THE *XYLELLA FASTIDIOSA* CELL SURFACE

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ABSTRACT

A common response of Gram-negative bacteria to environmental stress is to change the composition of their cell surface, particularly the protein composition of their outer membrane. These changes are known to have a profound effect on the sensitivity of Gram-negative bacteria to detergents, antibiotics, and bacteriophages. The goal of this project is to determine how environmental changes influence the protein composition of the *Xylella fastidiosa* (*Xf*) outer membrane. Our strategy has been to isolate the outer membrane fraction from *Xf* cells grown under different environmental conditions. The proteins in this fraction are then separated by one- or two-dimensional gel electrophoresis and their identity established by peptide mass fingerprinting. In this report, I have focused on experiments that examine the *Xf* outer membrane protein profile using one-dimensional gel electrophoresis. This analysis has allowed us to assign three outer membrane proteins to specific genes on the *Xf* chromosome. These gels have also allowed us to examine how the composition of the *Xf* outer membrane changes in response to environmental signals and the physiological state of the bacterial cell.

INTRODUCTION

Pierce's disease (PD) is a devastating disease of grapevines that is caused by the Gram-negative, endophytic bacterium *Xylella fastidiosa* (*Xf*). Although the specific details of the disease process are not fully understood, an important feature is the ability of this pathogen to colonize the xylem tissue of plants and the foregut of insect vectors (for a recent review, see 5). As with most pathogenic bacteria, successful colonization is dependent on the ability of planktonic *Xf* cells to adhere to the host cell surface and to form a microcolony (3, 4, 7). This surface-associated growth commonly leads to the formation of a biofilm. Biofilm-associated *Xf* bacteria constitute a major component of the bacterial biomass in the host tissue. In contrast, planktonic bacteria are less prevalent and are seen primarily as a mechanism for the bacteria to translocate from one surface to another.

The transition of bacteria from the planktonic to the biofilm-associated state involves profound physiological changes (3). The most obvious change is the production of an exopolysaccharide matrix, one of the distinguishing characteristics of a bacterial biofilm. However, the matrix-enclosed mode of bacterial growth requires many other changes, including changes in the protein composition of the bacterial cell envelope. In Gram-negative bacteria, these changes include differences in both the relative abundance of some major outer membrane proteins and the appearance or disappearance of specific high-affinity receptor proteins. This differential expression allows the bacteria to cope with the new environmental condition and with alterations in the nutrient supply.

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 (8). As a result, strategies designed to attack planktonic cells are usually not effective against biofilm-associated cells (3). Therefore, in order to develop effective methods for controlling the spread of *Xf*, it is important to obtain information concerning the protein composition of the *Xf* outer membrane and how the composition of this membrane changes in response to environmental signals and the physiological state of the bacterial cell.

OBJECTIVES

The goal of this project is to analyze the outer membrane proteome of *Xf* and to determine how the outer membrane protein profile changes in response to various physiological and environmental conditions. Our experiments are designed to address two objectives:

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

RESULTS

The primary focus of our research during this reporting period has been to analyze the outer membrane proteome of *Xf* and to assign the outer membrane proteins to specific genes on the *Xf* chromosome. In last year's Symposium Proceedings (6), we described our protocol for analyzing the protein profile of the *Xf* outer membrane. This protocol involves rupturing the *Xf* cells with a French pressure cell and isolating the outer membrane fractions by sucrose density gradient centrifugation. The proteins in this fraction are then analyzed using SDS-polyacrylamide (PAGE) gel electrophoresis. These gels have allowed us to quantitate the amount of the different proteins in the *Xf* outer membrane and to predict the sizes of the proteins based on their migration in the gels. Figure 1 shows a series of SDS-polyacrylamide gels, which reveal the outer membrane profile of *Xylella fastidiosa* strain Temecula 1. These Coomassie-stained gels indicate that there are at least 14-16 major proteins in the *Xf* outer membrane. The sizes of the outer membrane proteins range from 130K to 18K. (Proteins smaller than 18K would not have been detected in this series of experiments.).

