Vol.65: e22210440, 2022
https://doi.org/10.1590/1678-4324-2022210440
ISSN 1678-4324 Online Edition

Article - Biological and Applied Sciences

Microbial Biodiversity in Honey and Pollen Pots Produced by *Tetragonisca angustula* (Jataí)

Marcia Regina Beux[1,2*]
https://orcid.org/0000-0002-6174-392X

Jaqueline Leobet[4]
https://orcid.org/0000-0002-5139-9598

Suelen Ávila[1]
https://orcid.org/0000-0002-3776-1968

Fabiana Barbieri[5]
https://orcid.org/0000-0001-5656-4217

Monica Surek[3]
https://orcid.org/0000-0003-4048-3046

Sila Mary Rodrigues Ferreira[1,4]
https://orcid.org/0000-0001-6118-6089

Keliani Bordin[2]
https://orcid.org/0000-0003-1605-5690

Edvaldo Antonio Ribeiro Rosa[2]
https://orcid.org/0000-0001-6087-4365

[1]Universidade Federal do Paraná (UFPR), Programa de Pós-graduação em Alimentação e Nutrição, Curitiba, Paraná, Brasil; [2] Pontifícia Universidade Católica do Paraná, Escola de Ciências da Vida, Curitiba, Brasil; [3]Universidade Federal do Paraná (UFPR), Departamento de Farmácia, Curitiba, Paraná, Brasil. [4]Universidade Federal do Paraná (UFPR), Departamento de Nutrição, Curitiba, Paraná, Brasil; [5]Universidade Federal do Paraná (UFPR), Curitiba, Paraná, Brasil.

Editor-in-Chief: Paulo Vitor Farago
Associate Editor: Jane Manfron Budel

Received: 10-Jul-2021; Accepted: 22-Apr-2022.

*Correspondence: beuxmarcia@gmail.com; Tel.: +55-41-3360-4017. (M.R.B.)

HIGHLIGHTS

- Eight species of bacteria and three yeasts were identified in *T. angustula* products.
- *Bacillus* was the main bacteria present in pots of stingless bee honey and pollen.
- *S. meliponinorum* was the main yeast found in jataí honey, and *Z. bailli* in jataí pollen.
- Symbiotic relationship between microorganisms and honey and pollen pots

Abstract: The combination of nutritional and chemical factors in honey and pollen produced by *Tetragonisca angustula* (jataí) reveals a unique ecosystem that is conducive to developing microorganisms. However, there is a lack of information regarding the microbial species grown in the nest pots of this stingless bee species. The objectives of this study were to count, isolate and identify the microbiota associated with the pots of honey and pollen. The samples were collected from three jataí beehives. Microbiological analyses showed that the average total aerobic mesophiles ranged between 3.78 and 3.71 log CFU/g; lactic bacteria were 3.59 and 3.56 log CFU/g; and the yeast was 4.41 and 4.66 log CFU/g for the honey and pollen, respectively. There were four gram-positive cocci bacteria (*Staphylococcus saprophyticus* subsp. *bovis, S. vitulinus, S. kloosii* and *S. pasteuri*); two spore-forming gram-positive bacteria *(Bacillus pumilus* and *B. thuringiensis)*; and one gram-negative bacteria (*Pantoe agglomerans).* One lactic bacteria (*Leuconostoc mesenteroides* subsp. *mesenteroides)* was also identified. Three yeast species were isolated (*Starmerella meliponinorum, Candida magnoliae* and *Zygosaccharomyces bailli).* Bacillus was the main bacteria in jataí honey and pollen. *Z. bailli* was the dominant yeast in the pollen, and *S. meliponinorum* was the dominant yeast in the honey. *T. angustula* bees and their products (honey and pollen) are ecosystems that provide sustainable environmental

and technological integration. They also provide a selection of microorganisms that can be applied in the food, cosmetic and pharmaceutical industries.

Keywords: stingless bee honey; isolation; characterization.

