REPORT TYPE: Renewal Progress Report for CDFA agreement number 11-0148-SA.

PROJECT TITLE: Characterization of *Xylella fastidiosa* lipopolysaccharide and its role in key steps of the disease cycle in grapevine.

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REPORTING PERIOD: The results reported here are from work conducted July 2011 to present (March 2012).

INTRODUCTION

Xylella fastidiosa (Xf) is a Gram-negative bacterium comprised of subspecies that cause disease on several different economically important crops, such as grapevine, almond, and oleander. We are currently exploring the role of lipopolysaccharide (LPS) as both a virulence factor in grapevine and host specificity determinant for this pathogen. LPS is an integral part of the Gram-negative bacterial outer membrane and is primarily displayed on the outer surface of the cell, thereby mediating interactions between the bacterial cell wall and its environment. LPS is composed of three parts: 1) lipid A, 2) oligosaccharide core, and 3) O-antigen polysaccharide (**Figure 1**) (29). Both lipid A and the oligosaccharide core are highly conserved among all Gram-negative bacteria, whereas, the O-antigen can be varied even among subspecies. LPS (sometimes called "endotoxin") has been implicated as a major virulence factor in both plant and animal pathogens, such as *Escherichia coli*, *Xanthomonas campestris* pv. *campestris*, and *Ralstonia solanacearum* just to name a few (7, 11, 20). Because of its location in the outer membrane, LPS can also contribute to the initial adhesion of the bacterial cell to a surface or host cell (9, 22). Additionally, host perception of LPS is well documented and occurs in both plants and animals (23). The host immune system can recognize several regions of the LPS structure and can mount a defense response in response to bacterial invasion based on this recognition. Bacteria can also circumvent the host's immune system by altering the structure of the O-antigen portion of the LPS molecule or by masking it with capsular or exopolysaccharides.

We are focusing on the O-antigen portion of LPS in three *Xf* isolates that colonize different hosts: Temecula1, causal agent of Pierce's Disease (PD); M12, causal agent of almond leaf scorch (ALS); and Ann-1, causal agent of oleander leaf scorch (OLS). Based on previously reported investigations of LPS in host-pathogen interactions in other bacterial systems (14, 27), we identified two genes, *waaL* (PD0077) and *wzy* (PD0814) in the genomes of the three *Xf* isolates included in this study. These genes are predicted to encode proteins required for production of a full O-antigen moiety in *Xf*. Wzy is a putative O-antigen polymerase that plays a role in chain length determination of the O-antigen. WaaL is a putative O-antigen ligase that ligates the preformed O-antigen to the oligosaccharide core. Therefore, we predicted that mutants in *wzy* would produce a truncated O-antigen and that mutants in *waaL* would be completely lacking the O-antigen (**Figure 1**). Based on these alterations in the O-antigen, we hypothesized that we would see profound differences in virulence, surface attachment, aggregation, and insect transmission.

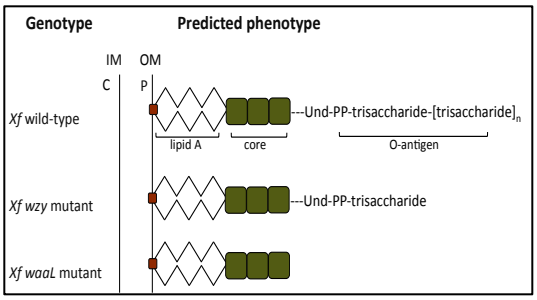

Figure 1. *Xf* strains lacking a functional Wzy (polymerase) are predicted to have truncated O-antigen with only the initiating polysaccharide unit. Strains lacking a functional WaaL (ligase) are predicted to lack the O-antigen. Und-PP = undecaprenylphosphate; IM = inner membrane; OM = outer membrane; C = cytoplasm; P = periplasm.

