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Original article

Novel lactic acid bacteria inhibiting *Paenibacillus larvae* in honey bee larvae*

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Abstract – We evaluated the antagonistic effects of newly identified lactic acid bacteria (LAB) in the genera *Lactobacillus* and *Bifidobacterium*, originating from the honey stomach, on the honey bee pathogen, *Paenibacillus larvae*. We used inhibition assays on agar plates and honey bee larval bioassays to investigate the effects of honey bee LAB on *P. larvae* growth in vitro and on AFB infection in vivo. The individual LAB phylotypes showed different inhibition properties against *P. larvae* growth on agar plates, whereas a combination of all eleven LAB phylotypes resulted in a total inhibition (no visible growth) of *P. larvae*. Adding the LAB mixture to the larval food significantly reduced the number of AFB infected larvae in exposure bioassays. The results demonstrate that honey bee specific LAB possess beneficial properties for honey bee health. Possible benefits to honey bee health by enhancing growth of LAB or by applying LAB to honey bee colonies should be further investigated.

American foulbrood / *Paenibacillus larvae* / Lactic Acid Bacteria / *Lactobacillus* / *Bifidobacterium* / inhibition

1. INTRODUCTION

Honey bees, *Apis mellifera* are the most important pollinating insect worldwide. Hence, apiculture has a great economic impact on insect pollinated crops throughout the world, and the health status of honey bees has become an important concern in many countries. There is a large diversity of microorganisms associated with honey bees (Gilliam, 1997) most of them commensals, but some are pathogens affecting adult bees and brood. American foulbrood (AFB) is a common bacterial disease affecting apiculture worldwide (Ellis and Munn, 2005). AFB is lethal to infected honey bee larvae, and can be devastating to colonies and apiaries causing serious financial losses in apiculture. The disease is caused by *Paenibacillus larvae* (Genersch et al., 2006), a spore-forming, Gram-positive rod-shaped bacterium that infects young larvae through ingestion of contaminated food. The bacterial spores germinate and proliferate in the midgut lumen before they start to breach the epithelium and invade the haemocoel (Yue et al., 2008). The colonization of the larval midgut has been suggested to be one of the key steps in the pathogenesis of *P. larvae* (Yue et al., 2008). Newly hatched larva can become infected by as few as 10 spores (Woodrow, 1942), but the dosage-mortality relationship is greatly influenced by larval age, genetic constitution and bacterial strain (Genersch et al., 2005). The spore concentrations needed to cause disease may vary with a factor of at least 20 (Ashiralieva and Genersch, 2006).

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Microbial commensals within host organisms are of great importance and mostly exist in harmony with the host. Moreover, the balance of the human microbial flora within the gastrointestinal tract may be important in the development of disease (Guarner, 2005). There are fewer commensals within insects compared to within humans, but their impact may be even more important. Ryu et al. (2008) discovered a mutualistic relationship between the endogenous gut flora and the fruit fly *Drosophila*, revealing that the fly's normal flora was sufficient to suppress the growth of pathogenic bacteria.

Bacteria belonging to lactic acid bacteria (LAB) are functionally related by their ability to produce lactic acid during homo- or heterofermentative metabolism (Klaenhammer et al., 2002). *Lactobacillus* and *Bifidobacterium*, two of the most important genera within LAB, are commonly found as commensals and are used as probiotics for humans and animals (Ouwehand et al., 2002). A novel flora composed of *Lactobacillus* and *Bifidobacterium* has recently been identified in the honey stomach of honey bees, *A. mellifera* (Olofsson and Vásquez, 2008; Vásquez et al., 2009). The phylogenetic analyses performed in both studies showed the LAB flora in the honey stomach to be composed of twelve different phylotypes. Furthermore, the findings revealed that honey bees and the novel LAB flora evolved in mutual dependence of one another; the LAB obtaining a niche in which nutrients are available, the honey bees and the honey in turn being protected by the LAB from harmful microorganisms (Olofsson and Vásquez, 2008).