GRAPHICAL ABSTRACT

INTRODUCTION

Stingless bees (SB) are ecologically important because they preserve plant biodiversity; they are considered to be the primary pollinator of Brazilian flora. It is estimated that the honey produced in one day results from at least one million interactions between flowers and bees. SB are eusocial bees that are pantropically distributed; they do not present a functional sting. SB colonies are perennial and can resist long periods of adversity through consuming food stored in the nests (pots containing honey and pollen). Nesting commonly occurs in exposed nests, and in pre-existing holes such as underground and tree cavities; however, SB can also be raised in urban environments using appropriate wooden boxes. Of these, *Tetragonisca angustula* (jataí) is the most adapted and well-known species [1–4].

Inside SB nests, brood combs are surrounded by pots of food used to maintain the nest. SB contain specific substances and enzymes that chemically transform and modify floral nectars from rich vegetation in native environments; the bees deposit, dehydrate, and store the nectar in pots. SB products are stored inside the nests to be used as sources of sugar and protein [1,4].

The honey and pollen from these bees contain bioactive substances that include phenolic compounds, amino acids, vitamin C, carotenoids and other minor compounds related to their antioxidant and antimicrobial potential. They are entirely different from the honey and pollen produced by bees of the genus *Apis mellífera* in terms of consistency, aroma, color and flavor [1,5].

The main microorganisms living in SB colonies are yeasts, molds, and bacteria [6–9]. It has been demonstrated that the microflora of bees can be used as a biocontrol agent, a potential probiotic, and as a producer of antimicrobial compounds and enzymes [7,8]. Microorganisms from SB can be beneficial because specific products may be produced by them, such as mead, honey-pollen jelly, or a creamy pollen milkshake [10]. Studies indicate that the honey produced by *Tetragonisca angustula* SB has antimicrobial activity against pathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli* [11], *Bacillus cereus, Pseudomonas aeruginosa*, and against yeasts such as *Candida albicans* [12]. in addition, this honey has shown excellent antioxidant capacity [13].

However, information regarding the association of some microorganisms (*Bacillus* spp., *Clostridium* spp., *Enterococcus* spp., *Fructobacillus* spp., *Lactobacillus* spp. and *Staphylococcus* spp.) with SB honey and pollen is only available for a few bee genera, namely *Heterotrigona itama; H. erythrogastra; Lepidotrigona terminata; Tetrigona apicalis; T. melanoleuca; T. bingami; Geniotrigona thoracica; Homotrigona fimbriata;* and *Melipona fasciata* [2,7,8,14–17]. Therefore, this study aimed to explore and characterize the diversity of microorganisms isolated from the sources of *Tetragonisca angustula* bees.

MATERIAL AND METHODS

Samples

The honey and pollen bees were collected from three jataí (*Tetragoniscaangustula*, Latreille, 1811) beehives at the Curitiba Meliponary, Paraná State, Brazil (25°02′40″ S; 49°16′23″ W), during April 2018 (Figure 1). The honey was collected from sealed storage pots in the nest by suction using disposable syringes, and the pollen was collected with sterile spatulas. This material was transferred to sterile flasks and then packed in a refrigerated box for transport to the laboratory.

