

DIFFERENT APPROACH TO THE INVESTIGATION OF BEEHIVE PRODUCTS: A NEW SOURCE OF MICROORGANISMS WITH POTENTIAL INDUSTRIAL APPLICATION

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Abstract: Beehive products have been known since ancient times and are used in medicine, food, and beverages. Honey, bee bread, propolis, pollen, royal jelly, and beeswax are valuable sources of active compounds that contribute to modern apitherapy and help combat civilization-related diseases. However, there is growing attention to the microbiota of beehive products, which vary among different products and depend on several factors, such as the environment surrounding the hive, the collection process of bee products, and their subsequent processing. Culture-dependent and culture-independent methods have been used to investigate the microbiota of bee products. Commonly identified microorganisms include lactic acid bacteria, spore-forming *Bacillus* species and related genera, osmophilic yeasts, and xerophilic molds. The microbiota of bee products holds underexplored potential for applications in industry and medicine, including as potential probiotics, sources of antimicrobial agents, and enzyme producers.

1. Introduction 2. Review methods 3. Bee products and their biological activity 3.1. Honey 3.2. Bee bread 3.3. Bee pollen 3.4. Propolis 3.5. Beeswax 3.6. Royal jelly 4. Microbial diversity of beehive products 4.1. Lactic acid bacteria and bifidobacteria 4.2. Endospore-forming bacteria 4.3 Other bacteria 4.4. Yeasts and molds 5. Industrial and biotechnological potential of the microbiota of beehive products 5.1. Beehive products as a source of probiotic microorganisms 5.2. Antimicrobial activity of beehive product-derived strains 5.3. Enzymatic potential of microbes from bee products 5.4. Fermentation capacity 6. Conclusion.

Keywords: antimicrobial activity, beehive products, enzyme, microbiota, probiotics;

1. Introduction

Since ancient times, honey, propolis, bee bread, and other beehive products have been valued for their use in medicine, food, and beverages (Crane and Kirk Visscher 2009). Their health-promoting properties form the basis of apitherapy (Weis et al. 2022), with antimicrobial activity of honey being one of the best-documented features (Stefanis et al. 2023). Beehive products have been explored for applications as antiviral and antibacterial agents, including against antibiotic-resistant bacteria (Sahlan et al., 2020; Nair et al., 2020), in wound healing (El-Kased et al. 2017), and as ingredients in cosmetics (Jodidio et al. 2024), with some studies also suggesting anticancer potential (Badolato et al. 2017).

Honey is mainly produced by honeybees (*Apis mellifera* L.) and other social insects. Many plants provide nectar or honeydew, which bees collect and process by evaporating water and adding salivary enzymes (Crane and Kirk Visscher 2009). Pollen is collected by moistening it with oral secretions and transferring it to the hive. These resources enable the production of honey, corbicular pollen, bee bread, propolis, and beeswax (Bogdanov 2016). Since the composition, flavor, and aroma of beehive products depend on botanical and geographic origin as well as weather conditions (Crane and Kirk Visscher 2009), numerous studies have examined their characteristics and functional properties worldwide. Research on apitherapy and bee products is continually growing, with new applications such as beehive air therapy, apilarnil use, immune-response

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bioactive molecules, and microbial secondary metabolites gaining increasing attention (El-Didamony et al. 2024). Traditionally, bee products have been recognized and studied mainly for their unique chemical composition and strong antimicrobial properties rather than for their associated microbiota. Nevertheless, they harbor diverse microbial communities, including lactic acid bacteria (LAB), spore-forming bacteria, yeasts, and molds, introduced from the surrounding environment, plants, the hive itself, and by the bees (Grabowski and Klein 2017).

Such microorganisms could be successfully utilized in industry. However, novel strains must fulfill the regulatory requirements defined by agencies such as the European Food Safety Authority (EFSA) and the United States Food and Drug Administration (FDA). Probiotics must be correctly identified and safe, and their beneficial effects should be supported by at least one clinical trial (Binda et al. 2020). Enzyme producers should be recognized as safe hosts and show that the enzymes are suitable for food or pharmaceutical use (Sutay Kocabaş et al. 2019; Pariza et al. 2001). Microorganisms producing antimicrobial compounds must be checked for unwanted toxins or resistance concerns. For food processing, the strains with Generally Recognized as Safe (GRAS) or Qualified Presumption of Safety (QPS) status are the best candidates for commercialization (Laulund et al. 2017).

The main goal of this review is to shift the focus from bee products themselves to their microbiota and to address two key questions: (i) what is currently known about the microbial diversity inhabiting bee products, and (ii) which functional properties of bee-product-associated microbiota are most relevant for potential industrial applications.

2. Review methods

The literature scoping aimed to analyze studies published between 2004 and 2024 on microorganisms isolated from bee products and their functional properties, focusing on LAB and spore-forming bacteria, while only briefly mentioning other groups. The literature was retrieved from two electronic databases, PubMed and Google Scholar, and supplemented by searches in the institutional Discovery catalogue of the Gdańsk University of Technology to identify additional relevant sources. The search algorithms consisted of various combinations of keywords including “bee products”, “honey”, “honey isolates”, “propolis”,

“bee bread”, “pollen”, “microbiota”, “lactic acid bacteria”, “spore-forming bacteria”, “probiotics”, “probiotic potential”, “enzymes”, “antimicrobial compounds” and “industrial application” using operators AND/OR to refine the results. The preliminary literature search was done for titles, abstracts, and keywords. The articles were selected for further evaluation if they provided information on microorganisms isolated from bee products or biochemical characterization and discussed potential applications. In addition, a brief overview of the most common applications and biological activities of bee products was included to provide general context and to outline their currently well-known uses.

3. Bee products and their biological activity

3.1. Honey

Research has established that honey consists of approximately 39.4% fructose, 28.2% glucose, 3.2% sucrose, and 8.5% other sugars. Honey contains approximately 18% water and some minor compounds such as minerals (0.36%), proteins (1.13%), organic acids (0.17-1.17%), which contribute to its acidity, vitamins, and enzymes (<0.1%) as well as phenolic compounds (0.1%) (Santos-Buelga and González-Paramás 2017). Recent studies have focused on comparing specific groups of functional compounds responsible for the health-promoting properties of honey, examining samples from a single region of the world or a single botanical source. Furthermore, more studies have focused on honey produced by honey-making insects other than those belonging to the genus *Apis* (Pimentel et al. 2022). For example, Costa dos Santos et al. published a paper on stingless bee eucalyptus honey, which is characterized by a higher water concentration (30-40 g · 100 g⁻¹). They quantified 13 compounds, five of which were identified for the first time in stingless bee honey (Costa dos Santos et al. 2022). Ten honey samples from the pre-Sahara region in Algeria were physiochemically characterized by Ben Amr et al., who reported an average water content of 17.48% and a mean pH of 4.08. Furthermore, they evaluated honey based on its total phenolic content, a group of compounds responsible for its antioxidant properties. Moreover, all studied samples were effective against *Staphylococcus aureus* and *Listeria innocua*, and some exhibited antibacterial activity against *Bacillus subtilis*, *Micrococcus luteus*, and *Escherichia coli* (Ben Amor et al. 2022). Ten Romanian honey samples were evaluated according

to their chemical attributes and antimicrobial potential. The researchers determined the average humidity, predominant microelements, as well as the average pH value, saccharide content, and the content of flavonoids and polyphenols, which are responsible for antioxidant activity. Moreover, research has revealed the antimicrobial effects of the tested honey samples on *E. coli*, *Listeria monocytogenes*, *Bacillus cereus* bacteria, and *Candida albicans* yeast, as well as their antioxidant activity (Pătruică et al. 2022).

3.2. Bee bread

Bee bread (naturally fermented pollen) is recognized as a functional product owing to its health-promoting properties resulting from the fermentation process, which enhances the digestibility and bioavailability of nutrients (Aksoy et al., 2024). Bee bread research focuses on bioactive and phenolic compounds, amino acid content, and organic acid and sugar concentrations. Eleven samples of bee bread from Turkey were investigated by Kolayli et al., who reported that the dry substances in bee bread mainly consisted of carbohydrates, proteins, and lipids, on average 68.18%, 19.61% and 6.43%, respectively. In addition, bee bread has a high mineral concentration and varies in total phenolic, flavonoid, and condensed tannin content, which, according to the authors, depends on sample origin. Findings indicate that *p*-coumaric acid is one of the primary phenolic compounds found among the 11 samples (Kolayli et al. 2024). *p*-Coumaric acid exhibits antioxidant, antiviral, mild antifungal, antibacterial activities, and a cytotoxic effect on cancer cells (Pei et al. 2016).

