

Honey Bees/Honey as Probiotic and Prebiotic Products

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Honey bees come from the family of Apidae and the genus *Apis*. *A. dorsata*, *A. mellifera*, *A. cerana*, *A. laboriosa*, *A. florea*, *A. andreniformis*, *A. koschevnikovi*, and *A. nigrocincta* are eight known species that can be found around the world. Honey bees are significant pollinators for cultivating crops for food production, ensuring the continuity of almost all life in this world. The honey bee's gut contains many microorganisms as its normal microbiota. Most are probiotics, made up of lactic acid bacteria (LAB) and Bifidobacterium, which are widely distributed in their digestive tract system. Probiotics were first described in 2013 by the International Scientific Association for Probiotics and Prebiotics (ISAPP) as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host". The scientific definition has been extensively applied around the globe. Probiotics enhance intestinal health and increase immune reaction by producing biological antimicrobial substances that can inhibit pathogens which caused digestive system imbalances in humans and animals.

probiotic prebiotic honey bee honey

1. Introduction

Many products produced by honey bees are useful to humans, including honey, [1][2] which is the most important and widely consumed bee product worldwide. Honey, a "natural sweet substance produced by *Apis mellifera* L. bees from the nectar of plants, secretions of living parts of plants, or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honeycomb to ripen and mature" [3], comes in two varieties namely: blossom/nectar honey and honeydew honey. Blossom honey is made from flowering plant nectar, whereas honeydew honey is manufactured from honeydew collected from various parts of a plant or other sap-producing plants and insects [4]. Honey can be divided into two categories: unifloral (monofloral) and polyfloral (multifloral). Unifloral honey is made primarily from one type of plant nectar and is identified through pollen analysis, which reveals dominant pollen from a single plant species. Polyfloral honey does not have dominant pollen from one plant species but has a mixture of pollen from several plants [5]. Due to its refined, one-of-a-kind, and distinct flavor, unifloral honey typically commands a higher market price than polyfloral honey. The premium quality of unifloral honey mostly depends on the exclusive geographical area or the special plant species, for example, the Manuka honey from New Zealand [6]. Honey may contain probiotics that have been transmitted from the guts of honey bees during the process of making honey and may remain alive for a certain period [7]. Thus, both honey bees and honey may provide potential probiotics for future use. The health benefits of honey concerning its probiotic bacteria are that the probiotics will help to revitalize and strengthen the immune system of the host against harmful environmental factors and pathogens, aid in digestion, detoxify harmful substances and provide essential nutrients [8].

Honey is mostly made up of sugars or carbohydrates such as fructose (32–44%), glucose (23–38%), and some other complex sugars (5–15%) including sucrose, maltose, lactose, raffinose, trehalose, erlose, gentiobiose, turanose, panose, melezitose, and kojibiose amongst others [9]. Besides carbohydrates, the quality and health advantages of honey are also ascribed to the various components it possesses, such as protein, organic acids, amino acids, vitamins, minerals, enzymes, and polyphenols [10]. Different varieties of honey may vary in their content due to the different sources derived, such as geographical area,

botanical origin, and bee species [9]. Blossom or nectar honey can be distinguished from honeydew honey by analyzing its carbohydrate concentration. Blossom honey contains higher concentrations of monosaccharides but is lower in trisaccharides (mainly melezitose, erlose, raffinose, and maltotriose) and other oligosaccharides compared to honeydew honey [11]. The honey's prebiotic properties are known to come from its indestructible carbohydrates that cannot be fermented by digestive enzymes in humans and are not taken up in the upper intestinal tract system. They are capable of improving and enhancing health in general and intestinal health in particular by stimulating the development and promoting metabolic activity of the typical residents of the colon [12]. Honey's prebiotic qualities can help probiotic microorganisms to flourish by supplying adequate nutrients. An increased number of probiotics may help to alleviate the total surface area for nutrient absorption, thus improving the health of the digestive system and enhancing resistance to pathogen infections [13]. These findings have sparked some ideas for conducting studies for further research on the natural microbiota of the bees' gut with probiotic properties as a disease defense mechanism to be used as prophylaxis to treat not only bees themselves but also other animals and humans [8].

2. Probiotic Properties of Honey Bees and Honey

Table 1 highlights the studies that have been performed in several countries on honey bees' guts and honey as the origin of potential probiotics. The majority of honey bee probiotics have been identified from *A. mellifera* spp., with a few from the *A. cerana* spp., and *A. dorsata* spp. Probiotics isolated from the honey bee gut were composed of diverse microorganisms including *Bifidobacterium* and lactic acid bacteria (LAB), as well as *Fructophilic lactic acid bacteria* (FLAB) which is a subgroup of LAB, yeasts, and other types of bacteria such as the *Bacillus* spp.

