MINISTERE DE L'ENSEIGNEMENT SUPERIEUR ET DE LA RECHERCHE SCIENTIFIQUE
UNIVERSITE AKLI MOHAND OULHADJ – BOUIRA
FACULTE DES SCIENCES DE LA NATURE ET DE LA VIE ET DES SCIENCES DE LA TERRE
DEPARTEMENT DE BIOLOGIE

Réf : ……./UAMOB/F.SNV.ST/DEP.BIO/2022

MEMOIRE DE FIN D'ETUDES

EN VUE DE L'OBTENTION DU DIPLOME DE MASTER

Domaine : SNV **Filière** : Sciences Biologiques
Spécialité : Microbiologie Appliquée

Présenté par :
BENLAKEHAL Manel

Thème

Isolement et purification phénotypique et identification génotypique des bactéries du microbiote intestinal de *l'Apis mellifera*.

Soutenu le 04/07/2023 (14 :30) **Devant le jury composé de :**

Nom et Prénom	*Grade*		
Mme MOUHOUNB C	*Professeur*	*Univ. de Bouira*	*Président*
Mr BOURNINE L	*MCA*	*Univ. de Bouira*	*Examinateur*
Mme DJENADI K	MCB	*Univ. de Bouira*	*Promoteur*
Mme AL AMIR LAIDOUCI H	*Maitre de Recherche*	*Institut Pasteur d'Alger*	*Co Promoteur*
Mme CHERIFI A	*MCB*	*Univ. de Bouira*	*Invité d'honneur*
Mr BERRAH A	*Docteur Vétérinaire*	*Institut Pasteur d'Alger*	*Invité d'honneur*
Mr BENDEDDOUCHE B	*Professeur*	*Institut Pasteur d'Alger*	*Invité d'honneur*

Année Universitaire : 2022/ 2023

MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH
AKLI MOHAND OULHADJ – BOUIRA UNIVERSITY
FACULTY SCIENCES OF NATURE AND LIFE AND EARTH SCIENCES
DEPARTMENT OF BIOLOGY

REF : ……./UAMOB/F.SNV.ST/DEP.BIO/2022

MASTER'S project

SUBMITTED FOR THE DEGREE OF MASTER "SECOND CYCLE LMD"

Field: Nature and Life Sciences Branch: Biological Sciences
Specialty: Applied Microbiology

By
BENLAKEHAL Manel

Research topic

Isolation, purification phenotypic and genotypic identification of bacterial gut microbiota from *Apis mellifera*

Supported: July 04th, 2023 (02:30pm) **Ahead of the Jury:**

Last and first name	*Grade*		
Ms MOUHOUNB C	*Professor*	*Bouira university*	*Chair*
Mr BOURNINE L	*MCA*	*Bouira university*	*Examiner*
Ms DJENADI K	*MCB*	*Bouira university*	*Supervisor*
Ms AL AMIR LAIDOUCI H	*Associate Professor*	*Pasteur Institute, Algeria*	Co-supervisor
Ms CHERIFI A	*MCB*	*Bouira university*	Guest of honour
Mr BERRAH A	*Doctor of Veterinary Medicine*	*Pasteur Institute, Algeria*	Guest of honour
Mr BENDEDDOUCHE B	*Professor*	*Pasteur Institute, Algeria*	Guest of honour

Scholar year: 2022/ 2023


بسم الله الرحمن الرحيم

Acknowledgements

First, I would like to thank God, the Almighty and Merciful, who has given me the courage, intelligence, and patience to humbly complete this work.

I would like to express my deepest thanks to the members of the jury: Professor MOUHOUB and Dr. BOURNINE. I consider myself extremely fortunate to have had the opportunity to benefit from your expertise and participation as jury members.

I would like to express my sincere gratitude to Dr. DJENADI Katia, my supervisor, who has been consistently by my side, showing attentive listening and exemplary availability throughout the realization of my project. I am grateful to her for her inspiration, motivation, and the precious time she has dedicated to guiding me. I also warmly thank Ms. AlAmir Hanane, my co-supervisor, for her valuable guidance, which has been of immeasurable importance.

I am infinitely and eternally grateful, deep within my being, to Dr. BERRAH Anis, to whom I owe immense gratitude. He has been there for me wholeheartedly, providing invaluable assistance in achieving my goal. It is thanks to him that I was able to take my first steps in the field of molecular biology. His generosity and unwavering support have been of paramount importance in my journey.

Not forgetting Professor BENDEDDOUCHE Badis, head of the Laboratory of Food, Water, and Environmental Bacteriology at the Pasteur Institute of Algiers, his passion for research and unwavering support have been an invaluable source of motivation throughout this project. His constant encouragement has driven me forward with determination. I am deeply grateful to him, and my gratitude towards him is and will remain eternal. His invaluable support has been an essential pillar of my success, and I am honored to have benefited from his precious expertise and unwavering generosity.

My deep gratitude goes to the entire team of the Laboratory of Food, Water, and Environmental Bacteriology, who warmly welcomed and supported me throughout these precious months of internship. In particular, I would like to express my sincere thanks to Ms. Nadjet, Amine, Ihssan, Fatima Zahra, Nora, Hamida, Mr. Djamel, Soumia, and many other members of this exceptional team. Their kind welcome, unwavering support, and family spirit will forever be engraved in my heart. I am infinitely grateful to have had the chance to meet them.

A huge THANK YOU to our dear Department Head, Dr. MAHDJOUB, for his unwavering support throughout my journey. A big thank you to Ms. MEDBOUA, Ms. CHERIFI, Ms. HAMID, Mr. ARAB, Mr. RAI and all those who have carefully overseen my education throughout my academic journey.

Dedicates

To my dear parents,

No dedication can truly express the extent of my respect, eternal love, and deep appreciation for the sacrifices you have made for my education and well-being.

I am grateful for all the support and love you have showered upon me since my childhood, and I hope that your blessings will always accompany me.

Thank you both a thousand times for everything. May the Almighty God protect you and grant you good health, happiness, and a long life.

To my dearest and one-of-a-kind brother, the second man in my life,

As a token of my sisterly affection, deep tenderness, and gratitude, I wish you a life filled with happiness and success. May God, the Almighty, protect you and keep you for us

To my beloved aunts, uncles, and extended family members of the BENLAKEHAL and SEDKAOUI families,

To my dearest and one-of-a-kind friend Sarah, may God keep you for me

I would like to express my appreciation and gratitude to all those who are dear to me and whom I unintentionally omitted to mention. To those who dedicate their lives to making this world a better place

Table contents

Acknowledgments
Dedicates
Abbreviations list
Table list
Figure list

Introduction.....1

Chapter I: Characterization of *Apis mellifera*.....4

I.1. *Apis mellifera* 5

I.2. Honeybee society 6

I.2.1. Bee queen 6

I.2.2. Drones 6

I.2.3. Bee workers 6

I.3. *Apis mellifera*, an important insect 8

I.3.1. Pollination 8

I.3.2. Honeybee's products 9

I.4. The differentiation of bees into queens and workers 10

Chapter II: Honeybees microbiota 13

II.1. Introduction to the gut microbiota in honeybees 13

II.1.1. Overview of the honeybee gut microbiota.....13

II.1.2. Importance of the gut microbiota for honeybee health 13

II.2. Honey bees microbiome diversity..... 13

II.2.1. Establishment of the gut microbiota in honeybees 13

II.2.2. The gut microbiome diversity within honeybee casts 14

II.3. Diversity of honey bee workers gut microbiota 15

II.4. Diversity and dynamics of gut microbiota in adult worker bees 17

II.5. The role of the microbiome in all individuals 18

II.5.1. Nutritional and physiological metabolic roles 19

II.5.2. The role of gut microbiome in bee health 19

II.6. Factors that disrupt the honeybee gut microbiota 20

II.6.1. Impact of biotic factors on honeybee gut microbiota 20

II.6.2 Impact of abiotic factors on honeybee gut microbiota.....21

II.7. Methods to study the diversity of the microbiome.....21
II.7.1. The monoculture and Metagenomic……21

Chapter III: Material and method……23
III.1. *Apis mellifera* gut sampling.....24
III.1.1. *Apis mellifera* samples …….....24
III.2. Extraction of the gut from the bee…….....31
III.3. Bacterial diversity of Apis mellifera gut microbiota ……31
III.3.1. Bacterial diversity counting ….....32
III.3.1.1. First trial …….....32
III.3.1.2. Second and third trials…….....32
III.3.2. Bacterial isolation and purification…….....33
III.3.3. Bacterial identification….....35
III.3.3.1. 16S DNA PCR amplification…….....35
III.3.3.1.1. Suspensions and reagent preparation….....37
III.3.3.2. Electrophoresis and visualization of UV PCR bands…….....37
III.3.2.3. The sequence treatment and analysis……..38

Chapter IV: Results and discussion……..... ...39
IV.1. Phenotypic evaluation and characterization of Bacterial diversity….....40
IV.1.1. Enumeration …….....40
IV.1.2. Isolation and purificatio.....41
IV.2. Phenotypic analysis…….....41
IV.2.1. Gram Staining Results…….....46
IV.2.2. Biochemical Evaluation …….....46
IV.3. Genotypic characterization…….....51

Chapter V: Conclusion ……56

Bibliographic references

Supplementary data

Review paper

Abstract

Abbreviations list

- **BHIB :** Brain Heart Infusion Broth
- **BET :** Ethidium Bromide
- **CFU :** Colony-Forming Unit
- **DNA** : Deoxyribonucleic Acid
- **GYC :** Glucose, Yeast extract, Calcium carbonate Agar
- **MEGA 6** : Molecular Evolutionary Genetics Analysis
- **MRS** : De Man, Rogosa, and Sharpe Agar
- **NCBI :** National Center for Biotechnology Information
- **PCA :** Plate Count Agar
- **PCR :** Polymerase Chain Reaction
- **RNA :** Ribonucleic Acid
- **SFB :** Selenite-Cystine Broth
- **TAE :** Tris-Acetate-EDTA
- **TBX :** Tryptone Bile X-Glucuronide Agar
- **TSA :** Tryptic Soy Agar
- **TSE :** Tryptone Sel Eau
- **VF :** Viande Foie
- **VRBG :** Violet Red Bile Glucose Agar

Table list

Table I : Different characteristics of our samples…….30

Table II : Volumes of reaction mixture preparation……..…….....36

Table III : Cycling instruction…….……. 37

Table IV : Enumeration of bacteria on PCA medium for samples…….....40

Table V: The development of Bouira isolates on different media……..42

Table VII: The development of Ouled Djellal isolates on different…….43

Table VI: The development of Mostaganem isolates on different media…….44

Table VIII : Phenotypic characteristics of bacteria fond in samples from Bouira region……..…….48

Table IX : Phenotypic characteristics of bacteria found in samples from Mostaganem region…….……..49

Table X : Phenotypic characteristics of bacteria found in samples from Ouled Djellal region……..50

Figure list

Figure 1 : The inhabitants of the hive…….…….. 7

Figure 2 : Differenciation of bees into queens and workers and their principal roles……..…….……...12

Figure 3 : Variability of major gut microbiota groups in different castes……..16

Figure 4 : Composition and special composition of bacterial communities within the Honeybee gut…….……..18

Figure 5 : The different sampling sites…….…… 26

Figure 6 : Area sampling 1 – Alessa -Ouled Rachad – Bouira.…….…. 27

Figure 7: Area sampling 2 – Ouled El Kheir – Mostaganem……...…… 28

Figure 8 : Area sampling 3 – Doucen – Ouled Djellal…….…….29

Figure 9 : Visualization of insert sequences on Electrophoresis gel : Analysis of band patterns…….48

Figure 10 : Phylogenetic tree representing the dominant family of bacterial isolates in our samples……..52

Introduction

The microscopic realm that exists within the digestive systems of living organisms conceals captivating enigmas that profoundly affect their well-being and vitality. Among these microorganisms, the intestinal microbiota holds a pivotal role in maintaining optimal health, not only for humans but also for animals. Its crucial significance is now acknowledged in terms of sustaining physiological equilibrium, warding off diseases, and even influencing behavior [1].

In this intricate world of minuscule diversity, honey bees (*Apis mellifera*) emerge as invaluable animal models, allowing us to unravel the complexities of the gut microbiota and its impact on individual health. A remarkable aspect of honey bees is their social structure, comprising three distinct castes: the queen, the workers, and the drones. Despite their distinctive phenotypes, these individuals share the same genetic makeup. However, it is through the mechanism of epigenetics that variations arise, not only in terms of morphology but also in the composition of the intestinal microbiota [1].

Around the world, numerous investigations have highlighted the diversity of the gut microbiome [2, 3, 4, 5]. Nevertheless, research on honey bee gut microbiota in Algeria remains limited. This country, known for its expansive landmass and diverse climate that encompasses a variety of landscapes and ecological conditions, provides a unique opportunity to investigate the interplay between honey bee gut microbiota and their environment. Among the studies carried out in Algeria on the gut microbiota of honey bees, a notable piece of research by Meriem et al. is the characterization of lactic acid bacteria strains isolated from Algerian honeybees and honey, exploring their potential probiotic and functional features for human use [6]. However, no study has yet addressed the identification of the gut microbiota of honey bees from different sites in Algeria, representing different ecosystems. This approach would enable us not only to study the variability of gut diversity but also to confirm the variability of ecosystems and their influence on this diversity. It would also enable us to estimate the sanitary quality of our bee samples.

Consequently, our study aims to delve into the complex world of the gut microbiota of honey bees in Algeria, focusing specifically on the worker species Apis mellifera. We aim to lift the veil on the mysteries surrounding its composition, paving the way for exciting future research.Therefore, our study aims to delve into the intricate world of honey bee gut microbiota in Algeria, focusing specifically on the *Apis mellifera*

worker species. We aspire to unveil the mysteries surrounding its composition, paving the way for exciting future research endeavors.

To explore the captivating diversity of the microbiota, we have employed both phenotypic and genotypic approaches. Phenotypically, we have utilized observational techniques to identify discernible characteristics of various microbial taxa. In parallel, at the genotypic level, we have targeted the V3 and V4 regions of the 16S RNAr gene, renowned for their ability to elucidate the taxonomic diversity of the microbiota. The results obtained will be further validated through metabarcoding analysis, which enables comprehensive examination of multiple genomic regions, providing a more holistic understanding of the studied microbiota.

This document is structured around five major chapters. The first two chapters highlight the importance of the model insect *Apis mellifera* and the crucial role of its gut microbiota. Various approaches are then presented to identify the intestinal bacterial community of our samples. The results obtained are then presented and discussed, leading to a conclusion.

The discoveries stemming from this comprehensive investigation could shed new light on the intricate interplay between honey bee gut microbiota and the ecological environment in Algeria. Moreover, they may have substantial implications for safeguarding bee health and preserving the ecosystem as a whole.

Chapter I
Characterization of *Apis mellifera*

I.1. *Apis mellifera*

Humans have always been fascinated by bees because of their complex social life, their incredible learning and memorizing abilities that allow them to efficiently navigate between the hive and the flowers.

The bee belongs to the order Hymenoptera, whose name comes from the Greek word hymen which means "membrane". It is a member of the Apidae family and the *Apis* genus [7]. The bees of this genus are characterized by a very social behavior and live in colonies, in hives or nests [7]. The most widespread is *Apis mellifera*, commonly known as the black bee and for its great importance to beekeeping [8].

The honey bee contributes to the survival of its colony by communicating the location of food sources through a complex wave dance [10].

Honey bees *Apis mellifera* have unique characteristics that make them an essential pollinator. They represent a considerable work force that can be stimulated by beekeepers [9].

According to Linné (1758), the domestic bee is classified as follows:

- **Kingdom**: *Animalia*
- **Phylum**: *Arthropoda*
- **Sub-branch**: *Pancrustacea*
- **Class**: *Insecta*
- **Order:** *Hymenoptera*
- **Sub-Order**: *Apocrita*
- **Family:** *Apidae*
- **Genus**: *Apis*
- **Species**: *Apis mellifera Linnaeus*

I.2. Honeybee society

Apis mellifera, as a social insect, lives in huge, well-organized societies in which cooperation and altruism are necessary for survival and communication is crucial. The colony is composed of hundreds of male drones, sterile workers ranging from 12,000 to 90,000 depending on the season, and a single queen [10, 11].

I.2.1. Bee queen

The queen is the largest bee in the colony and the only one capable of laying both fertilized eggs that will become workers and unfertilized eggs that will become drones [12] (See Figure 1). She makes mating flights outside the colony only once in her life with several drones, which will give her a sufficient supply of sperm to ensure the manufacture of workers for seven to eight years. (During this time, the sperm will remain perfectly alive in her genital tract) [12, 13, 14, 15, 16, 17]. After the mating flight, the queen returns to the nest to lay up to 2,000 eggs per day [12].

The queen is responsible for creating pheromones that control the behavior and growth of other bees in the hive [13, 18, 19, 20, 17]. If the queen dies or is unable to lay eggs, worker bees create a new queen by selecting a few larvae and feeding them, a special diet called royal jelly [12].

I.2.2. Drones

Bee drones are often called "Willi lazy" [21]. They appear in May and play a crucial role in the reproductive process called "flying sperm"[22, 23]. They copulate with the queen in the air and then die [12] (See figure 1). Drones that did not participate in the reproductive process are expelled from the hive in late July and die of starvation [24, 25].

Drones also have high thermogenic capacity [22, 23]. Due to their large body mass, they participate in the heat production of the colony under conditions of extreme heat stress [26]

I.2.3. Bee workers

Workers are sterile bees with non-functioning ovaries and are unable to fertilize [12]. Nonetheless, they fulfill many duties related to reproduction, such as cleaning the combs and feeding the larvae, as well as constructing combs, evaporating nectar, and guarding the hive. Most importantly, they are in charge of finding food and water for the colony [27] (See figure 1). Worker bees typically survive for around six weeks during summer and their roles change as they grow older, usually transitioning from nurse to shepherd. This phenomenon, in which behavior alters with age, is called "age polythism" [28].

Young worker bees begin as nurse bees, tending to the development of brood and secreting wax to build the honeycomb. As they grow older, they transform into field bees and begin to forage for nectar and pollen. Toward the end of their lives, they become guard bees, protecting the hive from intruders [28, 29].

Group cohesion is maintained by its ability to differentiate between nest members and non-nest members and to recognize the role of each individual, leading to a well-organized hive [30].

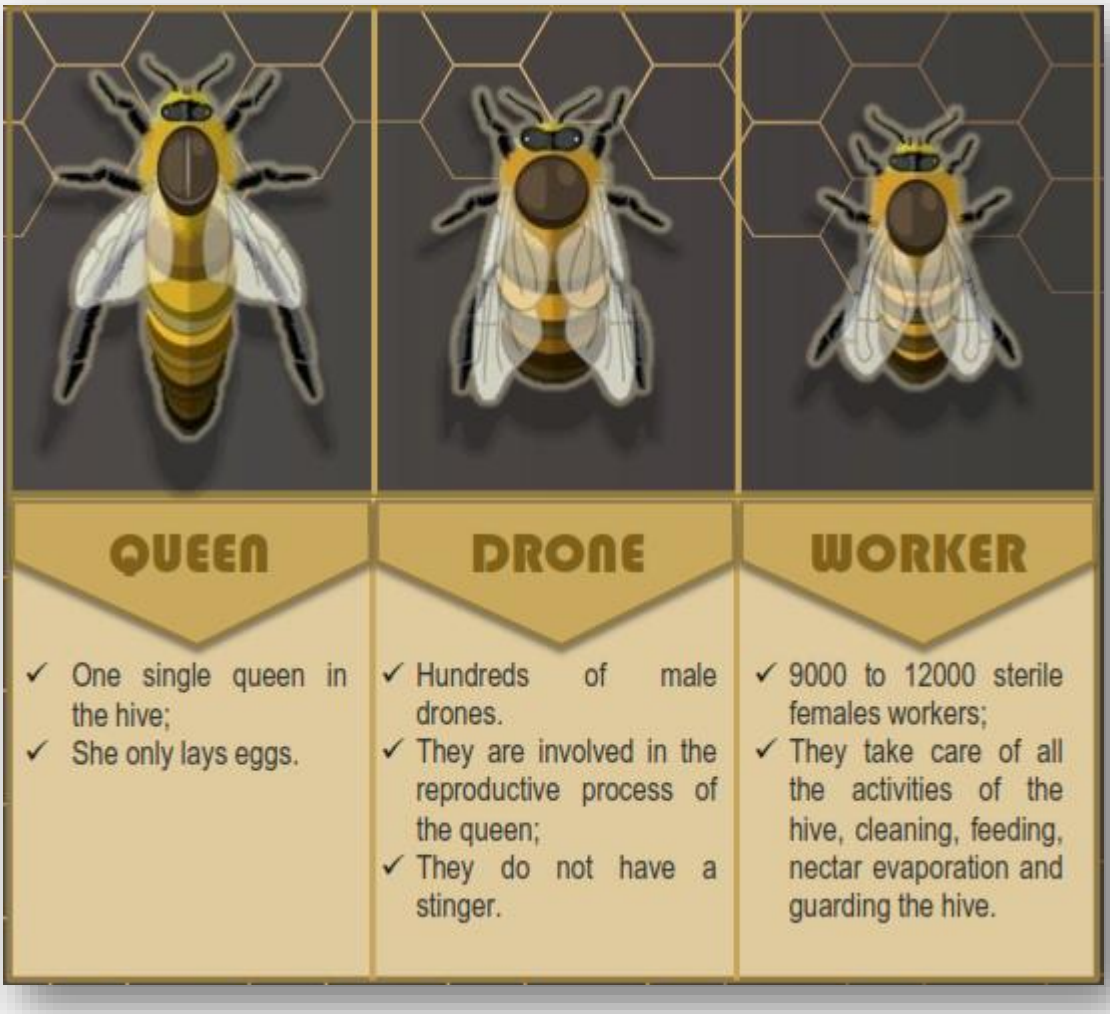


Fig1. The inhabitants of the hive

I.3. *Apis mellifera*, an important insect

Honeybees are key pollinators for many species of flowering plants, helping to sustain the plants that provide food for humans and animals. Without them, many fruits, vegetables and nuts would not exist [31].

In addition, honeybees produce honey, beeswax, and other products that have a variety of uses in industries such as cosmetics and medicine [31, 32, 33, 34].

I.3.1. Pollination

The interaction between plants and pollinators is one of the main forces that support biodiversity on Earth [35]; without pollinators, pollen and seeds cannot be delivered and flowering plants cannot reproduce [36].

In addition to being directly responsible for the maintenance and reproduction of flowering species, pollination also contributes to the survival of other elements of the ecosystem such as herbivores and seedeaters that depend on floral resources [31] The social insect species known as the honeybee *Apis mellifera* has successfully colonized many ecosystems around the world. It is essential for the pollination of wild and domestic plants, which has important ramifications for the global economy and the health of natural ecosystems [37]. Studies have shown how crucial this insect is as a pollinator in natural environments around the world and how important it is to protect them in order to preserve the genetic variety of local subspecies and their ecological role [38, 39, 40].

Honey bees appear to be the most frequent pollinators in natural habitats, accounting for 13% of floral visits on average, with 5% of plant species visited exclusively by *A. mellifera* [41], making them the most important group of pollinators, and their role as pollinators in natural and agricultural ecosystems is becoming increasingly evident and recognized [42, 43, 44]. This also demonstrates that honeybees can contribute to the maintenance of biodiversity in native flowering plant communities [45, 46, 47], thus confirming the words of Albert Einstein: **“**If the bee disappears from the face of the earth, man will not have more than four years to live**”**

I.3.2. Honeybee’s products

In addition to their role as pollinators, this insect has the exclusive ability to provide a wide range of valuable products to humans, such as wax, pollen, propolis, royal jelly and, especially, honey [31].

