



The promise of probiotics in honeybee health and disease management

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Abstract

Over the last decades, losses of bee populations have been observed worldwide. A panoply of biotic and abiotic factors, as well as the interplay among them, has been suggested to be responsible for bee declines, but definitive causes have not yet been identified. Among pollinators, the honeybee *Apis mellifera* is threatened by various diseases and environmental stresses, which have been shown to impact the insect gut microbiota that is known to be fundamental for host metabolism, development and immunity. Aimed at preserving the gut homeostasis, many researches are currently focusing on improving the honeybee health through the administration of probiotics e.g., by boosting the innate immune response against microbial infections. Here, we review the knowledge available on the characterization of the microbial diversity associated to honeybees and the use of probiotic symbionts as a promising approach to maintain honeybee fitness, sustaining a healthy gut microbiota and enhancing its crucial relationship with the host immune system.

Keywords Honeybee · Microbiota · Immune response · Probiotics · Biocontrol

Introduction

Animal pollinators provide an important ecosystem service helping a various range of plants to reproduce and ensuring the maintenance of plant species diversity and food production (Greenleaf and Kremen 2006; Klein et al. 2007; Winfree et al. 2008; Buchon et al. 2013). *Apis mellifera* is arguably one of the most considerable insect pollinators (Klein et al. 2007), due to the role it plays in the production of vegetables, fruits, and stimulating crops.

In last decades, abnormal bee mortality has been globally observed, especially in several European and North American countries (Aizen and Harder 2009), posing serious issues to the pollination service and, thus, crop yield (Steffan-Dewenter et al. 2005). Some wild bee species are currently vulnerable (Yeung et al. 2006; Oldroyd 2007), whereas others have suffered large contractions (Goulson et al. 2008). Many factors could be responsible for bee declines, including abiotic and biotic agents, as well as their combination. Among abiotic stressors, we can mention bee environment destruction, pesticide use and climate change (Barnett et al. 2007; Pettis et al. 2012), whereas biotic factors include infections caused by microsporidia parasites, such as *Nosema* spp. (Higes et al. 2006; Higes et al. 2008; Bromenshenk et al. 2010; Hatjina et al. 2011), spore-forming bacteria, such as *Paenibacillus larvae*, the causative agent of American foulbrood (AFB) (Bailey 1983; Dobbelaere et al. 2001), pathogenic fungi, such as *Ascosphaera apis*, parasitic mites, such as *Varroa destructor* (Le Conte et al. 2010; Rosenkranz et al. 2010) and viruses, such as Deformed Wing Virus (DWV). *V. destructor* is ultimately a vector of different viruses that can infect *Apis mellifera* (Rosenkranz et al. 2010). Considering bee importance, research on bee health has recently become a hot topic.

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To protect honeybees against diseases, beekeepers can adopt various intervention measures, e.g. the use of the antibiotics oxytetracyclin and fumagilin-B to control *P. larvae* and *Nosema* pathogens, respectively (Huang et al. 2013). However, the prolonged use of antibiotics causes several troubles, among which the emergence of antibiotic-resistant pathogens and, consequently, their use to treat honeybee diseases has been banned in many European countries. Another serious concern for honeybee health is the employment of neonicotinoids. These compounds can indeed contribute to the bee decline by acting in synergy with different factors, e.g., the shortage of floral resources and the presence of pathogens and parasites (Goulson et al. 2015). Thus, in 2013, the European Commission has banned the use of the three neonicotinoids clothianidin, imidacloprid, and thiamethoxam on the basis of a risk assessment evaluation (Regulation 2013). Currently, biocontrol research in apiculture is mainly focused on developing alternative strategies to maintain and improve bee health, for example by enhancing the honeybee immune system responsible for fighting against honeybee pathogens (Lourenço et al. 2013). In this review, we summarize the available information on bacterial diversity and interaction with the honeybee gut, focusing on the importance of probiotics' administration in maintaining and sustaining the health of this host which is crucial for the functioning of both agricultural and natural terrestrial ecosystems.

Diversity and roles of gut microbial communities of honeybees

Honeybee microbiota

Invertebrates are particularly interesting since they generally host simple gut microbial communities if compared to vertebrates. The complexity of human and, in general, mammalian systems has indeed led researchers to consider invertebrates as excellent models for studying the diversity, function, and interactions of the microbiome with the host (Erkosar and Leulier 2014; Engel et al. 2015; Prosdocimi et al. 2015; Saraiva et al. 2015; Kwong and Moran 2016; Zheng et al. 2017). The honeybee gut microbiota shows a number of similarities with the human intestinal microbial community, and thus, it has been recently proposed as an interesting experimental model (Zheng et al. 2018).