Table 1. Potential probiotics in the bees' gut and honey.

Probiotic	Source	Origin/Country	Reference
<i>Bifidobacterium</i> spp.	<i>Apis cerana japonica</i> gut	Tsukuba, Japan	[14]
<i>Bifidobacterium</i> spp., <i>Lactobacillus</i> spp., <i>Bacillus</i> spp.	<i>Apis cerana indica</i> gut	Samut-Songkhram, and Chumphon, Thailand	[15]
<i>Lactobacillus</i> spp.	<i>Apis cerana indica</i> gut	Karnataka, India	[16]
<i>Lactobacillus plantarum</i> , <i>Lactobacillus pentosus</i> , <i>Lactobacillus fermentum</i>	<i>Apis dorsata</i> gut	Terengganu, Malaysia	[17]
<i>Lactobacillus kunkeei</i> strains	Yigilca honey bee gut	Duzce, Turkey	[18]
<i>Lactobacillus plantarum</i> , <i>Lactobacillus paraplanitarum</i> , <i>Lactobacillus plantarum</i> strains	<i>Apis mellifera</i> gut	Menoua, Cameroon	[19] [20]
<i>Lactobacillus plantarum</i> strains	<i>Apis cerana indica</i> gut	Kerala, India	[21]
Lactic Acid Bacteria (LAB) genera: <i>Enterococcus</i> , <i>Lactobacillus</i> , <i>Micrococcus</i> , <i>Lactococcus</i> , <i>Streptococcus</i> ,	<i>Apis cerana indica</i> Fabricius, <i>Apis mellifera</i> Linnaeus, <i>Apis florea</i> Fabricius, & <i>Apis dorsata</i> Fabricius guts and honey	Tamil Nadu, India	[22]

Probiotic	Source	Origin/Country	Reference
<i>Pediococcus</i> , <i>Leconostoc</i>			
<i>Enterococcus faecalis</i> strains, <i>Lactobacillus brevis</i> , <i>Lactobacillus casei</i>	<i>Apis mellifera</i> gut	Cairo, Egypt	[23]
<i>Fructobacillus fructosus</i> strains, <i>Lactobacillus kunkeei</i> strains	<i>Apis mellifera mellifera</i> , <i>Apis mellifera ligustica</i> and hybridized bee guts, larvae and honey	Aland Island, Finland	[24]
<i>Lactobacillus kunkeei</i> strains (sixty-six strains), <i>Lactobacillus casei</i> (one strain), <i>Lactobacillus</i> spp. (five unidentified strains), <i>Fructobacillus fructosus</i> strains (eight strains), <i>Enterococcus</i> (five strains), <i>Bifidobacterium asteroides</i>	<i>Apis mellifera</i> gut	The Caucasus Mountains, and Kolkheti Valley, Georgia	[25]
<i>Lactobacillus kunkeei</i> strains, <i>Lactobacillus fructosus</i> strains	<i>Apis mellifera</i> gut	Lublin, Poland	[26]
<i>Lactobacillus kunkeei</i> strains, <i>Fructobacillus fructosus</i> strains	<i>Apis mellifera</i> Linnaeus gut	Pulawy, Poland	[27]
<i>Fructobacillus fructosus</i> , <i>Proteus mirabilis</i> , <i>Bacillus subtilis</i> , <i>Bacillus licheniformis</i> , <i>Lactobacillus kunkeei</i> , <i>Enterobacter kobei</i> , <i>Morganella morganii</i>	<i>Apis mellifera jemenitica</i> gut	Riyadh, Saudi Arabia	[28]
<i>Apilactobacillus kunkeei</i> strains	<i>Apis mellifera</i> Linnaeus gut	N/A	[29]
<i>Bacillus</i> spp.	<i>Apis cerana japonica</i> gut	Tsukuba, Japan	[30]
<i>Bacillus subtilis</i> strains	Honey bee gut and honey	N/A	[31]
<i>Bacillus licheniformis</i> , <i>Paenibacillus polymyxa</i> (<i>Bacillus polymyxa</i>), <i>Wickerhamomyces anomalus</i> , <i>Lachancea thermotolerans</i> , <i>Zygosaccharomyces mellis</i> ,	<i>Apis mellifera carnica</i> gut <i>Apis mellifera ligustica</i> gut	Giza, Egypt	[32]
<i>Lactobacillus kunkeei</i> strains, <i>Lactobacillus</i> spp.	Honey (<i>Apis dorsata</i>)	Kedah, Malaysia	[33]
<i>Leuconostoc mesenteroides</i> strains	Honey (<i>Apis mellifera</i>)	Algeria	[34]
Lactic Acid Bacteria (Species and subspecies not mentioned)	Honey (<i>Apis mellifera</i>)	Indonesia	[35]

