CALIFORNIA STATE SCIENCE FAIR
2010 PROJECT SUMMARY

Name(s)

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Project Number

S1705

Project Title

A New Rapid Processing Method for the Detection of Candidatus lieberibacter Bacteria in Psyllid Vectors

Abstract

Objectives/Goals

Greening (citrus), Psyllid Yellows (tomato) and Zebra Chip (potato) are diseases caused by 'Candidatus Lieberibacter' bacteria. Symptoms include yellow leaves, poor growth and unusable fruit. Vectored by psyllids and non-culturable, there is no treatment for infected plants which must be destroyed. Bacteria detection is done by DNA testing of the psyllid vector since bacteria in the plant is hard to detect. Currently, DNA analysis of the psyllid is labor intensive. This project's objective is to validate a new method for rapid processing of psyllid samples for the detection of the bacteria.

Methods/Materials

Three psyllid collection and DNA extraction protocols were tested for the amount of 'Ca. L. psyllaurous' DNA extracted from infected psyllids. In Exp. 1 (Standard method), psyllids were collected in ethanol and DNA was extracted using a MP kit Bio protocol. In Exp. 2, psyllids were collected in ethanol and DNA was extracted using a direct boil method. In Exp. 3, psyllids were smashed into Whatman #1 papers, the paper with the psyllid remains was boiled in the extraction buffer. Presence of 'Ca. L. psyllaurous' was tested by a Taqman based real time PCR and the results were confirmed by conventional PCR. The PCR product was cloned in TOPO TA vector and sequenced at UC Riverside. The serial dilutions from the plasmid were used for preparing a standard curve in real time PCR.

Results

Based on cycle threshold values psyllids collected on Whatman #1 paper with the direct boil DNA extraction had similar amounts of initial DNA as that of the standard protocol. Psyllids collected in ethanol and direct boiled, without lysing, in the extraction buffer yielded less DNA than the other two methods. The sequence of the conventional PCR product was 99% similar to the sequences of 'Ca. L. psyllaurous' from the Genbank database.

Conclusions/Discussion

A new method of sample processing using filter paper and DNA extracted by boiling was compared to a standard method. The new method of psyllid collection was found as efficient as the standard, less expensive and less complicated to use. The direct boil method of DNA extraction greatly reduces the labor and material cost. This study demonstrates an improved method of handling psyllid samples and extraction of bacteria, enabling efficient processing of samples reducing both time and cost of processing without compromising the sensitivity of the test results.

Summary Statement

A new rapid process for the detection of 'Candidatus Liberibacter' was validated quantitatively using real time qPCR in the psyllid vector which transmits the bacteria causing severe grove damage in citrus and loss of solanceaous crops.

Help Received

Used lab equipment at United States Department of Agriculture, Agriculture Research Service (USDA-ARS), National Clonal Germplasm Repository for Citrus and Dates in Riverside, under the supervision of Dr. Manjunath Keremane and Dr. Chandrika Ramadugu.