

Bacteriophage Isolation for *Elizabethkingia meningoseptica*

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Abstract:

Elizabethkingia meningoseptica is an antibiotic resistant bacteria that causes meningitis. Very little is currently known about this bacteria. To date, no bacteriophages have been isolated that affect *E. meningoseptica*. We attempted to isolate phages for *E. meningoseptica* from several environmental samples. We filtered our environmental samples, and plated the environmental samples with *E. meningoseptica* to see if any phages were present in that environmental sample. Because no known phages have been isolated for *E. meningoseptica*, if we manage to isolate any phages, we would be the first lab to do so.

Keywords: Bacteriophage, Bacteria, Meningitis, Antibiotic Resistance

Introduction

E. meningoseptica causes meningitis infections in hospitals, especially in the neonatal intensive care unit, and the long term care unit (Amer et al 2010, Weaver et al 2009). Unfortunately, this particular strain of bacteria is resistant to most common antibiotics, including penicillin. Because of this bacteria's resistance to antibiotics, an infection caused by the *E. meningoseptica* has a high mortality rate, especially in patients with compromised immune systems (Hsu 2010). While common in hospitals, this bacteria has also been found in the gut of fish, and in mosquitos in Asia (Ngwa et al 2013).

Although *E. meningoseptica* is resistant against most common antibiotics, it may still be susceptible to some bacteriophages. Currently, there are no phages isolated that affect *E. meningoseptica*. A bacteriophage is a virus that primarily parasitizes bacteria. By discovering a bacteriophage that affects *E. meningoseptica*, we would be one step closer to discovering what affects this bacteria. By discovering what *E. meningoseptica* is susceptible to, researchers will be one step closer to discovering how to eliminate the bacteria, once it infects

patients. Perhaps researchers could even learn ways to prevent *E. meningoseptica* infections before they happen. We hypothesize that if we take environmental samples from areas rich in bacteria, we will be able to isolate bacteriophages that, hopefully, affect *E. meningoseptica*.

Methods

Our first step was collecting environmental samples. We used an empty water bottle to collect water from Theta pond, a Ziploc bag to collect samples from a compost heap, and Dr. Canaan went to the water treatment plant and obtained a container full of sewage water. We chose these three environmental samples because all three places are common places where bacteria is found. As mentioned before, we hoped to find bacteriophages in areas that commonly contain bacteria.

Once we had samples, we used a centrifuge to separate the solids in our samples from the liquids. For our purposes, we only need the liquid portion, so as to mix better in solution with *E. meningoseptica*, and to pass through our filters. Once we obtained our environmental samples, we

filtered our samples. From our environmental samples, we drew up 10 mL with a syringe. We then attached a 0.8 microliter filter onto the syringe, and filtered the 10 mL into a separate container. From the 0.8 filtered solution, we drew up 5 mL using a syringe, and attached a 0.45 microliter filter onto the bottom. We then filtered the 5 mL into yet another container. Using this system, we had an unfiltered solution, a 0.8 microliter solution, and a 0.45 microliter solution.

Once we filtered our environmental samples, we mixed together the filtered solution with other components, which we then poured onto bacteria plates. The mixture we poured onto the plates were 3 mL soft agar, 1 mL environmental sample, 100 microliters bacteria, and 30 microliter of CaCl₂. We chose this to serve as our control because it is relatively harmless, and serves as a good positive, and negative control. The CaCl₂ was used to help the bacteriophages “bind” to the bacteria. Once we created our mixture, we made the plates. We used distilled water as a negative control for phage presence. We made plates including the unfiltered solution, 0.8 solution, and 0.45 solution. The plates were incubated at about 57°C for 24 hours. At 24 hours, we were able to observe growth on the plates for *E. meningoseptica*. In addition, our growth medium for *E. meningoseptica* was nutrient based agar. We used this agar because after trial and error, we observed this agar works best for the growth of *E. meningoseptica*. At the end of the 24 hour incubation period, we removed the plates from the incubator and observed the plates. We were hoping to observe clearing in the cultures on the plates. Clearing indicates that some the bacteria colonies have died in that area.

