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Basic / Sección Básica
Scientific Note / Nota científica

Genetic variation of *Bactericera cockerelli* Šulc. (Hemiptera: Triozidae) suggests new haplotype in México

Variación genética de *Bactericera cockerelli* Šulc. (Hemiptera: Triozidae) sugiere nuevo haplotipo en México

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Abstract: A new genetic variation and potential new haplotype of *Bactericera cockerelli* was identified based on the analysis of the mitochondrial region Cytochrome Oxidase subunit I of two populations from greenhouses in Villa Unión, Durango and Saltillo, Coahuila, Mexico. A variation was found in the base 247 of a 500 bp amplified of the *mt*COI gene, this change implies the substitution of an adenine (A) to a Guanine (G), this mutation was detected in the insects collect from greenhouses and in their progeny obtained under laboratory conditions. This genetic variant has a great potential to be a new haplotype of *B. cockerelli*, therefore, it was designated Mexico Haplotype 1 (MXH1) with GenBank accession number KX130767. A total of ten insects from the original collection and then from laboratory cages were evaluated for Lso infection and all tested negative.

Keywords: Lso, *mt*COI, MXH1, polymorphism, potato psyllid.

Resumen: Se identificó una nueva variante genética y un nuevo haplotipo potencial de *Bactericera cockerelli* basado en el análisis de la región mitocondrial Citocromo Oxidasa subunidad I de dos poblaciones provenientes de invernaderos de Villa Unión, Durango y Saltillo, Coahuila, México. Se identificó la variación de la base 247 de un amplificado de 500 pb del gen *mt*COI, cuyo cambio implica la sustitución de una adenina (A) por una Guanina (G), esta mutación se encontró en los insectos de la colecta inicial y en la progenie obtenida bajo condiciones de laboratorio. Esta nueva variante genética tiene un gran potencial como nuevo haplotipo de *B. cockerelli* por lo que se denominó México Haplotipo 1 (MXH1), con número de acceso GenBank KX130767. Un total de diez insectos de la colecta original y diez de las crías de laboratorio fueron evaluados para determinar la presencia de Lso y todos resultaron negativos.

Palabras clave: Los, mtCOI, MXH1, polimorfismo, psílido de la papa.

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Introduction

Bactericera cockerelli (Hemiptera: Triozidae) is an economic pest of plants of the family Solanaceae, mainly of potato (*Solanum tuberosum* L.) and tomato (*Solanum lycopersicum* L.) (Munyaneza 2010) crops. Symptoms including the yellowing of leaves are caused by direct feeding of the psyllids and by the transmission of '*Candidatus* Liberibacter solanacearum' (Lso) (Munyaneza *et al*. 2007; Liefting *et al*. 2009). Lso is a Gram-negative bacterium limited to the phloem (Munyaneza *et al*. 2007; Secor *et al*. 2009) and associated with zebra chip on potato and permanent yellowing disease on tomato (Munyaneza *et al*. 2009, 2010; Crosslin *et al*. 2011).

Genetic differentiation studies of *B. cockerelli* populations contributed to haplotypes determination according to simple nucleotide polymorphism (SNP) of mitochondrial gene cytochrome oxidase subunit I (*mt*COI), gave place to the determination of four haplotypes in different geographical regions of the United States: Western, Central, Northwestern and Southwestern (Swisher *et al*. 2012, 2013a, 2014). The Central haplotype was initially found in East of Mexico, through Texas, Kansas, Colorado, Nebraska, Wyoming, and North Dakota, through Latin

America, including El Salvador, Honduras, Mexico, and Nicaragua (Liu *et al*. 2006; Swisher *et al*. 2012, 2013a, b). The Western haplotype was found in California, Idaho, New Mexico, Oregon and Washington (Swisher *et al*. 2012, 2013a). The Northwestern haplotype has been found in Idaho, Oregon and Washington (Swisher *et al*. 2012, 2013a), and the Southwestern haplotype was found in Colorado and New Mexico states (Swisher *et al*. 2014). A previous study reported the presence of the Central haplotype in Mexico, Queretaro, Sinaloa and Toluca states (Swisher *et al*. 2013b). The purpose of this study was to learn about haplotype-based genetic variation of *B. cockerelli* by analyzing *mt*COI in two specific populations from Mexico and its Lso infection.

