UNDERSTANDING *XYLELLA FASTIDIOSA* COLONIZATION AND COMMUNICATION IN XYLEM LUMINA

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INTRODUCTION

Symptoms of Pierce's disease of grape caused by *Xylella fastidiosa* are generally recognized as being caused by restricted sap flow and resultant water stress due to plugging of xylem elements (Goodwin et al. 1988; Purcell and Hopkins 1996; Mollenhauer and Hopkins 1974). Such blockage is the result of massive bacterial aggregates and associated mucilage. 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* gene homologs. 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* strongly suggests that the bacterium produces AI and regulates genes in a density-dependent manner. This finding is intriguing because it suggests that a *luxI-luxR* type quorum-sensing regulatory system may be functioning in *X. fastidiosa* biofilm communities in xylem vessels.

The overall goal of the proposed research is to identify factors that affect colonization and plugging of grape xylem elements by *X. fastidiosa* and to use this information for development of effective control strategies for Pierce's disease. Our approach is to determine physical and chemical factors that influence *X. fastidiosa* attachment and colony development using an in vitro system, and to establish whether genes associated with these activities are regulated by quorum-sensing. The in vitro system that we propose has several advantages. It will allow the direct observation of bacterial community development in 'artificial' vessels microfabricated to possess topographies and chemistries similar to 'real' *in planta* vessels. We will be able to determine how physical and in some cases biological parameters affect biofilm formation and plugging induced by virulent and avirulent or weakly virulent strains. Furthermore, it will be possible to differentiate between plant-induced responses and those induced specifically by the pathogen.

OBJECTIVES

1. 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 flow rate during and following colonization
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

We have just received the funding for this project (October 2002).

REFERENCES

Arnold, J.W. 1999. Measurements of the surface topography of stainless steel related to bacterial attachment and biofilm formation. Poult. Sci. 78 (SUPPL. 1): 82.

Bremer, P.J., G.G. Geesey and B. Drake. 1992. Atomic force microscopy examination of the topography of a hydrated bacterial biofilm on a copper surface. Curr. Microbiol. 24: 223-230.

Caldwell, D. E. and J. R. Lawrence. 1988. Study of attached cells in continuous flow slide culture, p. 117–138. In J. W. T. Wimpenny (ed.), CRC handbook of laboratory systems for microbial ecology research. CRC Press, Inc., Boca Raton, Fla.

Cha, C., P. Gao, Y-C. Chen, P.D. Shaw and S.K. Farrand. 1998. Production of acyl-homoserine lactone quorum-sensing signals by Gram-negative plant-associated bacteria. Mol. Plant Microb. Int. 11: 1119-1129.

Fuqua, C., S.C. Winans and E.P. Greenberg. 1996. Census and consensus in bacterial ecosystems: The LuxR-LuxI family of quorum-sensing transcriptional regulators. Annu. Rev. Microbiol. 50:727-751.

Goodwin, P.H., J.E. De Vay and C.P. Meredith. 1988. Roles of water stress and phytotoxins in the development of Pierce's disease of the grapevine. Physiol. Mol. Pathol. 32:1-15.

Korber, D.R., A. Choi, G.M. Wolfaardt, S.C. Ingham and D.E. Caldwell. 1997. Substratum topography influences susceptibility of *Salmonella enteritidis* biofilms to trisodium phosphate. Appl. Environ. Microbiol. 63: 3352-3358

LeChevallier, M.W., T.M. Babcock and R.G. Lee. 1987. Examination and characterization of distribution system biofilms. Appl. Environ. Microbiol. 53: 2714-2724.

Mollenhauer, H.H. and D.L. Hopkins. 1974. Ultrastructural study of Pierce's disease bacterium in grape xylem tissue. J. Bacteriol. 119: 612-618.

Mollenhauer, H.H. and D.L. Hopkins. 1976. Xylem morphology of Pierce's disease-infected grapevines with different levels of tolerance. Physiol. Plant Path. 9: 95-100.

Purcell, A.H. and D.L. Hopkins. 1996. Fastidious xylem-limited bacterial plant pathogens. Annu. Rev. Phytopathol. 34: 131-151.

Ridgway, H.F and B.H. Olson. 1981. Scanning electron microscope evidence for bacterial colonization of a drinking-water distribution system. Appl. Environ. Microbiol. 41: 274-287.

Simpson, A.J. G., et al. 2000. The genome sequence of the plant pathogen *Xylella fastidosa*. Nature 406: 151-159.

Sternberg, C., B.B. Christensen, T. Johansen, A.T. Nielsen, J.B. Andersen, M. Givskov and S. Molin. 1999. Distribution of bacterial growth activity in flow-chamber biofilms. Appl. Environ. Microbiol. 65: 4108-4117.

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