PARATRANSGENESIS TO CONTROL PIERCE'S DISEASE: ISOLATION AND ANALYSIS OF ANTI-*XYLELLA* SINGLE CHAIN ANTIBODIES

Project Leader:
David Lampe
Department of Biological Sciences
Duquesne University
Pittsburgh, PA 19282

Project Director:
Thomas A. Miller
Department of Entomology
University of California
Riverside, 92521

Collaborators:
Carol Lauzon
Dept. of Biological Sciences
California State University, Hayward
Hayward, CA 94542

Blake Bextine
Dept. of Entomology
University of California
Riverside, 92521

Don Cooksey
Dept. of Plant Pathology
University of California
Riverside, 92521

Frank Richards
Yale Medical School
New Haven, CT 06520

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ABSTRACT
We have used a two methods to attempt to isolate single-chain antibodies (scFv) specific for the surface of the Temecula strain of *Xylella fastidiosa*. A method using whole *X. fastidosa* cells to pan a phage display scFv library was successful and we recovered one very specific scFv that strongly interacts with Temecula and less so with the Tulare strain of *X. fastidiosa*. A method using a purified surface protein (mopB) from Temecula yielded two scFvs that interacted with that protein, but failed to interact with intact cells. Intended uses for these scFvs in a paratransgenesis method to control Pierce's disease will be discussed.

INTRODUCTION
The glassy-winged sharpshooter (GWSS) is the principle vector of the xylem-limited bacterium *Xylella fastidiosa* (*Xf*), which causes Pierce's disease (PD) in grapes. Limiting the spread of this pathogen by rendering GWSS incapable of pathogen transmission (paratransgenesis) is a promising method of pathogen control. Paratransgenesis seeks to modify the phenotype of an organism indirectly, by modifying its symbiotic bacteria to confer vector-incompetence

Paratransgenic approaches to disrupt pathogen infection of humans are being developed by several groups. These include interference with the ability of triatomid bugs to transmit pathogens causing Chagas' disease (Beard, Dotson et al. 2001), interference with HIV attachment to its target cells in the reproductive tracts of humans (Chang, Chang et al. 2003), and the elimination of persistent *Candida* infections from biofilms in chronically infected patients (Beninati, Oggioni et al. 2000). Paratransgenesis has also been applied to deliver cytokines mammalian guts to relieve colitis (Steidler, Hans et al. 2000; Steidler 2001). Thus, the method has wide applicability.

Alcaligenes xylosoxidans denitrificans (Axd) is bacterial species that can colonize the GWSS foregut and cibarium, as well as various plant tissues, including xylem. It is non-pathogenic in insects and plants and is not known to be a pathogen in healthy humans. Given these characteristics, *Axd* has become the focus of our paratransgenesis efforts to control PD in grapes. Over the past two years we developed the technology to stably modify *Axd* by inserting genes into its chromosome. We have recently turned our attention to isolating factors that can specifically inhibit the transmission of *Xf* from GWSS.

We report here the isolation of a single chain antibody phage that is specific for the surface of *Xf* and appears to have some strain specificity. Other tests have shown that this antibody phage can inhibit the transmission of PD by sharpshooters under experimental conditions.

OBJECTIVES
1. Isolate single chain antibody (scFv) clones capable of binding to the surface of *Xylella fastidiosa* with high specificity either though the use of intact cells or purified surface proteins.
2. Test the strain specificity of these scFvs.

RESULTS
A. Single-chain antibodies (scFvs) as specific control agents of Xylella fastidiosa
We have spent the past year designing and implementing a strategy to isolate factors that are specifically targeted at *Xf* for use in a paratransgenesis approach to controlling PD. The greatest degree of specificity against PD is likely to be obtained using synthetic antibody constructs called single-chain antibodies, or scFvs. An scFv is composed of a single gene consisting of the variable regions of an antibody heavy chain and light chain fused together with a synthetic linker sequence. Genes for these scFvs are randomized at certain codons and are constructed in large libraries that can be "panned" with an antigen of interest to obtain specific binders.

B. Use of whole X. fastidiosa pv. Temecula to pan scFv libraries

Our target is the surface of *Xf* pv Temecula, therefore it made sense to use whole *Xf* to pan scFv libraries. We panned a commercially-available scFv phage library and obtained one very specific scFv that we call S1. This scFv does not interact with *Axd* or *Chryseomonas luteola*, two bacterial species that live in the gut of the GWSS and bacterial species that might be utilized in paratransgenesis. Interestingly, this particular scFv lacks a heavy chain region, thus the specificity is mediated only by the light chain. We do not yet know what structure or protein is bound by S1. S1 has been tested by Blake Bextine in GWSSs for its effect on disrupting the transmission of *Xf* to plants.

C. Use of genomic data and purified surface proteins

The S1 phage scFv was the only specific binder we obtained by screening whole cells, yet we would like to isolate multiple different scFvs in order to target different structures. This is possible by taking advantage of the available genomic and proteomic data for *Xf* to target specific surface proteins. The genomes of both the citrus variegated chlorosis (CVC) and Temecula (PD) strain of *Xf* have been sequenced (Simpson, Reinach et al. 2000; Bhattacharyya, Stilwagen et al. 2002). In addition, proteomic data is available for the CVC strain that documents which proteins are abundant and surface-exposed (Smolka, Martins et al. 2003). We targeted 5 surface exposed proteins (PilT, Type IV fimbriae, PilY1, hsf adhesin, and mopB) and used the Temecula genome sequence to identify homologues of them compared to the CVC strain.

We attempted to clone full-length genes for each target in *E. coli* fused to a maltose binding protein gene in order to aid in affinity purification. Only one of these constructs could be cloned (encoding mopB), probably because expression of the remainder of these are toxic in *E. coli*. A screen of the scFv library with purified Temecula mopB protein led to the isolation of two specific scFv clones. Neither of these, however, was able to bind to the surface of whole *Xf* pv Temecula cells in an ELISA, perhaps because the antigen that each scFv bound to was not exposed in whole cells. This remains a viable approach, but steps will be needed to express only parts of surface proteins that are actually surface-exposed in whole cells.

D. Strain specificity of scFvs.

We tested the S1 phage for its degree of strain-specificity. Below is a table based on a comparative ELISA using S1 and different *Xf* strains. Interestingly, some strain specificity appears to be present, which may reflect the known diversity of some *Xf* surface proteins (Bhattacharyya, Stilwagen et al. 2002).

CONCLUSIONS

We have isolated a scFv that has high specificity for the surface of the Temecula strain of *Xf*. This scFv is capable of interfering with the transmission of PD from GWSS to naïve host plants (pers. comm. B. Bextine). We are now working to move this scFv into candidate bacterial species for use in a paratransgenesis approach to controlling PD.

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