OP-110 [Bee Health]
Newly isolated bacteria increase the survival of honeybee larvae infected with Paenibacillus larvae

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Honeybees are essential pollinators while contributing to agriculture. Some risk factors, including pathogens, parasites, pesticides, and a reduction in pollen availability, affect the health of honeybees. American foulbrood (AFB) is a devastating bacterial disease affecting honeybee colonies caused by Paenibacillus larvae. Honeybee gut core microorganisms were usually selected and applied to inhibit various honeybee diseases including AFB. However, there were still many honeybee gut-related non-core bacteria with probiotic properties that have not been explored, and they show great application potential in the control of honeybee diseases. In this study, two strains 74-8 and FB-1 with inhibitory effects on P. larvae were isolated from the whole gut of honeybees, and the multiple probiotic properties and safety in honeybee larvae were tested to assess their potential use in the prevention of AFB. Leuconostoc citreum 74-8 and Latilactobacillus sakei FB-1 could inhibit P. larvae by producing acid in vitro and increased the survival rates of larvae infected with P. larvae by approximate 30 % in vivo. L. citreum 74-8 and L. sakei FB-1 could colonize honeybee larvae and significantly increase the abundance of dominant microorganisms. Meanwhile, L. citreum 74-8 and L. sakei FB-1 can also modulate the transcription of antimicrobial peptides (AMPs) apidaecin, abaecin, hymenoptaecin in honeybee larvae infected with P. larvae. L. citreum 74-8 and L. sakei FB-1 from honeybee guts exhibited probiotic characteristics and effectively antagonized AFB pathogen P. larvae through multiple mechanisms. In conclusion, this study presents valuable findings that contribute to the understanding of probiotic applications in honeybee health management and highlight a new strategy for the control of AFB.

Newly isolated bacteria increase the survival of honey bee larvae infected with *Paenibacillus larvae*

Reporter: Li Zhang

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Part 1

Background

Self Introduction

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Research field: bee health

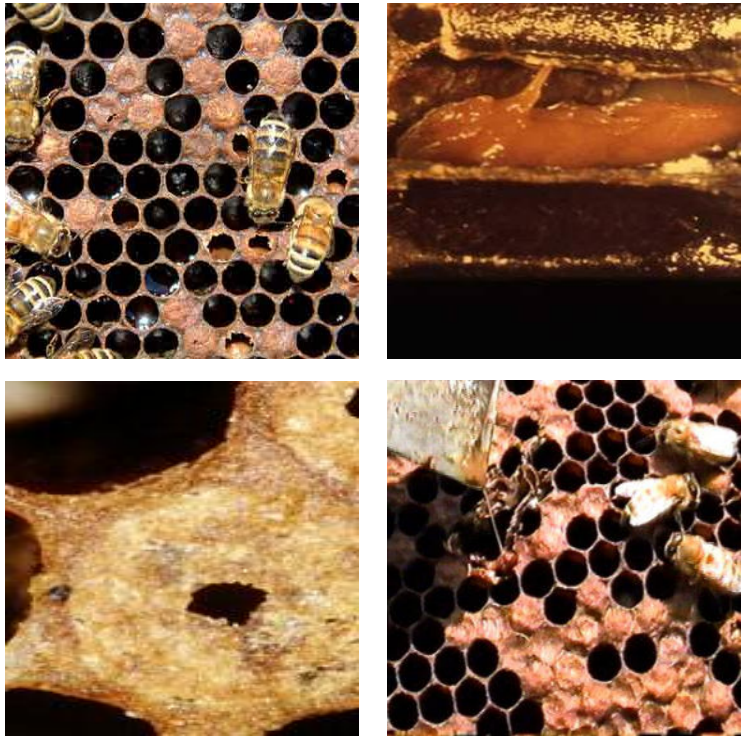
gut microorganism

Institute of apicultural research,

Chinese academy of agricultural sciences (IAS, CAAS)

1. Background

Pests of honye bee
(Evans & Schwarz. 2011)



American foulbrood disease (AFB)

Many pathogens affect the health of *Apis mellifera*

AFB is difficult to control as the pathogen produces resistant spores

1. Background

Strains	Source	References
Fructobacillus fructosus KY027123	Honey bee gut	
Proteus mirabilis KY027132	Honey bee gut	(AL-GHAMDI et al., 2018)
Bacillus licheniformis KY027142	Honey bee gut	
Enterobacter kobei KY027178	Honey bee gut	
Morganella morganii KY027186	Honey bee gut	
Lactobacillus reuteri ATCC23272	Commercial strain	(BABRUD et al., 2019)
Lactobacillus plantarum Lp39	Commercial strain	
Lactobacillus rhamnosus GR-1	Commercial strain	(DAISLEY et al., 2020)
Lactobacillus kunkeei BR-1	Honey bee hive	
Lactiplantibacillus plantarum P8	Honey bee gut	
L. plantarum P25	Honey bee gut	(IORIZZO et al., 2020b)
L. plantarum P100	Honey bee gut	
Bacillus megaterium	Commercial strain	(NOWOTNIK et al., 2024)
Bacillus pumilus	Commercial strain	
Bacillus licheniformis	Commercial strain	
Enterococcus lactis GL3	Larvae	(YE et al., 2025)

- The **probiotic properties** of bacteria:

  producing acid and antibacterial substance

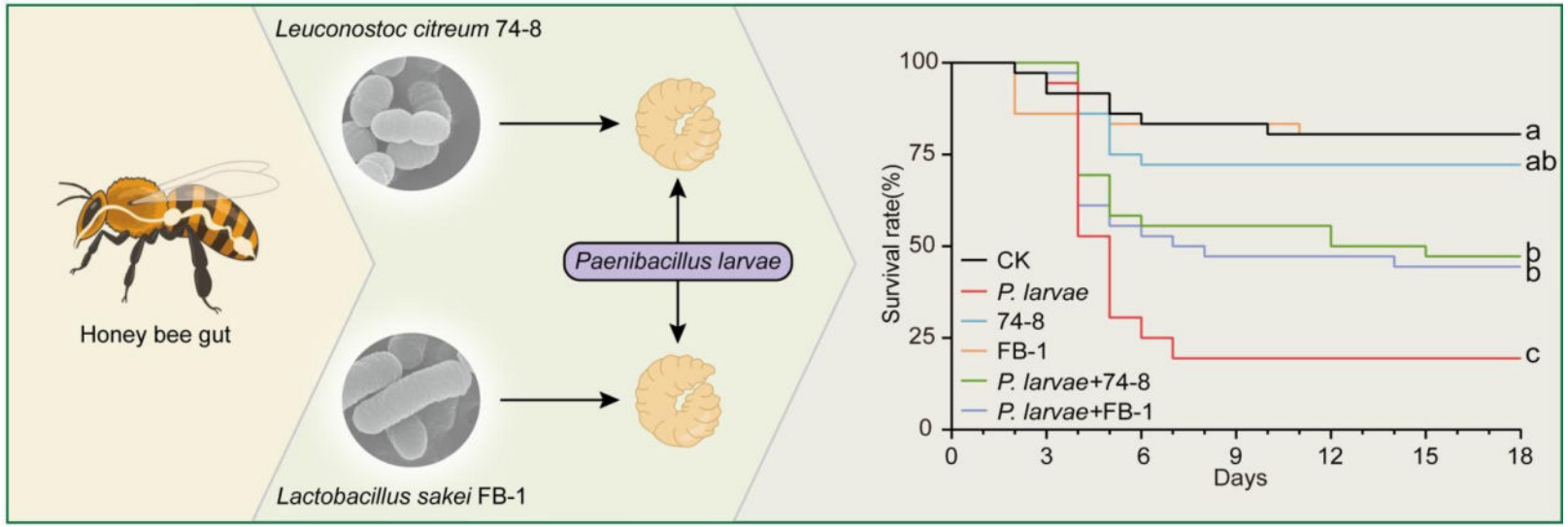
  auto-aggregation, co-aggregation and cell surface hydrophobicity

- The **interaction** between probiotics and honey bee still remains unclear

Bacteria, especially the probiotics from different resources show great potential in inhibiting AFB.

