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Research Article

## Antibacterial Activity of Bees Gut *Lactobacilli* against *Paenibacillus Larvae In Vitro*

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### Abstract

The aim of this study was to evaluate antimicrobial activity of bees gastrointestinal *Lactobacillus* spp. of against *Paenibacillus larvae*. Content of the intestinal tract was cultured for isolation of *Lactobacillus* spp. Gut homogenates were plated on de Man, Rogosa and Sharpe agar (MRS, Oxoid) plates and incubated for 48-72h at 30°C anaerobically. Then, the identification of isolates with MALDI-TOF MS Biotyper was done. The bacterial strains *Lactobacillus gasseri*, *L. amylovorus*, *L. kunkeei*, *L. fructivorans*, *Paenibacillus larvae* were isolated from gut content of bees. The disc diffusion method was used for the determination of antimicrobial activities of the *Lactobacillus* supernatant against two strains of *Paenibacillus larvae*. The best antimicrobial activity of *Lactobacillus* against *Paenibacillus larvae* from gut was found in *L. gasseri* supernatant. Lesser degree of antimicrobial activity against *P. larvae* was found in *L. kunkeei* supernatant. The strongest antibacterial activity against *P. larvae* CCM 4438 was found in *L. gasseri* and *L. amylovorus* and the least antibacterial activity was found in *L. fructivorans*.

**Keywords:** *in vitro*, antibacterial effect, bees gut microflora, *Paenibacillus larvae*.

### Introduction

*Paenibacillus larvae* is a Gram-positive, spore forming pathogen that causes the American foulbrood (AFB) in honey bee [1, 2]. *P. larvae* may form spore or exists as a vegetative cell depending on environmental conditions. For example, *P. larvae* forms resistant spores under nutrient deprivation with viability as long as 70 year [3]. *P. larvae* spores germinate only in the gut of honey bee larva host and pathogen needs

the honey bee larvae to complete the life cycle [2]. Newly germinated *P. larvae* proliferate with extreme bacteremia and death of the first or second instar larvae several days after infection [4]. *P. larvae* re-sporulate after consumption with formation of billions of spores, which are infectious spores are could be spread within the colonies by bees and beekeeping practices [5]. Gut bacteria stimulate the immunity of honey bee larvae, the stage, where organism is vulnerable to infection by different pathogens, and promote mounting of anti-pathogen immune responses [6]. Addition of probiotic bacteria into the larval food of honey bee led to the decreased the number of infected larvae with *Paenibacillus larvae* [7]. The aim of this study was to evaluate the effect of

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isolated gut bacteria from local honey bee *Apis mellifera* of Slovakia against two different strains of *Paenibacillus larvae*.

## Material and Methods

### Collection of *Apis mellifera* and isolation of gut bacteria

Colonies of *Apis mellifera* from wooden hives in an apiary at the the East Slovakia in July 2017 were studied. Foragers (n=10) and returning workers (n=10) of *A. mellifera* from each colony were aseptically taken at hive entrances and put into a 1.5 ml sterile tubes. For removal of gut, the digestive tracts were aseptically dissected, transferred into a tube with physiological solution and homogenized. Then the material was plated onto de Man, Rogosa and Sharpe agar (MRS, Oxoid) and incubated for 48-72h at 30°C. Bacterial colonies were identified according to size, colour and morphology [8].

### Sample preparation and MALDI-TOF MS measurement

Prior to identification, the bacterial colonies were subcultured on Nutrition agar (Oxoid) at 37°C for 18-24 h. One colony of each bacterial isolate was selected for screening. Subsequently, an analysis of the bacteria identification was performed using the Maldit TOF MS Biotyper described by Kluga et al. [9].

### Bacterial strains for testing

The bacterial strains *Lactobacillus gasseri*, *L. amylovorus*, *L. kunkeei*, *L. fructivorans*, *Paenibacillus larvae* were isolated from gut microflora of bees and *Paenibacillus larvae* CCM 4438 was collected from the Czech Collection of microorganisms (Brno, Czech Republic).