As revealed by 16S rRNA gene high-throughput surveys and metagenomics analysis, the adult honeybee gut consists of host-adapted, facultative anaerobic, and microaerophilic bacteria, which encompass nine bacterial species or phylotypes constituting the 95–99.9% of the bacterial community in almost all specimens (Martinson et al. 2011; Engel et al. 2012; Moran et al. 2012; Corby-Harris et al. 2014a; Kwong

and Moran 2016; Kwong et al. 2017; Bleau et al. 2020; Callegari et al. 2021; Su et al. 2022) (Fig. 1). Among these, five bacterial phylotypes represent the core microbiota, namely *Snodgrassella alvi* and *Gilliamella apicola* (two Gram-negative species from the Proteobacteria phylum), *Lactobacillus* Firm-4 and *Lactobacillus* Firm-5 (two Gram-positive species from the Firmicutes phylum) and *Bifidobacterium* species (from the Actinobacteria phylum) (Babendreier et al. 2006; Martinson et al. 2011; Bottacini et al. 2012; Kwong and Moran 2013). Other four, less abundant, bacterial phylotypes, members of the *Proteobacteria* phylum, are *Frischella perrarra*, *Bartonella apis*, *Commensalibacter* sp. (previously indicated as *Gluconobacter* Alpha 2.1; Bonilla-Rosso et al. 2019) and *Bombella apis* (previously indicated as *Acetobacteraceae* Alpha2.2, then formerly *Parasaccharibacter apium*; Smith et al. 2021) (Engel et al. 2013; Corby-Harris et al. 2014b; Kešnerová et al. 2016). These core and non-core bacterial taxa are generally acquired from surrounding (hive components) and transmitted by bee workers (Powell et al. 2014; Kwong and Moran 2016).

Anatomically, the adult gut is subdivided into various sections, i.e., crop, midgut, ileum and rectum (Fig. 2): these compartments are responsible for storing nectar, digestion and nutrient assimilation, waste excretion and water/salt reuptake, respectively. While few bacteria inhabit the first two compartments, the two distal sections are densely populated. Particularly, the crop harbours members of Enterobacteriaceae family, *L.* (= *Apilactobacillus*) *kunkeei* and *Ba. apis* (Corby-Harris et al. 2014a), while the midgut is often dominated by *G. apicola* and *Ba. apis* (Ludvigsen et al. 2015; Jia et al. 2016; Anderson and Ricigliano 2017). The ileum is instead inhabited by *S. alvi*, *G. apicola* and *Lactobacillus* Firm-5, while the rectum is dominated by the two Firmicutes phylotypes *Lactobacillus* Firm-5 and *Lactobacillus* Firm-4 and the Actinobacteria *Bifidobacterium* spp. (Martinson et al. 2012; Powell et al. 2014; Maes et al. 2016; Anderson and Ricigliano 2017) (Table 1).

Changes in the bacterial community structure and composition have been reported to occur in accordance to age, caste differentiation and season turnover. Dong et al. (2020) assessed the composition of the gut microbiota in workers at different age, reporting that their bacterial community is significantly altered with age progression. For instance, the gut microbiota at 1 day post-emergence is dominated by *Gilliamella*, *Frischella*, and *Snodgrassella*, while along the life cycle the proportions of these bacterial phylotypes change. Considering caste differentiation, the gut of adult workers is inhabited by a relatively stable array of bacterial phylotypes compared with the ones of males or queens (Kapheim et al. 2015; Tarpay et al. 2015).

Conversely to the adult gut, the larval one has been much less explored with regards to the microbiota characterization (Hroncova et al. 2019; Kowallik and Mikheyeva 2021;

