Diseases Caused by Bacteria and Phytoplasmas

First Report of *Xanthomonas citri* subsp. *citri* Causing Citrus Canker on Lime in Rio Grande do Norte, Brazil

L. C. S. Amancio,¹ A. D. B. Baia,¹ E. B. Souza,² R. Sales-Júnior,³ A. M. P. Negreiros,³ V. Q. Balbino,⁴ and M. A. S. Gama^{1,†}

¹ Universidade Federal Rural de Pernambuco, Departamento de Agronomia, Recife, 52171-900, Pernambuco, Brazil

² Universidade Federal Rural de Pernambuco, Departamento de Biologia, Recife, 52171-900, Pernambuco, Brazil

³ Universidade Federal Rural do Semi-Árido, Departamento de Ciências Agronômicas e Florestais, Mossoró, 59625-900, Rio Grande do Norte, Brazil

⁴ Universidade Federal de Pernambuco, Departamento de Genética, Recife, 50740-600, Pernambuco, Brazil

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Citrus canker caused by *Xanthomonas citri* subsp. *citri* is one of the most important citrus diseases in the world (Gottwald et al. 2002), mainly for citrus-producing countries with humid subtropical regions such as United States, Argentina, and Brazil, where losses may be significant (Behlau et al. 2020). In the state of Rio Grande do Norte (RN), Brazil, citrus production is expanding and shows social and economic importance for small farmers, which produced approximately 297 tons of lime in this state in 2019 (IBGE 2021). In December 2019, we observed symptoms of erumpent lesions with margins surrounded by yellow haloes on leaves and fruit of the lime (*Citrus aurantifolia* cv. Galego) (about 5% incidence) in a plantation located in the municipality of Mossoró, RN (05°12'21.1"S, 37°19'16"W). Samples were collected from the lime orchard, and five bacterial strains (CCRMXC01 to CCRMXC05) showing yellow, convex, mucoid colonies were isolated in a nutrient-yeast-dextrose-agar medium (NYDA). Pathogenicity tests were performed on sweet orange (*C. sinensis* cv. Pera) and lime (*C. latifolia* cv. Tahiti) seedlings. Four wounds per leaf (upper side) were carried out with an entomological pin and 10 µl of a bacterial suspension (10⁸ CFU ml⁻¹) were deposited on each wound. The negative control consisted of leaves treated with sterile distilled water (SDW). For each citrus species, we used four replicates per strain and one leaf with four wounds per replicate. Inoculated leaves developed erumpent lesions with margins surrounded by yellow haloes six

days after inoculation (DAI) in both citrus species, while leaves treated with SDW remained symptomless. Nine DAI, we reisolated the pathogen and performed rep-PCR (REP, ERIC, and BOX-PCR) analyses (Gama et al. 2018) with the strains inoculated and reisolated to confirm the identity of the strains and to fulfill Koch's postulates. The strains were stored at the Culture Collection Rosa Mariano (CCRM) of the Phytopathology Laboratory at the Universidade Federal Rural de Pernambuco. The five strains reisolated showed the same REP, ERIC, and BOX-PCR profiles as the strains used for inoculations. The molecular identification was performed sequencing the *dnaK*, *fyuA*, *gyrB*, and *rpoD* genes (Young et al. 2008). Each fragment was sequenced in both the forward and reverse directions. Using the BLASTn tool, we observed that sequences of the *dnaK* (GenBank MW218913 to MW218917), *fyuA* (MW218918 to MW218922), and *rpoD* (MW218928 to MW218932) genes of the strains CCRMXC01 to CCRMXC05 showed 100% identity with the sequences of these genes from the type strain (ICMP 24^T) and of other strains of *X. citri* subsp. *citri* (ICMP 21 and ICMP 7493), while sequences of *gyrB* (MW218923 to MW218927) of the former strains showed 100% identity with the *gyrB* sequence of the strains ICMP 24^T and ICMP 7493 and 99.85% identity with strain ICMP 21. This short variation in the sequence of the *gyrB* gene also may be observed among strains of *X. citri* subsp. *citri* available in NCBI (<https://www.ncbi.nlm.nih.gov/>). The phylogenetic analysis performed using Bayesian inference and the concatenated sequence of all the type or representative strains of species and pathovars of *Xanthomonas* available in GenBank showed that the strains CCRMXC01 to CCRMXC05 clustered together with strain ICMP 24^T with 1.0 posterior probability. To our information, this is the first report of *X. citri* subsp. *citri* causing citrus canker on lime in RN state, Brazil.

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[†]Indicates the corresponding author.
M. A. S. Gama; mas.gama@yahoo.com.br