UNDERSTANDING *XYLELLA FASTIDIOSA* COLONIZATION AND COMMUNICATION IN XYLEM LUMINA

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ABSTRACT
Flow cells that emulate xylem vessels have been microfabricated in silicon and in polydimethylsiloxane. *Xylella* cells in these artificial vessels are being studied for colonization and biofilm development.

INTRODUCTION
Pierce's disease of grape is caused by *Xylella fastidiosa*. Symptoms are generally recognized as being caused by restricted sap flow and resultant water stress due to plugging of xylem elements by live bacterial aggregates and associated mucilage (Goodwin et al., 1988; Purcell and Hopkins, 1996; Mollenhauer and Hopkins, 1974). It is not clear whether the extracellular polymeric mucilage is of bacterial and/or plant origin. Based on the analysis of the complete genome sequence of *X. fastidiosa*, gums produced by the *X. fastidiosa* are similar to the 'xanthan gums' produced by *Xanthomonas campestris* pv *campestris*, although they may be less viscous (Simpson et al., 2000). In addition, tylose development in xylem vessels in response to the presence of the bacterium further restricts sap flow (Mollenhauer and Hopkins, 1976). These general concepts *X. fastidiosa* pathogenicity are readily recognized, although it is not understood how the bacterium becomes established in the turbulent habitat of a 'fluid conduit' i.e., xylem vessels and tracheae. Bacterial spread through xylem elements is also poorly understood, albeit enzymatic degradation of pit membranes is thought to be involved (Mollenhauer and Hopkins, 1976). Colony formation is likely to be influenced by the physical constraints of the xylem element surface much like the formation of bacterial biofilms is influenced by surface characteristics (microtopography, chemistry, etc.) in other aqueous and fluid environments such as medical stints and prostheses, food handling equipment, and water supply systems (Ridgway and Olson, 1981; LeChevallier et al., 1987; Caldwell and Lawrence, 1988; Sternberg et al., 1999). Surface microtopography of these environments influence the temporal and spatial aspects of bacterial colonization (Bremer et al., 1992; Gorman et al., 1993; Korber et al., 1997; Arnold, 1999). Surfaces become colonized as cells (in this case bacteria) attach initially via physio-chemical forces, and ultimately with extracellular polysaccharides or ligand-mediated interactions. The end result is the establishment of biofilms consisting of bacteria in a polysaccharide matrix that provide a protective habitat that is conducive for continued cell growth and colony formation. The recently completed sequencing of the *X. fastidiosa* genome has revealed several open reading frames with putative functions that may be associated with bacterial colonization of xylem vessels and disease (Simpson et al., 2000). For example, at least one ORF with homology to the *luxR* family of transcriptional regulators has been identified (GenBank accession AAF83782). Such genes encode proteins (LuxR homologs) that when bound by acyl-homoserine lactone autoinducer molecules (AI), regulate transcription of diverse types of genes (Fuqua et al., 1996). Autoinducers are synthesized by enzymes that are encoded by *luxI* homologs or other synthase genes. The *luxI – luxR* regulatory system was first discovered in the marine bacterium *Vibrio fischeri*, however now related systems have been discovered in diverse species of bacteria including plant and animal pathogens (Cha et al., 1998). Autoinducers diffuse bi-directionally across bacterial membranes and reach concentrations for efficient activation of LuxR regulators in environments of high bacterial density. Thus the ability of AI to activate the LuxR regulators is a cell density-dependent response referred to as quorum-sensing or autoinduction. The discovery of *luxR* homologs in *X. fastidiosa* suggests that the bacterium produces AI or related signal molecules that may regulate genes that are associated with biofilm production in a density-dependent manner. This finding is intriguing because it suggests that a quorum-sensing regulatory system may be functioning in *X. fastidiosa* biofilm communities in xylem vessels.

OBJECTIVES

1. To understand how the physical parameters of xylem tracheae and vessels influence *Xylella fastidiosa* colonization. Toward this, we will evaluate colony formation, mucilage production, biofilm development, and evaluate flow rate during and following colonization. Our approach will be to use microfabricated 'artificial' vessels that mimic topologies and chemistries of 'real' *in planta* vessels.
2. Determine whether *X. fastidiosa* produces acyl-homoserine lactone autoinducer molecules that are involved in regulation of genes associated with ability to cause Pierce's Disease.