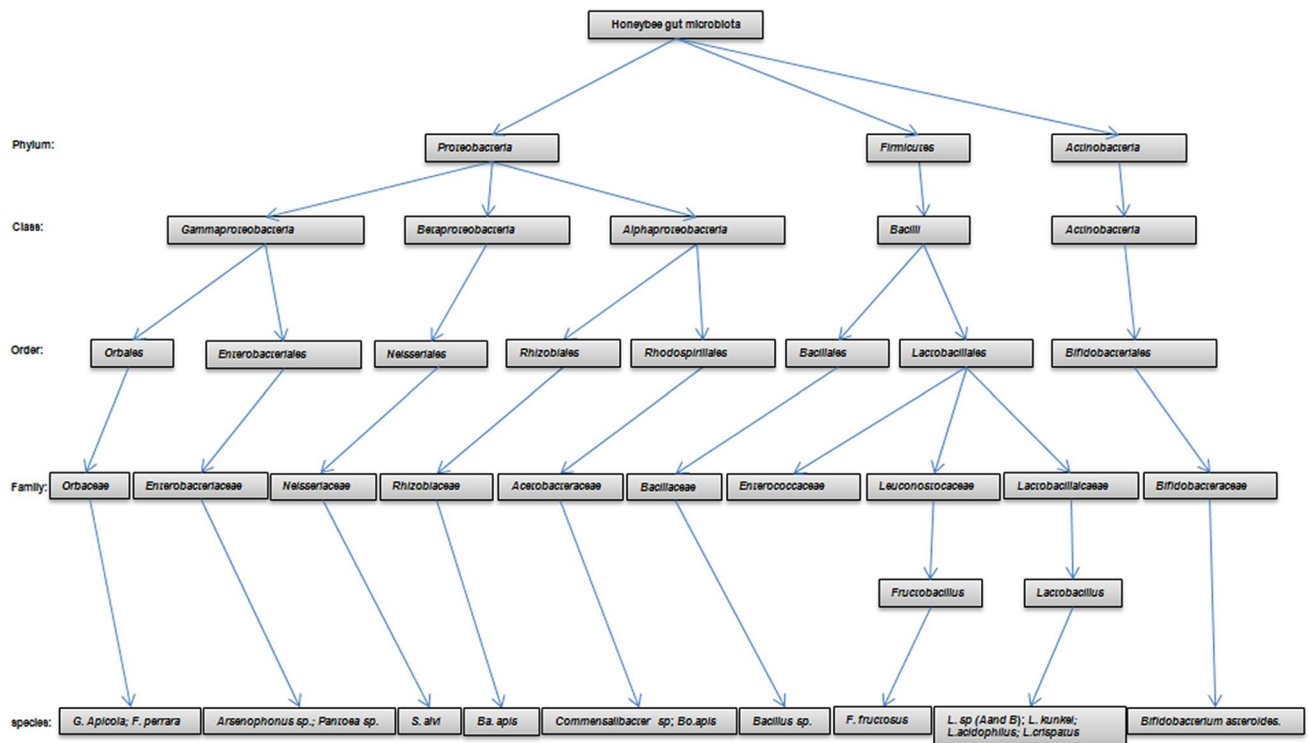


Fig. 1 The major commensal bacterial species living in adult honeybee and larval gut (Vojvodic et al. 2013; Corby-Harris et al. 2014a; Engel et al. 2014; Kwong and Moran 2016; Maes et al. 2016)

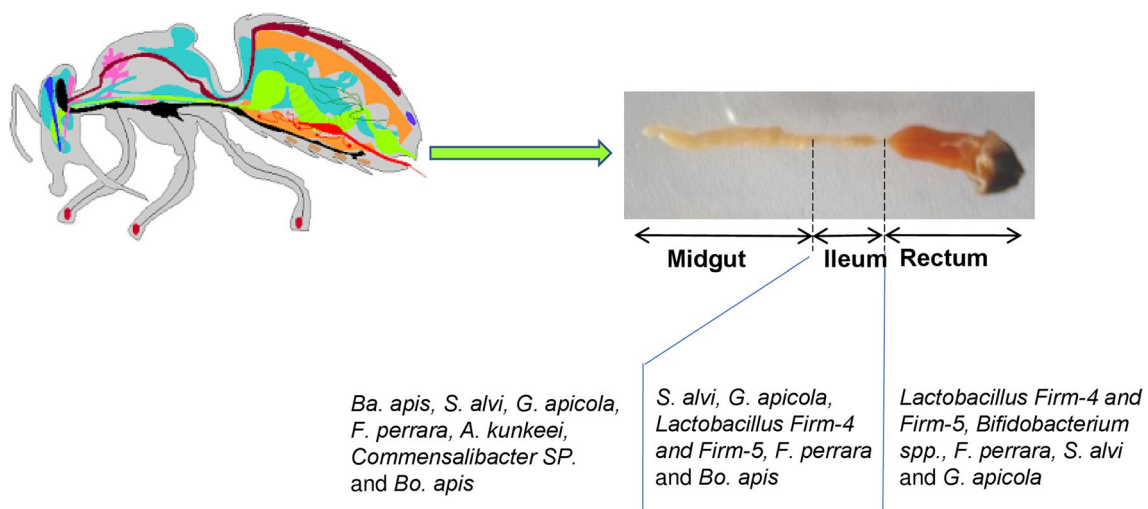


Fig. 2 The honeybee gut. The honeybee adult gut compartments inhabited by the host-adapted phylotypes (Jia et al. 2016; Kwong and Moran 2016; Maes et al. 2016; Anderson and Ricigliano 2017; Bonilla-Rosso and Engel 2018)

Daisley et al. 2020a). For instance, using an isolation-based approach, Vojvodic et al. (2013) showed that the larval gut is colonized by a number of bacterial groups previously found in the honeybee adult gut, i.e. *Bo. apis*, *L. kunkeei*, *Lactobacillus* sp. A (*Lactobacillus* Firm-4), *Lactobacillus* sp. B (*Lactobacillus* Firm-5), *S. alvi*, *Bifidobacterium*,

Fructobacillus fructosus and *Bacillus* spp. The diversity of the larval bacterial communities changes with the larval age: first and second larval instars are exclusively dominated by *Bo. apis*, whereas older instars are mainly inhabited by *Bo. apis*, *Lactobacillus* Firm-4, *Lactobacillus* Firm-5, *L. kunkeei* and *S. alvi* (Vojvodic et al. 2013) (Table 1).