Purpose

Select bacteria with multiple **probiotic properties** from honey bee gut to inhibit AFB and protect honey bee health.

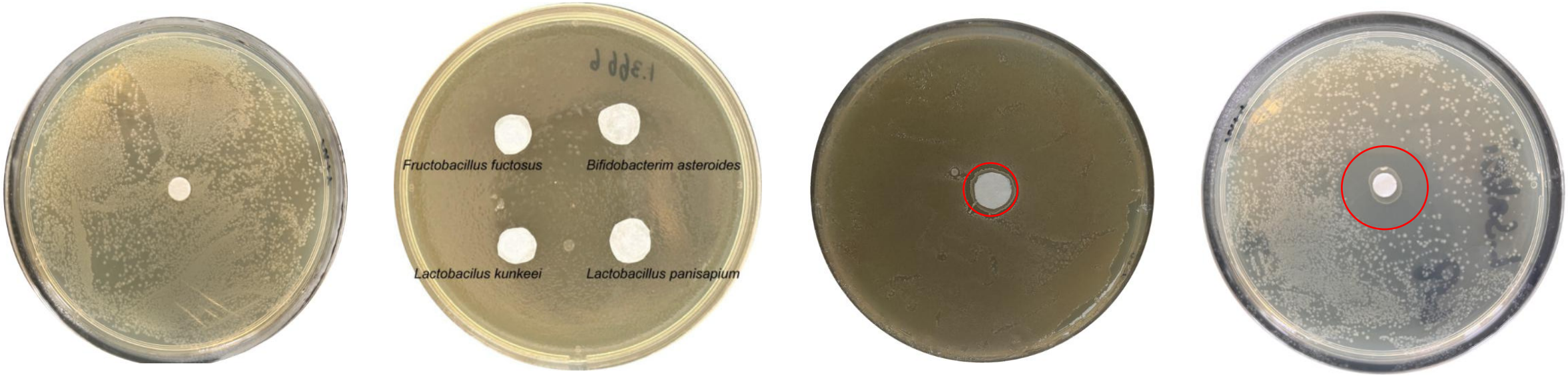


- Inhibit *Paenibacillus larvae in vivo*
- Determine the effectiveness of inhibiting AFB
- Detect probiotic properties
- Test safety to honey bee

Part 2

Results

2.1 Selecting strains that inhibit *P. larvae*

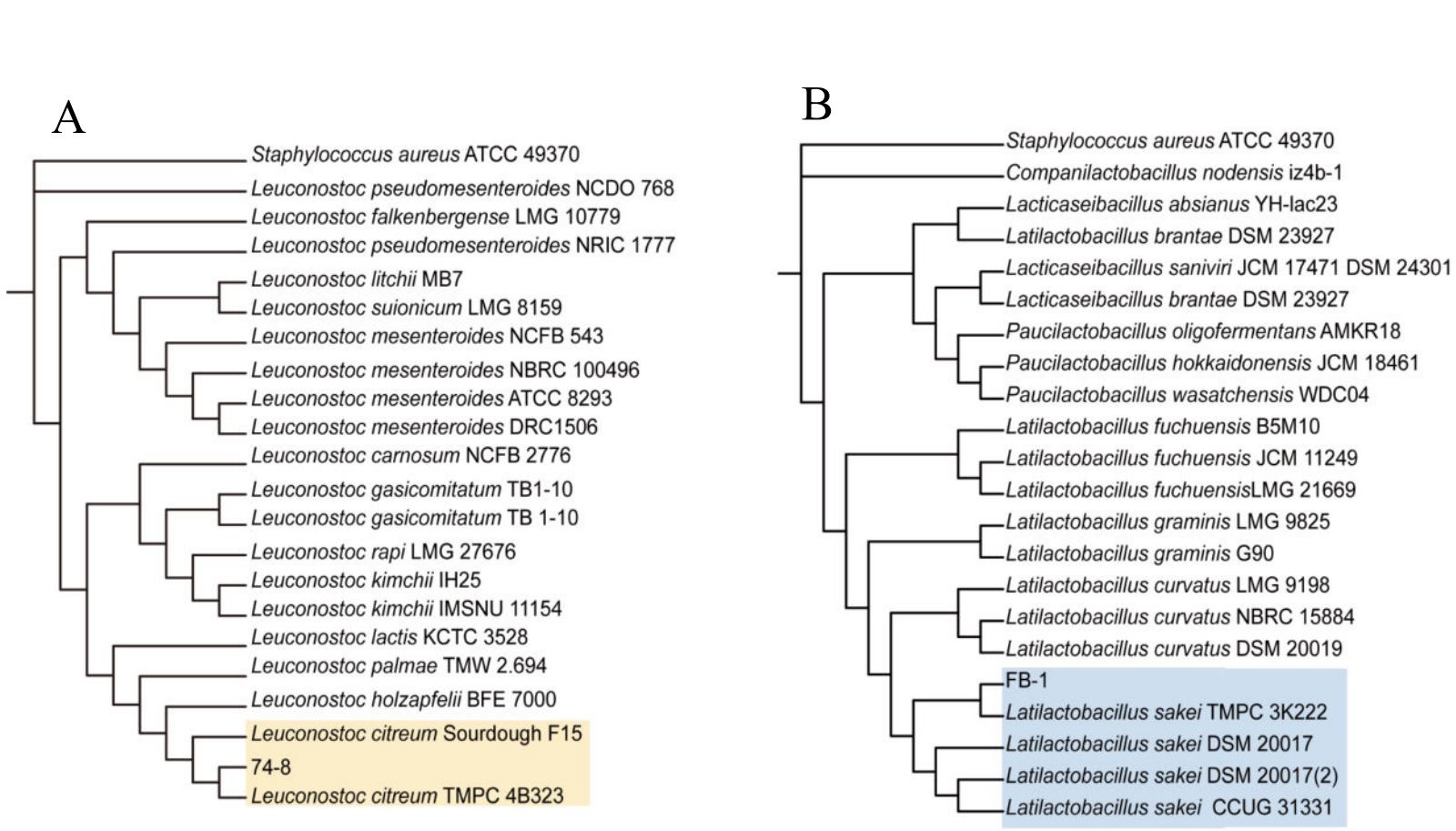


Control | Gut bacteria | 74-8 | FB-1

Inhibition of *P. larvae in vitro*

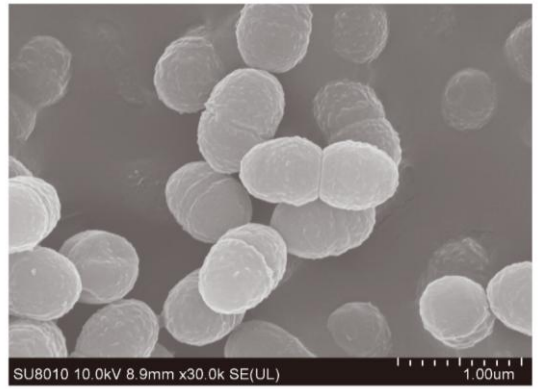
From several gut bacteria, strains 74-8 and FB-1with antimicrobial activity against *P. larvae* were selected. Their inhibition diameters were 8.31 mm and 27.99 mm, respectively.