Honey is perhaps the most well-known product of bees, and for good reason. It is a natural sweetener rich in antioxidants and with antibacterial properties [12].

The process of honey formation begins with the collection of nectar from plants. This nectar is then stored in the bee's honey stomach [47], where it undergoes enzymatic transformation aided by the addition of invertase [48]. The nurse bees collect the nectar and deposit it in the honeycomb, where it undergoes a ripening process that converts sucrose to glucose and fructose and evaporates water until its water content is reduced to about 17% [47, 48]. This process usually takes between one and three days before it is finalized by plugging the nectar-filled cells with beeswax [47].

Bee pollen is a complex combination of flower pollen, nectar, enzymes, and honey that bees collect and store in their hive [49]. It is a superfood rich in protein, vitamins and minerals [50, 51, 52, 53, 54].

Pollen is a microscopic grain-like structure found in the anther of stamens in angiosperms [55]. Worker honeybees attract hundreds, even thousands of pollen grains during their visits due to the weak electrostatic field generated between the (negatively charged) flower and the bee's body [56]. Subsequently, bees carry pollen as pellets and store it inside cells for later consumption to meet their protein needs and to synthesize jelly in their feeding glands.

Bee pollen is often used as a dietary supplement to boost energy and immunity, as well as to alleviate allergy symptoms and improve digestion [12, 57, 58, 59].

Royal jelly is a milky substance that is produced by honeybees and fed to the queen bee [60]. It is rich in nutrients, including vitamins, minerals, and amino acids, and is believed to have numerous health benefits [60, 61, 62, 63].

Royal jelly is often used as a dietary supplement to boost energy and immunity, as well as to improve skin health and reduce inflammation [64].

A current study by Guo et al [65] demonstrated the biologically active effects of royal jelly on the maintenance of biological functions such as lifespan, immunity,

obesity, memory, blood sugar, digestive system, and anticancer and antibacterial properties [63]. In addition, further information has been provided by Ahmad et al [61] on the biological and pharmaceutical properties of royal jelly, including antioxidant, antimicrobial, wound healing, anti-aging, anticancer, anti-inflammatory, immunomodulatory, anti-hypertensive, anti-hyperlipidemic, neurotrophic and estrogenic effects.

Propolis is a resinous substance commonly called "bee glue", collected by bees from plants and trees, buds and plant secretions [66]. Honeybees use this product to seal cracks, smooth the interior walls, and protect dead intruders inside the hive to stop their decay [67]. In addition, propolis protects the colony from disease due to its antiseptic and antimicrobial properties [66]. Propolis has long been used for its anti-inflammatory properties and to heal wounds, ulcers and injuries, as well as to promote tissue regeneration [68, 69, 70].

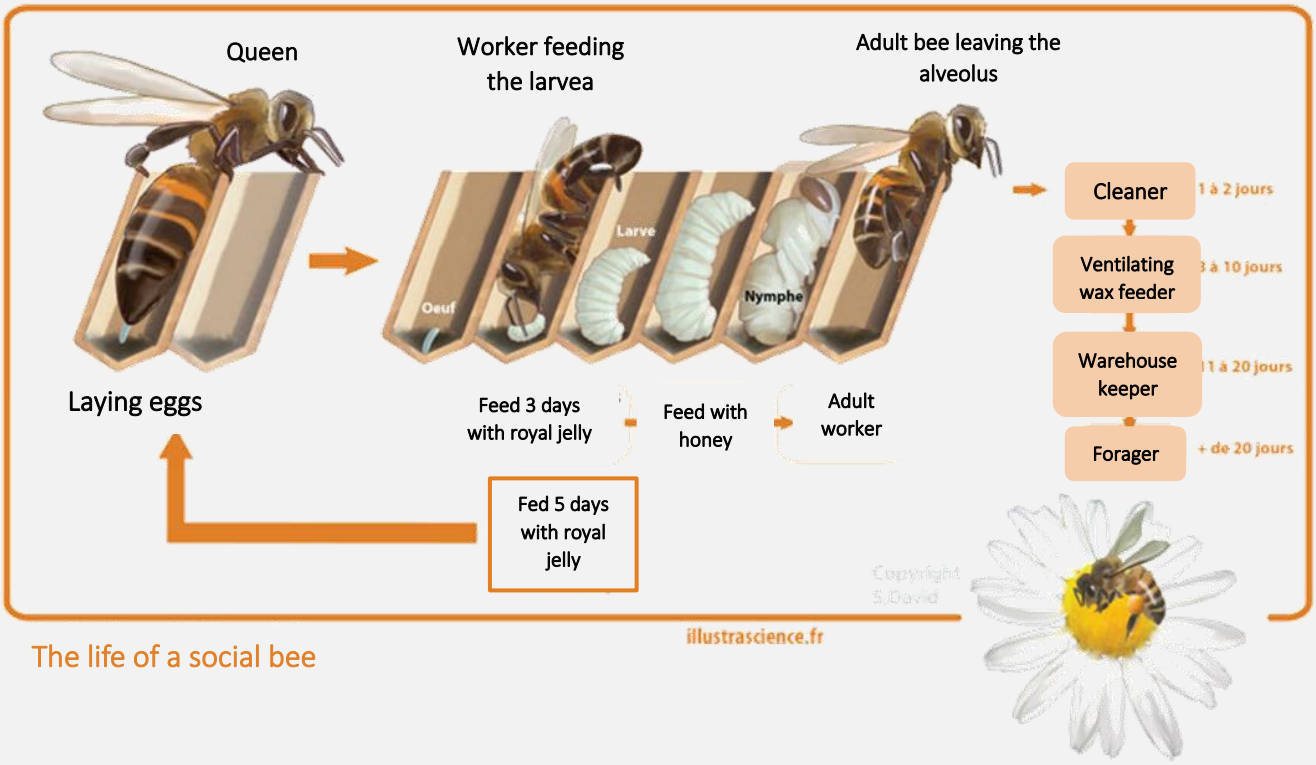
I.4.The differentiation of bees into queens and workers

Honey bees (*Apis mellifera*) use a diverse diet and a haplodiploid sex determination system to produce three different organizational outcomes from the same genome [71, 72].

Depending on food consumption throughout post embryonic development, two identical diploid embryos can mature into either a functionally sterile, short-lived worker or a highly reproductive, long-lived queen. Males, on the other hand, grow from unfertilized haploid eggs produced by a queen in specific cells. Since sex determination is genetically determined, drone larvae consume a different diet than worker larvae [72, 73].

In the post-embryonic stage, the differentiation between queen and worker is due to the feeding of the larvae [74]. At the beginning of their development, all larvae receive royal jelly [74]. However, from the third day onwards, some larvae are fed only with royal jelly and will become queens. Other larvae, fed with a mixture of royal jelly and bee bread, will become workers [74] (See Figure 2).

This nutritional difference from the third day onwards induces changes in gene expression and thus in the phenotype, determined by epigenetic phenomena [74, 75, 70, 76, 77]. The physiological processes that are at the origin of the differentiation of phenotypes can be metabolic variations (slowing down or speeding up), developmental variations (shape, speed, size), for example [78].



The life of a social bee

Fig2. Differentiation of bees into queens and workers and their principal roles (Site 01)

Chapter II
Honeybees microbiota

II.1. Introduction to the gut microbiota in honeybees

II.1.1. Overview of the honeybee gut microbiota

The gut microbiota refers to a complex system of beneficial microorganisms, including bacteria, yeast and fungi, that inhabit the bee gut. These microorganisms play a critical role in the regulation of various metabolic functions, such as glucose and lipid homeostasis, satiety, energy management, and vitamin production [79, 80, 81]. In addition, the microbiota is involved in the regulation of various biochemical and physiological processes through the production of metabolites and other substances [82].

It should be noted that the composition of microbial communities in the gut could vary significantly not only between different species, but also within the same species [83].

II.1.2. Importance of the gut microbiota for honeybee health:

Recent scientific findings provide enough evidence to suggest that the gut microbiome constitutes a new organ system in the human body, given its crucial role in maintaining human health and regulating various biological processes [84].

The gut microbiota in honeybees is vital for food digestion, nutrient production, and maintaining a healthy immune system [85, 86, 87, 88]. Studies have shown that a diverse gut microbiota is associated with improved honeybee health and resistance to pathogens [89, 90]. Exposure to pesticides or antibiotics can disrupt the gut microbiota and negatively impact honeybee health [91, 92, 88, 93] Understanding the importance of the gut microbiota is critical for promoting honeybee colony survival and productivity, which is essential for maintaining ecosystem services and food security [86, 94, 95].

II.2. Honeybees microbiome diversity

II.2.1. Establishment of the gut microbiota in honeybees

The social bees accommodate a microbiota that is both specialized and simple, arranged spatially within distinct compartments of their digestive system [96].

Honey bee workers experience four distinct developmental stages: egg, larva, pupa, and adult [97]. Throughout these stages, there is a significant variation in the absolute abundance of gut-associated bacteria [98].

Larvae initially acquire the intestinal microbiota through interactions with nurse workers and food. However, the composition of intestinal microbiota is temporary, as the larval exoskeleton, including the intestinal mucosa, is shed in early and late pupation stages [99]. This shedding process eliminates any bacteria that might have been present in the larval midgut before pupation [99]. The mature bee bites through the wax cap when metamorphosis is complete. Adult worker bees are almost bacterium-free when they emerge [100, 101, 102], while some germs may be acquired when they devour [103].

During the first three days following emergence, the typical gut microbiota is developed through social contacts with other workers. The quantity of bacteria in the intestine grows logarithmically until it reaches 10^8-10^9 bacterial cells four days after emergence [104].

II.2.2. The gut microbiome diversity within honeybee casts

Numerous studies have shown that the composition of the gut microbiome varies between different castes of bees, including queens, workers and drones [105] (See Figure 3). Interactions occur between these castes, as well as between them and pre-imaginal bees [106]. The lifestyle of the castes and the individual microbiota of honey bees are influenced by their interactions with the environment, the hive and other bees [107].

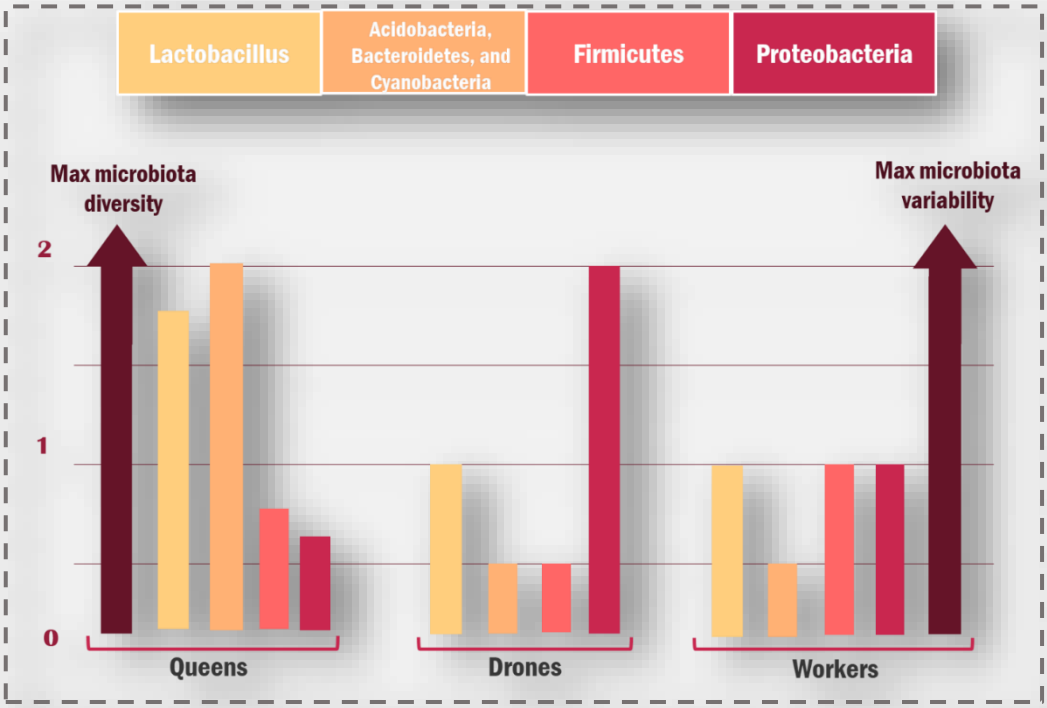
These studies also demonstrated greater microbiota diversity in queen bees compared to other groups [105], while worker bees showed greater variability than queen or drone samples (See Figure 3).

The queen bee microbiota was distinguished by the marked presence of *Acidobacteria, Bacteroidetes*, and *Cyanobacteria*, whereas these groups were significantly reduced or absent in worker bees and drones. In contrast, all bee samples contain *Proteobacteria* and *Firmicute*, although *Firmicutes* reach their maximum level with restricted diversity in drones. worker bees are characterized by a notable

abundance of should be noted that the queen bee may sometimes have low levels of *Lactobacillus* bacteria relative to other bees *Proteobacteria* and *Firmicute* [105] (See Figure 3).

It when she is old or ill [105], but in general, healthy queens have high levels of *Lactobacillus* relative to other groups [108].

Fig 3. Variability of major gut microbiota groups in different castes bees



II.3. Diversity of honey bee workers gut microbiota

Considering the variations in the gut microbiome of different bee castes, it is interesting to look specifically at the gut microbiota of worker bees.

Metagenomic studies based on 16S rRNA and total DNA of the gut community have revealed the presence of nine species of bacteria dispersed in the gut of worker bees, representing 95% to 99.9% of the bacteria present in almost all individuals [109, 110, 111]. Among these nine taxa present in the bee digestive tract, two species of Gram-negative *proteobacteria* are ubiquitous: *Snodgrassella alvi and Gilliamella apicola* [112, 113, 114]. In addition, two species of Gram-positive belonging to *Firmicutes* such as *Lactobacillus Firm-4 and Lactobacillus Firm-5*, are abundant and widespread in the rectum [112, 113] *Bifidobacterium asteroides* is also found in relatively small quantities compared to other bacteria species [115, 116]. These

microorganisms are considered essential in the bee gut, being commonly called the "core bacteria" [117]. Other species belonging to the *Proteobacteria*, less abundant or less stable, have been identified, such as *Frischella perrara, Parasaccharibacter apium, Bombella apis, Bombella mellum, Bombella favorum, Bartonella apis* and *Commensalibacter sp*. Two other species of the phylum Bacteroidetes have also been identified: *Apibacter mensalis* and *Apibacter adventoris* [113, 114, 115, 118, 119, 120].

The worker bee gut is composed of three distinct sections, each one harboring different bacterial communities (see Figure 4). The first section, called the crop, has a low presence of bacteria, being mainly colonized by species affiliated to the *Enterobacteriaceae*, *Lactobacillus kunkeei* and *P. apium*, which are identified in nectar and hive materials [121, 122, 123].

The second section, the midgut, is the site of food digestion and nutrient absorption. It contains the fewest bacteria among the parts of the digestive tract, with about 107 cells [124]. The dominant species in this section are *Gilliamella apicola, Snodgrassella alvi* and *Bartonella apis* [124, 125]. Other bacteria, such as *Parasaccharibacter apium* and *Lactobacillus kunkeei*, are also frequently found and are mainly derived from pollen and nectar [124]. The pylorus, a small segment just downstream of the midgut, is colonized by *Frischella perrara* (see Figure 4) [126].

The hindgut is divided into two distinct regions, the ileum and the rectum, which differ in their bacterial community composition [127]. The ileum is covered by a large biofilm dominated by major Gram-negative species such as *S. alvi, G. apicola*, as well as bacteria of the genus *Lactobacillus Firm-5 and Firm-4* [124] (See figure 4).

On another hand, the rectum plays a role in water absorption and fecal formation [127]. It is the place where bacteria are most abundant, with about 109 bacterial cells [128]. The rectum is mainly dominated by *Lactobacillus Firm-4*, *Lactobacillus Firm-5* and *Bifidobacterium* species [124] (See Figure4).

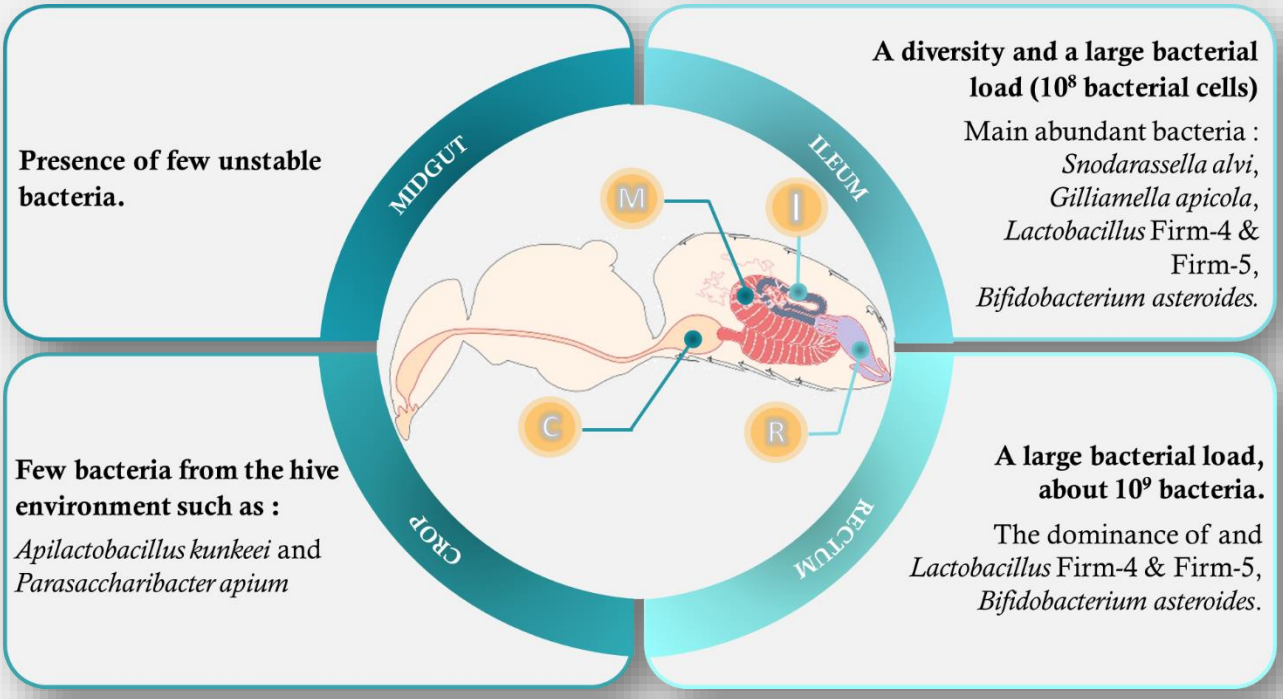


Fig4. The several main species of the bee gut microbiota and their distribution

II.4. Diversity and dynamics of gut microbiota in adult worker bees

The adult workers have a fairly consistent assemblage of gut bacteria compared to males or females [129, 130, 131], as well as compared to other insects in general [132, 133]. However, even workers of the same age in the same colony can have very different proportions of baseline gut species [134, 130, 135]. Changes in the relative proportions of core species can also occur in colonies due to age or season [129, 136]. The extent to which these changes are specific to particular geographic regions or conditions is unclear, in part because it is not possible to directly compare community profiles generated by different laboratories using different nucleic acid extraction and amplification protocols [137].

More mature foragers may have a lower abundance of core bacteria than young adult bees [129]. Several studies using 16S rRNA gene profiling have shown minor differences in gut community composition between colonies in different locations [134, 129, 138]. Regarding seasonal patterns, a 6-month longitudinal study of workers' guts revealed changes in the microbiome [136]; however, guts can produce erratic results due to their low bacterial content. Another study found minor differences in whole-gut communities between fall and spring foragers [139].

II.5. The role of the microbiome in all individuals

The significance of the species comprising the intestinal microbiome has been demonstrated in several studies, much like the human microbiota, which is involved in most metabolic and immune functions of the body. Therefore, it is assumed that the intestinal microbiome of bees is equally important. Consequently, studies have been conducted based on laboratory experiments that expose bees to various factors and monitor changes in the quality of the intestinal microbiome, thus affecting the overall functioning of the organism. As a result, several important roles have been identified for the intestinal microbiota of bees. It is evident that the intestinal microbiome of bees plays a vital role in their overall health and functioning [140, 141, 142, 143, 144, 145, 146, 147, 148].

II.5.1. Nutritional and physiological metabolic roles

The nutritional requirements of honeybees are exclusively fulfilled through the consumption of nectar and pollen. Analysis of the bee gut microbiota through metagenomic studies has uncovered two fundamental functions performed by the honeybee gut microbiota: nutrient synthesis and biomass decomposition. These roles are vital for the breakdown and utilization of the ingested food sources [149].

Extensive research has been conducted by subjecting bees to experiments involving an imbalanced diet containing insufficient amounts of essential nutrients such as vitamins and amino acids. These studies have focused on investigating the nutritional function, including nutrient synthesis. The findings suggest that honeybee endosymbionts play a crucial role in producing nutrients that are not readily available in their dietary sources [150].

Both the gut microbiome and the host bee possess the capability to release cellulolytic enzymes for the decomposition and catabolism of biomass. Studies indicate that the presence of microbial activity enhances the efficiency of these processes, leading to more effective breakdown and utilization of biomass [150].

The ability of core honeybee microbiome species like *Lactobacillus* species, *G. apicola*, and *Bifidobacterium* to metabolize a variety of plant carbohydrates and related

compounds, including pectin, a significant constituent of pollen's inner wall, has been shown through genomic and metabolic studies. According to Zheng et al., *G. apicola* which mostly inhabits the intestinal compartment, is in charge of breaking down pectin into galacturonate, which is a significant pectin degradation by product. These intestinal microbes also assist in the detoxification of secondary plant chemicals, lipid and protein digestion, and protein synthesis [151, 152].

Furthermore, these studies have provided evidence that these bacteria have a primary capability for metabolizing mannose, a key component of nectar. In addition, intestinal symbionts play a crucial role in converting plant exudates and buds into propolis, as well as facilitating the fermentation process that transforms nectar into honey. Moreover, they contribute to the preservation of honey's freshness [153, 154].

II.5.2. The role of gut microbiome in bee health

Similar to the impact of the gut microbiome on human immune function and overall health, recent studies have revealed the significant role of the gut microbiota in honeybees [155]. Notably, research has demonstrated that disruptions in the gut microbiota caused by the antibiotic tetracycline render honeybees more vulnerable to the opportunistic bacterium Serratia, consequently diminishing their survival rates [157].

An increase in the abundance of the parasite *Lormaria passim* has been observed when there is a disturbance in the composition of the gut microbiota [156]. Moreover, feeding bees with aged pollen leads to higher mortality rates due to elevated levels of the *Nosema fungus*. This dietary change also results in significant alterations in the composition of gut endosymbionts, leading to the development of a highly imbalanced microbiome. Consequently, the dysbiosis within microbiome may compromise gut resistance to internal pathogens [158].

These research findings, supported by monocolonization tests and microbiota transplants, establish a clear link between the gut microbiota and their host, honeybees, and demonstrate a relationship between pathogen resistance and host benefits. Moreover, investigations have revealed that the gut microbiomes play a role in regulating insect competence by modulating the gut environment to inhibit parasite

growth and stimulate the host immune system. The production of antimicrobial peptides by the gut microbiota further contributes to the control of bacterial and parasitic infections [159, 160].

However, the extent to which these effects act as priming responses to enhance pathogen resistance remains to be fully elucidated. In this context, the mucosal immune system faces the challenge of simultaneously carrying out two contradictory tasks. On one hand, it needs to tolerate the presence of the gut microbiota to avoid eliciting detrimental systemic immune responses. On the other hand, these microbes play a crucial role in maintaining intestinal homeostasis through various mechanisms, such as the production of peptidoglycans, lipopolysaccharides, flagellins, and other factors [161, 162].

