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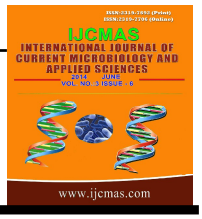

Original Research Article

Contribution to identification of the microflora of the digestive tract and pollen of Algerian honeybees: *Apis mellifera intermissa* and *Apis mellifera sahariensis*

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A B S T R A C T

The microorganisms associated to the two Algerian subspecies honeybees *A.m.intermissa* and *A.m.sahariensis* were characterized. The identification of the microflora associated with the digestive tract and pollen was made two seasons during the year 2012, namely in January -February (winter test) and May (spring test). The research of this microflora was realized by the digestive tract, stored pollen (bee bread) and pollen baskets. The Isolated bacteria belong to the genera *Enterobacter*, *Pantoae, Pseudomonas,* and *Lactococcus* and to genera neighbors, *Lactobacillus* and *Streptococcus*. All yeast strains are likely to belong to the Saccharomyces genera and the molds are identified genera *Penicillium, Aspergillus, Cladosporium*, *Alternaria* and *Mucor*. According to the biochemical and physiological characters that are found, certain bacteria may be involved in the degradation of the components of the two extern layers of pollen, be ingested by the bees with food and shared with other individuals of the hive by the phenomenon of trophallaxis. The presence of yeasts and molds can be explained by the close relationship as maintenance honeybee with its environment, soil and flowers.

Keywords

A.m.intermissa, *A.m.sahariensis*, microflora, digestive tract, bee bread, pollen baskets, bacteria, yeasts and molds.

Introduction

Honey bees are of great ecological and economic importance as pollinators of many crop and wild plants (Free, 1993). It plays an important economic role as a carrier of beekeeping (honey, royal jelly, pollen, propolis and wax) and agriculture by providing a quantitative and qualitative crop growth. It also plays a role in ensuring environmental sustainability of many wild plant species. The power of the honeybee is mainly pollen, nectar of flowers and honeydew produced by aphids. Social apoid species require pollen as the main protein source (Haydak, 1968, 1970; Dobson & Peng, 1997).

Bacteria associated with bees are widely distributed in soil, water and air, stored

bee food and surface of plants (Glinski and Jorosz, 1992). Microbial communities in honeybee intestines have been studied (Gilliam & Valentine, 1976; Gilliam & Morton, 1978; Gilliam *et al*., 1990; Gilliam, 1997). The normal microflora is obtained from consumption of pollen, other food, and through contacts with older bees in the colony (Glinski and Jarosz, 1995). The study of microorganisms associated with *Apis mellifera* were worn on the identification of bacteria of the Enterobacteriaceae family, Bacillus genera Lactobacillus, Pseudomonas, Bifidobacterium, Corynebacterium, Streptococcus and Clostridium as well as fungi and yeasts (Jeyaprakarsh *et al*., 2003 ; Kačăniovă *et al*., 2004). Many studies have shown that these organisms represent a natural microflora that is specific to the environment of the pollen (the atmosphere, soil, and the flower of kindness) and also the digestive tract and the mandibular secretions of the honeybee working. These studies were conducted in the United States (Gilliam et *al*., 1988), Slovakia (Rada et *al*., 1997), Iran (Ebrahimi et *al*., 2005) , Colombia (Duberney et *al*., 2006) and Russia (Kačăniovă *et al*., 2004; Lyapunov et *al*., 2008 ; Kačániová et *al*.,2009).

All these researchers have demonstrated the presence of two outer layers in the pollen which are proved unpleasant to the honeybee, and the presence of bacteria, yeasts and molds allowed the latter to acquire proteolytic enzymes. As a result, it was interesting to conduct this study in our country, especially with the existence of two local subspecies of honeybees namely *Apis mellifera intermissa* and *Apis mellifera sahariensis*. The aim of our study was to determine of several specific of microorganism in the midgut and pollen of winter and spring honeybees.

Materials and Methods

Worker bees were collected from the experimental apiary of the Department of Agricultural Zoology of the National School of Agricultural El- Harrach (ENSA) of Algiers. The collected workers bees are belonging to two subspecies Algerian honeybees. *Apis mellifera intermissa* comes from the plain of Mitija and *Apis mellifera sahariensis* comes from area Ben Zireg the wilaya of Becharin south of Algeria. These hives are not subject or transhumance or treatment with pesticides. The colony used must have a satisfactory condition and no visible disease symptoms. In order to gain insight on the microbiota associated with bee pollen and mile during the season. Harvesting honeybees and pollen (pollen baskets and pollen bread) was performed during the year 2012.