If clearing is observed, we would perform a serial dilution, in which we take

draw up the area of clearing with a micropipette, and mix it with 10 mL of water. We mix and plate that, and add 10 more mL, and so on until we have 7 different dilutions. With a serial dilution, we

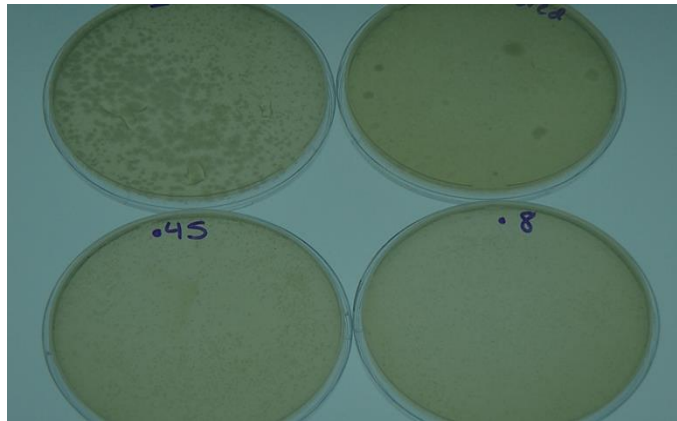


Figure 1 - This figure depicts the bacteria plates from the Theta pond environmental samples. There is no evidence of clearing.

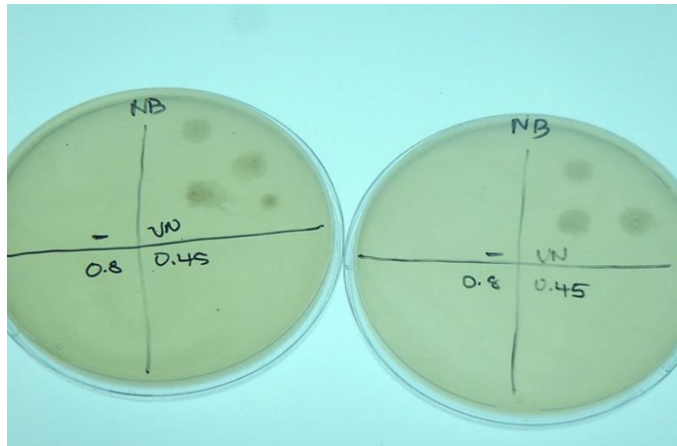


Figure 2 - This figure depicts the bacteria plates from the compost heap environmental samples. There is no evidence of clearing.

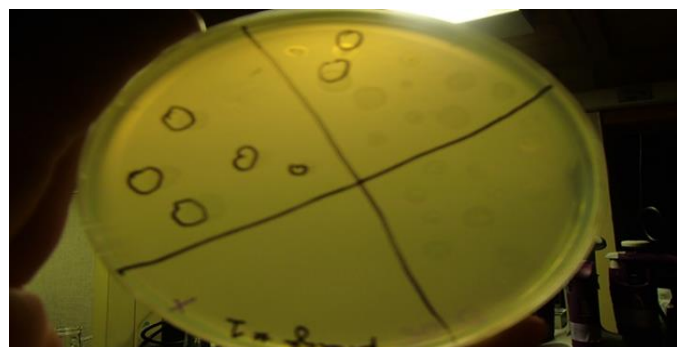


Figure 3 - This image depicts the first sewage trial with .45 microliter filter. Circles indicate clearing.

plate each of these solutions, and hope to observe clearing at each stage. This indicates that a phage is causing the clearing.



Figure 4 - This image is an up close photo of the .45 microliter filtered sewage water from our second trial. No clearing is evident.

