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BACTERIAL ECTOMICROFLORA OF *VARROA DESTRUCTOR*, ECTOPARASITE OF HONEYBEE, COLLECTED IN THE APIARY OF BOUMERDES (ALGERIA)

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ABSTRACT

Varroa destructor Anderson and Trueman (Acari: Varroadae), previously known as *Varroa jacobsoni*, is an important pest of the honeybee, *Apis mellifera* L. It has been causing severe damage to populations of this species worldwide in recent years. The aim of this work was the isolation and identification of the bacterial ectomicroflora of the *Varroa destructor*, an ectoparasite of the bee (*Apis mellifera* L). Samples of *Varroa* were collected from the location of Boumerdes (situated in northern Algeria) beehive summer debris. The ectoparasitic honeybee *Varroa* was disinfected with 70% ethanol and then it was spread in nutrient agar plates. For the isolation and identification of the bacteria, the macroscopic and microscopic characters were done according to Bergey's manual of systematic Bacteriology. Biochemical characteristics were tested by using API 20E galleries (Biomerieux). The experiments were performed twice. The results of the preliminary study showed that the ectoparasite harbored seven genera of bacteria: *Staphylococcus* sp (3), *Bacillus* sp (2) and *Pseudomonas* sp (2). The colonies of *Staphylococcus* are Gram positive, mobile, coccoid shaped, aero-anaerobic and with a positive catalase. *Bacillus* are Gram-staining-positive rods, mobile, endospore forming, aerobes or facultative anaerobic and can produce catalase and oxidase. *Pseudomonas* bacteria are Gram-negative, oxidase-positive, strict aerobic and non-spore forming.

Key words: *Apis mellifera L, Varroa destructor, bacterial ectomicroflora, beehive summer debris.*

INTRODUCTION

Varroa destructor (Anderson and Trueman, 2000) is the most destructive and important pest in beekeeping wordwide. The mite originates in Asia whose natural host is *Apis cerana* (Le Conte *et al*., 2010). On the new host, *Apis mellifera*

(Hymenoptera: Apidae), *Varroa destructor* induce several damages including the disturbance of the morphological, biochemical and immunological parameters (Weinberg and Madel, 1985; Daly *et al.,* 1988, Marcangeli *et al*., 1992; Contzen *et al.,* 2004; Yang and Cox-Foster, 2005; Belaïd and Doumandji, 2010; Belaïd *et al*., 2017). Others effects were caused by the microflora transmitted by the obligatory ectoparasite of the honeybee (*Apis mellifera* L). The mite can transmit American foulbrood (De Rycke *et al*., 2002).), the sacbrood virus (Bailey, 1991), acute paralysis virus (Ball, 1985, 1988; Ball and Allen, 1988) by inoculating virus particles into the haemolymph of honeybees. The haematophagous is also one of the vectors of fungi (Benoit *et al*., 2004) and several bacteria (Gliński and Jarosz, 1990 a, 1992). To our knowdelge, there are a few studies have been made about the bacteria of the *Varroa* (Majboroda *et al*., 2013; Vanikova *et al*., 2014; Maddaloni and Pascual, 2015). The purpose of the study was to determine the bacteria ectomicroflora of the *Varrao destructor* in *Apis mellifera intermissa* in Boumerdes (Algeria).

MATERIAL AND METHODS

Collection of *Varroa* mites

Samples of adult female mites of *Varroa destructor* naturally fall were obtained from *Apis mellifera intermissa* colonies in summer placed in apiary of the Boumerdes (situated in northern of Algeria) beehive summer debris. The ectoparasitic honeybee *Varroa* was disinfected with 70% ethanol and then it was spreading in nutrient agar plates. The experiments were performed twice.

Isolation and identification of bacteria

For the isolation and identification of the bacteria, the macroscopic and microscopic characters were done according to the Bergey's manual of systematic Bacteriology (Holt *et al*., 1994). Biochemical characteristics were tested by using API 20E galleries (Biomerieux).

RESULTS AND DISCUSSION

In the work, a total of seven strains were isolated from the ectoparasitic mite *Varroa* destructor collected from different location in Boumerdes (Figure 1, Table 1 and 2).