LAB are known to be good producers of antimicrobial substances such as organic acids, hydrogen peroxide and antimicrobial peptides (de Vuyst and Vandamme, 1994). However, there is a clear variation in the production of antimicrobial substances and other beneficial qualities between the different species and genera within LAB (Ouwehand et al., 2002; Pfeiler and Klaenhammer, 2007). Therefore, it is important to investigate the potential antagonistic properties of the recently discovered honey bee stomach LAB against specific honey bee pathogens.

The purpose of the present study was to investigate if the newly identified *Lactobacillus* and *Bifidobacterium* phylotypes inhibit growth of *P. larvae* and have an effect on AFB disease development in infected honey bee larvae.

2. MATERIALS AND METHODS

2.1. *Paenibacillus larvae* strains

Four strains of *P. larvae* representing four different genotypes based on PCR of conserved motifs in bacterial repetitive ERIC elements described by Genersch and Otten (2003) were used in the inhibition experiment (described below). Two of these isolates were commercially available reference or type collection strains: LMG 16252 (ERIC III), LMG 16247 (ERIC IV). The remaining two strains were isolated from samples of Swedish honey bee brood with symptoms of AFB (field strains) deposited at the Culture Collection of the University of Gothenburg as CCUG 48979 (ERIC I) and CCUG 48972 (ERIC II). The properties of the different genotypes were described by Genersch et al. (2006). The field isolates of *P. larvae* were also used for experimentally infecting larvae in bioassays. Bacterial strains were cultivated on MYPGP-agar plates as described by Nordström and Fries (1995), and incubated at 35 °C and 5% CO_2 for 10–14 days to obtain sporulation. Fresh spore suspensions were prepared by suspending bacterial colonies in sterile 0.9% NaCl. A direct microscopic count of the spore suspensions was made in a Helber Bacteria Counting Chamber (Hawksley) using a phase-contrast light microscope (400x). Spore suspensions with concentrations of 5×10^4 and 5×10^5 spores per milliliter (total microscopic count) were prepared fresh for each experiment. This corresponds to a viable count of approximately 5×10^3 and 5×10^4 colony forming units (cfu) per mL given that 10% of the *P. larvae* spores germinate on solid MYPGP media (Forsgren et al., 2008). The solutions were further diluted in the larval food reaching final counted concentrations of approximately 5×10^3 and 5×10^4 spores per mL ($\leqslant 5 \times 10^2$ and $\leqslant 5 \times 10^3$ cfu) respectively (Tab. I).

2.2. Honey bee specific LAB

Twelve previously found phylotypes derived from the honey stomachs of honey bees (Olofsson and Vásquez, 2008; Vásquez et al., 2009)

Table I. Different treatment groups in the bioassay and the final concentration of *P. larvae* and LAB in the larval food. Groups 2 and 5 received LAB supplemented food in connection with *P. larvae* spores (time of infection = day 0) and all the following days (ff). Groups 3 and 6 received LAB supplemented food day two post-infection (post inf.) and following days (ff).

Group	*P. larvae*	LAB	Timing
1	5×10^3 spores/mL	-	
2	5×10^3 spores/mL	5×10^4 bacteria/mL	day 0 ff
3	5×10^3 spores/mL	5×10^4 bacteria/mL	day 2 post inf. and ff
4	5×10^4 spores/mL	-	
5	5×10^4 spores/mL	5×10^4 bacteria/mL	day 0 ff
6	5×10^4 spores/mL	5×10^4 bacteria/mL	day 2 post inf. and ff
Control	-	-	

were used in the study. *Lactobacillus kunkeei* (Fhon2), *Bifidobacterium asteroides* related phylotypes (Hma3, Bin7 and Bin2), *Bifidobacterium coryneforme* (Bma6) and six *Lactobacillus* phylotypes (Hon2, Hma2, Biut2, Bma5, Hma8, Hma11 and Bin4). Individual LAB were inoculated into different broths. Phylotypes Bin2, Bin7, Hma3, Bma6 and Hma11 were inoculated into LCM (*Lactobacillus* Carrying Media) (Efthymiou and Hansen, 1962). Fhon2 was inoculated into MRS (de Man, Rogosa and Sharpe, Oxoid) broth supplemented with 20% fructose. Phylotypes Hon2, Bma5, Hma2, Biut2, Hma8 and Bin4 were inoculated into MRS broth (Oxoid) supplemented with 0.5% L-cysteine. The tubes with the LAB were incubated anaerobically at 35 °C for 3 days. A mixture containing all twelve phylotypes in approximately the same amounts with a total concentration of 10^7 bacteria/mL was prepared and used in the exposure bioassays.