Materials and methods

Adult insects of *B. cockerelli* were collected from tomato greenhouses in Villa Union, Durango (23°57′12″N, 104°02′21″W) and Saltillo, Coahuila (25°21′17″N, 101°02′17″W), Mexico in the summer of 2015. Insect samples were collected with an entomological aspirator directly from the plant and stored in plastic containers with 70% ethanol inside a cooler.

DNA was extracted from five insects individually from each location, using the technique described by Doyle and Doyle (1990). Primers CO1 F3 and CO1 R3 were used to amplify a region of 500 bp from mitochondrial gene Cytochrome C Oxidase subunit I (Liu *et al*. 2006; Swisher *et al*. 2012) by endpoint PCR. 4 µL of Hot Start Taq DNA Polymerase (Gen Script), 0.5 µL of each primer at 10 µM and 50 ng of DNA were used in the PCR reaction for a final volume of 15 µL. Thermocycler conditions: initial denaturation of 98°C for 30 s; 30 cycles of 98°C for 10s, 56°C for 20s and 72°C for 30s; and a final extension at 72°C for 7min. PCR products were sequenced directly using the CO1 F3 and CO1 R3 primers in both directions by Macrogene, USA. The products were aligned by MEGA 7 and analyzed in BLAST® (NCBI 1988), by comparing them with Western (GenBank accession number JQ708095), Central (Gen Bank accession number JQ708094), Northwestern (GenBank accession number JQ708093) and Southwestern (Gen Bank accession number KC305359) *B. cockerelli* haplotypes. For amino acid translation, SIB ExPASy Bioinformatics Resources Portal (Artimo *et al*. 2012) was used with the genetic code setting for invertebrate mitochondria.

For each location, one female and one male were placed to reproduce on tomato seedlings in entomological cages, under a photoperiod of 14/10h light/darkness. From each cage, five insects from the first generation were collected and subjected to DNA extraction and DNA barcoding as described above. Five psyllids from initial collections and five psyllids from laboratory colonies were tested for Lso infection by conventional PCR using universal primers for Liberibacter species LG774 F and LG1463 R (Morris *et al*. 2017) and Lso specific primers Lso TX 16/23 F and Lso TX 16/23 R (Ravindran *et al*. 2011). Experiments to determine Lso acquisition and transmission by this populations were not performed.

Results

DNA was extracted individually from insects from each locality to amplify and sequence the *mt*COI gene in order to genetically identify two populations of *B. cockerelli* collected inside greenhouses from geographically distinct localities. Rearing cages were established under laboratory conditions from a female and a male from each of the sampled localities, obtaining the F1 on which the molecular identification procedure was repeated through barcoding to confirm the DNA sequence obtained and its heritability. The presence of Lso in the same genetically identified insects was evaluated to determine if they were active vectors of this bacterium. Five sequences of *B. cockerelli* were obtained from each one of the two locations sampled. All sequences were similar and had a single genetic variation at base number 247 of the 500 bp amplicon of the *mt*COI gene (Fig. 1). This change involves the replacement of an Adenine for a Guanine. This genetic variation is related to a potential transitional mutation in the mitochondrial region of *B. cockerelli* compared with the previously described Central haplotype. The protein product is not modified by the nucleotide change, indicating that it is a silent mutation in the analyzed populations. These results were confirmed through the analysis of progeny (F1) from laboratory established cages by sequencing five individuals from each of the two different colonies, obtaining same genotypic variation in the position 247 of the *mt*COI gene. This new genetic variant was tentatively designated as Haplotype 1 Mexico (MXH1), GenBank Accession number KX130767.

A total of twenty psyllids (ten from initial collect and ten from laboratory cages) were tested for Lso detection, were all individuals of *B. cockerelli* MXH1 haplotype tested negative for Lso infection.