3.3. Bee pollen

Bees collect flower pollen, mix it with a small amount of secretion from salivary glands or nectar, and such pollen loads are transferred to hives, where it is fragmented and stored in the combs as a reservoir of nutrients (Komosinska-Vassev et al. 2015). The main components of pollen are proteins (22.7%), carbohydrates (30.8%), fructose, glucose, and lipids. The 1.6% on average consists of flavonoids, leukotrienes, catechins, and phenolic acids. Moreover, it is a source of vitamins and organic acids (e.g., pantothenic, nicotinic, folic, biotin, rutin, and inositol), macro- and micronutrients, and nucleic acids, specifically ribonucleic

acids. Overall, approximately 200 different substances were found in the bee-collected pollen. Therefore, a wide range of functional properties of pollen have been described (Komosinska-Vassev et al. 2015). The sunflower bee-collected pollen extract was found to contain compounds that were active against mycotoxigenic molds. The results indicated the potent inhibitory effect of hydroxycinnamic acid amides naturally occurring in bee pollen on *Aspergillus niger*, *Fusarium culmorum*, and *Penicillium vercosum* (Kyselka et al. 2018). Another study focused on the antimicrobial activity of pollen solutions against five bacterial strains (*E. coli* CCM 3988, *Enterococcus raffinosus* CCM 4216, *Paenibacillus larvae* CCM 4483, *Brochotrix thermosphacta* CCM 4769, and *Pseudomonas aeruginosa* CCM 1960) and five species of filamentous fungi belonging to the *Aspergillus* genus (*A. flavus*, *A. fumigatus*, *A. niger*, *A. ochraceus*, and *A. versicolor*). The greatest inhibition zone was observed for the growth of *P. larvae*; however, all tested microorganisms were inhibited (Fatrcová-Šramková et al. 2016). Another study carried out on mice analyzed the effects of bee pollen, yeast-fermented bee pollen, and wall-broken bee pollen on fasting blood glucose, insulin, and lipid levels, hepatic gene expression related to oxidative stress, and oxidant parameters in the liver, which are correlated with metabolic syndrome. The effect of bee pollen on the gut microbiome of mice was also analyzed. In summary, supplementation with bee pollen positively affected metabolic syndrome and promoted health (Yan et al. 2021).

3.4. Propolis

Propolis or 'bee glue' is produced by *A. mellifera* from exudates collected from, e.g., tree buds, sap flows, leaves, branches, and bark, mixed with bee saliva and beeswax. It is a waxy substance composed of 50% lipids, 30% wax, 10% aromatic oils, 5% pollen, and 5% other substances, such as amino acids, vitamins, and minerals. The adhesive properties of bee glue are used to protect the hive and seal the honeycombs (Bobiş 2022).

Propolis extract has many activities, including antioxidant, antibacterial, antinociceptive, antiviral, anticancer, neuroprotective, antidiabetic, and cardioprotective. Therefore, studies have focused on describing its constituents (over 500) and, more importantly, on corresponding health-promoting attributes (Bobiş

2022; Zullkiflee et al. 2022). A clinical trial conducted on 66 patients with type 2 diabetes demonstrated the beneficial effects of propolis. However, the study did not investigate the specific compounds responsible for this positive outcome (Samadi et al. 2017). Demir et al. conducted a preliminary study and concluded that propolis can reduce cell proliferation and is a promising candidate for future anticancer drug development (Demir et al. 2016). The ethanolic extract of Polish propolis was characterized by total phenolic and flavonoid compounds, and the total phenol content was estimated at 116.16-219.41 mg/g of extract, and flavonoids at 29.63-106.07/g of extract. Moreover, the research provided data on the antioxidant activity, effect on human red blood cells, and antibacterial and antifungal activities of the examined samples. The results indicated high human red blood cell protection activity, diverse antimicrobial activity against *L. monocytogenes*, *B. cereus*, *E. coli*, and *Salmonella enterica* subsp. *enterica* serovar Enteritidis, *P. aeruginosa*, *Klebsiella pneumoniae*, *Saccharomyces cerevisiae*, and *C. albicans*, as well as moderate antiviral activity against HPV (Woźniak et al. 2022). Miryan et al. carried out a clinical trial investigating the effect of propolis supplementation on irritable bowel syndrome (IBS). The study showed that daily intake of propolis can improve the severity of pain-related symptoms (Miryan et al. 2022).

3.5. Beeswax

Specialized wax glands of worker bees secrete beeswax as a liquid that hardens in the air. Insects use it to seal and build combs of cells inside the hive (Crane and Kirk Visscher 2009). The materials used by bees to produce wax are mostly carbohydrates (fructose, glucose, and sucrose); however, alkyl esters of monocarboxylic acids, free fatty acids, and hydrocarbons are the main components (Bogdanov 2004). The use of beeswax by humans dates back to ancient Egypt. However, the primary purpose for which this product is currently used has not changed significantly. Beeswax is used in cosmetics as a thickener or emulsifier combined with other components. Moreover, it is effective in treating dermatological conditions such as dermatitis (Nong et al. 2023). Beeswax exhibits antimicrobial properties against, e.g., *S. aureus* and *Staphylococcus epidermidis*, *E. coli*, *P. aeruginosa*, and *C. albicans* (Ghanem 2011; Kacániová et al. 2012), as well as *Aspergillus* spp. (Kacániová et al. 2012).

3.6. Royal jelly

Young worker bees, unable to fully digest honey, produce royal jelly, a white to yellow gelatinous glandular fluid with a faint phenolic scent. It is a nutrient for larval bees for up to 3 days. In addition, royal jelly is the only food available to queen bees, which prolongs their lifetime. Nevertheless, royal jelly composition is influenced by bee species, climate, and harvesting season, and it is more stable than other bee products. Bee milk contains 60-70% water, 12-15% proteins, 10-16% sugar, 3-6% fats, vitamins, and minerals (Kayshar et al. 2024). Additionally, royal jelly, owing to its high concentration of bioactive compounds, exhibits antibiotic, anticancer, antioxidant, anti-inflammatory, and neuroprotective properties and helps combat overweight (Oršolić and Jazvinščak Jembrek 2024). For example, Sharif and Darsareh (2019) conducted a clinical trial that indicated that an 8-week daily royal jelly intake helps with menopausal symptoms, such as hot flashes, sleep difficulties, anxiety, or exhaustion (Sharif and Darsareh 2019). Another clinical trial of royal jelly was conducted in older adults to investigate whether RJ promotes healthy aging. The overall outcomes proved that royal jelly could supplement aging people due to its ability to enhance immunity (Bouamama et al. 2021).

4. Microbial diversity of beehive products

The microbial diversity of bee products is influenced by their high sugar concentration, low pH, and limited water activity, which favor osmophilic and osmotolerant microorganisms, such as the yeast *Zygosaccharomyces* (Čadež et al., 2015; Rodríguez-Andrade et al., 2019), spore-forming bacteria (López and Alippi 2019), and LAB, which inhabit sugar-rich environments (Endo and Salminen 2013). Moreover, the characteristics of the bee product microbiota, including the nutrient composition, reflect environmental changes. The harvesting region, time of year, and relative abundance of plant families visited by bees significantly influence the composition of both the bacterial and fungal communities (Tiusanen et al. 2024). The microbiota of beehive products changes over time as the products mature (Detry et al. 2020; Friedle et al. 2021). Furthermore, some genera are commonly found and widely reported to be isolated from bee products, whereas others have been identified primarily through metagenomic research. The methodological approach-

es varied among the studies. Some studies have focused on the isolation and identification of specific microbial genera previously detected in bee products and their differentiation (Endo and Salminen 2013; López and Alippi 2007). Other studies have analyzed the composition of the entire microbiota, either with or without microorganism isolation, emphasizing variations in microbiota among samples from different sources or insect genera (Iurlina and Fritz 2005; Sinacori et al. 2014; Pomastowski et al. 2019; Dimov et al. 2021). Nevertheless, a growing number of studies are being conducted to contribute to the valuable collection of data for further investigation of these microorganisms and their potential industrial applications, particularly as novel microorganisms continue to be isolated and identified (Jojima et al. 2004; Saksinchai et al. 2012; Wang et al. 2018; Hilgarth et al. 2021).

4.1. Lactic acid bacteria and bifidobacteria

LAB belong to the bacterial order Lactobacillales, including genera such as *Lactobacillus*, 23 new genera consisting of species assigned to the genus *Lactobacillus* until 2020 (Zheng et al. 2020), *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Oenococcus*, *Sporolactobacillus*, *Tetragenococcus*, *Vagococcus*, and *Weissella*. LAB are a group of Gram-positive, catalase-negative, non-sporulating microorganisms tolerant of acidic conditions. LAB are isolated from diverse sources, including fermented foods and beverages, dairy products, fruits, flowers, coffee, cocoa beans, and the gastrointestinal tracts of fish and bees (Meruvu et al., 2023). A special group of LAB is fructophilic lactic acid bacteria (FLAB), which prefer fructose over glucose as a carbon source. The representatives of FLAB are *Fructobacillus* spp. and *Apilactobacillus kunkeei*, which is a former member of the genus *Lactobacillus*, now reclassified (Endo and Salminen 2013; Zheng et al. 2020). *Bifidobacteria* are Gram-positive, non-motile, non-spore-forming actinobacteria that typically have a Y- or V-shaped rod morphology. Historically, they were classified as LAB due to their ability to produce lactic acid. However, genomic and phylogenetic analyses have shown that they belong to the phylum Actinobacteria, clearly distinct from the Firmicutes-derived LAB (Turrone et al. 2011). The LAB, FLAB, and *Bifidobacterium* spp. have been commonly identified in all bee products in culture-dependent and culture-independent studies.