Table 1 Principal studies describing the bacterial diversity in the adult and larval honeybee gut

Study	Honey bee samples	Bacterial taxa (in order of abundance)
Martinson et al. 2011	Adults	<i>Snodgrassella</i> , <i>Lactobacillus</i> sp. (A and B), <i>Bifidobacterium</i> , and <i>G. apicola</i> (Gamma 1)
Martinson et al. 2012	Adults	<i>Lactobacillus</i> sp. (A and B), <i>Bifidobacterium</i> , <i>S. alvi</i> , <i>G. apicola</i> , <i>F. perrara</i> , and <i>Bo.apis</i>
Moran et al. 2012	Adults	<i>G. apicola</i> (Gamma 1), <i>G. apicola</i> (Gamma 2), <i>S. alvi</i> (Beta), <i>Lactobacillus</i> sp. (A and B), <i>Bifidobacterium</i> , <i>Bo.apis</i> , and Enterobacteriaceae
Vojvodic et al. 2013	1st instar larvae	<i>Bo.apis</i> and <i>L. kunkeei</i>
	2nd instar larvae	<i>Bo.apis</i> and <i>Lactobacillus</i> sp. B (Firm-5)
	3rd instar larvae	<i>Bo.apis</i> , <i>L. kunkeei</i> , <i>Lactobacillus</i> sp. (A and B), <i>Bifidobacterium</i> , <i>F. fructosus</i> , and <i>Bacillus</i> sp.
	4th instar larvae	<i>Bo.apis</i> , <i>L. kunkeei</i> , <i>Lactobacillus</i> sp. (A and B), <i>F. fructosus</i> , and Neisseriaceae
	5th instar larvae	<i>Bo.apis</i> , <i>L. kunkeei</i> , <i>Lactobacillus</i> sp. (A and B), <i>Bifidobacterium</i> , Neisseriaceae, and <i>Bacillus</i> sp.
Corby-Harris et al. 2014a	Adults (pollen foragers)	Acetobacteraceae Alpha 2.1 (<i>Commensalibacter</i> sp.), <i>Lactobacillus</i> sp. (A and B), <i>G. apicola</i> (Gamma 1), <i>S. alvi</i> (Beta), and <i>Bifidobacterium</i>
Engel et al. 2014	Adults	<i>G. apicola</i> , <i>S. Alvi</i>
Kwong et al 2017	Adults	<i>Lactobacillus</i> sp. (A and B), <i>Snodgrassella</i> , <i>Gilliamella</i> , <i>Bifidobacterium</i> , <i>Ba.apis</i> , and <i>Frischella</i>
Bleau et al. 2020	Adults (nurse bee)	Acetobacteriaceae (<i>Bombella</i> sp., <i>Commensalibacter</i> sp.), Bifidobacteriaceae, Enterobacteriaceae (<i>Arsenophonus</i> sp., <i>Pantoea</i> sp.), Lactobacillaceae, Neisseriaceae (<i>Snodgrassella</i> sp.), Orbaceae (<i>Frischella</i> sp., <i>Gilliamella</i> sp.), Rhizobiaceae (<i>Bartonella</i> sp.) and Enterococcaceae
	Adults (foragers)	<i>Gilliamella</i> , <i>Snodgrassella</i> , <i>Lactobacillus</i> sp. (A and B), <i>Bifidobacterium</i> , <i>Frischella</i> , <i>Commensalibacter</i> , <i>Bombella</i> , <i>Apibacter</i> , and <i>Bartonella</i>
Dong et al. 2020	1 day poste mergence	<i>Gilliamella</i> , <i>Frischella</i> , and <i>Snodgrassella</i>
	3 day poste mergence	<i>Lactobacillus</i> , <i>Bifidobacterium</i> , and <i>Commensalibacter</i>
	12 day poste mergence	<i>Gilliamella</i> , <i>L. kunkeei</i> and <i>Bartonella</i>
	25 day poste mergence	<i>Lactobacillus helsingborgensis</i> , and <i>Bifidobacterium</i>
	Between 19 and 25 day poste emergence	<i>Bacteroides</i> , <i>Escherichia-Shigella</i> , and Porphyromonadaceae
Tola et al. 2020	Adults (foragers)	<i>Gilliamella</i> , <i>Snodgrassella</i> , <i>Lactobacillus</i> sp. (A and B), <i>Bifidobacterium</i> , <i>Frischella</i> , <i>Commensalibacter</i> , <i>Bombella</i> , <i>Apibacter</i> , and <i>Bartonella</i>
Callegari et al.2021	Adults (foragers)	<i>Lactobacillus</i> sp. (A and B), <i>Bifidobacterium</i> , <i>Gilliamella</i> , <i>Snodgrassella</i> , <i>Bartonella</i> , <i>Bombella</i> , <i>Frischella</i> , <i>Apibacter</i> , and <i>Commensalibacter</i> ...