2.2 Identification of 74-8 and FB-1



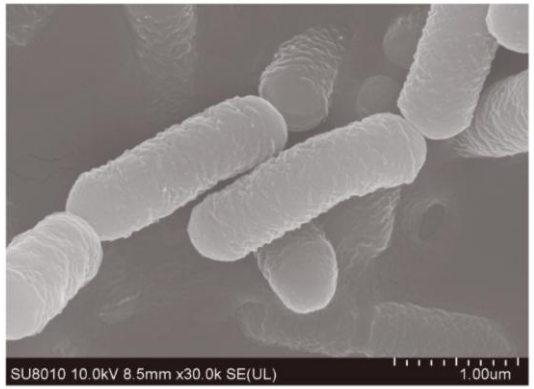
Phylogenetic tree of 74-8 and FB-1

C



Leuconostoc citreum 74-8

D

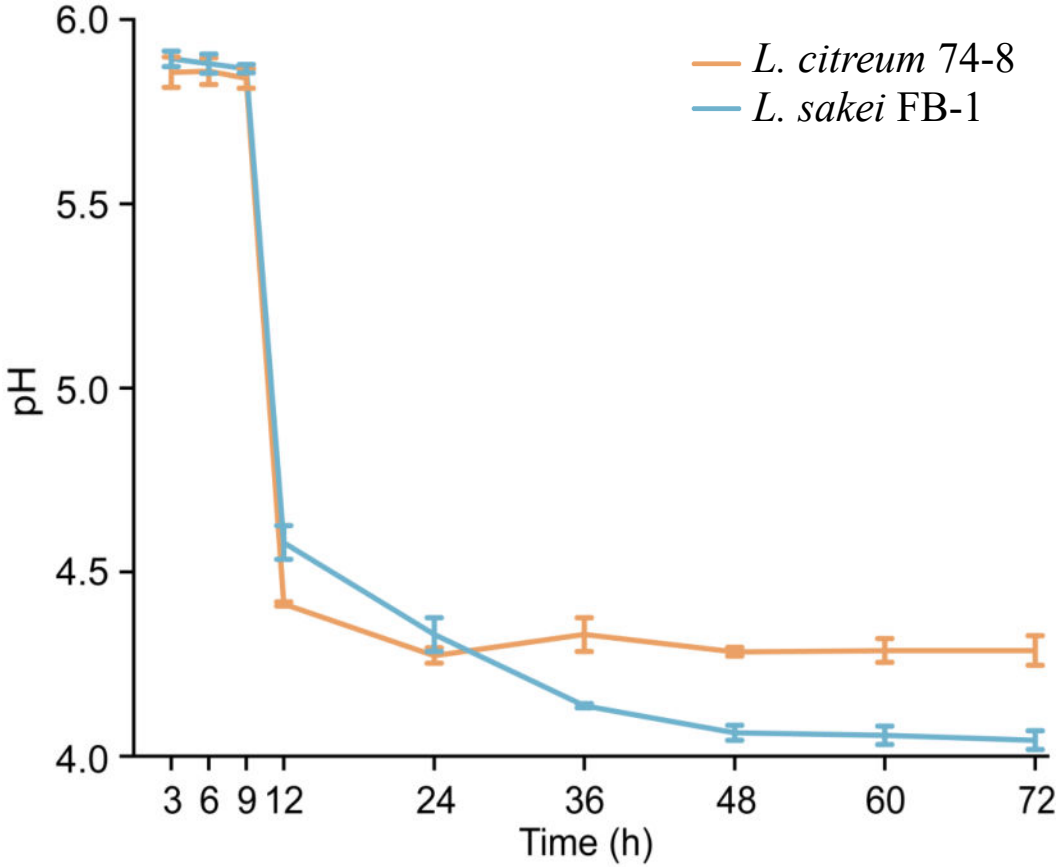


Latilactobacillus sakei FB-1

SEM images

74-8 and FB-1 were identified as *Leuconostoc citreum* and *Latilactobacillus sakei*, respectively.

2.3 Detect probiotic properties： produce acid



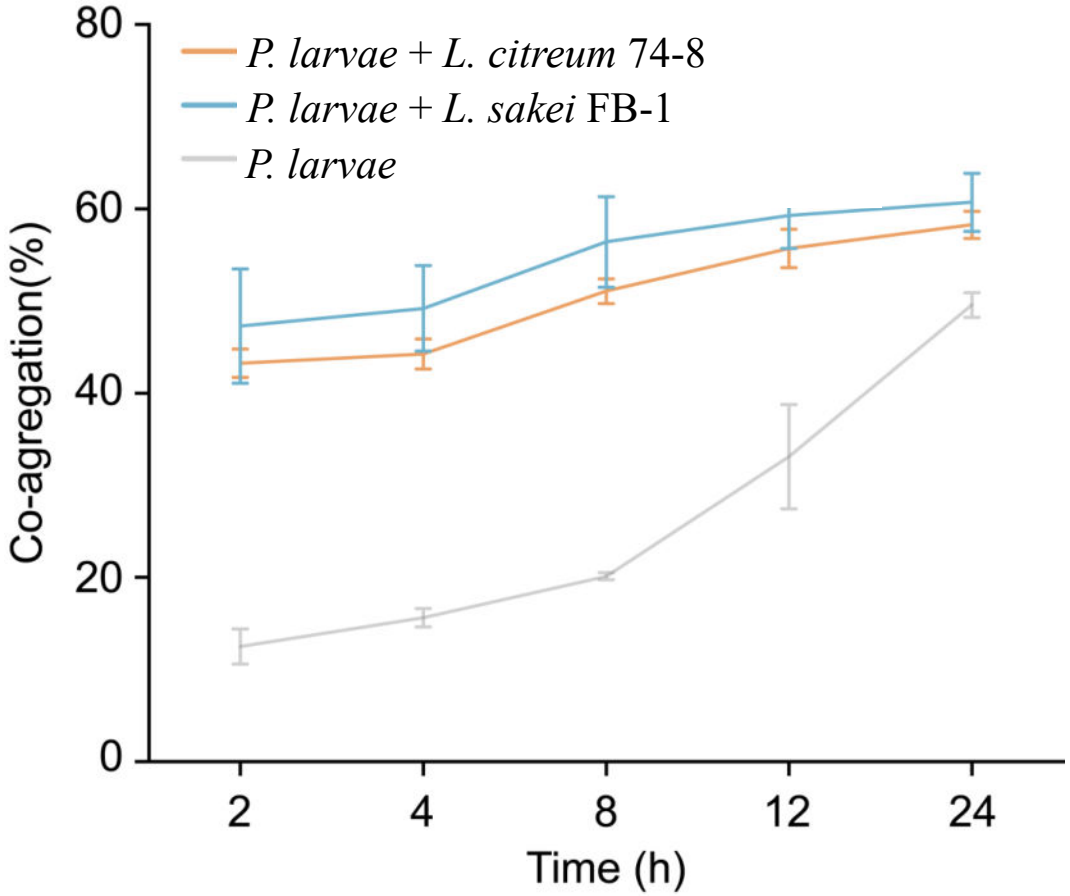
pH of *L. citreum* 74-8 and *L. sakei* fermentation broth

- Acid is thought to be important for the inhibition of *P. larvae*.
- Both *L. citreum* and *L. sakei* showed excellent acid-producing capabilities.

