

# Probiotics and postbiotics: a promising prophylactic measure for American foulbrood and European foulbrood diseases of honey bees

Naheed Mojjani<sup>1\*</sup>, Masoumeh Bagheri<sup>2</sup>, Sumel Ashique<sup>3</sup>, Seyede Hanieh Hosseini<sup>4</sup>, Abrar Hussain<sup>5</sup>, Mojtaba Moharrami<sup>1</sup>, Azadeh Zahmatkesh<sup>1</sup>, Mehran Moradi<sup>4\*</sup>

<sup>1</sup> Razi Vaccine and Serum Research Institute (RVSRI), Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran; <sup>2</sup> Department of Pharmaceutical Technology, Bharat Technology, Uluberia, India; <sup>3</sup> Bengal College of Pharmaceutical Sciences and Research, Durgapur, India; <sup>4</sup> Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran; <sup>5</sup> International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan.

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## Abstract

American foulbrood (AFB) and European foulbrood (EFB) diseases caused by *Paenibacillus larvae* and *Meliscoccus plutonius* are prevalent honeybee brood diseases that pose significant economic challenges to the apiculture industry globally. Antibiotic treatment has led to the emergence of antibiotic-resistant strains, encouraging the search for alternative and safe measures to effectively control these diseases. Honeybee gut microbiomes have proven effects on all spectra of honeybee health by enhancing resistance to several diseases via immune modulation and the production of different antimicrobial metabolites. The major part of the gut microbiota is identified as probiotic bacteria, which are live microorganisms that, when administered in adequate amounts, confer health benefits to the host. Probiotics have shown promising health benefits for honeybees. Honeybee gut probiotics provide protection via the production of different metabolites (postbiotics), such as hydrogen peroxide, vitamins, organic acids, free fatty acids, bacteriocins, neurotransmitters, secreted bio-surfactants, and reactive oxygen species. Vast numbers of these gut bacteria and their postbiotics have wide-spectrum antibacterial effects on AFB and EFB. This review highlights the significance of the honeybee gut microbial community, its probiotic potency, and the role of postbiotic metabolites as safe prophylactic measures for preventing AFB and EFB diseases in honeybees.

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## Introduction

Honey bees play a vital role in the production of certain beneficial agents such as wax, honey, pollen, propolis and royal jelly, and are economically considered the most valuable insect pollinators. Globally, around 90.00% of wild flowering plants rely on animal pollination for reproduction, with non-bee insects contributing 25.00 - 50.00% of floral visits to key crops,<sup>1</sup> highlighting the importance of these insects. The honey bee gut is considered as a significant reservoir of diverse microorganisms (microbiota) and genetic information with important implications for bee health, ecology and research.<sup>2</sup> The gut microbiota of honey bees is dominated by a small number of bacterial species that are specific to bee guts offering a unique opportunity to study host-microbe interactions.<sup>3</sup>

Biotic factors, such as parasites and pathogens, can significantly impact the gut microbiota of honey bees. For example, infection with *Nosema ceranae*, a microsporidian parasite, has been shown to alter the abundance of several bacterial species in the bee gut.<sup>4</sup> While, abiotic factors, particularly pesticides and antibiotics, also play a significant role in disturbing the honey bee gut microbiota.<sup>4</sup> Antibiotic treatments, while effective against bacterial infections, can cause collateral damage to the gut microbiome, potentially leading to long-lasting consequences for host health.<sup>5</sup> American foulbrood (AFB) and European foulbrood (EFB) are prevalent, highly contagious bacterial infections affecting honeybee brood, leading to substantial colony losses and posing serious economic challenges to beekeeping worldwide. These diseases lead to decreased hive viability, reduced honey production and substantial economic losses for beekeepers.<sup>6</sup>

### \*Correspondences:

Naheed Mojjani. PhD

Razi Vaccine and Serum Research Institute (RVSRI), Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran

E-mail: n.mojgani@rvsri.ac.ir

Mehran Moradi. DVM, PhD

Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran

E-mail: m.moradi@urmia.ac.ir



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Traditional treatments for AFB- and EFB-infected honey bee colonies often involve the use of antibiotics.<sup>7</sup> Although initially effective against these diseases, the efficacy of antibiotics has waned due to the emergence of resistant bacterial strains that have acquired resistance genes or mechanisms against these molecules.<sup>8</sup> Also, conventional antibiotic residues can accumulate in wax, bee bread and honey, and further migrate to brood and adult honey bees, adversely affecting the overall health of the colony.<sup>9</sup> To address these challenges, there is a growing need to develop new natural products that can effectively treat AFB and EFB diseases in honey bees without promoting the development of resistant bacterial strains or leaving toxic residues in beehives or beekeeping products (Tables 1 and 2).

