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NOTA CIENTÍFICA

First report of *Kurthia gibsonii* in the digestive system of *Apis mellifera* in an indigenous community in the south of Mexico

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Abstract

The insect digestive system is an outstanding niche for recognizing and understanding features and interactions with unique microbiota. *Apis mellifera* play an important role in honey production and pollination, but their populations have decreased over time, so their study and comprehension are vital. Specimens were collected to study the culturable microbiota of the digestive system in an indigenous community in southern Mexico. Digestive samples of bees from four apiaries were randomly analyzed and cultured, and Gram-positive bacilli were identified through conventional biochemical tests and with molecular markers. *Kurthia gibsonii* was identified in 7.5% (6/80) of the cases, and the sequences obtained were reported in GenBank. This is the first report of *K. gibsonii* in the digestive system of bees in apiaries in southern Mexico.

Keywords: *Apis mellifera,* digestive system, *Kurthia gibsonii*

Primer reporte de *Kurthia gibsonii* en el aparato digestivo de *Apis mellifera* en una comunidad indígena del sur de México

Resumen

El sistema digestivo de los insectos es un nicho excepcional para reconocer y comprender características e interacciones particulares con una microbiota única. *Apis mellifera* juega un papel importante en la producción y polinización de la miel, pero sus poblaciones han disminuido con el tiempo por lo que su estudio y comprensión es vital. Se recolectaron especímenes para estudiar la microbiota cultivable del sistema digestivo en una comunidad indígena del sur de México. Se analizaron aleatoriamente muestras digestivas de abejas de cuatro apiarios, se cultivaron e identificaron bacilos Gram positivos mediante pruebas bioquímicas convencionales y marcadores moleculares. Se identificó *Kurthia gibsonii* en el 7,5% (6/80) de los casos y las secuencias obtenidas se reportaron en Genbank. Este es el primer reporte de *K. gibsonii* en el sistema digestivo de abejas en apiarios en el sur de México.

Palabras clave: *Apis mellifera*, *Kurthia gibsonii*, sistema digestivo

Beekeeping is an essential socioeconomic and ecological activity worldwide, highlighting the studies in Mexico. Indigenous communities have practiced ancestral and sustainable beekeeping for thousands of years and using modern modifications, like substituting native bees for European bees (Gupta, 2014). The Amuzgos are one of the most representative ethnic groups of Mexico; they are in Oaxaca and the area in Costa Chica from the State of Guerrero. They inhabit a region with enriched vegetation and template climate. Their economy depends on the trade of agricultural products and, particularly in beekeeping, an element of their ancestral and cultural use and custom. This last practice has been adapted into modern elements only to increase production and be competitive (INPI, 2020).

Currently, because of the notable consequences of anthropogenic activities, the conservation and study of the local species of honeybees have increased in importance. Moreover, the comprehension of the bacterial microbiota in honey bees has been a significant factor in their health and conservation. For this reason, cultivable bacteria isolated from bee gut microbiota have been an unexpected result in various studies. Bacterial genera such as *Pseudomonas*, *Streptococci*, *Micrococci*, *Lactobacillus*, *Klebsiella*, *Proteus*, *Yersinia*, *Bifidobacteria*, *Corynebacterium*, *Bacillus*, and *Clostridium* (García *et al.*, 2006) have been detected. The current literature links *Kurthia gibsonii* as an emerging pathogen in secondary infections, together with other well-known pathogenic genera causing severe damage in farm animals (Lozica *et al.*, 2022) and other insects.

In this study, 95 beehives were analyzed in four sites from the northern area of the Amuzga indigenous community of Zacualpan in Southern Mexico (place **1**: 16°45'11.5" N, 98°17'25.7" W; place **2**: 16°45'02.2" N, 98°17'19.5" W; place **3**: 16°45'26.1" N, 98°18'53.0" W, and place **4**: 16°45'07.1" N, 98°16'52.9" W) (Figure 1). For each place, three beehives were considered, according to the NOM-001-ZOO-1994 norm. A morphological comparison was made using the size and proportion of the bees, according to the Ortiz *et al.* (2004)

Figure 1. Characteristics of the apiaries of *Apis mellifera* in Southern México. a) Place 1, b) Place 2, c) Place 3, and d) Place 4.

Table 2. List of bacteria isolated by conventional and molecular methods.

Site	Strain	UFC/ml	Carbohydrate Fermentation	Identified Strains	Identity Value (%)	GenBank Access number
1	S1I	$1x10^3$	-	*Kurthia gibsonii* strain KH2	99.58	OP824654
2	S2H	$1x10^3$	Glucose	*Bacillus cereus* strain CLF3	99.88	OP824658
3	S3GA	$1x10^3$	-	*Kurthia gibsonii* TY-06	99.76	OP824657
	S3GB	$>15x10^3$	Glucose/Lactose	*Kurthia gibsonii TY-06*	99.79	OP824659
4	S4D	$3x10^3$	Glucose	*Kurthia gibsonii* TY-06	99.83	OP824656
	S4F	$1x10^3$	-	*Kurthia gibsonii* TY-06	99.35	OP824653
	S4G	$>15x10^3$	-	*Kurthia gibsonii* strain KH2	99.68	OP824655

criteria.