Olofsson and Vásquez isolated bacteria, which until 2020 were classified as *Lactobacillus* spp. (Zheng et al. 2020), and *Bifidobacterium* spp. from fresh raspberry flower honey and honeybees. They also examined the microbiota of flowers in the hive surroundings to compare the microbiomes of the different samples. The study revealed that honey's LAB most likely originates from the honeybee's digestive tract. They established that the dominant species among bees were bacteria closely related to *A. kunkeei*, which explains the expected presence of this species in honey. Surprisingly, notable differences were observed in the microbiota of samples originating from hives of different sizes – only honey from standard-sized hives contained both *A. kunkeei* and *Bifidobacterium asteroides* (Olofsson and Vásquez 2008). *A. kunkeei* is a LAB species repeatedly reported in honey (Jacinto-Castillo et al. 2022), bee bread (Lozo et al. 2015), bee-collected pollen (Uçar et al. 2022), and propolis (Casalone et al. 2020). Moreover, another study investigating honey samples from different stingless bee species supported the hypothesis that the LAB microbiota in honey primarily originates from the bee's honey stomach rather than from flowers. Analysis of various stingless bee species revealed significant variations in the abundance and diversity of LAB among honey samples. Additionally, the physicochemical properties of honey influenced bacterial presence. According to Andrade-Velásquez et al. seven LAB strains were isolated, three of which were identified as *Fructobacillus pseudoficulneus*, one as *Fructobacillus tropaeoli* and three as potentially novel species within the genus *Fructilactobacillus* (Andrade-Velásquez et al. 2023).

Belhadj and coworkers isolated 567 pure cultures of LAB from raw bee pollen grains. Further identification of ten selected strains revealed the highest similarity to *Lactiplantibacillus plantarum* (basonym *Lactobacillus plantarum*) (Zheng et al. 2020), *Limosilactobacillus fermentum*, *Limosilactobacillus ingluviei*, both previously classified as *Lactobacillus* (Zheng et al. 2020), *Ligilactobacillus acidipiscis* (formerly *Lactobacillus acidipiscis*) (Zheng et al. 2020), *Lactococcus lactis*, *Pediococcus pentosaceus*, and *Weissella cibaria* (Belhadj et al. 2014). Moreover, studies based on culture-independent methods have also reported the presence of LAB, including *Lactobacillus* spp. (Friedle et al. 2021; Laconi et al. 2021; Ghosh et al. 2022;), *Lactococcus* spp. (Friedle et al. 2021; Uçar et al. 2022), and *Streptococcus* spp., in bee pollen (Ghosh et al. 2022). Another study explored the naturally occurring LAB in honey, bee bread, bee

pollen, and royal jelly. A total of 42 isolates were identified, representing the following genera: *Enterococcus* (23.8%), *Micrococcus* (18.8%), *Streptococcus* (13.8%), *Pediococcus* (13.8%), *Lactobacillus* (10.0%), according to the classification valid until 2020 (Zheng et al. 2020), *Lactococcus* (10.0%), and *Leuconostoc* (10.0%) (Mathialagan et al. 2018).

Research on the microbiota of bee products has led to identifying novel LAB. Seventeen isolates, with some strains proposed as potentially new species, were obtained from the honeycomb of *Apis dorsata* (Olofsson and Vásquez 2008; Mathialagan et al. 2018). This study reinforces the hypothesis that honeycomb microbiota is a natural reservoir of LAB, with promising applications in probiotic development and food technology. Moreover, LAB strains could play a crucial role in improving bee health and contribute to sustainable apiculture practices (Tajabadi et al. 2013). Furthermore, an investigation of the *Apis cerana* bee bread microbiota collected in China enabled the characterization of a novel species, *Lactobacillus panisapium*, distinct from previously known *Lactobacillus* species. The strain was identified based on 16S rRNA gene sequencing and the housekeeping genes *rpoA* and *pheS*. Further phylogenetic analysis revealed that the closest relatives were *Lactobacillus bombicola*, *Lactobacillus apis*, and *Lactobacillus helsingborgensis* (Wang et al. 2018).

4.2. Endospore-forming bacteria

The term “aerobic endospore-forming bacteria” (SFB) refers to *Bacillus* spp. and related genera, including *Geobacillus*, *Paenibacillus*, *Lysinibacillus*, and *Brevibacillus*, which were previously classified within the genus *Bacillus* (Logan and Halket 2011). Most SFB are widely distributed in natural environments, and many species from various SFB genera are commonly isolated from and identified in bee products due to their ability to form spores that can withstand the harsh conditions of these environments (Tsadila et al. 2023). The SFB isolated from bee products continues to attract the interest of scientists who use various identification methods. An increasing number of studies are focusing on the *Bacillus* genus and related bacteria, not only in the context of the safety of bee products but also for their potential applications. Sinacori and coworkers, in their study, determined the microbiota composition of 38 nectar and honeydew honey samples from different geographical and botanical origins. They isolated 423 spore-forming, Gram-positive bacteria, prelimi-

narily considered *Bacillus* spp. Furthermore, the bacteria were investigated by REP-PCR; the (GTG)₅ and BOXA1R profiles were combined, and the comparison among all isolates recognized 42 distinct clusters. 16S rRNA identification with RFLP analysis of representative strains revealed species including *Paenibacillus polymyxa*, *Bacillus simplex*, *Bacillus licheniformis*, *B. subtilis*, *B. cereus*, *Bacillus thuringiensis*, *Bacillus amyloliquefaciens*, *Bacillus megaterium*, and *Bacillus pumilus*. Moreover, the last three species were found in various samples, regardless of their botanical origin; however, the highest number of isolates belonged to *P. polymyxa* and *B. amyloliquefaciens* (Sinacori et al. 2014). The *B. amyloliquefaciens* was isolated in different studies from bee-derived samples such as honey and identified based on the sequence of the 16S rRNA gene (Pajor et al. 2018), as well as from bee pollen and identified through the BioLog Gen III microplates (Dinkov 2017), whereas *P. polymyxa* was reported to be isolated from bee bread and identified based on the 16S rRNA gene (Lozo et al., 2015). Moreover, *B. pumilus* was isolated from honey and identified using MALDI-TOF/MS (Pomastowski et al., 2019), based on morphology (Iurlina and Fritz 2005), using BioLog Gen III microplates (Dinkov 2017), and sequencing of the 16S rRNA gene (Lozo et al. 2015; Pajor et al. 2018; Tsadila et al. 2021). Thirty fresh bee pollen samples from Argentina have been investigated (Alippi et al. 2022). The study aimed to isolate aerobic SFB using HiChrome *Bacillus* agar. Strains were further morphologically and biochemically characterized, and PCR-RFLP was performed to assign isolates to specific species. A total of 73 strains were purified, including *B. amyloliquefaciens*, *B. cereus*, *Bacillus clausii*, *Bacillus coagulans*, *B. licheniformis*, *B. megaterium*, *B. pumilus*, *B. simplex*, *B. subtilis*, *B. thuringiensis*, *Paenibacillus alvei*, *Paenibacillus amylolyticus*, *P. larvae*, *P. polymyxa*, *Lysinibacillus sphaericus*, and *Rummeliibacillus stabekisii*. The study did not report any correlation between bacterial composition and geographic origin. However, *B. cereus* was the most prevalent species (50%), followed by *B. megaterium* and *B. subtilis* (40% each). A metagenomic study of bee pollen conducted in 2022 also reported the presence of *Bacillus* among the microbiota of bee pollen (Ghosh et al., 2022; Uçar et al., 2022). Furthermore, *Bacillus* genera isolated from pollen were identified through the system BioLog Gen III microplates (Dinkov, 2017) and 16S rDNA (Salomón et al., 2024). The presence of *B. subtilis* and *B. licheniformis* in pollen samples has been reported by Lozo et al. (2015. More-

over, various identification methods, such as MALDI-TOF/MS (Pomastowski et al. 2019), 16S rRNA gene sequencing (Sinacori et al. 2014; Lozo et al. 2015; Tsadila et al. 2021; Peřka et al. 2023), and metabolic fingerprinting (Sadik and Ali 2012) have been applied to distinguish *B. subtilis* recovered from honey, bee bread, and bee pollen samples. *B. cereus* is commonly isolated from bee products (Iurlina and Fritz 2005; Ashour et al. 2018; Pomastowski et al. 2019; Tsadila et al. 2021). A study by López and coworkers aimed to assess the phenotypic and genotypic diversity of *B. cereus* strains isolated from Argentinian honey samples. Their findings revealed that 27% of the honey samples contained *B. cereus*, while other *Bacillus* species were found in 14% of the samples. The study further evaluated the isolates' biochemical properties and morphological characteristics, as well as restriction fragment length polymorphism (RFLP) analysis of the 16S rRNA gene. The presence of *B. cereus* in honey has been attributed to various sources, including pollen, honeybees, wax, beekeeping equipment, and environmental dust (López and Alippi 2007).

In a separate study, the same authors focused on *B. megaterium*, which is frequently identified among the microorganisms present in the microbiota of bee products (Lozo et al. 2015; Pomastowski et al. 2019; Casalone et al. 2020; Tsadila et al. 2021). They isolated 52 strains from honey samples from Argentina, Brazil, France, and Mexico. Their findings indicate a high degree of genetic and phenotypic diversity among *B. megaterium* isolates, suggesting multiple potential sources of bacterial contamination, similar to their conclusions regarding *B. cereus*. Moreover, both studies highlighted that honey can harbor microbial communities that may pose potential consumer risks. Consequently, regular monitoring of microbial contamination in honey is recommended to ensure its safety (López and Alippi 2009).

