

Isolation of Mycobacteriophage from Local Soil

Christina Kling

Faculty Advisor: Donna Rhoads-Frost

Department of Biology and Environmental Science

College of Arts and Sciences

Abstract:

The goal of this experiment was to isolate bacteriophage from local soil that were specific for *Mycobacterium smegmatis* (*M. smegmatis*), a common model organism for *Mycobacterium tuberculosis*, the bacterial pathogen that causes tuberculosis. Soil was collected near large bodies of water, such as the Mill River in Hamden and East Rock Park and the Branford Supply Ponds. Soil extracts were prepared using phage buffer and co-plated with *M. smegmatis* using brain-heart infusion top agar. Phages were identified on these plates based on the presence of replicable clear zones (plaques) on the bacterial lawn. Two probable phage were isolated from soil samples obtained near the Mill River in Hamden. The DNA of these phage can be sequenced to confirm that they are indeed mycobacteriophage, and then pure lysates of these phage can be used to study the toxins that are produced when *M. smeg* s 0.076 Tw 0.916 0 Td [(E)-4(.)-4(c)-3(o)-7(li)]TJ /TT1 1 Tf -0.009 Tc 0.009 Tw (,)Tj /TT2 1 Tf 0.006 T

multidrug resistant or extremely drug resistant tuberculosis and for these people, there is little that can be done.² Research into finding bacteriophage that can be used not just to diagnose tuberculosis, as is done now³, but to ultimately cure resistant drug-resistant tuberculosis infections

Results:

Two phages appear to have been isolated from soil collected near the Mill River in Hamden, CT. The two phages were isolated and purified based on plaque morphology. One phage produced pin-prick shaped plaques that were less than 100 micrometers in diameter.

Tc 0a9Oh586.446w 21.34.84 T8()JTJ -0.0064 Tc 0.3JTJ09 -1.3e (at)-i12(e)4(8)18(d)-tived6 T

the various toxins produced during the lysing process that lead to sepsis in animal phage treatment trials. If the identities of these toxins can be determined, it might be possible to find a way to mitigate their physiological effects to reopen the possibility of using phage to treat tuberculosis. In addition to looking at directly mitigating the effects of any toxins released upon lysing, various methods of delivering phage can be examined to determine if there is a way to administer phage at a rate in which the body can deal with the amount of bacteria being lysed.

Acknowledgements:

This project was funded by the Summer Undergraduate Research Fellowship at the University of New Haven. Thank you to the faculty in the Department of Biology and Environmental Science and the Department of Chemistry and Chemical Engineering for providing additional materials and guidance.

References:

1. Phage Therapy Center. Available from http://www.phagetherapycenter.com/pii/ParentServlet?command=static_home
2. Pommerville, J.C. Alcama's Fundamentals of Microbiology 9th edition, March 8, 2010. Joans and Bartlett Publishers
3. Mole RJ, Maskell T. *Phage as a diagnostic—the use of phage in TB diagnosis*. J Chem Technol Biotechnol 2001;76:683–8.
- 4.