <i>P. l. larvae</i> from adult honeybee and honey samples.....	40
3.1.1. Detection of <i>P. l. larvae</i> from adult honeybee samples.....	40
3.1.2. Detection of <i>P. l. larvae</i> from honey samples.....	41
4. Isolation and preparation of the probiotic lactic acid bacteria (LAB).....	42
5. DNA preparation and manipulation.....	43
5.1. Bacterial DNA isolation.....	43
5.1.1. DNA isolation from <i>P. l. larvae</i> and LAB, cultured colonies.....	43
5.1.2. Bacterial DNA isolation from honeybee workers.....	43

Subject	Pages
5.1.3. Bacterial DNA isolation from honey.	44
5.2. PCR amplification of 16S-rRNA gene.....	45
5.2.1. PCR primers and reaction mixtures..	45
5.2.2. The PCR Temperature profiles.....	48
5.3. Agarose gel electrophoresis.....	49
5.4. Sequence analysis.....	50
6. Inhibition bioassays against <i>P. l. larvae</i> bacterial spores.....	51
6.1. Lactic acid bacteria (LAB) inhibition bioassay.....	51
6.2. Oxytetracycline (Terramycine) antibiotic inhibition bioassay.....	52
6.3. Royal jelly inhibition bioassay.....	53
IV. Results.....	55
1. AFB clinical diagnosis (field symptoms).....	55
2. Identification and characterization of <i>P. l.</i> <i>larvae</i> , AFB bacterial pathogen.....	56
2.1. Bacterial isolates.....	56
2.2. Morphology of <i>P. l. larvae</i> bacterial colony.....	57
2.3. Microscopic identification of <i>P. l. larvae</i> bacteria.....	57

Subject	Pages
2.4. Biochemical identification of <i>P. l. larvae</i> bacteria.....	58
2.4.1. Holst milk test.....	58
2.4.2. Catalase test.....	58
3. Field experiment observations and samples collected results.....	59
3.1. Field experiment observations.....	59
3.2. Detection of <i>P. l. larvae</i> from adult honeybee and honey samples.....	59
3.2.1. Honeybee and honey samples bacterial cultivation results.....	59
3.2.2. Honeybee and honey samples bacterial DNA KAT-PCR results.....	66
4. Isolation and cultivation results of the probiotic lactic acid bacteria (LAB).....	70
5. PCR amplification and sequencing results....	72
5.1. PCR amplification of 16S-rRNA genes...	72
5.2. Sequencing results.....	73
6. Inhibition bioassay results.....	92
6.1. Lactic acid bacteria (LAB) inhibition bioassay.....	92
6.2. Oxytetracycline (terramycine) and Royal jelly inhibition bioassays.....	94

Subject	Pages
V. Discussion.....	97
I. Early Diagnosis of AFB.....	100
II. Treatment of the honeybee American Foulbrood (AFB) disease.....	106
III. Conclusions.....	116
IV. Recommendations.....	117
VI. Summary.....	118
VII. References.....	122
VIII. Arabic Summary.....	153

LIST OF TABLES

	pages
Table (1): PCR reaction components and their volumes for KAT primers.....	46
Table (2): PCR reaction components and their volumes for 16s rRNA primers.....	47
Table (3): A summary of cultivation results for detection of <i>P. l. larvae</i> from honey and honeybee samples collected from infected and control colonies for the first 4 weeks post-infection.....	65
Table (4): KAT-PCR detection of <i>P. l. larvae</i> from honey and honeybee samples collected from infected and control colonies for the first 4 weeks post-infection.....	69
Table (5): Inhibition of <i>P. l. larvae</i> bacterial spores, American Foulbrood (AFB) bacterial pathogen, by seven honeybee lactic acid bacteria (LAB) phylotypes and their combination, oxytetracycline (Terramycine) antibiotic and royal jelly.....	96