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REVIEW

Prospects of probiotics in beekeeping: a review for sustainable approach to boost honeybee health

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Abstract

Honeybees are vital for global crop pollination, making indispensable contributions to agricultural productivity. However, these vital insects are currently facing escalating colony losses on a global scale, primarily attributed to parasitic and pathogenic attacks. The prevalent response to combat these infections may involve the use of antibiotics. Nevertheless, the application of antibiotics raises concerns regarding potential adverse effects such as antibiotic resistance and imbalances in the gut microbiota of bees. In response to these challenges, this study reviews the utilization of a probiotic-supplemented pollen substitute diet to promote honeybee gut health, enhance immunity, and overall well-being. We systematically explore various probiotic strains and their impacts on critical parameters, including survival rate, colony strength, honey and royal jelly production, and the immune response of bees. By doing so, we emphasize the significance of maintaining a balanced gut microbial community in honeybees. The review also scrutinizes the factors influencing the gut microbial communities of bees, elucidates the consequences of dysbiosis, and evaluates the potential of probiotics to mitigate these challenges. Additionally, it delineates different delivery mechanisms for probiotic supplementation and elucidates their positive effects on diverse health parameters of honeybees. Given the alarming decline in honeybee populations and the consequential threat to global food security, this study provides valuable insights into sustainable practices aimed at supporting honeybee populations and enhancing agricultural productivity.

Keywords Probiotics · Honeybees · Honeybee health · Bee colony · Immunomodulator · Infections · Microbiota

Introduction

Honeybees being one of the most important crop pollinators in the world increase the productivity and quality of crops through cross pollination (Sihag 1986; Kumar et al. 2021). Bees attribute to around 80% of global pollination out of which around 6.1% of crop land is pollinated by honeybees (Aizen and Harder 2009). Honeybees alone contribute to approximately $ 20 billion crop production annually in the US by functioning as a chief pollinator. Research also shows that planned honeybee pollination can enhance seed production and quality of crops, leading to increased yields and improved crop quality. It was also found that planned honeybee pollination resulted in a higher number of seeds per pod, increased seed weight, and higher yields compared to open field conditions (Singh et al. 2016).

Honeybees produce honey, bee bread, propolis, venom, wax, and royal jelly that are required during their life cycle (Cornara et al. 2017; Kurek-Gorecka et al. 2020). The queen bee feed upon the royal jelly whereas the drone and worker larva feed upon royal jelly, honey, and pollen (Melliou and Chinou 2014; Vezeteu et al. 2017). Proper nutritional supplementation is required for the growth and development of the bees and its deficit may cause viral and fungal diseases (Iorizzo et al. 2020). For proper development and reproductive functioning in bees, proteins, vitamins, minerals, and lipids are required which are predominantly found in pollen (Danner et al. 2017) that are essential for the development of the hypopharyngeal gland (Hoover and Ovinge 2018). A honeybee diet comprises of mainly water, nectar, and pollen. The nectar collected from flowers is the source

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of carbohydrates and the pollen is source of protein for bees (Agarwal et al. 2023). Sometimes, when the natural sources of food are not available to the bees during the dearth period, pollen supplementary diets play a crucial role in providing essential nutrients to the bees (Ullah et al. 2021a).

Now a days, beekeepers are facing twice the colony losses, affecting the pollination and food security all over the world. The parasitic and pathogenic infections induce serious colony losses, deteriorating the health of bees (Ricigliano et al. 2022a). Colony collapse disorder is a great concern, which causes high mortality and causes a drastic decrease in the population of adult bees (Royan 2019). One of the major reasons for the loss of bee colonies is infections caused by different pathogens like *Paenibacillus larvae,* Varroa mites and *Nosema cerana* (Royan et al. 2007; Evans and Schwarz 2011). *Melissococcus plutonius* cause major damage to bee keeping industry worldwide (Forsgren 2010). The most common method to combat these infections caused by different pathogens that lead to colony loss, is the use of antibiotics, although their consumption can be harmful to bees as well as humans (Vasquez et al. 2012) and can also cause antibiotic resistance. Hence, rather than using antibiotics, pollen substitute diets supplemented with probiotics can be implemented to increase the gut health of honeybees. Better gut health indicates presence of beneficial bacteria in the gut of bees, which serve various immunomodulatory functions so that they can fight bacterial infections. A well-balanced diet is important to maintain the health of bees. Plant-based pollen substitute diets usually are protein rich, but decrease the life span of bees; hence there is a great interest in feeding probiotics like *Lactobacillus, Bifidobacterium*, or other beneficial bacteria to the honeybees (Kaznowski et al. 2005).

The review aims to investigate various probiotic supplementation and their effects on health of bees. We will explore how probiotics, including various species such as *Lactobacillus*, *Bifidobacterium*, *Bacillus*, *Pediococcus*, and *Enterococcus*, contribute to enhancing the survival rate, colony strength, immunity, honey production, royal jelly production and promoting the overall well-being of these vital pollinators. These findings provide valuable insights into implementing probiotics to enhance overall well-being in bee populations.

Microbiota of honeybees

Microbiota of an organism refers to the beneficial microorganisms like bacteria that inhabit in different body parts of an organism and play several roles for proper functioning of the body and establishing health of an organism. Microbiota of an organism perform various metabolic, immunomodulatory, anticarcinogenic and anti-inflammatory activities (Pascale et al. 2018), and plays a crucial role to induce host immune response (Molloy et al. 2012). There are more than nine bacterial species that colonize the gut of honeybees, mostly found in the hindgut of honeybees (Kwong and Moran 2016). The hindgut of mature worker bees consists of 10^8 to 10^9 healthy bacterial cells (Powell et al. 2014). The composition of microbiome differs amongst the adult worker bees and adult queen bee (Tarpy et al. 2015; Kapheim et al. 2015) as the newly emerged worker bees usually lack the gut microbiota (Dong et al. 2020), and they gain this microbiota after a few days of their emergence by social interaction with nurse bees through oral trophallaxis (Martinson et al. 2012; Motta et al. 2018). The gut microbes are at abundance post 3 to 5 days of adult emergence (Li et al. 2017). Lactic acid bacteria are a dominant species of bacteria found inside the honeybee's gut. Decrease in lactic acid bacteria and increase in proteobacteria indicates a dysbiosis in the gut of honeybees (Daisley et al. 2020a).

Microbiota along the gut of honeybee

The abundance in microbial communities increases from crop to rectum of gut of the honeybees (Fig. 1). It indicates that the diversity of organisms is least at the foregut and is most abundant in the hindgut (Lofgren et al. 2019). Symbiotic bacteria and fungi constitute to the main microbial communities present in the gut of bees.