Thus far, we have demonstrated that mutations in *wzy* and *waaL* do affect O-antigen biosynthesis and cause a visible change in the O-antigen profiles as seen in **Figure 2**. LPS was extracted from Temecula1 WT, *wzy,* and *waaL* using a hot-phenol extraction method (28) and then subjected to Tris-Tricine polyacrylamide gel electrophoresis (PAGE). Mutations of these loci in *Xf* Temecula1 had a significant reduction in O-antigen in the *waaL* mutant strain and a lack of O-antigen in the *wzy* mutant strain (**Figure 2**). The *waaL* gene is annotated as an O-antigen ligase, an enzyme that ligates the assembled O-antigen to the core LPS and we expected that a *waaL* mutant would completely lack smooth LPS (O-antigen). The *wzy* gene is annotated as an O-antigen polymerase, an enzyme involved in chain length determination of the O-antigen, and we expected a mutation in *wzy* to result in a truncated O-antigen. However, we observed essentially the reverse of what we expected. The *wzy* mutant lacked O-antigen and the *waaL* mutant still produced residual amounts of O-antigen (**Figure 2**). This suggested two things to us: 1) that Wzy may be functioning as a ligase rather than a polymerase and 2) there is a protein(s) that shares a redundant function with WaaL. It is not uncommon for enzymes in the LPS biosynthetic pathway to be "cross annotated" as either O-antigen ligases or polymerases due to the structural similarities of the proteins. Both WaaL and Wzy possess a Wzy_C domain that belongs to Pfam 04932, a family of proteins that includes the O-antigen ligases. Therefore, it is reasonable to assume that Wzy is actually behaving as an O-antigen ligase rather than as the annotated O-antigen polymerase. Both mutant phenotypes were restored by introducing *waaL* or *wzy* into their respective mutant genomic backgrounds using the chromosomal complementation vector, pAX1Cm (17) (**Figure 2**).

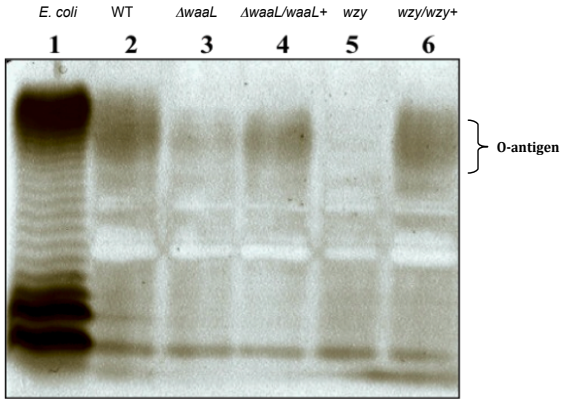

Figure 2. Mutations in key enzymes of the *Xf* LPS biosynthetic pathway result in reduction or abolishment of O-antigen. Temecula1 lacking a functional WaaL (lane 3) had less O-antigen than the wild-type (lane 2) while Temecula1 lacking Wzy (lane 5) appeared to have no distinguishable O-antigen moiety. Complementation of these mutants restored O-antigen quantity to near that of the wild-type parent (lanes 4 and 6). LPS was extracted from cells normalized to cell density using a modified hot phenol method and analyzed on a 4, 12% discontinuous Tricine-PAGE gel and silver stained (26). (1) *E. coli* standard, (2) Temecula1 wild-type, (3) Temecula1*waaL*, (4) Temecula1*waaL*/*waaL*+, (5) Temecula1*wzy*, (6) Temecula1*wzy*/*wzy*+.

Following the confirmation of the change in the O-antigen profiles for the *waaL* and *wzy* mutants, we then tested these strains for differences in virulence *in planta*. Temecula1 O-antigen mutant strains were inoculated into grapevine, cv. 'Thompson Seedless' according to the method of Hill and Purcell (1995) (12). Plants were rated weekly on a disease index scale of 0-5, based on PD symptom development, with 0 being healthy and 5 being dead or dying (10). PD symptoms were detected 4 weeks after inoculation, similar to other greenhouse PD virulence studies conducted in Riverside County. We observed no difference in disease progress between the Temecula1 *waaL* mutant and the wild-type strains. In contrast, the Temecula1 *wzy* mutant was significantly delayed in causing PD symptoms on grapevine, and after 11 weeks of incubation, it did not cause the extensive PD development

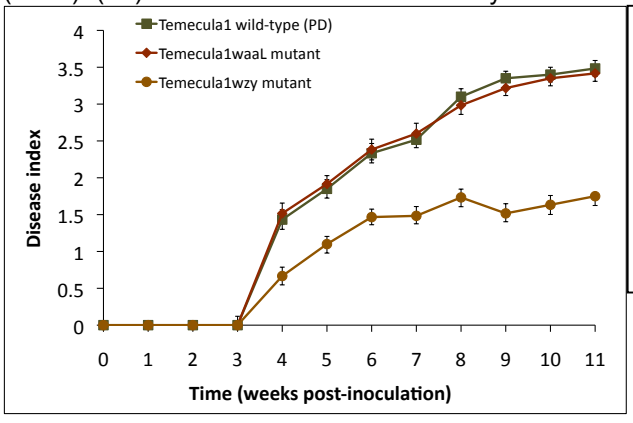

Figure 3. Disease progress of *Xf* Temecula1 wild-type and O-antigen mutant strains on grapevine cv. 'Thompson Seedless'. The *wzy* mutant strain lags behind the wild-type in causing leaf scorching symptoms and does not cause wild-type levels of PD throughout the disease progress curve. The *waaL* mutant is as virulent as wild type. Data are means of three independent assays with 10 replications each. Bars represent standard error.