Probiotics are live microorganisms that have significant health benefits to the host when consumed in sufficient amounts.<sup>10</sup> These live bacteria have long been studied as natural, non-toxic compounds that can control the spread of diseases.<sup>9</sup> Lactic acid bacteria (LAB), such as *Lactobacillus*, *Bifidobacterium*, and *Enterococcus* are the major bacterial genera used as probiotics to modulating intestinal homeostasis and promoting gut health.<sup>11</sup> They inhibit the growth of pathogenic bacteria while promoting beneficial flora in the gastrointestinal tract. Probiotics isolated from animals and humans have significant importance for various applications across different species.<sup>12</sup> In addition to other sources, probiotics have been isolated from honey bees.<sup>13</sup> Studies have shown that certain LAB isolated from honey bees can have beneficial effects on bee health and reduce pathogen prevalence.<sup>14</sup> Some researchers suggest probiotics may boost the innate immune response against microbial infections and help maintain a healthy gut microbiota in bees.<sup>15</sup> Specific probiotic strains such as *Lactiplantibacillus plantarum* and *Apilactobacillus kunkeei* have demonstrated *in vitro* inhibition of bee pathogens that cause AFB and EFB.<sup>16</sup>

The AFB is caused by the Gram-positive bacterium *Paenibacillus larvae* (formerly known as *Bacillus larvae*), while EFB is another brood disease caused by *Melissococcus plutonius*.<sup>6</sup> *Paenibacillus larvae* is a spore-forming and flagellated bacterium and cause a highly contagious disease that affects the larval and pupal stages of honey bees. This disease is primarily transmitted through the exchange of contaminated bee equipment between colonies as well as by drifting bees.<sup>55</sup> It may occur frequently and spread rapidly,<sup>56</sup> and several factors can affect the clinical signs of AFB such as the genotype, stage of the disease and the strength of the bee colony.<sup>57</sup> Despite the worldwide occurrence, EFB is not notifiable in any country.<sup>58</sup> The reduced virulence of *M. plutonius* is associate with main factor allowing certain larvae to successfully reach the pupation stage. The EFB-infected colonies showed capped and uncapped cells that were irregularly scattered on the brood frame. According to the

symptoms, the color of larvae infected with EFB changes from white to yellow, brown and even grayish-black depending on the severity of the disease.<sup>59</sup> *Melissococcus plutonius* reproduces in the larval gut, therefore, larvae that survive can infect other larvae with their feces as they pupate.<sup>54</sup>

Keeping in mind these potential results and elaborating on the significant role of apiculture and the honey bee industry in the economic development of a country, less is known about natural and cost-effective ways of controlling these diseases. Hence, this review aimed to provide an up-to-date knowledge regarding the use of probiotics and postbiotics in the treatment of AFB and EFB diseases.

**Strategies to the control of AFB and EFB.** Various strategies have been explored to control AFB and EFB in honey bees. Antibiotics, particularly oxytetracycline have been widely used to treat AFB and EFB. However, their effectiveness is diminishing due to the emergence of resistant bacterial strains.<sup>60</sup> This has led to the exploration of alternative antibiotics such as tylosin and lincomycin which have shown promise in laboratory studies.<sup>60</sup> However, several natural and safe antimicrobials such as nanoparticles and natural products<sup>61,62</sup> have been investigated for their role in improving honey bee health. These agents help prevent diseases, avoid harmful residues in honey bee products, enhance colony survival and reduce honey bee mortality.<sup>9,63</sup> Biotics such as probiotics and postbiotics are novel alternatives.

The health effects of gut microbiota are dependent on live probiotic cells, the inanimate cell (paraprobiotics) and the metabolites (postbiotics). Paraprobiotics specifically refer to inactivated microbial cells of probiotics, either intact or ruptured, while postbiotics are microbial metabolites such as short-chain fatty acids (SCFAs), organic acids, bacteriocins, peptides and exopolysaccharides (EPS) which are produced during cell growth.<sup>64</sup> The antimicrobial metabolites produced by LAB species can be categorized into low molecular weight and higher molecular weight compounds.<sup>65,66</sup> Bacteriocins are post-translationally modified antibacterial substances that are mostly produced by Gram-positive bacteria and have antibacterial potential.<sup>67</sup> *Bacillus* strains, especially *B. subtilis*, have shown *in vitro* antimicrobial activity against important honeybee pathogens such as *P. larvae* and *A. apis*.<sup>40</sup> One of the key compounds produced by these *Bacillus* strains is surfactin, a cyclic lipopeptide that effectively inhibits *P. larvae*.<sup>40</sup> Mojgani *et al.*<sup>54</sup> studied the antibacterial effects of LAB metabolites against bacterial agents responsible for AFB and EFB diseases. According to their findings, metabolites produced by probiotic bacteria can exhibit significant antibacterial activity against *M. plutonius*. Likewise, the postbiotics of LAB species showed similar antibacterial activity and inhibited the growth of *M. plutonius* indicating the potential area of research that focuses on the use of

postbiotics as a safe antimicrobial and cost-effective strategy for to prevent honey bee diseases.<sup>54</sup> Previously, other studies also demonstrated the significance of postbiotics in the control of several honey bee diseases.<sup>9</sup>

**Table 1.** Important features of honey bee gut microbes and their activity against *Paenibacillus larvae* and *Melissococcus plutonius*.

