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Identification of *Buchnera* sp., symbiont of *Toxoptera aurantii*

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RESUMEN

Los áfidos son insectos que se alimentan del floema, los cuales debido a la falta de aminoácidos esenciales en la savia floématica, han desarrollado simbiosis con bacterias. Afidos identificados como Toxoptera aurantii, *afectando plantas de* Macadamia integrifolia *en Cuba, fueron analizados para la presencia de* Buchnera aphidicola, *con el objetivo de lograr una mejor caracterización de los mismos y un posible método de control. La amplificación por PCR mostró la presencia de la bacteria simbionte en todas las poblaciones de áfido detectadas. El análisis filogenético indicó que la bacteria detectada podría ser una nueva especie perteneciente al género* Buchnera, *y constituye el primer informe de esta bacteria en* T. aurantii.

Palabras claves: *áfido, simbiosis, plaga, filogenia, Buchnera*

ABSTRACT

Aphids are phloem-feeding insects which have developed symbiotic association with bacteria to acquire amino acids lacking in the phloem. Toxoptera aurantii *affecting* Macadamia integrifolia *trees in Cuba were analyzed for the presence of* Buchnera aphidicola, *to characterize the aphids and find efficient management methods. The PCR amplification and sequencing showed that in all populations detected in every tree, was present the symbiotic bacteria. The phylogenetic analysis indicates that the bacteria detected could be new specie belonging to* Buchnera *genus, and is the first report of this bacterium in* T. aurantii.

Key words: *aphid, symbiosis, pest, phylogeny, Buchnera*

INTRODUCTION

Aphids (Hemiptera: Aphidoidea) are able to synthetize only nine of twenty essential amino acids, reason why the symbiotic relation with bacteria from *Buchnera* genus is vital. Those bacteria are capable to synthetize the amino acids lacking in the phloem [Douglas, 1998; Rusell *et al*., 2003].

Toxoptera aurantii (Boyer de Fonscolombe) (Homoptera: Aphididae), is an extremely polyphagous species, having been recorded in at least 190 genera in 80 families of plants plants [van der Goot, 1917; Essig, 1949; Bodenheimer, 1951; Leonard *et al*., 1971]. *T. aurantii* have been previously reported in Cuba [Jaraslau, 1974], mainly affecting citrus as vector of Citrus tristeza virus (CTV) [Batista *et al*., 2008].

The black aphid is an economically important pest for many crops in Cuba, mainly fruits like citrus, mangoes, macadamia nuts and cocoa, and the identification of the symbiotic bacteria in this aphid could be employed in management strategies.

MATERIALS AND METHODS

All aphids were collected in an *ex situ* tropical and subtropical fruit trees collection in Artemisa, Cuba. We collected aphids from ten Macadamia nut trees. DNA was extracted from 0.5 g of black aphids collected from every macadamia plant. The aphids were macerated with liquid nitrogen and incubated 30 minutes with 2.5 ml of extraction buffer (100 mMTris-base pH 8, 150 mMNaCl, 25 mM EDTA, 8 mMpolyvinylpyrrolidone and 50 mMcetyltrimethyl ammonium bromide). To avoid oxidation, we supplied buffer with 0.2 % β-mercaptoethanol. After incubation we precipitated by centrifugation 10000 rpm and transferred 900 µl into 2.0 ml clean Eppendorf

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tube. We added 900 µl of chloroform: isoamylalcohol (24:1 proportion), shaking in vortex and centrifugation 10 000 rpm to separate phases; 800 µl of this solution was transferred into 1.5 ml clean Eppendorf tube and 500 µl of isopropanol was added. After 2 hours, the mixture was centrifuged 10 000 rpm; the supernatant was removed, and the pellet washed twice with 70 % ethanol. The pellet was dried and eluted in 30 µl of tri distilled sterile water. The obtained DNA was used as a template for a PCR assay. Universal primer pairs that target the bacteria 16S rRNA gene, FD1 and RP1 [Weisburg *et al*., 1991], were used for the reaction.