observed in plants inoculated with wild-type (as determined by an ANOVA analysis with Tukey's pairwise comparisons) (**Figure 3**). Plants inoculated with 1X PBS buffer control did not develop any PD symptoms. **This indicates that absence of the O-antigen correlates with a profound defect in *Xf* virulence.**

In addition, we quantified bacterial population levels to assess any colonization differences between Temecula1 wild type and the O-antigen mutant strains that may be occurring *in planta*. We isolated bacteria from surface-sterilized petioles at 13 weeks post-inoculation and found that plants inoculated with the *wzy* mutant harbored significantly less bacteria than plants inoculated with WT Temecula1 (**Figure 4**). Populations were quantified by plate counts and the data were analyzed using a Wilcoxon rank test. However, the *waaL* mutants colonized the plants to similar levels as wild type *Xf*.

LPS is considered to be a Pathogen Associated Molecular Pattern (PAMP) that is recognized by the immune system of both plants and mammals causing the host to mount a defense response. One strategy that pathogens use to evade the host defense response is to mask the conserved portions of the LPS molecule (core and Lipid A) with a varying O-antigen or even exopolysaccharide. We speculate that the high molecular weight O-antigen chain in the *Xf* LPS molecule may serve to mask the rest of the LPS molecule from the host to avoid triggering a defense response, and the removal of this protective O-antigen may trigger the host defense response, resulting in reduced colonization and reduced disease symptoms as observed for the *wzy* mutant.

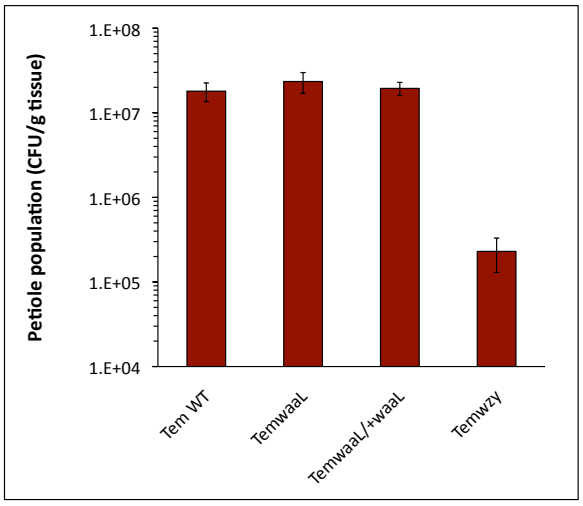

Figure 4. Xylem populations of the Temecula1 *wzy* mutant after inoculation of grapevine petioles are significantly less than those of the wild-type parent. Populations were quantified by plate counts. Five petioles from three independent inoculations. Bars represent the standard error of the mean.

OBJECTIVES

Objective 1: Expand the characterization of the LPS profiles from the grape, almond, and oleander strains of *Xf*.

Objective 2: Test attachment and biofilm formation phenotypes of *Xf* O-antigen mutants to the biologically relevant substrates, chitin and cellulose.

Objective 3: Test O-antigen mutants for insect transmissibility.

Objective 4: Test O-antigen mutants for increased vulnerability to environmental stress and antimicrobial compounds.

SUMMARY OF ACCOMPLISHMENTS AND RESULTS

Objective 1: Expand the characterization of the LPS profiles from the grape, almond, and oleander strains of *Xf*. A bacterium with only lipid A and core oligosaccharide is said to have “rough” LPS and one with lipid A, core oligosaccharide, and O-antigen is said to have “smooth” LPS. There can be both smooth and rough variants of the same bacterial species. We compared the LPS profiles of three *Xf* strains (Temecula1 (grape), M12 (almond), and Ann-1 (oleander)) by Tris-Tricine polyacrylamide gel electrophoresis (PAGE). This work is based on the hypothesis that there are strain specific differences among the variable O-antigen portion of the LPS molecules that contributes to the host specificity of these three isolates. We isolated LPS from all three *Xf* isolates that were grown on solid PW medium using a hot-phenol extraction method (28). The extracted LPS preparations were then subjected to Tris-Tricine

PAGE. These analyses have confirmed that all three strains possess smooth LPS (ie. O-antigen), which was previously unknown (**Figure 5**). The gels also revealed small shifts in the molecular weights of the smooth LPS for each strain, indicating a fundamental difference among the O-antigen chain length or composition.