Bacteria	Features	Effects against AFB & EFB	References
<i>Bacillus amyloliquefaciens</i> HTI-19 <i>Bacillus subtilis</i> HTI-23	High survivability in the artificially modified digestive tract systems, wide antimicrobial spectra, high cell hydrophobicity, and auto aggregation	NI	17
<i>Enterococcus faecium</i> SM21	Lactic acid production bacteriocin-like compound production	<i>In vitro</i> inhibition of AFB	18
<i>Lactobacillus kunkeei</i>	Show good gastrointestinal tract tolerance, high antibiotic susceptibility, high cell surface hydrophobicity, exhibit auto- and co-aggregation capacities	<i>In vivo</i> inhibition of AFB	19,20
<i>L. kunkeei</i>	Fructophilic lactic acid bacteria species with potential antibacterial activity	<i>In vitro</i> and <i>in vivo</i> inhibition of EFB	21,22
<i>Fructobacillus fructosus</i>	Show good gastrointestinal tract tolerance, high antibiotic susceptibility, high cell surface hydrophobicity, exhibit auto- and co-aggregation capacities	<i>In vitro</i> inhibition of AFB	20,23
<i>Lactiplantibacillus plantarum</i> KX519413 <i>L. plantarum</i> KX519414	Withstand gastrointestinal tract conditions, efficient production of exopolysaccharide, exhibit biofilm formation, and auto-aggregation	NI	24
<i>Apilactobacillus kunkeei</i>	Gut microbial community	<i>In vitro</i> inhibition of AFB	25
<i>A. kunkeei</i> strains	Native gut microbiota of honey bees	Decrease the mortality caused by <i>P. larvae</i> infection in larvae	26
Acja3 (Bacillus strain)	Antimicrobial compound production, adaption to the specific environmental conditions of the gut	<i>In vitro</i> inhibition of EFB	27
<i>Proteus Mirabilis</i> <i>Enterobacter Kobei</i> <i>Morganella Morganii</i> <i>Fructobacillus fructosus</i> , <i>L. plantarum</i> Lp39 <i>Lacticaseibacillus rhamnosus</i> GR-1 <i>L. kunkeei</i> BR-1	Antimicrobial compound production, adaption to the specific environmental conditions of the gut	<i>In vitro</i> inhibition of AFB	23
<i>Bacillus leicheniformis</i> <i>L. kunkeei</i>	Upregulation of immune-related gene expression	<i>In vitro</i> inhibition of AFB	28
<i>L. plantarum</i> <i>Lactobacillus. apis</i> <i>Lactobacillus apinorum</i> <i>Lactobacillus helsingborgensis</i> <i>Lactobacillus kimbladii</i> <i>Lactobacillus kullabergensis</i> <i>Lactobacillus mellifer</i> <i>L. apinorum</i> <i>L. apis</i>	Isolated from gut of <i>Apis mellifera jementica</i> in Saudi Arabia	<i>In vitro</i> inhibition of AFB	23
<i>Bifidobacterium asteroides</i>	Isolated from digestive tract of adult honey bees in Košice, Slovakia	<i>In vitro</i> inhibition of AFB	29
<i>L. plantarum</i> <i>Lactobacillus brevis</i>	Isolated from the digestive tract of honey bees, <i>A. mellifera</i> , <i>Apis andreniformis</i> and <i>Melipona beechei</i>	<i>In vitro</i> and <i>in vivo</i> inhibition of AFB and EFB	22,30
<i>Bifidobacterium longum</i> <i>Bifidobacterium indicum</i>	Isolated from stomach of <i>A. mellifera</i> in Czech Republic	<i>In vitro</i> inhibition of AFB and EFB	31
<i>Bacillus cereus</i> , <i>Bacillus megaterium</i> , <i>Bacillus licheniformis</i> , <i>Bacillus pumilus</i> , <i>B. subtilis</i> , <i>Brevibacillus laterosporus</i> <i>Stenotrophomonas maltophilia</i> , <i>Acinetobacter</i> sp., <i>Bacillus fusiformis</i> , <i>Brevibacillus formosus</i> <i>L. kunkeei</i> , <i>B. asteroides</i> related phylotypes, and <i>Lactobacillus phylotypes</i>	Isolated from gut of honey bee workers	<i>In vitro</i> and <i>in vivo</i> inhibition of AFB	32,33
<i>Bacillus isolates</i>	The hetero and homo fermentative isolates produce lactic acid	<i>In vitro</i> inhibition of AFB	29
<i>L. apis</i> HSY8_B25, <i>Lactobacillus panisapium</i> PKH2_L3, and <i>Lactobacillus melliventris</i> HSY3_B5	Terminal restriction fragment length polymorphism analysis of communities in the midguts of <i>A. mellifera</i> and <i>Apis cerana</i> honey bees in Thailand and their antibacterial activity against AFB	<i>In vitro</i> and <i>in vivo</i> inhibition of AFB	6
<i>L. plantarum</i>	Not studied for probiotic properties	<i>In vitro</i> inhibition of different strains of <i>P. larvae</i>	34
	Isolated from honey bee larvae	<i>In vitro</i> inhibition of different strains of <i>P. larvae</i>	35
	Not investigated for probiotic properties	<i>In vitro</i> and <i>in vivo</i> inhibition of AFB	6
	Synergistic action of mixed cultures against <i>P. larvae</i>	<i>In vitro</i> inhibition of AFB	29
	Isolated from digestive tract of the Japanese honey bee, <i>A. cerana japonica</i>	<i>In vitro</i> inhibition of AFB	36
	Honey bee gut bacteria	Prevented AFB and EFB in bees	37