Discussion

Genetic classification of *B. cockerelli* populations has allowed the identification of four haplotypes or genetically distinct populations. The Central, Western, Northwestern and Southwestern haplotypes have been differentiated by analysis of the 500 bp specific region of the mitochondrial gene Cytochrome Oxidase subunit I (Chapman *et al*. 2010; Swisher *et al*. 2012, 2013a, 2014). Haplotype differentiation is based on nucleotide variation within this region, ranging from one to 17 nucleotides difference between haplotypes, that is, one SNP difference between Western and Central haplotypes; whereas there are 16 and 17 SNPs differences between the Northwestern haplotype and the Central and Western haplotypes, respectively (Swisher *et al*. 2012). The Southwestern haplotype has two SNPs that are absent from the rest of the haplotypes (Swisher *et al*. 2014). The potential new MXH1 haplotype differs by two SNPs from the Western haplotype in positions 51 and 247; it differs by one SNP from the Central haplotype in position 247; by 18 SNPs from the Northwestern haplotype and by three SNPs from the Southwestern haplotype. The tentative MXH1 haplotype is closely related to the Central haplotype by having only one different base; the MXH1 has a Guanine and the Central haplotype has an Adenine in position 247. This change, as with the four haplotypes identified, does not generate a change in the amino acid sequence (Swisher *et al*. 2014) and it can be heritable to the progeny. To our knowledge, this is the first research work that identifies a new genetic variation in *B. cockerelli* from Mexico, where only the Central haplotype have been reported by Swisher *et al*. (2013b) in same states where MXH1 was found, however, the populations sampled by these authors were collected under open field conditions. Populations analyzed in this work come from greenhouses, where they probably had no contact with other populations or developed several generations in