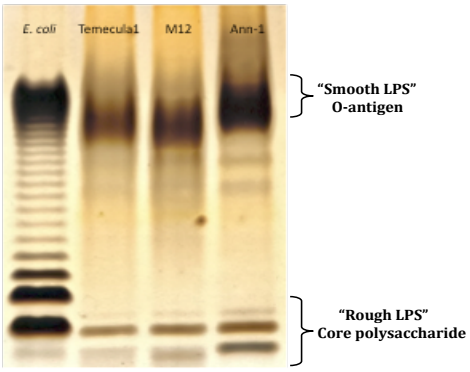

Figure 5. Tris-Tricine SDS-PAGE gel indicating the presence of both smooth (**O-antigen**) and rough (core polysaccharide) LPS in **1)** Temecula1 (PD), **2)** M12 (ALS), and **3)** Ann-1 (OLS) strains of *Xf*. LPS was extracted by the hot-phenol extraction method.

We are presently conducting a more detailed carbohydrate composition and linkage analysis of *Xf* LPS using Gas chromatography/Mass Spectrometry analysis and have begun our studies with the Temecula1 strain and its respective *wzy* mutant in collaboration with the Complex Carbohydrate Research Center (CCRC) at the University of Georgia, Athens. We have isolated total LPS from 100 mg of starting cell mass from wild-type Temecula1 and Temecula *wzy*. The CCRC is performing O-antigen purification using a mild acid hydrolysis technique and will perform carbohydrate composition and methylation linkage analysis. This will identify the exact carbohydrate constituents of the entire O-antigen and provide structural information about how each sugar is linked in the O-antigen carbohydrate chain. Following this analysis, we will move on to test the *waaL* mutant and the almond (M12) and oleander (Ann-1) isolates.

Host specificity plant assays. While there are likely several factors that contribute to host specificity of *Xf*, we would like to investigate if O-antigen is involved. *Xf* Temecula1 can colonize and cause disease in grape and, to some extent, in almond. *Xf* M12, an ALS strain, causes disease in almond and elicits some symptoms in grape (2). Moreover, *Xf* Ann-1 cannot cause disease in grape or almond and neither Temecula 1 nor M12 can cause disease in oleander. We speculated that removal or truncation of the O-antigen would affect the ability of Temecula1 to infect (and elicit scorch symptoms) on grape, almond, and oleander. Furthermore, perhaps changes in O-antigen may enable Ann-1 to become a pathogen of grape or almond while M12 and Temecula1 strains become pathogens of oleander.

We inoculated Temecula1 WT and the Temecula O-antigen mutant strains into host (grape) and non-host (almond and oleander) plants. We observed that the grape isolate (Temecula1 WT) can colonize and cause disease in almond, albeit, to a lesser extent than the WT Almond Leaf Scorch (ALS) isolate (M12), which is consistent with what other research groups have observed (1). Interestingly, the Temecula1 *waaL* mutant strain is **more virulent** in almond than its Temecula1 WT parent and is similarly virulent to the WT ALS isolate (M12). This suggests that an alteration in the O-antigen moiety of the LPS molecule does affect host specificity. The Temecula1 *wzy* mutant is similarly less virulent in almond as in grape. As expected, *Xf* Ann-1, the OLS isolate, does not cause scorch symptoms on almond (**Figure 6**).

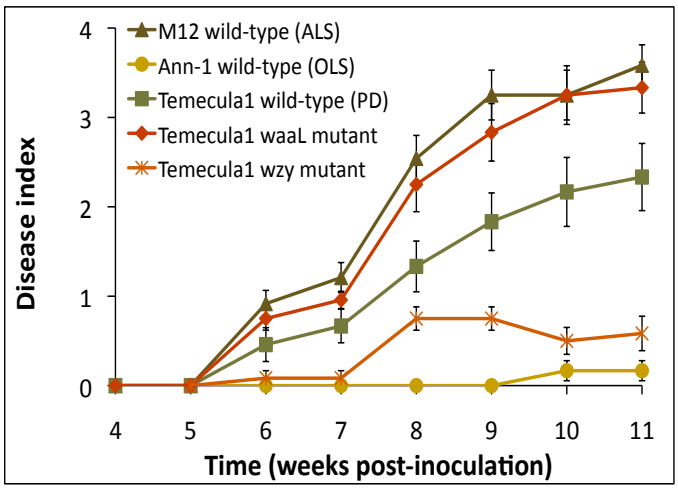

Fig. 6. Disease progress of M12 (ALS), Ann-1 (OLS) Ann-1, Temecula1 (PD) isolates, and Temecula1 O-antigen mutants in almond cv. 'Sonora'. Loss of WaaL increases virulence of a PD isolate of *Xf* in almond. Data are means of two independent assays with 6 replications each. Bars represent standard error.