In contrast, *Bacillus* spp. from beehive products can possess probiotic properties (Peřka et al. 2023), which will be further discussed in this review. Nevertheless, *Bacillus* spp. (including *B. subtilis*, *Bacillus velezensis*, *B. licheniformis*, as well as *B. pumilus* or *B. zhangzhouensis*, and *B. altitudinis* or *B. stratosphericus*) were recovered from Polish honey and bee bread samples. Based on the sequencing of the 16S rRNA gene, strains were classified into species. Interestingly, this research did not provide any information about the presence of genera other than *Bacillus*. However, the authors high-

lighted that the investigated samples were mature bee bread harvested from honeycomb cells, stored for about four months, and dried bee pollen. Thus, only highly resistant and spore-forming bacteria, such as *Bacillus* spp., could survive under those conditions (Peřka et al., 2021, 2023). *P. larvae* is a pathogenic species with a single known host: honeybee larvae (Ebeling et al., 2016). Thus, studies investigating bee products have often explicitly focused on the isolation of this microorganism. However, it is not the only species within the *Paenibacillus* genus identified in beehive products. *P. alvei*, *P. amylolyticus*, *P. polymyxa*, *P. xylanexedes*, and *P. illinoisensis* have been recovered from honey, bee bread, pollen, and propolis (Sinacori et al., 2014; Pajor et al., 2018; Pomastowski et al., 2019; Casalone et al., 2020; Salomón et al., 2024). Moreover, a metagenomic study detected *Paenibacillus* spp. in honey (Kňazovická et al., 2019), as well as in bee bread and pollen (Ghosh et al., 2022). Several species of *Lysinibacillus* have also been isolated from honey, for example, *L. boronitolerans* (Pomastowski et al., 2019), *L. fusiformis* (Pajor et al., 2018; Tsadila et al., 2021), *L. xylanilyticus* (Pajor et al., 2018), and *L. halotolerans*, which was isolated from propolis (Casalone et al., 2020). Studies focused on the safety of bee products consider the presence of pathogenic, spore-forming bacteria as a potential risk to consumers. *Clostridium botulinum*, associated with honey, may cause infant botulism. Research reports indicate that the number of spores varies between samples and does not always reach levels that pose a health risk. *C. botulinum* may grow in maturing honey but is inhibited when the sugar concentration becomes too high. However, there is insufficient knowledge regarding the effect of shelf life on this pathogen (Grabowski and Klein 2017).

4.3. Other bacteria

The microbiota of beehive products is also influenced by hive management practices, the collection process of bee products, and their subsequent processing. Thus, the microorganisms identified within the microbial community of bee products may originate from the human microbiota or other sources of contamination (Grabowski and Klein, 2017). Studies have reported that bee products contain microorganisms belonging to the families Enterobacteriaceae, Pseudomonadaceae, Staphylococcaceae, and Micrococcaceae (Dinkov 2017; Kňazovická et al. 2019).

4.4. Yeasts and molds

The ability of yeasts and molds to survive in high-sugar and low-water-activity environments results from complex survival strategies, including physiological, biochemical, and ecological adaptations. These mechanisms involve osmoprotectant accumulation, enzyme modifications, cell wall structural adjustments, metabolic flexibility, and evolutionary ecological strategies. Such adaptations enable yeasts to withstand osmotic stress, making them valuable for industrial applications such as food preservation, wine fermentation, and biotechnology (Snyder *et al.* 2019). Osmophilic yeasts (adapted to high osmotic pressure) and xerophilic molds (adapted to dry conditions) can survive in honey, bee bread, and bee pollen; thus, the presence of these microorganisms in bee products is commonly reported. Agarbati and coworkers investigated the yeast community in bee products, including corbicular pollen, bee bread, and propolis, as well as the microbiota of worker bees and flowers that served as their food source, with a focus on identifying stable yeast species and examining the seasonal variations in the microbial populations. Microorganisms were cultured and identified by sequencing the ITS1-5.8S-ITS2 rDNA region. The study revealed 51 species belonging to 27 genera, most classified within the phylum Ascomycota (20 genera). In winter, the most prevalent yeast in the bee gut was *Aureobasidium pullulans*. In contrast, species that remained stable across both winter and summer included *Meyerozyma guilliermondii*, *Debaryomyces hansenii*, *Hanseniaspora uvarum*, *Hanseniaspora guilliermondii*, and *Starmerella roseus*. A total of 16 genera were identified in the bee products, with the most abundant being *Metschnikowia* spp. (20%), *Starmerella* spp. (17%), *Filobasidium* spp. (15%), *Meyerozyma* spp. (10%), *Aureobasidium* spp. (7%), and *Debaryomyces* spp. (7%) (Agarbati *et al.* 2024).

Most species identified in beehive products were absent in the bee gut mycobiota. In flowers, 11 different genera of yeasts were identified. Moreover, yeasts belonging to *Aureobasidium*, *Filobasidium*, *Meyerozyma*, and *Metschnikowia* were present in flowers, bee guts, and bee products, likely due to the continuous exchange between the environment and bees in both directions (Agarbati *et al.*, 2024). Several studies have reported that some species belonging to the above-mentioned genera can be isolated from bee products. *M. guilliermondii* and *Starmerella lactis-condensi* were detected in bee pollen and honey (da Silva *et al.*, 2024),

as determined by MALDI-TOF/MS. *A. pullulans* and *D. hansenii* were isolated from honey (Sinacori *et al.*, 2014) and bee bread (Detry *et al.*, 2020). Several species belonging to the genus *Starmerella* have also been reported in bee bread (Detry *et al.*, 2020) and pollen (Agarbati *et al.*, 2024). The 47 honey samples, with water activity ranging from 0.6 to 0.86 and produced by 17 different species of stingless bees, were investigated to characterize yeast diversity. Moreover, the osmotolerance of the isolates was studied. Sixteen yeast species from 9 genera were identified using 26S rDNA sequencing. The dominant genera were *Starmerella* (mainly *S. etchellsii* and *S. apicola*) and *Zygosaccharomyces* (represented by *Z. mellis*, *Z. rouxii*, and *Z. siamensis*); however, the genera *Candida*, *Debaryomyces*, *Moniliella*, *Rhynchoagastrea*, *Torulaspora*, *Wickerhamiella*, and *Wickerhamomyces*, notably *W. sydowiorum*, which was identified in honey for the first time, were also reported. The authors concluded that osmotolerant yeasts capable of fermenting glucose and fructose were the most abundant among all the samples. Some of these species were found in samples from specific bee species, suggesting a close relationship between bees and their microbiota (Echeverrigaray *et al.* 2021). Nevertheless, *Zygosaccharomyces* spp. are widely distributed among beehive products and have been isolated from *Citrus* spp., *Carduus defloratus*, *Eucalyptus camaldulensis*, *Eriobotrya japonica*, as well as multifloral and honeydew honeys (Sinacori *et al.* 2014; Rodríguez-Andrade *et al.* 2019), and bee bread samples (Čadež *et al.* 2015; Detry *et al.* 2020; Agarbati *et al.* 2024).

A culture-dependent method was used to isolate yeasts and molds from royal jelly obtained directly from bees in Egypt, commercially available from local Egyptian sellers, and imported sources. The study reported no significant differences in the microbial populations among the samples. Further identification of the isolates based on morphological and physiological characteristics revealed two fungal genera: *Aspergillus* and *Penicillium*, with the latter occurring more frequently. However, the most abundant microorganism was *S. cerevisiae* yeast (Ashour *et al.* 2018). Several representatives belonging to the genera *Aspergillus* and *Penicillium* have also been recovered from different beehive products, including honey (Sadik and Ali 2012; Sinacori *et al.* 2014; Rodríguez-Andrade *et al.* 2019) and bee pollen (Sinpoo *et al.* 2017). Moreover, *A. flavus* was detected in bee bread (Bush *et al.*, 2024). Xerotolerant and xerophilic molds and yeasts were isolated

from 84 nectar and honeydew honey samples collected across Spain. The ability of the isolates to grow under low water-activity conditions was examined. Furthermore, all microorganisms were identified by sequencing the 28S nrRNA gene, and some were additionally characterized based on the ITS5/ITS4 fragment and the *BenA*, *CaM*, and *rpb2* genes. One hundred four fungal strains were recovered from the samples, representing 32 species belonging to 16 genera. The most common genera were *Aspergillus*, *Betisia*, *Candida*, *Eremascus*, *Monascus*, *Oidiodendron*, *Penicillium*, *Skoua*, *Talaromyces*, and *Zygosaccharomyces*. A new family, Helicoarthrosporaceae; two new genera, *Strongyloarthrosporum* and *Helicoarthrosporum*; and seven new species, namely *Strongyloarthrosporum catenulatum*, *Helicoarthrosporum mellicola*, *Oidiodendron mellicola*, *Skoua asexualis*, *Talaromyces basipetosporus*, *T. brunneosporus*, and *T. affinitatimellis*, have been proposed. The study confirmed that all fungal taxa isolated from honey were xerophilic and xerotolerant, meaning they can grow in high sugar concentrations and low water activity environments; however, only *Ascosphaera atra*, *Betisia alvei*, *Eremascus albus*, *S. catenulatum*, and *Xerochrysium xerophyllum* can be considered obligate xerophiles (Rodríguez-Andrade et al. 2019). Research on bee products has provided data on novel species of osmophilic microorganisms. Two new species of the genus *Candida* were isolated from raw honey in Thailand, characterized, and identified as *Candida lundiana* and *Candida suthepensis* (Saksinchai et al. 2012). In another study, 34 yeast strains were recovered from six honeycombs containing bee bread, and one isolate from a sample of polyfloral honey purchased from a beekeeper in Hungary. Representative isolates were identified and assigned to the genus *Zygosaccharomyces*, namely *Z. rouxii*, *Z. mellis*, and *Z. siamensis*. This study also reported that the isolate originating from honey and four isolates recovered from bee bread were closely related to *Z. gambellarensis*, but they exhibited obligate osmophilicity. As a result, after detailed biochemical and genetic characterization, a novel species *Zygosaccharomyces favi* was distinguished (Čadež et al. 2015).

