Structural Determination of Bacteriophage P22 Genome Ejection with Cryo-Electron Microscopy and UCSF ChimeraX

Antibiotic-resistant infections are becoming increasingly common in bacterial infections of all types. These infections significantly increase treatment cost, hospital stay length and patient mortality and morbidity. With the increase in antibiotic resistance, there is a need to find alternative treatments for bacterial infections. A possible treatment is bacteriophages. Bacteriophages (phages) are a group of viruses that can infect and treat antibiotic-resistant bacteria. Bacteriophage therapy is a compassionate treatment for antibiotic-resistant bacteria, but more research is needed before it can become a commonplace treatment. Bacteriophage infection initiation is a poorly understood part of phage ecology. The simplified steps of infection initiation are landing, phages attach to the bacterial membrane, pinning, baseplate attaches to the bacterial membrane, needle penetration, phage needle punctures the bacterial membrane, and genome ejection. This study focuses on the genome ejection aspect of phage infection initiation. Bacteriophage P22 is a phage commonly used as a model for phage assembly and infection. In previous studies, phage P22 was found to construct an "trans-envelope channel" that is essential for successful genome ejection during infection initiation. The specific proteins that comprise and construct this channel are unknown. The purpose of this study is to determine the structure and proteins that comprise the trans-envelope channel. The hypothesis is that the protein gp7 forms the trans-envelope channel. The independent variable is the treatment of *Salmonella typhimurium* with bacteriophage P22. The dependent variable is the *Salmonella typhimurium* infection and subsequent genome ejection. The controls are the *Salmonella typhimurium* samples that are not treated with bacteriophages. This study will be in-vitro and first, bacteriophage P22 will be amplified and grown on *Salmonella typhimurium* petri dishes. The *Salmonella typhimurium* will be grown in LB broth and after amplification, the bacteriophages will be centrifuged to isolate. Separately, *Salmonella typhimurium* colonies will be grown in the presence of arabinose. Arabinose is a chemical used to induce minicell growth. Minicells are significantly smaller than normal cells and are necessary for cryo-electron microscopy, the imaging technology used in this study, since they reduce sample thickness. The minicells will be centrifuged twice to isolate then mixed with 10mnm colloidal gold. The minicell-gold mixture will be poured onto holey carbon grids then frozen with liquid ethane then cooled with liquid nitrogen. The samples will then be imaged under an cryo-electron tomography (cryo-et). The software programs of IMOD, MotionCor2 and UCSF ChimeraX were used to create higher resolution cryo-ET images and for data analysis. IMOD is a program that will be used to determine the contrast transfer function (CTF) and defocus to enhance image resolution. MotionCor2 will be used to correct image artifacts caused by electron-beam induced sample motion. USFC ChimeraX will be used to visualize and manipulate viral structures in 3-D to replicate phage genome ejection in animated videos and label structures in static images. All parts of the study except for UCSF Chimera data analysis will be run by the mentor. The UCSF ChimeraX data analysis will be performed by the student. This study will bring more

understanding to the understudied topic of bacteriophage infection initiation and provide more information vital for bacteriophage therapy to become a commonplace treatment.