CALIFORNIA STATE SCIENCE FAIR
2012 PROJECT SUMMARY

Name(s)

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Project Number

S1903

Project Title

An Investigation of Chromosomally Integrated Bacteriophage in Candidatus liberibacter psyllaurous Bacteria and Its Effec

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 crops. Vectored by psyllids and fastidious, there is no treatment for infected plants which must be destroyed. Infected plants have varying expression of symptoms, with some areas appearing healthy while others clearly infected. This projects objective is to ascertain bacteriophages effect on the expression of disease virulence.

Methods/Materials

The Ca. L. psyllaurous bacterial genome contains two P4 bacteriophage regions. 75 primers, in 7 experiments examined the entire genome determining the amount of initial DNA found in symptomatic and non-symptomatic infected plants. 672 samples, with controls were evaluated using primers in both phage and non-phage regions and SYBR green with a ABI 7000 QPCR. Additionally, PCR product for Ca. L. psyllaurous was refined, the results were confirmed by conventional PCR, cloned in TOPO TA vector and sequenced at UC Riverside. Plasmid serial dilutions were used to prepare a standard curve.

Results

Amplification plots, melt curves, Cycle Threshold C(t) were evaluated for each sample. Primers which gave erroneous results were excluded. Sample c(t) value data was tabulated 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, more DNA was found in the symptomatic samples in phage region primers than non-symptomatic ones. Additionally, samples from winter seasons yielded overall less DNA than those taken in summer, both for phage and non-phage primer regions.

Conclusions/Discussion

As the non-symptomatic plants had more bacterial DNA than symptomatic ones, clearly 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 the phage#s transition from a lysogenic state. Samples taken from both summer and winter, yielded a greater amount of DNA in both phage and non-phage regions, indicating that the bacteria is more active when warmer. The presence of phage was demonstrated to clearly have an effect upon disease virulence, with evidence of a phages lytic state leading to more pronounced symptoms.

Summary Statement

The role of bacteriophage in disease expression for plants infected with #Candidatus Liberibacter psyllaurous# was validated, 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. Chandrika Ramadugu.