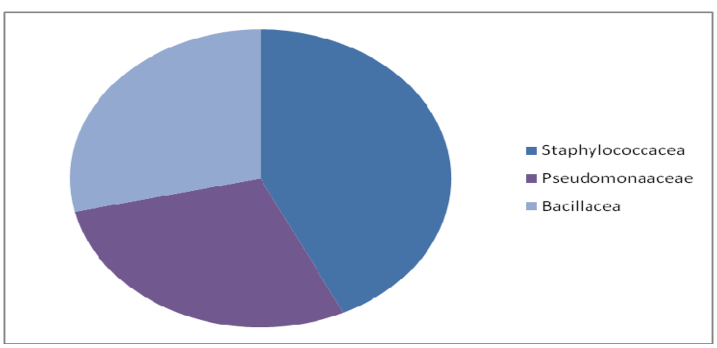

Figure 1: Percent distribution of bacteria isolates

According to the Bergey's manual of systematic Bacteriology (Holt *et al*., 1994), the results showed that the ectoparasite harbored 7 strains of bacteria: 3 belonging of Staphylococaccae (S1, S2 and S7) 42,85%, 2 of Bacillaceae (S3 and S4) and 2 of Pseudomonodaceae (S5 and S6) (Fig 1) 28,57%. The colonies S1, S2 and S7, the members of Staphylococcacea, isolated from the ectoparasite are Gram positive, mobile, coccoid shaped, aero-anaerobic and with a positive catalase. S3 and S4 colonies belonging to Bacillaceae are Gram-staining-positive rods, mobile, endospore forming, aerobes or facultative anaerobic and can produce catalase and oxidase. S5 and S6 are Gram-negative, oxidase-positive, strict aerobic and non-spore forming. The isolates of family Pseudomonadaceae detected during our studies and collected from *Varroa destructor* beehive summer debris were identified as *Pseudomonas* sp (Table 1).

Table 1. Morphological and physiological characteristics of isolated bacteria from *V. destructor*.

	S1	S2	S3	S4	S5	S6	S7
Shape	c	c	r	r	r	r	c
Gram strains	+	+	+	+	-	-	+
Endospore	-	-	+	+	-	-	-
Respiratory type	a	a	a	an	a	a	an
Oxidase test	-	-	+	+	+	+	-
Catalase test	+	+	+	+	+	+	+
Motility	+	+	+	+	-	-	+

(+): positive test ;(-): negative test; c: cocci; r: rods; a: aerobic; an: anaerobic.

Based on API 20E galleries (Biomerieux), the strains identified as *Bacillus* sp are positive for ortho-nitro-phénol-galactosidase, arginine di-hydroxylase, Ornithine décarboxylase, citrate utilization test, acetoin production, gelatinase but negative for indol production, urease and inositol. In our study, the colonies of *Pseudomonas* sp were positive for ortho-nitro-phénol-galactosidase, arginine di-hydroxylase, ornithine décarboxylase (ODC), citrate utilization test (CIT). The strains were capable of using Glucose, Mannose, Sorbitol, Rhamnose, Saccharose, Melibiose, Amydaline and Arabinose (Table 2).

Table 2. Biochemical characteristics of bacterial isolats

	S1	S2	S3	S4	S5	S6	S7
ONPG	+	+	+	+	+	+	+
ADH	+	+	+	+	+	+	+
LDC	-	-	-	+	-	+	-
ODC	+	+	+	+	+	+	+
CIT	+	+	+	+	+	+	+
H_2S Production test	-	-	-	-	-	+	-
URE	+	+	-	-	+	+	+

TDA	-	-	-	-	-	-	-
IND	-	-	-	-	-	-	-
VP	+	+	+	+	+	+	+
GEL	+	+	+	+	+	+	+
GL	+	+	+	+	+	+	+
MANE	+	+	+	+	+	+	+
INO	-	-	-	-	-	+	+
SOR	+	+	+	+	+	+	+
RHA	+	+	+	+	+	+	+
SAC	+	+	+	+	+	+	+
MEL	+	+	+	+	+	+	+
AMY	+	+	+	+	+	+	+
ARA	+	+	+	+	+	+	+

(+): positive test ;(-): negative test; Ortho-nitro-phénol-galactosidase (ONPG); Arginine di-Hydroxylase (ADH); Lysin di-Carboxylase (LDC); Ornithine décarboxylase (ODC); Citrate utilization test (CIT); Urease (URE); Tryptophane Désaminase (TDA ; Indol production (IND) ; Acetoin production (VP) ; Gelatinase (GEL) ; Glucose (GL) ; Mannose (MANE); Inositol (INO) ; Sorbitol (SOR) ; Rhamnose (RHA) ; Saccharose (SAC); Melibiose (MEL); Amydaline (AMY); Arabinose (ARA).