The pH values of *L. citreum* 74-8 and *L. sakei* FB-1 steadily decreased over a period of 72 h.

At the 72nd hour, the pH of *L. citreum* 74-8 and *L. sakei* FB-1 were 4.28 and 4.04 respectively.

2.3 Detect probiotic properties： co-aggregation



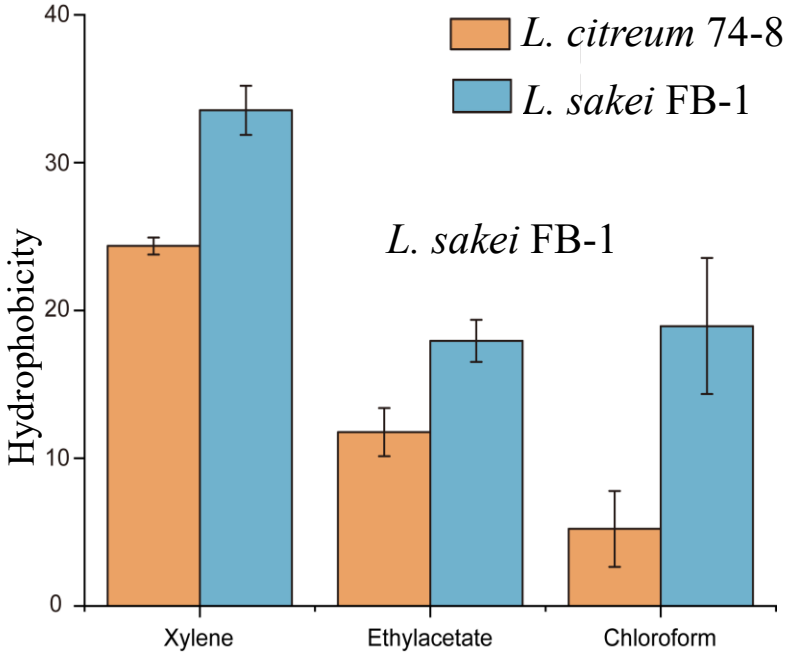
Co-aggregation of *L. citreum* 74-8 and *L. sakei* FB-1 with *P. larvae*

Co-aggregation characteristics suggest that bacteria may be able to form a physical barrier prior to pathogen colonization and thus interfere in this process.

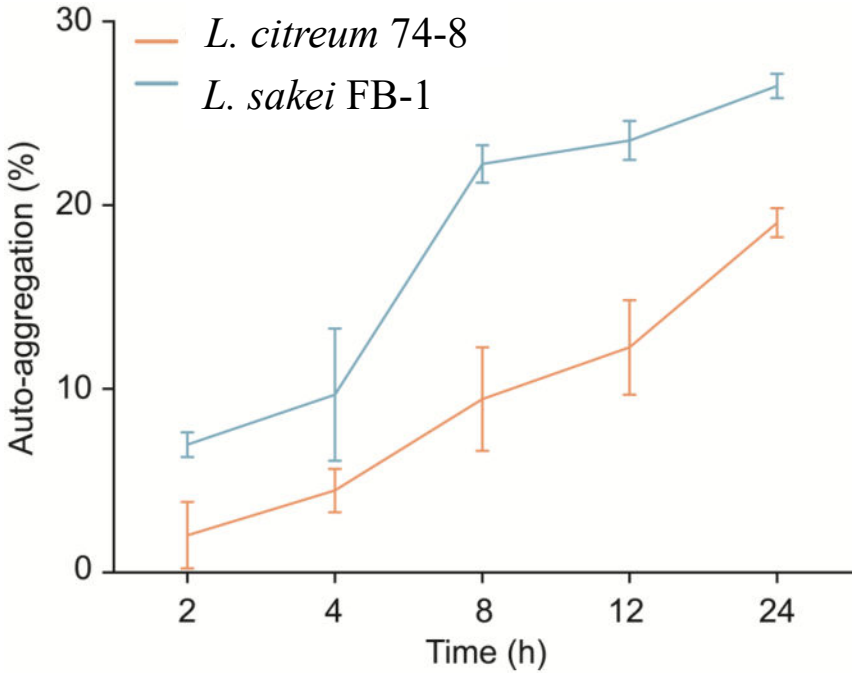
L. citreum 74-8 and *L. sakei* FB-1 might serve as a potential interfering agent for pathogenic bacteria to infest the intestinal tract in honey bees.

Both *L. citreum* 74-8 and *L. sakei* FB-1 exhibited the ability to co-aggregate with *P. larvae*.

2.3 Detect probiotic properties : hydrophobicity and auto-aggregation



Hydrophobicity *L. citreum* 74-8 and *L. sakei* FB-1



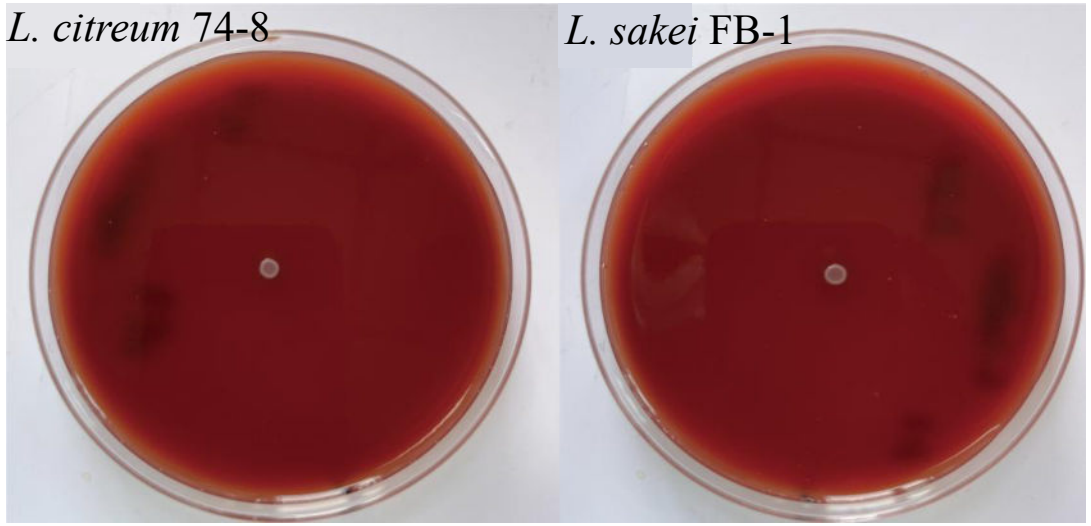
Auto-aggregation of *L. citreum* 74-8 and *L. sakei* FB-1

- Adhesion capacity of probiotics correlates with hydrophobicity and auto-aggregation.