NI: Not identified; AFB: American foulbrood; EFB: European foulbrood.

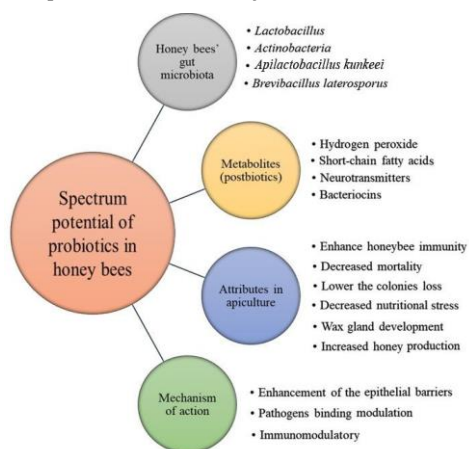
**Table 2.** Postbiotic metabolites produced by probiotic bacteria demonstrating inhibitory effects against *Paenibacillus larvae* and *Melissococcus plutonius*.

Probiotic bacteria	Postbiotic metabolites/ antimicrobial agents	Methods used for isolation of postbiotic	Effects against AFB & EFB	References
<i>Bacillus amyloliquefaciens</i> <i>Bacillus subtilis</i> <i>Bacillus pumilus</i> <i>Bacillus licheniformis</i> <i>Bacillus cereus</i> <i>Bacillus megaterium</i> <i>Brevibacillus laterosporus</i>	Iturin-like peptide		Inhibits <i>P. larvae</i>	
<i>Bacillus thuringiensis</i> subsp. <i>Entomocids</i> HD110	4.80 KDa bacteriocin (enterocin 110)	Centrifugation, filter sterilizations, and partial purification of enterocin	Inhibited 17 strains of <i>P. larvae</i>	39
<i>B. subtilis</i> and <i>Brevibacillus</i>	Lipopeptides surfactant			40
<i>B. laterosporus</i>	Other antimicrobial peptides			41
<i>B. cereus</i>	Subtilin (spaS) Bacillomycin (bmyB)	Plate inhibition assay	Inhibitory activity toward EFB	41
<i>B. megaterium</i> <i>B. subtilis</i>	Iturin (surfactin) bacilysin	Not purified		
<i>B. laterosporus</i> F5	Laterosporulin		Inhibitory activity towards AFB	42
<i>Lactobacillus acidophilus</i> and <i>Lacticaseibacillus casei</i>	Cell free supernatant	Centrifugation and filter sterilization	Invitro inhibitory effects against AFB	43
<i>Lactiplantibacillus plantarum</i>	Exopolysaccharides	ATR-FRTIR, size exclusion chromatography	Inhibitory effects against AFB	44
<i>L. plantarum</i> S7-10, S20-3, PT-23-1	Plantaricin	Ammonium sulfate precipitations (85.00%)		45,46
<i>B. thuringiensis</i>	Entomocin			
<i>Brevibacillus sp. strain</i> GI-9	Laterosporulin	hydrophobic interaction chromatography, reverse-phase HPLC, MALDI-TOF	Inhibitory effects against AFB	42,47
<i>Lactobacillus kunkeei</i> FF30-6	Kunkecin A	three-step chromatography procedure, reverse-phase HPLC, ESI-TOF MS	Antimicrobial activity against EFB	48
<i>Lactobacillus helsingborgensis</i>	Bacteriocin		Inhibitory activity towards AFB	49
<i>B. cereus</i>	BLIS		Inhibitory activity toward EFB	50
<i>Enterococcus faecium</i>	Enterocin	Whole-genome sequencing using the Illumina HiSeq platform	Inhibitory activity towards AFB	51
<i>L. kunkeei</i> Fhon2, <i>Lactobacillus apinorum</i> , <i>Lactobacillus mellis</i> , <i>Lactobacillus mellifer</i> , <i>Lactobacillus apis</i> , <i>Lactobacillus</i> <i>helsingborgensis</i> , <i>Lactobacillus melliventris</i> , <i>Lactobacillus kimbladii</i> and <i>Bifidobacterium</i> <i>asteroides</i> and <i>Bifidobacterium coryneform</i>	Major fatty acids detected	MALDI-TOF MS protein profiling, bacterial cell extracts were prepared from fresh colonies using the ethanol/formic acid extraction procedure		52
<i>Lactobacillus R4B<sup>T</sup></i> and <i>4C</i>	L-lactic acid	Capillary isotachopheresis method, D/L-lactic acid kit	Exhibits antimicrobial activity against AFB and EFB	31
<i>Bifidobacterium</i> AcjBF isolates	Lactic acid, formic acid, and acetic acid	API 50CH biochemical kit	Exhibited antagonistic effects against EFB	27