A lot papers are published on the subject of the viral transmission such Sacbrood bee virus (Ball, 1999a), *Acute Bee Paralysis Virus* (Faucon *et al.,* 1992; Ball, 1999a; Brodsgaard *et al.,* 2000), *Kashmir Bee Virus* (Ball, 1999 a ; Chen *et al.,* 2004; Nyguyen *et al.,* 2010), virus *Deformed Wing Virus* (Ball, 1999b; Bowen-Walker *et al*.; Tentcheva *et al.,* 2004; Chejanovsky *et al.,* 2010; Nyguyen *et al.,* 2010). But, they have a few numbers of data concerning the fungi and bacteria microflora of the *Varroa*. Hrabak (2003) and Benoit *et al*. (2004) reported the femal adults of the honeybee mite *Varroa destructor* have on their surface and have the potential to disperse fungal spores (conidia) throughout the bee colony (*Aspergillus flavus*, *Penicillium multicolor*, *Penicillium simplicissimum*, *Mucor ramosissimus*, *Mucor indicu*, *Mucor hiemalis* and *Ascosphaera apis)*. According to De Rycke *et al*., (2002), *Varroa destructor* is capable of transporting spores of *Paenibacillus larvae* (the American foulbrood agent) to the surface of its body. Based on the Gallery API 20 E (Bio-Merieux), Belaïd *et al*. (2018) found that the heamolymph worker honeybees parasitized by *Varroa destructor* was contamined by *Bacillus licheniformis, Bacillus mycoide*, *Bacillus coagulans, Brevibacillus chohinensis*, *Aeromonas hydrophila* and *Pantoa sp.* Glinski and Jarosz (1990 b) reported that using *Serratia marcescens*, microbiological assays have indicated that *Varroa jacobsoni* can harbour this indicator bacterium on its body surface and internally. According to Hubert *et al*. (2017), the location, time of year and degree of infestation by *Varroa* had significant effects on the composition of the bacteriome of honey bee workers. These authors found that varroosis are more important factor than *Nosema ceranae*, *Nosema apis* and *Lotmaria passim* infestation influencing the honey bee bacteriome and contributing to the changes in symbiotic bacterial taxa. In the colonies with high *Varroa* infestation levels (varroosis), the relative abundance of the bacteria *Bartonella apis* and *Lactobacillus apis* decreased. In contrast, an increase in relative abundance was observed for several taxa including *Lactobacillus helsingborgensis, Lactobacillus mellis, Commensalibacter intestini, and Snodgrassella alvi*.

Our preliminary investigations show the presence of several bacterial strains as *Bacillus* sp, *Pseudomonas* sp and *Staphylococcus* sp isolated from the honeybee external body of the parasitic mite *Varroa destructor* collected from Boumerdes beehive summer debris. According to Hrabak (2003), the genus *Staphylococcus albus* and *Enterobacter cloacae* were isolated from the external ectoparasite mite. Bacillacea (*Bacillus sp*) and Micrococcaceae were cited by Tsagou *et al*., (2004). According to Alquisira-Ramírez *et al.,* (2014), fifty-four *Bacillus*-like strains were isolated from dead *Varroa destructor* collected in 24 colonies of bees from seven apiaries. Many bacteria such *Morganella* sp, *Enterococcus* sp, *Pseudomonas* sp, *Rahnella* sp, *Erwinia* sp and *Arsenophonus* sp were identified by Hubert *et al*., (2015). Other bacteria microflora was also recorded by Maddaloni and Pascual (2015) (*Bacillus subtilis*, *Burkholderia*, *Pseudomonas syringae*, *Pantoa agglomerans*, *Pantoa vagans*, *Paenibacillus* wynnii, *Staphylococcus caprae, Bifidobacterium asteroids, Staphylococcus caprae, Micrococcus luteus* etc. and by Vanikova *et al*. (2015) (*Microbacteruim* sp and *Bacillus* sp). The most abundant bacteria in *Varroa* mites belonged to the family *Enterobacteriaceae*, especially the genera *Arsenophonus*, *Enterobacter* and *Proteus*. axon-specific *Enterobacteriaceae* and *Arsenophonus* probes also confirmed their localization in the cecum of *Varroa (*Pakwan *et al*., 2018). The external body of *Varroa destructor* is not only place where microorganisms could reside. The salivary glands and gut are also colonized by the microflora (Ball, 1997).

CONCLUSION

The preliminary study showed that the ectoparasitic mite, *Varroa destructor*, harbored 7 genera of bacteria: 3 belonging of Staphylococaccae, 2 of Bacillaceae and 2 of Pseudomonodaceae. The bacteria associated with the mite can play an important role in the phenomenon called Colony Collapse Disorder.

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