RESULTS

PCR product of expected size (~1250 bp) was obtained from *Toxoptera aurantii* collected from the ten plants. PCR products were purified (Wizard SV Gel and PCR Clean-Up System, Promega, Madison, WI, USA), cloned (pGEMT-Easy Vector, Promega), and two individual clones per insects group collected in every plant were sequenced by Macrogene, South Korea. Phylogenetic relationships were established between *Buchnera aphidicola* (*Toxoptera aurantii*) 16Sr RNA gene, partial sequence and those of *Buchnera sp*. previously identified (Mega 5.0, USA).

The FD1/RP1 sequences of bacterium were amplified from aphids collected from all plants. All sequences amplified were 100 % identical to each other. The consensus sequence (1095 nt) of the bacterium amplified from aphid was deposited in GenBank (Accession KF992834) and showed 96 % of sequence identity with those of the *Buchnera aphidicola*., including strains isolated from *Aphis fabae fabae* (AY518294), *Aphis craccivora* (EF614236), *Aphis gossypii* (JN989961) and *Schizaphis graminum* (NR_074512). The Blast results were confirmed by phylogeny (*Figure 1*) since the *Buchnera aphidicola* isolated from *T. aurantii* did not grouped with the other *Buchnera aphidicola* previously study.

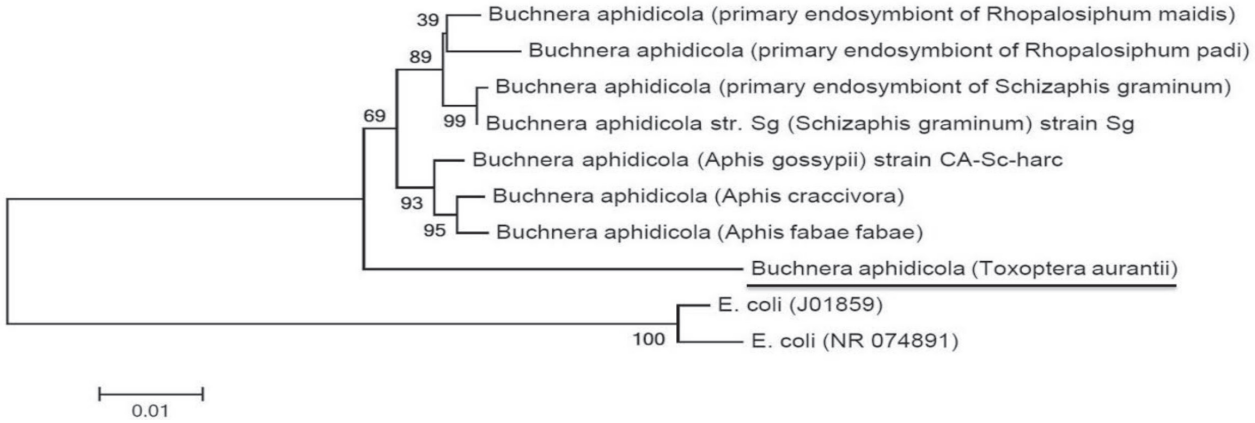

Figure 1. Phylogenetic tree based on the 16S rDNA sequences of the *Buchnera spin Toxoptera aurantii*, and other 16Sr reference *Buchnera aphidicola* isolated from other aphids. Bootstrap values obtained for 1000 replicates are shown above the branches. *Escherichia coli* was used as the outgroup to root the tree.

CONCLUSIONS

- In this study, was detected a tentative new species of *Buchnera* genus based mainly in the 16S rRNA sequences. This is the first report of the presence of bacterial symbiont in the black aphid *Toxoptera aurantii*.
- Taking into account that when sequence similarity is lower than 97.5 %, the organisms could belong to related species [Stackebrandtand Goebel, 1994], the symbiotic bacteria detected in *T. aurantii* could be new specie or at least a new strain. Other studies should be done to confirm this. The evolutionary relation between the aphids and the bacteriocytes (*Buchnera*) has to be taken into account to define new species [Shigenobu *et al*., 2000].

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