The gut bacteria play a pivotal role in achieving various essential objectives, including the regulation of nutrient digestion and overall physiological functions. The harmonious interaction between the microorganisms and the host's immune system is crucial for maintaining a well-functioning body. This intricate relationship also influences several important aspects, such as body size, weight gain, developmental rate, metabolism, stress susceptibility, stem cell activity, and wing area. All these factors contribute to the overall balance and well-being of the honeybee's body [137].

II.6. Factors that disrupt the honeybee gut microbiota

Like any other species, honeybees face pressure from various biotic and abiotic factors due to their ecological importance and the challenges they encounter. These factors can influence the composition and dynamics of the microorganism communities that constitute the gut microbiota of bees [163, 164].

II.6.1. Impact of biotic factors on honeybee gut microbiota

The gut microbiota of bees is significantly influenced by various pathogens, including those transmitted by parasites such as Varroa mites (*Varroa destructor*) and small hive beetles (*Aethina tumida*). Additionally, fungi such as Nosema ceranae and Nosema apis, as well as bacteria including *Paenibacillus larvae*, *Melissococcus plutonius*, and *Paenibacillus alvei*, and viruses such as *Sacciform* Brood Virus, Queen

Black Cell Virus, and Chronic Bee Paralysis Virus, have a profound impact on the bee's gut microbiota [165, 166, 167, 168].

II.6.2 Impact of abiotic factors on honeybee gut microbiota

Honeybees are exposed to various pesticides, including chlorothalonil, imidacloprid, and coumaphos, through contaminated nectar, pollen, and water. These pesticides can have detrimental effects on their health [169, 170, 171]. Moreover, they can disrupt the structure and function of the honeybee microbiome, leading to a decrease in beneficial gut bacteria and an increase in pathogenic microorganisms [172].

Additionally, honeybees face environmental challenges such as inadequate nutrition, pollution, reduced vitality and genetic diversity, and degradation of environmental quality. They also experience fluctuations in humidity, temperature, and light conditions [173, 174].

II.7. Methods to study the diversity of the microbiome

The significance of gut microbial communities for the overall health of animals, including humans and insects, has gained widespread recognition in recent years [175]. This recognition has been primarily driven by the development of advanced tools for studying microorganisms in non-laboratory environments, facilitated by two distinct methods.

II.7.1. The monoculture and Metagenomic

The monoculture method involves sterile collection of bee samples from different hives and developmental stages. These samples are then aseptically cultivated in specific media to isolate their microorganisms. Subsequent molecular tests, such as amplification of the 16S rRNA using the CTAB-Phenol-Chloroform DNA extraction method, are employed for the identification of the isolated bacteria [176].

In contrast, the metagenomic method entails sequencing the genomes of all bacteria present in the bee's gut. The DNA is extracted from the bee's gut, followed by sequencing and targeted amplification of the 16S rRNA or the entire genome. Bioinformatic assembly of the sequenced genome(s) is then performed, and the resulting sequences are analyzed [177]. This approach has provided insights into the

symbiotic capabilities of these bacteria and has enhanced our understanding of the functional and evolutionary genetic content of the specific gut microbiota in honeybees [176].

While traditional culture-based approaches were commonly used, they often yielded inaccurate and limited insights into microbial populations, particularly in complex habitats like the gut. This is primarily attributed to the difficulty of cultivating the majority of organisms found in these environments under laboratory conditions [177]. The culture-based approach focuses on identifying the approximately 1% of culturable, living microorganisms in a sample. These microorganisms can exhibit various colony shapes and traits, which are further investigated through phenotypic and molecular analyses for classification purposes [177]. In contrast, the metagenomic technique enables the identification of both cultivable and non-cultivable microorganisms, leading to improved classification and a more comprehensive identification of the microbial community [178].

Chapter III
Materials and methods

The aim of our work is to investigate on the Apis mellifera gut microbiota diversity from North of Africa (Algeria). During our investigations, we follow both cultural and molecular methods. For this purpose, the insect samples were harvested from Sir BENLAKEHAL's hives and our study was conducted between two laboratories. The harvest of the gut was performed in the Faculty of Medicine laboratory, Abderrahmane Mira Bejaia University. Subsequently, the cultural isolation and molecular analysis using the 16S rRNA PCR method were carried out at the Laboratory of Water and Food Bacteriology at the Pasteur Institute of Algiers, Algeria.

The initial step involved the meticulous extraction of the gut from the honeybees, which was conducted at the specialized laboratory of the Faculty of Medicine, Abderrahmane Mira University in Bejaïa. Precise techniques were employed to ensure the accurate extraction of samples.

Following the extraction, our research progressed to the Laboratory of Water and Food Bacteriology at the Pasteur Institute of Algeria, where the cultural isolation of the extracted samples took place. In this laboratory, we successfully isolated and cultivated the bacteria present in the digestive system, allowing for their comprehensive characterization.

Concurrently, we employed the molecular method of 16S rRNA PCR to amplify and genetically analyze the samples. And later the obtained results were confirmed by the metagenomic analysis. This molecular analysis provided detailed insights into the molecular diversity of the honeybees' digestive system.

By combining cultural and molecular approaches, our study has provided a comprehensive understanding of the diversity within the domestic honey bee Apis mellifera gut.

III.1. *Apis mellifera* gut sampling

III.1.1. *Apis mellifera* samples

During the autumn season (September to October, 2022), the samples were harvested from three areas in Algeria: north-central, northwest, and southeast (See table I and Figure 5). In the north-central zone, samples (Sample 01) were collected from the forested area of Allessa village, located in the Ouled Rachad district, which is part of the Bechloul municipality (See Figure 6). This village is situated approximately

thirty kilometers southeast of the Bouira province. In the northwest zone, samples (Sample 02) were collected from the Oued El Kheir region, situated in the central-southern part of the Mostaganem province (See Figure 7). Lastly, in the southeast zone, sampling (Samples 03) was harvested in the forage-rich region of Doucen, a municipality in the Ouled Djellal province (formerly Biskra municipality) (See Figure 8).

Fig5. The different sampling sites

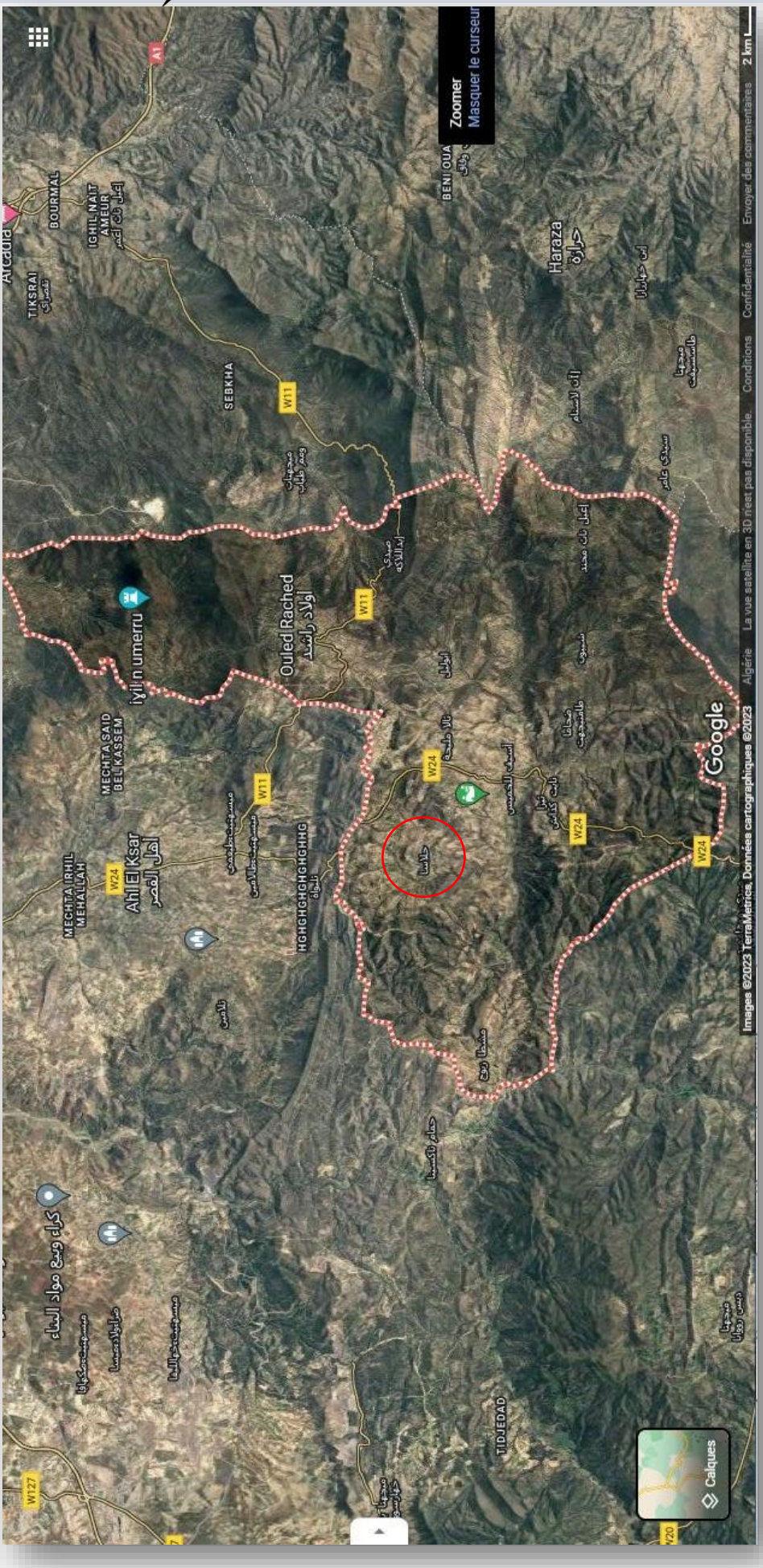


Fig6. Area Sampling 1 – Allessa – Ouled Rachad – Bouira.

Fig7. Area Sampling 2 – Oued El Kheir – Mostaganem

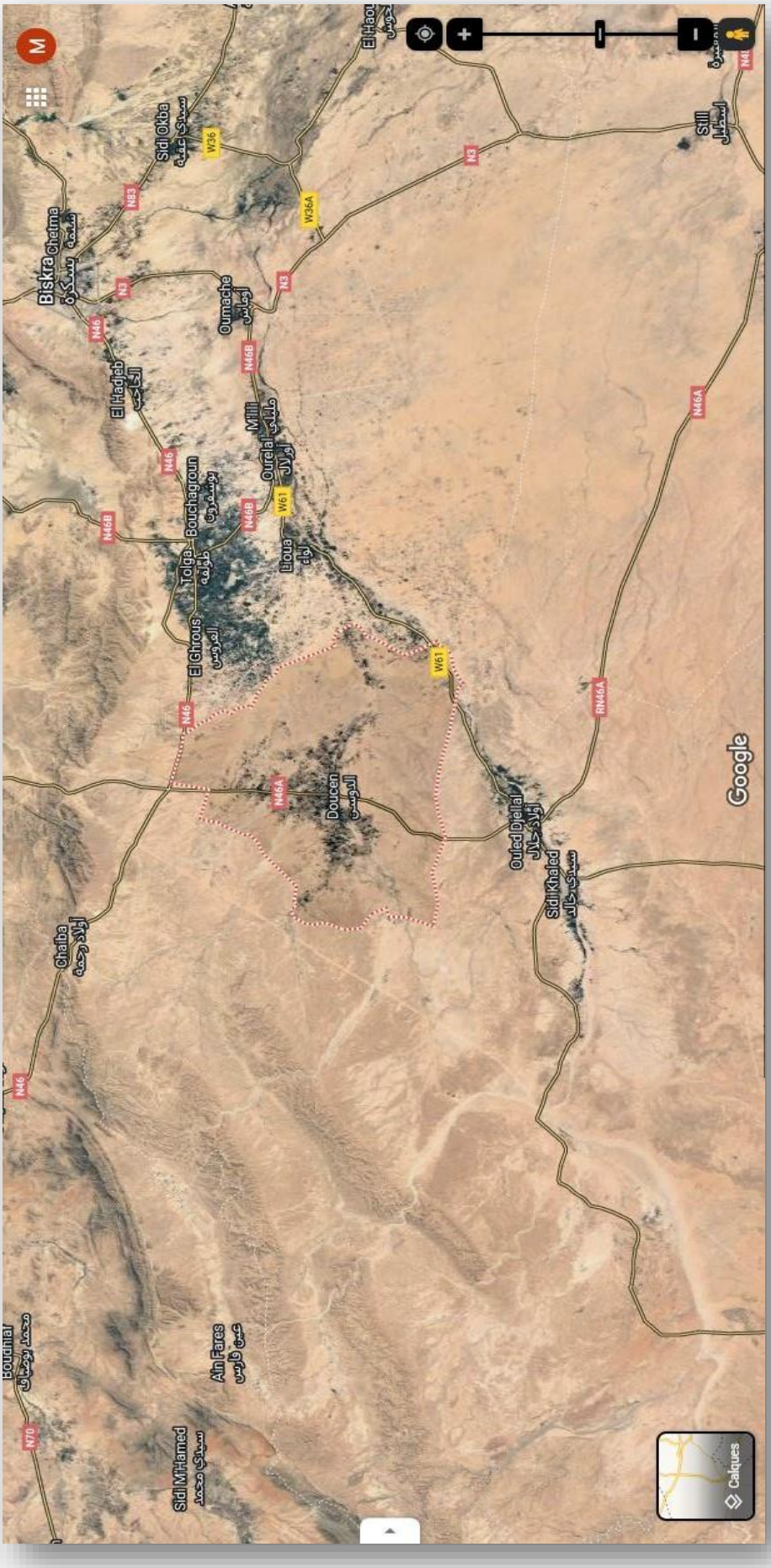


Fig8. Area Sampling 3 – Doucen – Ouled Djellal.

According to information (Table I) obtained from Mr. BENLAKEHAL: "Each bee hive is unique and has specific factors and characteristics that influence the health and productivity of the bees. As a beekeeper, it is our responsibility to provide our bees the optimal conditions for their well-being. Among these factors, we place great importance on climate, as bees are sensitive to variations in temperature and humidity. We also ensure that they have adequate food, with access to a variety of flowers and pollen sources to ensure a balanced nutritional intake. The condition of the hive is another crucial aspect, as we make sure it is disease-free, well-ventilated and spacious enough for the colony to thrive. Finally, we pay attention to the environment surrounding the hives, avoiding exposure to pesticides and promoting natural habitats conducive to pollination. By taking all these factors into account and providing the ideal conditions, we allow our bees to thrive and play their vital role in pollination and ecosystem preservation as well as productivity."

Attached are the characteristics of the bees sampled:

Table I. Different characteristics of our samples

Parameters / Samples	Climatic data	Feeding bees	The state of the hive	Treatment	Area	Location
Sample (1)	Dry and not very cold microclimate	Rosemary in small quantities (*Rosemarinus*)	Medium	Bayvarol	Forest region (Bouira)	36.374332° N, 4.091804° E
Sample (2)	Humid and not very cold microclimate	*Tamarix* dominance	Strong	Bayvarol	Forest region (Mostaganem)	35.985787° N, 0.420409° E
Sample (3)	Subtropical desert (hot and dry)	Medicago sativa (*Lucerne*)	Strong	Bayvarol	Forage region (Ouled Djellal)	34.4334426° N, 5.081450° E

The sampling was carried out by Mr. BENLAKEHAL with precision following the sequential steps outlined below: First, the jars were previously filled halfway with absolute ethanol, which is a commonly used preservative for the preservation of biological samples. Then, once in the field, the jar was opened in front of a randomly selected hive in the collection area. At this point, the flying bees were captured and introduced into the jar. The ethanol in the jar preserves the bees and prevents degradation of the microbial samples in their gut.

After filling the jars with the flying bees, they were sealed tightly. Then, the jars were stored in a refrigerator at a temperature of 4°C. This low-temperature storage step is important to maintain the stability of the samples and preserve the microorganisms in them until later analysis. Keeping the jars under proper refrigeration conditions prevents deterioration of the microbial samples and ensures their integrity for subsequent studies.

III.2. Extraction of the gut from the bee

In order to launch the investigations on the gut microbiome diversity we extracted the gut from the insect's body. Under septic conditions we carried out the desiccation of the insect body and the gut was collected into sterile Eppendorf tubes containing a mixture of heart brain broth and glycerol (50/50) (v/v). Then, the tubes were conserved at 4°C until the investigations process.

III.3. Bacterial diversity of *Apis mellifera* gut microbiota

In this section, we will outline our methodological study approach to isolate, purify and identify the diversity of the gut microbiome in our bee samples. To do so, our work has been divided into two distinct fields of study: a microbiological cultural study and a molecular study involving the identification of our isolates.

The culture-based microbiological study consists of culturing the samples on specific culture media in order to isolate the different bacteria present in the bee gut. Subsequently, these isolates were purified and subjected to biochemical tests such as catalase and oxidase, as well as microscopic observation in the fresh state and using Gram stains.

On the other hand, the molecular study involves the use of 16S RNA sequencing techniques to identify the different bacterial strains present in the samples. This method allows a more accurate and complete identification of the microbial diversity in the bee gut.

III.3.1. Bacterial diversity counting

This section deals with the preparation of stock suspensions from bee digestive tracts, followed by their plating on specific culture media. This method allows the isolation of the different bacterial strains present in bee digestive tracts.

III.3.1.1. First trial

The experiment began by performing a first test on samples from the first region (Bouira). For this purpose, the digestive tubes of five samples were crushed in Eppendorf, thus allowing the preparation of the stock suspensions.

Then, from these stock suspensions, a series of dilutions was performed to obtain different concentrations, ranging from 10^{-1} to 10^{-4}. This was done by adding 100 µL of the stock suspension into 900 µL of appropriate sterile TSE buffer, following a precise dilution scheme.

Each stock suspension thus diluted was inoculated into plate count agar (PCA) medium, which were used for the enumeration of microorganisms (see Table I in supplementary data 2). A total of twenty plates were used for this assay. Inoculation was performed by using a rake pipette and dropping a 100 µl volume of each suspension into each PCA medium. It is important to note that all manipulations were performed aseptically to avoid external contamination and to ensure reliable results.

Once inoculation was complete, the PCA plates were placed in an incubation set at a constant temperature of 30°C. The plates were left to incubate for 72 hours to allow growth and development of the microorganisms in the suspensions.

III.3.1.2. Second and third trials

The experimental protocol was replicated in a similar manner on samples from the other regions, namely Mostaganem and Ouled Djellal. However, adjustments were made to the number of dilutions and plates used due to the relatively low microbial load observed in these samples.

For the samples from the Mostaganem region, three different dilutions were prepared from the stock suspensions. Each dilution was made by adding 100 uL of the stock suspension in a volume of 900 uL of TSE, following a precise dilution scheme. Then, these three diluted suspensions were inoculated into PCA medium, allowing the presence and growth of microorganisms to be assessed in each dilution. A total of three PCA plates were used for each sample from the Mostaganem region (see Table I in supplementary data 2).

For the Ouled Djellal samples, due to the even lower microbial load observed in the previous trials, only one dilution was prepared from the parent suspension. This dilution was carried out in the same way as for the other samples. Subsequently, the diluted suspension was inoculated into a single PCA dish, allowing detection and enumeration of the microorganisms present (see Table I in supplementary data 2).

All manipulations, including preparation of the dilutions, inoculation into the PCA plates and incubation at 30°C for 72 hours, were performed aseptically to prevent external contamination and ensure reliable results.

III.3.2. Bacterial isolation and purification

To isolate and purify the bacteria, the protocol started with the preparation of the stock suspensions from the five samples from the Bouira region. The digestive tubes of each sample were carefully crushed with a sterilized metal rod to release the bacteria present. To optimize the bacterial load, a volume of 100 µl of each stock suspension was taken and inoculated into 1 mL of BHIB broth contained in Eppendorf tubes. These Eppendorf tubes were then placed in an oven set at 37°C, thereby promoting bacterial growth for a period of 24 hours.

The following day, the incubated Eppendorf tubes were collected and processed for the preparation of appropriate dilutions. A volume of 100 µL of each incubated suspension was taken with a sterile micropipette and carefully placed in 900 µl of TSE solution. This step resulted in a 10^{-1} dilution, reducing the initial concentration of bacteria.

From this 10^{-1} dilution, a new 100 µL sample was taken and placed in Petri dishes containing different selective culture media. For each medium, the flooding method was used, involving the addition of the precise volume of the diluted sample over the entire surface of the medium using a sterilized rake pipette. The selective media used included GYC, MRS, Chapman, Macconkey, VRBG, Baird Parker, TSA and TBX. However, for VF and SFB media, specific dilution volumes of 500 µL and 1 mL respectively were added.

Once the petri dishes were inoculated, they were scored and placed in an oven adapted to the temperature and duration of each selective medium. Appropriate incubation times were observed to allow growth and development of the bacteria, as well as the formation of characteristic colonies on the selective media.

The entire protocol was carried out under strict aseptic measures to avoid cross-contamination and ensure the reliability of the results obtained.

After 24 hours of incubation, a 100 µL volume of the dilution from the SFB tubes was inoculated onto plates containing Hektoen medium and then incubated at 37°C for 24 hours.

The bacterial colonies that developed in the petri dishes were then observed and evaluated to provide valuable information on the presence and abundance of bacteria in the samples from the Bouira region.

The same protocol was applied to samples from the other two regions, Mostaganem and Ouled Djellal. However, adjustments were made to the dilutions due to the bacterial load observed in the samples from the Bouira region, for which additional dilutions were performed for the samples from the Mostaganem and Ouled Djellal regions. Thus, the number of dilutions was increased until a dilution of 10-3 was reached. This would allow to obtain bacterial concentrations more adapted for an accurate and reliable reading of the results.

Once bacterial growth was assured in the culture dishes, including GYC, MRS, Chapman, Baird Parker, TSA, and PCA media, bacterial colonies were purified by transferring a separate colony from each dish to the same appropriate selective media. The plates were then incubated under conditions appropriate for each medium.

After confirming the purity of the dishes, one colony from each purified dish was sub-cultured with a Pasteur pipet and placed in a tube containing 5 ml of BHIB. The tubes were incubated at temperatures appropriate for each isolate for 24 hours.

The next day, the tubes were retrieved from the ovens and 5 mL of 99.6% glycerol was added. The tubes were then carefully vortexed with a Pasteur vortexer and divided into 2 mL cryotubes for short-term storage at 4 °C for future use.

It is worth noting that all manipulations during this purification step were conducted aseptically to prevent cross-contamination and ensure reliable results.

III.3.3. Bacterial identification

We used a biochemical procedure for identification of our preserved isolates, which included Gram stain, catalase, and oxidase tests, as well as fresh observation for initial characterization of isolates. Next, a selection of isolates was made for identification using 16S RNA.

To complete this process, we used colony-based PCR and specialized primers targeting stable regions of the 16S gene (V3 and V4). The size of the amplified genes was approximately 200 base pairs. This method allowed us to obtain unique DNA sequences for each isolate.

The discovered DNA sequences were then subjected to bioinformatics processing to be compared with reference databases. This allowed us to determine the bacterial species corresponding to our isolates based on their genetic similarity.

It is important to note that all steps of this protocol were performed according to good laboratory practices and using aseptic technique to ensure the reliability of the results.

