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## PCR DETECTION OF THE RICKETTSIA ASSOCIATED WITH PAPAYA BUNCHY TOP DISEASE

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**ABSTRACT.** Papaya bunchy top (PBT) disease is a major limiting factor in the production of papaya (*Carica papaya* L.) in the American tropics. We recently discovered a small, rod-shaped, gram-negative bacterium within the latex-producing cells (laticifers) of PBT-affected, but not healthy, papaya plants. Phylogenetic analysis based on the sequences of four genes indicated that bacterium is a member of the  $\alpha$ -subdivision of the *Proteobacteria* and of the genus *Rickettsia*. Primers for detection of the PBT bacterium by the polymerase chain reaction were designed to amplify a 705 bp fragment of the putative gene for the flavoprotein subunit of succinate dehydrogenase. Using these primers, a single PCR product of the expected size was obtained with DNA extracts from all 12 PBT-affected papaya plants including seven from Puerto Rico and five from Costa Rica; whereas, no amplification of the DNA fragment was detected for any of the 13 DNA extracts from individual healthy plants including six from Florida and seven from Puerto Rico. Further evidence of the widespread occurrence of the association between PBT and the PBT bacterium was obtained when DNA extracts from papaya samples from Barbados, Dominica, Grenada, Jamaica, St. Lucia, and St. Vincent tested positive by the PCR assay. The rickettsia was also detected by PCR in the leafhopper vector, *Empoasca papayae*, collected in several different papaya plantings affected by PBT in Puerto Rico. The PCR assay appears to provide the first specific means of detection for PBT.

## INTRODUCTION

Papaya bunchy top (PBT) disease is a major limiting factor in the production of papaya (*Carica papaya* L.) in the American tropics. The cause of the disease has been uncertain. Recently, a small, rod-shaped, gram-negative bacterium was discovered within the latex-producing cells (laticifers) of PBT-affected, but not healthy, papaya plants (Davis, et al., 1996). Attempts to isolate this fastidious bacterium in axenic culture were unsuccessful, impeding identification of the bacterium and verification of its pathogenicity to papaya plants. Nevertheless, the discovery was quite unique, providing the first evidence for a leafhopper-transmitted, laticifer-inhabiting, plant pathogenic bacterium.

Portions of genes corresponding to those for 16S rRNA, the flavoprotein subunit of succinate dehydrogenase (SdhA), citrate synthase (GltA), and the 17 kDa rickettsial common antigen were isolated from the non-cultivable bacterium from diseased plants and sequenced (unpublished). Comparative sequence analyses of the genes consistently indicated that the bacterium is a member of the  $\alpha$ -subdivision of the *Proteobacteria* and of the genus *Rickettsia*. *Rickettsia* have been found naturally in arthropods and can be pathogenic to man and other vertebrates. *Rickettsia*-like bacteria have been found in association with some plant diseases

(Davis, 1991), however, this was the first evidence of its kind implicating a true *Rickettsia* as a plant pathogen.

Here we report the development of a polymerase chain reaction (PCR) procedure for detection of the PBT-associated bacterium. The rickettsia was detected by PCR in diseased, but not healthy, papaya tissues and in the leafhopper vector, *Empoasca papayae*, providing further evidence of the possible etiological role of the bacterium in the disease.

## MATERIALS AND METHODS

To obtain DNA extracts from papaya leaf petioles which were enriched for bacterial DNA, plant juices containing the PBT-associated bacterium were expressed from ca. 3-mm cross-sections with a garlic press and collected in 1.5-ml tubes at 1 ml per tube on ice. The extracts were then centrifuged at 325 x g for 5 minutes, and the supernatants collected in a fresh tube. One-half ml of 35% sucrose in DNA extraction buffer (100 mM Tris-HCl (pH 8.0), 50 mM EDTA (pH 8.0), 500 mM NaCl) was layered under the extract in each tube, and the preparations were centrifuged at 16,000 x g for 10 minutes. Pellets were resuspended in 500  $\mu$ L of DNA extraction buffer, and DNA extracted as described (Dellaporta, et al., 1983). DNA from healthy plants was prepared in the same manner. Adult *E. papayae* were ground individually in 20  $\mu$ L of TAE buffer (40-mM TRIS-acetate, 1-mM EDTA, pH 8.0), and the brie incubated in a boiling water bath for 10 minutes, frozen rapidly in liquid nitrogen for 2 minutes, thawed at room temperature and centrifuged at 12,000 x g for 5 minutes. The supernatants were then used as samples for PCR analysis.