Symptoms of oleander leaf scorch develop ten months post-inoculation, and we are currently awaiting the results from host specificity

studies conducted in this host plant. We are beginning to observe initial leaf scorch symptoms in the oleander plants that were inoculated in June of 2011 and are beginning to rate disease progress in those plants. We are in the process of constructing the corresponding *waaL* and *wzy* mutants in the M12 and Ann-1 isolates of *Xf*. We will repeat the host specificity studies this year with the mutants in all three isolates.

Objective 2: Test attachment and biofilm formation phenotypes of *Xf* O-antigen mutants to the biologically relevant substrates, chitin and cellulose. Biofilm formation is an important component of the plant-microbe interaction. To test the role of LPS in *Xf* biofilm formation, we quantified the Temecula1 *waaL* and *wzy* mutant strains in two biofilm related behaviors: 1) surface attachment and 2) cell-cell aggregation. Both of these phenotypes are critical early steps in the formation of a mature biofilm. We hypothesized that LPS may contribute to these behaviors because of its location and abundance in the outer membrane. Glass is a hydrophilic surface and the polysaccharide components (ie. pectin, cellulose, hemicellulose) that make up the xylem primary cell wall are also highly hydrophilic. This similarity allows us to extrapolate our *in vitro* results to what might be occurring in portions of the plant xylem tissue that have an abundance of exposed primary cell wall polysaccharides, such as the pit membranes that separate one xylem vessel from another. Interestingly, when grown in glass tubes, the Temecula1 *wzy* mutant aggregated less, but attached more to a glass surface (**Figure 6**). The *wzy* mutant was also significantly less virulent *in planta* which may be a result of its hyperattachment phenotype causing it to adhere more strongly to the xylem primary cell wall, which does not allow it to move as efficiently throughout the plant.

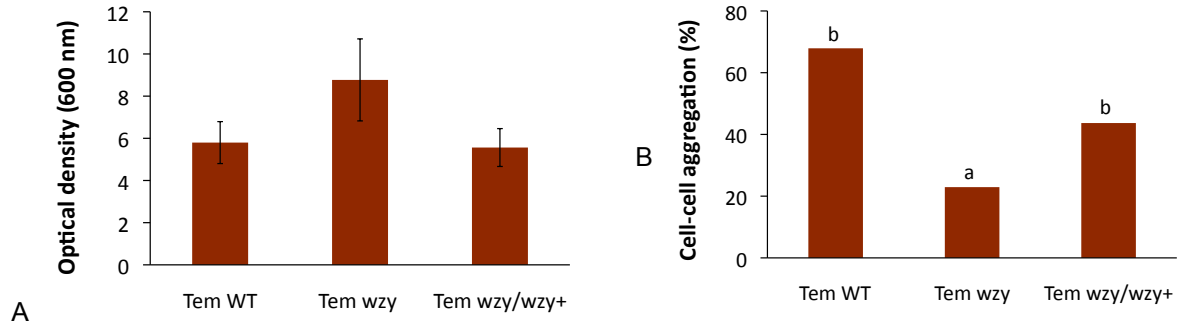

Figure 7. Surface attachment and cell-cell aggregation of the O-antigen mutant, *wzy*. A) The Temecula1*wzy* mutant cells attach to a solid surface to a greater extent than the wild-type parent. Attachment assays involved crystal violet staining of cells attached to a glass surface at the medium/air interface (2) after 7 days of incubation at 28°C, 100 rpm. **B)** The Temecula1*wzy* mutant cells are reduced in the ability to aggregate to each other compared to the wild-type. Aggregation assays reflect the proportion of the total cell population that remains in culture after 10 days of static incubation (3). At least three independent assays were performed in triplicate. Bars represent standard error of the mean.

We are currently testing the *waaL* mutant for attachment to glass. In addition, we are expanding the attachment studies to the more biologically relevant substrates, chitin and cellulose, for both mutants. In brief, for the chitin binding studies, we are taking advantage of commercially available chitin-coated magnetic beads (New England Biolabs; Ipswich, MA), and we are quantifying the ability of the Temecula1 WT, *waaL*, *wzy* and their respective complemented strains to attach to these chitin coated beads using a Syto9 fluorescent dye specific for bacteria. Fluorescent counts correlate with cell number and indicate whether or not the mutants attach to chitin as well as the WT. We are also using a semi-quantitative assay to determine if O-antigen is involved in attachment to grapevine cellulose fibers isolated from healthy grapevine petioles. Cellulose fibers will be incubated by a slow spin centrifugation and washed three times with 5ml of PD3 or XFM media to remove any unattached *Xf* cells. The *Xf*-cellulose fiber complex will be stained with the Syto9 fluorescent stain as described above and then visualized using the Leica SP2 Confocal Microscope available for use in the UCR core microscope facility on a recharge basis. Attached *Xf* cells will be quantified using the MCID software package also available in the UCR core microscopy facility.

