First report of '*Candidatus Liberibacter solanacearum*' in Ecuador and in South America

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Received: 11 June 2019 / Accepted: 29 January 2020 / Published online: 3 February 2020
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Abstract

'*Candidatus Liberibacter solanacearum*' (CaLso) haplotypes A and B, is a phloem-limited proteobacterium widely associated with Zebra chip (ZC) disease in potato tubers. In June 2019, symptoms such as yellowing and upward rolling in leaves and browning of vascular tissues and streaks at the medullary area in tubers were observed at potato fields located at the Pichincha province, Ecuador. In addition, high populations of *Bactericera cockerelli* were associated with the occurrence of these symptoms. CaLso haplotype A was detected in infected potato fields and the psyllid, *B. cockerelli* using conventional PCR. DNA sequence analysis confirmed CaLso in infected potato plants and insect tissues. To our knowledge this is the first report of CaLso haplotype A associated with potato and *B. cockerelli* at the Pichincha province, Ecuador and South America, where potato represents an important agricultural commodity.

Keywords *Solanum tuberosum* · *Bactericera cockerelli* · Haplotype A · Pichincha

'*Candidatus Liberibacter solanacearum*' (CaLso) haplotypes A and B, is an endogenous, phloem-limited proteobacterium widely associated with Zebra chip (ZC) disease in potato tubers (Munyanze et al. 2007). This disease is transmitted by a potato-tomato psyllid, *Bactericera cockerelli* (Hemiptera: Trioziidae), an important pest of Solanaceae (Munyanze et al. 2007). The disease associated with CaLso and its vector has caused millions of dollars in losses to potato and other solanaceous crops in North and Central America, and New

Zealand (Munyanze 2012). In Ecuador, *B. cockerelli* was reported for the first time by Castillo et al. (2019) causing serious economic losses to potato producers. Presumably, the psyllid was accidentally introduced from Guatemala, Honduras or Nicaragua, Central America (Jackson et al. 2009; Bextine et al. 2013 and Munyanze et al. 2013), where the presence of the insect was previously reported. Although *B. cockerelli* is present in Ecuador, the presence of CaLso has not been reported.

To detect CaLso, two potato samples (M6-P-Pi and M7-P-Pi) were collected from plants showing leaf symptoms such as yellowing and upward rolling, and tubers showing browning of vascular tissues and streaks at the medullary area, in June 2019 (Fig. 1). In addition, sixty adults of *B. cockerelli* separated in two composite samples (M1-P and M2-P) were collected in the same potato fields for analysis. DNA was extracted from leaf midribs using the Fungi/Yeast Genomic DNA Isolation Kit (Norgen Biotek Corp., ON, Canada). To apply this protocol to plant tissues, 200 mg of leaf midribs were macerated in 1 ml of Lysis Buffer L using a freeze mortar. DNA from insects was extracted using the Puregene® Blood Core Kit A (Qiagen, MD USA), according to the manufacturer's instructions. To amplify the partial sequence of 16S ribosomal RNA gene, conventional PCR reactions were conducted using specific primers for CaLso detection (CLi.po.F/OI2c) (Secor et al. 2009). AccuPrime High Fidelity Taq

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Fig. 1 Symptoms observed in potato plants infected with '*Ca. L. solanacearum*' at the Pichincha province, Ecuador. **A)** Leaf yellowing and upward rolling; **B)** Tubers showing browning of vascular tissues and streaks in the medullary area

DNA polymerase (Invitrogen, CA USA) was used for PCR, according to manufacturer's instructions. Negative 'water' controls and potato healthy plant samples were also included. All PCR products were analysed on a 2% agarose gel and the positive PCR products were purified using PureLink® PCR purification Kit (Invitrogen, CA USA).

Both, potato and insects samples analysed produced amplicons with an expected size of 1078 bp with primers CLi.po.F/OI2c. No amplicons were obtained with negative samples (water and healthy potato plants). All positive PCR products from potato and insect samples were sequenced at commercial facilities (Macrogen, MD USA). Sequence and BLAST analysis showed a 100% homology with '*Candidatus Liberibacter solanacearum*' (GenBank accession

numbers: FJ957896 from Mexico and EU918197 from United States). Alignment analysis of all sequences of CaLso revealed two conserved single nucleotide polymorphism (SNP) mutations (g.212 T > G and g.581 T > C), confirming that the sequences belong to CaLso haplotype A. DNA sequences were deposited at NCBI with GenBank accession numbers: MN396642 (M6-P-Pi), MN396643 (M7-P-Pi), MN031249 (M1-P) and MN031250 (M2-P).

Phylogenetic trees were constructed using partial sequences of 16S rRNA gene using Maximum Likelihood method with 2000 bootstrap value. DNA sequences obtained from infected plants and insects in June 2019, grouped with CaLso, with a bootstrap value of 75% compared to several members of the clade (Fig. 2). To our knowledge, this is the

Fig. 2 Phylogenetic tree inferred from 16S rDNA sequences using the Maximum Likelihood method, obtained from 17 members of '*Candidatus Liberibacter* spp.' group, including '*Candidatus Phytoplasma solani*' clone 5043 and strain 121/09 as outgroups. Codon-based MUSCLE alignment function was used to align the

sequences. Bootstrap values are shown next to the branches. GenBank accession numbers are indicated in parentheses, black dots indicate samples analysed in this study: insect samples M1-P and M2-P; infected potato samples M6-P-Pi and M7-P-Pi

first molecular report of CaLso haplotype A from Pichincha province, Ecuador, and the first report of CaLso in potato and in the potato-tomato psyllid, *B. cockerelli* in South America, where potato represents an important agricultural commodity.

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