Honeybee bacterial community also undergoes seasonal variations (Kwong and Moran 2016). Winter honeybees host a more abundant community with lower diversity in comparison with nurses and foragers, showing higher levels of *Bartonella* and *Commensalibacter* (Kešnerová et al. 2020). Similarly, it has been found that also in tropical or subtropical climates, i.e. where honeybees emerge constantly along the year, nurses show a higher gut microbiota diversity in spring and a lower one in summer and winter: *Lactobacillus* spp. dominate in spring, while *Gilliamella* and *Snodgrassella* are more abundant in summer and winter (Castelli et al. 2022). Differently from Kešnerová et al. (2020), no variation in the total bacterial abundance was significantly reported through the year in nurses sampled in tropical or subtropical climates (Castelli et al. 2022).

Honeybee gut is also inhabited by yeasts and other fungal partners, on which so far not so many studies have been devoted compared to the ones performed on the bacterial

component of the honeybee microbiota (Cox-Foster et al. 2007; Ludvigsen et al. 2020; Callegari et al. 2021). Acquired during food intake, fungal populations are less abundant than bacterial ones in the host gut, but nevertheless, they have been suggested to have a role in food digestion and to help the establishment of spatial and trophic interactions among gut members (Callegari et al. 2021). Yeasts such as *Hanseniaspora* and *Starmerella* have been found to dominate the midgut, ileum and rectum compartments of Italian honeybees, whereas *Zygosaccharomyces* are mainly associated to honeybees collected in Saudi Arabia, displaying a different vegetation in the sampling areas (Callegari et al. 2021).

Microbiota functions in the honeybee gut

The microbiota of adult honeybees plays many crucial functions in the host, e.g. supporting meal digestion,

improving the innate immune response against pathogens and parasites, enhancing host development, modulating host behaviour and protecting the honeybee against microplastic contamination (Engel et al. 2015; Kwong and Moran 2016; Zheng et al. 2017; Wang et al. 2021). The presence of the bacterial community is essential for the honeybee, as it promotes the acquisition of insect weight through its metabolism and hormonal signalling, further influencing the insect behaviour (Zheng et al. 2017). The abundance of the core bacterium *S. alvi* in ileum has been positively related to bee survival and development (Maes et al. 2016), whereas the occurrence of *F. perrara* in the pylorus area has been correlated with scab formation, highlighting that the host mounts an immune response and a melanisation cascade against this bacterium, thus suggesting its recent acquisition or evolution as a symbiont (Engel et al. 2015). Indeed, when honeybees are fed with aged diets, the increase of *F. perrara* in the ileum, with a simultaneous decrease of *S. alvi*, is related to an increased mortality of honeybees (Maes et al. 2016).

Numerous studies have also shown that Gram-positive bacteria isolated from honeybee larvae and adults are active against bee pathogens thanks to the production and release of antibacterial substances. Particularly, *Brevibacillus* spp., *Bacillus*, and lactic acid bacteria (*L. kunkeei*, *L. acidophilus* and *L. crispatus*) exhibit inhibitory effects on the growth of *P. larvae* (Evans et al. 2006; Yoshiyama and Kimura 2009; Kačániová et al. 2021). Finally, both *Betaproteobacteria* and *Gammaproteobacteria* have been proven to possess a defensive role towards the invasion of the parasite *Chritidia bombi* in bumble bees (Koch and Schmid-Hempel 2011).

Biotic and abiotic factors of honeybee gut dysbiosis

Gut dysbiosis refers to the intestine microbial imbalance which leads to negative host physiological and functional changes (Hamdi et al. 2011). To understand this abnormal mechanism, the interaction between the intestinal microbes and host fitness needs to be carefully investigated. So far, only a few works have shown that the loss of host performance or function is directly related to the gut microbial variation, such as an altered proportion among core gut species, or a displacement of the core microbiota by opportunistic/pathogenic microorganisms (Anderson and Ricigliano 2017). Considering that many types of biotic and abiotic stresses, as well as the interplay among them, are known to affect honeybee fitness, it is hence essential to investigate their link with the bee gut dysbiosis (Anderson and Ricigliano 2017).

