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PP-095 [Bee Health]

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Paenibacillus melissococcoides: a new pathogen of honey bee brood?

Dainat Benjamin[1], Charrière Jean-Daniel[1], Dietemann Vincent [1,2]
1. Swiss Bee Research Centre, Agroscope, Bern – Liebefeld, Switzerland ; 2. Department Ecology Evolution, University of Lausanne, Switzerland

Introduction

We recently described a new bacteria found in a brood-diseased colony: *Paenibacillus melissococcoides* [1].
We then investigated whether this bacterium was pathogenic for honey bee brood by testing **Koch's postulates** [2].

Results

Koch's postulates

The microorganism must be isolated from a diseased organism… ✓

…and grown in pure culture ✓

The cultured microorganism should cause disease when introduced into a healthy organism. ✓

The microorganism must be reisolated from the experimentally inoculated, diseased host … ✓

…and identified as identical to the original causative agent. ✓

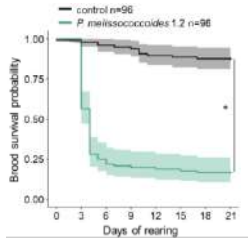
Our observations

P. melissococcoides found in
- adults of symptomatic colonies without *M. plutonius*
- nutritive jelly fed to larvae of EFB-diseased colonies
- larvae and adult workers of EFB-diseased colonies

P. melissococcoides was not found in healthy colonies.

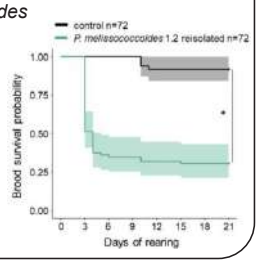
P. melissococcoides was grown in pure culture

inoculation of healthy larvae reared in vitro led to death



P. melissococcoides was reisolated from an inoculated dead larva and grown in pure culture

the cultured *P. melissococcoides* led to death



MALDI-TOF MS

Organism	Score[x]
P. Melissococcoides	2.28
P. Melissococcoides	2.29

[x] value interpretation:
3.00–2.30: highly probable species identification;
2.29–2.00: secure genus identification, probable species identification
* P<0.05

Discussion

Our results indicate that *P. melissococcoides* is pathogenic to honey bee brood in laboratory conditions at natural concentrations[2]. There is as yet no strong evidence that *P. melissococcoides* triggers a disease at the colony level on its own but first evidence in field idiopathic cases suggests that colony level pathogenicity is possible.

Methods

Bacteria were grown on Basal medium for four days at 36°C under oxic conditions [1].
Freshly hatched larvae were reared in vitro [3] . Differences in brood survival between the inoculated and control groups were tested using log-rank tests for two group comparisons in R, version 4.1.0.
MALDI-TOF MS from Bruker were used following the Biotyper MSP Identification Standard Method.

1. Ory F, Dainat B... & Dietemann V, 2023. *Paenibacillus melissococcoides* sp. nov., isolated from a honey bee colony affected by European foulbrood disease. *Int. J. of system. Evol. Microbiol.*, 73(4), 005829.
2. Ory F, Dainat B,... & Dietemann V, 2025. Ecology and pathogenicity for honey bee brood of recently described *Paenibacillus melissococcoides* and comparison with *Paenibacillus dendritiformis*, *Paenibacillus thiaminolyticus*. *Env. Microbiol. reports*, *17*(3), e70089.
3. Crailsheim et al., 2013. Standard methods for artificial rearing of *Apis mellifera* larvae. In V Dietemann; J D Ellis; P Neumann (Eds) The COLOSS BEEBOOK, Volume I: standard methods for Apis mellifera research. *J. Apic. Res.* 52(1).

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Benjamin J. Dainat[1], Jean-Daniel Charrière[1], Vincent Dietemann[2]
[1]Swiss Bee Research Centre, Agroscope Liebefeld, Bern, Switzerland
[2]Swiss Bee Research Centre, Agroscope Liebefeld, Bern, Switzerland, Department of Ecology and Evolution, University of Lausanne, Lausanne, Switzerland

Because honey bee colonies contain many individuals in close physical contact, they constitute fertile grounds for numerous pathogens to multiply and spread. Among these, the two globally distributed brood bacteria Paenibacillus larvae and Melissococcus plutonius are the causative agents of American (AFB) and European foulbrood (EFB) diseases, respectively. By affecting larvae, these bacteria can lead to colony weakness and possibly to death. In Switzerland, high numbers of EFB cases were reported each year, leading to increased research efforts into the epidemiology of EFB. During such research, an atypical bacterium was found in addition to M. plutonius in an apiary affected by EFB. It turned out to be a new species, which we named Paenibacillus melissococcoides. As this new bacterium was discovered in a brood diseased colony, we investigated whether it was present in larval jelly and in larvae, and tested, in vitro, whether it was pathogenic to the brood. To better understand the interaction between bacteria of the genus Paenibacillus and A. mellifera, we also compared the virulence of P. melissococcoides with that of two closely related Paenibacillus species, which were previously found to be associated with honey bees. In this presentation, we review what we have learned to date on the ecology and pathogenicity of this species and discuss whether P. melissococcoides is a new honey bee pathogen. The discovery of this new species brings new insights into EFB epidemiology and the suite of bacteria associated with it, as well as a better understanding of the ecology of Paenibacillus species when they encounter honey bees. We argue that P. melissococcoides can be classified as an EFB secondary invader, but because of a few cases where it was found in brood diseased colonies in the absence of M. plutonius and P. larvae, P. melissococcoides may be a brood pathogen in its own right, occasionally obscuring the diagnosis of foulbroods.