Survey of *Erwinia amylovora*, causal agent of fire blight, from apple and pear orchards in Utah for streptomycin resistance

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Abstract

Fire blight caused by the bacterium *Erwinia amylovora* results in millions of dollar in losses worldwide. It is the most important disease problem for apple and pear growers in Utah. Currently the only effective management strategy is application of the antibiotic streptomycin. In 2006, resistant isolates were detected in an apple orchard in Utah County. To determine the distribution of resistant isolates, samples collected in 2006, 2007, 2010 and 2011 from orchards across Utah were tested for resistance to streptomycin. Isolates were screened at 0, 100 and 1000ppm of streptomycin. Bacteria were spread on LB agar. A hole was punched from the agar and the streptomycin solution was pipetted into the well. After 24 hours a bacteria-free zone around the well was observed for sensitive isolates but not for resistant isolates. The majority of resistant isolates were found in Utah County where most orchards are located. The resistance in most Utah isolates is caused by a mutation in the rpsL gene but at least one isolate acquired a plasmid containing streptomycin resistance genes.

Introduction

Fire blight is a disease of apples, pears and other rosaceous plants. The disease is caused by the bacterium *Erwinia amylovora.* The bacteria survive the winter in cankers and enter the plant through natural openings or wounds. Fire blight is the most important disease problem for Utah apple and pear growers. To manage the disease streptomycin is used as the most effective treatment. In recent years, streptomycin-resistant isolates have been found across the United States including Utah (1;2). There are at least two mechanisms for resistance development: 1. a single base pair mutation in the *rpsL* gene. With the mutation streptomycin can no longer bind to S12 protein encoded by the *rpsL* gene and 2. the acquisition of a plasmid containing StrA-StrB gene complex.

Figure 3: Gala tree branch with an oozing canker

Figure 1: Gala tree infected with fire blight

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20060144 GTATGTACTCGTGTGTACACGACTACCCCTAGAAAACCGAACTCCGCACTGCGTAAAGTG
2007053  GTATGTACTCGTGTGTACACGACTACCCCTAGAAAACCGAACTCCGCACTGCGTAAAGTG
20110701 GTATGTACTCGTGTGTACACGACTACCCCTAGAAAACCGAACTCCGCACTGCGTAAAGTG
20110604 GTATGTACTCGTGTGTACACGACTACCCCTAGAAAACCGAACTCCGCACTGCGTAAAGTG
2011165  GTATGTACTCGTGTGTACACGACTACCCCTAGAAAACCGAACTCCGCACTGCGTAAAGTG
20110600 GTATGTACTCGTGTGTACACGACTACCCCTAGAAAACCGAACTCCGCACTGCGTAAAGTG
2006132  GTATGTACTCGTGTGTACACGACTACCCCTAAAAAACCGAACTCCGCACTGCGTAAAGTG
2006121  GTATGTACTCGTGTGTACACGACTACCCCTAAAAAACCGAACTCCGCACTGCGTAAAGTG
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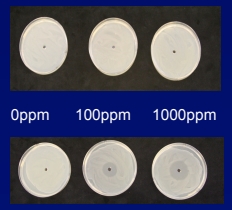
Figure 5: Sequenced isolates, red indicates resistant and purple sensitive to streptomycin

Figure 6: Stars indicate counties that were sampled for *E. amylovora*



0ppm 100ppm 1000ppm

Figure 4: Above is a resistant streptomycin test; below is a sensitive streptomycin test

Materials and Methods

E.amylovora isolates were streaked onto CCT plates. The bacteria were boiled in water for 3-4 minutes to release DNA. PCR was conducted, using *E.* amylovora-specific primers (3) to verify the ID. The DNA bands were extracted from electrophoresis gels (Qiagen Gel Extraction Kit) and sequenced at The University of Arizona genetics core lab. Sequences were compared with *E. amylovora* sequences in the NCBI Genbank database. Each isolate was tested for streptomycin resistance. In addition, isolates from 2006 and 2007 from the same locations were also tested. The bacteria were quantified to a concentration of $1x10^8$ bacteria. 100 microliters of the bacterial suspension were spread onto LB plates. A 10mm hole was cut in the center of each plate and filled with diluted streptomycin. For each isolate there were 3 plates: water control, 100ppm, and 1000ppm of streptomycin. After 24 hours plates were evaluated. A halo indicated the isolate was sensitive and no halo indicated resistance to streptomycin. The *rpsL* gene of resistant isolates was sequenced and compared to sensitive isolates to determine if a mutation was present. *rpsL* sequences were edited and then aligned in Clustal W.

Results and Discussion

I isolated *E. amylovora* from approximately 107 symptomatic trees from Utah County and 90% of the isolates were tested for the rpsL gene. From those 90% all of them showed the same mutation in the gene on codon 43. 15 trees were sampled from the Cache County area and only one had *Erwinia.* The 2006 and 2007 isolates were sent off for sequencing and came back with the same mutation. Majority of the isolates in Utah had a mutation in codon 43 which results in streptomycin resistances. The mutation changes the amino acid from a lysine to an arginine. Comparing the recent 2011 isolate with the 2006 isolates it is confirmed that the mutation is persistent even after streptomycin has not been used for years. Few isolates have not been identified due to a different mutational mechanism.

References

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