Abiotic factors

Abiotic factors, such as the lack of food and pollen, pesticide exposure and climate change, can impose a stress to the honeybee, likely increasing its susceptibility to other stressors (Schwarz et al. 2015). Poor pollen diet increases the host sensitivity to pesticides, pathogens (such as viruses) and parasites (such as *Nosema*) (Huang et al. 2012; Maes et al. 2016). Maes and co-authors (2016) have also revealed that newly emerged bees fed with an aged diet show a bacterial intestinal dysbiosis with the increase of the opportunistic pathogen *F. perrara* and the decrease of the core bacterium *S. alvi*, which ultimately results in increased honeybee mortality, reduced thorax weight and increased loads of *Nosema* spp. (Maes et al. 2016). Recently, Li et al. (2022) have also demonstrated that reduction in pollen consumption, due to seasonal diet shifts, drives a variation of the gut community structure, resulting in the winter dominance of the non-core bacterium *Bartonella*, which may help the host to survival to winter conditions.

Honeybee habitat disruption, due to hive installation in greenhouses, results in the down regulation of immune-related and antioxidant system genes of the bees, leading to the increase of *Nosema* spp. loads in these immune-suppressed hosts which also accumulate oxidative damage (Morimoto et al. 2011). Additionally, bee colonies exposed to a low, sub-lethal level of the neonicotinoid imidacloprid experience increased levels of *Nosema* parasites (Pettis et al. 2012).

Biotic factors

When infected by *Nosema* parasites or other pathogens, such as the causative agents of AFB or European Foulbrood (EFB), a perturbation of the core gut microbiota associated to honeybees occurs (Hamdi et al. 2011). Obligate intracellular *Nosema* microsporidia represent a serious risk for the beekeeping sector (Higes et al. 2006, 2007). They can infect honeybees through faecal–oral and oral–oral routes of transmission: spores can be found in bee faeces, but also in pollen, and they can germinate in midgut and infect midgut epithelium cells (Higes et al. 2008; Genersch 2010; Fries 2010; BenVau and Nieh 2017). *Nosema*-infected honeybees show shortened lifespans, delayed development, and altered physiology, immunity and behaviour (Genersch 2010). Paris et al. (2017) have interestingly reported a decrease of reactive oxygen species (ROS) amounts and ROS damage in honeybees infected by *N. ceranae*; however, high levels of protein oxidation have been detected in honeybees infected by this parasite and exposed to the insecticide fipronil at the same time, suggesting that *Nosema* could have a role in increasing the toxicity of the insecticide. Moreover, infections with *N. apis* have been associated with the presence

of several viruses, i.e. the Black Queen Cell Virus (BQCV), the Bee Virus Y (BVY), and the Filamentous Virus (FV) (Fries et al. 2013). Abdi et al. (2018) have also reported a co-infection of honeybees from the same apiary with the three pathogens DWV, *N. apis*, and *N. ceranae*. Finally, in worker bees, poor pollen nutrition results in a reduced resistance against DWV and *Nosema* spp., while good protein supplementation can increase bee resistance to pathogens and parasites (Posada-Florez et al. 2019; Watkins de Jong et al. 2019; Huang 2012).

Affecting apiculture worldwide with severe economic losses for beekeepers, AFB is a honey bee brood disease caused by the highly contagious bacterium *P. larvae* (Genersch 2008). *P. larvae* spores, which can be transferred by adult nestmates to larvae during feeding activity, germinate inside the larval midgut within 24 h, after which they breach through the midgut wall (Evans 2004). López et al. (2017) have showed that larvae, co-exposed to *P. larvae* and to sub-lethal doses of pesticides (dimethoate or clothianidin), have higher mortality levels than larvae exposed solely to the bacterial pathogen. Interactions between pesticides used in beekeeping and pathogens of honeybees could hence have a more marked effect than what previously considered on colony health.

Dysbiosis has been also observed in hives with colony collapse disorder (CCD) symptoms: comparing healthy bee colonies with CCD-suffering ones, higher relative abundance of α -Proteobacteria and Firmicutes have been found in honeybees from the first ones (Cox-Foster et al. 2007). Similar results have been also reported by Cornman et al. (2012). In this light, great efforts need to be carried out to unravel the variation of the honeybee gut microbiota when challenged by different stressors, and combination of them, to understand stress-gut microbiome interplay. Moreover, alternative treatments should take into consideration the possibility to restore the indigenous microbial community perturbed by stressors.