<i>Limosilactobacillus reuteri</i> ATCC23272	Cell free supernatant	Centrifugation and filter sterilization	Inhibition of <i>P. larvae</i> biofilm	53
<i>Apilactobacillus kunkeei</i> <i>L. plantarum</i>	Cell free supernatant of other metabolites	Centrifugation and filter sterilizations	Inhibitory activity on vegetative forms of <i>P.</i> <i>larvae</i> and <i>M.</i> <i>plutonius</i>	16
<i>Lacticaseibacillus rhamnosus</i> , <i>L. apis</i> <i>L. plantarum</i> <i>Pediococcus acidilactici</i>	Organic acids and BLIS	Centrifugation and filter sterilizations	Exhibited antagonistic effects against EFB	54

BLIS: Bacteriocin like inhibitory substance; ATR-FRTIR: Fourier transform infrared spectroscopy; HPLC: High-performance liquid chromatography; ESI-TOF MS: Electrospray ionization time-of-flight mass spectrometry; MALDI-TOF: Matrix-Assisted Laser Desorption/Ionization-Time of Flight; MS: mass spectrometry; NMR: Nuclear magnetic resonance; HRMS: High-resolution mass spectrometry; NI: Not identified).

**Probiotic microbiota of honey bees.** Symbiotic microorganisms that colonize the gut in balanced proportions are known to maintain and modulate the health of bees. The LAB has as a crucial role in the gut microbiota of honey bees.<sup>6,22</sup> The composition of LAB in honey bee gut microbiota can vary across regions.<sup>68</sup> This highlights the importance of studying microbiome diversity in different indigenous bee species and regions to fully understand the role of LABs in honey bee health. Toutaiee *et al.*<sup>69</sup> investigated LAB isolated from the gut of honey bees *Apis mellifera*. The findings showed that the honey bee gut was a reservoir of *Lactobacillus acidophilus*, *Lacticaseibacillus casei*, *L. plantarum*, *Lactobacillus apis*, *Enterococcus faecium*, and *Pediococcus acidilactici*, with antibacterial effects against *Listeria monocytogenes*, *Escherichia coli*, *Enterococcus faecalis*, and *Streptococcus mutants*.

To date, approximately 45 LAB species have been isolated and identified from bees and their products and approximately 30.00% of the bacteria present in the whole gut of honey bees belong to the genera *Lactobacillus* and *Bifidobacterium*.<sup>34</sup> By acquiring niches in which nutrients are available, LAB species protect honey bees and their honey from pathogenic bacteria. The gut microbiota of honey bees plays a crucial role in up-regulating the expression of antimicrobial peptides (AMPs) such as apidaecin and hymenoptaecin, which are essential components of the bee innate immune system. Studies have shown that bees with a normal gut microbiota exhibit increased expression of these AMPs in gut tissue compared to bees lacking gut microbiota.<sup>34</sup> This up-regulation of AMP expression is a host mechanism to regulate the microbiota and potentially improve the immune system against future pathogenic infections. Probiotics, particularly those derived from the honey bee gut microbiota, show promise in supporting honey bee health by lowering mortality and colony loss, decreasing nutritional stress, enhancing resistance to disease and growth stimulation.<sup>6,23,28,70</sup> Figure 1 illustrates the different aspects of probiotic and honey bee associations.