Microbiota of foregut

Bombella apis and *Lactobacillus kunkeei* are commonly found in the crop region of the honeybee's foregut (Kwong and Moran 2016). The overall abundance of microbial biomass is low in the foregut of bees.

Microbiota of midgut

Snograssella alvi is present evenly throughout the midgut of bees whereas *Gliamella apicola* is found close to pylorus region. *Bombella apis* is also found in the bee's midgut (Martinson et al. 2012). The pylorus region of midgut has the presence of *S. alvi* and *F. perrara* (Smutin et al. 2022).

Microbiota of hindgut

The hindgut of bees consists of ileum and rectum. The microbial communities found in ileum are *Snograssella alvi, Gliamella apicola, Lactobacillus Firm-4* and *5* and *Lactobacillus kunkeei* (Martinson et al. 2012; Kwong and Moran 2016; Bonilla-Ross and Engel 2018). The rectum has the presence of *Lactobacillus Firm-4* and *5, Bifidobacterium* and *B. asteroids* (Chen et al. 2021). Other non-core bacteria like Bartonella apis and Commensalibacter spp. are also found in

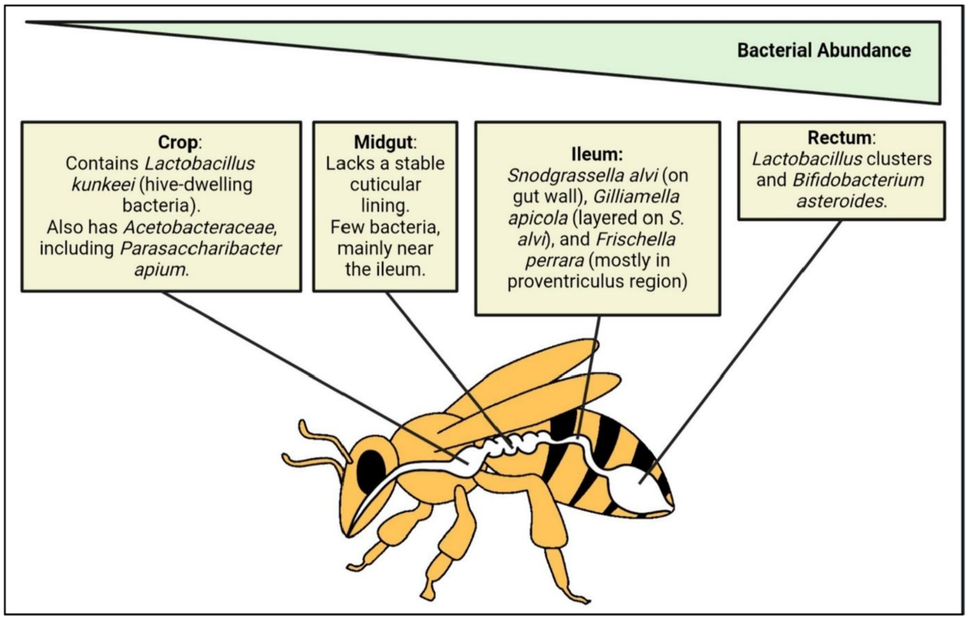

Fig. 1 Microbiota along the gut of bees: Diversity of microorganisms found in foregut, midgut and hindgut of honeybees. Figures shows bacterial abundance increases along the gut from foregut to the hindgut

the rectum (Moran et al. 2012; Martinson et al. 2012; Engel et al. 2015; Kwong and Moran 2016).

Functional role played by microbiota of honeybees

The gut microbiota in insects plays a crucial role in their development, growth, reproductive functions, and metabolism (Wang et al. 2020). Additionally, it aids in the efficient absorption of nutrients and the synthesis of essential nutritional compounds (Pernice et al. 2014). These endosymbionts can produce nutrients which are absent in food (Shi et al. 2010). Due to their fermentation property, the gut microbiota helps in conversion of nectar into honey (Silva et al. 2017). Gut microbiota interacts with the receptors present on the cells and signal the pathways associated to cell survival and death, cellular replication, and inflammatory response (Evans et al. 2006; Valentini et al. 2014). The gut microbiota also influences the neurophysiological aspects of insect through gut microbiota- brain axis (Westfall et al. 2018; Leger and McFrederick 2020). The social behaviour of bees is also regulated by their gut microflora (Vernier et al. 2020). Studies indicate that this microbiome plays a crucial role in immune functioning and metabolism of bees and help in growth and development of bees (Raymann and Moran 2018). Bacteria like *G apicola, Lactobacillus* and *Bifidobacterium* have metabolic capabilities that help in carrying out various metabolic activities inside the body of bees (Ellegaard et al. 2015; Zheng et al. 2016). Studies suggest that microbiome of bees have a positive impact on insulin and vitellogenin signalling, weight gain and gut size of bees (Zheng et al. 2017). Different strains of bacteria found in the gastrointestinal tract possess genes that help in carbohydrate metabolism in bees as indicated by genomic studies (Kwong et al. 2014; Lee et al. 2015). The microbiota also has a role in fighting the infections, as the bees having the entire gut community produce more antimicrobial peptides (Kwong et al. 2017).

Factors altering the microbiota of honeybees

Numerous biotic and abiotic factors contribute to dysbiosis in honeybee microbiomes, including exposure to antibiotics, plant protection products, stress, diet (Ludvigsen et al. 2015), immune responses to viral, fungal, and bacterial infections, aging, and the transition to foraging (Fig. 2) (Corby-Harris et al. 2014; Anderson et al. 2016). Use of antibiotics is the major reasons for the dysbiosis. Bees in bee keeping industry are usually exposed to antibiotics to prevent pathogenic infections. In the US, oxytetracycline is the commonly used antibiotic for this purpose (Tian et al. 2012). The exposure to tetracycline causes a disbalance in size and composition of the microbiome which result in more opportunistic infections and high mortality in bees (Raymann et al. 2017). Not only the antibiotics but also some pesticides have an adverse effect on the microbiome of bees (Kakumanu et al. 2016). In the agricultural industry, several insecticides, fungicides, and herbicides are used which can be toxic to pollinators like honeybees and can lead to alteration in gut of such organisms (Favaro et al. 2023). The chemicals present in plant protection products like herbicides, fungicides and insecticides alter the composition of the microbiome of bees (Table 1) (Hotchkiss et al. 2022). Chlorothalonil, a widely used fungicide in agriculture, influences the lifespan and nutritional efficiency of bees (Kakumanu et al. 2016) by altering gut microbial communities, resulting in dysbiosis