Probiotic	Source	Origin/Country	Reference
<i>Bacillus</i> spp.	Commercial honey (Libya, Saudi Arabia and Egypt)	N/A	[36]
<i>Bacillus subtilis</i> , <i>Brevibacillus brevis</i> , <i>Bacillus megaterium</i> strains, <i>Lactobacillus acidophilus</i>	Local honey	Iran	[37]
<i>Bacillus subtilis</i> strains <i>Bacillus endophyticus</i>	Mountain honey Persimmon honey (commercial)	Nigeria Egypt	[38]
<i>Bacillus</i> spp.	Honey	China	[39]
<i>Bacillus subtilis</i> , <i>Bacillus mycoides</i> , <i>Bacillus thuringiensis</i> , <i>Bacillus amyloliquefaciens</i> , <i>Bacillus velezensis</i>	Raw honey (Polyfloral)	Romania	[40]
<i>Gluconobacter oxydans</i>	Honey (<i>Apis cerana indica</i>)	Tamil Nadu, India	[41]
<i>Saccharomyces cerevisiae</i> strains, <i>Meyerozyma guilliermondii</i>	Raw honey (<i>Apis dorsata fabricius</i>)	Ratchaburi, Thailand	[42]

4. Bogdanov, S. Honey types. In Book of Honey; Scribners: Hunter, NY, USA, 2011; Chapter 6; pp. 1–5.
5. Ajibola, A. Novel insights into the health importance of natural honey. *Malays J. Med. Sci.* 2015, 22, 7–22. N/A = not available.
6. Johnston, M.; McBride, M.; Dahiya, D.; Owusu-Apenten, R.; Nigam, P.S. Antibacterial activity of Manuka honey and its components: An overview. *AIMS Microbiol.* 2018, 4, 655–664. Bifidobacterium and its components: An overview. *AIMS Microbiol.* 2018, 4, 655–664. The LAB. One study has shown the isolation of several *Bifidobacterium* species from the Japanese honey bee (*Apis cerana japonica*) gut. The isolates were 7. Luchese, R.H.; Prudencio, E.R.; Guerra, A.F. Honey as a functional food. In Honey Analysis; INTECH Open shown to be strongly linked to *bifidobacteria* obtained from the European honey bees, implying that these bacteria are Science: London, UK, 2017; Chapter 13; pp. 287–307. peculiar to the honey bee species.
8. Kechagia, M.; Basoulis, D.; Konstantopoulou, S.; Dimitriadi, D.; Konstantina, G.; Skarmoutsou, N.; Fakiri, LAB is the most dominant probiotic isolated from the *Apis mellifera* 2018, 2018, 481651. *Environ. Health Perspect.* 2018, 126, 1–11. *Environ. Health Perspect.* 2018, 126, 1–11. many studies conducted earlier (refer to *Table 1*). Various strains of LAB were harvested from the guts of *A. mellifera*, *A. cerana*, *A. dorsata*, and *A. florea* species. In 9. Meo, S.A.; Al-Asiri, S.A.; Mahesar, A.L.; Ansari, M.J. Role of honey in modern medicine. *Saudi J. Biol. Sci.* most of the studies, the microorganisms were identified using the PCR technique with gene sequencing. *Lactobacillus* spp. 2017, 24, 975–978. was recovered from the digestive tract of *A. cerana indica* in various Karnataka locales [16]. Other *Lactobacillus* species such 10. Bobasa, O.; Mojica, A.; Rain, B.; Lallemand, W.; Represa, E.; Sánchez, D.; Ruiz, C.; Sánchez, A.; Sánchez, P.; Martínez, J. Cruz for 51.2% of the study. was the first to highlight the presence of *Lactobacillus* spp. in the intestinal tract of the honey bee gut (*A. dorsata*). The chemical composition and health-promoting properties of *Food Chem.* 2020, 325, 126870. be dependent on the 11. De-Melo, A.A.M., De Almada-Muradian, L.B.; Sancho, M.T.; Pascual-Maté, A. Composition and properties of *Apis mellifera* honey: A review. *J. Apic. Res.* 2017, 57, 5–37. There have also been findings of the presence of *Bacillus* sp., in honey. Earlier in 2012, Esawy and colleagues [36] isolated 12. Mohan, A.; Qureshi, S.; Gutierrez, M.; Lopez, N.; Gao, Y.; Shu, Q. Effect of honey in improving the gut microbiota balance. *Food Qual. Saf.* 2017, 1, 107–115. tolerance to pH and bile salts with varying degrees of viability, resistance to 0.3% bile and pancreatic enzyme (except for one isolate), and negative results for hemolytic testing. The isolates also demonstrated antioxidant and 13. Patrulca, S.; Dumitrescu, G.; Popescu, R.; Fillmon, N.M. The effect of prebiotic and probiotic products used antimicrobial properties, suggesting that they could flourish in the intestinal tract and function as potential antibiotic producers in feed to stimulate the bee colony (*Apis mellifera*) on intestines of working bees. *J. Food Agric. Environ.* [36] 2013, 11, 2461–2464.