RESULTS AND CONCLUSIONS

Results

Development of artificial xylem vessels (flow chambers). Flow chambers of four different size dimensions have been designed and microfabricated. Positive masters for the chambers were either fabricated directly in semiconductor grade silicon wafers using photolithography and reactive ion etching procedures or as negative masters on similar silicon wafers

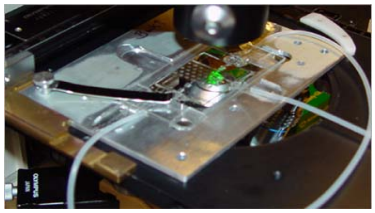

Figure 1. PDMS 'flow chamber' mounted on microscope stage with connected media supply tubing.

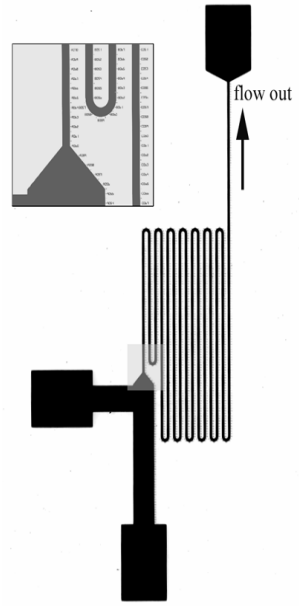

Figure .2. Schematic of 14 cm long flow chamber. Channel is 100 □m wide.

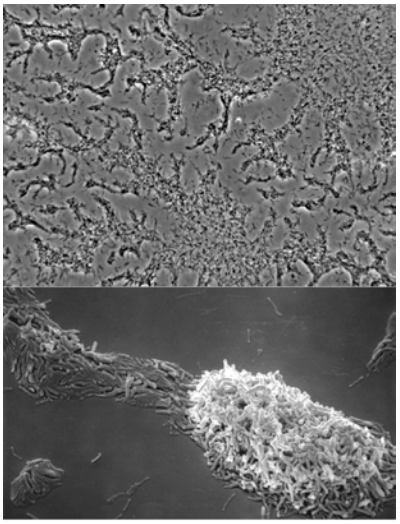

Figure 3. Xylella cells colonizing PDMS (LM and SEM images)

with patterned SU-8 photoresist providing the relief pattern from which subsequent chambers were fabricated. In either instance, the final chambers were made of polydimethylsiloxane (PDMS) which when sealed with a cover glass after plasma etching constituted a complete flow chamber (Figs. 1, 2). Chamber dimensions varied from 4-14 cm in length, 100 μm deep, and 50, 100, and 1000 μm in width. Introduction of media and *Xylella* cells into the chambers was controlled with a syringe pump. Once cell attachment to the pre-chamber surface was complete, non-attached cells were flushed from the chamber and sterile media was pulled continuously through to emulate plant xylem fluid flow. Temporal and spatial changes in *Xylella* cell distribution, colony development, and morphology are being assessed.

Adhesion and attachment of Xylella to inert surfaces. A range of surface treatments were examined for adhesion and attachment of *Xylella* cells in preparation for use in the xylem flow chambers. Glass cover slips were treated with various silanes to create surface chemistries of specific affinities (Table 1). In addition PDMS and cellulose were also examined. *Xylella* cells adhered to and colonized best to diphenyldichlorosilane, and dimethyldichlorosilane. PDMS was also very efficiently colonized (Fig. 3). Temporally, attachment of *Xylella* cells occurred nearly as soon as contact with the surfaces were made. Attachment was either at the polar ends or along the length of the cells appeared, in the short term at least, to be secure and irreversible. Highest concentrations of cells appeared at the air-liquid interface, although a significant number of cells attached submerged in the growth media as well.

Table 1. Attachment of *Xylella* cells to various substrata

Substratum	Attached cells
polydimethylsiloxane	++++
muffled glass	+
dialysis membrane	+
bis(2-hydroxyethyl)-3-aminopropyltriethoxysilane	+
3-aminopropyltriethoxysilane	+ ½
diphenyldichlorosilane	++
n-octadecyltrichlorosilane	+++
dimethyldichlorosilane	+++

Biofilm development dependency on initial cell concentration. Colonization and biofilm development (as determined by crystal violet staining) occurred on inert polyproplylene and polystyrene surfaces over a period of one week. Biofilms density was affected by the initial concentration of cells that were added to the medium. For example, cultures initiated from a cell suspension of OD_{600}= 0.1 had a significantly denser biofilm than from those initiated from OD_{600} = 0.7.

Assays to select adhesion-deficient mutants are currently being done.

Conclusions

The results of this investigation will result in understanding temporal and spatial aspects of *Xylella* colonization and movement to new sites.

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