5. Industrial and biotechnological potential of the microbiota of beehive products

Beehive products are a valuable source of microorganisms with industrial and biotechnological potential. These microorganisms, including bacteria and

yeasts, exhibit diverse functionalities applicable to healthcare, food production, and biotechnology. This chapter highlights the industrial and biotechnological applications of bee-product-associated microbiota, emphasizing their usefulness in probiotic design, antimicrobial agent and enzyme production, and food processing.

5.1. Beehive products as a source of probiotic microorganisms

The term “probiotics”, first introduced in the 1960s, is currently defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (Hill et al. 2014; ISAPP 2023). This definition emphasizes three essential criteria: viability of the microbial strain, administration in sufficient quantity, and a demonstrated beneficial effect on the host. Only strains that fulfill these requirements and have been rigorously evaluated for safety and efficacy can be classified as probiotics. Such microorganisms are increasingly applied in functional foods, dietary supplements, and pharmaceutical preparations. The selection of probiotic strains is currently based on multiple criteria, including genetic and phenotypic stability, host-associated stress resistance (tolerance to gastric acidity and bile salts, resistance to salivary, gastric and pancreatic enzymes), adhesion to intestinal epithelial cells, antimicrobial activity (production of antimicrobial metabolites, competition to pathogens), safety assessment, and host-associated functional properties such as immunomodulatory capacity, secretion of functional molecules (antioxidants, enzymes, short-chain fatty acids, vitamins), and other more specific activities (de Melo Pereira et al., 2018). The genera most frequently associated with probiotic properties include *Bifidobacterium* (*B. adolescentis*, *B. animalis*, *B. bifidum*, *B. breve* and *B. longum*) and members of the family *Lactobacillaceae*, which include, among others, *Lactocaseibacillus* (*L. casei*, *L. paracasei*, *L. rhamnosus*), *Lactiplantibacillus* (*L. plantarum*), *Lactobacillus* (*L. acidophilus*, *L. gasseri*, *L. johnsonii*), *Ligilactobacillus* (*L. salivarius*), *Levilactobacillus* (*L. brevis*), and *Limosilactobacillus* (*L. fermentum*, *L. reuteri*) - all formerly belonging to the genus *Lactobacillus*. The yeast *Saccharomyces boulardii* and some *Bacillus* species are also used as probiotics (Hill et al., 2014; de Melo Pereira et al., 2018; Zheng et al. 2020; Guarner et al. 2024).

A wide range of studies has reported several LAB, including FLAB species, to be present in bee products

and has examined their probiotic properties. Since some LAB are already recognized as well-established probiotics, it is scientifically justified to consider bee products - naturally rich in LAB - as reservoirs for potential probiotic strains. In addition, bee products also represent a niche for *Bacillus* and related genera, as well as yeasts, which have likewise been investigated for probiotic traits in several studies. This section discusses the key functional characteristics that microorganisms must exhibit to be defined as probiotics. It evaluates how LAB, *Bacillus* spp., and other isolates derived from bee products perform with respect to these essential criteria.

5.1.1. Tolerance to gastrointestinal tract conditions

Survival under gastrointestinal (GI) tract conditions is one of the requirements for defining a microorganism as a probiotic. A probiotic must be administered in a viable form and survive exposure to harsh environments such as low gastric pH, gastric and pancreatic enzymes, and bile salts. Only under these conditions can it reach the small intestine, where it can deliver positive effects. Therefore, *in vitro* tests of acid and bile tolerance, often combined with simulated oral, gastric, and intestinal conditions, serve as an important tool for screening potential probiotic strains.

A. kunkeei demonstrated tolerance to low pH and bile salts. The study showed that the growth inhibition of *A. kunkeei*, isolated from bee bread and bee pollen, ranged from 10–60% at pH 4, while under bile stress conditions, it reached 60–80% (Ispirli and Dertli 2021). Another isolate survived at 74% after 1.5 h at pH 2 with 0.3% bile salts, comparable to the reference *L. rhamnosus* ATCC7469 (Ebrahimi et al. 2021). *L. plantarum* tolerated simulated digestion, including lysozyme, gastric pepsin (pH 3 and 2), and intestinal bile salts (0.3%) with pancreatin. However, survival dropped to ~52% under gastric conditions and partially recovered to ~63% after intestinal exposure (De Simone et al. 2023). *F. fructosus* behaved similarly, showing ~55% survival in gastric conditions and ~68% after intestinal exposure in the same model (De Simone et al., 2023). In another study, 14 isolates, including *F. fructosus*, *A. kunkeei*, *Lactobacillus kimbladaii*, and *Lactobacillus kullabergensis*, showed survival rates ranging from 33–83% at pH 2 and 69–99% at pH 3, with tolerance to 0.3% bile salts for 4 hours and final viability ranging from 53% to 86% (Meradji et al. 2023). Screening of 39 LAB isolates from

Iranian honey revealed that all strains survived pH 3.3 for three hours, while only two isolates of *A. kunkeei* and one of *F. fructosus* were bile-resistant (Lashani et al. 2020). Thirteen Iranian honey isolates belonging to *L. rhamnosus*, *L. acidophilus*, and *L. plantarum* showed variable tolerance to gastrointestinal tract conditions. Eleven strains survived at pH 4; however, only two *L. plantarum* strains survived at pH 2, tolerated 0.3% bile salts, and were resistant to gastric juice and pepsin (Abadi et al. 2023). *F. fructosus*, *Lactobacillus crustorum*, *Lactobacillus mindensis*, *Lactobacillus musae*, and *Leuconostoc mesenteroides* showed high survival at pH 3 (91.7–99.9%) and in 0.3% bile salts for four hours (Mohammad et al. 2020).

Besides LAB, studies have also reported *Bacillus* and related genera in bee products, which have been screened for probiotic properties. *B. megaterium* isolated from Iranian honey, among seventeen other bacteria, exhibited the best survival rates and was able to withstand more than four hours at pH 2, maintaining over 50% viability under acidic stress. In the same study, bile salt resistance was observed in *B. subtilis*, which was unable to tolerate low pH (Razmgah et al. 2016). Two strains of *B. subtilis* in another study showed high tolerance to acidic conditions, with >90% survival at pH 3.5 for three hours, and full survival in 0.3% bile salts and pancreatic enzymes (Hamdy et al. 2017). Another study confirmed the survival of *B. subtilis* under low pH conditions. The study included *B. endophyticus* and two *B. subtilis* strains. One *B. subtilis* strain survived at a rate >90% at pH 2 for up to 24 h, while the other tested strains withstood the same conditions with survival rates above 70%. All tested strains tolerated pancreatic enzymes and bile salt environments (Abdel Wahab et al. 2018). Over 85% of *B. amyloliquefaciens* cells survived at pH 2 and in 0.3% bile salts for three hours (Zulkhairi et al., 2019). In another study, *Bacillus* spp. isolates were highly tolerant to pancreatic enzymes and bile salts (Esawy et al., 2012).

Enterococcus faecalis tolerated pH 3 with survival rates of 91–99% and resisted 0.3% bile salts for four hours (Mohammad et al. 2020). *Gluconobacter oxydans*, an acetic acid bacterium, showed 100% survival at pH 5 and 55% at pH 2 after three hours, and tolerance up to 2% bile salts (Begum et al. 2015).

Yeasts isolated from honey (*Z. rouxii*, *Candida* sp., *S. pombe*, *R. ruineniae*, *C. lusitaniae*, and *M. chrysoperlae*) survived at pH 3.5 and 37 °C. In a dynamic *in vitro* digestion model, *S. pombe*, *Z. rouxii*, and *M. chrysoperlae* maintained counts >10⁶ CFU/mL throughout oral,

gastric, and intestinal phases, confirming robust tolerance (Machado et al. 2024).

5.1.2. Properties related to intestinal colonization

Microorganisms' ability to colonize the gastrointestinal tract is considered an important functional property of probiotic candidates. Such colonization depends on their ability to adhere to intestinal epithelial cells, form aggregates, and interact with the host mucosa, which prolongs their residence time in the gut and enhances competitive exclusion of pathogens while supporting host–microbe interactions (Hill et al. 2014; ISAPP 2023).

