

**Title: LIBRARY CONSTRUCTION AND SEQUENCING OF NOVEL BACTERIOPHAGE**

**Performance Sites: LBRN/INBRE Molecular and Cellular Biology Core (MCBC)  
LSU School of Veterinary Medicine**

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### **Abstract**

*Agrobacterium tumefaciens* is a gram-negative, rod-shaped pathogenic bacterium that induces crown galls in a wide range of dicotyledonous plants. *A. tumefaciens* possesses a Ti plasmid that can transfer DNA from the plasmid into plant cells causing an up-regulation of hormones responsible for growth and cell division and resultant crown gall formation. Bacteriophages that infect *A. tumefaciens* have been isolated in our laboratory and preliminary analyses of their plaque morphology, host range specificity, homo/heteroimmunity, and DNA restriction enzyme digestion patterns have been completed. This proposal seeks funds to support the genomic library construction and sequencing of a set of these isolates utilizing the molecular core facilities available through the LSU School of Veterinary Medicine. The resultant genomic data will be used to screen for the efficacy of employing one of these phage isolates as a novel bio-control strategy for *Agrobacterium* infections in economically important plants.

## Research Plan

### Introduction

*Agrobacterium tumefaciens* is a native soil microorganism that has the ability to infect plant tissue. While not all plants are susceptible to *Agrobacterium* infection, the production of galls within agronomically important plants, such as grapes and stone fruit is known to reduce yield. In recent years, members of the agricultural community have begun to look for alternatives to pesticide or herbicide application to control such infections. As such, many alternative therapies, including phage therapy have emerged as possible prescriptions for bacterial diseases, both in humans and in plants. In their lytic cycle, bacteriophage infection results in new virion construction and host cell lysis. As such, lytic phages can be considered ideal candidates for use in active phage therapy as these phages represent natural predators of their bacterial hosts.

### Specific Aims and Methods of the Proposed Project

Our laboratory has gathered preliminary data concerning the physical characterization of five novel *Agrobacterium* phages (currently designated as JP1 – JP5). The objective of our initial work was to prepare high quality DNA for eventual genome sequencing and to test the bio-control merit of selected isolates. As an initial step, we wish to sequence JP1 and JP5, a lytic and a temperate phage, respectively. All the phages isolated and characterized thus far come from a single organic soil sample that was enriched for *Agrobacterium* phage growth. JP1 consistently displayed lytic plaque morphology (Figure 1). Three of the five phages displayed consistent lysogenic (turbid) plaques. All five phages are of the siphoviridae morphotype with long, non-contractile tails (Figure 2). The host range of all five phages have been determined using *A. tumefaciens* c58, heat cured (non-infective C58) A136, *A. tumefaciens* TT134, and B6 (the host of the previously described PB2 phage), and two strains of *A. rhizogenes* 100 and 117. All JP phages are specific to *A. tumefaciens* c58. We also created a lysogen culture of *A. tumefaciens*-JP1 and tested relative immunity with the other JP phages. All were homoimmune to (not able to infect) the JP1 lysogen, indicating a close genetic relationship between all of the isolates.



**Figure 1: JP1 Plaque morphology at a  $10^{-3}$  dilution.**

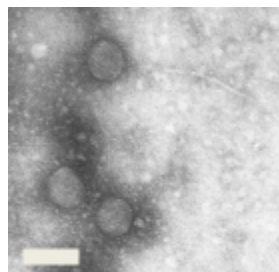


**Figure 2: JP1 phage structure through transmission electron microscopy. Scale bar = 100 nm.**

*Agrobacterium* phages isolated by others have been characterized through similar physical analyses, but few have been subjected to genetic characterization. One phage, PB2, was isolated in 1982 and its DNA subjected to restriction enzyme digestion to map the cleavage points of the genome (Sollitti and Chesney, 1987). As assurance that JP1 – JP5 were not relatives of PB2, we subjected the DNA of our phage isolates to the same enzyme panel used previously on PB2. None of our phages produced the same restriction digest pattern as PB2. Clearly, more detailed knowledge of these genomes is important for future therapeutic uses as well as, understanding phage genome organization within organisms that have well defined and pathogenically important plasmid systems.

Since August 2008, the Biology Department at the University of Louisiana at Monroe has been involved in a classroom initiative associated with Howard Hughes Medical Institute in which

numerous bacteriophage of *Mycobacterium smegmatis* have been isolated and genetically characterized. To date, 2532 phages have been isolated and characterized, while 374 have been sequenced and 223 of those genomes have been annotated and submitted to GenBank. Therefore, the pattern and layout of the *Mycobacterium* phage genome is well understood and can be used for comparison to *Agrobacterium* phage genome organization. As a member of the Phage Hunters Program, ULM has access to all experimental wet lab protocols (phage isolation, purification, DNA restriction digest analysis) and multiple bioinformatics tools required for the analysis and annotation of sequenced genomes.

As next generation DNA sequencing technology becomes more widely available, the ability of individual research laboratories to perform their own library construction becomes more necessary. The main objective of this proposal is to gain proficiency in the requisite DNA library preparation techniques required for Ion Torrent sequencing. Although we will specifically focus on the *Agrobacterium* phage isolates during this period of residence at LSU, our intention is to extend our library construction efforts to bacteriophage DNA samples from other host systems. For example, over the past five years, the ULM Phage Hunters Program has isolated ~100 phages from *Mycobacterium smegmatis*, but fewer than 10 of them have been sequenced to date. Since several of the *M. smegmatis*-specific phages have been shown to be capable of infecting pathogenic species of the *Mycobacterium* genus, specifically *M. tuberculosis*, sequencing and comparative genomic study of additional isolates is necessary to identify potential candidates for therapeutic delivery agents.

#### **Timeline**

- May, 2013 – Run QC gels of phage isolates to ensure good quality/quantity of DNA preparations. Additional DNA isolation from high-titer lysates will be performed if necessary.
- June, 2013- Library construction and Ion Torrent Sequencing at LSU
- July, 2013
- July, 2013 - Begin bioinformatics analyses of sequenced phage genomes.  
Extend library construction efforts to other phage samples of interest.