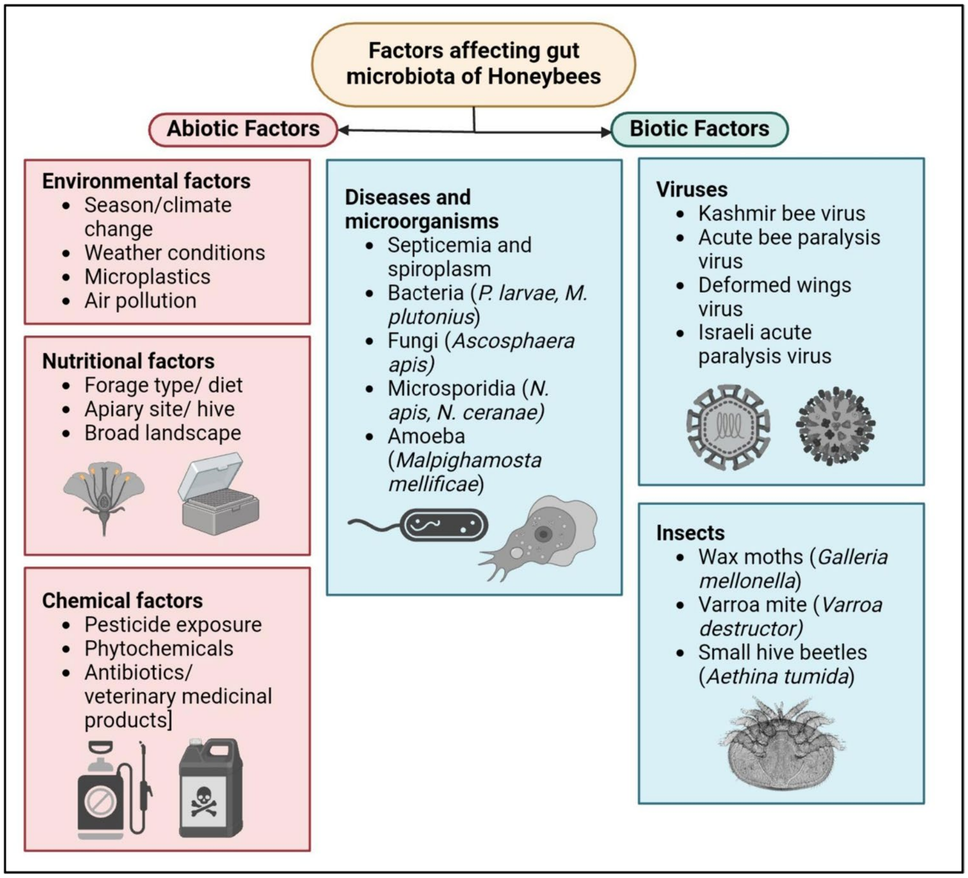

Fig. 2 Factors altering microbiota of honeybees: Several abiotic and biotic factors like exposure to chemicals, environmental factors and biological factors can alter the microbiota of honeybees

Table 1 Chemical factors affecting the gut microbiota of bees and their harmful effects

Factors	Harmful effects on gut microbiota	References
Chlorothalonil	Decreased abundance of *Lactobacillus* species in the gut of bees	Rouze et al. 2019
Glyphosate	Decreased abundance of gut microbiota which leads increased exposure to xenobiotics	Motta et al. 2018; Motta et al. 2020
Thiacloprid	Decrease in abundance of microbiota	Liu et al. 2020
Nitenpyram	Caused compromised immunity and metabolic disorders/changes in bees	Zhu et al. 2020
Tetracycline	Decreased survival rate Increase susceptibility to opportunistic pathogens Increased abundance of antibiotic residues in bee products	Bonilla-Rosso and Engel 2018
Tylosin tartrate	Increased rate of behavioural disorders	Ortiz-Alvarado et al. 2020
Particulate matter	Influenced microbial communities abundance along the gut of honeybees	Mutlu et al. 2018
Heavy metals	Negative impacts reported on microflora of bees	Costa et al. 2019; Rothman et al. 2019
Micro-plastics	Micro-plastics accumulation in gut of bees leads to decreased diversity of gut microflora	Wang et al. 2021

(Wu et al. 2022). Glyphosate, a component of plant protection products (PPPs), has been demonstrated to be lethal to certain microbial species in the gut of honeybees (Motta et al. 2018, 2020). Getting exposed to PPPs increase the occurrence of *N. ceranae* and other viral infections in honeybee resulting in high mortality of bees (Vidau et al. 2011; Raymann and Moran 2018).

Effects of dysbiosis in bees

Poor nutrition is a primary cause of disruptions in the gut microbiota, leading to infections and increased mortality in bees (Maes et al. 2016). Dysbiosis is also a consequence of antibiotic usage to combat infections and exposure to toxins present in pesticides employed by farmers to safeguard their crops against pests. This dysbiosis significantly impacts the development of worker bees, influencing vital developmental gene expressions such as vitellogenin (Schwarz et al. 2016). Moreover, it adversely affects the immune system, as these beneficial bacteria play a crucial role in stimulating immune system pathways and acting as immunomodulators (Schwarz et al. 2016; Kwong et al. 2017; Emery et al. 2017). The gut microbiota plays a pivotal role in shielding bees from the toxic effects of chemicals found in plant protection products (PPPs), and dysbiosis may amplify the toxicity associated with these PPPs (Almasri et al. 2022). Disruptions in the gut microbiota contribute to the deterioration of bee health, leading to colony loss, increased susceptibility to infections, reduced sealed brood area, diminished colony strength, and lower production of bee products. Probiotics offer a potential treatment for addressing this dysbiosis.

Probiotics versus antibiotics to fight against bee infections

An interplay among various diseases and environmental factors impacts the gut microflora of honeybees. Currently studies are focusing on maintaining gut homeostasis by the administration of probiotics that boost the innate immunity of bees against pathogenic infections (Abdi et al. 2023). Several factors like migration, limitation of food and use of pesticides in fields result in a disbalance in microbiota of bees, which as a result weakens the immune system of bees (Ullah et al. 2021b; Iorizzo et al. 2022; Ye et al. 2023). The immune system weakening may lead to several pathogenic infections in bees. To combat these infections, antibiotics were the first choice as therapeutic agents. Although it is the most basic method to fight against bacterial infections, antibiotic use also led to resistance against antibiotics (Goderska et al. 2008). The microbiota of bees supports the digestion of food and maintains a healthy weight in adult bees (Kwong et al. 2017). Antibiotic use disrupts the microflora of adult bees. These bees suffer from low nutrition level and have a beforehand transition to foragers from nurse bees. Hence the gut bacteria regulate the behavioural development in bees (Zheng et al. 2017; Ortiz-Alvarado et al. 2020). There is a continuous effect of antibiotics on the composition and the size of gut microflora of bees (Raymann et al. 2017). Exposure to antibiotics decreases the regulation of several genes important to maintain survivorship of bees (Li et al. 2019).

