CONTROL OF PIERCE'S DISEASE THROUGH DEGRADATION OF XANTHAN GUM

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ABSTRACT
Endophytic xanthan gum-degrading bacteria isolated by enrichment culture were characterized for their mode and dynamics of xanthan degradation, colonization of plants, and interactions with *Xylella fastidiosa*. In vitro, growth and biofilm production by the endophytic xanthan degrader from oleander, *Acinetobacter johnsonii* GX123, was enhanced by xanthan gum as a substrate and by cells of *X. fastidiosa* added to a minimal medium. Xanthan gum was degraded rapidly during log-phase growth the this endophyte, and viscosity was reduced almost to non-detectable levels. GX123 colonized stems and leaves of oleander systemically, and systemic colonization was enhanced by co-inoculation with *X. fastidiosa*. Its effects on symptom expression are still under investigation.

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 (Katzen et al, 1998), probably contributing to bacterial adhesion, aggregation, and plugging of xylem. The published genome sequence of *X. fastidiosa* (Simpson et al., 2000; Bhattacharyya, A., et al. 2002; Van Sluys et al., 2003) revealed that this pathogen also has genes for xanthan gum production. In Pierce's disease, xanthan gum is likely to contribute to plugging of the grapevine xylem (Keen et al., 2000) and possibly to the aggregation of the bacterium in the mouthparts of the glassy-winged sharpshooter. Because of its importance as an industrial thickener and emulsifier, xanthan gum synthesis and degradation has been studied extensively (Becker et al., 1998). Bacteria that produce xanthan-degrading enzymes have been isolated from soils by several researchers, using enrichment techniques with xanthan gum as the sole carbon source (Sutherland, 1987; Ruijssenaars et al., 2000).

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.

Previously, we used modified xanthan gum that mimicks *Xylella* xanthan from a *Xanthomonas* mutant as the sole carbon source for enrichment culture from Pierce's disease infected grapevines. The xanthan gum biosynthetic operon in the *Xylella* genome is different than the bacterium from which commercial xanthan gum is prepared, *Xanthomonas campestris*. However, it is not feasible to produce enough xanthan gum for our studies from the slow-growing *Xylella fastidiosa*, so we genetically modified a strain of the fast-growing *Xanthomonas campestris* to produce a modified xanthan gum that is predicted to have the same chemical structure as that from *Xylella*. This was accomplished by deleting the *gumI* gene from the biosynthetic operon. We reported last year that over 100 bacterial strains were initially recovered from 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 were further characterized.

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

Characterization of endophytic bacteria that degrade xanthan gum

The xanthan-degrading bacteria included both gram-negative and gram-positive bacteria, as identified by fatty acid methyl ester analysis and 16S rDNA sequencing. One particular strain (GX123) with high xanthan-degrading activity but no cellulase activity that was isolated from oleander was identified as *Acinetobacter johnsonii* and was characterized in more detail. In batch culture, addition of 0.02% xanthan gum to a minimal medium allowed growth of GX123 in vitro and greatly enhanced biofilm formation by this strain. The measured relative viscosity of xanthan gum rapidly decreased during log-phase growth of GX123 from a value of 7.6 to 2.3 over 24 hours and to 0.2 by 56 hours. Addition of cells of *Xylella fastidiosa* instead of xanthan gum also greatly enhanced both growth and biofilm formation by strain GX123.

Colonization of oleander plants by *Acinetobacter johnsonii* GX123 was examined with and without coinoculations with *Xylella fastidiosa*. In single inoculations, the endophyte spread several centimeters upward from stem inoculation points to achieve populations of 3×10^3 CFU/g after 7 days and 4×10^5 CFU/g after 21 days. It also spread to the first leaf above the inoculation point in each inoculated plant by 7 days (3.2×10^3 CFU/g) and was up to 2×10^5 CFU/g by 21 days in the first leaf. GX123 was not detectable 4 cm below the inoculation point 7 days after inoculation, but after 21 days, it was present at 3.2×10^4 CFU/g at this distance below the inoculation point. However, it was never detected as far as 8 cm below the inoculation point in the single inoculations, even after following populations for 105 days. In co-inoculations with *X. fastidiosa*, the endophyte generally reached higher populations levels in stems and leaves above the inoculation points and spread downward further and more quickly. Unfortunately, Pierce's disease symptoms have not yet developed in these plants, so that the effect of the endophyte on disease expression has not yet been determined.

CONCLUSIONS

The xanthan gum-degrading endophyte *Acinetobacter johnsonii* GX123 is a good candidate as a possible biocontrol agent for Pierce's disease, since it effectively degrades xanthan gum and successfully colonized plants above and below inoculation points, including systemic leaves. The enhancement of growth of this endophyte in vitro, as well as in plants, when *X. fastidiosa* was co-inoculated suggests that it benefits from the presence of this bacterium, possibly growing on the xanthan gum present on and released from *X. fastidiosa* cells. Its effects on symptom expression are still under investigation.

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