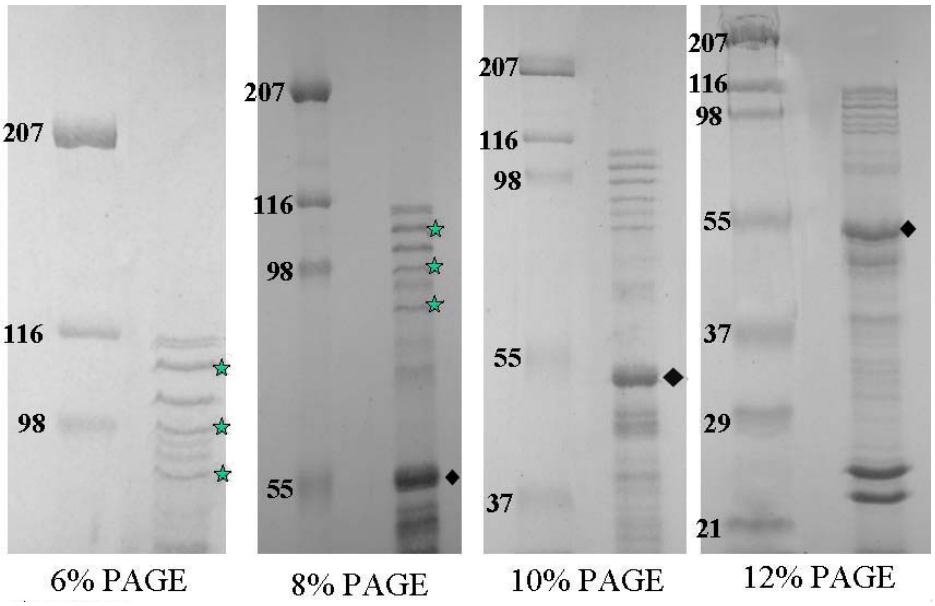

Figure 1: The outer membrane profile of *Xylella fastidiosa* strain Temecula 1. Proteins in these gels were identified using Coomassie blue stain. The numbers indicate the size of molecular weight standards and their migration on the different percentage gels (left lane). On each gel, the outer membrane proteins from *Xf* Temecula 1 are present in the right lane. The diamonds indicate the location of the MopB protein on the different percentage gels. The stars indicate the locations of the three excised bands, which contained a unique protein based on the MALDI-TOF spectra.

The most abundant outer membrane protein is the MopB protein, which has been characterized by George Bruening and his colleagues (2). Using their purified MopB protein, we have been able to determine the location of the MopB protein relative to other proteins in our outer membrane profiles. (MopB is indicated by the diamonds in Figure 1). The next step in our analysis was to assign additional proteins to specific genes on the *Xf* genome. For these experiments, we separated the proteins in the outer membrane fractions on preparative SDS-PAGE gels and excised five distinct bands from the gels. The proteins in each band were then subjected to trypsin digestion and the resulting fragments were analyzed by MALDI-TOF-MS at the UC Davis Molecular Structure Facility. The resulting information was analyzed using MS-Fit at Protein Prospector (UCSF; http://prospector.ucsf.edu). Analysis of the bands at ~114K and ~104K indicated that more than one protein was present in the excised gel fragment. In contrast, the other three bands contained unique proteins. This allowed us to assign these three outer membrane proteins to specific genes on the *Xf* chromosome (10). The locations of the bands containing these proteins are indicated by the stars in Figure 1.

The largest of the three proteins is ~108K and corresponds to PD1283. PD1283 is predicted to encode a 958 amino acid protein and has been classified as a TonB-dependent receptor protein. The second protein is ~98K and corresponds to PD0326. PD0326 is predicted to encode a 784 amino acid protein and shows homology to the outer membrane protein/protective antigen OMA87. Based on this homology, PD0326 is also called the *oma* gene in some databases. The third protein is ~90K and corresponds to PD0528. Interestingly, this gene is classified in many databases as an inner membrane. However, our analysis of this protein using relatively new computer algorithms suggests that PD0528 encodes a beta barrel outer membrane protein (1). This assignment is more consistent with our fractionation results, which indicate that the PD0528 protein is a major component of our *Xf* outer membrane fraction.

Our analysis of the outer membrane fractions using one-dimensional (1-D) gels illustrates the validity and power of our approach for assigning outer membrane proteins to specific genes on the *Xf* chromosome. However, it was not possible to completely separate all of the outer membrane proteins using 1-D gels. To overcome this problem, we are analyzing our

outer membrane fractions using two-dimensional (2-D) gel electrophoresis with the assistance of our cooperator Linda Bisson and a graduate student in her laboratory, Paula Mara. This technique separates proteins based on their isoelectric points (p*I*) and their apparent molecular weights. In our initial experiments, we identified over 40 well-separated spots and have analyzed these gels using Phoretix proteome analysis software. This software has allowed us to make a tentative assignment of molecular weights and isoelectric points to many of the predominant proteins. To confirm the identification of some of the ambiguous spots, we plan to cut out these spots and identify the proteins using MALDI-TOF-MS as described above. Although we are still working out some technical details, using 2-D gels will allow us to determine the relative abundance of each of the outer membrane proteins under different environmental conditions (the focus of Objective 2). These gels will also provide us with a proteome map for *Xf* Temecula 1 outer membrane, which we can then compare to the published whole-cell protein map for *Xf* CVC (9).

CONCLUSIONS

Proteins on the bacterial cell surface play an important role in the ability of pathogenic bacteria, such as *Xf*, to induce the disease state. During the past year, we have used one-dimensional gel electrophoresis to examine the *Xf* outer membrane profile and have assigned three proteins to specific genes on the *Xf* chromosome. We have also been developing a protocol for analyzing the *Xf* outer membrane proteome using two-dimensional gels. Once these technical details have been worked out, we will be in the position to examine how different physiological and environmental signals affect the relative abundance of specific *Xf* outer membrane proteins. This information should provide valuable insights into the role of the outer membrane proteins in *Xf* virulence and identify potential new targets that may help in the development of effective strategies for controlling the spread of PD.

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