The extraction method of the intestinal tract of the bee was made according to Carreck *et al*. (2013). The tissue was macerated in 0.9% saline solution into a mortar under sterility conditions. Only 600 µl of the solution was transferred into plastic tubes with glycerol solution at 10% v/v (1 ml) and stored at -80 °C to produce a backup of the sample.

Each sample was diluted 1:1000 into trypticase soy agar by mass streaking and made in duplicate. Petri dishes were incubated from 24 to 48 h at 28-30°C temperature. Colony-forming units were counted and measured 24 to 48 hours after inoculations. All colonies were studied by macroscopic and microscopic morphology using Gram stain. Solely Gram-positive strains were studied by biochemical tests based on carbohydrate metabolism: galactose, lactose, raffinose, mannose, cellobiose, saccharose, maltose, glucose, sorbitol, and trehalose, according to Dhameliya *et al.*, (2020).

Genomic DNA from the selected strains was extracted according to Adame-Gómez *et al.*, (2019). Subsequently, PCR (Polymerase Chain Reactions) with the universal primers 27F (5'-AGAGTTTTGATCCTGG CTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') were made according to previous reference (Tajabadi *et al*. 2013). A total volume of 13 µl of the master MIX was prepared with 6.25 µl, 4.75 µl of water-free nucleases, 0.5 µl of primers, and 1 µl of the DNA sample using the protocol described in Adame-Gómez *et al.,* (2019). The PCR products were analyzed and visualized in an agarose gel electrophoresis. PCR products were extracted using GeneJET extraction gel (Thermo Scientific®) and sequenced in the Institute of Biotechnology from the National Autonomous University of Mexico.

The obtained sequences were analyzed as Fasta formatted in MEGAX program version 10.1 (Wong *et al.*, 2021) to correct undistinguished sequences. The resulting sequences were compared with other genes obtained from BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Altschul *et al.* 1990) and downloaded from GenBank (www.ncbi.nlm.nih.gov/genbank).

Phylogenetic analysis was made using the sequences obtained and similar sequences of BLAST naming genera and species (Table 1), using the Neirghbor-Joining method with ~ 658 nucleotides from each sequence. The Jukes and Cantor (1969) matrix was applied, and all the processes were computed using MEGA (Wong *et al.*, 2021).

Four apiaries were visited and analyzed in the Zacualpan community, where the honey production was evaluated: 150 liters in place one, 180 liters in

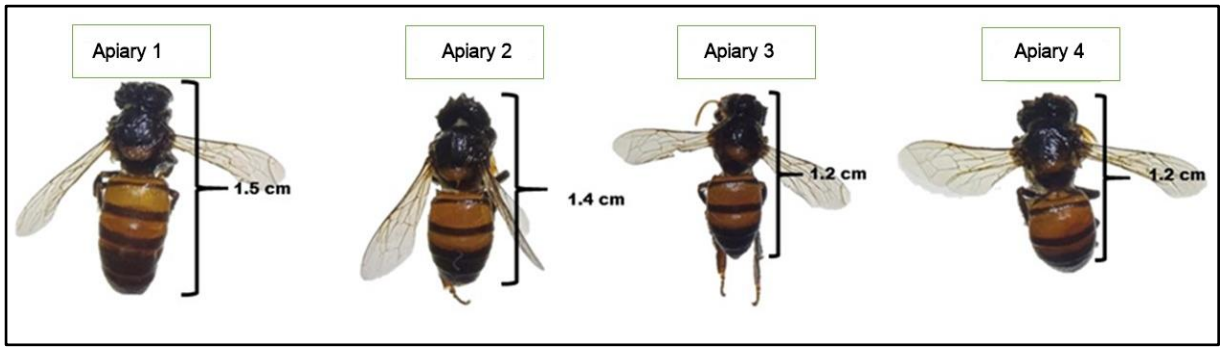

Figure 2. Comparatives from physical properties of some selected bees.