PBT-specific PCR primers were designed in this study for the *sdhA* gene of the PBT bacterium (Genbank accession U76909) as follows: Forward primer PBTF1, AAAGGTTCTGATTGGTTAGGTG (nt 67 to 88); the reverse primer PBTR1, ATCTTTATGCTCTCCAACCTCCTC (nt 749 to 771). For PCR, a 20- $\mu$ L reaction mixture (20 mM Tris (pH 8.3), 1.5 mM MgCl<sub>2</sub>, 25 mM KCl, 0.01% gelatin, 0.05% Tween 20, 100  $\mu$ M of each dNTPs, 20 pmol primer, 1.5 U Taq DNA polymerase and approximately 1 ng/ $\mu$ L sample DNA as a template) was overlaid with 10  $\mu$ L of liquid wax (M. J. Research). The PCR parameters were 40 cycles of 94°C for 1 minute, 52°C for 1.5 minute, 72°C for 1 minute and final extension 72°C for 10 minutes. The PCR products were visualized by electrophoresis in 0.7% agarose gel in TAE buffer.

## RESULTS AND DISCUSSION

The hypothesis that PBT is caused by a bacterium was based largely on the premise that the same bacterial species is constantly associated with PBT-affected but not healthy plants (Davis, et al., 1996). Identification of the bacterium was based largely on its morphological characteristics because more specific means were not available. To further test the hypothesis that a PBT-associated rickettsia causes PBT, we designed PCR primers to amplify a 705-bp fragment of the putative *sdhA* gene of the PBT bacterium. Using these primers, a single PCR product of the expected size was obtained with DNA extracts from all 12 PBT-affected papaya plants tested including seven from Puerto Rico and five from Costa Rica; whereas, no amplification of the DNA fragment was detected for any of the 13 DNA extracts from individual healthy plants including six from Florida and seven from Puerto Rico. Further

evidence of the widespread occurrence of the association between PBT and the PBT bacterium was obtained when DNA extracts from papaya samples from Barbados, Dominica, Grenada, Jamaica, St. Lucia, and St. Vincent, which had been obtained from various contributors in a previous study (Davis et al., 1996), tested positive by the PCR assay.

PBT is spread in nature by the leafhopper, *Empoasca papayae* Oman (Adsuar, 1946), but virtually nothing else is known about insect vector relationships or the mechanism of transmission. The presence of the PBT bacterium in *E. papayae* from papaya orchards affected by PBT in Puerto Rico was investigated. The putative *sdhA* gene of the PBT bacterium was detected by PCR in 24 of 40 and 7 of 12 individual adult *E. papayae* from orchards near Isabela and Lajas, respectively.

Our results clearly indicate that a rickettsial plant pathogen might cause PBT. This indicates that in vitro culture of the pathogen in cell-free medium is not likely to be productive but opens new avenues for research. Much remains to be learned about many aspects of the disease and its pathogen including its epidemiology and insect vector relationships. Such information should lead to the development of better management practices for PBT. Studies on the PBT bacterium might also shed light on the biology and evolution of rickettsial pathogens of animals and arthropods. It is also interesting to contemplate whether or not other similar bacteria inhabit plants in nature, with or without causing plant diseases. Such bacteria might have been easily overlooked due to their fastidious nature and unique ecological niche.

## REFERENCES

- Adsuar, J. 1946. Transmission of papaya bunchy top by leafhoppers of the genus *Empoasca*. Science 103:316.
- Davis, M. J., Kramer, J. B., Ferwerda, F. H., Brunner, B. R. 1996. Association of a bacterium and not a phytoplasma with papaya bunchy top-disease. Phytopathology 86: 102-109.
- Davis, M. J. 1991. Fastidious bacteria of plant vascular tissue and their invertebrate vectors. In: Balows A, Trupér HG, Dworkin M, Harder W, Schleifer KH (eds) The Prokaryotes, A Handbook on the Biology of Bacteria, Ecophysiology, Isolation, Identification, Applications, vol. 4. Berlin, Heidelberg, New York: Springer-Verlag, pp. 4030-4049.
- Dellaporta, S., Wood, J., and Hicks, J. B.. 1983. A plant DNA miniprep: Version II. Plant Mol. Biol. Rep. 1:19-21.

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