2.3. Honey bee material

Worker larvae (first instar) were collected and grafted from *A. mellifera* colonies in the apiary at the Swedish University of Agricultural Sciences in Uppsala, Sweden and used in the exposure bioassays.

2.4. Inhibition assay

Agar media was prepared for each LAB phylotype (phylotype Bin4 was not included in this assay). MRS agar (pH 6.2) supplemented with 0.5% L-cysteine was used for phylotypes Hon2, Hma2, Bma5, Hma8 and Biut2. MRS agar was used for phylotypes Bin2, Bin7, Hma3, Bma6 and Hma11. Tomato juice agar (Oxoid) was used for phylotype Fhon2 and the reference strain *Lactobacillus kunkeei* YH-15^T (CCUG 53901). LAB was grown individually in different broths as described above. Filter paper disks were impregnated with the individual LAB phylotypes at a final concentration of 10^6 (for Bma6, Hon2, Hma2), 10^7 (Fhon2, Hma8, Biut2, Hma11), and 10^8 (Bin2, Hma3, Bin7, Bma5). Each disk was centered on the prepared agar media. In addition, a filter paper disk was impregnated with a suspension containing a mixture of all LAB. The filter disks were then placed on MRS agar media. The agar media were incubated anaerobically at 35 °C for 12 hours. Soft agar of MYPGP was prepared as previously described (Nordström and Fries, 1995). The temperature of the soft agar was adjusted to 42 °C. *P. larvae* spores (ERIC I, ERIC II, ERIC III and ERIC IV) were mixed with the soft agar, poured into the agar media containing the grown LAB and incubated at 35 °C and 5% CO_2 for 7 days. Inhibition of *P. larvae* strains by LAB was defined as the distance of the paper disks and the first line of *P. larvae* growth.

2.5. Exposure bioassays

To rear larvae in vitro a protocol by Aupinel et al. (2005) was followed with minor modifications. Worker honey bee larvae were reared in 48-well tissue culture plates on a diet consisting of 50% royal jelly (v/v) (Stakich, Inc., Bloomfield Hills, MI, USA), and 50% of an aqueous solution of D-glucose (12%) and D-fructose (12%). The sugar was dissolved in sterile deionised water and the sugar solution was autoclaved at 100 °C for 40 minutes and kept frozen in aliquots. Before any experiment, the required amount of diet was prepared and

then stored at +4 °C for the duration of the experimental feeding. Before grafting, each plastic well was supplied with 50 μL of the pre-warmed diet. The control group was provided with uninfected diet while the experiment groups were either provided larval diet spiked with known amounts of *P. larvae* spores (groups 1 and 4; Tab. I) or *P. larvae* spores mixed with the LAB mixture (groups 2 and 5). First instar worker larvae (less than 24 hours) were grafted from larval combs and transferred to the surface of the larval diet of the different treatments. Twenty-four hours post exposure to spore containing diet; all larvae were transferred to wells containing a diet without *P. larvae* spores, but were supplemented with LAB to determine if the timing of LAB supplements affected the activity against *P. larvae*. Two groups of larvae were initially placed on food with *P. larvae* spores without addition of LAB, but received LAB supplemented food from day two post-infection (groups 3 and 6, Tab. I). Fifty μL of the supplemented diet was provided to the larvae once a day using a scaled sterile syringe. The tissue culture plates were kept in plastic boxes with a wire mesh insert. The boxes were filled with a bottom layer of 15.5% glycerol in 0.45% methyl benzethonium chloride in order to prevent fungal growth. The larvae were maintained in an incubator at 35 °C with a relative humidity of 96%. On the 7th day, when the larvae generally begun to defecate and spin their cocoon, the 48-well plates were transferred to boxes where the relative humidity was kept at 80% through a saturated solution of NaCl allowed to cover the bottom. The boxes were kept in an incubator at 35 °C until the experiment was finished 14 days post-infection. Throughout the experiment dead larvae were removed daily and cultured on agar plates to verify presence or absence of *P. larvae*.