L. plantarum and *F. fructosus* demonstrated moderate adhesion to Caco-2 cells, with 5.3% and 7.5% adhesion rates, respectively. Importantly, neither strain exhibited cytotoxic effects on the epithelial cell line, confirming their safety in this *in vitro* model (De Simone et al. 2023). A study conducted by Ispirli and Dertli reported a level of autoaggregation close to 100% for *L. plantarum* and *F. fructosus* and nearly 100% of hydrophobicity for *F. fructosus* strains. *A. kunkeei* displayed variable but generally high colonization-related traits. Autoaggregation capacity ranged from 50% to 80%, while cell surface hydrophobicity varied between 30% and 70% (Ispirli and Dertli, 2021; Meradji et al. 2023). The adhesion ability and autoaggregation capacity of *A. kunkeei* isolates from Iranian honey were comparable to those of the reference strain *L. rhamnosus* ATCC7469, with autoaggregation exceeding 80% and cell surface hydrophobicity of approximately 33% (Ebrahimi et al. 2021). *L. mesenteroides* expressed high autoaggregation and strong hydrophobicity, indicating good potential for adhesion and colonization (Ispirli and Dertli, 2021). In general, LAB isolates recovered from bee products demonstrated high colonization potential, with autoaggregation rates ranging from 40% to 100% and hydrophobicity values between 30% and 100% (Mohammad et al. 2020; Ebrahimi et al. 2021; Meradji et al. 2023).

B. subtilis showed strong adhesion ability to intestinal epithelial-like cells, with adhesion rates ranging from ~30% to 87%, depending on the strain tested (Hamdy et al. 2017; Abdel Wahab et al. 2018). *B. amyloliquefaciens* also exhibited notable colonization-related traits: autoaggregation capacity of about 84% while cell surface hydrophobicity was 57% (Zulkhairi Amin et al. 2019). These features suggest a strong potential for persistence and competition with pathogens in the intestinal environment.

Yeast strains isolated from bee products also demonstrated excellent colonization-related characteristics. *S. pombe* showed the highest autoaggregation capacity (100%), followed by *M. chrysoperlae* (94%) and *Z. rouxii* (89.8%). Other isolates, including *Candida* sp., *C. lusitaniae*, and *R. ruineniae*, also exhibited strong autoaggregation abilities (Machado et al., 2024). These results indicate that bee product–derived yeasts may display strong adhesion-related properties, broadening the diversity of microorganisms with probiotic potential.

5.1.3. Antipathogenic activity

Antimicrobial properties are advantageous for probiotic candidates because they support colonization resistance, reduce pathogen burden, and help maintain intestinal homeostasis. These effects are typically assessed through *in vitro* antagonism assays against pathogenic microorganisms. Although such properties do not replace clinical validation, they provide mechanistic evidence supporting the probiotic potential of candidate strains (Hill et al. 2014; FAO/WHO 2002).

A. kunkeei strains isolated from bee bread, bee pollen, and honey in four independent studies inhibited the growth of at least one pathogen belonging to either Gram-positive bacteria (*B. cereus*, *S. aureus*) or Gram-negative bacteria (*E. coli*, *K. pneumoniae*, *P. aeruginosa*, *Salmonella Typhimurium*, *Salmonella enterica*, *Salmonella Enteritidis*, *Shigella flexneri*) (Lashani et al., 2020; Ebrahimi et al. 2021; Ispirli and Dertli 2021; Meradji et al. 2023). Cell-free culture supernatants of *L. plantarum* showed antagonistic activity against seven foodborne pathogenic bacteria, with the biggest inhibition zones up to 26 mm against *S. flexneri*, 21mm against *E. coli*, and 20 mm against *S. enteritidis* and *S. aureus* (Lashani et al. 2020). The same authors tested 17 LAB strains, with the most potent inhibitory effect observed against *S. flexneri* (Lashani et al. 2020). In another study, honey isolates identified as *L. plantarum* and *F. fructosus* reduced foodborne pathogenic bacteria (*E. coli*, *L. monocytogenes*, *S. aureus*) viability in co-culture assays. Inhibition was expressed as a percentage of growth reduction, ranging between 25% and 40% depending on the strain and pathogen (De Simone et al. 2023).

B. subtilis isolates from three different studies demonstrated antagonistic activity against pathogens, including Gram-positive bacteria (*B. cereus*, *S. aureus*), Gram-negative bacteria (*S. typhi*, *E. coli*, *P. aeruginosa*),

as well as yeast and molds belonging to *Candida* spp. and *Aspergillus* spp. (Razmgah et al., 2016; Hamdy et al., 2017; Abdel Wahab et al., 2018). Razmgah et al. also reported inhibition of *Streptococcus agalactiae*, *Streptococcus pyogenes*, *Enterococcus faecalis*, *Shigella dysenteriae*, and *L. monocytogenes* by *B. subtilis* (Razmgah et al. 2016). *Bacillus* isolates recovered from honey were observed to inhibit multiple enteric pathogens, including *B. megaterium*, *B. subtilis*, *E. coli*, *Enterobacter cloaca*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus*, and *C. albicans*, with inhibition zones comparable to those of erythromycin (Esawy et al. 2012).

Yeasts isolated from bee products showed antagonistic effects against pathogenic bacteria. *M. chrysoperlae*, *S. pombe*, and *Z. rouxii* inhibited the growth of *E. coli* and *S. aureus*. Moreover, *M. chrysoperlae* and *S. pombe* exhibited antimicrobial activity against *Salmonella enteritidis* (Machado et al. 2024).

5.1.4. Antibiotic susceptibility

Antibiotic susceptibility testing is a crucial step in the safety evaluation of probiotic candidates. According to FAO/WHO (2002) and ISAPP consensus, antibiotic resistance carried by mobile genetic elements is of most significant concern, as it can be horizontally transferred to pathogens, whereas resistance due to chromosomal mutations or intrinsic mechanisms is not always considered exclusionary (Gueimonde et al. 2013; Hill et al. 2014). For this reason, both phenotypic and genotypic assessments are recommended.

Studies reported that LAB isolates remained susceptible to β -lactams, including ampicillin, cefoxitin, erythromycin, and chloramphenicol (Lashani et al. 2020; Meradji et al. 2023), penicillin (Ebrahimi et al., 2021; Ispirli and Dertli, 2021), as well as ribosome-targeting antibiotics namely erythromycin and chloramphenicol (Lashani et al. 2020; Ispirli and Dertli 2021; Meradji et al. 2023). However, *A. kunkeei* isolates frequently displayed resistance to ribosome-targeting antibiotics such as streptomycin and kanamycin (Ispirli and Dertli, 2021), tetracycline and gentamicin (Meradji et al. 2023), as well as nucleic acids synthesis inhibitors and β -lactams, including oxacillin and penicillin (Meradji et al., 2023). *F. fructosus* and *L. plantarum* were generally resistant to streptomycin and kanamycin (Ispirli and Dertli, 2021) and oxacillin, penicillin, vancomycin, and nalidixic acid (Meradji et al. 2023).

Honey-derived *B. subtilis* strains (recognized as GRAS) have been reported to be susceptible to β -lactams (penicillin, ampicillin, chloramphenicol, erythromycin), glycopeptides (vancomycin), and ribosome-targeting antibiotics (streptomycin and kanamycin) (Razmgah et al. 2016; Abdel Wahab et al. 2018). Some isolates were also resistant to rifampicin and aminoglycosides, but remained sensitive to chloramphenicol, tetracycline, and erythromycin (Esawy et al. 2012; Hamdy et al. 2017; Abdel Wahab et al. 2018). However, not all bacterial species belonging to the genus *Bacillus* have GRAS status; nevertheless, studies demonstrate their susceptibility to certain antibiotics, e.g., penicillin, erythromycin, and tetracycline (Razmgah et al. 2016). On the other hand, some isolates identified as genus *Bacillus* exhibit resistance to amoxicillin, penicillin, and ampicillin (Esawy et al. 2012; Razmgah et al. 2016).

5.1.5. Other safety criteria

Beyond antibiotic susceptibility, further safety criteria are required to define the probiotic suitability of bee product-derived isolates. These include hemolysis testing, screening for virulence genes, and cytotoxicity assays. The most favorable outcome is γ -hemolysis, which is considered safe for humans, while α or β -hemolysis is undesirable and disqualifies strains from probiotic use (FAO/WHO 2002; Hill et al. 2014).

LAB and FLAB isolates were consistently reported as γ -hemolytic (Meradji et al. 2023; Abadi et al. 2023) with no cytotoxic effects observed in Caco-2 cells (De Simone et al. 2022).

Bacillus spp. demonstrated more variable profiles. While some *Bacillus* isolates were γ -hemolytic (Esawy et al. 2012), other *Bacillus* spp. showed α - or β -hemolysis, and several strains carried putative virulence determinants, raising potential safety concerns (Razmgah et al., 2016; Zulkhairi Amin et al., 2019).

Yeasts from bee products, including *S. pombe*, *Z. rouxii*, *M. chrysoperlae*, *Candida* sp., *R. ruineniae*, and *C. lusitaniae*, were not assessed for hemolysis or virulence factors in detail. However, in available studies, they did not show cytotoxic effects and survived gastrointestinal stress (Machado et al. 2024). Further targeted safety evaluation remains necessary to confirm their probiotic potential.

5.1.6 Conclusion

One of the key requirements for considering a microorganism as a probiotic is its safety profile, and species-level identification of isolates is one of the first mandatory steps. According to ISAPP, microorganisms belonging to the same species as accepted probiotics are recognized as safe, which provides them with a more straightforward pathway to probiotic status. Nevertheless, probiotic potential and safety often differ substantially between strains of the same species. For this reason, clinical trials remain indispensable, and it cannot be assumed that all isolates of a given species automatically qualify as probiotics.