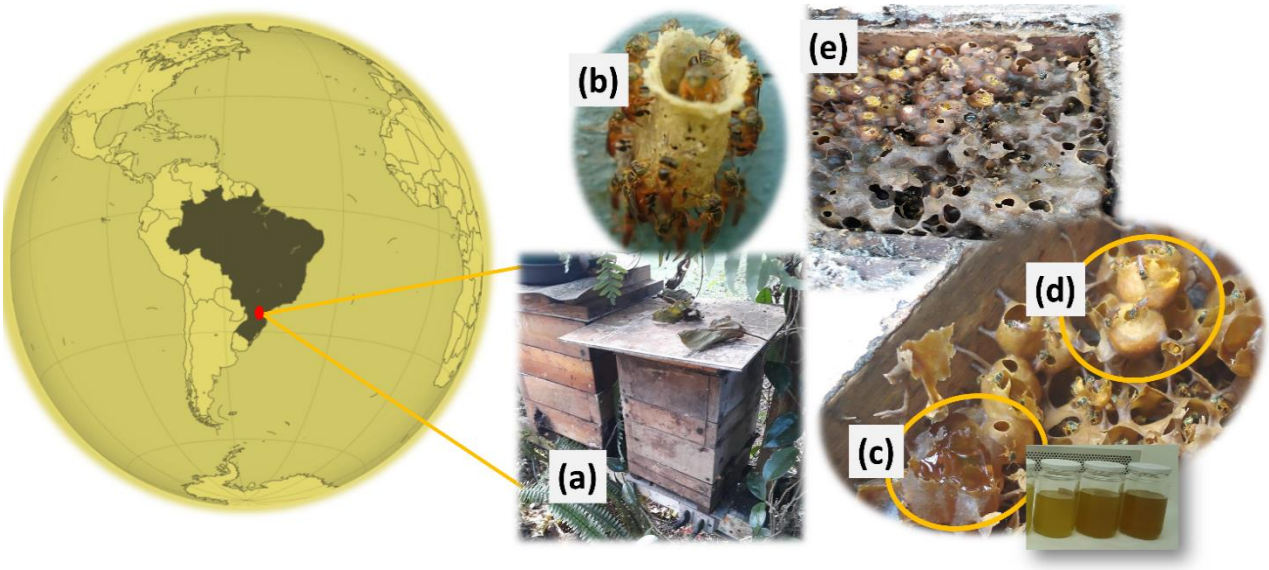

Figure 1. (a) Location of Curitiba Meliponary (25°02′40″ S; 49°16′23″ W) of (b) jataí (*Tetragonisca angustula)* where the (c) honey and (d) pollen samples were collected from (e) stingless bee beehives.

Microbial counts and isolation

A quantity of 1 g of each stingless bee honey sample (TaM1, TaM2, and TaM3) and pollen (TaP1, TaP2, and TaP3) was weighed aseptically and homogenized with 9 mL of sterile NaCl (145 mM) and then vortexed for 30 s. Aliquots of 100 µL were transferred and spread over standard plate count agar (PCA), Man, Rogosa and Sharpe agar (MRS), Sabouraud dextrose agar (SDA), and M-Green agar (MGA). PCA isolations were incubated at 36 °C for 48 h and 20% pO_2. The MRS isolations were set at 36 °C for 48 h, 10% pO_2 and 10% pCO_2. The isolations in SDA and MGA were incubated at 25 °C for five days and 20% pO_2. The counts obtained in PCA (total count of mesophilic bacteria), MRS (count of lactic bacteria), SDA, and MGA (yeast count) were expressed in log CFU/g.

Bacteria identification

Priming was performed using Gram's differential stain, with subsequent biochemical, physiological and antimicrobial resistance tests.

The gram-positive cocci were subjected to the following hemolysis assays; production of catalase, coagulase, and DNAse; glucose oxidation/fermentation; resistance to bacitracin and novobiocin; hydrolysis of arginine and urea; butylene-glycolic fermentation; acetoin production; nitrate reduction; and fermentation of salicin, sucrose, trehalose, lactose, L-arabinose, D-mannitol, xylose, D-ribose and D-mannose.

The sporulated gram-positive bacilli were subjected to the following hemolysis assays; production of catalase, amylase, gelatinase, lecithinase and caseinase; tolerance to NaCl (0, 2, 5, 7, and 10%); butylene-glycolic fermentation; nitrate reduction; fermentation of D-glucose, D-mannose, D-mannitol, L-arabinose, D-xylose, salicin and m-inositol; growth at pH 6 and 5 °C; and the presence of parasporal crystal.

The gram-negative bacilli were subjected to the following oxidase assays; production of gas in glucose, H_2S, indole, DNAse, urease, phenylalanine deaminase, ß-D-galactosidase, lysine decarboxylase and ornithine decarboxylase; motility; esculin hydrolysis; use of citrate; acetoin production; butylene-glycolic

fermentation and mixed acid fermentation; fermentation of arabinose, sucrose, mannose, melibiose, sorbitol, mannitol, adonitol, galactose, inositol and lactose; and hydrolysis of arginine.

The lactic acid bacteria were subjected to the following hemolysis tests; production of catalase and gas; hydrolysis of arginine; tolerance to 10% ethanol; growth at 37 °C; and fermentation of D-xylose, L-arabinose, maltose, raffinose, galactose, salicin, D-mannose, D-mannitol, ribose and cellobiose.