A total of 1200 larvae were used in five repeated experiments.

2.6. Statistical analysis

The response variable in this study was the proportion of infected larvae, hence the effects from our experimental variables was tested using a generalization of the ANOVA test using binomial errors and a logit link function. The generalized ANOVA was used to evaluate the influence of LAB, and the timing and doses of *P. larvae* spores on the proportion of infected larvae. The statistical evaluations were performed using R (R Development Core Team, 2005).

3. RESULTS

3.1. Inhibition

The combination of all eleven LAB phylotypes resulted in total inhibition (no visible growth) of all four investigated *P. larvae* strains (Tab. II). The individual LAB phylotypes and the reference strain*L. kunkeei*^{T} showed different inhibition properties against *P. larvae* strains (Tab. II). Two *Lactobacillus* phylotypes (Biut 2 and Hma 11) individually inhibited of all four represented genotypes. *B. coryneforme* (Bma 6) totally inhibited the growth of *P. larvae* genotype ERIC III and IV (reference strains) whereas there was no growth inhibition of the isolate of ERIC I genotype and only partial inhibition of genotype ERIC II. *Lactobacillus* phylotypes BMA 5 totally inhibited growth of *P. larvae* genotypes I-III, but resulted in a 1.9 cm inhibition zone of *P. larvae* genotype ERIC IV (Fig. 1).

3.2. Exposure bioassays

The overall effect from adding the LAB mixture to the larval food was a significantly reduced number of infected larvae when pooled data from all experiments, irrespective of the infective dose, were analyzed (P = 0.007, Fig. 1). The positive effect from adding LAB to the groups fed larval food with higher doses of *P. larvae* (groups 5 and 6) was highly significant (P = 0.005, Fig. 2), however the difference among the groups fed lower spore doses (group 2 and 3) was not (P = 0.198, Fig. 1).

The ANOVA analysis on the effect of the time LAB was added to the feed showed no significant differences on the proportion of infected larvae in any of the treated groups. The effect of adding LAB in combination with *P. larvae* at the time of spore administration was not significantly different from adding LAB to the food 48 hours post infection and throughout the feeding period.

The total mortality of the uninfected control group larvae was less than 17% in all the presented experiments.

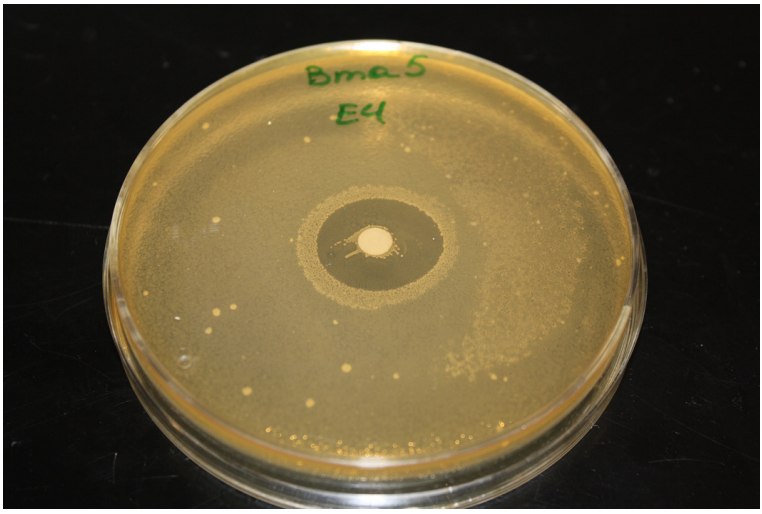

Figure 1. Inhibition from honey bee LAB phylotype Bma 5 (EF187242) on *Paenibacillus larvae* reference strain (LMG 16247) genotype ERIC IV.