Limitations of available treatments aimed at controlling honeybee pathogens

Chemical treatments against honeybee pathogens

Antibiotics have been used to manage AFB and EFB, since the early 1950's (Kochansky et al. 2001). Up to 2005, oxytetracycline (OTC), also known as Terramycin (trade name), was the only authorized antibiotic used against these two foulbrood diseases in the United States (Gochnauer 1951; Kochansky et al. 2001). Then, in consequence of recurrent findings and increasing concerns for the appearance of oxytetracycline-resistance strains of *P. larvae*, in 2005 the use of an alternative antibiotic, i.e. the

macrolide tylosin, also known as Tylan (trade name), was formerly accepted by the U.S. Food and Drug Administration (Alippi 2000; Evans 2004; Yoshiyama and Kimura 2009; Tian et al. 2012). Indeed, high incidences of tetracycline/oxytetracycline resistance genes have been reported in the gut bacterial community of American honeybees both using molecular tool, as well as through the isolation of tetracycline-resistant *G. apicola* and *S. alvi* isolates (Tian et al. 2012). On the other hand, following a more precautionary approach antibiotics in Europe have been banned for apiculture. Besides the emergence of pathogenic resistant strains, antibiotics have indeed many disadvantages, e.g., the inefficacy against pathogenic spores, the negative effect on bee vitality and longevity, and the presence of chemical residues in pollen, bee wax and honey, affecting consequently honey safety and quality (Genersch 2010; Barrasso et al. 2018). Currently, bacterial resistance to antibiotics, and more general to antimicrobials, is considered a serious risk for human, animal and environmental health (Perry and Wright 2013; Larsson and Flach 2022).

On the other hand, in Canada and USA, beekeepers manage *Nosema* microsporidia control taking advantage of the only commercially registered antibiotic, i.e. fumagillin bicyclohexyl ammonium, also known as Fumagilin-B (trade name), produced by Medivet Pharmaceuticals Ltd. (Williams et al. 2008). Conversely, antibiotic treatments on *Nosema*-infected hives are not allowed in Europe (Fries 2010).

Effects of biocidal treatments on honeybee gut

Similarly to humans and livestock, the use of antibiotics has been shown to cause gut dysbiosis in honeybees, resulting in the increase of insect mortality and host susceptibility to opportunistic pathogens' invasion both in hive and in laboratory experiments (Bulson et al. 2021; Raymann et al. 2017; Soares et al. 2021; Aljedani 2022). Particularly, adult workers treated with tetracycline hosted a disturbed gut microbiota, in terms of size and composition (Raymann et al. 2017; Daisley et al. 2020b). Raymann et al. (2017) have indeed documented smaller bacterial communities in antibiotic-treated bees than in non-treated ones, with a reduction of the core phylotypes *Bifidobacterium*, *Lactobacillus* Firm-4, *Lactobacillus* Firm-5 and *Ba. apis*, and an increase of the relative abundance of *G. apicola*. Persisting for long periods of time, antibiotics inducing dysbiosis can lead to the increase of non-bacterial pathogens such as *Nosema* spp. and viruses (Koch and Schmid-Hempel 2011; Schwarz et al. 2016; Raymann et al. 2017). Moreover, honeybees exposed to pesticides (e.g., chlorothalonil) have perturbed native gut communities (Kakumanu et al. 2016).

The promise of probiotics

The term “probiotic” has been used with different meanings over the years. In 1965, it was formerly used to describe substances secreted by microorganisms, able to stimulate the growth of other microorganisms (Lilly and Stillwell 1965). Currently, it is used to indicate microorganisms with beneficial effects for humans and animals: the commonly used definition indicates probiotics as dietary supplements of “live microorganisms that when administered in adequate quantities confer a health benefit on the host” (Fuller 1992; Schrezenmeir and de Vrese 2001; Texts 2001; Johnston et al. 2016; Perdigon and Maldonado Galdeano 2017; Hill et al. 2014) by enhancing its intestinal microbial balance (Rasic 1983; Johnston et al. 2016). Therefore, to understand probiotics’ functioning, knowledge of the microbial diversity and ecology of the intestinal tract is pivotal (Smoragiewicz et al. 1993).

In humans, probiotics are used for the treatment of clinical conditions characterized by abnormal gut microflora and impaired intestinal mucosal barrier functions, favouring mechanisms of pathogen elimination and, generally, stimulating the immune response (Salminen et al. 1998; Gourbeyre et al. 2011; Johnston et al. 2016). Negative effects of antibiotic treatments can be indeed reduced by the administration of probiotic strains such as *Lactobacillus* and *Bifidobacterium* ones (Colombel 1987; Gorbach et al. 1987; Kujawa-Szewieczek et al. 2015; Härtel et al. 2017; Baud et al. 2020). Probiotic bacteria (e.g., *Lactobacillus* and *Bifidobacterium* strains) have been also shown to interact with the gut microbiome to strengthen the immune system, being effective in reducing not only bacterial replication, but also viral one (Bozkurt and Quigley 2020; Mirzaei et al. 2021). However, if probiotics have been considered an economical and safe alternative for the cure of human viral diseases, there are no data on the use of probiotics against honeybee viral infections. Further research is thus needed to investigate if probiotics could be used by beekeepers for the treatment of viral diseases.

