N. Price - Report to the American Vineyard Foundation – February 2002

I. Project Title

Xylella fastidiosa bacterial polysaccharides with a potential role in Pierce's disease.

II. Principal Investigator(s), Cooperator(s):

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III. Summary:

Xylella fastidiosa are gram-negative bacteria that occur in the xylem of PD-infected grape plant, and are transmitted by xylem-feeding insects; leafhoppers and sharpshooters. Our hypothesis is that bacterial polysaccharides (EPS and LPS) produced by X. fastidiosa may be causative agents of Pierce's disease on grapes.

1. We have chemically analyzed isolated PD-infected grape xylem sap and Xylella cultures for Xylella EPS and LPS. We have found no evidence for the production of a "xanthan-like" EPS gum. We concluded that the symptoms of Pierce's disease are not due to a buildup of a Xylella ESP gum in infected vines. Physical occlusion of infected xylem tissue is therefore more probably due to adhering and clumping bacteria cells, and/or to the production of an abundant LPS.

2. Our research has identified a Xylella cell-wall LPS (lipopolysaccharide), composed of polymeric D-galactose and L-rhamnose sugars. Significantly, Xylella LPS from the grape strain is structurally different to LPS isolated from orleander-specific Xylella.

3. Perhaps most important, we have shown than an antibody raised against the Xylella grape-pathogen recognizes the purified LPS from this strain, but does not detect LPS from the orleander Xylella or from control bacteria. This antibody also detects the Xylella LPS in PD-infected grape sap isolated from plants in the field.

Our results suggested that an Xylella LPS antibody kit could be developed as a early diagnostic for Pierce's disease infected vines, that could be used by the non-specialist in the field. With this goal in mind, we are presently developing a quantitative, color-based LPS-antibody dot blot assay for the early detection of PD.

IV. Objective(s) and Experiments Conducted to Meet Stated Objective(s):

The prioritized research objectives were stated as:-

1. To structurally characterize bacterial exopolysaccharide(s) from the xylem sap of PD-infected and non-infected vines (using two grape varieties, Chardonnay and Carbernet), and to ascertain its potential involvement in Pierce's disease.
2. To structurally characterize lipopolysaccharide O-antigen from Xylella fastidiose grape pathovar. isolated from the xylem of PD-infected and non-infected vines (two varieties, Chardonnay and Cabernet).
3. To structurally characterize exopolysaccharides and O-antigen from Xylella fastidiosa grown in culture, and to assess potential changes in response to changing growth conditions and/or cultures additives.

Objective 1. has been met by Dr. Kirkpatrick's lab at UC Davis. PD-infected and uninfected xylem sap are isolated, freeze-dried and shipped to the Price lab for chemical analysis. Objectives 2 and 3, chemical characterization of Xylella LPS and EPS by GC-MS, TLC, LC-MS and NMR are undertaked by the PI's lab.

V. Summary of Major Research Accomplishments and Results (by Objective):

We have chemically analyzed isolated xylem sap and Xylella cultures for Xylella EPS and LPS. However, we have found no evidence for the production of a "xanthan-like" EPS gum. Neither of the techniques used (GC-MS, TLC, LC-MS or NMR) detected EPS either in lab cultures of Xylella or grape xylem sap exudates. EPS production was also monitored during the vine growing season, and again was NOT detected.

Xylella LPS (lipopolysaccharide) has been purified for the first time, has been analyzed by SDS-PAGE, and detected in-gel with a Xylella-specific polyclonal antibody. Significantly, the antibody recognizes the grape-specific (Temecula) LPS, but not LPS from an orleander strain or control bacteria.

Compositional analysis (GC-MS alditol acetates) of the Temecula LPS found equimolar D-Gal and L-Rha. In contrast, the orleander-strain produced an LPS containing far less L-Rha.

We conclude that the grape stain produces an L-rhamnose rich LPS, and that the rhamnose residues are selectively recognized by the Xylella antibody. This antibody also detected LPS in infected grape sap, suggesting the possibility of producing a antibody-based diagnostic kit for field analysis of the Pierce's disease

VI. Outside Presentations of Research:

A paper and talk of some of the results from this research were presented at the CDFA Symposium on Pierce's Disease held in San Diego in December 2001. Further results on the chemical composition of the Xylella LPS, and the LPS-antibody specificity have been obtained since that meeting took place.