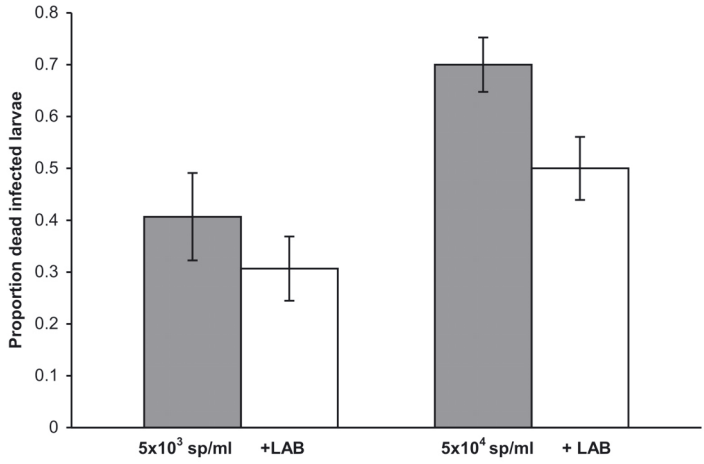

Figure 2. Proportion infected larvae from all five experiments (total number infected after 14 days). Data presented as a mean for groups fed the lower dose *P. larvae* (group 1); groups fed the lower dose *P. larvae* and LAB (groups 2 and 3); groups fed the higher dose *P. larvae* (group 4); groups fed the higher dose *P. larvae* and LAB (groups 5 and 6). The difference between groups fed the higher dose of *P. larvae* was significant ($P = 0.005$). The mortality in the uninfected control group was less than 17% in all experiments.

Table II. Inhibition of four *P. larvae* strains (ERIC I-IV) by eleven honey bee specific LAB phylotypes. Phylogenetic trees are shown in previous work (Olofsson and Vásquez, 2008; Vásquez et al., 2009).

Lactobacillus/Bifidobacterium	*Paenibacillus larvae*			
phylotypes	ERIC I	ERIC II	ERIC III	ERIC IV
Bma 6	no inhibition zone	2 cm inhibition zone	total inhibition	total inhibition
Bin 7	total inhibition	0,5 cm inhibition zone	1,7 cm inhibition zone	2,2 cm inhibition zone
Hma 3	total inhibition	total inhibition	1 cm inhibition zone	2,8 cm inhibition zone
Biut 2	total inhibition	total inhibition	total inhibition	total inhibition
Bin 2	total inhibition	no inhibition zone	3 cm inhibition zone	no inhibition zone
Hma 11	total inhibition	total inhibition	total inhibition	total inhibition
Hma 2	total inhibition	2 cm inhibition zone	total inhibition	total inhibition
Bma 5	total inhibition	total inhibition	total inhibition	1,9 cm inhibition zone
Hma 8	total inhibition	3 cm inhibition zone	2 cm inhibition zone	total inhibition
Fhon 2	1,8 cm inhibition zone	no inhibition zone	1 cm inhibition zone	1,8 cm inhibition zone
Hon2	total inhibition	total inhibition	total inhibition	1 cm inhibition zone
Combination of all phylotypes	total inhibition	total inhibition	total inhibition	total inhibition
Lactobacillus kunkeii^{T}	no inhibition zone	no inhibition zone	2 cm inhibition zone	no inhibition zone

