CHARACTERIZATION OF FIMBRIAE PRODUCTION AND ATTACHMENT OF FIMA$^-$ AND FIMF$^-$ MUTANTS OF *XYLELLA FASTIDIOSA* IN VITRO

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INTRODUCTION

Xylella fastidiosa is a Gram-negative bacterium which causes serious diseases of plants such as Pierce's disease (PD) of grape (*Vitis vinifera* L.), citrus variegated chlorosis (CVC), and almond leaf scorch and colonizes many other plant hosts as well as insect vectors (Purcell 1997). The complete genome sequences of several strains of this organism are now available and provide the base material to study the function of most genes in this organism. *X. fastidiosa* is known to produce fimbriae to attach unipolarly to cell surfaces (Purcell et al. 1979, Feil et al. 2002). The fimbrial genes are clustered in an operon containing 6 open reading frames (ORFs) (Bhattacharyya et al. 2002). Several of these ORFs have been identified to have homology with genes of other organisms that were shown to be involved in the production of fimbriae (Bhattacharyya et al. 2002). Fimbriae- and pili-mediated attachment of bacteria to host tissues is important for bacterial colonization and pathogenicity (Hultgren et al. 1996). We investigated the role of fimbriae and adhesins in the virulence of *X. fastidiosa* to plants such as grape. The aim of this study was to determine the importance of fimbriae on the attachment of *X. fastidiosa* to xylem vessels. Two fimbrial mutants, FimA$^-$ and FimF$^-$ (a homolog to the adhesin MrkD) were produced and further characterized (Feil et al. 2002). Pathogenicity test showed that the mutants were still virulent in grapes. Research is still underway to determine to what extent the process of colonization of plants is altered in FimA$^-$ and FimF$^-$ mutants. We expect that the speed with which the cells move through the plant and the time before symptom development is altered in the mutants; detailed measures of pathogen populations of the wild-type and mutant strains is underway in inoculated plants to determine these features. We described here the results of several attachment assays used to further characterize the attachment of these mutants compared to the attachment of the wild type to various substrates.

OBJECTIVES

1. Determine the role of fimbriae in the attachment of *Xylella fastidiosa* to grape xylem vessels.
2. Identify compounds that either enhance or inhibit the production of fimbriae.

RESULTS AND CONCLUSIONS

The large majority of site-directed mutants in *X. fastidiosa* obtained after introducing FimA or FimF genes modified to contain an insertion of a kanamycin resistance marker gene into the Temecula strain using a pUC18-based suicide plasmid have been the result of double recombination events. While this is a very fortuitous result given that we obtain a very high frequency of gene knockouts in our mutagenesis strategy, such results are unexpected given that in most other bacteria gene replacement occurs via a process that first generated single recombination events leading to cis-merodiploid strains. We are currently testing whether the high frequency of apparently simultaneous double recombination events is due to a linearization of the in-coming plasmid DNA.

To further characterize the attachment of the fimbrial mutants, FimA$^-$ and FimF$^-$ we chose glass and balsa wood as substrates for the assays. The attachment of the mutants to these substrates was compared with the attachment of the wild-type parental strain. Several media were also compared to determine if attachment to substrates was dependent on the nature of aqueous medium in which the cells were suspended. Fluorescence microscopy revealed that adhesion to glass and aggregate formation was greatly reduced for the mutants compared to the wild-type cells. Wild-type cells formed aggregates of large size at occasional sites on both glass slides and on wood surfaces. Most of the attached cells were found within such aggregates; very few cells were attached as solitary cells to the surfaces. In contrast, almost no cell aggregates were observed in the FimA$^-$ and FimF$^-$ mutant strains, and few solitary cells also had adhered to the surfaces. To determine the amounts of cells remaining attached to glass or wood, we quantified the amount of protein as an estimate of the number of cells present in a sample. The greatest reduction of attachment using this assay was found when FimA$^-$ cells were grown in a low nutrient medium, whereas in the PW medium the attachment was similar for the mutants and wild-type cells. These results suggest that pili play an important role in attachment of *X. fastidiosa* cells to each other to form aggregates, and that pili may play

little role in attachment to other surfaces. Since cell masses are a main feature of *X. fastidiosa*-infected xylem vessels, the self-aggregation of the pathogen conferred by pili may be an important virulence factor. The self-association of cells of *X. fastidiosa* should also influence the extent to which cells move through the plant and contribute to blockage of water movement, thus influencing symptom development.

When scanning electron microscopy (SEM) was used to examine wild-type and FimA$^-$ and FimF$^-$ mutants, we observed that fimbriae production between the cells and the balsa wood for the wild type cells was enhanced when cells were grown in a low nutrient medium. Examination of the FimA- mutant with SEM showed that fimbriae production was rare and that the fimbriae length was much reduced from that of wild-type cells grown under similar conditions.

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