VII. Research Success Statements:

The stated hypothesis is that bacterial polysaccharides produced by X. fastidiosa may be causative agents of Pierce's disease on grapes. The initial aim was to collect xylem exudate from PD-infected and non-infected Chardonnay and Carbernet vines (in the Kirkpatrick lab), and to set up EPS and LPS carbohydrate extraction and analysis (in the PI's lab). This requires the use of a specialized pressure cell, housed in Dr. Kirkpatrick's laboratory at UC, Davis. The sap is freeze-dried and shipped to the Price lab for carbohydrate analysis and DOC-PAGE analysis. We have isolated and analyzed 28 xylem exudate samples from PD-infected and control vines, and 12 samples of culture exudate from Xylella fastidiosa grown in laboratory culture. This sampling (done by Ms. Roper) represents the backbone of the project.

Carbohydrate Analysis

The Xylella Genome project identified 9 genes with homology to the xanthan gum biosynthetic cluster of Xanthomonas campestris, i.e. gumBCDEFHJKM, but lacked gumGIL. In Xanthomonas these gum genes direct the synthesis of a highly viscous exopolysaccharide gum (xanthan). The production of a comparable gum by Xylella in the xylem of infected vines would likely block the plants' water uptake system. The lack of gumGIL in the Xylella genome suggested that its EPS gum should be closely analogous to xanthan gum, but lacking the terminal mannose (Man). Our initial aim was to determine whether xanthan lacking this outermost Man residue would still form a viscous polymerized gum likely to clog xylem vessels, or whether the intrinsic viscosity would be lost. We report that the gel viscosity was unaffected by removal of the outermost mannose, and if produced in the xylem of vine plants, even at relatively low concentration (0.5% w/v), it would very likely clog them.

Hydrolysis/Methanolysis

Controlled hydrolysis of xanthan (or the predicted Xylella gum) produces a characteristic disaccharide, GlcA-(beta-1,2)-Man, which is therefore be characteristic of this type of polysaccharide and useful as a "fingerprint" diagnostic. TLC analysis of commercial xanthan revealed the predicted GlcA-□1,2-Man, and the structures was confirmed by alditol acetate GC-MS. The analyses were then applied to the grape xylem sap exudates collected by Ms Roper. We did not detect any "xanthan-like" gum by GC-MS or TLC in these samples. To confirm this, the xylem saps were analyzed by proton NMR, and although sucrose and glucose were found, again there is no evidence for the proposed Xylella EPS gum.

Xylella Lipopolysaccharide

LPS is the outermost polysaccharide layer of gram-negative bacteria. Xylella LPS may mediating recognition and adhesion between the bacteria and host plant, or the bacteria and the insect vector. Xylella has the full genetic compliment required for LPS biosynthesis. We have identified both ADP-heptose synthetase (rfaD) and two heptosyltransferase (rfaC, rfaF) genes suggesting that Xylella has a LPS core structure □Hep-1,3-□Hep-1,5-□KDO-2,6-lipidA, similar to E. coli or Salmonella. Other genes (rfbBCD) likely determine the synthesis of dTDP-L-rhamnose. We have now purified the LPS from Xylella grape, orleander and almond strains. Alditol acetate analysis indicates that the grape strain (Temecula) LPS consists of equimolar D-galactose and L-

rhamnose, and that the Gal/Rha ratio is significantly lower in the orleander strain. We have analyzed the LPS by SDS-PAGE, and see obvious differences in gel mobility. Moreover, an antibody raised to the grape Xylella recognizes the grape strain LPS on gel blots. Importantly, the antibody also recognizes Xylella LPS from the infected xylem saps, but not the uninfected controls. LPS from the orleander strain (and LPS from a control bacteria, Klebsiela) are not recognized by the antibody. It is our recommendation that an antibody to purified LPS from the grape strain has potential as a field tool for the diagnosis of Pierce's disease infection.

VIII. Funds Status:

Funds from the American Vineyard Foundation are being used to support a Research Assistant, Ms Billyana Tsvetanova, in my laboratory, and in partial support of an RA, Ms Caroline Roper, in Bruce Kirkpatrick's laboratory at UC, Davis. Our present round of funding ends in May 2002.