4. DISCUSSION

Our results demonstrated a strong inhibitory effect of the combined honey bee stomach LAB flora and of two *Lactobacillus* phylotypes (Hma11 and Biut2) on the in vitro growth of *P. larvae* (Tab. II). Furthermore, the results clearly demonstrate that addition of LAB to young honey bee larvae exposed to *P. larvae* spores decreases the proportion of larvae that succumb to AFB infection. Thus, our results strongly suggest that probiotic bacteria linked to the honey bee stomach have important implications for honey bee pathology in general and for AFB tolerance in particular. Evans and Armstrong (2005, 2006) have previously shown antagonistic interactions between honey bee symbionts such as *Bacillus* spp. against *P. larvae*. However, in their studies no interaction was shown for any of the honey stomach LAB flora. Members of the genera *Lactobacillus* and *Bifidobacterium* are well known beneficial bacteria widely used as probiotics to modulate the composition of the microflora in order to protect the host from infections (Ouwehand et al., 2002). Considering the colonization of the larval midgut as a key factor in the pathogenesis of *P. larvae* (Yue et al., 2008) and the demonstrated strong inhibitory effect from LAB on *P. larvae* growth, it is not surprising that the addition of the honey bee specific LAB have an impact on the development of the infection. In nature, food given to young honey bee larvae contains reduced sugars not present in the mandibular or hypopharyngeal glands producing this food (Gontarski, 1960). Probably such carbohydrates originate from the honey stomach (Townsend and Shuel, 1962) and could serve as a source for LAB to young larvae. As the worker larvae grow older (> 3 days), the worker brood food also contains pollen (Haydak, 1970). Recently, it was demonstrated that the honey stomach LAB flora probably plays a key role in the production of bee bread from bee pollen (Vásquez and Olofsson, 2009). Bee bread, having a pH between 3.8 and 4.3 and containing organic acids, was apparently fermented by the honey stomach LAB flora when added to bee pollen via regurgitated nectar from the honey stomach (Vásquez and Olofsson, 2009). This discovery shed light on how the honey bees standardize the production of bee bread and shows that the viable LAB flora is present in significant numbers in fresh bee bread. The LAB and their metabolites are probably transferred to the larvae already with the first food since the larval food contains carbohydrates not found in the food producing glands (Gontarski, 1960). A further boost with LAB is likely administered when the older larvae also are fed the bee bread. Such modification of the gut microflora may hamper the germination and proliferation of the *P. larvae* spores in the larval midgut, thus preventing the infection. It has been demonstrated that organic acids at approx. pH 4.2 produced by LAB inhibit germination of spore forming bacteria (de Vuyst and Vandamme, 1994). Noticeably, during our study it was obvious that the royal jelly used when feeding the LAB to the larvae exacerbates a negative influence on the LAB, preventing their growth in less than 24 hours (data not shown) probably due to its low pH (4.0). However, the fact that addition of fresh LAB to the larval food significantly reduced the proportion of *P. larvae* infected larvae suggests that the larval gut conditions allow LAB to be antagonistic to the pathogen.

The results also demonstrate that not all LAB inhibit *P. larvae*, as the reference strain (*L. kunkeei*, CCUG 53901) showed only partial inhibition of the *P. larvae* strain ERIC III-genotype (Tab. II). It is known that LAB produce organic acids by their metabolism and a variety of antagonistic substances including metabolic end products, antibiotic-like substances and bactericidal proteins (de Vuyst and Vandamme, 1994). Furthermore, LAB possess different qualities at the species and strain level and some of these antagonistic qualities may be situated on unused genes or plasmids. Nevertheless, the 16S rDNA sequence of both the type strain of *L. kunkeei* and the honey stomach isolates *L. kunkeei* Fhon2 are exactly the same with no single nucleotide difference within the entire 16S rRNA gene (Olofsson and Vásquez, 2008). This means that the type strain and the isolates from the honey stomach are the same at the strain level regarding the 16S rRNA gene. However, there

were clear differences between these isolates when we investigated the entire chromosome and its protein production (unpublished data). The fact that the reference strain did not inhibit *P. larvae* may demonstrate that the inhibition properties of *L. kunkeei* are dependent on other factors than organic acids. Total inhibition of all four *P. larvae* strains seems to be achieved by a combination of the factors mentioned above such as type of metabolic end products, production of antibacterial peptides or bacteriocins and hydrogen peroxide, but further investigation is needed to document these products. It is obvious that the individual LAB phylotypes inhibit *P. larvae* strains differently (Tab. II). The results suggest that the entire LAB flora may work in a synergistic manner against *P. larvae* and possibly also on other deleterious microorganisms. The differences between LAB produced antimicrobial substances and the inhibition ability of single LAB members should be further investigated in vivo as we know that the LAB members differ in their metabolic pathway (unpublished data).

