CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)

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Project Number

31580

Project Title

Saving Citrus Trees: Detecting Bacteria Associated with Citrus Greening Disease in Asian Citrus Psyllids

Abstract

Objectives/Goals

Citrus greening disease (or huanglongbing, HLB) was introduced in the Western hemisphere in 2004, has already destroyed about a third of the industries in both Florida and Brazil, and has spread to several countries including Mexico. Asian citrus psyllid (ACP, Diaphorina citri), the vector of the disease, was introduced in San Diego in 2008, has spread to several counties and is threatening to invade California commercial citrus. This project was aimed at comparing different methods of psyllid DNA extraction and developing a rapid high throughput technique for detection of HLB associated fastidious bacterium, Candidatus Liberibacter asiaticus.

Methods/Materials

Psyllids maintained on HLB infected plants in a containment facility in Florida were shipped in 95% ethanol in accordance with regulations and stored frozen. DNA extractions were made from 48 to 96 single psyllids using three different methods: 1) Qiagen Blood and Tissue kit (QBT, commonly used method), 2) MP BIO kit (MPB, used in the host laboratory) and 3) Qiagen magattract high throughput method (QMAG). The samples were analyzed by standard Taqman-based multiplex real-time PCR assay targeting 16s ribosomal DNA of the HLB bacterium and the wingless gene of the psyllid. Selected samples were tested by conventional PCR and the 1167 bp product was cloned and sequenced for confirmation.

Results

HLB associated bacteria were detected in 33%, 14%, and 7% by QMAG, QBT, and MPB, respectively. Analysis of internal control DNA showed that only QMAG extractions contained psyllid DNA in 100% of the samples, but not those extracted by QBT (23%) and MPB (82%) methods. The first attempt using QMAG method showed cross contamination among samples. The issue was resolved by changing liquid handling methods during extraction. The use of several controls (DNA extraction control, non-target bacterial sample extraction control, no template PCR control and positive plasmid PCR control) helped to validate the assay.

Conclusions/Discussion

There is an intensive effort in California to prevent and monitor the spread of HLB by testing both psyllids and plants. Since a very low percentage of psyllids is known to carry the bacteria, large numbers of psyllids need to be tested to enable early detection and eradication of the pathogen. An improved, sensitive high throughput psyllid DNA extraction method was developed which may be useful for monitoring the HLB associated bacteria.

Summary Statement

An improved high throughput method of DNA extraction from Asian citrus psyllids was developed to facilitate efficient monitoring and early detection of citrus greening disease.

Help Received

Research was done in the United States Department of Agriculture, National Clonal Germplasm Repository for Citrus and Dates, Riverside, CA. Dr. Richard Lee and members of the laboratory generously provided facilities and guidance.