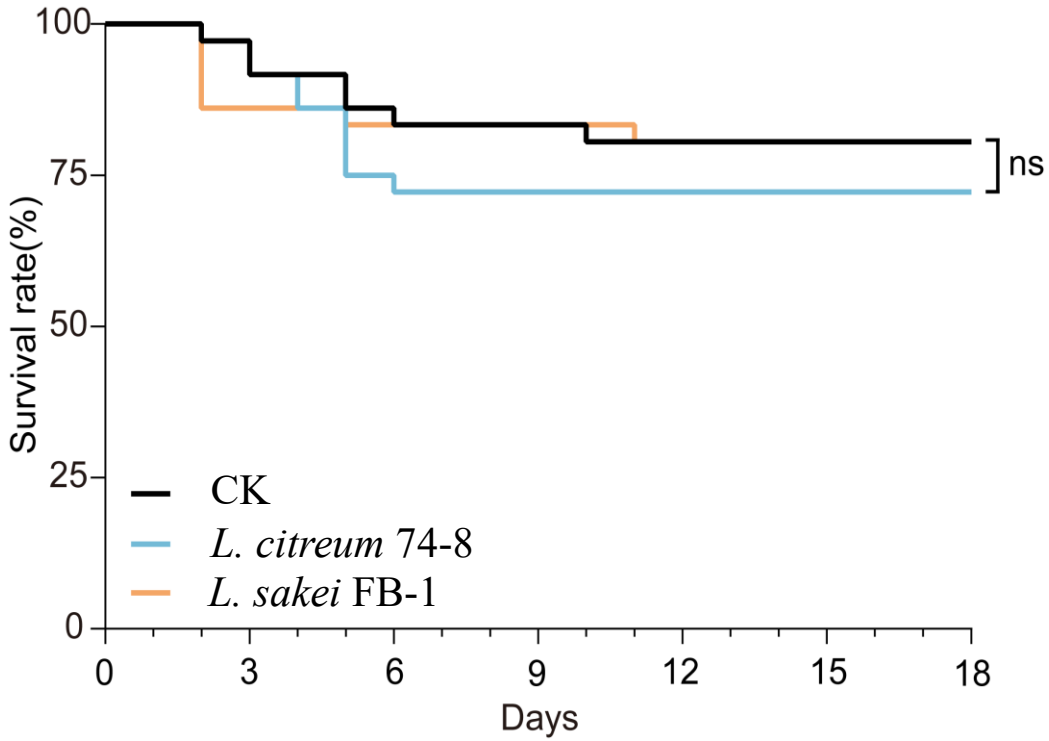
L. citreum 74-8 and *L. sakei* FB-1 possess hydrophobic and auto-aggregation properties, so they show adhesive capabilities and were able to colonize in gut.

L. citreum 74-8 and *L. sakei* FB-1 were hydrophobic. Their auto-aggregation progressively increased over time, *L. sakei* FB-1 showed higher auto-aggregation ability.

2.4 Test safety to honey bee



Hemolytic activity of *L. citreum* 74-8 and *L. sakei* FB-1

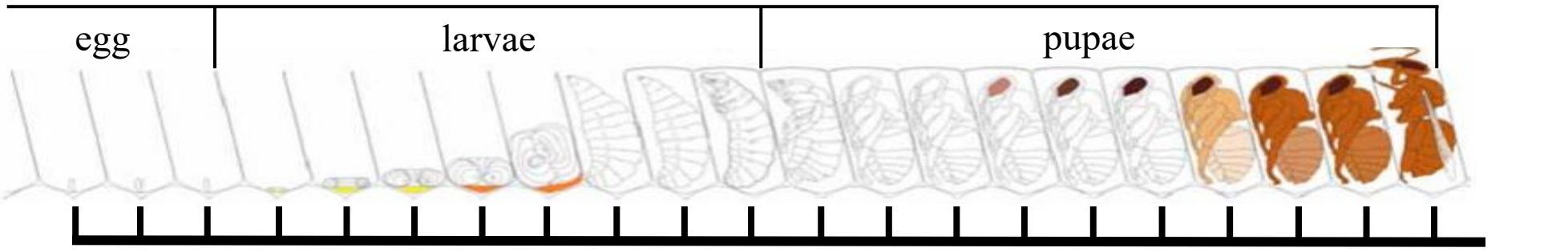


The survival rates of the larvae fed with *L. citreum* 74-8 and *L. sakei* FB-1

- *L. citreum* 74-8 and *L. sakei* FB-1 were not hemolytic.
- The survival rates of the larvae fed with *L. citreum* 74-8 or *L. sakei* FB-1 were not different from those of the control group.
- *L. citreum* 74-8 and *L. sakei* FB-1 were identified as safe strains.

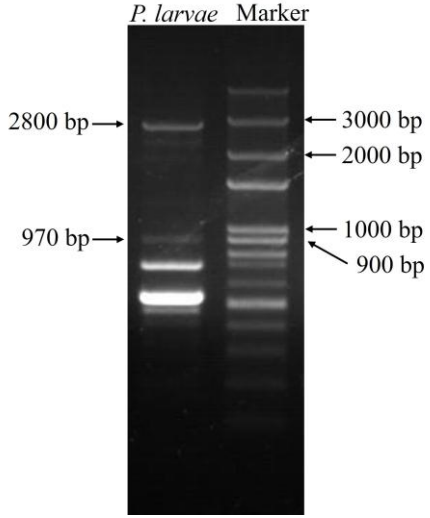
L. citreum 74-8 and *L. sakei* FB-1 demonstrate great potential in inhibiting AFB