Earlier field observations demonstrate that *P. larvae* infections may be present in honey bee colonies without producing clinical symptoms, and may be reduced from detectable to non-detectable levels (Fries et al., 2006; Lindström and Fries, 2005). Actually, an earlier field study indicated an inhibition of *P. larvae* (ERIC I) from LAB when sampling honey bees obtained from a regular apiary in Sweden (Olofsson and Vásquez, 2008). Our results appear to shed light on these field observations since we demonstrate LAB inhibition of *P. larvae* growth on agar plates and decreased mortality in larvae from AFB when fed LAB. This suggests that the *P. larvae* inhibition of the honey stomach LAB obtained in this work may be functional also at the colony level in the field.

The ability to inhibit other honey bee pathogens should be further investigated to document the full extent of the probiotic properties of the LAB flora of honey bees. In addition, strains within *Lactobacillus* and *Bifidobacterium* possess properties for enhancing the immune response in humans (Nova et al., 2007) and in honey bees (Evans and Lopéz, 2004) which could further increase their importance to honey bee health. Furthermore, it is important to investigate if management practices, such as antibiotic administration, may influence the probiotic flora and potentially lower the threshold for disease establishment.

We can conclude that the newly identified probiotic *Lactobacillus* and *Bifidobacterium* phylotypes exhibit strong inhibitory effects on *P. larvae* growth, and can to some extent prevent symptom development of AFB in honey bee larvae infected by *P. larvae*.

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Une nouvelle bactérie lactique inhibant le développement de *Paenibacillus larvae* chez les larves d'abeille.

loque américaine / *Paenibacillus larvae* / bactérie lactique / *Lactobacillus* / *Bifidobacterium* / inhibition du développement

Zusammenfassung – Neue Milchsäurebakterien, die *Paenibacillus larvae* in Honigbienenlarven hemmen. Die Amerikanische Faulbrut (AFB) ist eine Krankheit, die junge Honigbienenlarven befällt. Sie ist eine der schädlichsten Bienenkrankheiten und hat große ökonomische Bedeutung für die Imkerei weltweit. Der Erreger der AFB ist das sporenbildende Bakterium *Paenibacillus larvae*, das den Mitteldarm junger Larven durch kontaminiertes Futter befällt. Die Besiedelung des larvalen Mitteldarms stellt einen der Schlüsselfaktoren für die Pathogenese von *P. larvae* dar und bestimmte Zusammensetzungen der Mikroflora des Darmes könnten das Wachstum des Krankheitserregers unterdrücken. Kürzlich wurden eine neue Flora von Milchsäurebakterien (LAB) der Gattungen *Lactobacillus* und *Bifidobacterium* aus dem Honigmagen der Bienen beschrieben. LAB sind zwar bekannt für die Produktion von antimikrobiellen Substanzen, jedoch gibt es Variationen bezüglich der nutzbringenden Eigenschaften zwischen Arten und Gattungen. In dieser Untersuchung wurde der antagonistische Effekt der Honigbienen-LAB auf *P. larvae* beurteilt. Wir verwendeten Hemmtests auf Agarplatten

und Biotests mit Honigbienenlarven, um diese Effekte zu untersuchen. Die individuellen LAB-Phylotypen zeigten unterschiedliche Hemmeigenschaften gegenüber auf Agarplatten wachsenden *P. larvae*, während eine Kombination aller 11 LAB-Phylotypen sogar eine totale Hemmung (kein sichtbares Wachstum mehr) von *P. larvae* bewirkte. Eine Zugabe des LAB-Mix zum Larvenfutter reduzierte signifikant die Anzahl an AFB-infizierten Larven im Biotest.

Die Ergebnisse zeigen, dass die für Honigbienen spezifischen LAB nutzbringende Eigenschaften für die Bienengesundheit besitzen. Der mögliche Nutzen einer Applikation von LAB in Bienenvölkern sollte untersucht werden.

Amerikanische Faulbrut / *Paenibacillus larvae* / Milchsäurebakterien / *Lactobacillus* / *Bifidobacterium* / Hemmung

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