THE *XYLELLA FASTIDIOSA* CELL SURFACE

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ABSTRACT
The Gram-negative bacterium *Xylella fastidiosa* is the causative agent of Pierce's disease of grapevines. Many of the strategies proposed to control the spread of this bacterium are dependent on the ability of a particular compound to get into the cell by crossing the outer membrane. The goal of our project is to identify the outer membrane proteins of *X. fastidiosa* and to examine how different physiological and environmental signals influence the protein composition of the outer membrane. Our strategy has been to isolate the outer membrane fraction and to analyze the proteins in this fraction using one- and two-dimensional gel electrophoresis. Comparisons of the outer membrane protein profiles for cells grown under different environmental and physiological conditions will increase our understanding of the *X. fastidiosa* cell surface as well as provide important information concerning the role of the outer membrane proteins of *X. fastidiosa* in the development of Pierce's disease.

INTRODUCTION
The causative agent of Pierce's disease is the Gram-negative bacterium *Xylella fastidiosa,* which is a member of the gamma subgroup of the Proteobacteria and is phylogenically related to the *Xanthomonads* (for a review, see Hopkins and Purcell, 2002). *X. fastidiosa* is highly specialized and is capable of multiplying in both the foregut of xylem-feeding insects, such as the glassy-winged sharpshooter and in the xylem system of the host plant. The ability of *X. fastidiosa* to thrive in both the insect foregut and the xylem suggests that the bacterium has evolved regulatory mechanisms that help it to cope with the unique stresses experienced in these two very different ecological niches.

A common response of Gram-negative bacteria to any stress is to change the composition of their cell surface, particularly the protein composition of their outer membrane. The outer membrane is the outermost continuous structure on the bacterial cell surface and serves as a selective barrier between the cell and the external environment. Changes in the protein composition of the outer membrane are known to have a profound effect on the sensitivity of Gram-negative bacteria to detergents, antibiotics, and bacteriophages. Therefore, in order to develop effective methods for controlling the spread of *X. fastidiosa*, it is important to obtain information concerning the protein composition of the *X. fastidiosa* outer membrane and how the composition of this membrane changes in response to environmental signals.

OBJECTIVES
The overall goal of this proposal is to analyze the outer membrane proteome of *X. fastidiosa* and to determine how the proteome profile changes in response to various physiological and environmental conditions. This project will focus on the following two objectives:

1. Identify the major outer membrane proteins of *X. fastidiosa* and assign them to a specific gene on the *X.fastidiosa* chromosome.
2. Determine how the protein composition of the *X. fastidiosa* outer membrane is influenced by environmental signals and signals from the infected grapevine.

RESULTS
Identifying the major outer membrane proteins of *X. fastidiosa*.
Our strategy for analyzing the *X. fastidiosa* outer membrane is similar to the strategy that was successfully used to analyze the outer membrane proteome of *Caulobacter crescentus* (Phadke et al. 2001). For this analysis, we used two strains: the strain Temecula 1 provided by B. Kirkpatrick and the strain Stags Leap provided by A. Walker. Both strains were still virulent based on assays performed in these laboratories. We grew the two strains in 1.0 liter of PD3 media for 7-10 days at 28^{o}C. The cells were harvested and broken using a French pressure cell. The outer membrane fractions were isolated by sucrose density gradient centrifugation and then the proteins in this fraction were analyzed using one- and two-dimensional gel electrophoresis.

In our initial experiments, the outer membrane fractions were analyzed using 1-D SDS-polyacrylamide gels. These gels allowed us to quantitate the amount of the different proteins in the outer membrane for the two strains and to predict the sizes of the proteins based on their migration in the gels. These experiments revealed that there were between 8-10 major proteins and 16-18 minor proteins in the *X. fastidiosa* outer membrane. The sizes of these proteins range from 130 kD to 18 kD. (Proteins smaller than 18 kD would not have been detected in this series of experiments.) Our comparison of the outer membrane profiles of the Temecula and Stags Leap strains indicated that the protein composition of their outer membranes is very similar. We did observe slight differences in the amounts of some of the minor outer membrane proteins for the two strains. This may reflect slight variations in how these two strains respond to environmental conditions. However, a more detailed analysis is needed before we can draw any conclusions about these differences.