Yeast identification

The yeasts were identified by growth at 37 °C in the presence of 1% acetic acid, 50% glucose; production of urease and lysine decarboxylase; assimilation of glucose, xylose, rhamnose, melibiose, sucrose, starch, maltose, salicin, mannitol, cellobiose, raffinose, ribose, glycerol, trehalose, inositol, galactose, lactose and arabinose; and fermentation (glucose, sucrose, maltose, raffinose, trehalose, cellobiose, starch, galactose, xylose, lactose and melibiose).

RESULTS

The microbial examination of the stingless bee (SB) honey and pollen showed eight bacteria; one was gram-negative and seven were gram-positive. Only two of them were spore-forming, and one was lactic bacteria. Three yeast species were also isolated. The microbial counts for the samples of honey and pollen are shown in Table 1. The microbiological analyses demonstrated that the average total aerobic mesophiles ranged between 3.78 and 3.71 log CFU/g; the lactic bacteria were 3.59 and 3.56 log CFU/g, and the yeast was 4.41 and 4.66 log CFU/g for the honey and pollen, respectively.

A significant diversity in the microorganisms was observed; the bacteria that were found were *Staphylococcus saprophyticus* subsp. *bovis*; *S. vitulinus; S. kloosii; S. pasteuri; Bacillus pumilus; B. thuringiensis; Pantoea agglomerans;* and *Leuconostoc mesenteroides* subsp. *mesenteroides.* The yeasts that were identified were *Starmerella meliponinorum; Candida magnolia;* and *Zygosaccharomyces bailli* (Table 1).

Table 1. Microflora isolated from pots of stingless bee honey (TaM) and from pollen (TaP)

Groups		Identified species	Samples					
			TaM1	TaM2	TaM3	TaP1	TaP2	TaP3
Bacteria	Cocci Gram +	*Staphylococcus saprophyticus* subsp. *bovis*	+	-	+	-	-	-
		S.vitulinus	-	+	-	-	-	-
		S. kloosii	-	-	+	-	-	-
		S.pasteuri	-		+	-	-	-
	Sporulated Bacillus Gram +	*Bacillus pumilus*	+	+	+	-	+	-
		B. thuringiensis	-	-	-	+	-	+
	Bacillus Gram -	*Pantoea agglomerans*	-	-	-	-	-	+
	Latic	*Leuconostoc mesenteroides* subsp. *mesenteroide*s	-	-	+	-	-	-
	Yeast	*Starmerella meliponinorum*	+	-	+	-	-	+
		Candida magnoliae	-	-	-	+	-	-
		Zygosaccharomyces bailli	-	-	-	-	+	+

The analyzed honey samples revealed the presence of *S. saprophyticus* subsp*. bovis; S.vitulinus; S. kloosii;* and *S. pasteuri*; however, *Bacillus* sp. was the most common bacteria detected in the SB honey and pollen. *Bacillus pumilus; B. thuringiensis;* and. *Bacillus pumilus* were present in all the honey samples. *B. thuringiensis* was present in two pollen samples*,* and *Pantoea agglomerans* was also isolated in our study*.*

S. meliponinorum; C. magnoliae; and *Z. bailli* were identified, confirming the presence of yeasts in these micro-communities. *S. meliponinorum* was found in two jataí honey samples and one pollen sample. *Z. bailli* was found in two pollen samples.

DISCUSSION

Biodiverse microorganisms can originate from the digestive tracts of bees; from the nectar and floral pollen, from sources external to the nest, and from secondary contamination from humans, equipment, containers, wind, and dust [8]. Studies have shown that the intestines of bees contain 1% yeast, 27% gram-positive bacteria (*Bacillus; Bacteridium; Streptococcus;* and *Clostridium* spp.), and 70% gram-negative

bacteria (*Achromobacter; Citrobacter; Enterobacter; Erwinia; Escherichia coli; Flavobacterium; Klebsiella; Proteus;* and *Pseudomonas*) [18]. The SB honey samples showed a higher incidence of bacteria and yeast than the pollen samples. The predominance of this microbial group could have been correlated to higher acidity, moisture content, and water activity, which are characteristics of SB honey that encourage the growth of bacteria [3,17,18].