Objective 3: Test O-antigen mutants for insect transmissibility. In this objective, we are comparing sharpshooter transmission rates for wild type versus either the *waaL* or *wzy* mutant. We are using the artificial feeding sachet technique developed by Killiny and Almeida (15) because differences in strain multiplication *in planta* may confound estimates of sharpshooter acquisition efficiency, which depends on plant infection level (13). The artificial sachet technique is extremely useful because it allows us to normalize all strains to the same starting cell density in the individual feeding sachets, thereby, avoiding any *in planta* multiplication differences. We initially began these studies using the highly abundant Glassy-Winged Sharpshooter (GWSS) (*Homalodisca vitripennis*) species in Riverside county. In collaboration with Dr. Matt Daugherty (UCR, Dept. of Entomology), we successfully reared a clean (*Xf*-free) GWSS colony and conducted preliminary sharpshooter transmission studies. However, due to the inherently low *Xf* transmission rates for the GWSS, we have opted to proceed with the Blue-Green Sharpshooter (BGSS) (*Graphocephala atropunctata*) which has a much higher rate of transmission than the GWSS. This will allow us to more accurately quantify differences in acquisition and transmission rates between the WT and either mutant. In collaboration with Dr. Thomas Perring (UCR, Dept. of Entomology), we have initiated a BGSS colony. We have also obtained grapevine seeds from Foundation Plant Services (UCD) to propagate the seedlings necessary for these studies. We anticipate having these experiments up and running in the next few weeks.

Objective 4: Test O-antigen mutants for increased vulnerability to environmental stress and antimicrobial compounds. The outer membrane of a bacterial cell is strongly influenced by its environment, and variation in the O-antigen portion of the LPS molecule can aid in the adaptation or tolerance to different environmental stresses such as oxidative stress, temperature, and resistance to antimicrobial peptides (3, 4, 21, 25). We are investigating if the absence or truncation of the O-antigen affects tolerance to environmental stress and antimicrobial compounds by testing the WT Temecula1, *waaL,* and *wzy* mutants for increased susceptibility to three environmental stresses: 1) oxidative stress, 2) cold temperature, and 3) treatment with antimicrobial peptides.

We rationalized that LPS is likely involved in resistance to oxidative stress because of its physical location in the outer leaflet of the bacterial cell membrane. During the plant infection process, bacteria encounter oxidative stress in the form of reactive oxygen species (ROS). ROS can be a product of the elicitation of the host defense response or a by-product of normal plant metabolism and development (23). In any case, oxidative stress is detrimental to the bacterial cell, and the cells must have a mechanism to cope with this environmental insult. We hypothesize that LPS, in particular the high molecular weight O-antigen chain, provides some protective effect to the cells and that the *waaL* and *wzy* mutants will be more vulnerable to ROS than the WT Temecula1 strain. We performed a simple disc inhibition assay protocol as previously described (18) to test sensitivity to peroxide. In brief, either the WT Temecula1, *wzy* and complemented strains were plated in PD3 top agar. A Whatman paper disk impregnated with 100 µM H_2O_2 was overlaid onto the top agar. Plates were incubated for 7 days at 28ºC and observed for zones of inhibition around the disk containing the H_2O_2. The diameter of the zone of inhibition correlates with sensitivity to H_2O_2. We observed that the *wzy* mutant was more sensitive to peroxide treatment as compared to the Temecula1 WT. We are currently testing the *waaL* mutant for sensitivity to peroxide.

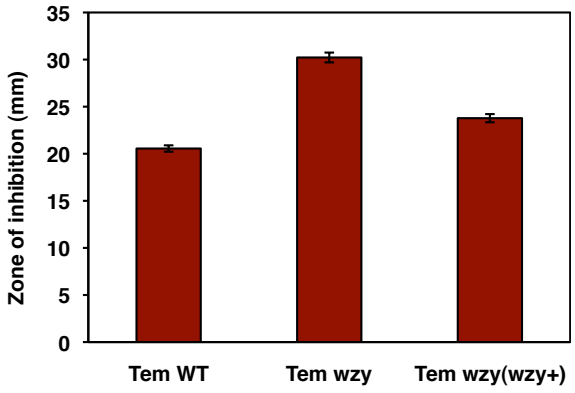

Figure 8. The role of O-antigen in the protection against oxidative stress. In the disk inhibition assay, the *Xf* Temecula1 *wzy* mutant strain was less tolerant of hydrogen peroxide stress than the wild-type parent or the complemented mutant strain. Three independent assays were performed in triplicate. Parenthetical values are the standard error of the mean.

