Diseases Caused by Bacteria and Phytoplasmas

First Report of *Xanthomonas albilineans* Causing Leaf Scald on Two Chewing Cane Clones in Zhejiang Province, China

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In recent years, outbreaks of leaf scald have been reported in two chewing cane clones, ‘Guangdong Huangpi’ and ‘Taoshang Guozhe’, in Zhejiang province, China. From May to July 2019, we collected symptomatic samples from Guangdong Huangpi (eight leaves and three stalks) from four farms in Wenling and Taoshang Guozhe (eight leaves and five stalks) from three farms in Ruian, Zhejiang province. Leaves in young plants exhibited white pencil-line streaks as well as partial or complete chlorosis of the leaf blade. Internal symptoms included an orange-red discoloration of the vascular bundles at the basal nodes of the stalk and discoloration extension into the internodes. Leaf and stalk tissues were used for bacterial isolation and purification on XAS medium, which is selective for *Xanthomonas albilineans* (Davis et al. 1994), using the streak plate method to obtain 24 isolates (Lin et al. 2018). Circular, convex, smooth, shiny, and yellow colonies were isolated from all the samples. The pathogenicity of two isolates, XaCN30 from Guangdong Huangpi and XaCN43 from Taoshang Guozhe, was confirmed with Koch’s postulates according to the protocol reported by Lin et al. (2018). The incidences of diseased plants (56 and 63%) were observed in individual host clones at 28 days postinoculation with isolates XaCN30

and XaCN43, respectively. Furthermore, all isolates were confirmed as *X. albilineans* via molecular methods. Polymerase chain reaction (PCR) amplification was conducted for all 24 isolates using the primer pairs XgyrB1F/XgyrB1R2 (Ntambo et al. 2019) and XAF1/XAR1 (Wang et al. 1999), which target the *gyrB* (encoding the β subunit of the DNA gyrase) and *abc* (encoding an ABC transporter) genes, and generate 904- and 608-bp amplicons, respectively. The PCR fragments were cloned into pMD19-T vector (TaKaRa, Dalian, China). For each isolate, three single colonies of transformed *Escherichia coli* DH5 α carrying the targeted fragment were sequenced. These sequences were deposited into GenBank with accession numbers MT776053 to MT776059 and MT776061 to MT776077 for *gyrB* gene and MT776098 to MT776104 and MT776106 to MT776122 for *abc* gene. Based on the two concatenated DNA sequences of our 24 isolates, compared with 27 previously reported *X. albilineans* isolates obtained from the GenBank database, pairwise sequence identity analysis revealed that all 24 isolates from Zhejiang province had 99.4 to 100% identity with each other, 99.6 to 100% identity with 14 published domestic isolates, and 98.3 to 100% identity with 13 foreign isolates. Furthermore, phylogenetic analysis with MEGA 7.0 (Kumar et al. 2016) showed that the isolates from Zhejiang province clustered into two distinct groups. One group consisted of 25 Chinese isolates (including all 11 isolates from Wenling) along with four isolates from the French West Indies (GPE PC73, GPE PC17, GPE PC86, and MTQ032) and one isolate from the United States (XaFL07-1), which were assigned to pulsed-field gel electrophoresis group B (Davis et al. 1997; Pieretti et al. 2012). A putative group was also proposed, which included all 13 isolates from Ruian, indicating that isolates from Ruian are distinct from the isolates isolated from other Chinese sugarcane-planting areas including Wenling. We conclude that leaf scald disease in local clones of chewing cane are caused by *X. albilineans* in Zhejiang province in China, which will be helpful for leaf scald management in chewing cane, a cash crop.

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