An experiment conducted by Besharati et al. (2024) aimed to assess the effects of probiotic, antibiotic, and combined probiotic plus antibiotic treatments on various parameters of honeybees. The results revealed that groups fed with probiotics exhibited an increase in colony population and enhanced honey production. Furthermore, this treatment led to a higher abundance of beneficial bacteria within the gut of bees. The honey produced by bees fed on probiotics showed an elevated concentration of fructose and glucose, coupled with a reduced amount of sucrose. Conversely, feeding honeybees with both probiotic and antibiotic treatments resulted in a decrease in *Escherichia coli* count within the bees' intestines. This dual treatment demonstrated a potential reduction in harmful bacteria. In a separate study conducted by Duan et al. (2021), it was observed that antibiotic treatment significantly reduced the expression of developmental, immunity, and metabolic genes in honeybee larvae. This reduction in gene expression correlated with a decrease in the overall fitness of the bees. The findings suggest a potential link between antibiotic treatment and adverse impacts on honeybee development, immunity, and metabolic processes. According to Li et al. (2017), eliminating the beneficial bacteria from the gut of bees using antibiotics negatively affect the immune system functioning by decreasing the expression of genes that code for antimicrobial peptides, which made bees highly susceptible to *Nosema* infection. Some of the most used antibiotics in apiculture industry are oxytetracycline, fumagillin, and tylosen (Piva et al. 2020). These antibiotics contain tetracycline which leads to antibiotic- resistance in several bacterial species (Tian et al. 2012). Oxytetracycline (OTC) is commonly used to prevent *Paenibacillus larvae* and *Melissococcus plutonius* infections in bees (Arbia and Babbay 2011). *P. larvae* has developed resistance against tetracycline (Murray and Aronstein. 2006). A study was conducted by (Daisley et al. 2020b) to see how treatment with three strains of *Lactobacillus* containing probiotic (LX3) after exposure to OTC impacts the recovery of gut microbiota, immunity, and productivity of hives. It was noted that treatment with LX3 lowers the *P. larvae* infections and enhance the immunity of bees post antibiotic treatment. Combination of both LX3 and OTC antibiotic treatment was found to be more effective as compared to antibiotic treatment alone. Tetracycline derived antibiotics like OTC alter the diversity of gut microflora in honeybees (Raymann et al. 2017; Raymann and Moran 2018)can be restored using LX3 supplementation. To prevent antibiotic resistance, their side effects such as; microbiota dysregulation and immune misfunctioning, probiotics now days are a preferred choice over antibiotics. The same is been corroborated from previous studies (Borgers et al. 2021) which authenticated this phenomenon and declare that use of antibiotics lead to a disruption in natural microflora of honeybees, which further can be restored using probiotics.

Delivery mechanisms for probiotic supplementation to honeybees

Delivery mechanism refers to various means through which the feeding of bees can be done. There are three types of delivery mechanisms commonly used to supplement probiotics to bees through food. These mechanisms are pollen patty infusion, sucrose syrup suspension and novel spray-based formulation (Fig. 3).

Pollen patty infusion

It consists of pollen alternatives like soy flour, brewer`s yeast, sucrose granules and sucrose syrup mixed all together. Further probiotics are added to this mixture with continuous stirring to form a homogenous mixture. The patty is poured between two wax paper sheets and kept inside the hive of bees. This method provides good nutritional benefits to the bees however the food can directly be consumed by only adult bees and not by the larvae on their own (Corby-Harris et al. 2014; Ricigliano et al. 2022b).

Sucrose syrup suspension

A standard sugar syrup solution is prepared by mixing sugar with water. To this sugar syrup, probiotics are added and the solution thus formed is kept inside the bee hives for supplementation. This method is the most used one as it is easy to apply however the osmotic stress in the solution may lead to lysis of bacterial cells present in it (Ptaszynska et al. 2016).

Spray based formulation

In a spray bottle, probiotics are added to the phosphate buffer solution in a very diluted concentration. This solution is sprayed all over the hive of the bees. This method ensures the proper distribution of food all over the hive and prevents the bacterial cell lysis caused due to osmotic stress (Liao and Shollenberger et al. 2003).

