CONTROL OF PIERCE'S DISEASE THROUGH DEGRADATION OF XANTHAN GUM

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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 variegated chlorosis (CVC) 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

Comparative genomics for investigation of xanthan production in different strains. To determine whether the xanthan gum from the Pierce's disease strain of *X. fastidiosa* is likely to be structured as we have predicted from the CVC strain sequence, we compared the available genomic sequences of the closely related almond leaf scorch strain and the more distantly related oleander leaf scorch strain. All of these *Xylella* strains have the same nine *gum* genes conserved, and they all lack *gumG, gumI,* and *gumL* that are found in *Xanthomonas*. In *Xanthomonas, gumI* is thought to add the terminal mannose residue on the sugar side chains of the xanthan polymer. *gumG* and *gumL* add acetylate and pyruvate to the terminal mannose. The lack of these three genes in *Xylella* suggests that its xanthan gum lacks the terminal mannose on the side chains, which is predicted to reduce its viscosity slightly.

Initial isolation of xanthan-degrading bacteria. We predicted that a potential source of bacteria that degrade xanthan gum would be in natural settings where xanthan gum is present, such as plants infected with *Xanthomonas campestris* or *X. fastidiosa*. Following the techniques used by industrial microbiologists studying xanthan degradation, we found that both grapevines infected with Pierce's disease and oleanders infected with oleander leaf scorch contain bacteria that are capable of degrading xanthan gum. These were obtained through enrichment in minimal broth media containing commercial xanthan gum as the only carbon source. After two serial transfers into fresh media, we plated these cultures on an agar medium containing xanthan and have obtained pure cultures of bacteria that grow on xanthan gum as the sole carbon source and clear the turbidity that the xanthan produces in the medium. We have several different types of bacterial xanthan degraders from these initial attempts. However, these bacteria were selected for degradation of commercial xanthan gum, which is produced from *Xanthomonas*. Therefore, we needed to repeat these isolations using xanthan gum from *Xylella,* which is predicted to differ chemically.

Enzymatic activity against xanthan from *Xylella*. Purifying significant quantities of xanthan from the slow-growing *X. fastidiosa* is not practical. Therefore, we have constructed a mutant of *Xanthomonas campestris* lacking the *gumI* gene that is responsible for adding the terminal mannose. A large section of the *gum* operon was cloned from *Xanthomonas*, and an antibiotic resistance cassette was inserted into a deletion in the *gumI* gene. This construction was used to introduce the mutation into wild-type *X. campestris* by homologous recombination. We are confirming that this strain produces xanthan that is chemically similar to that from *Xylella*. Viscosimetric assays are also being conducted to compare the viscosity of *Xylella* and *Xanthomonas* xanthan, as well as to provide a quantitative measurement for the activity of xanthan-degrading enzymes. Using this new source of modified xanthan gum, we are in the process of sampling symptomatic grapevines from diverse sources to develop a broad collection of xanthan-degrading bacteria. Other tests will be performed to characterize the nature of xanthan-degrading enzymes from these bacteria. We anticipate that we will recover both xanthanases that break the cellulosic backbone of the xanthan polymer, and xanthan lyases that cleave the sugar side chains.