2.5 Design of experiments



Oral administration of *P. larvae*, *L. citreum* 74-8 and *L. sakei* FB-1

Weighed and transferred to new cultural plates

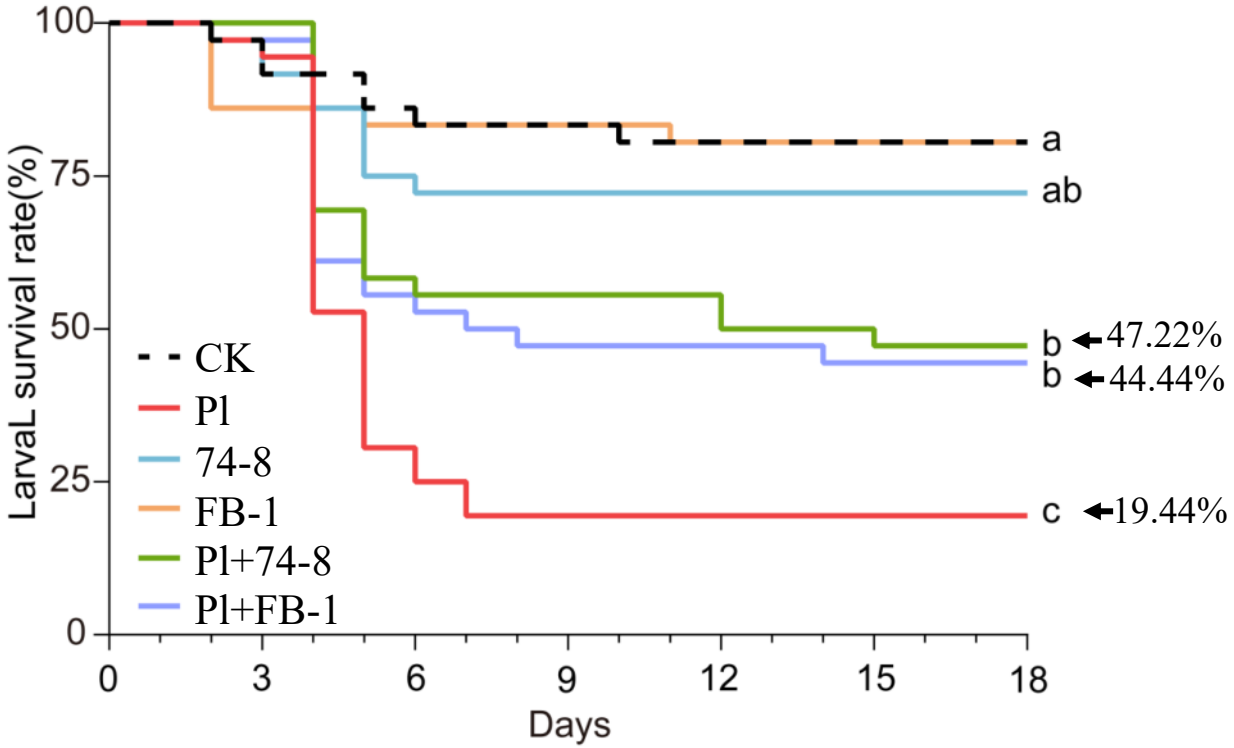


Genotyping of *P. larvae* by rep-PCR

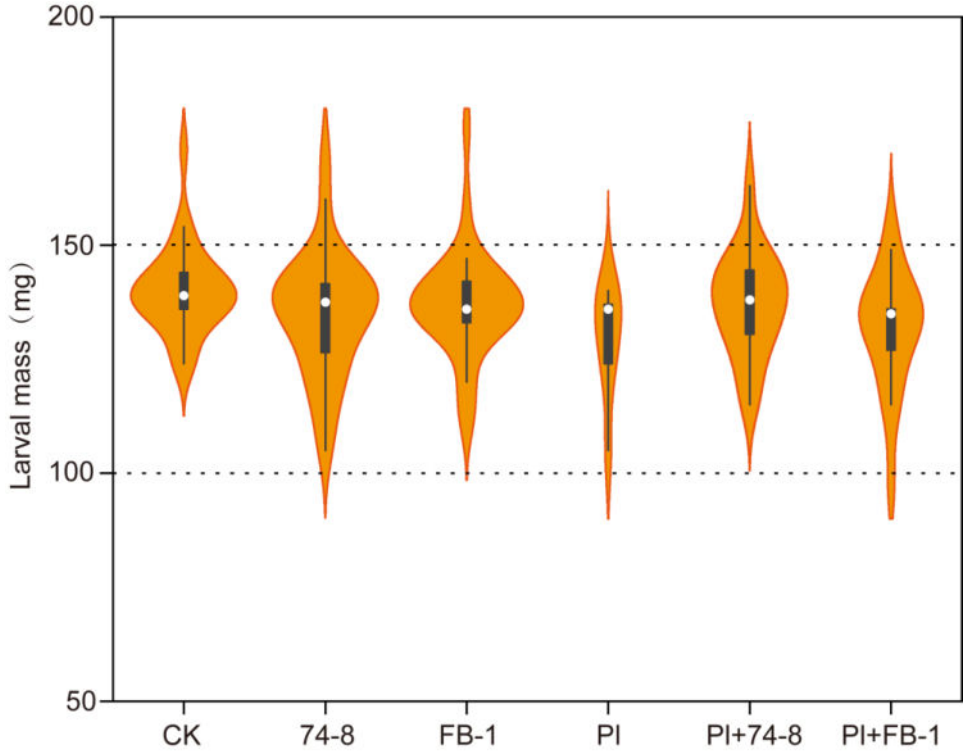
P. larvae larvae-specific ERIC pattern II

No.	Diets with different treatments	
1	Normal	CK
2	Spores of *P. larvae*	Pl
3	*L. citreum* 74-8	74-8
4	*L. sakei* FB-1	FB-1
5	Spores+*L. citreum* 74-8	Pl+74-8
6	Spores+*L. sakei* FB-1	Pl+FB-1

2.6 Inhibit AFB in the larvae



The survival rate of the larvae fed with *P. larvae*, *L. citreum* 74-8 and *L. sakei* FB-1