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15. Nonthapa, P.; Chanchao, C. *Pathogen detection and gut bacteria identification in Apis cerana indica in Thailand*. *Afr. J. Biotechnol.* 2015; 14, 3235–3247.

16. Pattaiahamaiah, M.; Reddy, M.S.; Brueckner, D. *Detection of novel probiotic bacterium Lactobacillus spp. in the workers of Indian honeybee, Apis cerana indica*. *Int. J. Environ. Sci.* 2012; 2, 1135–1143.

17. Tariq, M.; Iqbal, M.; Iqbal, M. *Molecular identification of Lactobacillus spp. isolated from the honey comb of the honey bee (Apis dorsata) by 16s rRNA gene sequencing*. *J. Apic. Res.* 2013; 52, 235–241.

18. Uğras, S. *Isolation, identification and characterization of probiotic properties of bacterium from the honey as human digestive enzymes do not possess β-glycosidases. Inulin, fructose-oligosaccharides (FOS), pyrodextrins, lactulose, and xylooligosaccharide are among the well-known prebiotics*. *Türk. Entomol. Derg.* 2017; 41, 253–261.

19. Kenfack, C.H.M.; Kaktcham, P.M.; Ngoufack, F.Z.; Wang, Y.R.; Yin, L.; Zhu, T. *Safety and antioxidant characterization of putative probiotic Lactobacillus strains from honey bees (Apis mellifera)*. *J. Adv. Microb.* 2018; 10, 1–18.

Table 2. Prebiotic potential of honey.

20. Kenfack, C.H.M.; Kaktcham, P.M.; Ngoufack, F.Z.; Wang, Y.R.; Yin, L.; Zhu, T. *Safety and antioxidant potential of bees*.

Probiotic	Sources of Prebiotic	Country	Key Findings	Reference
<i>Lactobacillus acidophilus</i> strains	Honey	India	<ul style="list-style-type: none"> Honey enhanced the coaggregation of <i>E. coli</i> with <i>L. acidophilus</i> NCDC 291 more than with <i>L. acidophilus</i> NCDC 13. 	olated [44]
<i>Lactobacillus acidophilus</i> , <i>Bifidobacterium bifidum</i>	Sesame honey (<i>Sesamum indicum</i>)	India	<ul style="list-style-type: none"> Both strains showed a higher capability of autoaggregation and hydrophobicity, and reduced autolytic activity with inulin compared to honey. 	om acid [45] 349–
<i>Lactobacillus acidophilus</i> , <i>Lactobacillus rhamnosus</i>	Chestnut honey	Turkey	<ul style="list-style-type: none"> Sesame honey (5%) exhibited selective and significant growth-supporting properties of the probiotics. Chestnut honey has positively impacted probiotic bacteria by increasing growth and modulating probiotic properties such as auto-aggregation and surface hydrophobia. 	a. Syst. ffa, G. in. [46] molecular ellifera L.
<i>Lactobacillus plantarum</i> strain	Wild honey (Polyfloral)	Cameroon	<ul style="list-style-type: none"> <i>L. plantarum</i> 29 V can survive for 28 days at 4 °C and 25 °C due to their 	nto sh [47]

28. Al-Ghamdi, A.; Khan, K.A.; Ansari, M.J.; Al-Masaudi, S.B.; Al-Kahtani, S. *Effect of gut bacterial isolates from Apis mellifera Jemenitica on Paenibacillus larvae infected bee larvae*. *Saudi J. Biol. Sci.* 2017; 25, 383–