Furthermore, these bacteria and yeasts prevent the growth of other microorganisms which are responsible for the deterioration of food storage pots through antimicrobial systems. High osmotic pressure, low water activity, low pH, acid medium, low protein content, low redox potential due to the high content of reducing sugars, and viscosity limiting the solubility of oxygen, may also inhibit the growth of microorganisms [19].

The genus *Staphylococcus* currently includes approximately 53 species and 28 subspecies, which are described according to their potential to produce coagulase. The *staphylococci* most frequently associated with human infection are *S. aureus; S. epidermidis;* S. *haemolyticus* and *S. saprophyticus* [20,21]. Four species of *Staphylococcus* were found in the samples (*S. saprophyticus* subsp*. bovis; S.vitulinus; S. kloosii;* and *S. pasteuri)*. Pucciarelli and coauthors [2] also verified the presence of *Staphylococcus* spp. in *T. angustula* honey. The aforementioned authors emphasized that this genus is rarely found in honey, whose antibacterial properties have been associated with the non-survival of this microorganism.

Bacillus sp. was the most common bacteria detected in both the SB honey and pollen, suggesting the existence of a symbiotic relationship between this bacterial genus and bees [2]. Bacillus bacteria are not inactivated by the inhibitory properties of honey, and grow well at pH six or higher [22].

Rosli and coauthors [17] studied the bacterial community of honey from eight different SB species (*Heterotrigona itama; Heterotrigona erythrogastra; Tetrigona apicalis; Lepidotrigona terminata; Tetrigona melanoleuca; Tetrigona bingami; Geniotrigona thoracica;* and *Homotrigona fimbriata*).They identified eight bacterial phyla, 71 families, 155 genera, and 70 species using 16S rRNA amplicon sequencing technology. Corroborating with the aforementioned study (and other researchers), the genus *Bacillus* is one of the bacterial genera most associated with SB colonies, followed by *Streptomyces* and *Lactobacillus* [2,7,8,15,16,23].

The genus *Bacillus* comprises rod-shaped gram-positive bacteria with the ability to form spores. There are 60 species of substantial genetic diversity, most of which are non-pathogenic. They can produce bacteriocins [24]. The roles of non-pathogenic microorganisms in honey bee colonies may include the biochemical contributions of intestinal microorganisms to honey bees, the conversion and preservation of pollen stored in comb cells, and resistance to disease [25].

Similarly, researchers isolated *Bacillus* bacteria in bee honey and pollen derived from different geographical and botanical origins. They found *B. pumilus; B. circulans; B. alvei; B. licheniformis; B. amyloliquefaciens; B. subtilis; B. cereus; B. thuringiensis; B. licheniformis; B. megaterium;* and *B. pumilus*. The authors emphasized that this bacterium could be considered a source of amylase, aiding in the degradation of sugars, and consequently contributing to the transformation of nectar into honey. *Bacillus* may produce digestive enzymes that act in the pre-digestion of stored food and the production of substances antagonistic to competing microorganisms, mainly organic acids and antibiotics. Acetic and lactic fermentation, which occurs in pollen and honey, are also performed by these bacteria [10,16,17,26].

We identified the genus *Pantoea* in one of the pollen samples. This strain has been previously found in the intestines of honey bees, in the hive environment, or on plant roots, leaves, and flowers [27,28]. According to Loncaric and coauthors [28] and Costa and coauthors [29], *P. agglomerans* has been studied for its commercial potential as a possible biological control agent in relation to the major post-harvest pathogens on pome and citrus fruits (against *E. amylovora*).

One of the honey samples evaluated in our study presented *Leuconostoc* spp., which are considered to be beneficial bacteria. *Leuconostoc* spp. are mesophilic, gram-positive and obligately heterofermentative cocci bacteria, which can transform glucose molecules into carbon dioxide, ethanol, and lactate. These bacteria play an essential role in food fermentation and preservation. They are used to improve the nutritional and organoleptic quality of food. Some leuconostoc bacteria produce exopolysaccharides, oligosaccharides, folate, riboflavin, vitamin K and bacteriocins [30,31].

