MANAGEMENT OF PIERCE'S DISEASE OF GRAPE BY INTERFERING WITH CELL-CELL COMMUNICATION IN *XYLELLA FASTIDIOSA*

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INTRODUCTION

Endophytic bacteria such as *Xylella fastidiosa (Xf)* colonize the internal tissues of the host, forming a structure very similar to a fixed biofilm inside the plant. A key determinant of success for an endophyte is the ability to move within the plant, sending out "scouts" to colonize new areas within the host. We expect activities required for movement to be most successful when carried out by a community of cells since individual cells may be incapable of completing the feat on their own and may be detected and easily eliminated by the host. Cells assess the size of their local population via cell-cell communication and coordinately regulate the expression of genes required for such processes. Our study aims to investigate cell-cell communication in *Xf* to determine its role in colonization and pathogenicity in grapevines.

Xf shares sequence similarity with the plant pathogen *Xanthomonas campestris* pathovar *campestris (Xcc).* In *Xcc,* the expression of pathogenicity genes is controlled by the Rpf system of cell-cell communication, enabling a population of cells to launch a pathogenic attack in a coordinated manner (Barber et al. 1997). Two of the Rpf proteins, RpfB and RpfF, work to produce a diffusible signal factor (DSF; Barber et al. 1997). As the population grows, the local concentration of DSF increases. Other Rpf proteins are thought to sense the increase in DSF concentration and transduce a signal, resulting in expression of pathogenicity factors (Slater et al. 2000).

The *Xf* genome not only contains homologs of the *rpf* genes most essential for cell-cell signaling in *Xcc*, but also exhibits striking colinearity in the arrangement of these genes on the chromosome (Dow and Daniels 2000). Thus *Xf* likely employs a cell-cell signaling apparatus similar to that of *Xcc.* Based on our knowledge of density-dependent gene regulation in other species, we predict the targets of Rpf regulation would be genes necessary for colonizing the xylem and spreading from vessel to vessel. For example, expression of extracellular polysaccharides, cellulases, proteases and pectinases might be induced by the signal. Similarly, we would expect the density-dependent genes to be expressed during the time when a population of *Xf* is ready to move into uncolonized areas.

It is conceivable that cell-cell signal interference may be used by other organisms to inhibit density-dependent behaviors, such as pathogenicity or spreading through the habitat. Several recent studies indicate that other organisms can disrupt or manipulate the cell-cell signaling system of bacteria (Leadbetter and Greenberg 2000; Manefield et al. 1999). Examination of *Xf* population size in plants where *Xf* lives as an endophyte versus those in which *Xf* causes the xylem blockage symptoms of Pierce's disease demonstrates a positive relationship between population size and symptom development (Fry and Milholland 1990). We hypothesize that an interaction between *Xf* and other organisms, such as another endophyte or the host plant itself, may modulate density-dependent behaviors in *Xf* by interfering with cell-cell signaling.

OBJECTIVES

1. Characterize cell-cell signaling factors in *Xylella fastidiosa*.
2. Determine role of signaling factors on virulence and transmissibility of *Xylella fastidiosa*.
3. Identify degraders of signaling factors of *Xylella fastidiosa.*
4. Identify inhibitory analogs of signaling factors of *Xylella fastidiosa.*

RESULTS AND CONCLUSIONS

Objective 1. We have constructed "signal-sensing" strains of *Xcc* to determine whether *Xf* uses the same butyrolactone signal as *Xcc* (Figure 1). These strains carry a green fluorescent protein (gfp) gene under the control of a promoter that is up-regulated in response to the signal. We have successfully detected a signal from *Xf* using this system, however the response is much weaker than that of *Xcc* (Figure 2). We conclude that *Xf* may make high concentrations of the signal only under specific conditions, such as *in planta.* A second possibility is that the *Xf* signal differs slightly from the *Xcc* signal and cannot

fully activate the *Xcc* signal sensor except at high concentrations. To distinguish between these hypotheses, we are constructing signal-sensing strains of *Xf* using a gfp gene fused to promoters of *Xf* genes we believe should be up-regulated in response to the signal. These strains can be examined *in planta* as well as in culture to sort out the above-mentioned possibilities.