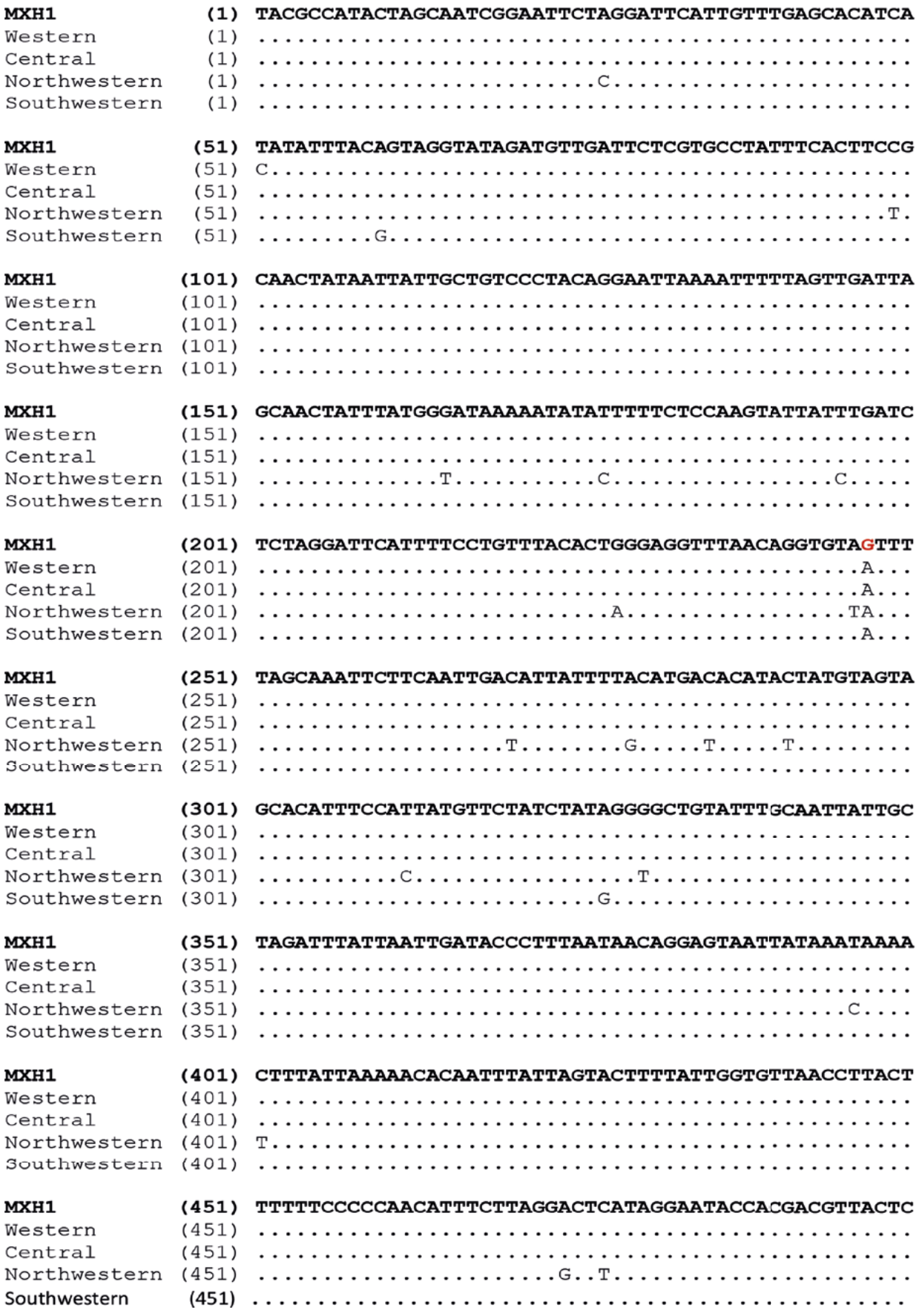

Figure 1. Comparison of DNA sequence from a 500 bp portion of *mt*COI gene obtained from *Bactericera cockerelli* MXH1 haplotype against Central, Western, Northwestern and Southwestern haplotypes. The MXH1 showing a new SNP (G) in position 247.

confined conditions. It is possible that the Central haplotype may have originated from the MXH1 haplotype or vice versa. Biological characteristics between haplotypes are influenced by geographic region, environment, host or endosymbionts (Nachappa *et al*. 2011; Cerna *et al*. 2021). Haplotype differences could determine vectoring efficiencies, fertility or interhaplotype fertility, fitness traits, host plant use, and overwintering capabilities (Mustafa *et al*. 2015), making necessary to study MXH1 biology, dispersion, and its interactions with other haplotypes.

All individuals of *B. cockerelli* MXH1 haplotype tested negative for Lso infection possibly due to its greenhouse origin, where they probably had no previous contact with Lso. This trait is shared with the Southwestern haplotype, which was also reported not to carry Lso (Swisher *et al*. 2014). However, Swisher *et al*. (2018) confirmed that the Southwestern haplotype can acquire and transmit Lso and declare not insect haplotype affects Lso transmission among Western, Central, Northwestern and Southwestern haplotypes. Therefore, MXH1 could also be an efficient vector of Lso.

Conclusions

These results show the existence of a new genetic variant of *B. cockerelli* with potential to be a new haplotype. More studies are needed to the determine the biological and ecological characteristics of this new haplotype and the differences with the Central, Western, Northwestern and Southwestern haplotypes in order to determine the origin and genetic relationship of this variation among populations, which will aid in designing psyllid management strategies and determining the pathogens it could vector, for example, Lso.

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Contribución de los autores

Mariana Beltrán Beache: elaboración de proyecto, diseño de metodologías, colecta e identificación de especímenes, análisis de datos y elaboración del manuscrito; J. Carlos Delgado Ortiz: colecta e identificación de especímenes, conservación de crías y elaboración de manuscrito; Y. María Ochoa Fuentes: diseño de metodologías, supervisión de actividades y elaboración de manuscrito; Ernesto Cerna Chávez; elaboración de proyecto para acceso a recursos económicos, diseño de metodologías, supervisión de actividades y elaboración de manuscrito.

Conflictos de interés

Los autores que participaron en esta publicación hicieron contribuciones significativas al manuscrito; todos los autores están de acuerdo y expresan que no hay conflictos de intereses en este estudio.