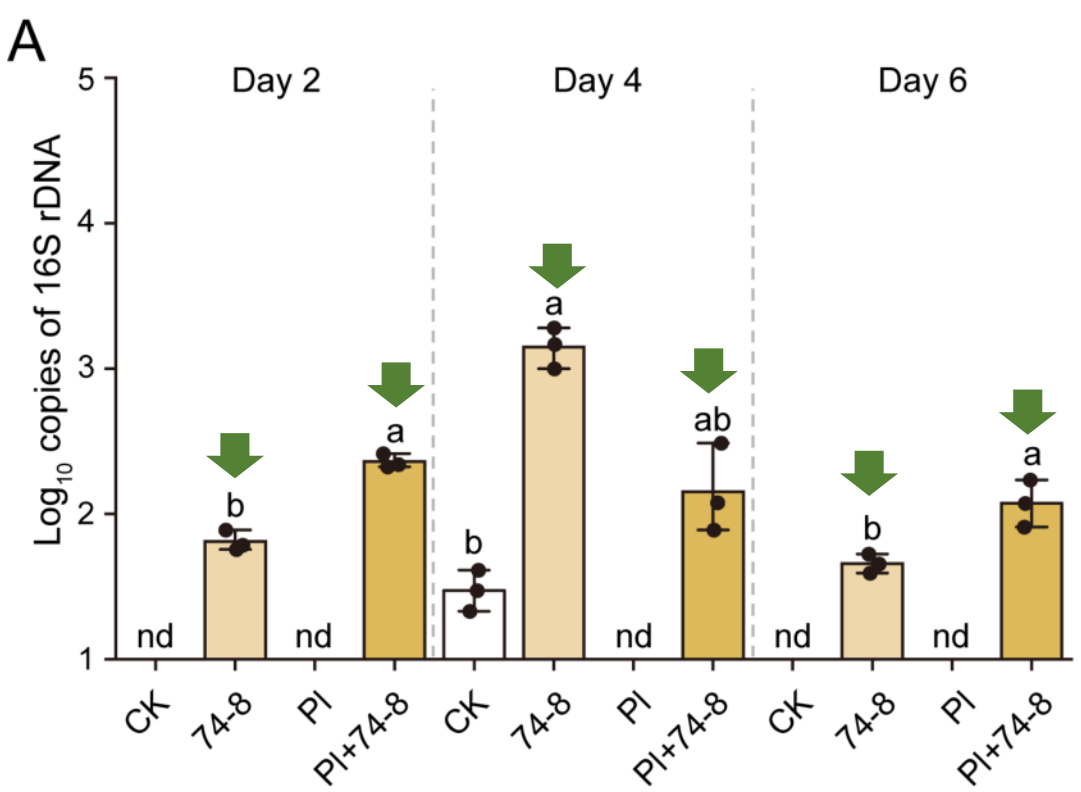


The weight of larvae on day 7

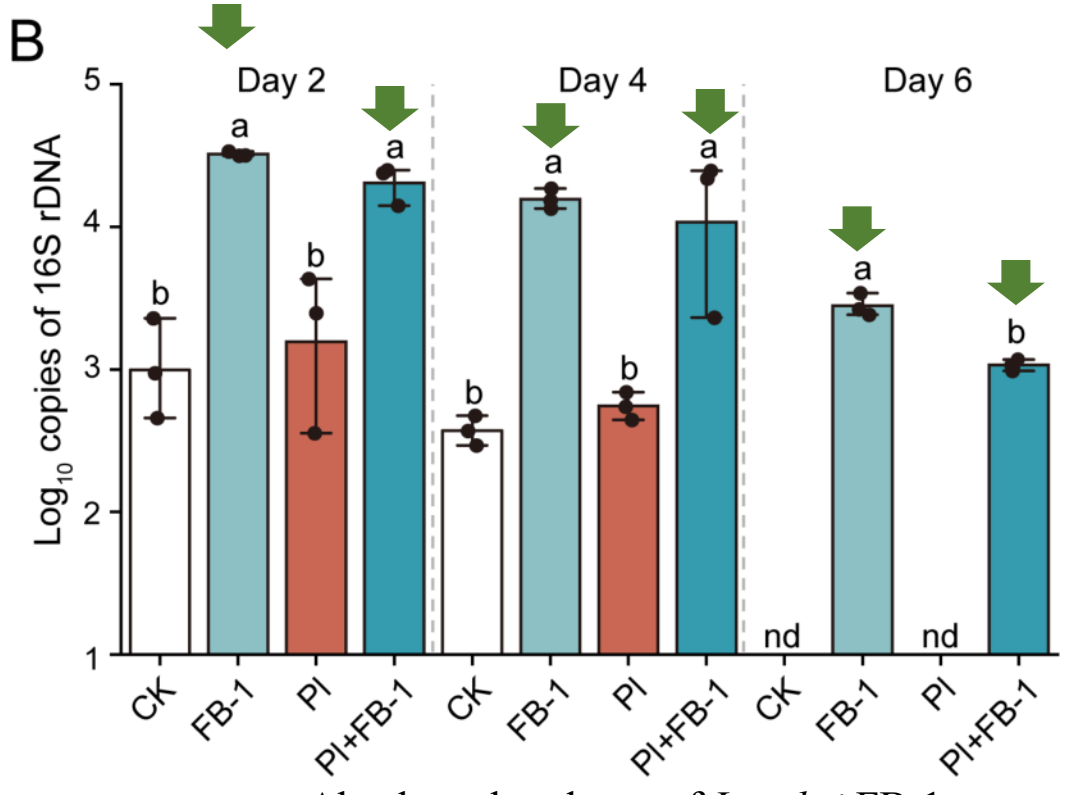
- Addition of *L. citreum* 74-8 or *L. sakei* FB-1 significantly increased the survival of honey bee larvae infected with *P. larvae*.
- There was no significant difference in larval weight among groups.

***L. citreum* 74-8 and *L. sakei* FB-1 can inhibit the AFB in the larvae.**

2.7 The abundance of *L. citreum* 74-8 and *L. sakei* FB-1



Absolute abundance of *L. citreum* 74-8

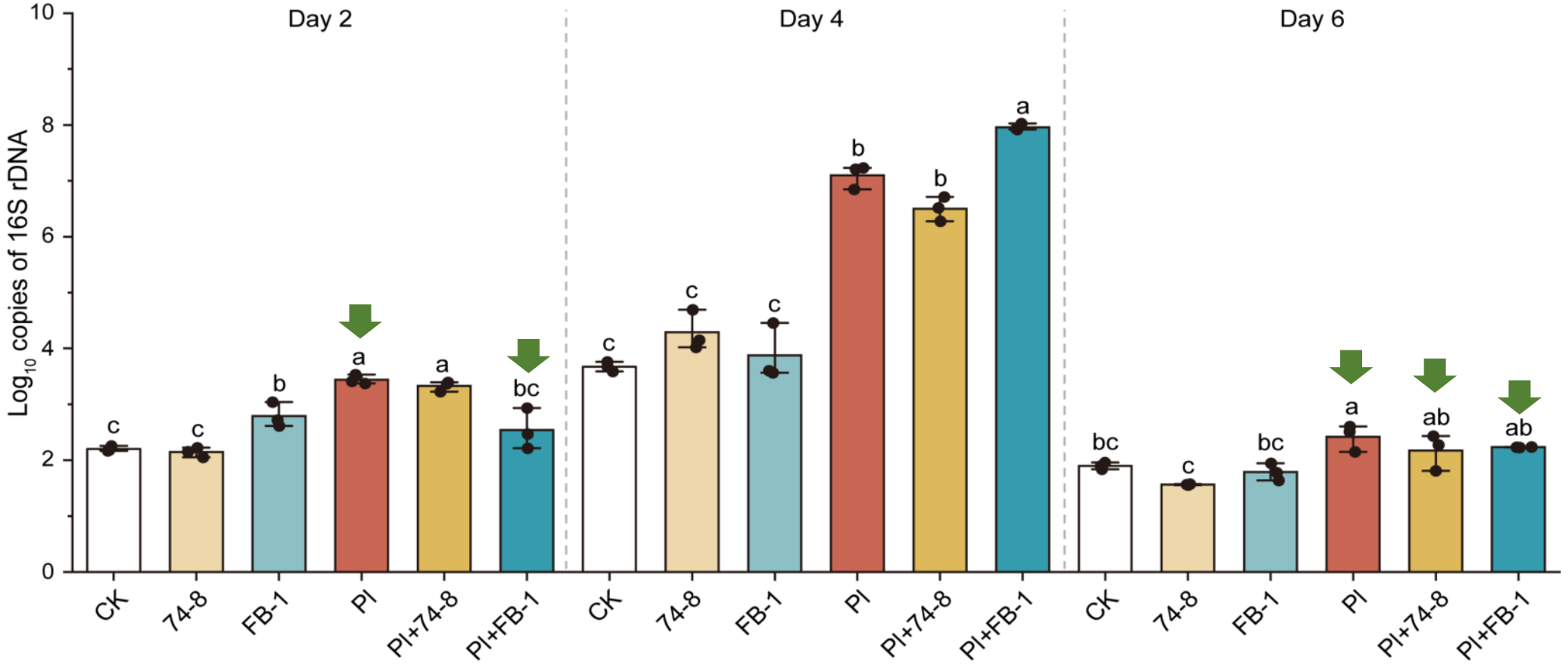


Absolute abundance of *L. sakei* FB-1

Honey bee larvae supplemented with *L. citreum* 74-8 or *L. sakei* FB-1 presented a significantly greater abundance of *L. citreum* 74-8 or *L. sakei* FB-1 than those of CK and Pl groups on day 2, day 4 and day 6.

L. citreum 74-8 and *L. sakei* FB-1 can colonize in the larvae, which is consistent with the results of self-aggregation and hydrophobicity.

