ROLE OF *XYLELLA FASTIDIOSA* ATTACHMENT ON PATHOGENICITY

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INTRODUCTION

Xylella fastidiosa is a gram negative bacterium which causes serious diseases of plants such as Pierce's disease (PD), citrus variegated chlorosis (CVC), or almond leaf scorch and inhabits many other insect and plant host (Purcell 1997). The control of plant diseases caused by this bacterium will ultimately require treating plants with chemical or biological methods, or manipulating plants genetically. In this proposal, we propose to identify inhibitors to the attachment and colonization processes of *X. fastidiosa*. A striking feature of *X. fastidiosa* is its polar attachment via the production of fimbriae (Kitajima et al. 1975, Purcell et al. 1979, Davis et al. 1981, Backus 1985, Purcell and Suslow 1988, H. Feil *unpublished data*). This is an adhesion mechanism that appears to be unique to *X. fastidiosa* and clearly requires traits special to this organism. This suggests the existence of either compounds or conditions that would prevent *X. fastidiosa* to form this polar fimbriae bundle and to attach to its host might confer disease resistance. A method of controlling *X. fastidiosa* would be to target these special traits that allow *X. fastidiosa* to adhere to its host. By interfering with the binding of *X. fastidiosa* to its host, these inhibitors would reduce *X. fastidiosa* virulence and therefore prevent the bacterium from establishing and causing disease.

OBJECTIVES

1. Determine the effects of targeted mutations of selected attachment genes (i.e. *fimA, pilH, pilS* and related genes) on *X. fastidiosa* attachment.

2. Determine differences in pathogenicity between *X. fastidiosa* attachment-deficient mutants and wild type PD strains.

3. Identify specific plant chemicals, pH, and various compounds that either promote or inhibit *X. fastidiosa* attachment in vitro and in plants.

RESULTS AND CONCLUSIONS

To achieve Objective 1, we produced site-specific mutants of *X. fastidiosa (Xf)* attachment gene. We improved on the method described in the transformation of the CVC strain of *Xf* to kanamycin resistance with plasmid *p*16Kori (Monteiro et al. 2001). In a similar fashion we disrupted the *fim*A gene of the *Xf* grape strain Temecula with the kanamycin resistant gene via homologous recombination (Figure 1). We are currently adding the *gfp* (green-fluorescent protein) gene to the vector plasmid to use it as a reporter gene for the *fimA* promoter. We were successful in producing *fimA*-disrupted transformants. It is the first time that a site directed transformation of a grape strain of *X. fastidiosa* has been demonstrated. The transformation efficiency was approximately 40 transformants per mg of plasmid DNA (*p*Vector). Figures 2 and 3 indicate that the transformants appear to be the result of a double crossing over event. The size of the *fim*A in the transformant corresponds to the size of the *fim*A gene to which was added the size of the Kanamycin gene. We are confirming the results using southern blot hybridization.

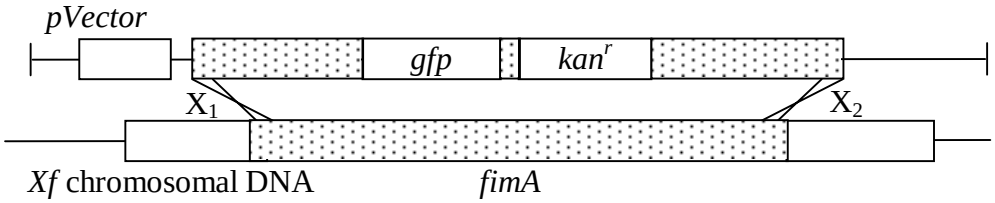

Figure 1: Schematic representation of the double cross over between the disrupted *fimA* gene of *p*Vector and the *fimA* chromosomal gene via homologous recombination.

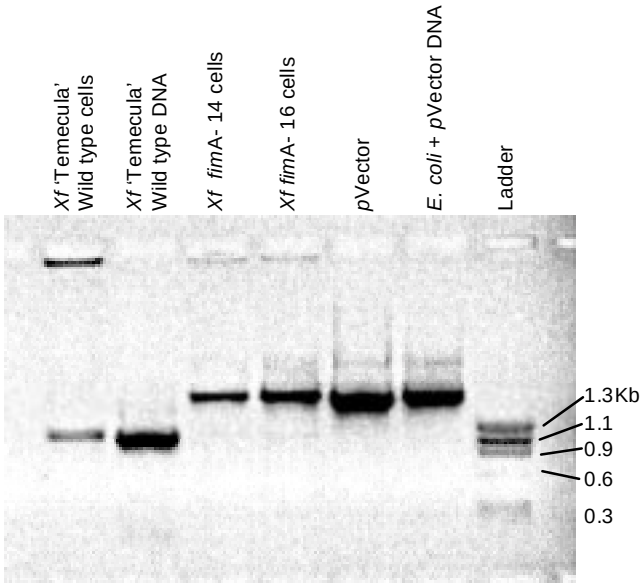

Figure 2: Agarose gel of PCR products using *FimA* primers

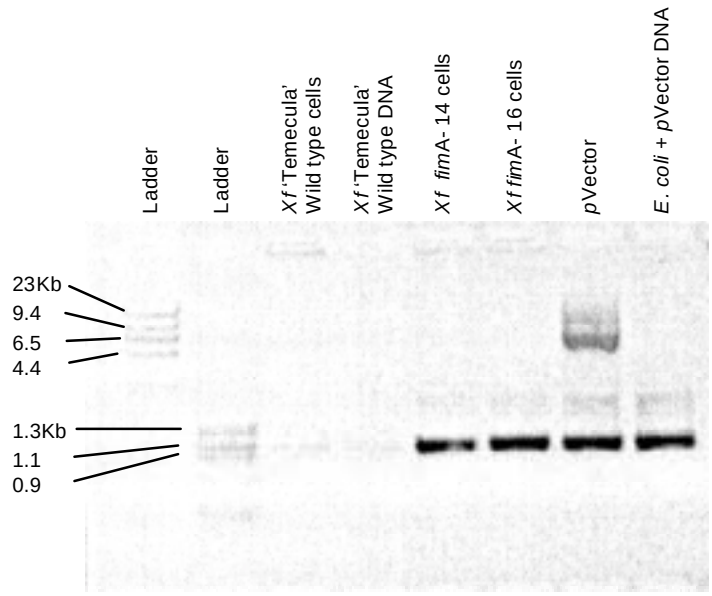

Figure 3: Agarose gel of PCR products using *Kan* primers

We are in the process of testing the *fimA*$^-$ transformants for pathogenicity by inoculation of grape plants. The nature of the *fimA* product is also investigated using SDS-PAGE of the supernatant of an agitated cell suspension of either wild type or *fimA*$^-$ mutants.

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