III.3.3.1. 16S DNA PCR amplification

To successfully conduct this study, a specific experimental protocol was established based on these data. It can be divided into several distinct steps:

The reaction was performed in a total volume of 25 µL reaction mixture, comprising 12.5 µL of double master mix (2xTaq polymerase - Algeria), 0.5 µL of each primer (10 µM), 9.5 µL of water, and 2 µL of bacterial suspension (See Table II). The amplification was carried out in a thermocycler (Biometra TRIO-Thermoblock from Pasteur Institute)

with an initial hot start cycle (3 min, 95°C), followed by 34 cycles of denaturation (30 sec, 95°C), annealing (30 sec, 55°C), extension (1 min, 72°C), and a final extension cycle (7 min, 72°C) (See Table III).

The 16S DNA sample underwent a sequencing procedure after being purified. To obtain the entire sequences of 16S RNA, we employed a sequencer. This sequencing was carried out following the appropriate protocols and parameters to ensure reliable and accurate results.

Table II: Volumes of reaction mixture preparation

COMPONENT	VOLUME
SUSPENSION	2 µl
PRIMER F (10 µM)	0,5 µl
PRIMER R (10 µM)	0,5 µl
2X MasterMix	12,5 µl
ddH2O	Up to 25 µl

Table III: Cycling instruction

INITIAL DENATURATION	DENATURATION	ANNELATION EXTENSION	FINAL EXTENSION
1 Cycle	34 Cycles		1 Cycle
95°C	95°C	55°C 72°C	72°C
5 mins	30 sec	30 sec 100-2000 bp/min	7 mins

III.3.3.1.1. Suspensions and reagent preparation

To begin with, a young culture was created using the selected isolates. The aim of this step was to stimulate development while ensuring isolate purity. The colony was then picked up at the tip of a sterile Pasteur pipette and transferred to a 500 mL Eppendorf tube. These Eppendorf tubes had previously been filled with 10 µL of ultrapure water.

We then designed our reaction mixture according to the above-mentioned protocol and the number of strains we intended to analyze.

After running the PCR according to the cyclic program described above, we recovered the PCR products obtained and proceeded to the next electrophoresis step.

III.3.3.2. Electrophoresis and visualization of UV PCR bands

To prepare the electrophoresis gel, we dissolved 3 g of basic agarose in 200 mL of TAE buffer in a clean vial, resulting in a 1.5% gel concentration. The vial containing the agarose solution was heated in a microwave oven in 30-second increments until the agarose was completely dissolved. After each increment, the solution was carefully stirred to ensure uniform dissolution. Once the agarose was dissolved, we added 6 µL of BET intercalant to the solution and gently stirred to disperse the intercalant well in the gel.

Next, we prepared the gel support by inserting strips to form the wells. The prepared gel solution was poured into the gel holder, then the gel was left to solidify for around 30 minutes at room temperature.

Once the gel had solidified, we placed it in an electrophoresis tank filled with TAE migration buffer. We loaded 10 µL of PCR product into the gel wells using a micropipette. In addition, a 1 kb molecular weight marker was loaded into a specific well to serve as a reference.

Next, we connected the electrophoresis tank to a power supply (APELEX) set at 100 V and let the samples migrate through the gel for one hour. After migration, the gel was exposed to a UV light source to visualize the DNA bands.

After ensuring visualization of the DNA bands at a size of 200 bp, the products were sent for sequencing.

With the sequences obtained, we were able to obtain information on the similarity and identification of the corresponding bacterial organisms.

In order to confirm the 16S DNA PCR results, metagenomic analysis was carried out by targeting the V3-V4 16S DNA region.

III.3.2.3. The sequence treatment and analysis

Once we received the complete sequences of 16S rRNA, we used the NCBI's GenBank resources (http://blast.ncbi.nlm.nih.gov/Blast.cgi) for further validation and comparison and we used the MEGA6 software (http://www.megasoftware.net/) to perform the sequences analysis and built the phylogenetic trees.

Chapter IV
Results and discussion

IV.1. Phenotypic evaluation and characterization of Bacterial diversity

In this study, sampling was carried out in autumn 2023, where we used a total of 15 bee samples, corresponding to 15 digestive tubes. More specifically, we extracted 5 digestive tubes from bees in the Bouira region, 5 digestive tubes in the Mostaganem region and 5 digestive tubes in the Ouled Djellal region.

To characterize the microbiota of these samples phenotypically, we carried out an enumeration using PCA medium to quantify the number of bacterial colonies present in the samples (see Table IV).

We then proceeded with isolation on specific media enabling the growth and identification of certain types of bacteria, which are then purified on the same appropriate specific media (Supplementary data 2).

Finally, we subjected the isolates obtained to phenotypic characterization tests, including Gram staining, catalase tests to assess the presence of the catalase enzyme and oxidase tests to detect oxidase enzyme activity (see tables VI, VII, VIII).

IV.1.1. Enumeration

The total number of bacteria recovered on PCA from the 15 samples collected from three distinct sites (Bouira, Mostaganem, and Ouled Djellal) was 425 CFU (see Table III). The Bouira region provided 73 CFU, or 17.18% of the total, to these samples. Mostaganem provided 145 CFU, accounting for 34.12% of the total. Finally, the Ouled Djellal region provided 207 CFU, accounting for 48.71% of the total (see Table V).

Table IV: Enumeration of bacteria on PCA medium for samples

REGION	BOUIRA	MOSTAGANEM	OULED DJELLAL
TOTAL NUMBER (UFC)	73	145	207

IV.1.2. Isolation and purification

With regard to isolation and purification, the bacteria obtained from bee samples (15) taken from three different sites respectively (Bouira, Mostaganem and Ouled Djellal) comprised 83 isolates, indicating a high level of bacterial diversity.

Of these 83 isolates, 13.25% grew on PCA medium, 22.9% on GYC medium, 20.48% on MRS medium, 21.7% on TSA medium, 13.25% on Chapman medium and 8.43% on Baird Parker medium.

These results indicate a diversity of bacterial strains isolated from bee samples, with varying preferences in terms of culture media favorable to their growth.

IV.2. Phenotypic analysis

Based on similarity between isolates, we selected 66 of the 83 for phenotypic characterization using an approach comprising a macromorphological study, a micromorphological study including Gram staining, and biochemical tests such as catalase and oxidase.

This phenotypic approach provided us with various aspects concerning the different selective media used, indicating the bacterial variety present in the gut microbiota of our bees (See table V, VI, VII).

Table V: The development of Bouira isolates on different media.

Lot	Isolates	GYC	MRS	PCA	CHAPMAN	BAIRD PARKER	TSA
Bouira	A1	+					
	A2	+					
	A3	+					
	A4	+					
	A5	+					
	A6	+					
	A7		+				
	A8		+				
	A9		+				
	A10			+			
	A11			+			
	A12				+		
	A13				+		
	A14				+		
	A15					+	
	A16					+	
	A17					+	
	A18						+
	A19						+
	A20						+

Table VI: The development of Mostaganem isolates on different media.

Lot	Isolates	GYC	MRS	PCA	CHAPMAN	BAIRD PARKER	TSA
Mostaganem	A21	+					
	A22	+					
	A23	+					
	A24	+					
	A25	+					
	A26	+					
	A27	+					
	A28	+					
	A29	+					
	A30		+				
	A31		+				
	A32		+				
	A33		+				
	A34		+				
	A35		+				
	A36			+			
	A37			+			
	A38				+		
	A39				+		
	A40				+		
	A41					+	
	A42					+	
	A43						+
	A44						+
	A45						+
	A46						+
	A47						+
	A48						+
	A49						+
	A50						+
	A51						+
	A52						+

Table VII: The development of Ouled Djellal isolates on different

Lot	Isolates	GYC	MRS	PCA	CHAPMAN	BAIRD PARKER	TSA
Ouled Djellal	A53	+					
	A54	+					
	A55	+					
	A56	+					
	A57		+				
	A58		+				
	A59			+			
	A60				+		
	A61				+		
	A62				+		
	A63					+	
	A64						+
	A65						+
	A66						+

IV.2.1. Gram Staining Results

Among the 66 characterized isolates, we observed a predominance of Gram-positive over Gram-negative bacteria among the 66 isolates characterized. More specifically, we found that 80.3% of isolates were Gram-positive, while 19.7% were Gram-negative.

To be more precise, we obtained 70% Gram-positive bacteria for isolates from the Bouira region, with 30% Gram-negative bacteria. In the Mostaganem region, we observed 92.3% Gram-positive bacteria and 7.7% Gram-negative bacteria. Finally, for isolates from the Ouled Djellal region, we obtained 78.8% Gram-positive and 21.4% Gram-negative bacteria (See tables VIII, IX, X).

IV.2.2. Biochemical Evaluation

Biochemical evaluation of our isolates provided additional information on the phenotypic characteristics of the bacteria studied. The results of the catalase and oxidase tests were analyzed to determine the presence or absence of these enzymes in the isolates.

With regard to the oxidase test, we observed that 18.91% of isolates were oxidase-positive, while 81.09% were oxidase-negative, for all three regions studied.

More specifically, in the Bouira region, 14.3% of isolates were oxidase-positive, while 85.7% were oxidase-negative. In the Mostaganem region, 33.3% of isolates were oxidase-positive, while 66.6% were oxidase-negative. Finally, in the Ouled Djellal region, 9.1% of isolates were oxidase-positive, while 90.9% were oxidase-negative (see tables VIII, IX, X).

To assess catalase, we examined the presence or absence of this enzyme in our bacterial isolates. Across the three regions studied, 89.28% of isolates were catalase-positive, while 10.72% were catalase-negative.

In the Bouira region, 88.24% of isolates were catalase-positive, while 11.76% were catalase-negative. In the Mostaganem region, 86.7% of isolates were catalase-positive, while 13.3% were catalase-negative. Finally, in the Ouled Djellal region, 92.9% of isolates were catalase-positive, while 7.1% were catalase-negative (See tables VIII, IX, X).

Based on the provided results, we can compare the three regions in terms of Gram staining, catalase, and oxidase activity as follows:

Comparing of results between Northwest and North-central regions:

- **Gram Staining:** Mostaganem had a higher proportion of Gram-positive bacteria (92.3%) compared to Bouira (70%). Bouira had a higher proportion of Gram-negative bacteria (30%) compared to Mostaganem (7.7%).
- **Catalase Activity:** Bouira had a slightly higher percentage of catalase-positive isolates (88.24%) compared to Mostaganem (86.7%). The catalase-negative isolates were slightly higher in Mostaganem (13,3%) compared to Bouira (11,76%).
- **Oxidase Activity:** Mostaganem had a significantly higher percentage of oxidase-positive isolates (33.3%) compared to Bouira (14.3%). The percentage of oxidase-negative isolates was higher in Bouira (85.7%) compared to Mostaganem (66.6%).

Comparing of results between North-central and Northern Sahara regions:

- **Gram Staining:** Ouled Djellal had a higher proportion of Gram-positive bacteria (78,8%) compared to Bouira (70%). Bouira had a higher proportion of Gram-negative bacteria (30%) compared to Ouled Djellal (21,4%).
- **Catalase Activity:** Ouled Djellal had a slightly higher percentage of catalase-positive isolates (92.9%) compared to Bouira (88.24%). The catalase-negative isolates were slightly higher in Bouira (11.76%) compared to Ouled Djellal (7.1%).
- **Oxidase Activity:** Ouled Djellal had a lower percentage of oxidase-positive isolates (9.1%) compared to Bouira (14.3%). The percentage of oxidase-negative isolates was higher in Ouled Djellal (90,9%) compared to Bouira (85,7%).

Comparison of results between Northern Sahara and Northwest regions:

- **Gram Staining:** Ouled Djellal had a lower proportion of Gram-positive bacteria (78.8%) compared to Mostaganem (92.3%). Mostaganem had a lower proportion of Gram-negative bacteria (7.7%) compared to Ouled Djellal (21.4%).
- **Catalase Activity:** Ouled Djellal had a slightly higher percentage of catalase-positive isolates (92.9%) compared to Mostaganem (86.7%). The catalase-

negative isolates were slightly higher in Mostaganem (13.3%) compared to Ouled Djellal (7.1%).

- **Oxidase Activity:** Ouled Djellal had a lower percentage of oxidase-positive isolates (9.1%) compared to Mostaganem (33.3%). The percentage of oxidase-negative isolates was lower in Mostaganem (66.6%) compared to Ouled Djellal (90.9%).

These comparisons highlight the variations in the proportions of Gram-positive and Gram-negative bacteria, as well as differences in catalase and oxidase activity among the three regions. The Mostaganem region had the highest proportion of Gram-positive bacteria, while the Bouira region had the highest proportion of Gram-negative bacteria. In terms of catalase activity, the Ouled Djellal region had the highest percentage of catalase-positive isolates, while the Mostaganem region had the highest percentage of catalase-negative isolates. For oxidase activity, the Mostaganem region had the highest percentage of oxidase-positive isolates, whereas the Bouira region had the lowest percentage of oxidase-positive isolates.

These findings provide insights into the bacterial diversity and metabolic capabilities within the gut microbiota of bees in the different regions.

Table VIII: Phenotypic characteristics of bacteria found in samples from the Bouira region

Lot	Isolates	Shape	Gram positive	Gram negative	Oxidase positive	Oxidase negative	Catalase positive	Catalase negative
Bouira	A1	ND	ND			(+)	(+)	
	A2	Diplococci		(+)	ND		(+)	
	A3	Cocci	(+)		(+)		(+)	
	A4	Clusters cocci	(+)		ND		(++)	
	A5	Small bacilli	(+)			(+)	(+)	
	A6	Bacilli	(+)			(+)	(+)	
	A7	Cocci	(+)			(+)	(++)	
	A8	Bacilli		(+)		(+)		(+)
	A9	Small bacilli		(+)		(+)		(+)
	A10	Cocci	(+)			(+)	(++)	
	A11	Cocci		(+)		(+)	(+)	
	A12	Cocci	(+)				(+)	(+++)
	A13	Bacilli	(+)			(+)	(++)	
	A14	Bacilli	(+)		ND		(++)	
	A15	Bacilli	(+)		ND		ND	
	A16	Bacilli	(+)		ND		ND	
	A17	Micrococci	(+)		ND		ND	
	A18	Bacilli	(+)			(+)	(+)	
	A19	Long chain bacilli		(+)	(+)		(+)	
	A20	Cocci		(+)		(+)	(+)	
%	30,3%	/	70%	30%	14,3%	85,7%	88,24%	11,8%

Table IX: Phenotypic characteristics of bacteria found in samples from the Mostaganem region

Lot	Isolates	Shape	Gram positive	Gram negative	Oxidase positive	Oxidase negative	Catalase positive	Catalase negative
Mostaganem	A21	ND	ND		ND		ND	
	A22	Bacilli	(+)		(+)		(+)	
	A23	Bacilli	(+)			(+)	(+)	
	A24	Bacilli	(+)			(+)	(++)	
	A25	Bacilli	(+)			(+)	(++++)	
	A26	Large bacilli	(+)		(+)		(+)	
	A27	Bacilli	(+)		(+)		(++)	
	A28	ND	ND		(+)		(++)	
	A29	Cocci		(+)		(+)	(+++)	
	A30	Small chain bacilli	(+)			(+)		(+)
	A31	ND	ND		ND		ND	
	A32	Bacilli	(+)		ND		(++)	
	A33	Chain bacilli	(+)		ND		(++)	
	A34	Bacilli	(+)			(+)	(+++)	
	A35	Cocci	(+)			(+)	(+++)	
	A36	ND	ND		ND		(++)	
	A37	Cocci	(+)		ND		(+++)	
	A38	Bacilli	(+)			(+)	(+)	
	A39	Bacilli	(+)		ND		(+)	
	A40	Cocci	(+)		ND		(+)	
	A41	ND	ND		ND		(+)	
	A42	ND	ND		ND		(+)	
	A43	Cocci	(+)		ND			(+)
	A44	Small bacilli		(+)	ND			(+)
	A45	Bacilli	(+)		ND		(++)	
	A46	Long bacilli	(+)		ND		(++)	
	A47	Bacilli	(+)		ND		(+)	
	A48	Large bacilli	(+)		ND		(++)	
	A49	Cocci	(+)		ND			(+)
	A50	Chain bacilli	(+)		ND		(++)	
	A51	Cocci	(+)		ND		(+++)	
	A52	Cocci	(+)		ND		(++)	
%	63,6%	/	92,3%	7,7%	33,33%	66,66%	86,7%	13,33%

Table X: Phenotypic characteristics of bacteria found in samples from the Ouled Djellal region

Lot	Isolates	Shape	Gram positive	Gram negative	Oxidase positive	Oxidase negative	Catalase positive	Catalase negative
Ouled Djellal	A53	Bacilli	(+)			(+)	(+++)	
	A54	Bacilli	(+)			(+)	(+++)	
	A55	Fine bacilli	(+)			(+)	(+++)	
	A56	Bacilli	(+)		(+)		(++)	
	A57	Small bacilli		(+)	ND		(++)	
	A58	Bacilli	(+)			(+)	(+)	
	A59	Bacilli	(+)		ND		(+)	
	A60	Bacilli	(+)			(+)	(+)	
	A61	Bacilli	(+)			(+)	(+)	
	A62	Bacilli	(+)			(+)	(+)	
	A63	Small bacilli		(+)	ND			(+)
	A64	Bacilli		(+)		(+)	(++)	
	A65	Small bacilli	(+)			(+)	(++)	
	A66	Large bacilli	(+)			(+)	(+)	
%	21,21%	/	78,6%	21,4%	9,1%	90,9%	92,9%	7,1%

IV.3. Genotypic characterization

Among the 66 isolates, we successfully sequenced the 16S rRNA gene for 49 isolates. Specifically, we identified 14 isolates from bees in the Ouled Djellal region, which accounted for 21.21% of the total isolates (refer to table IX). Additionally, we found 20 isolates from bees in the Bouira region; constituting 30.3% of the total isolates (refer to table VIII). Furthermore, 32 isolates were obtained from bees in the Mostaganem province; representing 63.6% of the total isolates (refer to Table IX).

As previously mentioned the studied DNA sequences are 200 bp in length and are specifically targeted by primers designed for the V3 and V4 regions. We confirmed this specificity by observing the resulting bands after electrophoresis and visualization under UV light using a 1 kb molecular marker. This size reference allowed us to estimate the size of the targeted DNA fragments, which approximately correspond to 200 bp, thus confirming their specific presence in the V3 and V4 regions (See figure 9).

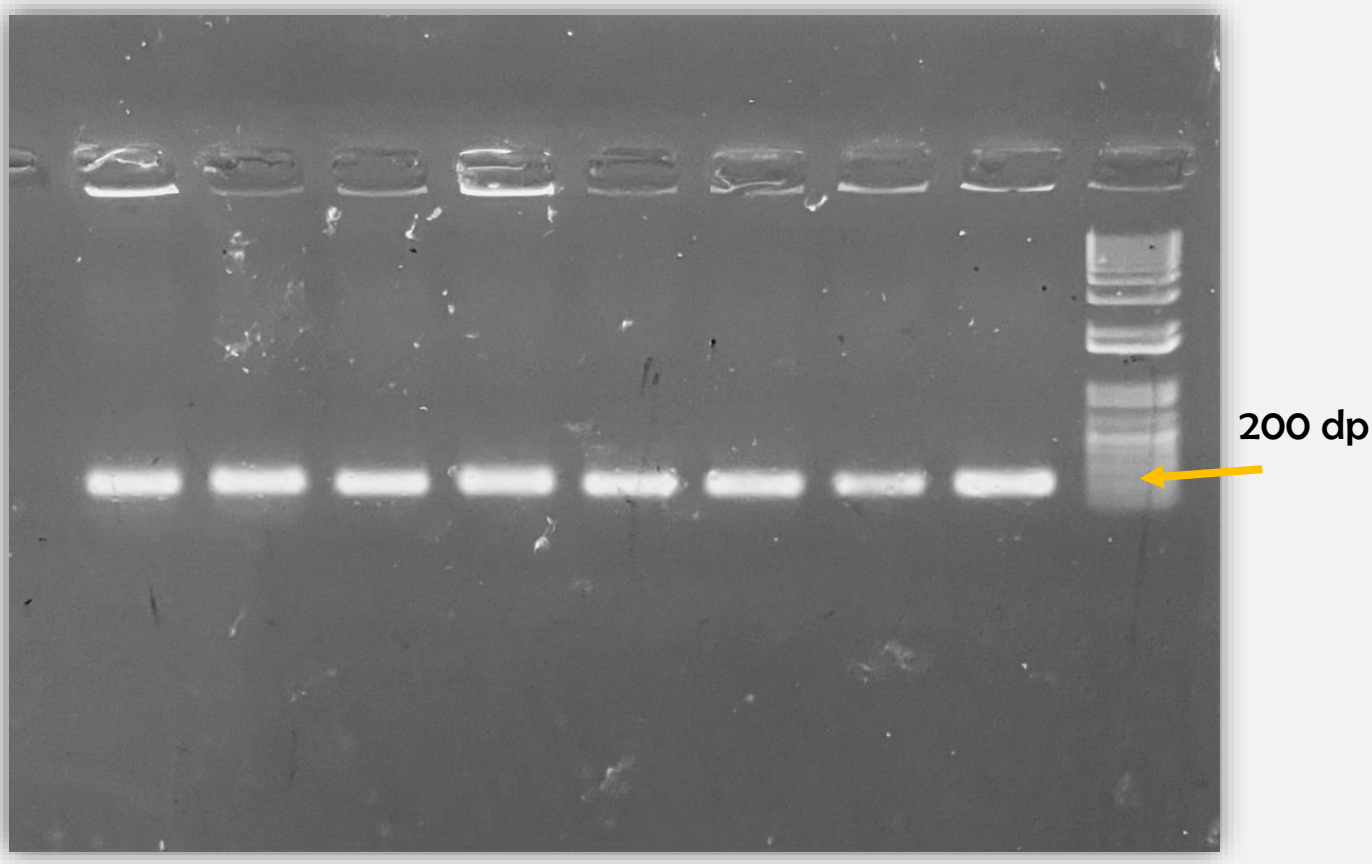


Fig9. Visualization of insert sequences on Electrophoresis gel: Analysis of band patterns

The application of the 16S rRNA PCR technique using the specific primer V3-V4 allow us to carry out the identification of 49 isolates, and confirm the predominant of bacterial belong to *Bacillaceae*, *Microbacteriaceae, Lactobacillaceae, Enterococcaceae, Neisseriaceae*, and *Pectobacteriaceae* in the three areas.