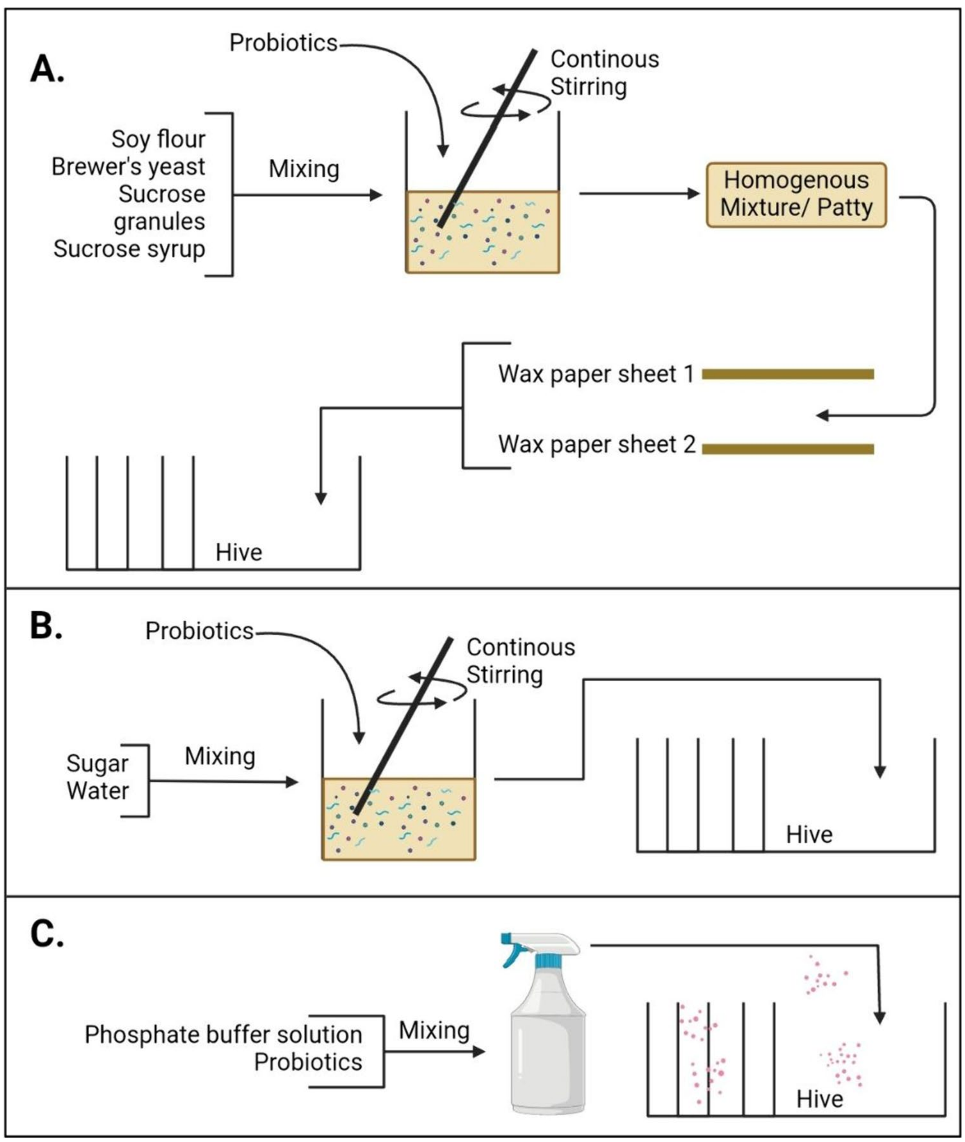

Fig. 3 Delivery mechanisms for probiotic supplementation to bees: **A**. represents pollen patty infusion method; **B**. represent sugar syrup suspension method; **C**. represent spray-based formulation for probiotic supplementation on bees

Effects of probiotics on health parameters of honeybees

Probiotics are the beneficial microorganisms that are found in gut of animals and help in digestion of food and are immune modulatory. They help the host fight pathogenic infections and improve their gut microbiota balance (Kaznowski et al. 2005). The most used probiotics are *Lactobacillus, Bifidobacterium, Pediococcus, and Bacillus* (Fig. 4) (Tomasik 2003). Probiotics help to induce immune response against infectious pathogens in honeybees (Evans and Lopez 2004). Studies indicate that non-infectious bacteria can produce antibacterial peptides like abaecin and defensin in the body of honeybees, which are responsible to stimulate the immune response in honeybees (Evans and Lopez 2004). Gut microbial disbalance lowers the body weight and development of honeybees and leads to early mortality of worker bees (Maes et al. 2016). This disbalance in the gut microflora of bees may lead to occurrence of various opportunistic infections which leads to decline in colony population (Anderson et al. 2017). In such cases, the probiotics may help to fight against pathogens and help in development of bees increasing their survival rate (Corby-Harris et al. 2014). The gut microflora of honeybees also produces organic acids, antimicrobial peptides and bacteriocins which help in defence against infectious pathogens (Audisio et al. 2011; Anderson et al. 2013; Endo et al. 2013; Corby-Harris et al. 2014). Beneficial bacteria in the gut of bees help to reduce the incidents of nosemosis and varroosis infections (Audisio 2016).

Probiotics have a positive influence on various health parameters of honeybees (Fig. 5). Probiotics help to increase the survival rate of bees by preventing pathogenic infections hence act as immunomodulators and increase the colony strength. It also leads to more honey and royal jelly production by increasing the expression of several genes. Probiotics supplementation raises the transcript level of several antimicrobial peptides showcasing their immunomodulatory activity and helps in efficient absorption of nutrients in the gut of bees. Probiotic supplementation helps to maintain the health of bees, and a healthier colony will produce more amounts of bee products and help in better crop yield by efficient pollination. Probiotics have a positive impact on health of bees.

Better survival rate of honeybees

Probiotics act as immunomodulators and prevent several pathogenic infections in bees. This leads to low colony loss due to infections and increases the survival rate of bees. Kaznowski et al. (2005) observed the effect of probiotics (Biogen-N and Trilac) on microflora present in the intestine of worker honeybees. It was revealed that probiotics favoured the survival of bees as the bee loss was observed to be 30 to 50% lower in the colonies fed with probiotics. In a similar study by Mishukovskaya et al. (2020), honeybees were given two types of probiotics to see their effect on honeybee colonies during wintering. The probiotics used were SpasiPchel containing *Bacillus subtilis* and PcheloNormosil containing *Lactobacillus* and *Bifidobacterium*. The results showed increased life span of bees, more colony strength, and a greater number of sealed broods. Probiotics are used as a substitute to antibiotics in prevention of bee infections. Probiotics help in growth and development of bee by producing important vitamins and amino acids. Lakhman et al. (2021) also conducted an experiment to see the effect of probiotics on viability of bees. In this experiment the bees were fed on sugar syrup with different concentrations of probiotics. The observations revealed an increased longevity of bees. Probiotic mixed with sugar syrup is hence proven to

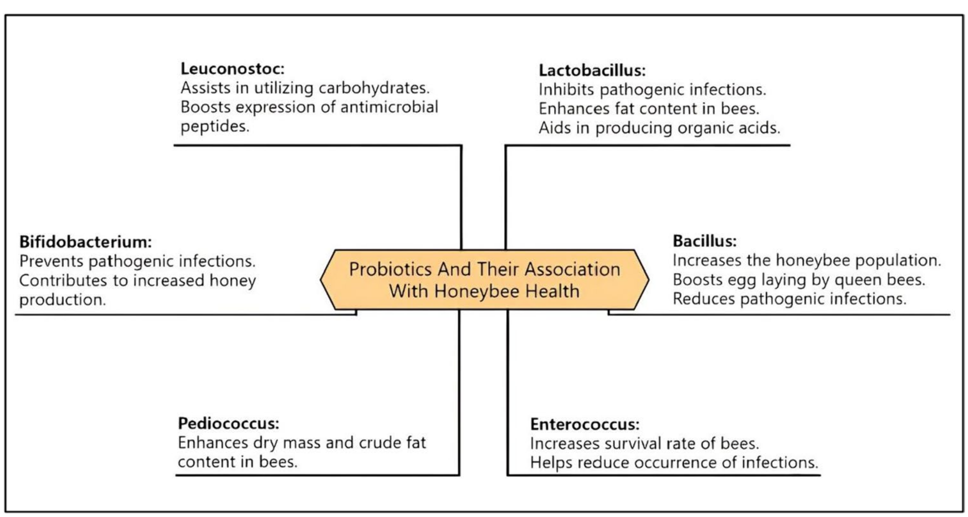

Fig. 4 Diversity of probiotics in the gut of honeybees and the functional role they attribute to boost honeybee health: Probiotics such as; *Bifidobacterium, Lactobacillus, Bacillus, Enterococcus, Leuconostoc* and *Pediococcus* are found in the gut of honeybee's and perform various metabolic activities to maintaining the overall well-being of honeybees

