Detection of *Candidatus Liberibacter asiaticus* from Wampee (*Clausena lansium* Skeels) by Nested PCR

Xiaoling Deng, Gen Zhou, and Huaping Li, Laboratory of Citrus Huanglongbing Research, Department of Plant Pathology, South China Agricultural University, Guangzhou, Guangdong 510642, People's Republic of China; and Jianchi Chen and Edwin L. Civerolo, San Joaquin Valley Agricultural Sciences Center, Crop Diseases, Pests, and Genetics Research Unit, USDA-ARS, Parlier, CA 93648

Corresponding author: Jianchi Chen. jichen@fresno.ars.usda.gov


Wampee (*Clausena lansium* Skeels) is native to southern China and Southeast Asia. Wampee trees are attractive, with grape-like fruits and a muscat taste and are popular in home gardens. Like other members of Rutaceae, wampee has long been suspected to have "yellow shoot" disease or Huanglongbing (HLB) and *Diaphorina citri*, the disease vector, was capable of a long-term survival on Wampee (2). The importance of wampee HLB is its potential to serve as a source of inoculum of the HLB pathogen, *Candidatus* Liberibacter spp., for sweet orange, mandarin, and other economically important citrus crops. Yet, the presence of *Ca*. Liberibacter spp. in wampee has not been confirmed with the 16S rDNA signature sequence that define this unculturable bacterium (1,3).

In August of 2006, we identified two wampee trees showing "yellow shoots" symptoms (Fig. 1A) adjacent to a mandarin orchard with HLB in Luoding City, Guangdong Province, People's Republic of China. Symptomatic leaves ranged from mottling and to yellowing (Fig. 1B). Leaf samples were collected, and DNA was extracted using the CTAB (cetyltrimethylammoniumbromide) method (4). DNA samples were assayed by nested-PCR. The general bacterial 16S rDNA primer set fDl/rD1 (AGA GTT TGA TCC TGG CTC AG / AAG GAG GTG ATC CAG CC) (1) was used for the outer round amplification. The PCR reaction (25 µl) mixture contained:10 mM Tris-HCl, pH 8.3; 50 mM KCl; 1.5 mM MgCl$_2$; 100 µM each dNTPs; 400 µM each primer, 1 U of *Taq* DNA polymerase and 1 µL of template DNA. Amplification was conducted with an initial denature at 96°C for 10 min, followed by 10 cycles consisting of: denaturing at 96°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s. Two µl of the amplicon were then used for inner round amplification using the same procedure but 35 PCR cycles with primer set OI1/OI2c (5' GCG CGT ATG CAA TAC GAG CGG CA 3' / 5' GCC TCG CGA CTT CGC AAC CCA T 3') specific for *Ca*. L. asiaticus (1,3). The amplified DNAs were resolved through agarose gel electrophoresis.

As shown in Fig. 2, a 1.1 kb amplicon was obtained from symptomatic but not asymptomatic leaf samples. Non-nested PCR using primer set OI1/OI2c-only did not yield any detectable DNA bands from either symptomatic or asymptomatic leaves. *Xba*I digestion yielded two fragments of 520 bp and 640 bp, characteristic to *Ca*. L. asiaticus. PCR amplicons were further sequenced to be 1,095 bp and shared a > 98% similarity to sequences of *Ca*. L. asiaticus in the GenBank database. No DNA was amplified with primer set GB1/GB2 (AAG TCG AGC GAG TAC GCA AGT ACT / CCA ACT TAA TGA TGG CAA ATA TAG) specific to *Ca*. L. americanus (5). We note that nested-PCR is necessary for *Ca*. L. asiaticus detection in wampee, suggesting low bacterial titer in this host. We recommend that eradication of wampee trees surrounding citrus orchards should be part of the overall management of citrus HLB.
Fig. 1. Huanglongbing symptoms in *Clausena lansium*: (A) “yellow shoots”; (B) comparison of symptomatic and asymptomatic leaves.

Fig. 2. Detection of *Candidatus Liberibacter asiaticus* by non-nested (using primer set OI1/OI2c-only) and nested PCR (using the general 16S rDNA primer set fDl/rD1 and then primer set OI1/OI2c) and results from Xba I digestion of PCR amplicons.

**Literature Cited**