**Fig. 1.** Potential role of probiotics in honey bees.

In research conducted by Pachla *et al.*<sup>20</sup> the probiotic and postbiotic characteristics of LAB obtained from the gastrointestinal system of *A. mellifera* were analyzed. The findings revealed that LAB-derived postbiotics could adapt to pH fluctuations within the honey bee digestive system, ensuring improved survival in the insect gut. Turong *et al.*<sup>36</sup> demonstrated that three specific *Lactobacillus* strains—*L. apis* HSY8\_B25, *Lactobacillus panisapium* PKH2\_L3, and *Lactobacillus melliventris* HSY3\_B5—exhibited promising probiotic properties. Additionally, these strains showed the capacity to suppress *P. larvae* infections in affected larvae. Another work identified two *L. plantarum* strains from Indian honey bees (*A. cerana indica*) with notable probiotic attributes such as resistance to acidic and bile environments, endurance under simulated digestive conditions as well as hydrophobicity and aggregation abilities.<sup>24</sup>

In addition to their antagonistic actions on honey bee diseases, LAB play a vital role in honey bees nutrition by synthesizing essential amino acids and vitamins during metabolic processes. They also aid in breaking down and fermenting complex carbohydrates and peptides in the honey bee diet making these nutrients more accessible to bees.<sup>16</sup> Additionally, LAB enzymes can detoxify certain carbohydrates that may be harmful to honey bees.<sup>71</sup> The LAB can enhance the innate immune system of honey bees and protect them against pathogens. Studies have shown that exposing honey bee larvae to LAB strains from genus *Lactobacillus* upregulates the expression of AMPs, boosting their immune response.<sup>72</sup>

In addition to LAB, some *Bacillus* spp. with probiotic properties have been identified in honey bees. Some of the most essential features of spore-forming *Bacillus* spp. include enhanced resistance to acidic and bile conditions, longer survival and shelf life, ability to produce AMPs (bacteriocins), stimulating immunity and adhesion abilities.<sup>73</sup> Sabaté *et al.* reported that *B. subtilis* strain Mori2 enhanced colony performance.<sup>40</sup> Additionally, *Bacillus amyloliquefaciens* HTI-19 and *B. subtilis* HTI-23, derived from Malaysian stingless bee honey, displayed probiotic potential such as broad antimicrobial activity against AFB and EFB diseases. Table 1 highlights antibacterial activity of various bacterial species on AFB and EFB infections.

**Postbiotics.** Postbiotics are bioactive metabolic substances produced by probiotics and other beneficial microorganisms either through secretion or as a result of cell lysis.<sup>9,64</sup> Similar to the effects of probiotics, research indicates that postbiotics provide comparable direct and significant health benefits to the host.<sup>66</sup> Some of the most important postbiotic metabolites include bacteriocins, hydrogen peroxide, enzymes, amino acids, vitamins, SCFAs, organic acids, EPS and surfactants.<sup>64</sup> Many studies have indicated the role of these bioactive compounds as therapeutic agents for managing diseases such as of EFB

and AFB and enhancing colony health in honey bees Table 2. Although the precise mechanism of action remains under investigation, it is believed that these compounds may exert their effects through three different mechanisms: i) direct pathogens inhibition, ii) enhancement of the epithelial barriers and iii) modulation of inflammatory and immune responses.<sup>9</sup> The role of different postbiotic metabolites produced by bacteria in honey bee guts and their antimicrobial performance on pathogens responsible for EFB and AFB in honey bees are discussed in following sections.

**Bacteriocins or bacteriocin like inhibitory substances (BLIS).** Bacteriocins are extracellular proteinaceous or peptide-antimicrobials produced by a vast number of probiotic bacteria.<sup>11,67</sup> Studies show that postbiotics of *Brevibacillus laterosporus*, a frequent bacterial species in the honey bee gut, inhibits the growth of *P. larvae*. Laterosporulin, a bacteriocin with a molecular mass of 5605.8 Dalton was discovered as the major component of the *B. laterosporus* postbiotics. Thermostability, pH tolerance and resistance to proteolytic enzymes are important properties of laterosporulins.<sup>47</sup> Additionally, other researchers have shown that bacteriocin could mediate *B. laterosporus* toxicity against *P. larvae* and could be implemented as a safe biological agent for honey bee protection against AFB disease.<sup>42</sup> In a study, Minnaard and Alippi<sup>50</sup> isolated two *Bacillus cereus* strains with specific inhibitory activity on *P. larvae*. According to their reports, the two bacterial strains could produce BLIS (BLISm6c and BLISm387) which were responsible for the inhibitory actions. However, the two BLIS were significantly different based on their molecular weights, antibacterial activity, minimal inhibitory concentration values and sensitivity to degradative enzymes. Their findings indicated that BLISm6c and BLISm387 could potentially control AFB.<sup>50</sup>