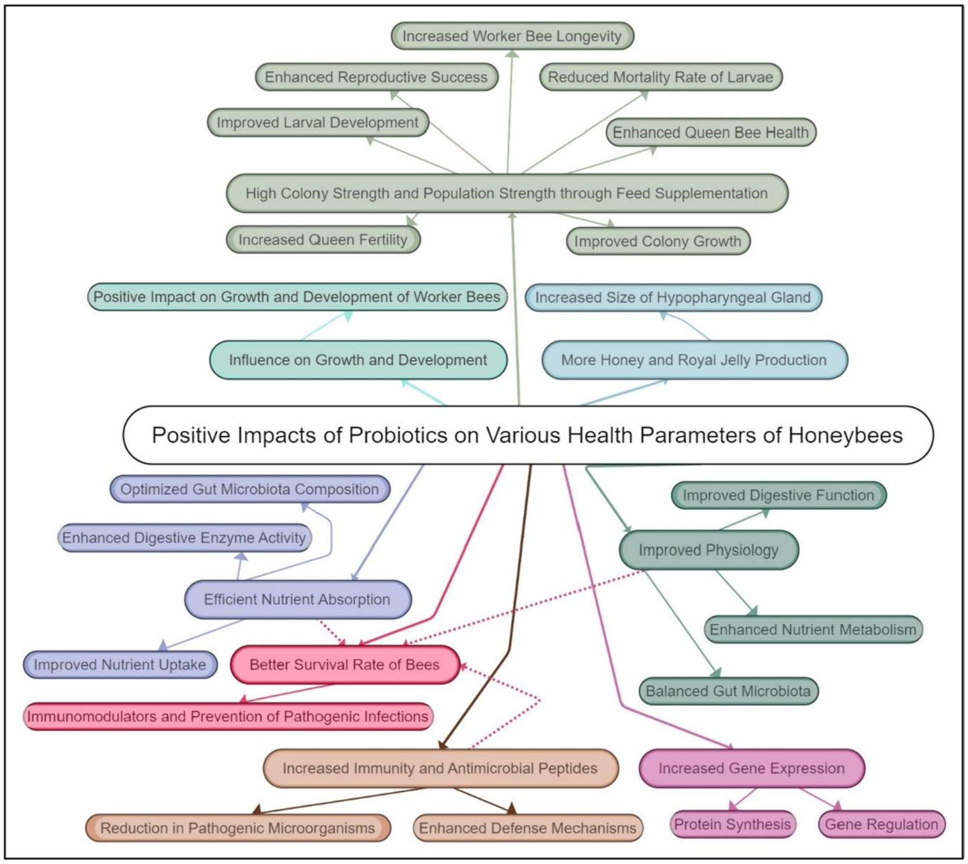

Fig. 5 Positive impacts of probiotics on health of honeybees: By maintaining balance in gut microbiota, probiotics attributes various positive impacts on health of honeybees

be a good alternative to antibiotics to increase the longevity of bees.

More honey and royal jelly production

Probiotics leads to increment in production of honey and royal jelly by increasing the size of hypopharyngeal gland and increasing the expression of several proteins like major royal jelly protein. A study was conducted by Fanciotti et al. (2017) to see the impact of *L. salivarius* A3iob on the yield of honey. *L. salivarius* is a bee gut associated strain of bacteria. The results showed an increase in honey yield by the bees fed on probiotic. Bees produced 2.3 to 6.5 times more honey as compared to control. *L. salivarius* A3iob was proven to positively impact the production of honey. A similar study was done to see the impact of organic acids and probiotic such as; *Lactobacillus rhamnosus* on the hypopharyngeal gland of honeybees by Hasan et al. (2022). The results revealed an increase in actinal surface area of hypopharyngeal gland of bees. A larger hypopharyngeal gland will produce more amount of royal jelly. Hassan et al. (2023) saw the effect of probiotic bacteria extracted from the gut of honeybees on the development of hypopharyngeal gland of nurse worker bees. Result showed a larger hypopharyngeal gland in bees fed on probiotics. All these studies indicate that probiotic supplementation increase size of the hypopharyngeal gland of honeybees.

Influence on growth and development

Probiotics influence the growth and development of honeybees. An experiment was conducted by Hasan et al. (2022) to study the effect of organic acid and probiotics in influencing the growth of worker bees under different conditions. The results showed significant gain of weight in groups given probiotics as compared to the control group. Increase in length of forewing was observed in experimental group. Hence the use of probiotics and organic acids positively affected the bees in terms of their growth and development. Mishukovskaya et al. (2023) studied the effect of probiotic supplementary feeding on bee colonies during winters. The probiotics used for the study were PcheloNormosil containing lactic acid bacteria and *Saccharomyces* and SpasiPchel additive containing 3 strains of *Bacillus subtilis*. Observations showed a higher egg laying by queen bees, increase in sealed brood area, increase in strength of colony and high honey yield in bees feeding on LAB and *Saccharomyces* supplemented probiotic. Hence the probiotics are beneficial in growth and development of honeybees.

High colony strength

Probiotics leads to increased egg laying capacity by queen bees and also enhanced sealed brood area therefore increasing the strength of the colony. An experiment was conducted by Audisio (2016) to see the potential of gram-positive bacteria for bees. The bacterial strains isolated from bees and honey were *Lactobacillus johnsonii* and *Bacillus subtilis*. Bees fed with these bacteria showed more egg laying by the queen bee, more honey yield, reduced incidents of nosemosis and varroosis infection and a higher number of bees in the colony. Garcia-Vicente et al. 2023 conducted a study to see the effect of feed supplementation with probiotics and postbiotics on population of bees. The results indicated that the postbiotic consumption enhanced bee strength, increased egg laying and bee population. It also decreased the *N. ceranae* and *V. destructor* infections. Hence feed supplemented with postbiotics can have a positive effect on health of bees.