Starmerella, Candida and *Zygosaccharomyces* frequently occur in SB pollen and honey; they provide sensory and conservation characteristics [10,24,32,33]. These species may have potential uses in biotechnology such as in the production of biosurfactants and other microbial polymers [34]. According to Camargo and coauthors [35], *Candida* yeast seem to dehydrate the pollen stored by *Ptilitrigone lurida* bees. This dehydration process is efficient in limiting deterioration and preventing phorids (Phoridae, Pseudohypocera) from consuming pollen, which can cause serious damage to the colonies of this bee species.

Floral visits by SB directly influence the frequency and abundance of yeasts in the nectar. The more attractive the characteristics of the composition of the nectar, the greater the probability of visitation and yeast cells being inoculated after opening the corolla of the flowers [36–38].

As was the case in our study, *S. meliponinorum* was previously identified in samples of honey, pollen and propolis of *T. angustula* bees [30,32,33,39], and *Zygosaccharomyces* sp. was found in association with adult bees of *M. quadrifasciata*. *S. meliponinorum* can ferment pollen after storage [10]. Species of *Zygosaccharomyces* are recognized as being osmophilic. Studies have shown that this yeast has high productivity of glycerol [40].

The microorganisms found in SB products (honey and pollen) are fundamental for the life of stingless bees, helping to keep colonies healthier and more productive. They can be explored as new sources of substances for humans (as a biocontrol agent, preservative, antibiotic, probiotic, and as a producer of antimicrobial compounds and enzymes).

CONCLUSION

The honey and pollen collected from three beehives of *T. angustula* native stingless bees were dominated by gram-positive bacteria, mainly *Bacillus pumilus and* B*. thuringiensis* species. From the three yeast species that were isolated, *Zygosaccharomyces bailli* was the dominant yeast in jataí pollen, and *S. meliponinorum* was the dominant yeast in jataí honey. It is hoped that this characterization study of bacterial and yeast diversity from the sources of *T. angustula* bees will indicate a pathway towards extending the biotechnological potential of future applications of these products (stingless bee honey and pollen) in the food, cosmetic and pharmaceutical industries.

Funding: This research received no external funding.
Acknowledgments: The authors would like to thank Prof. Harold Brand (meliponicultivator) for kindly supplying the honey and pollen samples. We also gratefully acknowledge the LABORCLIN-Group SOLABIA, for the supply of culture media. We are grateful for the infrastructure and technical resources provided by the Federal University of Paraná (UFPR) and the Pontifical Catholic University of Paraná (PUC/PR), Brazil. This study was supported by Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) by financial support and a postdoctoral fellowship awarded to S. Ávila (grant number 88887371865/2019-00).
Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study, in the collection, analysis and interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

REFERENCES

1. Oliveira FA, de Abreu AT, de Oliveira Nascimento N, Froes-Silva RES, Antonini Y, Nalini HA, et al. Evaluation of matrix effect on the determination of rare earth elements and As, Bi, Cd, Pb, Se and In in honey and pollen of native Brazilian bees (Tetragonisca angustula - Jataí) by Q-ICP-MS. Talanta. Elsevier; 2017; 162:488–94.
2. Pucciarelli AB, Schapovaloff ME, Kummritz S, Señuk IA, Brumovsky LA, Dallagnol AM. Microbiological and physicochemical analysis of yateí (Tetragonisca angustula) honey for assessing quality standards and commercialization. Rev Argent Microbiol. Elsevier; 2014; 46:325–32.
3. Schvezov N, Pucciarelli AB, Valdes B, Dallagnol AM. Characterization of yateí (Tetragonisca fiebrigi) honey and preservation treatments: Dehumidification, pasteurization and refrigeration. Food Control. Elsevier; 2020; 111:107080.
4. Patricia V, Oliverio V, Triny L, Favián M. Meliponini biodiversity and medicinal uses of pot-honey from El Oro province in Ecuador. Emirates J Food Agric. 2015; 27:502–6.
5. Pucholobek G, de Andrade CK, Rigobello ES, Wielewski P, de Toledo V de AA, Quináia SP. Determination of the Ca, Mn, Mg and Fe in honey from multiple species of stingless bee produced in Brazil. Food Chem. 2022;367.
6. Liberato M da CTC, Morais SM de, Magalhães CE de C, Magalhães IL, Cavalcanti DB, Silva MM de O. Physicochemical properties and mineral and protein content of honey samples from Ceará state, Northeastern Brazil. Food Sci Technol. 2013; 33:38–46.
7. Ngalimat MS, Rahman RNZRA, Yusof MT, Syahir A, Sabri S. Characterisation of bacteria isolated from the stingless bee, Heterotrigona itama, honey, bee bread and propolis. Peer J. 2019; 2019:1–20.
8. Ngalimat MS, Abd Rahman RNZR, Yusof MT, Amir Hamzah AS, Zawawi N, Sabri S. A review on the association of bacteria with stingless bees. Sains Malaysiana. 2020; 49:1853–63.
9. De Paula GT, Menezes C, Pupo MT, Rosa CA. Stingless bees and microbial interactions. Curr Opin Insect Sci. 2021; 44:41–7.