*Apilactobacillus kunkeei* which belongs to fructophilic LAB was considered a major component of the honey bee gut microbiota.<sup>34</sup> A recent investigation revealed that the *A. kunkeei* postbiotics exerted inhibitory effects on *M. plutonius*. For example, Zendo *et al.*<sup>48</sup> isolated and identified bacteriocin kunkecin A with a molecular mass of 4218.3 from *A. kunkeei* postbiotics through three-step chromatography as well as electrospray ionization time-of-flight mass spectrometry approaches. Based on spot-on-lawn assay results, these researchers found that *M. plutonius* was highly sensitive to the actions of kunkecin A, while *P. larvae* was tolerant of kunkecin A, therefore, kunkecin A was suggested to be a useful strategy for the control of EFB.<sup>48</sup>

**Short-chain fatty acids and organic acid.** Microbial symbionts residing in the gastrointestinal tract have the ability to generate a range of metabolites such as SCFAs, amino acids and neurotransmitters.<sup>64</sup> Organic acids and SCFAs (mainly acetate, propionate, and butyrate) are a

major class of postbiotic metabolites that have significant effects on host physiology. Honey bee microbiota produces organic acids through the fermentation of dietary nectar and pollen.<sup>74</sup> Based on this study, organic acids added to the feed of pollen-restricted bees significantly affected hindgut enteroendocrine signaling gene expression.<sup>74</sup> According to reports, honey bee core microbiota are active *in vivo* and via expressing critical key enzymatic genes, produce organic acids such as acetate, lactate, succinate and formate which influence gut epithelium functions.<sup>71,75</sup> Lee *et al.*,<sup>76</sup> by metatranscriptome analysis showed that *Actinobacteria*, *Bacilli* and  $\gamma$ -*Proteobacteria* played a crucial role in production of SCFAs, lactate, formate and acetate. Free fatty acids, with their amphipathic structures, interact with the bacterial cell membrane and create transient or permanent pores of variable sizes that lead to devastating effects on bacterial cells.<sup>77</sup> In addition, Free fatty acids can bind the carriers of the electron transport chain directly or insert into the inner membrane causing the electron carriers to move apart or be displaced entirely from the membrane. This results in reduced ATP production and the bacterium is deprived of an essential source of energy. Furthermore, their insertion into the bacterial inner membrane can increase membrane fluidity and lead to cell lysis.<sup>76</sup> These studies indicated that the honey bee gut microbiota, through the synthesis of a variety of organic acids, could enhance their antibacterial effects against Gram-negative and Gram-positive bacteria.<sup>78</sup>

Comprehensive metabolomics analyses have demonstrated that a wide variety of organic acids accumulate due to the activity of gut bacteria in honey bees, potentially playing a significant role in the host physiological processes.<sup>79</sup> Several LAB species have been well-studied for their ability to produce organic acids which may be strain- or species-specific.<sup>80</sup> In an experimental study by Killer *et al.*<sup>31</sup> *Lactobacillus* strains, including R4BT and R4C, exhibited *in vitro* antagonistic effects on AFB and EFB through lactic acid, propionic, acetic and valeric acid production. In another study, Olofsson *et al.*<sup>52</sup> showed that 13 LAB species isolated from honey bees could produce organic acids, however, in variable amounts. Similarly, *Limosilactobacillus reuteri* ATCC 23272 was shown to inhibit *P. larvae* owing to its organic acid-producing ability.<sup>53</sup>

**Hydrogen peroxide.** Olofsson *et al.*<sup>52</sup> reported that *Lactobacillus* strains (*L. apis* Hma11, *Lactobacillus kimbladii* Hma2, *Lactobacillus helsingborgensis* Bma5, *L. melliventris* Hma8, and *Lactobacillus kullabergensis* Biut2) isolated from honey bee stomach contents had antibacterial actions which was related to H<sub>2</sub>O<sub>2</sub>-producing ability. A substantial correlation was found between the level of endogenous hydrogen peroxide and the extent of bacterial growth inhibition by honey.