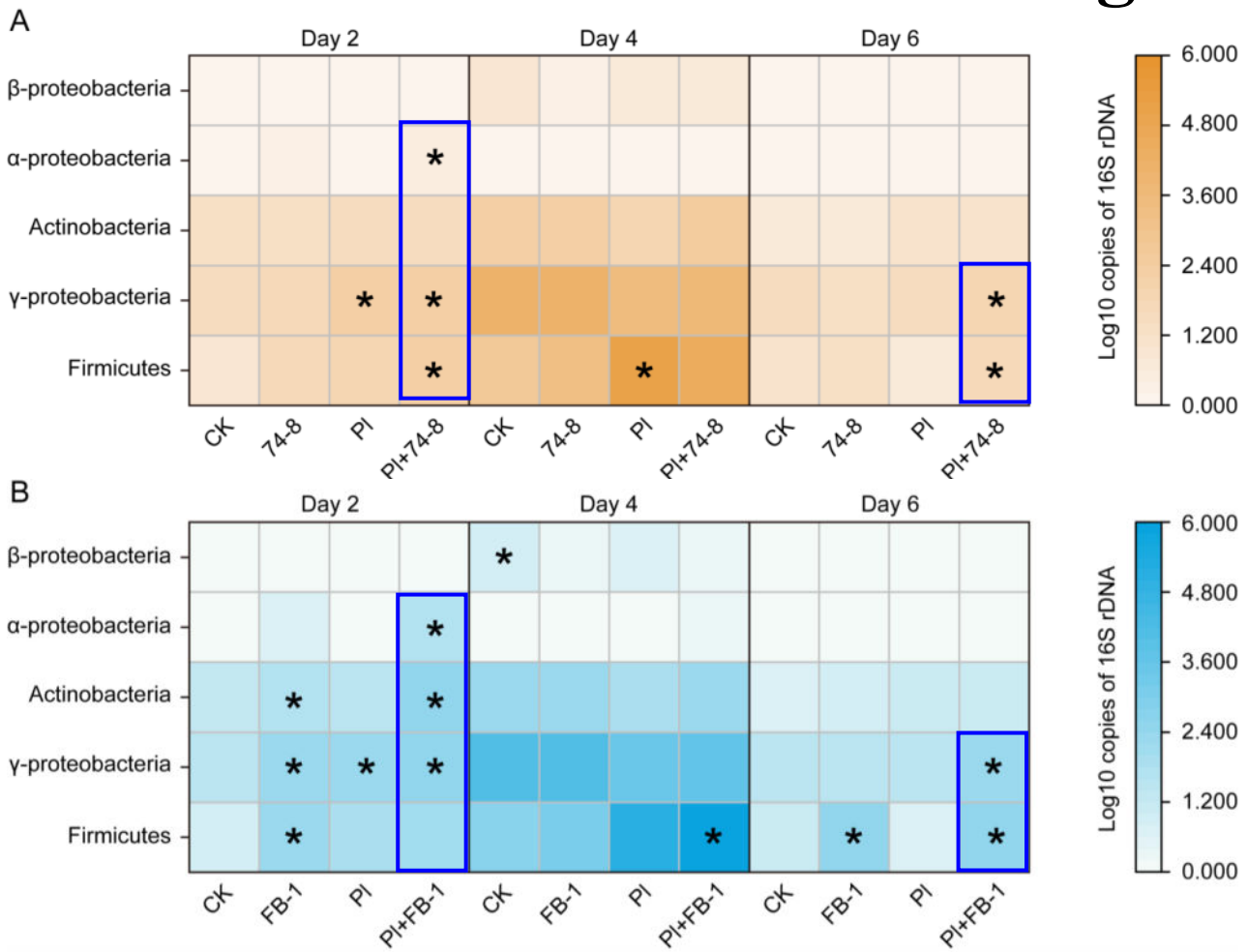
2.8 The abundance of *P. larvae*



Absolute abundance of *P. larvae*

- On day 2, *P. larvae* abundance was significantly lower in the Pl + FB-1 group than that in the Pl group;
- On day 6, *P. larvae* abundances were lower in the Pl + FB-1 and Pl +74-8 groups than that in the Pl group.

2.9 The abundance of core microorganisms

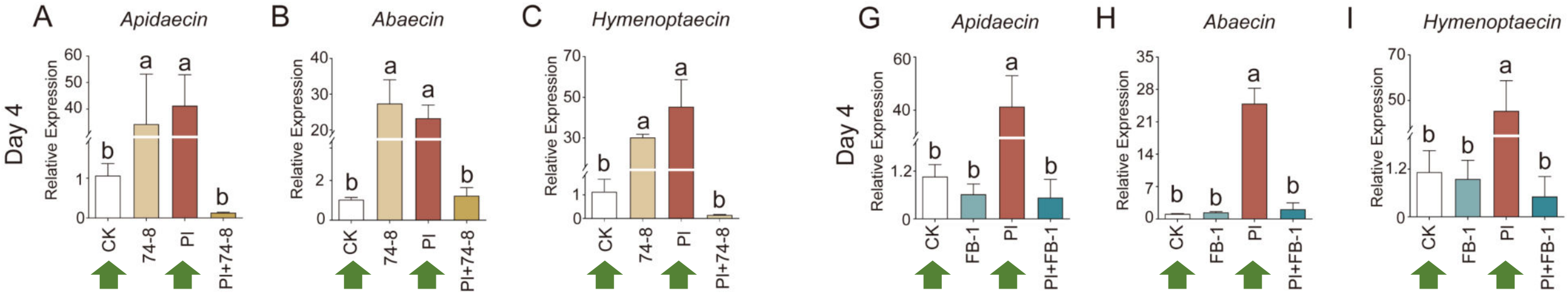


Absolute abundance of core microorganisms in larvae supplemented with *L. citreum* 74-8 (above) and *L. sakei* FB-1(below)

L. citreum 74-8 and *L. sakei* FB-1 could successfully colonize the intestinal tract of honey bee larvae, then inhibit *P. larvae* and were beneficial to the core bacteria.

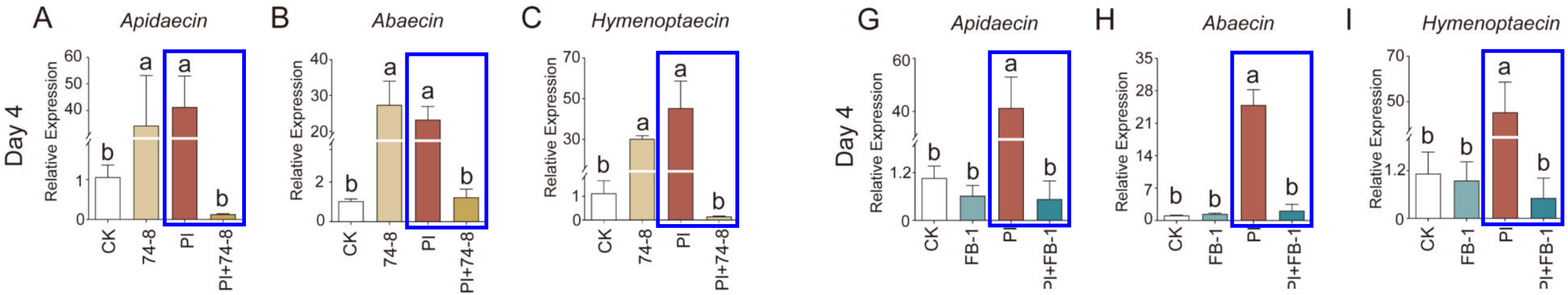
Supplementation of *L. citreum* 74-8 and *L. sakei* FB-1 could increase the abundance of some gut bacteria in honey bee larvae

2.10 Transcriptional levels of antimicrobial peptides



Larvae exposed to *P. larvae* showed strong transcriptional levels of antimicrobial peptides of abaecin, apidaecin and hymenoptacein.

2.10 Transcriptional levels of antimicrobial peptides



Inoculating larvae with *L. citreum* 74-8 and *L. sakei* FB-1 significantly reduced RNA levels of antimicrobial peptides in infected larvae

Part 03

Conclution

3. Conclutions

- Two strains with probiotic properties, *L. citreum* 74-8 and *L. sakei* FB-1, were isolated from the gut of honey bees；
- *L. citreum* 74-8 and *L. sakei* FB-1 increased the survival rate of honey bees infected with AFB from 19.44% to 47.22% and 44.44%, respectively;
- *L. citreum* 74-8 and *L. sakei* FB-1 can colonize the larval gut and enhance the abundance of core bacteria.
- *P. larvae* significantly induced the transcription of antimicrobial peptides (AMP) in larvae, while oral administration of *L. citreum 74-8* and *L. sakei* FB-1 stabilized the AMP expression.
- Future research should emphasize *in vivo* validation of these probiotics in real-world environments and explore their feasibility for integration into beekeeping practices.

Zhao Hongyang, Han Bo, Yang Sa, Zhao Danyang, **Zhang Li***, Dai Pingli*, 2025. Newly isolated bacteria increase the survival of honey bee larvae infected with *Paenibacillus larvae*. **Entomologia Generalis**.

Laboratory of Resource Insects Protection

Photo by the first author: Zhao Hongyang
Location: Saihanba, Hebei Province, China