10. Menezes C, Vollet-Neto A, Contrera FAFL, Venturieri GC, Imperatriz-Fonseca VL. The Role of Useful Microorganisms to Stingless Bees and Stingless Beekeeping. Pot-Honey A Leg Stingless Bees. 2013. p. 153–71.
11. Santos AC dos, Biluca FC, Braghini F, Gonzaga LV, Costa ACO, Fett R. Phenolic composition and biological activities of stingless bee honey: An overview based on its aglycone and glycoside compounds. Food Res. Int. 2021.
12. Demera JH, Angert ER. Comparison of the antimicrobial activity of honey produced by Tetragonisca angustula (Meliponinae) and Apis mellifera from different phytogeographic regions of Costa Rica. Apidologie. 2004; 35:411–7.
13. Rao PV, Krishnan KT, Salleh N, Gan SH. Biological and therapeutic effects of honey produced by honey bees and stingless bees: a comparative review. Rev Bras Farmacogn. Sociedade Brasileira de Farmacognosia; 2016; 26:1–8.
14. Gilliam M. Identification and roles of non-pathogenic microflora associated with honey bees. FEMS Microbiol Lett 155. 1997; 155:1–10.
15. Syed Yaacob SN, Huyop F, Kamarulzaman Raja Ibrahim R, Wahab RA. Identification of Lactobacillus spp. and Fructobacillus spp. isolated from fresh Heterotrigona itama honey and their antagonistic activities against clinical pathogenic bacteria. J Apic Res. Taylor & Francis; 2018; 57:395–405.
16. Aina F, Amin Z, Sabri S, Ismail M, Chan KW, Ismail N, et al. Probiotic Properties of Bacillus Strains Isolated from Stingless Bee (Heterotrigona itama) Honey Collected across Malaysia. Int J Environ Res Public Health. 2020; 17:1–15.
17. Rosli FN, Hazemi MHF, Akbar MA, Basir S, Kassim H, Bunawan H. Stingless Bee Honey: Evaluating Its Antibacterial. Divers Insects. 2020; 11:500.
18. Obakpororo EA, Swaroopa DR, Prakash MH. Antibacterial potential components of Bacillus species and antibiotics residues in branded and unbranded honey samples from Nigeria. African J Biotechnol. 2017; 16:58–64.
19. Gois GC, De Lima CAB, Da Silva LT, Evangelista-Rodrigues A. Composição do mel de Apis mellifera: requisitos de qualidade. Acta Vet Bras. 2013; 7:137–47.
20. Otto M. Staphylococci in the human microbiome: the role of host and interbacterial interactions. Curr Opin Microbiol. Elsevier Ltd; 2020; 53:71–7.
21. Hennekinne JA, De Buyser ML, Dragacci S. Staphylococcus aureus and its food poisoning toxins: Characterization and outbreak investigation. FEMS Microbiol Rev. 2012; 36:815–36.
22. Khan KA, Ansari MJ, Al-Ghamdi A, Nuru A, Harakeh S, Iqbal J. Investigation of gut microbial communities associated with indigenous honey bee (Apis mellifera jemenitica) from two different eco-regions of Saudi Arabia. Saudi J Biol Sci. King Saud University; 2017; 24:1061–8.
23. Promnuan Y, Kudo T, Chantawannakul P. Actinomycetes isolated from beehives in Thailand. World J Microbiol Biotechnol. 2009; 25:1685–9.
24. Silva MS, Rabadzhiev Y, Eller MR, Iliev I, Ivanova I, Santana WC. Microorganisms in Honey. Honey Anal. InTech; 2017.
25. Voulgari-Kokota A, McFrederick QS, Steffan-Dewenter I, Keller A. Drivers, Diversity, and Functions of the Solitary-Bee Microbiota. Trends Microbiol. Elsevier Inc.; 2019; 27:1034–44.
26. Sinacori M, Francesca N, Alfonzo A, Cruciata M, Sannino C, Settanni L, et al. Cultivable microorganisms associated with honeys of different geographical and botanical origin. Food Microbiol. Elsevier Ltd; 2014;38:284–94.
27. Leonhardt SD, Kaltenpoth M. Microbial communities of three sympatric Australian stingless bee species. PLoS One. 2014; 9:1–6.
28. Loncaric I, Heigl H, Licek E, Moosbeckhofer R, Busse HJ, Rosengarten R. Typing of Pantoea agglomerans isolated from colonies of honey bees (Apis mellifera) and culturability of selected strains from honey. Apidologie. 2009; 40:40–54.
29. Costa E, Usall J, Teixidó N, Delgado J, Viñas I. Water activity, temperature, and pH effects on growth of the biocontrol agent Pantoea agglomerans CPA-2. Can J Microbiol. 2002; 48:1082–8.
30. Daniel HM, Rosa CA, Thiago-Calaça PSS, Antonini Y, Bastos EMAF, Evrard P, et al. Starmerella neotropicalis f. a., sp. nov., a yeast species found in bees and pollen. Int J Syst Evol Microbiol. 2013; 63:3896–903.
31. Wang Y, Wu J, Lv M, Shao Z, Hungwe M, Wang J, et al. Metabolism Characteristics of Lactic Acid Bacteria and the Expanding Applications in Food Industry. Front. Bioeng. Biotechnol. 2021.