Results

Figures 1 and 2 showed no evidence of clearing. Figure 3 showed some evidence of clearing, indicated by the black circles. However, when we attempted the serial dilution for the areas of clearing, there was no evidence of clearing. Since the only plate we saw any clearing on was the 0.45 microliter filtered solution of sewage water, we obtained a second sample of the sewage water, and replicated the methods, in hopes of isolating bacteriophage from the second sample of sewage water. Unfortunately, this too showed no evidence of clearing, as seen in Figure 4.

Discussion

Our results indicate no evidence of bacteriophage presence in any of our environmental samples that affect *E. meningoseptica*. While we did see some clearing on the 0.45 microliter plate from the first sewage sample, we were unable to isolate a bacteriophage from these areas of clearing. Because the serial dilution did not yield any additional evidence of

bacteriophages, we hypothesize that the clearing was due to a microbe smaller than 0.45 microliters that affect *E. meningoseptica*. It is also possible that the clearing could be due to another bacteria smaller than 0.45 microliters that secreted an antibiotic that affected *E. meningoseptica*.

These results indicate that our environmental samples did not contain bacteriophages that affect *E. meningoseptica*. However, this does not mean that no phages exist. It is still possible that phages exist for this species, just not in this area of Oklahoma. We hypothesize that since *E. meningoseptica* is not ubiquitously found in Oklahoma, there would not be bacteriophages that affect *E. meningoseptica* in this area. It is possible that areas where this species is found naturally and more commonly, phages that infect *E. meningoseptica* can be found in that area, as well.

For example, *E. meningoseptica* is found more commonly in Taiwan and India. Since this species of bacteria is found more commonly there, it is possible phages that affect the species are found in these areas as well. More specifically, *E. meningoseptica* is common in long term health facilities (Lin et al 2009). This poses a threat in these areas when patients are transferred from these facilities, into facilities with immunocompromised patients (Weaver et al 2009). Future research teams could feasibly take our methodology, or a similar methodology, to these facilities with known cases of patients with *E. meningoseptica*. Hopefully, since the bacteria species was isolated in these facilities, a bacteriophage that affects *E. meningoseptica* can also be isolated in these areas.

Although our research team did not manage to isolate any phages that affect *E. meningoseptica* from our environmental samples, this question still remains an important, and interesting question. *E.*

meningoseptica is found in plants, soil, and water sources, especially in neonatal units. It can cause fatal infections in immune deficient patients (Amer et al 2011). Discovering what bacteriophages affect this species could lead to even more discoveries about *E. meningoseptica*.

Acknowledgements

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Literature Cited

- Hso, M., C. Liao, Y. Huang, C. Liu, C. Yang, K. Kao, and P. Hsuen. 2011. Clinical Features, Antimicrobial Susceptibilities, and Outcomes of *Elizabethkingia meningoseptica* (*Chryseobacterium meningosepticum*) Bacterium at a Medical Center in Taiwan. <http://link.springer.com/article/10.1007/s10096-011-1223-0/fulltext.html> Accessed 4/26/2015.
- Lin, Y., C. Chiu, Y. Chan, M. Lin, K. Yu, F. Wang, and C. Liu. 2009. Clinical and Microbiological Analysis of *Elizabethkingia meningoseptica* Bacteremia in Adult Patients in Taiwan. *Scandinavian Journal For Infectious Diseases*. 41(9): 628-634.
- Ngwa, C., V. Glockner, U. Abdelmonsén, M. Schenuermayer, R. Fischer, U. Hentschel, G. Pradel. 2013. 16S rRNA Gene – Based Identification of *Elizabethkingia meningoseptica* Bacterium of the Asian Malaria Vector *Anopheles Stephens* (Dipteria; Culicidae) with Antimicrobial Activities. *Journal of Entomology*. 50(2): 404-414.
- Weaver, K., R. Jones, R. Albright, Y. Thomas, C. Zanbrano, M. Costello, S. Havel, J. Price, S. Gerber. 2010. Acute Emergence of *Elizabethkingia meningoseptica* Infection Among Mechanically Ventilated Patients in a Long – Term Acute Care Facility. *Infection Control and Hospital Epidemiology*. 31(1).