

ISOLATION OF BACTERIOPHAGES SPECIFIC FOR *XYLELLA FASTIDIOSA*

Project Leader:

Michele M. Igo
Section of Microbiology
Division of Biological Sciences and
California Agricultural Experimental Station
University of California
Davis, CA 95616

Cooperators:

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

Bruce Kirkpatrick
Dept. of Plant Pathology
University of California
Davis, CA 95616

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ABSTRACT

This report gives an overview of the project. The goal of this project is to isolate a collection of viruses (phages) that can infect and replicate in *X. fastidiosa* (*Xf*). This collection will then be screened to identify phage exhibiting useful biological properties.

INTRODUCTION

The causative agent of Pierce's disease (PD) is the Gram-negative bacterium *Xylella fastidiosa* (*Xf*). *Xf* 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 (for recent reviews, see 4, 6, 7). The complex nature of the bacterial-host interactions that take place during the PD infectious cycle and the fastidious growth properties of *Xf* in the laboratory present a formidable challenge to researchers working with this bacterium. At present, there are only a few methods available to perform such basic operations as genetic exchange, mutant isolation, strain construction, and complementation. Further complications of working with *Xf* arise because of its slow generation time, its tendency to form aggregates, and its poor plating efficiency. Finally, few methods are available for disrupting the interaction between *Xf* and its hosts, which is a key component of the PD infectious cycle. As a result, there are currently no effective treatments to cure infected vines.

In other Gram-negative bacteria, bacteriophages, phage derivatives and phage components have played a major role in overcoming these issues (1, 3, 8). For example, phages have been used to move genetic markers between strains, for complementation, and as cloning vectors. In addition, phages have been used as diagnostic reagents to detect pathogenic bacteria, and as therapeutic agents in bacterial infections. Unfortunately, since not all phages possess exploitable properties, it is usually necessary to isolate a collection of phages that infect the bacteria of interest and then to screen the individual phages for desirable properties.

Based on studies of environmental samples, it has been estimated that there are $>10^{30}$ tailed phages in the biosphere and that phage typically outnumber bacterial cells 10 to 1 (2). These studies also revealed that phages could be found anywhere that their bacterial hosts are present. This observation has already proven true for *Xf*. Carol Lauzon and her colleagues have reported the presence of two phages associated with *Xf* from infected grapevines (5). The goal of this project is to isolate a collection of phages that are capable of infecting and replicating in *Xf* (Aim 1). These phages will then be screened individually to identify specific phages that have the potential to be used as genetic tools and for killing *Xf en planta* (Aim 2). Phages capable of moving genetic markers between *Xf* strains would give researchers in the field a powerful tool for investigating the properties of this unusual bacterium and establishing which parts of its genetic material make it such a deadly pathogen for certain varieties of grapes. Furthermore, phage or mixtures of phages capable of killing *Xf* would provide the tools necessary to determine the feasibility of using phage therapy to control the spread of PD.

OBJECTIVES

The primary goal of this project is to isolate a collection of phages as pure stocks and to screen this collection for phages that exhibit useful biological properties for studying and controlling the growth of *Xf*.

Specific Aim 1: Generate a collection of pure phage stocks that infect *Xf*.

1A) Collect environmental samples that potentially contain *Xf* specific phages.

1B) Isolate and obtain pure stocks of phages from the samples.

Specific Aim 2: Identify specific phage with potentially useful properties within our collection.

2A) Screen the collection to identify virulent phages.

2B) Screen the collection to identify generalizing transducing phages.

RESULTS AND CONCLUSIONS

The first goal of this project is to generate a collection of *Xf*-specific phages that exhibit different biological properties. To increase our chances of obtaining a diverse set of phages, we have collected samples from PD-infected grapevines growing in different vineyards in Northern California. Using infected grapevines as a source seems particularly promising based on the work of Dr. Lauzon and her colleagues (5). Our strategy has been to collect sap from infected vines and samples from the tissue of symptomatic plants. We have also collected soil samples from around infected grapevines to determine if the soil is a good source of *Xf*-specific phage. The next step in our analysis will be to determine if any of these samples contain phage that can infect *Xf*. As a starting point, we will use previously published protocols that have successfully been used to isolate phages from environmental samples for other Gram-negative bacteria.

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