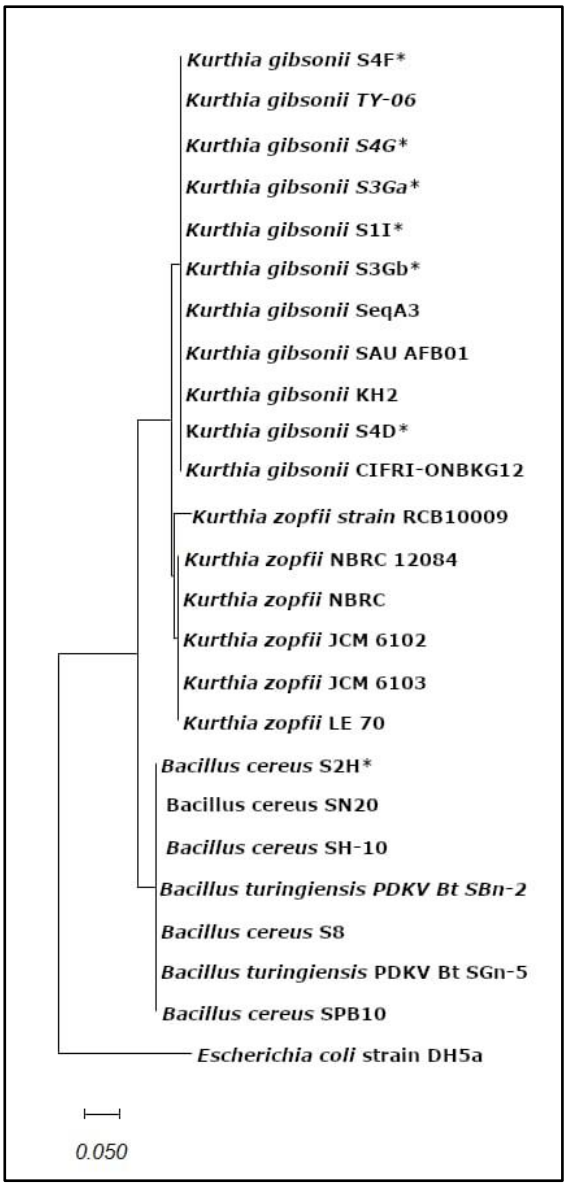

Figure 3. 16S rRNA Neighbor-joining phylogenetic tree from isolated strains in the digestive system from *Apis mellifera*. These sequences are obtained from the GenBank database, *E. coli* DH5α strain was added as an outgroup. The node value is presented as a percent after involving a bootstrap of 1000 replicates. The scale under the tree indicates the number of substitutions per nucleotide substitution. (*) Represent the strains obtained in these studies.

place two, 320 liters in place three, and only 6 liters in place four. In the review and macroscopic analysis of the bee population, four adult specimens were randomly sampled and observed to compare their morphometric structure and sizes. Images of each specimen are shown in Figure 2. According to previous references (Ortiz *et al.*, 2004), the specimen from Apiary 1 is the only one with regular proportions.

Seven of 80 bacterial strains were selected and isolated from the bee gut microbiome for their macro and microscopic characteristics, as well as by biochemical tests. These unknown strains appeared white, shiny, convex, and round 1 to 2 mm growing on LB and nutrient agar. Characterized as Gram-positive bacillus of short morphology, motile, with positive catalase and urease activity, growing at room temperature, some of these strains were capable of fermenting glucose and lactose (Table 1). Of the strains isolated, S2H was identified as *Bacillus cereus* with an identity value of 99.88%, Besides, S1I, S3GA, S3GB, S4D, S4F, and S4G strains correspond to *K. gibsonii* with a 99% identity value.

According to the phylogenetic analysis (Figure 3), the reported strains show low differences between the strains of *K. gibsonii* and *K. zopfii*, unlike the species *B. cereus* and *B. thuringiensis* where it is notable differences and substitution changes. This brings a discussion because concerning their microscopical aspects and properties *Kurthia* and *Bacillus* genera, have similar elements and properties.

Kurthia spp. is associated with the microbiome of some farm animals as chickens and pigs. The study of this relation has been recently reported as significative due to its biotechnological use in the processing and decoloring of triphenylmethane in textile effluents (Sani & Banerjee, 1999), into its infective role, producing zoonotic secondary infections (Kövesdi *et al.*, 2016), and as a symbiont in the carotenoids C30 production for the intestine of *Sympetrum frequens* (Koyanagi *et al.*, 2021).

Kurthia gibsonii has been reported as an emerging opportunistic pathogen in secondary coinfection in farm birds together with *E. coli* in pathologies that could cause chondronecrosis and osteomyelitis in lower joints (Lozica *et al.*, 2022). Due to the above, *K. gibsonii* species isolated in this study could become a bee emerging pathogen, or bees could be asymptomatic carriers. However, more studies are recommended to recognize its biology in bees.

The importance of the bee microbiota and its health has previously been reported. However, the finding of Kurthia genus in the hives may be a risk factor that compromises health or leads to stress, as suggested by Lorizzo *et al.* (2020). Understanding the bacterial microbiome of bees is an essential element in their health and relationship with the farm

itself because these bees are mainly involved in the pollination of many crops. Its population decline could cause severe problems in food production.

This is the first report of *K. gibsonii* in the digestive system of bees cultivated by the indigenous community in Southern Mexico.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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