Efficient absorption of nutrients

Probiotics found in the gut of the bees help in proper digestion and absorption of nutrients. Efficient absorption of nutrients influences the overall health of bees. Patruica and Mot 2012 fed the bees with prebiotics and probiotics for three weeks to see its effect on intestinal flora of bees. It was reported that there was a noticeable reduction in the number of bacteria in gut of bees, instead the gut of bees was colonised with healthy bacteria present in the probiotics. This resulted in better health status of bees as well as better bio productive index of bees. The effect of pre and probiotic supplementation was seen by (Patruica et al. 2013) on the intestine of worker bees. The results indicated that the probiotic fed bees showed more intestinal villi which help in the proper absorption of nutrients in bee's intestine.

Improved physiology

Probiotics help to increase the activity of several enzymes hence improving the physiology and metabolism of bees. Tlak Gajger et al. 2020 conducted an experiment to see the effect of probiotic treatment on immunological, therapeutic, and biochemical parameters of honeybees. The results showed reduction in *Nosema spp.* spore count, increase in colony strength, more carbohydrate concentration in haemolymph of experimental group, increase in trehalose concentration, increased protein and vitellogenin concentration, increase in diameter of hypopharyngeal gland and other positive physiological changes. Sokolova et al. (2022) studied the influence of *Bacillus* spp. and inactivated yeast on the physiology of *Apis mellifera.* The results showed that activity of proteases increased by 3.32 folds in gut of bees, activity of non-specific esterase increased by 2.16 folds in fat body, activity of glutathione-*S*-transferase increased by 2.64 folds in gut of bees and 1.69 folds in fat body of bees and the honey production increased by 1.5 folds.

Increased immunity

Probiotics contribute to immunomodulation through the production of antimicrobial peptides, thereby preventing pathogenic infections in bees. Royan (2019) studied the effect of mixture of *Lactobacillus* species on reduction of infections in bees. The experiment concluded that the *Bacillus subtilis* species raise the population of the colony and inhibits many pathogenic infections whereas the *Lactobacillus* sp. increase the fat content in bees body, helps to fight against pathogens, aids in production of organic acids and increases honey yield. *Bifidobacterium* also prevent pathogenic infection in bees. Daisely et al. 2020 fed the bees with probiotics to control American foulbrood infection caused by *Paenibacillus larvae*. Results showed a lower pathogenic load on bees. The use of probiotics reduced the pathogenic infections by increasing the immunity of bees and hence increased the survival rate of bees. In a similar experiment Padmashree et al. (2020) saw how probiotics influence the management of diseases and infections in honeybees. The results indicated decreased incidents of European foul brood infection and Thai sac brood infection. The brood comb area also increased. Borges et al. (2021) also conducted an experiment,where the *Nosema ceranae* infected bees were supplemented with probiotics and prebiotics. It was concluded that the probiotic containing *Enterococcus faecium* reduced *N. ceranae* infection rate and increased the survival rate of infected bees even more then healthy bees. Another similar study conducted by Klassen et al. (2021) concluded that protexin and naringenin significantly reduced the *Nosema ceranae* spores, decreased the incidents of infection, increased longevity significantly leading to increased honey production. Thus, decreased pathogenic infections revealed positive impact of probiotic supplementation on the immune functioning of honeybees.

Rise in gene expression and transcript level of antimicrobial peptide

Probiotics help to raise the gene expression and transcript level of antimicrobial peptides like abaecin and defensin which are mainly responsible for fighting against pathogens. This has also been corroborated from previous studies of Evans and Lopez 2004 who conducted an experiment to see the effect of feeding the non-infectious bacteria on the immune responsiveness of *Apis mellifera* larvae against a natural pathogen *Paenibacillus larva.* The observations revealed that the transcript level for abaecin and defensin was raised in case of experimental group which authenticated the study that non-infectious bacteria/ probiotics increased the amount of peptide abaecin during immune response to fight against the *Paenibacillus larva*. Probiotics like *Bifidobacterium* and *Lactobacillus* also increased the expression of abaecin in honeybees, helping in generating an immune response. Hence, they can be used as probiotics to increase the immunity of honeybees. Probiotics help to increase the expression of genes like MRJP1 and vitellogenin. Maruscakova et al. 2020 studies also reported how *Lactobacillus brebis* influences the immune responsiveness of bees by increasing gene expression encoding for different immune related components in intestine of bees. Results revealed that the probiotic had a positive impact on bees. It increased the gene expression for antimicrobial peptides (abaecin and defensin) and recognition receptors. There was an increased abundance of *Enterobacteria* and lactic acid bacteria inside the gut of bees. Huang et al. (2021) also reported the influence of probiotic *Leuconostoc mesenteroides* TBE-8 on bees and observed increased gene expression of MRJP1 by 1400folds, Vitellogenin by 20 folds, antibody peptide hymenoptaecin by 17 folds and Apidaecin by 7 folds after probiotic supplementation.

Several observations were made by scientists over the years by feeding different species of probiotics to the bees (Table 2). These studies indicate that probiotics like *Lactobacillus, Bacillus, Bifidobacterium, Enterococcus* etc. can be given in combination with one another to help improve the health status of honeybees.

Limitations to probiotic supplementation on bees

While probiotic supplementation positively influences honeybee health, there are notable limitations. Tlak Gajger et al. 2020 investigated the impact of varying probiotic concentrations on bee colonies. The study revealed that a 5% probiotic supplementation to sugar syrup increased colony strength, but as the concentration increased to 10%, it leads to honeybee mortality. It was also observed that bees exhibited higher diet consumption at a 2.5% probiotic concentration, but consumption rate decreased with higher probiotic concentrations. Thus, this study revealed that a high probiotic concentration in the diet may leads to reduced food intake and increased bee mortality. Additionally, Anderson et al. (2024) observed no significant correlation between probiotic supplementation and gut microbiota recovery following antibiotic treatment. Exposure to antibiotics like oxytetracycline and tylosin decreases the size and leads to a disbalance in gut microbiome of bees. Non- native probiotics were supplemented to such bees. The result showed no effect of probiotics on recovery of microbiota disbalanced due to antibiotic exposure. Hence the study showed that non- native probiotics are ineffective in treating antibiotic mediated dysbiosis in the gut microbiota of honeybees. Similarly, Damico et al. (2023) observed that the microorganisms present in probiotic supplementation were not found inside the gut of bees. Brown et al. (2022) observed that supplementation with probiotics and b- vitamins can affect the longevity of bees. It can enhance the longevity if given in a cautious amount although harmful effect of high concentration also exists. So from the literature it is concluded that currently there is a lack of knowledge regarding the concentration/ dosage, time of usage and safety considerations of probiotics to get its maximum benefits.