In addition, we are testing the *waaL* and *wzy* mutants for increased susceptibility to cold temperature and treatment with antimicrobial peptides. We chose these two treatments based on current strategies being evaluated for control of PD (5, 16). Antimicrobial peptides (AMPs) are a unique and diverse group of molecules that have potent antimicrobial effects even in very small doses (19). They are short peptide sequences composed of no more than 50 amino acids and can display broad-spectrum antimicrobial activity. There are some AMPs, such as polymyxin B, that have been shown to bind to LPS with high affinity (25).

We have chosen to test cecropin B because it has previously been identified to have antimicrobial activity against *Xf* (6). We have also chosen polymyxin B because its mode of action is to bind directly to LPS (25). Both peptides are readily available from Sigma-Aldrich, Inc. Antibacterial activity will be expressed as Minimum Inhibitory Concentrations (MIC), the concentration at which no growth is observed. We are initially conducting these experiments in liquid cultures, but the results will be confirmed by testing on solid PD3 medium overlaid with a Whatman paper disk containing the different concentrations of the AMP (cecropin B or polymyxin B) being tested. The MICs will be calculated by measuring the diameter of the inhibition zones around the disc containing the AMP. In all cases, the cultures will be incubated at 28°C and monitored for growth over the course of 7-10 days.

Cold temperatures have been associated with the geographic distribution of Pierce's disease (Purcell, 1977; Purcell, 1980). The effects of cold temperature-mediated therapy of *Xf*-infected grapevines is an ongoing area of research, and certain aspects of this phenomenon are currently being exploited as possible control strategies for PD (16, 24). Bacteria can modify their LPS in response to temperature (3, 21). These temperature-induced modifications of LPS are generally regarded as an adaptive response to the cells surrounding environment.

We are basing our assay on previously established protocols (8). In brief, we will first grow *Xf* on solid PD3 medium for 7 days at 28°C. Cells will be harvested and resuspended to an $OD600_{nm}$=0.1 (approx. $1x10^7$ cfu/ml) in PD3 medium. 500 µl aliquots will be incubated at temperatures of -5°C, 0°C, 5°C, 15°C, and 28°C. 100 µl aliquots will be removed daily for seven days, serially diluted, and plated onto PD3 solid medium to obtain cfu/ml counts. In order to avoid contamination via the opening and closing of tubes, the experimental setup will have enough tubes to allow for destructive sampling at each time point. Survivability curves will be generated based on the data obtained from the plate counts.

We anticipate that both the antimicrobial peptide and cold temperature studies will be completed by September of 2012.

PUBLICATIONS PRODUCED AND PRESENTATIONS MADE

Publications

Clifford, J. and Roper, M.C. *In preparation*. The role of O-antigen chain length in biofilm formation, virulence and host colonization for *Xylella fastidiosa*, causal agent of Pierce's disease of grapevine. To be submitted to *Molecular Plant Microbe Interactions*.

Clifford, J. and Roper, M.C. 2011. The role of lipopolysaccharide in virulence, biofilm formation, and host specificity of *Xylella fastidiosa. Phytopathology,* 101:S38.

Roper, M. 2011. Characterization of *Xylella fastidiosa* lipopolysaccharide and its role in key steps of the disease cycle in grapevine. California Department of Food and Agriculture. p.139-145. Pierce's Disease Research Symposium, Sacramento, CA.

Roper, M. 2010. The role of lipolysaccharide in virulence, biofilm formation, and host specificity of *Xylella fastidiosa*. California Department of Food and Agriculture. p.139-145. Pierce's Disease Research Symposium, San Diego, CA.

Roper, M. 2009. The role of lipolysaccharides in virulence, biofilm formation, and host specificity of *Xylella fastidiosa*. California Department of Food and Agriculture. p.106-111. Pierce's Disease Research Symposium. Sacramento, CA.

Presentations

Clifford, J. and Roper, M.C. 2011. Characterization of *Xylella fastidiosa* lipopolysaccharide and its role in key steps of the disease cycle in grapevine. Presented as a poster at the Annual Pierce's Disease Research Symposium, Sacramento, CA.