The digestive tract is removed by pulling on the last abdominal segments of bee pollen baskets and bee bread are handled in an aseptic area to avoid contamination from the outside environment

Cultivation

This work was carried out the preparation of stock solutions to the digestive tract, pollen baskets and bee bread. The latter will be cultured to find microorganisms involved in the process of digestion of the worker bee. Microorganisms are sought and counted the family Enterobacteriaceae, Streptococcaceae (Lactococcus, Strepotococcus), the genera Pseudomonas, Lactobacillus, Staphylococcus, yeasts and molds (Jeyaprakash et al, 2005; Duberney et al., 2006; Lyapunov et al., 2007; Kačániová et al., 2009).. The culture media used are selective and can isolate the desired micro-organisms (Table 1).

According Branger et al., (2007), count on solid medium based on the principle that each bacterium after incubation gives rise to a detectable colony macroscopically and only colonies of greater than 15 and lower than 300 numbers will be considered.

Bacteriology and Pocket Atlas of Microbiology (Hart, 1999). This identification is based on the macroscopic appearance, microscopic (Gram stain) and biochemical (API 20 Gallery) (Prescott *et al*., 2003; Meyer *et al*., 2004; Denis *et al.*, 2007; Freney *et al*., 2007). The identification of yeasts appealed to the study of cultural characteristics, morphological, cellular and biochemical. Morphological study and cellular is using microscopic observations of the shapes of cells, their mode of vegetative reproduction and their ability to filamentation. Unlike bacteria and yeast, the morphological study microscopic enough to determine the type of mold and isolated it by making sowings by buttons on media sound studies for growth and sporulation of fungi (Peberg ,1987; Barnett & Hunter, 1999).

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Results and Discussion

Winter test

The results for the microorganisms found in the digestive tract and bee bread of two local bee races are shown in Table 2

In the digestive tract, four (04) strains of Enterobacteriaceae are identified. Two strains belonging to the genus Pantoea SPP2 with probabilities of 75.22% and 47.75%. Other strains belong to the species Enterobacter gergoviae, with probabilities of 51.69% and 51.69%. The presence of this bacterial group was confirmed by Rada *et al*. (1997) and Ebrahimi *et al*. (2005). Possession to the same genera of Enterobacteriaceae was explained by the practice of trophallaxie is made between the worker bees (Hansen et al., 2004). We noted the presence of Staphylococcus sp in the gastrointestinal tract of the Saharan bee and the absence in the honeybee Tellian, unlike the results obtained by Kačániová *et al*., (2009), which are 3.37 log cfu / g.

Thirteen (13) fungal strains are purified. The identification revealed eight strains belonging to the genus Penicillium, Aspergillus, three strains and two strains in the genus Mucor and Cladosporium. These same molds are found by Gilliam *et al.* (1989) at the bee bread. During this test, we did not find pollen loads.

Spring test

The results for the microorganisms found

in the digestive tract, balls of pollen and bee bread (yellow and purple) of the two races of bees are represented in Table 3. The spring test focused on the identification of the digestive tract, three strains of *Pseudomonas sp*. These strains have the ability to assimilate glucose and sucrose into fructose degrade exceptionally three isolated strains of Pseudomonas can assimilate glucose and other sugars. This same character was described by Kačániová et al. (2009). Three of Staphylococci strains belonging to the species *Staphylococcus lentus* with probability 99,7 % and 99,8 % for each species and strain *Staphylococcus xylopsus* with a probability of 99,3 %.

Lactobacillus sp is present only in the gut of the Tellian bee with equal to 3,96 log cfu / g number . The study by Rada et al. (1997) revealed a number between 0,54 and 7,24 log cfu / g number. Knowing the origin of this flora is exclusively from their nutrition (pollen). This flora is variable according to the seasons and the interaction with the host (Smolska Szymczewska, 1989). The absence of enterobacteria and lactobacilli at the balls of pollen is reported. The presence of staphylococci is also noted but with an insignificant number. Two strains were isolated belonging to the total aerobic mesophilic flora, and one strain of *Pseudomonas sp* with the same characters previously reported. This result is confirmed by Duberney *et al*. (2006). These same authors have reported the ability of Pseudomonas to use the carbohydrates present in the pollen, in particular sucrose, and glucose as an energy source.