The safety evaluation of candidate strains requires the absence of hemolytic activity, virulence factors, and acquired/mobile antibiotic resistance genes, confirmed through both phenotypic and genotypic analyses. While intrinsic, non-transferable resistance linked to natural species physiology does not exclude a candidate, acquired and horizontally transferable resistance, such as to tetracyclines, macrolides/MLS, β -lactams, or aminoglycoside-modifying enzymes, is considered disqualifying. Importantly, resistance is not necessary for probiotic effectiveness during antibiotic therapy; clinical guidance instead recommends separating probiotic administration from antibiotic intake to minimize inactivation.

Although many bacterial species belonging to LAB have been granted GRAS status, studies have nevertheless reported resistance to antibiotics such as aminoglycosides (streptomycin, kanamycin) and tetracycline (Mohammad et al. 2020; Ispirli and Dertli 2021; Meradji et al. 2023).

The occurrence of aminoglycoside and tetracycline resistance is problematic, particularly because the underlying mechanisms were assessed phenotypically rather than genotypically, leaving uncertainty about whether the resistance is intrinsic or linked to mobile genetic elements. Studies investigating bee product-derived *Bacillus* spp. also revealed resistance traits, particularly to β -lactams and glycopeptides (Razmgah et al. 2016; Hamdy et al. 2017).

For a strain to be recognized as a probiotic, a clinically demonstrated, strain-specific health benefit remains essential (Hill et al., 2014; ISAPP, 2023). The bee product-derived isolates described in the reviewed publications have been primarily evaluated through *in vitro* tests of gastrointestinal survival, colonization traits, and antimicrobial activity. While these proper-

ties are relevant screening criteria, they do not replace clinical validation or meet all criteria for probiotic strains. Therefore, the available results point to probiotic potential and highlight directions for future studies, which should include determining host-associated functional properties such as anticancer, antidepressant, anti-diabetic, anti-obesity, and other effects.

5.2. Antimicrobial activity of beehive product-derived strains

The antimicrobial potential of isolates derived from bee products does not always align with studies on their probiotic properties. Instead, researchers focus on identifying novel compounds active against pathogenic species. This effort is driven by the need to discover new molecules that could serve as antibiotics, biocontrol agents, or food additives to prevent or control pathogens, especially those resistant to commonly used antimicrobial agents. *Bacillus* spp. and LAB, including those derived from bee products, are known for their antimicrobial potential. Consequently, many studies are investigating the antagonistic activity of different bacteria isolated from bee products against pathogenic microorganisms.

A total of 163 isolates, including *Bacillus* spp., *Paenibacillus* spp., *Lysinibacillus* spp., *Microbacterium* spp., and *Staphylococcus* spp., were recovered from 11 Polish honey samples. Their antimicrobial activity against *S. aureus*, *S. epidermidis*, *E. coli*, *L. monocytogenes*, *P. aeruginosa*, and *C. albicans* was tested. Most isolates with antimicrobial activity, effective against several pathogens, belonged to the genus *Bacillus*. Interestingly, only strains classified as *B. pumilus* inhibited the growth of the yeast *C. albicans*. Moreover, one *Paenibacillus* spp. strain, originating from buckwheat honey, inhibited the growth of *S. epidermidis*, *E. coli*, and *L. monocytogenes*. Furthermore, *Lysinibacillus* spp. were active against *L. monocytogenes*, *Microbacterium* sp. inhibited the growth of *S. epidermidis*, and *Staphylococcus pasteurii* was an antagonist of *P. aeruginosa* (Pajor et al. 2018). The results are consistent with those obtained by Pelka and coworkers, who focused on the antimicrobial activity of *Bacillus* spp. derived from bee bread and bee pollen. *Bacillus* spp. (*B. subtilis*, *B. licheniformis*, and *B. sonorensis*) isolated from raw honey and royal jelly limited the growth of *M. luteus* (Pelka et al. 2021). Moreover, among all the isolates, *B. licheniformis* was active against *Mycobacterium smegmatis*. The growth inhibition was probably achieved by

producing the antimicrobial compound lichenicidin (Martín-González et al. 2023). This appears to be an important discovery because *M. smegmatis* is a model organism for studying pathogenic bacteria belonging to the genus *Mycobacterium* (Sparks et al. 2023). Additionally, high antimicrobial efficacy against methicillin-resistant *S. aureus* (MRSA) was documented for peptides produced by *B. velezensis* strains isolated from honey, bee bread, and propolis (Baharudin et al., 2021). The same authors revealed the susceptibility of *B. cereus*, *Vibrio parahaemolyticus*, *V. alginolyticus*, *Aeromonas hydrophila*, and *Alcaligenes faecalis* to peptides derived from *B. velezensis* (Baharudin et al., 2021). Moreover, food-isolated fungi *Aspergillus fumigatus*, *A. niger*, *Cladosporium* spp., *Syncephalastrum* spp., and *C. albicans* were sensitive to the lipopeptide compound iturin A produced by the two *B. velezensis* strains isolated from honey (Xiong et al., 2022). The *B. subtilis* found in honey synthesized a cyclic lipopeptide surfactin. The compound exhibited high antagonistic activity against *L. monocytogenes*, including strains resistant to enterocins (Sabaté and Audisio 2013). *B. subtilis* from a study conducted by Lee and coworkers produced bacillomycin F (Lee et al., 2008). This peptide was tested against the fungus *Byssochlamys fulva* H25, a heat-resistant, difficult-to-manage, causative agent of food spoilage. The molecule was active against this pathogen, as well as other pathogens such as *Eurotium amstelodami*, *A. niger* 2270, *Monascus* sp., and *Rhizopus oligosporus*. Antifungal and antibacterial activities of *B. amyloliquefaciens* were also evaluated. A study investigating this species revealed activity against *C. albicans*, *E. coli*, and *S. aureus*, with inhibition zones comparable to antifungal fluconazole and antibacterial agents (Jia et al. 2020), as well as *Botryosphaeria dothidea*, *A. niger*, *Mucor racemosus*, *Fusarium oxysporum*, and *Penicillium citrinum* (Li et al. 2016). Moreover, it has been shown that the lipopeptide iturin A produced by *B. amyloliquefaciens* inhibited the growth of *A. niger* (Wang et al. 2024). In addition to *Bacillus* spp., several other bacterial genera are active against specific pathogens. A study by Lee and coworkers revealed that *P. polymyxa* isolated from honey was a producer of polymyxin E1 (Lee et al. 2009). This compound exhibited a strong inhibitory effect against *P. larvae*, a common honeybee pathogen. Moreover, the bacterial isolate was active against Gram-negative human pathogens (*Bordetella bronchiseptica*, *E. coli*, *Pseudomonas* spp., and *K. pneumoniae*), as well as Gram-positive pathogens and food spoilage

causative agents (*B. cereus*, *L. monocytogenes*).

LAB recovered from honey, particularly *L. plantarum*, *L. curvatus*, and *Pediococcus acidilactici*, exhibited antagonistic activity against *Candida* spp. (Bulgasem et al. 2016). Moreover, *L. acidophilus* strains produced compounds that inhibited the growth of multiple resistant Gram-positive bacteria, including *S. aureus*, *S. epidermidis*, and *B. subtilis* (Aween et al. 2012). Additionally, *Enterococcus faecium* isolated from Argentine honey produced a substance that inhibited bacteriocin-resistant *L. monocytogenes* (Ibarguren et al. 2010).

Most studies investigating bee product-derived microorganisms with antimicrobial properties have been limited to *in vitro* assays, typically demonstrating inhibition of selected bacterial or fungal pathogens. While these findings provide important first evidence of antimicrobial potential, they do not fulfill the requirements necessary for industrial application. Strain-level identification by whole-genome sequencing and comprehensive safety profiling remains essential to exclude virulence factors, transferable antibiotic resistance, or toxin production. Moreover, regulatory frameworks require the characterization and purification of active compounds, toxicological evaluation, validation in food matrices, animal models, or field trials, and stability and reproducibility of production. These critical steps are still missing in the majority of available studies, and until they are addressed, bee product-derived strains and their metabolites cannot be regarded as ready-to-use antimicrobial agents but rather as promising candidates for further development (Sutay Kocabaş et al. 2019; Pariza et al. 2001).

5.3. Enzymatic potential of microbes from bee products

Enzymes are biological catalysts widely utilized in the food and beverage, textile, pulp and paper, cosmetic, and pharmaceutical industries. The microorganisms present in beehive products represent an underexplored source of various enzymes. Several studies have revealed that microorganisms associated with bee products exhibit proteolytic, amylolytic, lipolytic, esterolytic, and cellulolytic activities (Ngalimat et al. 2019; Pełka et al. 2021). Investigating microorganisms derived from beehive products may lead to the discovery of novel biocatalysts.