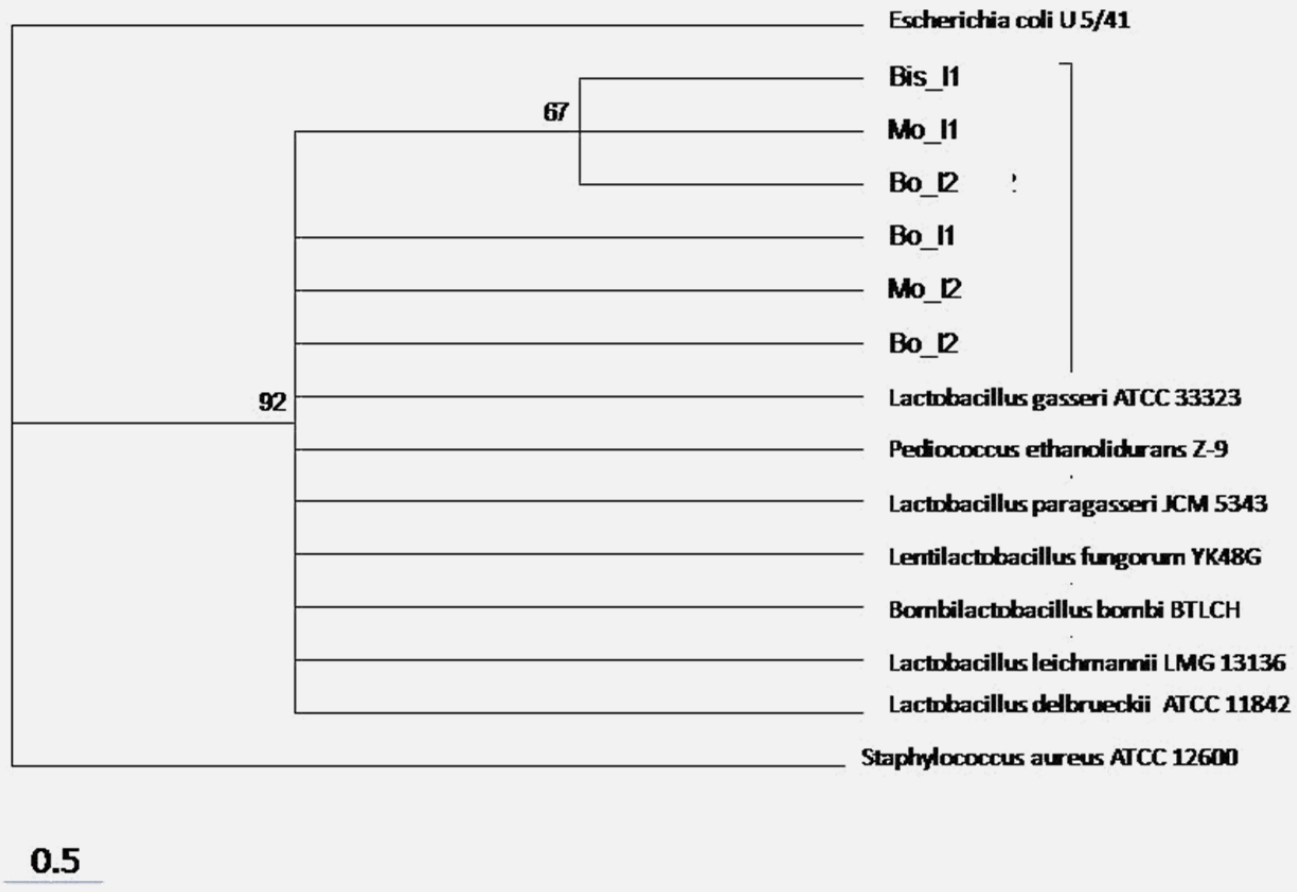


Fig10. Phylogenetic tree representing the dominant family of bacterial isolates in our samples

Figure 10 shows a phylogenetic tree representing the evolutionary relationships between different species of the dominant Lactobacillaceae family, based on 16S rRNA gene sequence analysis. The isolates used in this study come from three distinct sites: Bouira, Mostaganem and Ouled Djellal.

The phylogenetic tree reveals significant diversity among Lactobacillaceae isolates from the three sites. Several distinct clusters are formed, indicating the

presence of different species within this family. Despite belonging to the same family, these isolates represent distinct species.

Apis mellifera a prominent crop pollinator, it expresses an important role in food production and environment preservation. The honey bee, a social insect that harbours a core gut microbiota, which account for a high bacterial proportion [179].

We conducted an initial characterization of the prevalent diversity of the gut microbiota in worker honey bees in northern Algeria, specifically in Bouira (36.374332° N, 4.091804° E), Mostaganem (35.985787° N, 0.420409° E), and Ouled Djellal (34.4334426° N, 5.081450° E). This helped us determine the impact of different geographical areas on bacterial community diversity and the relative abundance of bacterial members.

In the results of our project, we found that bacterial diversity varies between the Bouira, Mostaganem, and Ouled Djellal regions, in contrast to other studies that have revealed relatively consistent gut microbiota across populations and geographies worldwide [180]. Since the gut microbiota has a profound effect on bee health, this study lays the foundation for a better understanding and identification of bee gut flora in different sites in Algeria and thus a better understanding of the impact of biotic and abiotic factors on the variability of this diversity and its health implications.

The obtained results revealed that the total number of bacteria present in honey bees from Ouled Djellal (207 CFU) is higher than in other samples of honey bees from the Bouira and Mostaganem regions. This is presumed to be due to poor release of bacterial cells from glycerol for the initial isolation.

Regarding the diversity of the core microbiota on different selective media, bees from the Mostaganem region exhibited high diversity. Our results align with those published by Tola et al.; bees from coastal regions host relatively high diversity of the central microbiota [181]. This suggests that the coastal ecosystem provides a more favorable habitat for the growth of beneficial bacteria, allowing them to surpass environmental and opportunistic bacteria.

In our study, the composition of the honey bee gut microbiota showed a high prevalence of Gram-positive bacteria. Specifically, we found that 80.3% of the isolates were Gram-positive, while 19.7% were Gram-negative. Furthermore, among all

bacterial isolates (83 isolated strains), 13.25% grew on PCA medium, 22.9% on GYC medium, 20.48% on MRS medium, 21.7% on TSA medium, 13.25% on Chapman medium, and 8.43% on Baird Parker medium. Among this obtained diversity, the approaches applied in our study revealed the abundance of *Lactobacillaceae*, *Bifidobacteriaceae*, and *Neisseriaceae*. Our results do not corroborate the findings of Bleau et al. They observed a negative correlation between *Enterobacteriaceae* and *Lactobacillaceae*, *Orbaceae*, and *Neisseriaceae* [182]. This implies that the honey bee colonies from which we sampled the specimens are in a healthy state, a confirmation provided by Sir BENLAKEHAL.

It is established that in honey bees, there is a positive correlation between *Enterobacteriaceae* and dysbiosis, indicating unhealthy colonies. The beneficial bacteria from the families we found in our samples play a role in the honey bee's innate immune system by promoting the production of antimicrobial peptides. These peptides have the potential to hinder the growth of *Enterobacteriaceae*, which could explain the antagonistic relationship observed compared to other studies [182, 183].

Within our findings related to the Bouira region, we observed a limited incidence of Staphylococci, specifically *Staphylococcus aureus* from benchtop contaminations, and *Staphylococcus epidermidis* from small parts of exoskeletons adhered to the digestive tubes of our bee samples. These observations indicate a restricted presence of these two types of Staphylococci in our sample from the Bouira region.

It is important to note that the results we obtained from the cultivable bacterial diversity were confirmed by a comprehensive taxonomic metagenomic study.

In this study, we provide an initial characterization of the intestinal microbiota of *Apis mellifera* in Algeria. We have shown that the major members of the bee's intestinal microbiota can vary depending on the environmental conditions in which the bee is situated. Our findings highlight the importance of future studies on the intestinal microbiota of bees in Algeria, which will contribute to understanding the role of individual members and the overall community in bee health. A better understanding of this bacterial biodiversity will not only continue to position the honeybee as an important model for research on the intestinal microbiota but could also help address global challenges such as bee decline. Additionally, this understanding will help

establish the honeybee as a crucial ecological bio-indicator for assessing ecosystem health and improving pollination services.

Chapter V
Conclusion

The aim of our research is to investigate the diversity of the intestinal microbiome of honeybees in different regions of Algeria, namely Bouira, Mostaganem, and Ouled Djellal, representing different ecosystems. Our investigations have revealed a significant microbial diversity. According to the obtained results, we have observed a high proportion of isolated Gram-positive bacteria, which is confirmed by the genotypic approaches following 16S rRNA PCR methods. Furthermore, these methods have also demonstrated that the intestinal tract of *Apis mellifera* can harbor a substantial proportion of bacteria belonging to the *genera Bacillaceae, Microbacteriaceae, Lactobacillaceae, Enterococcaceae, Neisseriaceae, and Pectobacteriaceae*.

The results we obtained unveil the variability of the intestinal microbiota of bees from one region to another, highlighting the influence of the ecosystem on this diversity. Moreover, the results indicate the good health of our honeybee samples, as evidenced by the dominance of Gram-positive bacteria, particularly the species *Lactobacillus.* Once on the fields we confirmed our finding by examining and looking on the history of the Sir BENLAKEHAL's honeybee.

Given the important role of honeybees in ecosystem preservation, the study of the intestinal microbiome of *Apis mellifera* has attracted the attention of researchers worldwide. Many of them emphasize the diversity and mutualistic relationship between the host and its microbiome.

In Algeria, research on the intestinal microbiota of honeybees is still limited. Therefore, it is crucial for us to advance and identify the bacterial species hosted by *Apis mellifera*, as well as to define the relationship between bacterial species, their roles, and their effects on the physiology and immunity of Apis mellifera. This research will provide insights into the intestinal microbiota of humans and serve as a significant animal model.

Bibliographic references

[1] Kwong, W. K., & Moran, N. A. (2016). Gut microbial communities of social bees. Nature reviews. Microbiology, 14(6), 374–384.

[2] Guo, J., Wu, J., Chen, Y., Evans, J. D., Dai, R., Luo, W., & Li, J. (2015). Characterization of gut bacteria at different developmental stages of Asian honey bees, Apis cerana. Journal of invertebrate pathology, 127, 110-114.

[3] Romero, S., Nastasa, A., Chapman, A., Kwong, W. K., & Foster, L. J. (2019). The honey bee gut microbiota: strategies for study and characterization. Insect molecular biology, 28(4), 455-472.

[4] Anjum, S. I., Shah, A. H., Aurongzeb, M., Kori, J., Azim, M. K., Ansari, M. J., & Bin, L. (2018). Characterization of gut bacterial flora of Apis mellifera from north-west Pakistan. Saudi journal of biological sciences, 25(2), 388-392.

[5] Jones, J. C., Fruciano, C., Hildebrand, F., Al Toufalilia, H., Balfour, N. J., Bork, P., ... & Hughes, W. O. (2018). Gut microbiota composition is associated with environmental landscape in honey bees. Ecology and evolution, 8(1), 441-451.

[6] Meradji, M., Bachtarzi, N., Mora, D., & Kharroub, K. (2023). Characterization of Lactic Acid Bacteria Strains Isolated from Algerian Honeybee and Honey and Exploration of Their Potential Probiotic and Functional Features for Human Use. Foods, 12(12), 2312.

[7] ROMAN, P. 2009. Les abeilles et la fabrication du miel, Éd. de l'Astronome.

[8] CLÉMENT Henri et coll, Le traité Rustica de l’apiculture, Paris, Éditions Rustica, 2e édition, 2006, p.12.

[9] Hilmi M, Bradbear N & Mejia D (2012). Beekeeping and sustainable livelihoods - Food and Agriculture Organization of the United States.

[10] Shihao Dong, Tao Lin, James C. Nieh, Ken Tan. Social signal learning of the waggle dance in honey bees. Science, 2023 DOI: 10.1126/science.ade1702.

[11] Glenny W., Cavigli I., Daughenbaugh K.F., Radford R., Kegley S.E., Flenniken M.L. Honey bee (Apis mellifera) colony health and pathogen composition in migratory beekeeping operations involved in California almond pollination. PLoS ONE. 2017;12:e0182814.

[12] Hewlett S.E., Wareham D.M., Barron A.B. Honey bee (Apis mellifera) sociability and nestmate affiliation are dependent on the social environment experienced post-eclosion. J. Exp. Biol. ;221:eb173054.

[13] Chauvin, P. R. (1987). La ruche et l'homme. Fance: CALMANN-LEVY

[14] Comparison studies of instrumentally inseminated and naturally mated honey bee queens and factors affecting their performance. Apidologie, 38, 390–410.

[15] El-Niweiri, M. A. A., & Moritz, R. F. A. (2011). Mating in the rain? climatic variance for polyandry in the honeybee (Apis mellifera jemenitica). Population Ecology, 53, 421–427.

[16] Heidinger, I. M. M., Meixner, M. D., Berg, S., & Büchler, R. (2014). Observation of the mating behavior of honey bee (Apis mellifera L.) queens using radio-frequency identification (RFID): Factors influencing the duration and frequency of nuptial flights. Insects, 5, 513–527.

[17] Lensky, Y., & Demter, M. (1985). Mating flights of the queen honey bee (Apis mellifera) in a subtropical climate. Comparative Biochemistry and Physiology A, 81, 229–241.

[18] Tibor, I., Szabo, I., Mills, P. F., & Heikel, D. T. (1987). Effects of honeybee queen weight and air temperature on the initiation of oviposition. Journal of Apicultural Research, 26, 73–78.

[19] Moritz, R. F. A., & Kuhnert, M. (1984). Seasonal effects of artificial insemination of honey bee queens (Apis mellifera L.). Apidologie, 15, 223–231.

[20] Naumann, K., Winston, M. L., Slessor, K. N., Prestwich, G. D., & Webster, F. X. (1991). Production and transmission of honey bee queen (Apis mellifera L.) mandibular gland pheromone. Behavioral Ecology and Sociobiology, 29, 321–332.

[21] Queen substance dispersal by messenger workers in honeybee colonies. Behavioral Ecology and Sociobiology, 5, 391–415.

[22] Bonsels, W. (1912). Ein Roman für Kinder (1.-3. Auflage). Berlin, Leipzig: Die Biene Maja und ihre Abenteuer; p. 178 S. 1912. 8°.

[23] Kovac H, Stabentheiner A. Thermografische Messung der Körpertemperatur von abfliegenden und landenden Drohnen und Arbeiterinnen (Apis mellifera carnica Pollm.) am Nesteingang. Mitt. Dtsch. Ges. Allg. Angew. Entomol. 2004;14:463–466.

[24] Stabentheiner A, Kovac H, Schmaranzer S. Thermal Behaviour of Honeybees During Aggressive Interactions. Ethology. 2007;113:995–1006

[25] Amiri E., Strand M.K., Rueppell O., Tarpy D.R. Queen quality and the impact of honey bee diseases on queen health: Potential for interactions between two major threats to colony health. Insects. 2017;8:48.

[26] Lee K.V., Goblirsch M., McDermott E., Tarpy D.R., Spivak M. Is the brood pattern within a honey bee colony a reliable indicator of queen quality? Insects. 2019;10:12.

[27] Harrison JM. Roles of individual honeybee workers and drones in colonial thermogenesis. J. Exp. Biol. 1987;129:53–61.

[28] (Glenny W., Cavigli I., Daughenbaugh K.F., Radford R., Kegley S.E., Flenniken M.L. Honey bee (Apis mellifera) colony health and pathogen composition in migratory beekeeping operations involved in California almond pollination. PLoS ONE. 2017;12:e0182814.

[29] Seeley, Thomas. (1982). Adaptive significance of the age polyethism schedule in honeybee colonies. Behavioral Ecology and Sociobiology. 11. 287-293.

[30] Johnson BR. Division of labor in honeybees: form, function, and proximate mechanisms. Behav Ecol Sociobiol. 2010;64(3):305-316.

[31] Hewlett S.E., Wareham D.M., Barron A.B. Honey bee (Apis mellifera) sociability and nestmate affiliation are dependent on the social environment experienced post-eclosion. J. Exp. Biol. 2017;221:eb173054.

[32] Villalba A., Maggi M., Ondarza P.M., Szawarski N., Miglioranza K.S.B. Influence of land use on chlorpyrifos and persistent organic pollutant levels in honey bees. Bee bread and honey: Beehive exposure assessment. Sci. Total Environ. 2020;713:136554.

[33] Djema, O., & Djouad, L. (2020). Miel: Composition, propriétés et utilisation en industrie alimentaire (Doctoral dissertation, Université Mouloud Mammeri).

[34] Lechaux, D. (2013). Le miel et la cicatrisation des plaies.

[35] El-Seedi, H. R., Eid, N., Abd El-Wahed, A. A., Rateb, M. E., Afifi, H. S., Algethami, A. F., ... & Khalifa, S. A. (2022). Honey bee products: Preclinical and clinical studies of their anti-inflammatory and immunomodulatory properties. Frontiers in Nutrition, 8, 761267.

[36] MacInnis, G., & Forrest, J. R. (2019). Pollination by wild bees yields larger strawberries than pollination by honey bees. Journal of Applied Ecology, 56(4), 824-832.

[37] Ollerton, J. Pollinator Diversity: Distribution, Ecological Function, and Conservation. Annu. Rev. Ecol. Evol. Syst. 2017, 48, 353–376.

[38] Ollerton, J.; Winfree, R.; Tarrant, S. How many flowering plants are pollinated by animals? Oikos 2011, 120, 321–326.

[39] Potts, S.G.; Petanidou, T.; Roberts, S.; O'Toole, C.; Hulbert, A.; Willmer, P. Plant-pollinator biodiversity and pollination services in a complex Mediterranean landscape. Biol. Conserv. 2006, 129, 519–529.

[40] Requier, F.; Garnery, L.; Kohl, P.L.; Njovu, H.K.; Pirk, C.W.W.; Crewe, R.M.; Steffan-Dewenter, I. The Conservation of Native Honey Bees Is Crucial. Trends Ecol. Evol. 2019, 34, 789–798.

[41] Fontana, P.; Costa, C.; Di Prisco, G.; Ruzzier, E.; Annoscia, D.; Battisti, A.; Caoduro, G.; Carpana, E.; Contessi, A.; Dal Lago, A.; et al. Appeal for biodiversity protection of native honey bee subspecies of Apis mellifera in Italy (San Michele all'Adige declaration). Bull. Insectology 2018, 71, 257–271.

[42] Hung, K.-L.J.; Kingston, J.M.; Albrecht, M.; Holway, D.A.; Kohn, J.R. The worldwide importance of honey bees as pollinators in natural habitats. Proc. R. Soc. B Biol. Sci. 2018, 285, 20172140.

[43] Hung K.J., Kingston J.M., Albrecht M., Holway D.A., Kohn J.R. The worldwide importance of honey bees as pollinators in natural habitats. Proc. Bio. Sci. 2018;285:20172140. doi: 10.1098/rspb.2017.2140.

[44] Ollerton, J.; Winfree, R.; Tarrant, S. How many flowering plants are pollinated by animals? Oikos 2011, 120, 321–326.

[45] Földesi, R.; Howlett, B.G.; Grass, I.; Batáry, P. Larger pollinators deposit more pollen on stigmas across multiple plant species—A meta-analysis. J. Appl. Ecol. 2021, 58, 699–707.

[46] Garibaldi, L.A.; Steffan-Dewenter, I.; Winfree, R.; Aizen, M.A.; Bommarco, R.; Cunningham, S.A.; Kremen, C.; Carvalheiro, L.G.; Harder, L.D.; Afik, O.; et al. Wild Pollinators Enhance Fruit Set of Crops Regardless of Honey Bee Abundance. Science 2013, 339, 1608–1611.

[47] Fontana, P., Costa, C., Di Prisco, G., Ruzzier, E., Annoscia, D., Battisti, A., Caoduro, G., Carpana, E., Contessi, A., Dal Lago, A., et al. (2018). Appeal for biodiversity protection of native honey bee subspecies of Apis mellifera in Italy (San Michele all'Adige declaration). Bulletin of Insectology, 71, 257-271..

[48] Hung, K.-L.J.; Kingston, J.M.; Albrecht, M.; Holway, D.A.; Kohn, J.R. The worldwide importance of honey bees as pollinators in natural habitats. Proc. R. Soc. B Biol. Sci. 2018, 285, 20172140.

[49] Mallinger, R.E.; Gaines-Day, H.R.; Gratton, C. Do managed bees have negative effects on wild bees?: A systematic review of the literature. PLoS ONE 2017, 12, e0189268.

[50] Ball D.W. The chemical composition of honey. J. Chem. Edu. 2007;84:1643. doi: 10.1021/ed084p1643.

[51] LiverTox: Clinical and Research Information on Drug-Induced Liver Injury [Internet]. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012-. Bee products: beeswax, bee pollen, propolis. 2022 May 1. PMID: 35593876.

[52] Campos, M. G., Bogdanov, S., de Almeida-Muradian, L. B., Szczesna, T., Mancebo, Y.,Frigerio, C., et al. (2008). Pollen composition and standardisation of analytical methods. Journal of Apicultural Research, 47, 154–161.

[53] Ghosh, S., & Jung, C. (2017). Nutritional value of beecollected pollens of hardy kiwi, Actinidiaarguta (Actinidiaceae) and oak, Quercus sp. (Fagaceae). Journal of Asia-Pacific Entomology, 20, 245–251.

[54] Mărgăoan, R., Mărghitaş, L. A., Dezmirean, D. S., Dulf, F. V., Bunea, A., Socaci, S. A., et al. (2014). Predominant and secondary pollen botanical origins influence the carotenoid and fatty acid profile in fresh honeybee-collected pollen. Journal of Agricultural and Food Chemistry, 62, 6306–6316.

[55] Melo, A. A. M., Estevinho, M. L. M. F., Sattler, J. A. G., Souza, B. R., da Silva Freitas, A., Barth, O. M., et al. (2016). Effect of processing conditions on characteristics of de-hydrated bee-pollen and correlation between quality parameters. LWT-Food Scienceand Technology, 65, 808–815.

[56] Thakur, M., & Nanda, V. (2018b). Exploring the physical, functional, thermal, and tex-tural properties of bee pollen from different botanical origins of India. Journal of Food Process Engineeringe12935.

[57] Steven, A. (2014). Pollen – a microscopic wonder of plant kingdom. International Jounal of Advanced Research in Biological Sciences, 1, 45–62.

[58] Clarke, D., Morley, E., & Robert, D. (2017). The bee, the flower, and the electric field: Electric ecology and aerial electroreception. Journal of Comparative Physiology. A, Neuroethology, Sensory, Neural, and Behavioral physiology, 203, 737–748.

[59] Di Pasquale, G., Salignon, M., Le Conte, Y., Belzunces, L. P., Decourtye, A., Kretzschmar, A., & Alaux, C. (2013). Influence of pollen nutrition on honey bee health: Do pollen quality and diversity matter? PloS One, 8.

[60] Vezeteu T.V., Bobiş O., Moritz R.F.A., Buttstedt A. Food to some. poison to others—honeybee royal jelly and its growth inhibiting effect on European Foulbrood bacteria. MicrobiologyOpen. 2016;6:e00397

[61] Melliou E., Chinou I. Studies in Natural Products Chemistry. Vol. 43. Elsevier; Amsterdam, The Netherlands: 2014. Chapter 8—Chemistry and bioactivities of royal jelly; pp. 261–290.

[62] Melliou, E.; Chinou, I. Chemistry and Bioactivities of Royal Jelly. J. Agric. Food. Chem. 2005, 53, 261–290.

[63] Ahmad, S.; Campos, M.G.; Fratini, F.; Altaye, S.Z.; Li, J. New Insights into the Biological and Pharmaceutical Properties of Royal Jelly. Int. J. Mol. Sci. 2020, 21, 382.

[64] Uversky, V.N.; Albar, A.H.; Khan, R.H.; Redwan, E.M. Multifunctionality and intrinsic disorder of royal jelly proteome. Proteomics 2021, 21, 2000237.

[65] Melliou, E.; Chinou, I. Chemistry and Bioactivity of Royal Jelly from Greece. J. Agric. Food Chem. 2005, 53, 8987–8992.

[66] Salazar-Olivo L.A., Paz-González V. Screening of biological activities present in honeybee (Apis mellifera) royal jelly. Toxicol. In Vitro. 2005;19:645–651.

[67] Guo, J.; Wang, Z.; Chen, Y.; Cao, J.; Tian, W.; Ma, B.; Dong, Y. Active components and biological functions of royal jelly. J. Funct. Foods 2021, 82, 104514.

[68] Papa, G., Maier, R., Durazzo, A., Lucarini, M., Karabagias, I. K., Plutino, M., Bianchetto, E., Aromolo, R., Pignatti, G., Ambrogio, A., Pellecchia, M., & Negri, I. (2022). The Honey Bee Apis mellifera: An Insect at the Interface between Human and Ecosystem Health. Biology, 11(2), 233.

[69] Damodaran, T. Propolis. In Nutraceuticals; Elsevier: Amsterdam, The Netherlands, 2021; pp. 795–812.

[70] Ghisalberti, E.L. Propolis: A Review. Bee World 1979, 60, 59–84.

[71] Miklos, G. L. G. & Maleszka, R. Epigenomic communication systems in humans and honey bees: From molecules to behavior. Horm Behav 59, 399–406 (2011).