Clifford, J. and Roper, M.C. 2011. The role of lipopolysaccharide in virulence, biofilm formation, and host specificity of *Xylella fastidiosa.* Presented as a poster at the Annual Meeting of the American Phytopathological Society, Honolulu, HI.

Clifford, J. and Roper, M.C. 2010. The role of lipopolysaccharide in virulence and host specificity of *Xylella fastidiosa*. Presented as a poster at the Annual Pierce's Disease Research Symposium, Sacramento, CA.

Clifford, J. and Roper, M.C. 2009. The role of lipopolysaccharide in virulence and host specificity of *Xylella fastidiosa*. Presented as a poster at the Annual Pierce's Disease Research Symposium, San Diego, CA.

RESEARCH RELEVANCE STATEMENT

The focus of this project is to further understand the molecular mechanisms governing *Xf* virulence. We are working toward understanding the role of the O-antigen component of LPS in contributing to virulence and those behaviors associated with xylem colonization, such as host cell wall attachment and cell-cell aggregation required for biofilm formation. In addition, the broad host range, but stringent host specificity, of *Xf* provides an opportunity to study the molecular mechanisms underlying the essential traits that lead to host specificity observed for this pathogen. There are likely numerous bacterial traits that contribute to host specificity of *Xf*, and we hypothesize that O-antigen plays a role. Most importantly, we have demonstrated that O-antigen in an important factor in PD development and host colonization and because of its abundance in the outer membrane, LPS may provide a target for disease control.

LAYPERSON SUMMARY

Xylella fastidiosa (*Xf*) is a bacterium capable of colonizing many different plant hosts. It is the causal agent of Pierce's Disease of grapevine (PD), which has caused major losses for the California grape industry. *Xf* also causes disease in other economically important crops, such as almond, citrus, and oleander. While all identified *Xf* isolates belong to the same species, some isolates can cause disease in one host, but not the other. For example, oleander isolates do not incite symptom development in grapevine and vice versa. One major goal of this project is to understand the bacterial characteristics that dictate host specificity. This research is particularly focused on elucidating the role of the *Xf* cell surface component, lipopolysaccharide (LPS), in the pathogenic interaction between the grapevine, almond, and oleander hosts. Because LPS plays an important role in several host-pathogen interactions, we are investigating the contribution of LPS in allowing *Xf* to colonize its host. LPS imparts traits that may contribute to pathogenesis, such as the ability to attach to host cell walls. Thus far, certain components of the LPS molecule have proven to be essential for the *Xf* interaction with its host, which together with its abundance on the bacterium's cell surface makes it a logical target for disease control. Antimicrobial compounds that disrupt or retard LPS biosynthesis exist which make bacteria more susceptible to other stresses. Potentially, these compounds could be used in combination with other anti-*Xf* compounds to control disease.

STATUS OF FUNDS

There will be approximately $17,400 remaining in this fund for year one of this two year project as of June 30, 2012 ($16,700 in salary and $700 in travel). There is a portion of the salary funds remaining because postdoctoral associate, Dr. Jennifer Clifford, who was the primary personnel on this project, recently left the Roper laboratory (December 2011) for a position at the USDA, Horticultural Crops Research Unit at Oregon State University. Therefore, a portion of the funds allocated for her salary remained in the fund beginning January 2011. I have reassigned the personnel in my laboratory to maintain certain aspects of the project in Dr. Clifford's absence. This includes Ph.D. student, Ms. Lindsey Burbank, who is conducting the *in vitro* assays described in Objectives 2 and 4. Therefore, a portion of her salary has fallen under the umbrella of this grant fund and I would like to request that I be granted an extension on year 1 of this fund to continue paying Ms. Burbank for the summer of 2012 (approximately $6,000). She will continue working on this project at that time as well as train the new Ph.D. student described below.

In the meantime, I have identified a talented young graduate student, Ms. Jeannette Rapicavoli, to work on this project as the main focus of her Ph.D. dissertation. She joined my laboratory in Winter quarter (January) of 2012. Ms. Rapicavoli's stipend and fees are currently being covered by a Dean's fellowship awarded to her by UCR but I will need to begin covering her summer stipend as of June 2012 and stipend+fees as of September 2012. I would like to request an extension on the remaining salary

funds associate with year one of this project to cover Ms. Rapicavoli's summer stipend (approximately $6,000) and a portion of her stipend+fees for Fall quarter 2012 (approximately $4,700).

I would also like to request an extension of the remaining travel money ($700) to travel to the Annual Meeting of the Annual Phytopathological Society in August 2012 in Providence, RI. I have been invited to give a talk on the data we have collected for this project and will need to cover airfare and lodging.

INTELLECTUAL PROPERTY

Thus far, there is no intellectual property associated with this project.

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