Amylases from *Bacillus* species isolated from honey have been investigated in several studies. *B. amyloliquefaciens* BH1 (Du et al., 2018) and *B. atrophaeus*

NRC1 (Abd-Elaziz et al. 2020) produced α -amylase. Both enzymes were produced, purified, and partially characterized. *B. amyloliquefaciens* BH1 α -amylase exhibited the highest activity and good stability at pH 7, but was labeled under alkaline or acidic conditions, with an optimum temperature of activity at 40 °C (Du et al., 2018), whereas *B. atrophaeus* NRC1 AmyI α -amylase was most active at pH 6 and 50 °C. This enzyme was stable at temperatures below 50 °C, but it quickly lost activity at higher temperatures. Moreover, AmyI demonstrated the ability to degrade starch, amylopectin, amylose, and glycogen (Abd-Elaziz et al., 2020). α -Amylase and β -amylase were also produced by *B. atrophaeus* AMA6 and *B. velezensis* AMA2, respectively. Optimization of enzyme production conditions revealed a temperature shift from 40 °C to 45 °C and a pH shift from 7 to 6 when cells were immobilized in alginate beads. The amylases produced by immobilized cells were more stable at alkaline pH and in the presence of detergents than enzymes secreted by free cells, which is valuable for evaluating possible potential practical applications (Esawy et al., 2025). The halophilic dextranase was produced by *B. subtilis* NRC-B233 isolated from Saudi Arabian Kashmiri honey. The optimal conditions for enzyme production were: 37 °C, pH 9.0, and 32 hours of incubation in a medium containing 5% starch and 2% peptone as the carbon and nitrogen sources, respectively. Moreover, enzyme production increased upon the addition of 0.175 mM CrCl_3 . The partially purified dextranase exhibited maximum activity at pH 9.2 and 70 °C and remained fully stable at 75 °C for one hour. Furthermore, enzyme activity increased approximately fourfold in 10% NaCl. The dextranase from *B. subtilis* NRC-B233 was capable of degrading glycosidic linkages in dextran, starch, amylopectin, inulin, and cellulose; thus, it can be potentially applied in the processing of polysaccharides. Its stability under harsh conditions suggests that dextranase produced by *B. subtilis* NRC-B233 may be suitable for use in the pharmaceutical and environmental industries (Mansour et al. 2011). Surwania and coworkers recovered eight bacterial isolates exhibiting proteolytic activity from honey. *Aeromonas hydrophila* strain YL17 was identified as the most efficient protease-producing isolate. The optimal conditions for enzyme production were as follows: 30 °C, pH 9.0, 48 hours with shaking, in a medium containing 1% maltose and 1% soybean powder as carbon and nitrogen sources, respectively. The crude protease in the post-cultivation medium was maximally active at pH 9.0 and 30 °C, and

the addition of hydrogen peroxide enhanced its activity. Moreover, the alkaline protease from *A. hydrophila* YL17 retained 97% of its initial activity in the presence of 1% Ariel. Finally, *A. hydrophila* YL17 protease was evaluated for its effectiveness in removing bloodstains in detergent formulations, enhancing washing performance compared to the detergent alone (Surwania et al. 2018). *B. sonorensis* AB7 and *B. licheniformis* CG1 were isolated from raw honey samples collected in South England, and the study revealed that these isolates could grow on chicken feathers as the sole carbon and nitrogen sources. *B. licheniformis* CG1 exhibited 2.6 times higher keratinase activity than *B. sonorensis* AB7. Moreover, the enzyme from *B. licheniformis* CG1 was active within a pH range of 6 to 9 (Martín-González et al. 2023). These results suggest that this enzyme could be an attractive candidate for feather waste bioconversion, detergent formulation, leather processing, and animal nutrition; however, further research is required. Silva et al. investigated the collagenase production by *Trichosporon* sp. strain 7V isolated from bee pollen collected in Brazil. The strain was selected from 11 yeast isolates showing collagenolytic activity. The highest collagenase activity was observed after 72 h of yeast cultivation at 28 °C using an agitation intensity of 160 rpm, pH 5.5, and substrate (gelatin) concentration of 4.0 g/L. The highest ability to hydrolyze type I bovine collagen by *Trichosporon* sp. 7V enzyme was observed at pH 6, 25 °C, and a substrate concentration of 7.5 g/L (da Silva et al. 2022). This biocatalyst could be valuable in biomedical applications such as wound healing and fibrosis treatment. Moreover, controlled collagen hydrolysis is required in leather processing and the food industries. Thus, this enzyme can potentially be applied in these industrial sectors. A xylose-utilizing yeast strain was isolated from a honey sample and identified as *C. tropicalis* YHJ1. The isolate could grow in culture media containing up to 600 g/L xylose and showed stress tolerance toward various chemical agents, namely 1 M NaCl, 16% (w/v) ethanol, 10 mM H_2O_2 , and 15 mM caffeine. The highest xylitol concentration was detected in the medium containing 300 g/L xylose. Furthermore, three genes involved in the initial steps of xylose metabolism were identified, and two NADPH-dependent xylose reductase *xy11* gene mutants were constructed. The wild-type XR, XR_S279L, and XR_S279N were expressed in *E. coli*, purified, and their activity with NADPH was evaluated. The most active was the wild-type XR, confirming that the stress-tolerant *C. tropicalis* YHJ1 can be used

for xylitol production from xylose-containing plant biomass hydrolysates (Kim et al. 2019).

5.4. Fermentation capacity

Bee products are a valuable source of fermenting microorganisms, such as LAB and yeasts. Ghesti and coworkers described the production of a Catharina Sour beer using a traditional method, comparing it to the use of bee pollen as a source of LAB. Traditional Catharina Sour beer production uses commercially available *Lactobacillus* to impart a specific and characteristic lactic acidity. This study reported better impressions of beer produced with bee pollen, which was evaluated based on aroma, flavor, and color. The authors concluded that bee pollen could serve as a source of microorganisms for sour beer production, allowing the development of new methods and beer styles (Ghesti et al. 2023). In a study described by Silva and coworkers, stingless bee pollen and honey were used as sources of fermenting microorganisms. Among the 55 isolated yeast strains, two *S. cerevisiae* strains could withstand a 10% (v/v) ethanol concentration and exhibited a higher growth rate than commercially utilized strains in a medium containing 10% (m/v) glucose.

Additionally, they could grow in a medium with 50% glucose, and in the presence of sodium or potassium metabisulfite (additives used to prevent bacterial growth during the production of fermented beverages) at commercially relevant concentrations. Finally, the *S. cerevisiae* isolates produced ethanol and glycerol under typical wine and mead production conditions, particularly at low pH (Silva et al. 2020). In another study, Srimeena and coworkers screened yeasts derived from honey for mead production. The selected *S. cerevisiae* YR5 showed good growth in a medium containing 20% (w/v) glucose, 20% (w/v) fructose, and 250 mg/L SO₂. It also exhibited measurable growth in a medium containing 10% (v/v) ethanol (Srimeena et al. 2013). Therefore, it can be concluded that *S. cerevisiae* strains derived from bee products are suitable for producing beverages with an alcohol level of up to 10%.

Although using bee products as a source of fermenting microorganisms, LAB, and yeasts imparts a unique flavor and aroma to alcoholic beverages, this approach causes numerous technological challenges. Because each bee product has a complex and unique microbiota, dependent on the botanical source, season, weather, etc., achieving reproducible results in the long term is impossible. Furthermore, bee prod-

ucts contain undesirable components, such as mold spores and *C. botulinum* spores, which can negatively impact the quality and safety of the final fermentation product. Therefore, it seems more rational to use bee products as the origin of strains further used in industry as well-defined starter cultures. On the other hand, reproducing a complex consortium of microorganisms as a starter culture can be very difficult and requires extensive research.

6. Conclusions

Despite being an ecological niche with demanding conditions for microbial survival, bee products harbor a diverse microbiota. Regardless of the identification methods employed by different authors, the results consistently indicate that bee products serve as a habitat for microorganisms belonging to LAB, including FLAB, spore-forming bacteria, yeasts, and molds. While bee products continue to attract considerable interest, an increasing number of studies are focusing on the potential of their associated microbiota. However, most of the reports discussed in this review are limited to preliminary screenings, providing only indicative evidence of the promising—yet insufficiently characterized—potential of microorganisms isolated from honey, pollen, and bee bread. This underscores the need for more comprehensive functional and applied investigations.

Future research should address several critical gaps that currently limit the translation of bee product-associated microbiota into practical applications. In the case of probiotic candidates, robust clinical trials confirming their health-promoting effects are still missing, yet such evidence is indispensable according to ISAPP guidelines. For producers of antimicrobial compounds, it is essential to move beyond demonstrations of antagonism toward pathogens and to conduct systematic evaluations of the safety and toxicological profile. In particular, the application of microbial enzymes remains restricted to laboratory-scale studies. In contrast, industrial implementation requires scalable production, cost-effective downstream processing, regulatory approval, and consistent product quality and stability. Moreover, bee product-derived microorganisms, including *S. cerevisiae* and LAB strains, have demonstrated promising fermentation capacity, suggesting potential as novel starters in beverage production. However, challenges such as strain standardization, reproducibility, and safety validation

must be addressed before these microorganisms can be fully integrated into industrial processes. Importantly, microorganisms intended for probiotic, enzymatic, or fermentative applications must comply with international safety and quality frameworks: probiotics require clinical validation according to ISAPP, enzyme producers must meet GRAS (FDA) or QPS (EFSA) safety standards supported by genomic and toxicological assessments, and fermentative strains must adhere to Codex Alimentarius and ISO guidelines ensuring technological performance, genetic stability, and reproducibility under GMP and HACCP conditions.

Despite these challenges, the microbiota of bee products represents a highly promising research subject, offering a rich and underexplored reservoir of microorganisms with biotechnological potential. Continued exploration of this niche is therefore likely to yield novel microbial producers and applications of considerable industrial and scientific relevance. The microbiota associated with beehive products, including unculturable microorganisms, are an abundant source of genes encoding enzymes and peptide antimicrobial compounds, which can be efficiently produced in heterologous expression hosts with confirmed safety status.

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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