THE ROLE OF CELL-CELL SIGNALING IN HOST COLONIZATION BY *XYLELLA FASTIDIOSA*

Project Leader:
Steven Lindow
Department of Plant and Microbial Biology
University of California
Berkeley, CA 94720

Cooperators:
Alexander Purcell
Department of Environmental Science Policy & Management
University of California
Berkeley, CA

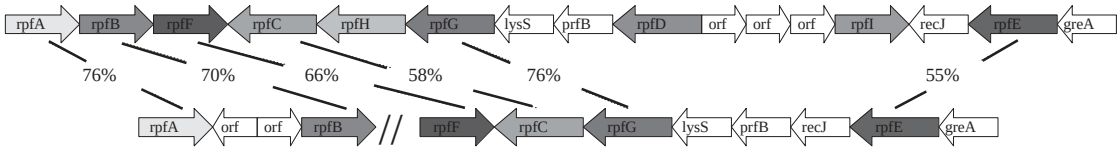
Karyn Lynn Newman
Department Plant and Microbial Biology
University of California
Berkeley, CA

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. Cells secrete extracellular polysaccharides that may serve to enhance surface attachment, to protect the cells from host defenses or to serve as a matrix to concentrate nutrient ions (Denny, 1995). But what happens when the local environment can no longer support further population growth? 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. For a xylem-restricted species such as *Xf*, movement must be from vessel to vessel, which would require degrading and traversing the pit membranes connecting them. We expect activities such as degradation of a pit membrane 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. Therefore, cells must have a means by which to assess the size of their local population, and to coordinately regulate the expression of genes required for such processes only at the appropriate time. Our study aims to investigate cell-cell communication in *Xf* to determine its role in colonization and pathogenicity in grapevine.

Xf shares sequence similarity with the plant pathogen s. *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). The *rpf* (regulation of pathogenicity factors) genes of *Xcc* encode the components of a cell-cell communication system. Two of the Rpf proteins 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. Furthermore, the *Xf* genome



Arrangement of rpf *genes on the chromosome of* Xcc *(top) and* Xf *(bottom). Numbers in parentheses indicate amino acid identity. Slashes mark a break in continuity.*

lacks homologs of the *luxI/luxR* genes and other genes shown to be involved in production and perception of AHLs (Dow and Daniels, 2000). Thus it is likely that *Xf* 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 of the plant.

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. To determine the chemical identity of the signal used in cell-cell signaling in *Xf*.

2. To investigate which behaviors are controlled by signaling factors.

3. To isolate other strains of bacteria that are capable of interfering with cell-cell signaling in *Xf*.

RESULTS AND CONCLUSIONS

Objective 1. We have obtained and are in the process of testing "signal sensing" strains of *Xcc* to determine whether *Xf* uses the same butyrolactone signal as *Xcc*. These strains carry a reporter gene under the control of a promoter that is up-regulated in response to the butyrolactone signal factor produced by *Xanthomonas*. We are also in the process of constructing plasmids for generating signal sensing strains of *Xf* using a reporter gene fused to promoters of genes we believe should be up-regulated in response to the signal in Xf.

Objective 2. We are in the process of constructing strains of *Xf* in which the *rpfB* gene, which is required for production of the signal in *Xcc* is knocked out. These will be subjected to phenotypic analysis and tested for their ability to infect and move within host plants. We are also determining the patterns of transcriptional regulation of genes in the rpf operon as well as other genes that are mostly likely involved in pathogenicity such as cellulases and polygalacturonases. The regulation of the genes is being assessed by producing fusions of these genes upstream from a promoterless *gfp* reporter gene. The gene fusions are being introduced into the chromosome via the use of integrative plasmids of partial stability that allow gene replacement in *Xf*. The plasmids that we are developing for this purpose possess features that should allow integrative recombination into target genes in the chromosome of *Xf* but also permit sensitive estimation of target genes by use of reporter gene fusions protected by transcription terminators.

Objective 3. We have collected grapevines from Pierce's Disease affected vineyards. We are in the process of recovering bacteria that grow inside these vines by 4 different methods to generate a comprehensive collection of grapevine endophytes. These endophytes are being cultured on 5% TSA. The each strain will be tested for the ability to interfere with cell-cell signaling in *Xcc* and *Xf* in an agar assay using diffusible factor-regulated genes using the signal sensing strains from objective 1.

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