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

وأوحي ربك الي النحل أن أتخذي من الجبال بيوتا

ومن الشجر ومما يعرشون ﴿٦٨﴾ ثم كلي من كل

الثمرات فاسلكي سبل ربك ذللا يخرج من بطونها

شراب مختلف ألوانه فيه شفاء للناس إن في ذلك

لآية لقوم يتفكرون ﴿٦٩﴾

صدق الله العظيم

سورة النحل أيه (٦٨ – ٦٩)

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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.

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