[72] Maleszka, R. The social honey bee in biomedical research: realities and expectations. Drug Discovery Today: Disease Models 12, 7–13 (2014)

[73] Hrassnigg, N. & Crailsheim, K. Differences in drone and worker physiology in honeybees (Apis mellifera). Apidologie 36, 255–277 (2005).

[74] (Ashby R, Forêt S, Searle I, Maleszka R. MicroRNAs in Honey Bee Caste Determination. Sci Rep. 2016 Jan 7;6:18794.

[75] Kucharski, R., Maleszka, J., Foret, S. & Maleszka, R. Nutritional control of reproductive status in honeybees via DNA methylation. Science 319, 1827–1830 (2008).

[76] Foret, S., et al. (2012). DNA methylation dynamics, metabolic fluxes, gene splicing, and alternative phenotypes in honey bees. Proceedings of the National Academy of Sciences of the United States of America, 109, 4968-4973.

[77] Spannhoff, A., et al. (2011). Histone deacetylase inhibitor activity in royal jelly might facilitate caste switching in bees. EMBO Reports, 12, 238-243.

[78] Kucharski, R., Foret, S., & Maleszka, R. (2015). EGFR gene methylation is not involved in Royalactin controlled phenotypic polymorphism in honey bees. Scientific Reports, 5, 14070.

[79] Greiner, T., & Bäckhed, F. (2011). Effects of the gut microbiota on obesity and glucose homeostasis. Trends in Endocrinology & Metabolism, 22, 117-123.

[80] LeBlanc, J. G., et al. (2013). Bacteria as vitamin suppliers to their host: A gut microbiota perspective. Current Opinion in Biotechnology, 24, 160-168.

[81] Gagliardi, A., et al. (2018). Rebuilding the gut microbiota ecosystem. International Journal of Environmental Research and Public Health, 15, 1679.

[82] Agus, A., Planchais, J., & Sokol, H. (2018). Gut microbiota regulation of tryptophan metabolism in health and disease. Cell Host & Microbe, 23, 716-724.

[83] Nicholson, J. K., et al. (2012). Host-gut microbiota metabolic interactions. Science, 336, 1262-1267.

[84] Singh, R., Sharma, P., & Kaur, J. (2020). Gut microbiome: a new organ system in the body. In Microbial Cell Factories and Biotechnology: Principles and Applications (pp. 167-179). IntechOpen.

[85] Engel, P., & Moran, N. A. (2013). The gut microbiota of insects—diversity in structure and function. FEMS Microbiology Reviews, 37(5), 699-735.

[86] Kwong, W. K., & Moran, N. A. (2016). Gut microbial communities of social bees. Nature Reviews Microbiology, 14(6), 374-384.

[87] Kwong, W. K., Medina, L. A., & Moran, N. A. (2017). Beneficial microbes defend against a host's systemic oxidative stress. Proceedings of the National Academy of Sciences, 114(36), E7463-E7470.

[88] Raymann, K., Coon, K. L., & Moran, N. A. (2018). Glyphosate perturbs the gut microbiota of honey bees. Proceedings of the National Academy of Sciences, 115(41), 10305-10310.

[89] Raymann, K., Bobay, L. M., & Moran, N. A. (2017). Antibiotics reduce genetic diversity and frequencies of core taxa in the honeybee gut microbiome. Molecular Ecology, 26(23), 4855-4865.

[90] Li, J. H., et al. (2019). Gut microbiota composition and functional capacity of Africanized honey bees (Apis mellifera) from distinct geographic locations. Microbial Ecology, 78(4), 833-844.

[91] Wu, Y., et al. (2019). Neonicotinoid-induced impairment of odor coding in the honeybee. Current Biology, 29(13), 2347-2353.

[92] Motta, E. V., Raymann, K., & Moran, N. A. (2018). Glyphosate perturbs the gut microbiota of honey bees. Royal Society Open Science, 5(12), 180091.

[93] Retschnig, G., et al. (2017). Inside honeybee hives: impact of natural propolis on the ectoparasitic mite Varroa destructor and viruses. Journal of Insect Physiology, 96, 40-50.

[94] Moran, N. A., Hansen, A. K., Powell, J. E., & Sabree, Z. L. (2012). Distinctive gut microbiota of honey bees assessed using deep sampling from individual worker bees. PLoS One, 7(4), e36393.

[95] Potts, S. G., Imperatriz-Fonseca, V., Ngo, H. T., Aizen, M. A., Biesmeijer, J. C., Breeze, T. D., ... & Vanbergen, A. J. (2016). Safeguarding pollinators and their values to human well-being. Nature, 540(7632), 220-229.

[96] Zheng, H., Powell, J. E., Steele, M. I., Dietrich, C., & Moran, N. A. (2017). Honeybee gut microbiota promotes host weight gain via bacterial metabolism and hormonal signaling. Proceedings of the National Academy of Sciences of the United States of America, 114(18), 4775–4780.

[97] Oertel, E. (1930). Metamorphosis in the honeybee. Journal of Morphology, 50, 295–339.

[98] Martinson, V. G., Moy, J., & Moran, N. A. (2012). Establishment of characteristic gut bacteria during development of the honeybee worker. Applied and Environmental Microbiology, 78(8), 2830–2840.

[99] Jay, S. C. (2015). The development of honeybees in their cells. Journal of Apicultural Research, 2, 117–134.

[100] Martinson, V. G., Moy, J., & Moran, N. A. (2012). Establishment of characteristic gut bacteria during development of the honeybee worker. Applied and Environmental Microbiology, 78(8), 2830–2840.

[101] White, P. B. (1921). The normal bacterial flora of the bee. Journal of Pathology and Bacteriology, 24, 64–78.

[102] Gilliam, M. (1971). Microbial sterility of the intestinal content of the immature honey bee, Apis mellifera. Annals of the Entomological Society of America, 64, 315–316.

[103] Schwarz, R. S., Moran, N. A., & Evans, J. D. (2016). Early gut colonizers shape parasite susceptibility and microbiota composition in honey bee workers. Proceedings of the National Academy of Sciences of the United States of America, 113(34), 9345–9350.

[104] Powell, J. E., Martinson, V. G., Urban-Mead, K., & Moran, N. A. (2014). Routes of acquisition of the gut microbiota of the honey bee Apis mellifera. Applied and Environmental Microbiology, 80(23), 7378–7387.

[105] Alam, A., Musarat, L., Wazed, A., & Hemmick, L. (n.d.). What are the Microbiome Profiles of Bees in a Nonproductive Hive? Science Education: SEPA. Mentor: Dr. Lucinda Hemmick. Longwood High School, Middle Island, NY. CSH Press. Barcode Long Island.

[106] Rangberg, A.; Diep, D.B.; Rudi, K.; Amdam, G.V. Paratransgenesis: An Approach to Improve Colony Health and Molecular Insight in Honey Bees (Apis mellifera)? Integr. Comp. Biol. 2012, 52, 89.

[107] Miller, D.L.; Parish, A.J.; Newton, I.L. Transitions and transmission: Behavior and physiology as drivers of honey bee-associated microbial communities. Curr. Opin. Microbiol. 2019, 50, 1–7.

[108] Raymann, Kasie and Moran, Nancy A. “The role of the gut microbiome in health and disease of adult honey bee workers.” Current Opinion in Insect Science 2018, 26:97-104.

[109] Moran NA, Hansen AK, Powell JE, Sabree ZL. Distinctive gut microbiota of honey bees assessed using deep sampling from individual worker bees. PloS One. 2012;7:e36393. 2012.

[110] Jeyaprakash A, Hoy MA, Allsopp MH. Bacterial diversity in worker adults of Apis mellifera capensis and Apis mellifera scutellata (Insecta: Hymenoptera) assessed using 16S rRNA sequences. J Invertebr Pathol. 2003;84:96–103.

[111] Corby-Harris, V., Maes, P., & Anderson, K. E. (2014). The bacterial communities associated with honey bee (Apis mellifera) foragers. PLoS ONE, 9(4), e95056.

[112] Zheng, H., Powell, J. E., Steele, M. I., Dietrich, C., & Moran, N. A. (2017). Honeybee gut microbiota promotes host weight gain via bacterial metabolism and hormonal signaling. Proceedings of the National Academy of Sciences, 114(20), 4775-4780.

[113] Jones, J. C., Fruciano, C., Hildebrand, F., Al Toufalilia, H., Balfour, N. J., Bork, P., ... Hughes, W. O. (2017). Gut microbiota composition is associated with environmental landscape in honey bees. Ecology and Evolution, 8(1), 441-451.

[114] Tola, Y. H., Waweru, J. W., Hurst, G. D., Slippers, B., & Paredes, J. C. (2020). Characterization of the Kenyan honey bee (Apis mellifera) gut microbiota: A first look at tropical and Sub-Saharan African bee associated microbiomes. Microorganisms, 8(2), 172.

[115] Bleau, N., Bouslama, S., Giovenazzo, P., & Derome, N. (2020). Dynamics of the honeybee (Apis mellifera) gut microbiota throughout the overwintering period in Canada. Microorganisms, 8(9), 1146.

[116] Kwong, W. K., & Moran, N. A. (2016). Gut microbial communities of social bees. Nature Reviews Microbiology, 14(6), 374-384.

[117] Kešnerová, L., Emery, O., Troilo, M., Liberti, J., Erkosar, B., & Engel, P. (2020). Gut microbiota structure differs between honeybees in winter and summer. The ISME Journal, 14(3), 801-814.

[118] Dong, Z. X., Li, H. Y., Chen, Y. F., Wang, F., Deng, X. Y., Lin, L. B., ... & Guo, J. (2020). Colonization of the gut microbiota of honey bee (Apis mellifera) workers at different developmental stages. Microbiological Research, 231, 126370.

[119] Kešnerová, L., Moritz, R., & Engel, P. (2016). Bartonella apis sp. nov., a honey bee gut symbiont of the class Alphaproteobacteria. International Journal of Systematic and Evolutionary Microbiology, 66(2), 414-421.

[120] Hilgarth, M., Redwitz, J., Ehrmann, M. A., Vogel, R. F., & Jakob, F. (2021). Bombella favorum sp. nov. and Bombella mellum sp. nov., two novel species isolated from the honeycombs of Apis mellifera. International Journal of Systematic and Evolutionary Microbiology, 71(4), 004908.

[121] Corby-Harris, V., et al. (2014). Origin and effect of Alpha 2.2 Acetobacteraceae in honey bee larvae and description of Parasaccharibacter apium gen. nov., sp. nov. Applied and Environmental Microbiology, 80(23), 7460-7472.

[122] Anderson, K. E., et al. (2013). Microbial ecology of the hive and pollination landscape: bacterial associates from floral nectar, the alimentary tract and stored food of honey bees (Apis mellifera). PloS ONE, 8(12), e83125.

[123] Anderson, K. E., et al. (2014). Hive-stored pollen of honey bees: many lines of evidence are consistent with pollen preservation, not nutrient conversion. Molecular Ecology, 23(22), 5904-5917.

[124] Anderson, K. E., Ricigliano, V. A. (2017). Honey bee gut dysbiosis: a novel context of disease ecology. Current Opinion in Insect Science, 22, 125-132.

[125] Ludvigsen, J., et al. (2015). Shifts in the midgut/pyloric microbiota composition within a honey bee apiary throughout a season. Microbes and Environments, 30(3), 235-244.

[126] Engel, P., Bartlett, K. D., & Moran, N. A. (2015). The Bacterium Frischella perrara Causes Scab Formation in the Gut of its Honeybee Host. mBio, 6(3), e00193-15.

[127] Ellis, J. D. (2015). The internal anatomy of the honey bee. Dadant & Sons.

[128] Raymann, K., & Moran, N. A. (2018). The role of the gut microbiome in health and disease of adult honey bee workers. Current Opinion in Insect Science, 26, 97-104.

[129] Hroncova, Z., et al. (2015). Variation in honey bee gut microbial diversity affected by ontogenetic stage, age, and geographic location. PloS ONE, 10(3), e0118707.

[130] Kapheim, K. M., et al. (2015). Caste-specific differences in hindgut microbial communities of honey bees (Apis mellifera). PloS ONE, 10(4), e0123911.

[131] Tarpy, D. R., Mattila, H. R., & Newton, I. L. (2015). Development of the honey bee gut microbiome throughout the queen-rearing process. Applied and Environmental Microbiology, 81(9), 3182-3191.

[132] Engel, P., & Moran, N. A. (2013). The gut microbiota of insects - diversity in structure and function. FEMS Microbiology Reviews, 37(5), 699-735.

[133] Yun, J. H., et al. (2014). Insect gut bacterial diversity determined by environmental habitat, diet, developmental stage, and phylogeny of host. Applied and Environmental Microbiology, 80(17), 5254-5264.

[134] Moran, N. A., Hansen, A. K., Powell, J. E., & Sabree, Z. L. (2012). Distinctive gut microbiota of honey bees assessed using deep sampling from individual worker bees. PloS ONE, 7(4), e36393.

[135] Powell, J. E., Martinson, V. G., Urban-Mead, K., & Moran, N. A. (2014). Routes of acquisition of the gut microbiota of the honey bee Apis mellifera. Applied and Environmental Microbiology, 80(23), 7378-7387.

[136] Ludvigsen, J., et al. (2015). Shifts in the midgut/pyloric microbiota composition within a honey bee apiary throughout a season. Microbes and Environments, 30(3), 235-244.

[137] Kwong, W., & Moran, N. (2016). Gut microbial communities of social bees. Nature Reviews Microbiology, 14(6), 374-384.

[138] Tozkar, C. Ö., et al. (2015). Metatranscriptomic analyses of honey bee colonies. Frontiers in Genetics, 6, 100.

[139] Corby-Harris, V., Maes, P., & Anderson, K. E. (2014). The bacterial communities associated with honey bee (Apis mellifera) foragers. PLoS ONE, 9(4), e95056.

[140] Engel, P., & Moran, N. A. (2013). Functional and evolutionary insights into the simple yet specific gut microbiota of the honey bee from metagenomic analysis. Gut Microbes, 4(1), 60-65.

[141] Anderson, K. E., & Ricigliano, V. A. (2017). Honey bee gut dysbiosis: A novel context of disease ecology. Current Opinion in Insect Science, 22, 125-132.

[142] Biedermann, L., & Rogler, G. (2015). The intestinal microbiota: Its role in health and disease. European Journal of Pediatrics, 174(2), 151-167.

[143] Nicholson, J. K., et al. (2012). Host-gut microbiota metabolic interactions. Science, 336(6086), 1262-1267.

[144] Zheng, H., Steele, M. I., Leonard, S. P., Motta, E. V. S., & Moran, N. A. (2018). Honey bees as models for gut microbiota research. Lab Animal, 47(10), 317-325.

[145] Dong, Z. X., et al. (2020). Colonization of the gut microbiota of honey bee (Apis mellifera) workers at different developmental stages. Microbiological Research, 231, 126370.

[146] Pernice, M., Simpson, S. J., & Ponton, F. (2014). Towards an integrated understanding of gut microbiota using insects as model systems. Journal of Insect Physiology, 69, 12-18.

[147] Cianci, R., Pagliari, D., Piccirillo, C. A., Fritz, J. H., & Gambassi, G. (2018). The microbiota and immune system crosstalk in health and disease. Mediators of Inflammation, 2018, 2912539.

[148] Sekirov, I., Russell, S. L., Antunes, L. C. M., & Finlay, B. B. (2010). Gut microbiota in health and disease. Physiological Reviews, 90, 859-904.

[149] Seeley, T. D. (2014). Honeybee Ecology: A Study of Adaptation in Social Life: A Study of Adaptation in Social Life. Princeton University Press.

[150] Shi, W., Syrenne, R., Sun, J.-Z., & Yuan, J. S. (2010). Molecular approaches to study the insect gut symbiotic microbiota at the "omics" age. Insect Science, 17, 199-219.

[151] Zheng, H., Powell, J. E., Steele, M. I., Dietrich, C., & Moran, N. A. (2017). Honeybee gut microbiota promotes host weight gain via bacterial metabolism and hormonal signaling. Proceedings of the National Academy of Sciences, 114, 4775-4780.

[152] Jing, T.-Z., Qi, F.-H., & Wang, Z.-Y. (2020). Most dominant roles of insect gut bacteria: Digestion, detoxification, or essential nutrient provision? Microbiome, 8.

[153] Silva, M. S., Rabadzhiev, Y., Renon Eller, M., Iliev, I., Ivanova, I., & Santana, W. C. (2017). Microorganisms in honey. Honey analysis. IntechOpen, S233-S258.

[154] Pachila, A., Ptaszyńska, A. A., Wicha, M., Oleńska, E., & Małek, W. (2017). Fascinating fructophilic lactic acid bacteria associated with various fructose-rich niches. Annals of the University Mariae Curie-Sklodowska Medica, 72, S41-S50.

[155] Zheng, H., Steele, M. I., Leonard, S. P., Motta, E. V., & Moran, N. A. (2018). Honey bees as models for gut microbiota research. Lab Animal, 47(11), 317-325.

[156] Schwarz, R. S., Moran, N. A., & Evans, J. D. (2016). Early gut colonizers shape parasite susceptibility and microbiota composition in honey bee workers. Proceedings of the National Academy of Sciences, 113, 9345-9350.

[157] Raymann, K., Shaffer, Z., & Moran, N. A. (2017). Antibiotic exposure perturbs the gut microbiota and elevates mortality in honeybees. PLoS Biology, 15, e2001861.

[158] Maes, P. W., Rodrigues, P. A. P., Oliver, R., Mott, B. M., & Anderson, K. E. (2016). Diet-related gut bacterial dysbiosis correlates with impaired development, increased mortality, and Nosema disease in the honeybee (Apis mellifera). Molecular Ecology, 25, 5439-5450.

[159] Azambuja, P., Garcia, E. S., & Ratcliffe, N. A. (2005). Gut microbiota and parasite transmission by insect vectors. Trends in Parasitology, 21, 568-572.

[160] Cirimotich, C. M., Ramirez, J. L., & Dimopoulos, G. (2011). Native microbiota shape insect vector competence for human pathogens. Cell Host & Microbe, 10, 307-310.

[161] Evans, J. D., Aronstein, K., Chen, Y. P., Hetru, C., Imler, J. L., Jiang, H., ... & Hultmark, D. (2006). Immune pathways and defence mechanisms in honey bees Apis mellifera. Insect Molecular Biology, 15, 645-656.

[162] Yiu, J. H. C., Dorweiler, B., & Woo, C. W. (2016). Interaction between gut microbiota and toll-like receptor: From immunity to metabolism. Journal of Molecular Medicine, 95, 13-20.

[163] Jones, J. C., Fruciano, C., Hildebrand, F., Al Toufalilia, H., Balfour, N. J., Bork, P., Engel, P., Ratnieks, F. L. W., & Hughes, W. O. (2017). Gut microbiota composition is associated with environmental landscape in honey bees. Ecology and Evolution, 8, 441-451.

[164] Muñoz-Colmenero, M., Baroja-Careaga, I., Kovačić, M., Filipi, J., Puškadija, Z., Kezić, N., Estonba, A., Büchler, R., & Zarraonaindia, I. (2020). Differences in honey bee bacterial diversity and composition in agricultural and pristine environments—A field study. Apidologie, 51, 1018-1037.

[165] Genersch, E., & Aubert, M. (2010). Emerging and re-emerging viruses of the honey bee (Apis mellifera L.). Veterinary Research, 41(6), 54.

[166] Higes, M., Martín-Hernández, R., Garrido-Bailón, E., García-Palencia, P., Meana, A., & del Nozal, M. J. (2008). Honeybee colony collapse due to Nosema ceranae in professional apiaries. Environmental Microbiology Reports, 1(2), 110-113.

[167] Genersch, E., Forsgren, E., Pentikäinen, J., & Fries, I. (2006). Reclassification of Melissococcus plutonius as a later synonym of Enterococcus plutonius corrig., a cause of European foulbrood of honey bees. Environmental Microbiology, 8(7), 1034-1039.

[168] Ellis, J. D., & Munn, P. A. (2005). The small hive beetle, Aethina tumida: A review. Bee World, 86(1), 21-35.

[169] Bommuraj, V., Chen, Y., Birenboim, M., Barel, S., Shimshoni, J. A. (2021). Concentration- and time-dependent toxicity of commonly encountered pesticides and pesticide mixtures to honeybees (Apis mellifera L.). Chemosphere, 266, 128974.

[170] Fisher, A., DeGrandi-Hoffman, G., Smith, B. H., Johnson, M., Kaftanoglu, O., Cogley, T., Fewell, J. H., & Harrison, J. F. (2021). Colony field test reveals dramatically higher toxicity of a widely used mito-toxic fungicide on honey bees (Apis mellifera). Environmental Pollution, 269, 115964.

[171] Miotelo, L., Mendes Dos Reis, A. L., Malaquias, J. B., Malaspina, O., & Roat, T. C. (2021). Apis mellifera and Melipona scutellaris exhibit differential sensitivity to thiamethoxam. Environmental Pollution, 268, 115770.

[172] Kakumanu, M. L., Reeves, A. M., Anderson, T. D., Rodrigues, R. R., & Williams, M. A. (2016). Honey bee gut microbiome is altered by in-hive pesticide exposures. Frontiers in Microbiology, 7, 1255.

[173] Neumann, P., & Carreck, N. L. (2010). Honey bee colony losses. Journal of Apicultural Research, 49, 1-6.

[174] Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., & Kunin, W. E. (2010). Global pollinator declines: trends, impacts and drivers. Trends in Ecology & Evolution, 25, 345-353.

[175] Clemente, J. C., Ursell, L. K., Parfrey, L. W., & Knight, R. (2012). The impact of the gut microbiota on human health: an integrative view. Cell, 148, 1258-1270.

[176] Watkins, J., & Jian, X. (1997). Cultural methods of detection for microorganisms: recent advances and successes.

[177] Hugenholtz, P., & Tyson, G. W. (2008). Microbiology: metagenomics. Nature, 455(7212), 481-483.

[178] Lapidus, A. L., & Korobeynikov, A. I. (2021). Metagenomic data assembly–the way of decoding unknown microorganisms. Frontiers in Microbiology, 12, 613791.

[179] Corby-Harris, V., Maes, P., & Anderson, K. E. (2014). The bacterial communities associated with honey bee (Apis mellifera) foragers. PloS one, 9(4), e95056.

[180] Moran, N.A.; Hansen, A.K.; Powell, J.E.; Sabree, Z.L. Distinctive gut microbiota of honey bees assessed using deep sampling from individual worker bees. PLoS ONE 2012, 7, e36393.

[181] Tola, Y. H., Waweru, J. W., Hurst, G. D. D., Slippers, B., & Paredes, J. C. (2020). Characterization of the Kenyan Honey Bee (Apis mellifera) Gut Microbiota: A First Look at Tropical and Sub-Saharan African Bee Associated Microbiomes. Microorganisms, 8(11), 1721.

[182] Bleau, N., Bouslama, S., Giovenazzo, P., & Derome, N. (2020). Dynamics of the Honeybee (Apis mellifera) Gut Microbiota Throughout the Overwintering Period in Canada. Microorganisms, 8(8), 1146.

[183] Kwong,W.K.; Mancenido, A.L.; Moran, N.A. Immune system stimulation by the native gut microbiota of honey bees. R. Soc. Open Sci. 2017, 4, 170003.

Electronic references:

Site (01): IllustraScience. (2023). From Les abeilles. Retrieved from https://illustrascience.fr/les-abeilles/.