Figure 1. Signal sensor strain overlaid on a wild-type Xcc colony (left) or rpfB (center) or rpfF mutants.

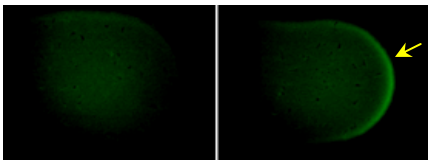

Figure 2. Signal sensor growing to the left of PWG extract (left) or Xf extract. Green fluorescence indicates signal. presence.

Objective 2. We have constructed strains of *Xf* Temecula in which the *rpfB* and *rpfF* genes, which are each required for production of the signal in *Xcc,* are knocked out. These mutants were constructed using exchange of the wild-type allele for a deleted copy carrying an antibiotic resistance gene on a suicide plasmid (Figure 3). In contrast to other reports of recombination into the *Xf* genome, we obtain almost exclusively double recombinants in the primary transformants after only 7 days of incubation on plates. We are testing *rpfB* and *rpfF* mutant strains for their ability to infect and move within host plants and to cause Pierce's disease symptoms. Our preliminary evidence indicates that neither of these genes is strictly required for virulence as mutant strains cause symptoms similarly to the wild type. However, these genes may play a role in modulating disease progress because the timing of symptom development differs between mutant and wild-type strains. Further characterization of infected plants is underway to investigate the mechanism behind these differences. We are in the process of testing transmissibility of the mutant strains by an insect vector. In addition, we are testing the mutants for signal production using the *Xcc* signal sensor. To better direct our analyses, we have constructed a strain of *Xf* that constitutively expresses Gfp in order to bring the *in planta* growth habit of *Xf* during symptom formation into sharper focus (Figure 4). By observing differences in colonization between symptomatic and asymptomatic samples we will have a clearer image of the mechanism of symptom formation and the best strategies for preventing it.

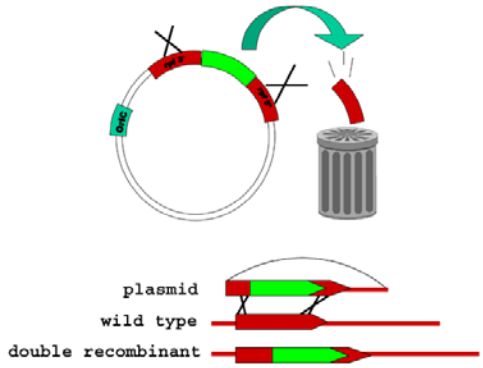

Figure 3. Gene knockout strategy using allelic exchange.

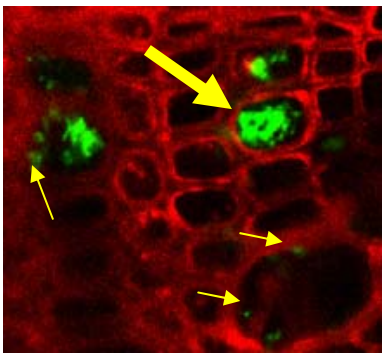

Figure 4. Gfp-labeled Xf viewed inside the live petiole of a grapevine by confocal microscopy. Large arrow indicates a large aggregate of cells. Small arrows point to individual cells or small groups. Red color is due to auto-fluorescence of the grapevine.

Objectives 3 and 4. We have collected grapevines from vineyards affected by Pierce's disease as well as tomato and cruciferous crop plants infected with the signal-producing pathogens *Xanthomonas campestris* pv *vessicatoria* and *Xcc,* respectively. We have recovered bacteria from inside these samples to generate a comprehensive collection of endophytes that grew in contact with the signal molecule. These endophytes are being tested for the ability to interfere with cell-cell signaling in *Xf* in an assay using the signal-sensing strains from Objective 1. We have thus far isolated several strains that are weakly inhibitory and are through about one-third of our collection.

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