32. Teixeira ACP, Marini MM, Nicoli JR, Antonini Y, Martins RP, Lachance MA, et al. Starmerella meliponinorum sp. nov., a novel ascomycetous yeast species associated with stingless bees. Int J Syst Evol Microbiol. 2003; 53:339–43.
33. Rosa CA, Lachance MA, Silva JOC, Teixeira ACP, Marini MM, Antonini Y, et al. Yeast communities associated with stingless bees. FEMS Yeast Res. 2003; 4:271–5.
34. Accorsini FR, Mutton MJR, Lemos EGM, Benincasa M. Biosurfactants production by yeasts using soybean oil and glycerol as low cost substrate. Brazilian J Microbiol. 2012; 43:116–25.
35. Camargo JMF, Garcia MVB, Júnior ERQ, Castrillon A. Notas prévias sobre a bionomia de Ptilotrigona lurida (Hymenoptera, Apidae, Meliponinae): associação de leveduras em pólen estocado. Bol do Mus Para Emílio Goeldi Série Zool. 1992; 8:391–5.
36. Bisui S, Layek U, Karmakar P. Determination of Nectar Resources through Body Surface Pollen Analysis: A Study with the Stingless Bee Tetragonula iridipennis Smith (Apidae: Meliponini) in West Bengal, India. Sociobiology. 2021.
37. Heil M. Nectar: Generation, regulation and ecological functions. Trends Plant Sci. 2011; 16:191–200.
38. Herrera CM, De Vega C, Canto A, Pozo MI. Yeasts in floral nectar: A quantitative survey. Ann Bot. 2009; 103:1415–23.
39. Stefanini I. Yeast-insect associations: It takes guts. Yeast. 2018; 35:315–30.
40. Dušková M, Ferreira C, Lucas C, Sychrová H. Two glycerol uptake systems contribute to the high osmotolerance of Zygosaccharomyces rouxii. Mol. Microbiol. 2015. p. 541–59.