Supplementary data

Supplementary data 1

The phenotypic and genotypic materials and products used:

- Agarose (itwreagents)
- Electrophoresis screen (Sony)
- Electrophoresis system (tank + generator) (APELEX)
- Electrophoresis transilluminator (Bioblock)
- EtBr (Pasteur)
- Forward primer (WIRAGEN, Algeria)
- Fuchsin (Pasteur)
- Laboratory water bath (MEMMERT)
- Lugol (Pasteur)
- Master Mix PCR (WIRAGEN, Algeria)
- Microwave (Menumaster)
- MilliQ water (Microbiotech)
- Molecular biology hood (Holten)
- Purple dye (Pasteur)
- Reverse primer (WIRAGEN, Algeria)
- The TAE Buffer (LCH chimie)
- Thermocycler PCR (Biometra)
- Vortex (TechnoKartell)

Supplementary data 2

Table I: Bacterial enumeration on PCA medium

Bouira	BOF1	BOF2	BOF3	BOF4	BOF5
10-1	0	0	2	1	14
10-2	0	0	0	6	4
10-3	0	0	contaminants	0	0
10-4	0	0	0	0	0
Mostaganem	ME1	ME2	ME3	ME4	ME5
10-1	0	3	2	21	1
10-2	0	1	3	3	0
10-3	contaminants	33	2	2	0
Ouled Djellal	BID1	BID2	BID3	BID4	BID5
10-1	4	1	23	0	55
10-2	0	0	0	0	0

Review paper

***Apis mellifera* gut microbiome bacteria: harmless or beneficial health effect**

Authors:

Manel BENLAKEHAL [(1)], Katia DJENADI [(2)(1)], Assia CHERIFI [(1)(3)], Anis BERRAH[(4)], Zakia CHERIFI[(1)], Hanane AL AMIR [(4)], Ourdia SADEDDINE ZENNOUCHE [(6)], Badis BENDEDDOUCHE[(4)], Chafika SAYAH-MOUHOUB[(1)(6)].

Affiliations

(1) Faculty of Natural and Life Sciences and Earth Science Akli Mohand Oulhadj University, Bouira (UAMOB), 010000, Algeria.

(2) Applied Biochemistry Laboratory, Faculty of Natural and Life Sciences, Abderrahmane Mira University, Bejaia 06000, Algeria.

(3) Laboratory for the production, protection and safeguard of endangered species and crops. Faculty of Biological Sciences and Agricultural Sciences. Mouloud Mammeri University, Tizi-Ouzou. Algeria

(4) Laboratory of Food, Water, and Environmental Bacteriology, Pasteur Institute of Algiers, 01 Route du Petit Staoueli, Algiers, Algeria.

(5) Laboratory of Applied Zoology and Animal Ecophysiology, Faculty of Natural and Life Science, University´ of Bejaia, Bejaia, Algeria;

(6) Management and evaluation of natural resources and quality assurance. Faculty of Natural and Life Sciences and Earth Science Akli Mohand Oulhadj University, Bouira (UAMOB), 010000, Algeria.

Corresponding author

*k.djenadi@univ-bouira.dz

In Press

Abstract

Apis mellifera is an important pollinator species for wild flora and agricultural production. This small insect is threatened by several factors including high exposition to parasite and pathogen organism. Nowadays numerous scientist highlighted on the fact that microbiome of adult honeybees plays an important role for bee health. The gut of *Apis mellifera* adult workers are dominated by species that can have an impact on metabolism, immune function, growth and development and protection against pathogen. The presence of any other pathogenic bacteria in their gut flora can disturb the physiology and immune system of the honeybees. Overall, we can conclude that gut microbiome has an important role in the honeybee health.

Keywords:

Apis mellifera, gut microbiome, bacterial diversity, honeybee health

In Press

I. Introduction

There are approximately 1.06 million identified species of insects on the Earth's surface. **[1].**These insects play several roles depending mainly on their genetic information and their gut microbiome diversity, which varies according to their social community and certain biotic and abiotic factors. *Apis mellifera* represents one of the most important insects due to its enormous economic value in agriculture. Moreover, it is related to its pollinating action, which ensures the fertilization of more than 80% of the floral species **[2].**In addition, the valuable products provided by this insect: honey, pollen, royal jelly, wax and propolis, which correspond to the important natural products currently used by human for his biological proprieties. The multiple levels at which the honeybee expresses there adaptations to its environment represent one of the richest sources of study and knowledge among all organisms. The obtained results highlighted on diverse perspectives to understand the physiology and preserve this insect "*Apis mellifera*" **[3].** Despite bees' ecological and economic importance, and rising worry over diminishing bee numbers, the function of their gut microbiome in colony health and nutrition remains unclear**.** In the bowel of the honeybee *Apis mellifera* has a distinct microbial community consisting of a taxonomically restricted set of bee-specific social species. Which is important in a range of metabolic processes, the control of many biochemical and physiological systems, and the operation of the bee's immune system, which varies according to its environment and the different factors surrounding it **[4]**

There are numerous studies regarding the ecology, physiology and pathology related to *Apis mellifera*. However, few studies reported as per known on honeybee microbiome especially *A. mellifera*. Therefore, the aim of this review is to provide a brief overview on the

In Press

diversity within *A. mellifera* microbiome, his role, function, and give a highlight on the factors that may influence on the microbiome diversity.

II. Apis mellifera a fascinating insect

III. Throughout history, honeybees have captivated human interest with their remarkable abilities. In addition to being tireless producers of honey and wax, they are also skilled architects and play a crucial role in crop pollination. Even in modern times, bees continue to surprise us with new scientific discoveries.

Apis mellifera is one of the most common floral visitors in natural habitats worldwide, accounting for 13% of floral visits in all networks, and is considered the only visitor to 5% of plant species **[8]**. Honeybees live in big communities with a complex organization in which the colony is mainly composed of a single queen, hundreds of drones (males), and 9000 to 12000 sterile workers whose numbers vary according on the season, **[14 ,15].** Workers are in charge of all tasks that lead to reproduction, including cleaning, larvae feeding, nectar evaporation, and hive maintenance. In particular, they are in charge of foraging to give food and water to the colony **[14].** The functions of the queen are limited exclusively to laying eggs. While the drones copulate with the queen in the air and then die. Drones who have not taken part in the reproductive process are evicted from the hive and die of starvation **[16, 17].**In the simplest terms, the nutrition of bees is based on nectar that provides those carbohydrates and pollen, which presents a source of proteins, lipids and other micronutrients **[18].** Their magical products know bees: namely honey, bee pollen, propolis, beebread, royal jelly, beeswax and bee venom, which play various functions in their life cycle and are considered antimicrobial and anti-inflammatory used in human treatments **[44, 45].**

In Press

IV. Apis mellifera gut microbiome bacterial diversity:

Apis mellifera fascinating insect with an important microbial diversity within his gut. The insect develops massive colonies with thousands of non-breeding female workers, hundreds of male drones, and only one breeding queen **[5].** The honeybees gut has an important role. It contribute in the storage and transport of nectar to the hive. The honeybee microbiota is present in different parts of the gut, including the crop between the esophagus and ventricle **[6, 7].**Based on the results of 16S rDNA gut community and total DNA metagenomic studies, the researchers found that there were primarily nine species of bacteria dispersed in the workers' guts. Five bacterial species have been found in almost all bees, including two

omnipresent Gram-negative species, which are members of the phylum *Proteobacteria* (*Snodgrassella alvi* and *Gilliamella apicola*) **[8, 9, 10]** (See figure 02). Furthermore, two Gram-positive species from the phylum *Firmicutes* are very abundant and widespread (*Lactobacillus Firm*-4 and *Lactobacillus Firm*-5). They occupy the distal rectum **[8, 9].** *Bifidobacterium asteroids* also found in relatively at low abundance compared to others bacterial species **[11, 12].** These are the most essential microorganisms in the honeybee's gut, or the so-called "core bacteria" **[13].** In addition, less abundant or less stable species of the *proteobacteria* phylum such as *Gammaproteobacteria Frischella perrara*; the *AlphaproteobacteriaParasaccharibacter apium*, *Bombella apis Bombella mellum*, *Bombella favorum*, *Bartonella apis* and *Commensalibacter sp* werealso identified**[14, 11, 9, 10, 15, 16]** (See figure 03).As well, two other species from the phylum *Bacteroidetes* have been identified (*Apibacter mensalis* and *Apibacter adventoris*) **[17, 18, 55, 56, 57, 58, 59, 60, 61, 62, 63].**These species are the best-known and most important honeybee gut microbiome **[19].** They we identified followed by two different methods including monoculture and metagenomic methods. The monoculture method consists on taking samples of bees in different hives and at different stages of growth in an aseptic way. Then isolate freshly their bacteria in selective media. After that, identify the isolate following molecularly essays. Once the DNA extracted according to the CTAB-Phenol-Chloroform method, the identification of bacterial isolates is carried out byRNA16S amplification **[20].** While metagenomic method is based on the sequencing of all the genome of, the bacteria harvested fromhoneybee gut. After DNA extraction, the sequencing and targeted amplification of the RNA16S or all the genome carried out following bioinformatics assembly of the sequenced genome(s). Finally,the obtained sequences were analyzed **[21].** The metagenomic method allows us to understand the functional and the genetic evolution of this characteristic intestinal microbiota and to predict the symbiotic capacities of these bacteria in the honeybee **[21].**While, the cultural method consists on the identification of the cultured microorganisms. They represent 1% from the whole gut microbiota and may appear in different forms of colonies and in different aspects. Those characteristics are then complemented by other phenotypic and molecular studies to allow their classification. While the metagenomic method allows us to identify cultured and uncultured microorganisms. In fact, this technic provides us a better identification of the population and therefore a better classification **[22].**

In Press

V. Role and function of *Apis mellifera* gut microbiome :

The importance of the species constituting the gut microbiome has been demonstrated in several studies including the human microbiota. The microbiome known to be involved in most metabolic and immune functions of the body. In fact, we assume that the gut microbiota of honeybee is equally important. Therefore, studies have been based on laboratory experiments that expose bees to various factors and monitoring changes in the quality of the gut microbiome, thus the functioning of its organism. As a result, several important roles have been identified for the bee gut microbiota **[23, 24, 25, 26, 27, 28, 29, 30, 31, 32].**Honeybees obtain all theirs nutrients from a very specific diet, composed exclusively of nectar and pollen. Honeybee gut microbiota metagenomic investigations shown that nutrient biosynthesis and biomass decomposition are two well-established roles of the honeybee gut microbiota, hence its involvement in the catabolism and degradation of these foods **[33].**Nutritional function or nutrient biosynthesis has been extensively studied in experiments involving bees fed an unbalanced diet; low in essential nutrients such as vitamins and amino acids. The results of these investigation show that honeybee endosymbionts contribute in the production of nutrients that are not found in food**.** Regarding biomass catabolism and deconstruction, the release of cellulolytic enzymes is a common trait between the gut microbiome and the bee host. Although, research showing that microbial activity improves the efficiency of these processes **[34].**Genomic and metabolic studies on the microbiome species from honeybee core including, *Lactobacillus*and *Bifidobacterium* species and *G. apicola* indicate theirs abilities to metabolize a wide range of plant carbohydrates and related compounds, such as pectins known as one of the major component of the polleninner wall (Table 1). Zheng and his co-workers revealed that in the intestinal compartment mainly colonized by *G. apicola*¸ an accumulation of galacturonate compound as the major degradation product of pectin is observed. In addition, these intestinal bacteria contribute in the digestion of lipids and proteins as well as to the detoxification of secondary plant compounds **[35, 36].**Zheng and his co-workers studies demonstrated the ability of these microorganisms to digest mainly mannose, which is a major compound of nectar (Table 1). Moreover, due to their fermentation capabilities, intestinal symbionts have an impact on the conversion of plant buds and exudates into propolis and nectar into honey. They are also in charge of the freshness of honey **[37, 38].**

In Press

Recent research has revealed that the gut microbiome of honeybees has a similar role **[39].**It has been shown that disruption of the gut microbiota with the antibiotic tetracycline, honeybees will have a low survival rate due to their increased vulnerability to the opportunistic germ such as *Serratia* **[41].**When the assembly of the gut microbiota is disrupted loads of the parasite *Lormaria passim* increase **[40].**Moreover, feeding aged pollen to honeybee causes increased mortality due to very high *Nosema fungus* loads, as well as significant changes in the composition of gut endosymbiont composition through the development of a significantly dysbiotic microbiome that may decrease gut resistance to intrinsic pathogens **[42].**

These findings reveal a link between gut microbiota and theirs host “honeybees”, as well as pathogen resistance and host advantages. Gut microbiomes have also been found to contribute in the modulation of insect competence by altering the gut environment, to restrict parasite development or to stimulate a host immune response while creating antimicrobial peptides that control parasites and bacterial infections **[43, 44].** The extent to which these effects may act as priming reactions that boost pathogen resistance has not yet proven. In this case, since the microbiota occupies the mucosal immune system, it is in charge of carrying out two opposing functions. It must tolerate the microbiota inhabiting the gut and prevent the induction of harmful systemic immune responses. While at the same time controlling the charge of microorganisms to avoid adverse reactions through a variety of ways. These microbes regulate intestinal homeostasis using substances such as peptidoglycans, lipopolysaccharides, flagellins and others **[45, 46].**The intestinal microbiota participates in the achievement of several objectives, mainly these two above-mentioned functions that are very complementary. The regulation of nutrient digestion and a good physiology only modulate the proper functioning of the microbes and thus a good functioning of the immune system. This relationship influences the host's body size, weight gain, developmental rate, and metabolism, sensitivity to stress, stem cell activity and wing area. All these variables create a good balance in the honeybee's body.

In Press

VI. Factors modulating the microbiome development and functions:

Any species is confronted with a pressure of biotic and abiotic factors. Even, honeybee that occupies such an important place in our natural environment. Numerous factors can potentially influence the communities of microorganisms that make up the honeybee gut

microbiota **[47, 48].**The main factor influencing the honeybee gut microbiome are biotic factors, including the pathogen germs able to induce infections and pathologies. Some of them are parasitic such as Varroa (*Varroa destructor*), others may causes infestation by the small hive beetle (Aethina tumida). Also bacteria such as *Paenibacillus larvae*, *Melissococcus plutonius* and *Paenibacillus alvei*. Moreover, viruses are also responsible of honeybee infections, including the *Sacciform Brood* Virus, the Queen Black Cell Virus and the Chronic Bee Paralysis Virus. In addition, scientists identified fungus able to infect honeybee such as *Nosema ceranae* and *Nosema apis*.

On another hands, abiotic factors are also involved in the gut microbiome modulation. Honeybees are exposed to many pesticides (e.g., chlorothalonil, imidacloprid and coumaphos) through contaminated nectar, pollen and water. Which contribute to significant adverse health effects **[49, 50, 51]** and unfavorable changes in the structure and function of the honeybee microbiome, resulting in a reduction in beneficial gut bacteria effect and an increase in pathogenic microorganisms infections**[52].**Moreover, honeybee faced to other factors that contribute to the weakening of their colonies, including environmental stresses like malnutrition, pollution, lack of vitality and genetic diversity or the depletion of environmental quality, as well as humidity, temperature and light **[53, 54].**

In Press

VII. Conclusion

Honeybees are the main pollinators and have an important economic role, their mortality and morbidity has attracted attention of scientists in order to understand the origin of this decline. Research conducted so far has shed light on the honeybee gut microbiota that plays a similar role to the human gut microbiota. It may be responsible for the quality of life and health of the honeybee. This seems to be influenced by different factors that decrease its efficiency and role in the host. All this finding has motivated researchers to study this microbiota in order to improve bee health and decrease the loss of these important pollinators.

VIII. References

[1] DANKS, Hugh V., SMITH, Andrew BT, FOOTTIT, R. G., et al. Insect biodiversity in the Nearctic region. Insect biodiversity science and society, 2017, vol. 1, p. 47-63

[2] Hung, K-L.J. et al. 2018. The worldwide importance of honey bees aspollinators in natural habitats. Proc. R. Soc. B. 285(1870): 2017–2040.Doi: 10.1098/rspb.2017.2140.

[3] WINSTON, Mark L. The biology of the honey bee. harvard university press, 1991

[4] Engel, P., Martinson, V. G., & Moran, N. A. (2012). Functional diversity within the simple gut microbiota of the honey bee. Proceedings of the National Academy of Sciences, 109(27), 11002-11007.

[5] Glenny, W.; Cavigli, I.; Daughenbaugh, K.F.; Radford, R.; Kegley, S.E.; Flenniken, M.L. Honey bee (Apis mellifera) colony health and pathogen composition in migratory beekeeping operations involved in California almond pollination. PLoS ONE 2017, 12, e0182814.

[6] Vásquez, A.; Forsgren, E.; Fries, I.; Paxton, R.J.; Flaberg, E.; Szekely, L.; Olofsson, T.C. Symbionts as major modulators of insect health: Lactic acid bacteria and honeybees. PLoS ONE 2012, 7, e33188.

[7] Corby-Harris, V.; Snyder, L.A.; Schwan, M.R.; Maes, P.; McFrederick, Q.S.; Anderson, K.E. Origin and effect of Alpha 2.2 Acetobacteraceae in honey bee larvae and description of Parasaccharibacter apium gen. nov., sp. nov. Appl. Environ. Microbiol. 2014, 80, 7460–7472

[8] Zheng, H.; Powell, J.E.; Steele, M.I.; Dietrich, C.; Moran, N.A. Honeybee gut microbiota promotes host weight gain via bacterial metabolism and hormonal signaling. Proc. Natl. Acad. Sci. USA 2017, 114, 4775–4780

[9] Jones, J.C.; Fruciano, C.; Hildebrand, F.; Al Toufalilia, H.; Balfour, N.J.; Bork, P.; Engel, P.; Ratnieks, F.L.W.; Hughes, W.O. Gut microbiota composition is associated with environmental landscape in honey bees. Ecol. Evol. 2017, 8, 441–451.

[10] Tola, Y.H.; Waweru, J.W.; Hurst, G.D.D.; Slippers, B.; Paredes, J.C. Characterization of the Kenyan honey bee (Apis mellifera) gut microbiota: A first look at tropical and Sub-Saharan African bee associated microbiomes. Microorganisms 2020, 8, 1721.

[11] Bleau, N.; Bouslama, S.; Giovenazzo, P.; Derome, N. Dynamics of the honeybee (Apis mellifera) gut microbiota throughout the overwintering period in Canada. Microorganisms 2020, 29, 1146.

In Press

[12] Kwong, W.K.; Moran, N.A. Gut microbial communities of social bees. Nat. Rev. Microbiol. 2016, 14, 374–384.

[13] Kešnerová, L.; Emery, O.; Troilo, M.; Liberti, J.; Erkosar, B.; Engel, P. Gut microbiota structure differs between honeybees in winter and summer. ISME J. 2020, 14, 801–814.

[14] Dong, Z.X.; Li, H.Y.; Chen, Y.F.; Wang, F.; Deng, X.Y.; Lin, L.B.; Zhang, Q.L.; Li, J.L.; Guo, J. Colonization of the gut microbiota of honey bee (Apis mellifera) workers at different developmental stages. Microbiol. Res. 2020, 231, 126370.

[15] Kešnerová, L.; Moritz, R.; Engel, P. Bartonella apis sp. nov., a honey bee gut symbiont of the class Alphaproteobacteria. Int. J. Syst. Evol. Microbiol. 2016, 66, 414–421.

[16] Hilgarth, M.; Redwitz, J.; Ehrmann, M.A.; Vogel, R.F.; Jakob, F. Bombella favorum sp. nov. and Bombella mellum sp. nov., two novel species isolated from the honeycombs of Apis mellifera. Int. J. Syst. Evol. Microbiol. 2021.

[17] Kwong, W.K.; Moran, N.A. Apibacter adventoris gen. nov., sp. nov., a member of the phylum Bacteroidetes isolated from honey bees. Int. J. Syst. Evol. Microbiol. 2016, 66, 1323–1329.

[18] Kwong, W.K.; Steele, M.I.; Moran, N.A. Genome sequences of Apibacter spp., gut symbionts of Asian honey bees. Genome Biol. Evol. 2018, 10, 1174–1179.

[19] Kešnerová L., Emery O., Troilo M., Liberti J., Erkosar B., Engel P. Gut microbiota structure differs between honeybees in winter and summer. ISME J. 2020;14:801–814.

[20] Watkins, J., & Jian, X. (1997). Cultural methods of detection for microorganisms: recent advances and successes.

[21] Lapidus, A. L., & Korobeynikov, A. I. (2021). Metagenomic data assembly–the way of decoding unknown microorganisms. Frontiers in Microbiology, 12, 613791.

[22] Hilton, S. K., Castro-Nallar, E., Pérez-Losada, M., Toma, I., McCaffrey, T. A., Hoffman, E. P., ... & Crandall, K. A. (2016). Metataxonomic and metagenomic approaches vs. culture-based techniques for clinical pathology. Frontiers in microbiology, 7, 484.

In Press

[23] Zheng H., Powell J.E., Steele M.I., Dietrich C., Moran N.A. Honeybee gut microbiota promotes host weight gain via bacterial metabolism and hormonal signaling. Proc. Natl. Acad. Sci. USA. 2017;114:4775–4780.

[24] Engel P., Moran N.A. Functional and evolutionary insights into the simple yet specific gut microbiota of the honey bee from metagenomic analysis. Gut Microbes. 2013;4:60–65.

[25] Anderson K.E., Ricigliano V.A. Honey bee gut dysbiosis: A novel context of disease ecology. Curr. Opin. Insect Sci. 2017;22:125–132.

[26] Biedermann L., Rogler G. The intestinal microbiota: Its role in health and disease. Eur. J. Pediatr. 2015;174:151–167.

[27] Nicholson J.K., Holmes E., Kinross J., Burcelin R., Gibson G., Jia W., Pettersson S. Host-gut microbiota metabolic interactions. Science. 2012;336:1262–1267.

[28] Zheng H., Steele M.I., Leonard S.P., Motta E.V.S., Moran N.A. Honey bees as models for gut microbiota research. Lab. Animal. 2018;47:317–325.

[29] Dong Z.X., Li H.Y., Chen Y.F., Wang F., Deng X.Y., Lin L.B., Zhang Q.L., Li J.L., Guo J. Colonization of the gut microbiota of honey bee (Apis mellifera) workers at different developmental stages. Microbiol. Res. 2020;231:126370.

[30] Pernice M., Simpson S.J., Ponton F. Towards an integrated understanding of gut microbiota using insects as model systems. J. Insect Physiol. 2014;69:12–18.

[31] Cianci R., Pagliari D., Piccirillo C.A., Fritz J.H., Gambassi G. The microbiota and immune system crosstalk in health and disease. MediatorsInflamm. 2018;2018:2912539.

[32] Sekirov I., Russell S.L., Antunes L.C.M., Finlay B.B. Gut microbiota in health and disease. Physiol. Rev. 2010;90:859–904. doi: 10.1152/physrev.00045.2009.

[33] Seeley TD: Honeybee Ecology: A Study of Adaptation in Social Life: A Study of Adaptation in Social Life. Princeton University Press; 2014.

[34] Shi, W.; Syrenne, R.; Sun, J.-Z.; Yuan, J.S. Molecular approaches to study the insect gut symbiotic microbiota at the “omics” age. Insect Sci. 2010, 17, 199–219

[35]. Zheng H, Powell JE, Steele MI, Dietrich C, Moran NA: Honeybee gut microbiota324

In Press

promotes host weight gain via bacterial metabolism and hormonal signaling. Proc325

Natl Acad Sci USA 2017, 114:4775-4780.

[36] Jing, T.-Z.; Qi, F.-H.; Wang, Z.-Y. Most dominant roles of insect gut bacteria: Digestion. detoxification or essential nutrient provision? Microbiome 2020, 8.

[37]. Silva, M.S.; Rabadzhiev, Y.; Renon Eller, M.; Iliev, I.; Ivanova, I.; Santana, W.C. Microorganisms in honey. Honey analysis. IntechOpen 2017, S233–S258.

