

## CONTROL OF PIERCE'S DISEASE THROUGH DEGRADATION OF XANTHAN GUM

### Project Leader:

Donald A. Cooksey  
Department of Plant Pathology  
University of California  
Riverside, CA 92521

### Cooperators:

Neal L. Schiller  
Division of Biomedical Sciences  
University of California  
Riverside, CA

**Reporting Period:** The results reported here are from work conducted from January 1, 2002 to December 31, 2002.

### INTRODUCTION

Pierce's disease of grapevine and other leaf scorch diseases caused by *Xylella fastidiosa* are associated with aggregation of the bacteria in xylem vessels, formation of a gummy matrix, and subsequent blockage of water uptake. In the closely related pathogen, *Xanthomonas campestris*, xanthan gum is known to be an important virulence factor, probably contributing to bacterial adhesion, aggregation, and plugging of xylem. The recently published genome sequence of the citrus strain of *X. fastidiosa* revealed that this pathogen also has genes for xanthan gum production. This project is to identify bacteria that produce xanthan-degrading enzymes to target this specific virulence factor of *X. fastidiosa*. This approach has the potential to significantly reduce damage caused by Pierce's disease in grapes and potentially in other hosts of *X. fastidiosa*, such as almonds and oleander. If xanthan gum is important in the aggregation of the pathogen in the insect vector, then our approach may also reduce the efficiency of transmission of Pierce's disease. Our first approach will be to develop endophytic bacteria that produce these enzymes in the xylem of grapevines, but another approach is to engineer grape plants to produce these enzymes. Through the cloning and characterization of genes encoding xanthases and xanthan lyases, we will facilitate possible efforts to transform grapevines to produce these enzymes.

### OBJECTIVES

1. Characterize xanthan-degrading enzymes from endophytic bacteria isolated from grape
2. Explore applications of naturally-occurring endophytic bacteria that produce xanthan-degrading enzymes for reduction of Pierce's disease and insect transmission
3. Clone and characterize genes encoding xanthan-degrading enzymes for enzyme overproduction and construction of transgenic endophytes and plants

### RESULTS AND CONCLUSIONS

#### ***Production of xanthan gum for enrichment of xanthan-degrading bacteria:***

The sequence of the xanthan gum biosynthetic operon in the genome sequence of the agent of Pierce's disease, *Xylella fastidiosa*, is different than the bacterium from which commercial xanthan gum is prepared, *Xanthomonas campestris*. This suggests that *Xylella* xanthan gum is chemically different, and we therefore wished to produce xanthan gum from *Xylella* for our enrichment studies. However, as described in our original proposal, it is not feasible to produce enough xanthan gum for our studies from the slow-growing *Xylella fastidiosa*. As proposed, we instead genetically modified a strain of the fast-growing *Xanthomonas campestris* to produce xanthan gum with the same chemical structure as that from *Xylella*. This was accomplished by deleting the *gumI* gene from the biosynthetic operon. Our genetic construction was confirmed, and we have produced significant quantities of xanthan gum from this mutant strain. The modified gum is still viscous, but has a measurable decrease in viscosity compared with gum isolated from the wild-type strain of *Xanthomonas*.

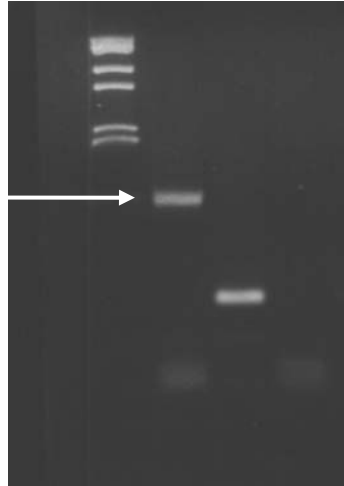
#### ***Enrichment for bacteria that degrade xanthan gum:***

We used the modified xanthan gum from the *Xanthomonas* mutant described above as the sole carbon source for enrichment culture from Pierce's disease infected grapevines. To isolate the endophytic bacteria, we collected 200 grapevine samples infected with Pierce's disease in Temecula and Bakersfield and 100 oleander samples infected with leaf scorch disease in Riverside. Individual tissue segments were placed into sterile test tubes with 10 ml of 1% NaOCl solution with 0.1% tween 20. Surface-disinfected pieces were aseptically transferred through three washes of 10 ml of sterile PBS (phosphate buffered saline). To check for surface contamination, 0.1ml of the third wash for each sample is transferred to 5ml of Tryptic soy broth medium and incubated at room temperature on a rotary shaker for 2 days. Surface-disinfected pieces were macerated with PBS with 0.1% tween 20 using mortars and pestles. Suspensions were transferred to minimal media with xanthan gum as the sole carbon source and incubated at room temperature on rotary shaker for 7 days. Cultures were centrifuged, and the viscosity of their supernatant was measured. Cultures that had a decreased viscosity were transferred to fresh media and incubated for 3 days. This enrichment step was repeated twice. Cultures were finally spread on solid media with xanthan gum as the sole carbon source, and individual colonies were streaked to purity on fresh plates. Pure cultures were tested for reduction of viscosity of xanthan gum as measured with an Ostwald capillary viscosimeter. Over 100 bacterial strains were initially recovered from these enrichment experiments, and 11 were subsequently confirmed to effectively degrade xanthan gum. These strains were then tested for cellulase activity. Degradation of the cellulosic backbone of the xanthan polymer would be desirable, but we do not want enzymes that recognize and degrade plant cellulose. Six of the strains had low or non-detectable cellulase activity and will be further tested for biological control efficacy in plants.

***Expression of the xanthan gum operon in Xylella fastidiosa:***

To support our hypothesis that xanthan gum is produced in infected plants, we have initially tested whether the xanthan gum operon is expressed. RT-PCR was performed with primers directed toward the first gene of the xanthan gum biosynthetic operon, *gumB*, with RNA extracted from different strains of *Xylella fastidiosa*. *gumB* mRNA was detected from some, but not all, strains of *X. fastidiosa* grown *in vitro*. Xanthan gum and other virulence factors may be produced at high levels only in plants when bacterial populations have reached a high density. Extraction of RNA from infected plants is in progress to study expression of the xanthan gum operon *in planta*.

*gumB* mRNA detected by RT-PCR  
from *Xylella fastidiosa* culture



**FUNDING AGENCIES**

Funding for this project was provided by the USDA Animal and Plant Health Inspection Service.