**Exopolysaccharides.** The EPS production and biofilm formation by LAB could be an effective strategy against biofilms and colonization of several Gram-negative and Gram-positive pathogenic bacteria such as *P. larvae* (*L. plantarum*) which play a crucial role in fresh pollen transformation into bee bread and have been localized in the honey bee gut.<sup>81</sup> In an experimental study by Iorizzo et al.<sup>82</sup> the capacity of EPS production in *L. plantarum* isolated from honey bee guts was assessed. Their findings illustrated that in De Man–Rogosa–Sharpe broth at 37.00 °C under aerobic conditions, all tested *L. plantarum* strains produced EPS, however, this ability was highly variable among different *L. plantarum* strains.<sup>82</sup>

**Lipopeptide biosurfactants.** Biosurfactants are surface-active amphiphathic molecules of microbial origin that are produced by many probiotic species.<sup>83</sup> These biological compounds are divided into five major classes based on their chemical composition and microbial origin.<sup>84</sup> Among these classes, lipopeptide biosurfactants, surfactins and iturins, which are secondary metabolites of many probiotic species have shown antibacterial, antifungal and antiviral activities.<sup>85</sup> Some *Bacillus* species can produce one type of lipopeptide biosurfactant, whereas, a few produce two or three types of lipopeptides.<sup>86</sup> These surfactants have been shown to possess a broad spectrum of antimicrobial and surface-active properties. The anti-AFB properties of lipopeptide biosurfactants have been previously reported.<sup>87</sup> Similarly, Benitez et al.<sup>88</sup> showed that *B. amyloliquefaciens* produced antimicrobial compounds such as lipopeptides that are active on *P. larvae*. It has been demonstrated that lipopeptide surfactants synthesized by *B. subtilis* C4, from honey samples inhibit the causative agent of AFB.<sup>40</sup> Sabaté et al. demonstrated that surfactin produced by a *B. subtilis* isolate from *A. mellifera* gut in Argentina inhibited the growth of *P. larvae*.<sup>40</sup>

Although there are some concerns regarding the potential risks associated with the overuse of probiotics and postbiotics in honeybee populations, there are no reports on this and various factors are considered when recommending suitable dosages including the type of probiotic microorganisms, dosage forms, delivery methods and the number of strains involved. Therefore, it is challenging to generalize a single dosage as sufficient to elicit probiotic effects across different hosts, including humans, animals and insects such as honeybees. Few studies have evaluated the effects of different probiotic doses on the control of diseases in honeybees. Borges et al.<sup>89</sup> investigated the effect of various probiotic doses on *N. ceranae* spores in honeybees. While these researchers only showed that higher doses of probiotics were less effective in mitigating the effects of the disease in honeybees, no other side effects or effects of high doses on bee populations were shown. Similarly, not much data are available regarding a fixed dose of postbiotics in honeybees that

could be considered a standard effective dose, and different studies have analyzed different concentrations of postbiotics depending on the strain used and the method of preparation of the postbiotics. Hence, further detailed investigations are required to determine a safe and efficacious dose. Owing to the dose-response effects of probiotics and postbiotics, it is highly recommended to conduct more field trials in honeybees to determine the efficacious doses of these natural ingredients with minimal side effects on the health of these insects.

To date, there have been no reports suggesting that probiotics can disrupt the microbiota of the host. Probiotics are beneficial bacteria derived from the host microbiota, and their consumption fosters a favorable environment within the microbiota, thereby, providing health benefits to the host. Research indicates that probiotics help maintain a balanced gut microbial community in honeybees and may alleviate dysbiosis in these organisms.<sup>90</sup>

**Conclusion and future prospective.** The AFB and EFB diseases are prevalent honey bee diseases that pose significant economic challenges to the apiculture industry globally. The application of probiotics and postbiotics in honey bee health is a promising approach for AFB and EFB. Probiotics, particularly LAB and *Bacillus* spp., have demonstrated significant potential in enhancing honey bee immunity, reducing mortality, and improving colony health. Postbiotics, including bacteriocins, SCFAs, and organic acids, offer additional antimicrobial benefits providing a natural and safe alternative to traditional antibiotics for AFB and EFB. Current findings highlight the importance of maintaining a balanced gut microbiota in honey bees and spotlight the potential of microbial-based interventions in apiculture that could be implemented by beekeepers to control AFB and EFB diseases. Future research should focus on optimizing the formulation and novel delivery method of probiotics and postbiotics to ensure their efficacy and stability in beekeeping practices. There is also a need for large-scale field trials to validate the long-term benefits of this biotics under diverse conditions. Additionally, exploring the synergistic effects of combining probiotics with other natural antimicrobial agents could further enhance their effectiveness. As the global decline in honey bee populations continues to threaten ecosystems and agriculture, hence, the development of sustainable, non-toxic strategies such as probiotics and postbiotics will be crucial in safeguarding these vital pollinators.

### Conflicts of interest

All authors have seen and agreed to the contents of the article and have no conflicts of interest associated with this work, including specific financial interests, relationships, and affiliations relevant to the subject of the article.

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