Future perspectives

Future studies can be done to find out the most potential probiotic which has least negative impact or side effects on the health of honeybees. Bees can be fed on different concentrations of probiotics to find out the concentration with maximum consumption and most beneficial effects. Different concentration of sugar syrup (10%, 20%,50% sugar solution) can also be used to find the best consumed one. Studies can be done to see the effect of probiotics on different bee pathogens that cause infections in bees, and further use those probiotics to maintain healthy bee colonies in beekeeping industry. Probiotics can be used to mitigate nutritional and environmental stress on bees. Research can be done to see how probiotic supplementation can enhance the antimicrobial properties of honey, which can further be used by humans as natural medicine to fight against various infections. Honey produced by the bees supplemented with probiotics can be used as a source of probiotic for humans and other animals and its effects can be compared to honey produced by non-probiotic supplemented bees. Further probiotic supplementation can be used to recover gut microbiota dysbiosis caused in bees due to antibiotic exposure. Probiotics can be used as a substitute to antibiotics to combat bee infections. Further studies can be done to find the most suitable dosage, time of probiotic supplementation and mechanism behind its mode of action to get maximum benefits.

Conclusion

Honeybees are the crucial pollinators of agricultural crops but nowadays bee keepers are facing twice the colony losses which are affecting the crop production, agriculture industry and food security. These colony losses are due to different pathogenic and parasitic infections, exposure to antibiotics and chemicals used in plant protection products like pesticides. There are several pathogens like *Paenibacillus larvae, Varroa mites* and *Nosema cerana* that cause diseases in bees

Table 2 Summary of different probiotic supplemented diets and observations made

Sno	Probiotics used	Parameters studied	Observations made	References
1	*Bifidobacterium infantis, B. longum, Lactobacillus rhamnosum, L. acidophilus, L. reuteri*	Immune response of honeybees against *Paenibacillus larvae*	Raised transcript level of abaecin and defensin	Evans and Lopez. 2004
2	*Lactobacillus, Pediococcus acidilactici, Bifidobacterium bifidium, Enterococcus faecium*	Intestinal flora and chemical composition of bees	Better survival rate of bees, Higher dry mass and crude fat in bees	Kaznowski et al. 2005
3	*Lactobacillus acidophilus, Bifidobacterium lactis*	Effect on intestinal microflora of bees	Increased beneficial bacterial population in gut of bees, Improved health status of bees, Better bio productive index of bees	Patruica and Mot 2012
4	*Lactobacillus acidophilus, Bifidobacterium lactis, Lactobacillus casei*	Effect on intestine of worker bees	Increased intestinal villi, Enhanced efficient absorption of nutrients	Patruica et al. 2013
5	*Lactobacillus johnsonii,Bacillus subtilis*	Various health aspects of bees	Increased egg laying capacity of queen bee, Higher honey yield, Reduced nosemosis and varroosis infections, Increased colony strength/ bee population	Audisio 2016
6	*L. salivarius*	Honey yield	Increase in honey production	Fanciotti et al. 2017
7	*Lactobacillus, Bacillus subtillis, Bifidibacterium*	Reduction of infection in bees	Increased egg laying capacity of queen bees, Decreased rate of pathogenic infections, Increased fat content in bee body, Increased honey yield	Royan 2019
8	*Lactobacillus brebis*	Immune response and gut microbiota of bees	Increased gene expression for antimicrobial peptides and recognition receptors, Increased gut flora of entero-bacterium and lactic acid bacteria	Maruscakova et al. 2020
9	*L. plantarum, L. rhamnosus,L. kunkeei*	To counter *Paenibacillus larvae* infection in honeybees	Lowered pathogenic infection, Increased immunity and Increased survival rate of honeybees	Daisely et al. 2020
10	*Bacillus subtilis, Lactobacillus, Bifidobacterium*	Effect on bee colonies during wintering	Increased life span/survival rate of honeybees, Increased colony strength, Increased number of sealed broods area	Mishukovskaya et al. 2020
11	*Enterococcus faecium*	Immunity against *Nosema ceranae* infection in bees	Reduced infection in bees, Higher survival rate of bees	Borges et al. 2021
12	*Leuconostoc mesenteroides*	Expression of different genes	Increased production of MRJP1 by 1400folds, vitellogenin by 20 folds, antibody peptide- hymenoptaecin by 17 folds and apidaecin by 7 folds	Huang et al. 2021
13	*Enterococcus faecium*	Impact on *Nosema ceranae* infection	Reduction in *Nosema ceranae* spore count, Increased honey production, Increased longevity, Increased colony strength/ population of adult bees	Klassen et al. 2021

Table 2 (continued)

Sno	Probiotics used	Parameters studied	Observations made	References
14	*B. subtillis,B. licheniformis*	Honeybee physiology and honey production	Increased activity of protease, Increased activity of non-specific esterases, Increased activity of glutathione-*S*-transferases, Increased honey production	Sokolova et al. 2022
15	*Lactobacillus rhamnosum, Bacillus clausii, Lactobacillus brevis*	Growth and development	Increased length of body, Increased length of forewing	Hasan et al. 2022
16	*Lactobacillus rhamnosum*	Hypopharyngeal gland	Increased actinal surface area of hypopharyngeal gland and hence increased royal jelly production	Hasan et al. 2022
17	*Lactobacillus*	Strength and health status of bees during spring	Enhanced bee strength, Increased bee population, Increased egg laying capacity, Reduced pathogenic infections in honeybees	Garcia-Vicente et al. 2023
18	*Enterococcus faecalis,Lactobacillus casei, Lactobacillus brevis*	Measurement of hypopharyngeal gland of worker bees	Larger hypopharyngeal gland and hence increased production of royal jelly	Hassan et al. 2023
19	LAB, *Saccharomyces, Bacillus subtilis*	Bee colony during spring	Incresed egg laying capacity of queen bees, Increased sealed brood area, Increased strength of colony, Increased honey yield	Mishukovskaya et al. 2023

and are responsible for loss of bee colonies. These infections can be prevented by enhancing the immunity of bees by feeding them on different probiotics like *Lactobacillus, Bifidobacterium, Pediococcus, Enterococcus, Bacillus* etc. Probiotics aids in maintenance of stability in the microbiota of bees and induce an immune response against the pathogens, prevent infections, increase longevity of bees, and increase the survival rate of bees. It also improves honey yield. Hence supplementations with probiotics have proved positive impacts on health aspects of honeybees. We can supplement probiotics to bees to maintain healthier colonies. Further we can see the effect of probiotics on different microbial infections on bees and prevent the occurrence of these infections. Studies can be done to see the influence of probiotics on the quality and quantity of bee products.

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Conflict of interest The authors declare that there is no known competing financial interest or personal relationship that could have appeared to influence the work in this manuscript.

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