Probiotic	Sources of Prebiotic	Country	Key Findings	Reference
2			ability to resist lower pH and the presence of oligosaccharides (fructo- and gluco-oligosaccharides) in honey recognized as prebiotics.	G.; Di aluation
3			- Hypercholesterolemic rats treated with honey containing <i>L. plantarum</i> 29 V showed an increase in HDL-cholesterol level and lowers total cholesterol, LDL-cholesterol, triglycerides and atherosclerosis index in serum.	, against 129.
3				Ir, N.M. solated
3	<i>Lactobacillus acidophilus</i> , <i>Lactobacillus gasseri</i> , <i>Lacticaseibacillus casei</i> , <i>Lacticaseibacillus rhamnosus</i> , <i>Lactiplantibacillus plantarum</i>	Fir, strawberry tree, ivy, tree of heaven, sulla, cardoon, rhododendron honey (Commercial, organic, monofloral honey)	- Fir, ivy, and sulla honey (1% and 2%) stimulate the growth of all the probiotics tested with various actions compared to more specific cardoon honey.	ota of [48] s and
3	<i>Bifidobacterium longum</i> strains, <i>Bifidobacterium breve</i> , <i>Bifidobacterium bifidum</i>	Agmark grade honey	- Honey showed a prebiotic effect on all isolates, especially on <i>B. longum</i> at 3% and 5% honey.	[49] t. Foods
3	<i>Bifidobacterium bifidum</i> and <i>Lactobacilli</i>	Clover honey (Unprocessed and sterilised)	- Increased <i>B. bifidum</i> colony counts were observed in all honey-supplied group (Group A-5 g, B-10 g, and C-15 g honey), with group B, showing a significant rise in comparison with the control.	[50] W bacillus
3	Bifidobacteria	Buckwheat honey	- Buckwheat honey assists in propagating native Bifidobacteria and prohibits the growth of the pathogenic bacterium in the gut system.	[51] among I. Agric. M.T.; st biota.
4	N/A	Manuka honey (MGO™)	- Honey-containing oligosaccharides inhibited <i>P. aeruginosa</i> (52%), <i>E. coli</i> O157:H7 (40%) and <i>S. aureus</i> (30%) in the cancer cells.	[52] I.G.; Appl.

41. Begum, S.B.; Roobia, R.R.; Karthikeyan, M.; Murugappan, R. Validation of nutraceutical properties of honey and probiotic potential of its innate microflora. *LWT-Food Sci. Technol.* 2015, 60, 743–750.

42. Zahoor, F.; Sooklim, C.; Songdech, P.; Duangpakdee, O.; Soontorngun, N. Selection of potential yeast probiotics and a cell factory for xylitol or acid production from honeybee samples. *Metabolites* 2021, 11, 312.

Probiotic	Sources of Prebiotic	Country	Key Findings	Reference
Microbiota of the mice gut	Jarrah honey	China	- Honey helps to retain more water in the faecal and relieves constipation and suppresses the growth of <i>Desulfovibrio</i> .	[53] 0, 66, 2, 2,
N/A	Giant Willow Aphid honeydew honey (<i>Tuberolachnus salignus</i>)	New Zealand	- A high concentration of melezitose can act as a prebiotic for the human digestive system since it is not hydrolysed by acid and is only partially hydrolysed by α -glucosidase.	[54] terial and
<i>Limosilactobacillus reuteri</i>	Manuka honey (Drapac DrKiwi AMF5, AMF10, AMF15 and AMF20)	New Zealand	- High sugar and oligosaccharides contributed to higher probiotic cell biomass of AMF20, but no obvious pattern in biomass with a decrease in AMF concentration.	[55] 2021, 24, F. their effect

on selected pathogenic and probiotic bacteria. *Microorganisms* 2021, 9, 1694.

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Both types of honey, either monofloral or polyfloral, can serve as good prebiotic sources in foods. Honey is combined with dairy products derived from the fresh milk of cows [56–57,58,59,60], goats [61–62], camels [57], and buffaloes [63] to produce yogurt.

54. Sneath, R.M.; Manley-Harris, M. *Composition and potential as a prebiotic functional food of a manuka honey.* Some researchers utilized kiwifruit milk powder to produce yogurt [64–65,66]. As for the food dairy products [67], soy milk [68,69] and hydrolyzed soybean extract [67] were chosen to replace the animal's milk. The most common starter culture

55. Mohan, A.; Gutierrez-Maddox, N.; Meng, T.; He, N.; Gao, Y.; Shu, Q.; Quek, S.Y. *Manuka honey with varying levels of active manuka factor (AMF) ratings as an anaerobic fermentation substrate for*

Limosilactobacillus reuteri DPC16.

Fermentation 2021, 7, 128.

for cultivating Bifidobacteria strains of diverse subspecies [64,65]. Saudi Arabian raw honey [56], black locust honey [57], Kerala

56. Raynes, A.; H. *Enhancement of probiotic bioactivity by soybean prebiotics to prevent bio-fermented milk culture.* *Soy* 2012, 9, 2246–2253.

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