The four strains of yeast belonging to the genus *Saccharomyces sp*. Mold identified by microscopic characters belong to the genera *Penicilliun sp, Aspergillus sp, Mucor sp* and *Cladosporium sp.*The analyzed bee bread is two colors (yellow and purple) and is made from a single hive that of the Saharan honeybee. The cultivated microorganisms are not presented with a significant number of Enterobacteriaceae and *Staphylococcus sp*. Two strains of *Pseudomonas sp*, two strains of total aerobic mesophilic flora are noted. Three strains of yeast *Saccharomyces sp* and ten fungal strains belonging to the genera: *Aspergillus sp* (three strains), *Alternaria sp* (three strains), *Cladosporium sp* (two strains), *Penicillium sp* (a stem) and *Mucor sp* (strain) are identified. The same results are reported by Gilliam *et al.* (1988). Depending on the color of the pollen stuffed into the pockets of executive's waxes hive of Saharan honeybee, we investigated the microflora associated with bee bread yellow and purple.

This search revealed the presence of bacteria, yeasts and molds. A non-significant number of Enterobacteriaceae and Staphylococci is signaled at the two rolls of bees. These results are confirmed by Duberney *et al*. (2006). According Guilliam (1979), Pseudomonas has the ability to provide secondary metabolites such as enzymes which can induce the maturation of the pollen, which increases the nutritional value and the availability of amino acids and possibly participation in the digestion . This explains their presence at the yellow and purple bee bread. Lactic acid bacteria are microorganisms capable of degrading xylose, galactose and arabinose own pollen for bees and indigestible (Zucoloto *et al*., 1977; Jones *et al*., 1986). The outbreak of process of lactic fermentation of pollen uses any microorganisms capable of fermenting sugars.

Yeasts are counted among the most capable of the fermentation microorganisms. We isolated and purified three strains of yellow and purple bee bread. The three strains have a reproduction by budding, capable of filamentation and they have a pseudomycelium. The identification of biochemical and physiological characteristics showed that the three strains have the ability to degrade they possessed lysine and another nitrogen source as lysine. We can deduce that the three strains are likely to belong to the genus *Saccharomyces sp* (Galzy *et al*., 1980; Rippon, 1988). Molds are found in the number of ten (10) strains. The identification of these strains revealed that three strains belong to the genus *Aspergillus sp.* Four strains belong to the genus *Alternaria sp* and each of the remaining strains belongs respectively to the genera *Penicillium sp*, *Cladosporium sp* and *Mucor sp*. These microorganisms were identified as (Barnett & Hunter, 1999).

Table.1 Solid culture media and conditions used for the detection and enumeration of microorganisms.

Microorganisms sought	**Media used**	**Incubation temperature**	**Incubation time**
Enterobactereae	Desoxycolate agar	37°C	48 h
Mesophilic aerobic flora	Nutrient agar/ PCA	32°C	72 h
Lactobacillus sp	MRS	37°C	48 h
Streptococcaceae	M17	37°C	48 h
Staphylococcus sp	Baird Parker	37°C	72 h
	Chapman		
Pseudomonas sp	King B	32°C	48 h
Yeast	Sabouraud with chloramphenicol, PDA	28°C	3 days
Mold	Sabouraud with chloramphenicol, OGA	28°C	3 at 5 days

Table.2 Results of the microorganisms looked for during the winter tes

Microorganisms	**digestive tract**		**Bee bread**	
	A.m.intermissa	*A.m.sahariensis*	*A.m.intermissa*	*A.m.sahariensis*
Enterobacteraea	4,07	UNC	/	/
Mesophilic aerobic flora	3,79	UNC	3,25	3,81
Staphylococci	-	+	/	/
Pseudomonas	UNC	UNC	/	/
Lactic bacteria	UNC	UNC	/	/
Mold	/	/	(3,04 ≈ 3,89)	4,53

Table.3 Results of the desired microorganisms in the spring test

Microorganisms	digestive tract		Bee bread		balls of pollen
Microorganisms	*A.m. intermissa*	*A.m. sahariensis*	*A.m. sahariensis*		*A.m. sahariensis*
			Purple	Yellow	
Enterobacteraea	UNC	UNC	+	+	-
Total aerobic mesophilic flora	UNC	UNC	UNC	UNC	
Staphylococci	3,46	3,25	+	+	+
Pseudomonas	3,44	UNC	UNC	UNC	UNC
Lactobacillus	3,96	-	UNC	UNC	-
Lactococcus and related genera	UNC	3,47	UNC	UNC	-
Mold	/	/	UNC	UNC	UNC
Yeats	/	/	UNC	UNC	UNC

Unit: log cfu / g; (UNC): uncountable, (-): Absence, (+): Presence; (/):Unrealized

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