[38]. Pachila, A.; Ptaszy´nska, A.A.; Wicha, M.; Ole´nska, E.; Małek, W. Fascinating fructophilic lactic acid bacteria associated with various fructose-rich niches. Ann. Univ. Mariae Curie Sklodowska Med. 2017, 72, S41–S50

[39] Zheng, H., Steele, M. I., Leonard, S. P., Motta, E. V., & Moran, N. A. (2018). Honey bees as models for gut microbiota research. Lab animal, 47(11), 317-325.

[40] .Schwarz RS, Moran NA, Evans JD: Early gut colonizers shape parasite susceptibility and microbiota composition in honey bee workers. Proc Natl Acad Sci USA 2016, 113:9345-9350.

[41] .Raymann K, Shaffer Z, Moran NA: Antibiotic exposure perturbs the gut microbiota and elevates mortality in honeybees. PLoS Biol 2017, 15:e2001861.

[42]. Maes PW, Rodrigues PAP, Oliver R, Mott BM, Anderson KE: Diet-related gut bacterial dysbiosis correlates with impaireddevelopment, increased mortality and Nosema disease in the honeybee (Apis mellifera). Mol Ecol 2016, 25:5439-5450.

[43] . Azambuja, P.; Garcia, E.S.; Ratcliffe, N.A. Gut microbiota and parasite transmission by insect vectors. Trends in Parasitology 2005, 21, 568–572.

[44]. Cirimotich, C.M.; Ramirez, J.L.; Dimopoulos, G. Native microbiota shape insect vector competence for human pathogens. Cell Host Microbe 2011, 10, 307–310.

[45]. Evans, J.D.; Aronstein, K.; Chen, Y.P.; Hetru, C.; Imler, J.L.; Jiang, H.; Kanost, M.; Thompson, G.J.; Zou, Z.; Hultmark, D. Immune pathways and defence mechanisms in honey bees Apis mellifera. Insect Mol. Biol. 2006, 15, 645–656.

In Press

[46]. Yiu, J.H.C.; Dorweiler, B.; Woo, C.W. Interaction between gut microbiota and toll-like receptor: From immunity to metabolism. J. Mol. Med. 2016, 95, 13–20.

[47] Jones J.C., Fruciano C., Hildebrand F., Al Toufalilia H., Balfour N.J., Bork P., Engel P., Ratnieks F.L.W., Hughes W.O. Gut microbiota composition is associated with environmental landscape in honey bees. Ecol. Evol. 2017;8:441–451.

[48] Muñoz-Colmenero M., Baroja-Careaga I., Kovačić M., Filipi J., Puškadija Z., Kezić N., Estonba A., Büchler R., Zarraonaindia I. Differences in honey bee bacterial diversity and composition in agricultural and pristine environments—A field study. Apidologie. 2020;51:1018–1037

[49]. Bommuraj, V.; Chen, Y.; Birenboim, M.; Barel, S.; Shimshoni, J.A. Concentration- and time-dependent toxicity of commonly encountered pesticides and pesticide mixtures to honeybees (Apis mellifera L.). Chemosphere 2021, 266, 128974.

[50]. Fisher, A.; DeGrandi-Hoffman, G.; Smith, B.H.; Johnson, M.; Kaftanoglu, O.; Cogley, T.; Fewell, J.H.; Harrison, J.F. Colony field test reveals dramatically higher toxicity of a widely used mito-toxic fungicide on honey bees (Apis mellifera). Environ. Pollut. 2021,269, 115964.

[51]. Miotelo, L.; Mendes Dos Reis, A.L.; Malaquias, J.B.; Malaspina, O.; Roat, T.C. Apis mellifera and Melipona scutellaris exhibit differential sensitivity to thiamethoxam. Environ. Pollut. 2021, 268, 115770.

[52]. Kakumanu, M.L.; Reeves, A.M.; Anderson, T.D.; Rodrigues, R.R.; Williams, M.A. Honey bee gut microbiome is altered by in-hive pesticide exposures. Front. Microbiol. 2016, 7, 1255.

[53] Neumann, P., and Carreck, N.L. (2010). Honey bee colony losses. J. Apic. Res. 49, 1–6.

[54] Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O., and Kunin, W.E. (2010). Global pollinator declines: trends, impacts and drivers. Trends Ecol. Evol. 25, 345-353.

[55] Ellegaard KM, Engel P. Genomic diversity landscape of the honeybee gut microbiota. Nat Commun 2019;10(1):446.

[56] Huang Y, Niu B, Gao Y, Fu L, Li W. CD-HIT Suite: a web server for clustering and comparing biological sequences. Bioinformatics 2010;26(5):680–2.

In Press

[57] Gu S, Fang L, Xu X. Using SOAPaligner for short reads alignment. Curr. Protoc. Bioinform. 2013;44(1):11.11.1-7.

[58] Dixon P. VEGAN, a package of R functions for community ecology. J Veg Sci 2006;14(6):927–30.

[59] Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD, et al. Picante: R tools for integrating phylogenies and ecology. Bioinformatics 2010;26(11):1463–4.

[60] R Core Team. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2017.

[61] Zhao Y, Jia X, Yang J, Ling Y, Zhang Z, Yu J, et al. PanGP: a tool for quickly analyzing bacterial pan-genome profile. Bioinformatics 2014;30(9):1297-9.

[62] Kanehisa M, Goto S. KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res 2000;28(1):27–30.

[63] Lombard V, Golaconda Ramulu H, Drula E, Coutinho PM, Henrissat B. The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Res 2014;42(D1):D490–5.

In Press

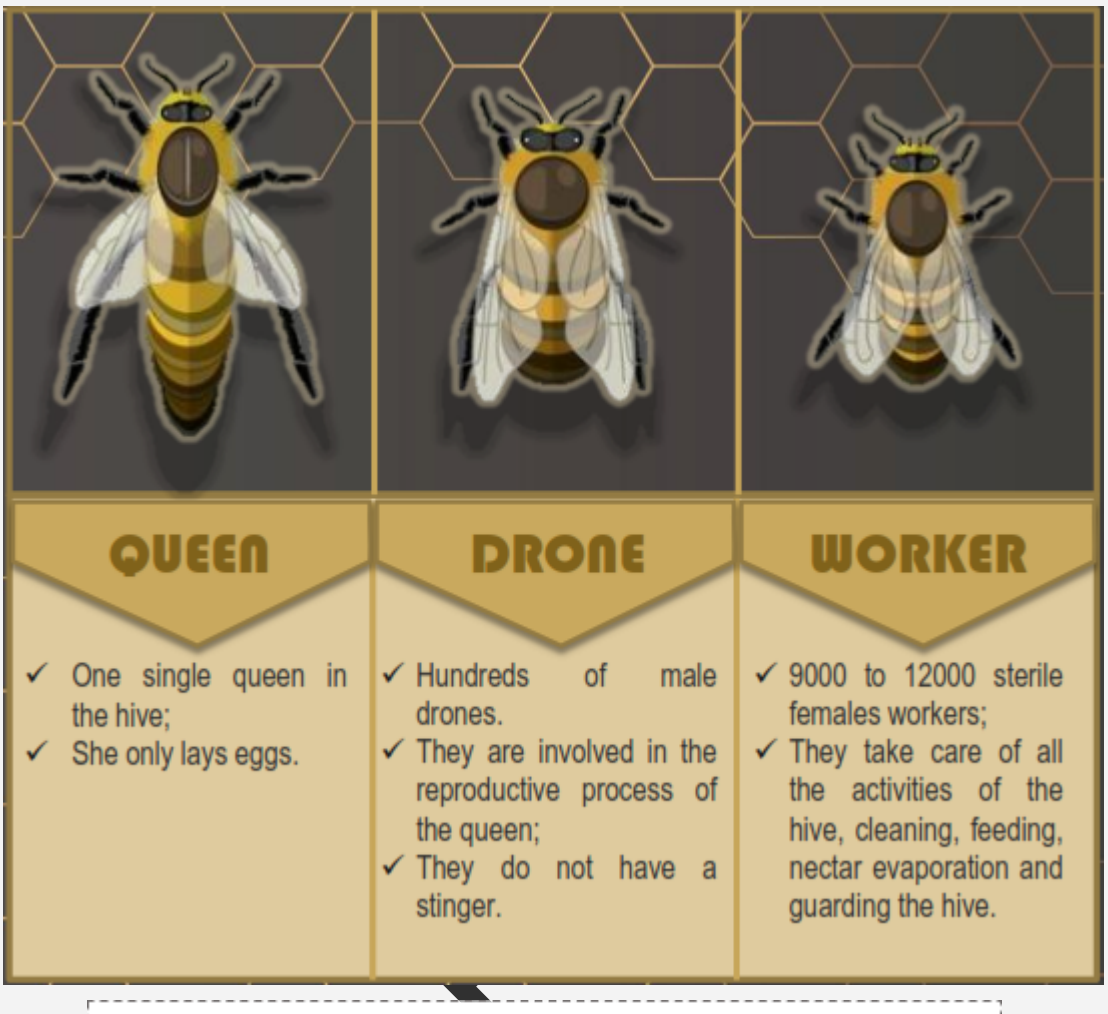


Fig1. The inhabitants of the hive

In Press

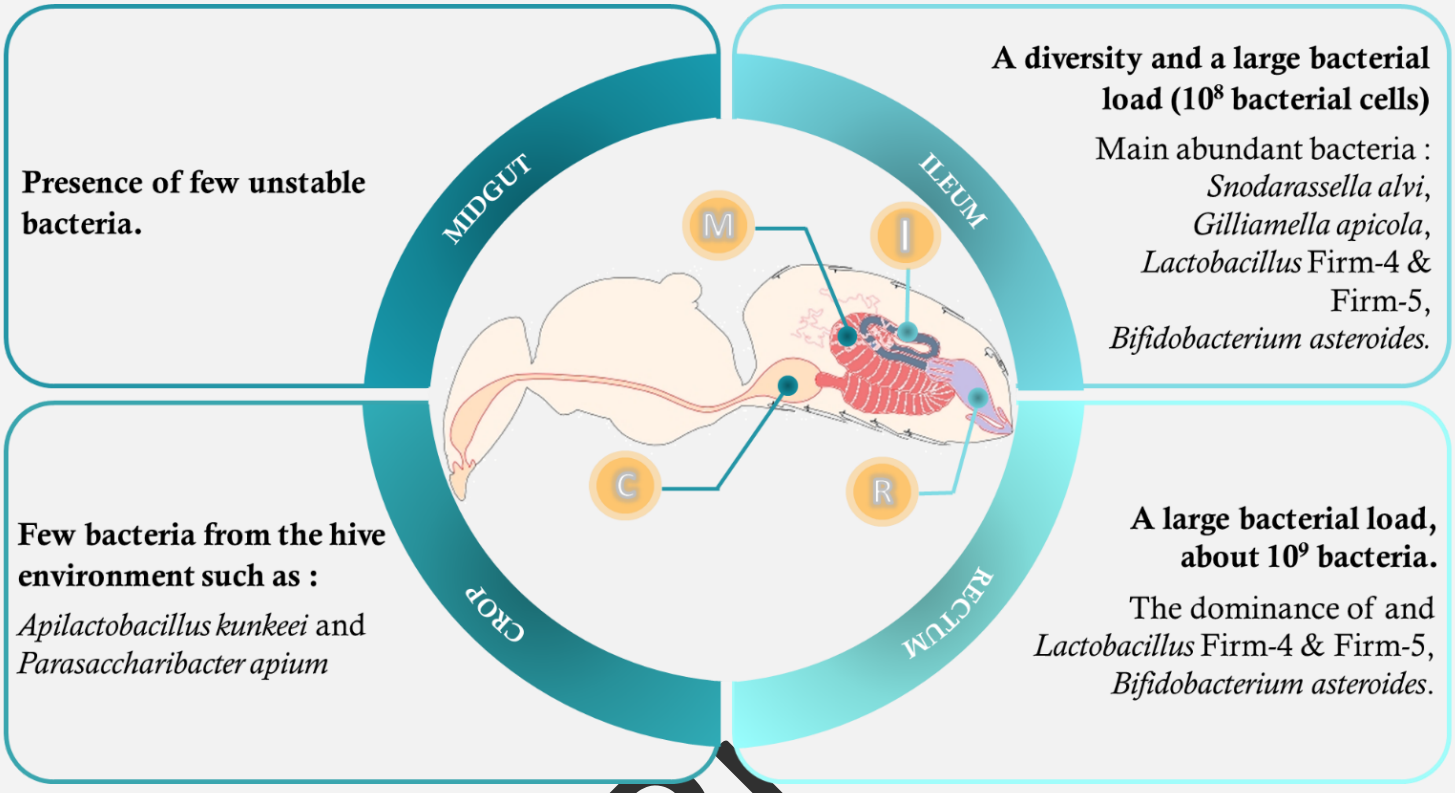


Fig2: The several main species of the bee gut microbiota and their distribution

in Pr

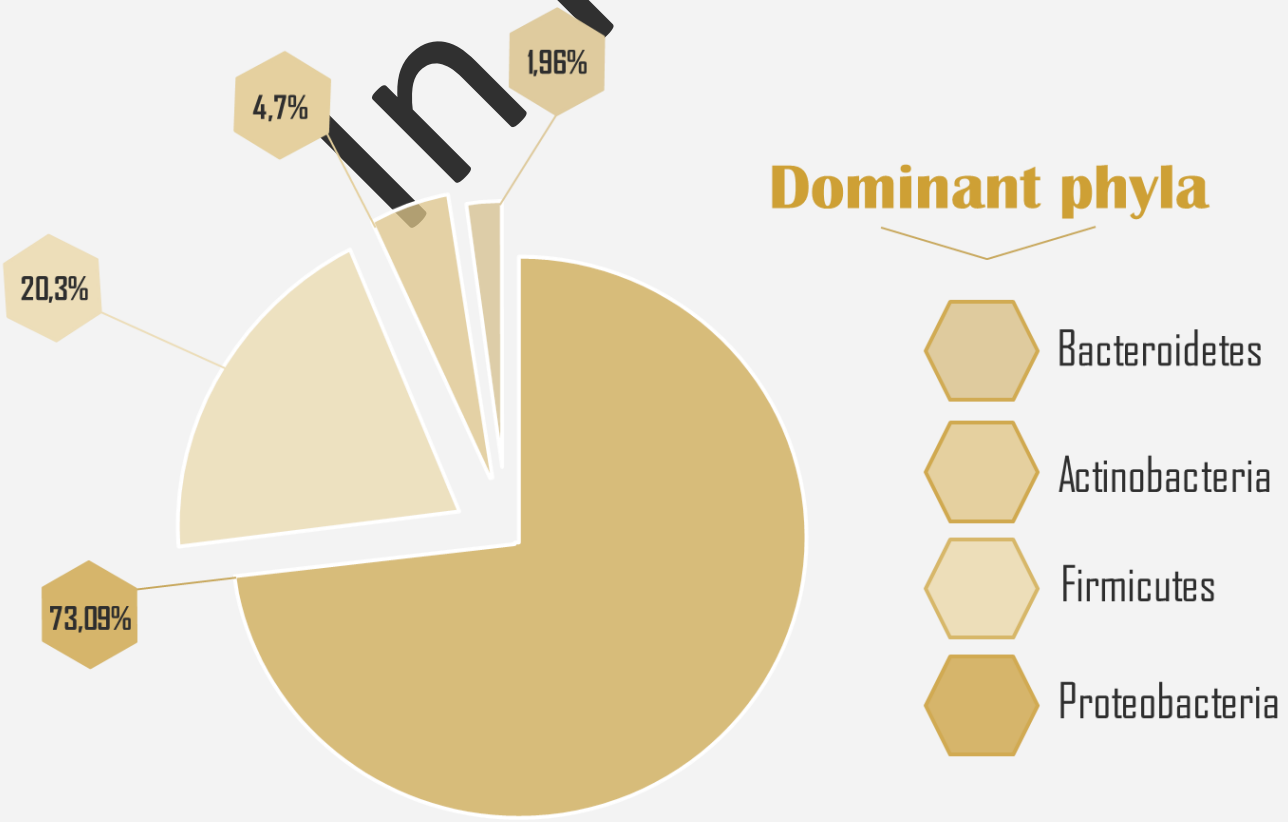


Fig3: Dominant phyla presented in a circular diagram

Parts of the gut	Main species	Phylum / Family	Functions
RECTUM	*Lactobacillus clusters*	*Lactobacillaceae.*	• Play a central role in the catabolism of carbohydrates and thus in the nutrition of their hosts.
	Bifidobacterium asteroides	*Actinobacteria*	
ILEUM	*Snodarassella alvi*	Neisseriaceae	• Protection against parasites and pathogens by producing toxins that attack them.
	Gilliamella apicola	Orbaceae	
	Lactobacillus Firm-4 Firm-5	Lactobacillaceae	• **Food degradation:** each bacterium has the ability to degrade a particular type of sugar and particular nutrients, e.g. *G. apicola* which is able to digest pectin present on the surface of pollen as well as carbohydrate processing (*G. apicola* has a striking enrichment in carbohydrate processing genes)
	Bifidobacterium sp	Bifidobacteriaceae	
	Frischella perrara	Orbaceae	
	Bartonella apis	Rhizobiaceae	
	Parassacharibacter apium	Alphaproteobacteria	
	Bombella apis	Acetobacteraceae	
	Apibacter adventoris	Bacteroidetes	
	Apibacter mensalis	Bacteroidetes	
MIDGUT	Few unstable bacteria.	/	/
CROP	*Apilactobacillus kunkeei*	Firmicutes	Located between the esophagus and ventriculus, and used for storage and transport of nectar to the hive; also called stomach or sack
	Parasaccharibacter apium	Alphaproteobacteria	

Table 1. The main species colonizing the digestive system of the honeybee and their functions

Abstract :

In order to gain a better understanding of the intriguing diversity within this microbiota and to address specific inquiries, researchers have turned their attention to Apis mellifera as a valuable animal model. The aim of our study is to identify and evaluate the bacterial diversity within the worker bee gut microbiota from different area in Algeria (Bouira, Mostaganem, and Ouled Djellal). The enumeration on PCA medium revealed 425 bacterial CFU from the whole harvested samples, with a distribution of 17.18% in Bouira, 34.12% in Mostaganem, and 48.71% in Ouled Djellal. Among the 83 obtained bacterial isolates, a wide range of diversity was observed, with variations in their growth preferences depending on the culture media used. Out of the 66 isolates, phenotypic analysis demonstrated that 80.3% of the isolates were Gram-positive, while 19.7% were Gram-negative. Furthermore, biochemical tests indicated that 18.91% of the isolates were oxidase-positive and 89.28% were catalase-positive. Significant differences in bacterial composition were observed among the studied regions, particularly concerning the prevalence of Gram-positive, oxidase-positive, and catalase-positive bacteria. Additionally, the application of the 16S rRNA PCR technique allow us to carry out the identification of 49 of the isolates, and confirm the predominant of bacterial belong to *Bacillaceae*, *Microbacteriaceae*, *Lactobacillaceae*, *Enterococcaceae*, *Neisseriaceae*, and *Pectobacteriaceae*. These results were further supported by the application of the molecular metabarcoding method. Overall, this research has provided valuable insights into the diversity of the gut microbiota of worker bees in Algeria, opening the doors for numerous future research endeavors in this field.

Keywords : Gut microbiota, *Apis mellifera*, Bacterial diversity, Algerian bees.

Résumé :

Afin de mieux comprendre la diversité intrigante de ce microbiote et de répondre à des questions spécifiques, les chercheurs se sont tournés vers *Apis mellifera* en tant que modèle animal précieux. Le but de notre étude est d'identifier et d'évaluer la diversité bactérienne au sein du microbiote intestinal de l'abeille ouvrière provenant de différentes régions d'Algérie (Bouira, Mostaganem, et Ouled Djellal). Le dénombrement sur milieu PCA a révélé 425 UFC bactériennes sur l'ensemble des échantillons récoltés, avec une répartition de 17,18% à Bouira, 34,12% à Mostaganem, et 48,71% à Ouled Djellal. Parmi les 83 isolats bactériens obtenus, une grande diversité a été observée, avec des variations dans leurs préférences de croissance en fonction des milieux de culture utilisés. Sur les 66 isolats, l'analyse phénotypique a montré que 80,3% des isolats étaient Gram-positifs, tandis que 19,7% étaient Gram-négatifs. En outre, les tests biochimiques ont indiqué que 18,91% des isolats étaient positifs à l'oxydase et 89,28% à la catalase. Des différences significatives dans la composition bactérienne ont été observées entre les régions étudiées, notamment en ce qui concerne la prévalence des bactéries Gram-positives, oxydases-positives et catalases-positives. En outre, l'application de la technique PCR de l'ARNr 16S nous a permis d'identifier 49 des isolats et de confirmer la prédominance des bactéries appartenant aux *Bacillaceae, Microbacteriaceae*, *Lactobacillaceae, Enterococcaceae, Neisseriaceae et Pectobacteriaceae.* Ces résultats ont été confirmés par l'application de la méthode de métabarcoding moléculaire. Dans l'ensemble, cette recherche a fourni des informations précieuses sur la diversité du microbiote intestinal des abeilles ouvrières en Algérie, ouvrant la voie à de nombreuses recherches futures dans ce domaine.

Mots-clés : Microbiote intestinal, *Apis mellifera*, Diversité bactérienne, Les abeilles algériennes

ملخص

من أجل اكتساب فهم أفضل للتنوع المثير للاهتمام داخل هذه الميكروبات ومعالجة استفسارات محددة، حول الباحثون انتباههم إلى *Apis mellifera* كنموذج حيواني قيم. الهدف من دراستنا هو تحديد وتقييم التنوع البكتيري داخل ميكروبيوتا أمعاء النحل العامل من منطقة مختلفة في الجزائر (البويرة ومستغانم وأولاد جلال). كشف التعداد على متوسط PCA عن 425 وحدة حرارية بكتيرية من العينات المحصودة بالكامل، مع توزيع 17.18٪ في البويرة، و 34.12٪ في مستغانم، و 48.71٪ في أولاد جلال. من بين 83 عزلة بكتيرية تم الحصول عليها، لوحظت مجموعة واسعة من التنوع، مع اختلافات في تفضيلات النمو اعتمادًا على الوسائط الثقافية المستخدمة. من بين 66 عزلة، أظهر التحليل الظاهري أن 80.3٪ من العزلات كانت إيجابية الغرام، بينما كانت 19.7٪ سلبية الغرام. علاوة على ذلك، أشارت الاختبارات الكيميائية الحيوية إلى أن 18.91٪ من العزلات كانت إيجابية الأوكسيداز و 89.28٪ كانت إيجابية الكاتالاز. لوحظت اختلافات كبيرة في التركيب البكتيري بين المناطق المدروسة، لا سيما فيما يتعلق بانتشار البكتيريا إيجابية الغرام وإيجابية الأوكسيداز وإيجابية الكاتالاز. بالإضافة إلى ذلك، يسمح لنا تطبيق تقنية S rRNA PCR16 بإجراء تحديد 49 من العزلات، وتأكيد أن الغالب من البكتيريا ينتمي إلى *Bacillaceae* و *Microbacteriaceae* و *Lactobacillaceae* و *Enterococcaceae* و *Neissteriaceae* و *Pacecticetceace* تم دعم هذه النتائج بشكل أكبر من خلال تطبيق طريقة الميتاباركود الجزيئية بشكل عام، قدم هذا البحث رؤى قيمة حول تنوع ميكروبيوتا الأمعاء للنحل العامل في الجزائر، مما فتح الأبواب للعديد من المساعي البحثية المستقبلية في هذا المجال.

الكلمات الرئيسية: ميكروبيوتا الأمعاء، *Apis mellifera*، التنوع البكتيري، النحل الجزائري.