With regards to insects and in particular to honeybees, recent studies have suggested that non-pathogenic bacteria could be used in probiotic treatments to enhance insect growth and population and to prevent diseases (Crotti et al. 2013; Audisio 2017; Grau et al. 2017). Interestingly, in *Drosophila melanogaster*, the presence of specific indigenous gut bacteria, such as *L. (=Lactiplantibacillus) plantarum* (Storelli et al. 2011) and *Acetobacter pomorum* (Shin et al. 2011; Storelli et al. 2011), regulate larval growth and body size via activation of the insulin pathway (Buchon et al. 2013). Among the bacterial groups frequently found in the honeybee microbiota, lactic acid bacteria (LAB) are attracting a great interest (Olofsson

and Vásquez 2008; Crotti et al. 2010; Buchon et al. 2013; Daisley et al. 2020a; Daisley et al. 2020b; Daisley et al. 2022). *Lactobacillus* and *Bifidobacterium* are certainly two of the most important genera normally found as commensals and used as probiotics for both humans and animals (Ouweland et al. 2002; Bozkurt and Quigley 2020).

In last years, great efforts have been devoted to study honeybee response after the exposure to probiotic microorganisms, considering both the elicitation of the innate immune system, which ultimately act to maintain homeostasis in case of a pathogen attack, and the probiotic ability to counteract directly a pathogen (Evans and Lopez 2004; Olofsson and Vásquez 2008; Kwong et al. 2017; Daisley et al. 2022). For instance, experiments carried out with *Lactobacillus* and *Bifidobacterium* strains showed a stimulation of the innate immune response of the bee and a positive contribution for the host fitness in presence of a pathogen (Evans and Lopez 2004; Daisley et al. 2020a; Daisley et al. 2020b; Iorizzo et al. 2022).

Evans and Lopez (2004) have orally administered to bees a mix of bacterial species belonging to the genera *Bifidobacterium* and *Lactobacillus* (*B. infantis*, *B. longum*, *L. (=Lactocaseibacillus) rhamnosus*, *L. acidophilus*, and *L. (=Limosilactobacillus) reuteri*, observing the ability of these non-symbiotic bacteria to induce a strong immune response with high levels of the antimicrobial peptide (AMP) abaecin, which could help the larvae to overcome pathogens’ attacks (Evans and Lopez 2004; Olofsson and Vásquez 2008). Moreover, some native bacterial species (e.g., originating from the honeybee stomach) have shown antagonistic activity against pathogens (Olofsson and Vásquez 2008; Zendo et al. 2020). Strains of *Lactobacillus johnsonii* (CRL 1647, AJ5, and IG9) and *Bacillus subtilis*, isolated from honeybee gut and honey samples, showed a beneficial effect on bee colony health against *P. larvae* and *N. apis* (Sabaté et al. 2009; Audisio et al. 2011, 2015; Vásquez et al. 2012; Lazzeri et al. 2020). *B. subtilis* subsp. *subtilis* Mori2, obtained from a honey sample, favoured bee performance, reducing the prevalence of *Nosema* and *Varroa* (Sabaté et al. 2012). Again, Daisley et al. (2020a) showed that a mix of three selected lactobacilli strains (*L. rhamnosus* GR-1, *L. plantarum* Lp39, and *L. kunkeei* BR-1, namely LX3, including non-symbiotic and healthy bee hive derived bacteria) could improve bee survival against AFB causative agent, inhibiting the pathogen and beneficially modulating the bee innate immune response. Moreover, the administration of these strains have been shown to mitigate the gut dysbiosis and immune dysregulation induced by the application of oxytetracycline (Daisley et al. 2020b).

The reported biological effects of probiotics might be due to several factors e.g. the ability of probiotics to produce active compounds or organic acids against pathogens, to elicit the immune response through AMPs expression, to

competitively exclude the pathogens and to suppress collaborative interactions (Lee and Salminen 2009; Endo and Salminen 2013; Kanmani et al. 2013; Muñoz-Atienza et al. 2014; Torres et al. 2016; Daisley et al. 2022). Yoshiyama et al. (2013) have indeed reported that the effect of LAB against *P. larvae* could be due to the release of organic acids.

Concluding remarks

Honeybee fitness is challenged by different biotic and abiotic stresses, which pose serious threats not only for honeybee individuals, but also for the ecosystem and crop yield. In this context, research interest is currently focused on the exploitation of probiotic formulations aimed at maintaining honeybee fitness and boosting its immune response. Probiotic products thus represent promising solutions for the sustainable management of honeybee health, but proper probiotics must be selected and tested in order to achieve this purpose considering all the different stressors and that, up to now, only limited studies have been performed with managed colonies. Moreover, further investigations should be carried out to decipher the mechanisms of competition and collaboration established between probiotics and native gut strains.

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Declarations

Conflict of interest The authors have declared that no competing interests exist.

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