CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

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Project Number

S1908

Project Title

Chromosomally Integrated Bacteriophage in Candidatus liberibacter Bacteria & Its Effect on Plant Disease Expression Yr2

Abstract

Objectives/Goals

Citrus Greening citrus, Tomato Psyllid Yellows and Potato Zebra Chip are all severe diseases caused by Candidatus Lieberibacter bacteria. Vectored by psyllids and nonculturable, there is no treatment for infected plants which must be destroyed, resulting in significant crop loss worldwide. Infected plants show varying expression of symptoms, with some areas appearing healthy with others infected. This project#s objective is to determine the effect of chromosomally integrated bacteriophage in Ca. Liberibacter and its effect on the expression of disease virulence.

Methods/Materials

DNA was extracted (Qigene) from over 20 healthy, infected but completely non-symptomatic, infected symptomatic and infected but with non-symptomatic shoots on symptomatic plants. Using qPCR (ABI 7500), the extracted DNA was evaluated with 70 primers in both phage and nonphage regions of this genome determining the quantity of DNA found in 3,500 samples during 37 experiments. C(t) values were evaluated in both phage regions, non-phage regions with each plant sample type. Each primer was evaluated individually over the all the same plant samples, and results were indexed against the 16s rRNA primer for comparison.

Results

Amplification plots, melt curves, Cycle Threshold C(t) were evaluated for each of the 3,500 samples. Primers which gave erroneous results were excluded. Sample c(t) value data was analyzed and graphed for analysis.Infected non-symptomatic plants yielded more initial bacterial DNA than symptomatic samples when considering primers over the entire genome and in non-phage regions. However, significantly more DNA was found within symptomatic samples using phage region primers than in the non-phage region primers.

Conclusions/Discussion

With this bacterium and the psyllid vector now endemic to the United States and most other world regions, studying the mechanism of disease virulence within this pathogen is extremely important. As the non-symptomatic plants had more bacterial DNA than symptomatic ones, results indicate the bacteriophage became lytic , destroying the bacterial cells in the symptomatic samples. In phage primer regions, more bacterial DNA was found in symptomatic plants, giving further evidence to phage transition from a lysogenic to lytic state, demonstrating that the presence of bacteriophage does increase disease virulence.

Summary Statement

The role of bacteriophage in disease expression was validated for plants infected with Candidatus Liberibacter, which causes severe diseases in citrus and solanaceous crops, indicating that lytic phage causes increased disease virulence.

Help Received

Experiments were conducted at the 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. Richard Lee. My parents provided transportation.