### Detection of antibacterial activity of lactobacilli

Culture of *Lactobacillus* after 24h of incubation in MRS broth were centrifuged at 5500g for 10 min at 4°C and 0.1 ml of the supernatant was used for detection of antibacterial activity. The suspension of tested *Paenibacillus larvae* in saline (0.1 ml of 10<sup>5</sup> cfu/ml) was spread on Mueller Hinton Agar (MHA, Oxoid). Filter paper discs (6 mm in diameter) were impregnated with 15 µl of supernatant and placed on the inoculated agars. Agars were incubated at 4°C for 2h and at 37°C for 24 h. All the tests were performed in triplicate. Filter discs impregnated with a 10 µl of distilled water were used as the negative, but antibiotic (amikacin 10 µg and gentamicin 10 µg) as the positive control.

## Statistical analyses

For *Lactobacillus* and *Paenibacillus* the mean and standard deviation of inhibition zones were calculated.

## Results and Discussions

The composition of the gut bacterial communities of social insects has been shaped by coevolution. The social behaviour of honeybees provides favourable conditions for the exchange of the symbiont microbes [10] and these microorganisms are efficiently transmitted between bee colony members and their different generations. The gut microbial composition of insects, and especially honeybees, is unique, harbouring highly genetically diverse, niche-adapted bacterial species [11]. The model of symbiosis between honey bees and their bacterial microflora could inspire ideas of how to exploit this microflora for the protection of the host's health. The question of whether endogenous bacterial symbionts suppress honeybee pathogenic bacteria has been asked and analyzed for many times. However, no direct or clear evidence supporting this hypothesis has ever been found. The use of exogenous lactic acid bacteria (LAB) as an alternative active flora has also been proposed [12,13]. Particular attention has been paid to testing exogenous LAB strains that produce bacteriocins. The mortality of *in vivo* infected bee larvae was studied previously to investigate whether the gut bacteria (*L. kunkeei*, *P. mirabilis*, *E. kobei*, *M. morgani*, *F. fructosus*, *B. licheniformis*, and *B. subtilis*) are capable of reducing the lethal effects of *P. larvae* infection [14].

In bees gut, four bacterial strains of *Lactobacillus* were isolated (*Lactobacillus gasseri*, *L. amylovorus*, *L. kunkeei*, *L. fructivorans*). From one bees colony *Paenibacillus larvae* was isolated. The scores of isolated bacteria showed in Table 1.

**Table 1**  
MALDI-TOF scores of isolated microorganisms

Isolated bacteria	Score
<i>Lactobacillus gasseri</i>	2.174
<i>Lactobacillus amylovorus</i>	2.206
<i>Lactobacillus kunkeei</i>	2.283
<i>Lactobacillus fructivorans</i>	2.076
<i>Paenibacillus larvae</i>	2.416

The antibacterial activity of *Lactobacillus* against bees gut *Paenibacillus larvae* is shown in Table 2.

Table 2

Antimicrobial activity of *Lactobacillus* supernatant against *Paenibacillus* larvae

Isolated bacteria	<i>Paenibacillus larvae</i> from gut	<i>Paenibacillus larvae</i> CCM4438
<i>Lactobacillus gasseri</i>	6.67±1.53	5.67±1.53
<i>Lactobacillus amylovorus</i>	6.00±1.00	5.67±2.08
<i>Lactobacillus kunkeei</i>	5.00±1.00	5.33±0.58
<i>Lactobacillus fructivorans</i>	5.33±1.53	5.00±1.00
Amikacin	10.97±0.49	10.37±0.67
Gentamicin	7.83±0.55	7.53±0.06

The maximal antimicrobial activity was found for *L. gasseri* supernatant (6.67±1.53 mm). The minimal antimicrobial activity against *P. larvae* was found for *L. kunkeei* supernatant (5.00±1.00 mm). The strongest antibacterial activity against *P. larvae* CCM 4438 was found for *L. gasseri* and *L. amylovorus* (5.67±1.53 resp. 5.67±2.08 mm) and the weakest antibacterial activity for *L. fructivorans* supernatant (5.00±1.00 mm).

Our results were in accordance with Forsgren et al. [7], who demonstrated that the LAB, including *L. kunkeei* from honey bee gut, had strong inhibitory effect on *in vitro* growth of *P. larvae*.

### Conclusions

Probiotic effects of four guts *Lactobacillus* isolated from the local honey bees of Slovakia were evaluated for their inhibitory activity against *P. larvae*. *Lactobacillus gasseri*, *L. amylovorus*, *L. kunkeei* and *L. fructivorans* can influence the growth of *P. larvae* isolated from bee. Bacteria belonging to *Lactobacillus* and some of their metabolites are getting significant importance in apiculture.

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