Although our initial analysis of the outer membrane fractions using 1-D gels provided valuable information about the *X. fastidiosa* outer membrane, it was not possible to completely separate all of the proteins using this method. Recently, we have begun to analyze our outer membrane fractions using 2-D gel electrophoresis. This technique separates proteins based on their isoelectric points (p*I*) and their apparent molecular weights. In our first series of experiments, we identified over 40 well-separated spots. We are currently in the process of analyzing our 2-D gels using Phoretix proteome analysis software. This software will allow us to make a tentative assignment of molecular weights and isoelectric points to the predominant proteins. The 2-D gels will also allow us to determine the relative abundance of each of the outer membrane proteins. Finally, these gels will provide us with an outer membrane protein map for *X. fastidiosa* Temecula 1 that can be compared to the published whole-cell protein map for *X. fastidiosa* CVC (Smolka et al. 2003).

Assigning the outer membrane proteins to specific genes on the X .fastidiosa chromosome

Our analysis of the proteins present in the outer membrane fraction has provided us with important information concerning the molecular weights and isoelectric points of these proteins. The next step has been to assign these proteins to specific genes on the *X. fastidiosa* chromosome. This step has been greatly facilitated by the recent sequencing and annotation of the *X. fastidiosa* Temecula 1 genome (Van Sluys et al., 2003) and by the availability of the published whole-cell protein map for *X. fastidiosa* CVC (Smolka et al., 2003). The use of these resources has allowed us to tentatively assign many of our outer membrane proteins to specific genes on the *X. fastidiosa* Temecula 1 chromosome.

To confirm the identification of some of the ambiguous spots, we are using mass spectrometry. Using this technique, we have successfully assigned one of our outer membrane proteins to a specific gene on the *X. fastidiosa* chromosome. For this analysis, we ran the outer membrane fraction on a preparative SDS-polyacrylamide gel and excised one of the distinct bands from the gel. The protein in this band was then subjected to trypsin digestion and the resulting fragments were analyzed by MALDI-TOF-MS at the UC Davis Molecular Structure Facility. Based on this analysis, we were able to assign this protein to a specific gene on the *X. fastidiosa* chromosome. Given our success with this protein, it should be possible for us to begin assigning other proteins to specific genes.

Determining how the protein composition of the X. fastidiosa outer membrane is influenced by different environmental and physiological signals

We have also begun to look at the outer membrane profiles of *X. fastidiosa* grown under different environmental and physiological conditions in the laboratory. To date, we have used 1-D gels to compare the outer membrane profiles when the strains were grown: (1) in rich versus defined medium, (2) in liquid medium versus on plates, (3) to exponential phase versus stationary phase. Our results indicate that the abundance of some outer membrane proteins does not change. This is particularly true of proteins that are predicted to have a structural role in maintaining cell surface integrity. However, the abundance of other proteins is more sensitive to changes in the growth conditions. It is highly likely that some of these changes will have profound effect on cell permeability and on the sensitivity of *X. fastidiosa* to detergents, antibiotics, and bacteriophages.

CONCLUSIONS

Proteins on the bacterial cell surface play an important role in the ability of pathogenic bacteria to induce the disease state. During the past year, we have focused on developing methods for studying the protein composition of the *X.fastidiosa* outer membrane and have begun to examine how different physiological and environmental signals affect the relative abundance of these proteins. We are now in the position to examine how changes in the abundance of specific proteins correlate to changes in virulence. This information should provide insights into the role of the outer membrane proteins in *X. fastidiosa* virulence and identify potential new targets that may help in the development of effective strategies for controlling the spread of Pierce's disease.

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