Isolation and Characterization of Specific Bacteriophages for *Pseudomonas aeruginosa* and *Staphylococcus aureus* from Wastewater from a Treatment Plant in Mayaguez, Puerto Rico.

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ABSTRACT

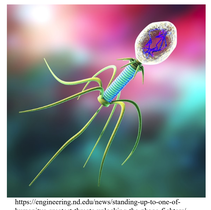
Pseudomonas aeruginosa and *Staphylococcus aureus* are multidrug resistant pathogenic bacterium considered by the CDC as a serious threat with urgent treatment needs. Carbapenems are last resort broad-spectrum antibiotics used to treat resistant bacteria. However, resistant strains produce carbapenemases to evade antibiotics making them difficult to treat. An alternative treatment that has been revisited experimentally involve the use of phage therapy (PD), which employs the specificity and bactericidal properties of bacteriophages to target specific strains. This research seeks to isolate *P. aeruginosa* and *S. aureus* bacteriophages. Given these bacteria are found in wastewaters and bacteriophages can survive with their host, samples from a wastewater treatment plant in Mayagüez, Puerto Rico were collected and used as sampling sites. To increases the bacteriophages concentration, an enrichment was performed by inoculating wastewaters filtrate with their respective hosts *(P. aeruginosa (ATCC 19660)* and *S. aureus (ATCC 25923))*, after amplification, the presence of the phages was confirmed using bacterial lawn-spotted test and plaque assays. Bacteriophages were successfully isolated, being necessary to dilute the sample to 10^{-6}-10^{-12} to avoid concurrent lysis in the plaque assay. The estimated bacteriophages in the lysate of *P. aeruginosa* was 2.5 x 10^{12} pfu/mL, and the average diameter of the plaques ranged from 0.8 to 1.0 mm. However, the estimated bacteriophages in the lysate of *S. aureus* was 1.87 x 10^{6} pfu/mL, and the diameter of the plaques was 0.4mm. The specificity test of the isolated bacteriophages of *P. aeruginosa* and *S. aureus* was tested with a bacterial group that included certifies strains of *P. aeruginosa*, *S. aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella aerogenes*, *Bacillus subtilis,* and *Salmonella diarizonae*. The presence of plaques was only found in their respective host. The morphological analysis of the isolated bacteriophages will be determined using TEM. The *P. aeruginosa* bacteriophage genetic material have been successfully extracted to further perform molecular analysis, including genome sequencing, restriction and in silico analysis. These findings confirm the presence of bacteriophages in the environment tested, allowing to test its potential used as bioprospect in phage therapy to antibiotic resistance strains of *P. aeruginosa* and *S. aureus.*

BACKGROUND

The increase in antibiotic resistance is commonly attributed to the excessive use of antibiotics and especially the improper use, additionally, it is very expensive to develop new antibiotics. This research has been very limited mainly due to lack of interest from the private sector and the rapid adaptability of microorganisms.[1] According to the 2019 CDC's Antibiotic Resistance Threats Report, more than 2.8 million antibiotic-resistant infections occur in the U.S. each year, and more than 35,000 people die as a result. [6] To focus on which species of bacterial pathogens are highly resistant to multiple antibiotics, we get carried away by the term ESKAPE, which means E for *Enterococcus faecium*, S for *Staphylococcus aureus*, K for *Klebsiella pneumoniae*, A for *Acinetobacter baumannii*, P for *Pseudomonas aeruginosa* and E for Enterobacter species.[2]

Pseudomonas aeruginosa and *Staphylococcus aureus* are multi-resistant pathogenic bacterium considered by the CDC as a serious threat with urgent treatment needs. The residual water of the treatment plants can act as a vector for the propagation or reappearance of many highly pathogenic microorganisms, as they are, *P. aeruginosa* and *S. aureus* . Viruses are pathogenic particles that infect specific cells and use its host cellular machinery for replication. Viruses that attack specifically bacteria are named bacteriophages, are unable to reproduce independently without their host.[4] It has been recognized that bacteriophages in recent years have several potential applications in modern biotechnology: as alternatives to antibiotics and for the detection of pathogenic bacteria.[3] The use of bound lytic phages that are host-specific for bacteria could provide targeted therapy that leaves human cells intact, with less damage to the flora, unlike some antibiotics that can cause host toxicity.[5]

The use of phage therapy (PD), employs the specificity and bactericidal properties of bacteriophages to target specific strains. The main purpose of this investigation is the isolation and characterization of specific bacteriophages for *P. aeruginosa* and *S. aureus* from wastewater from a treatment plant in Mayaguez, Puerto Rico and used as sampling sites, for the future test its potential used as bioprospect in phage therapy to antibiotic resistance strains of *P. aeruginosa* and *S. aureus.*



https://engineering.nd.edu/news/standing-up-to-one-of-humanitys-greatest-threats-unlocking-the-phage-fighters/

OBJECTIVES

- Isolation of specific bacteriophages of *Pseudomonas aeruginosa* and *Staphylococcus aureus* from wastewater treatment plant in Mayagüez, Puerto Rico.
- Morphological characterization of isolated viruses and perform specificity tests.

METHODS

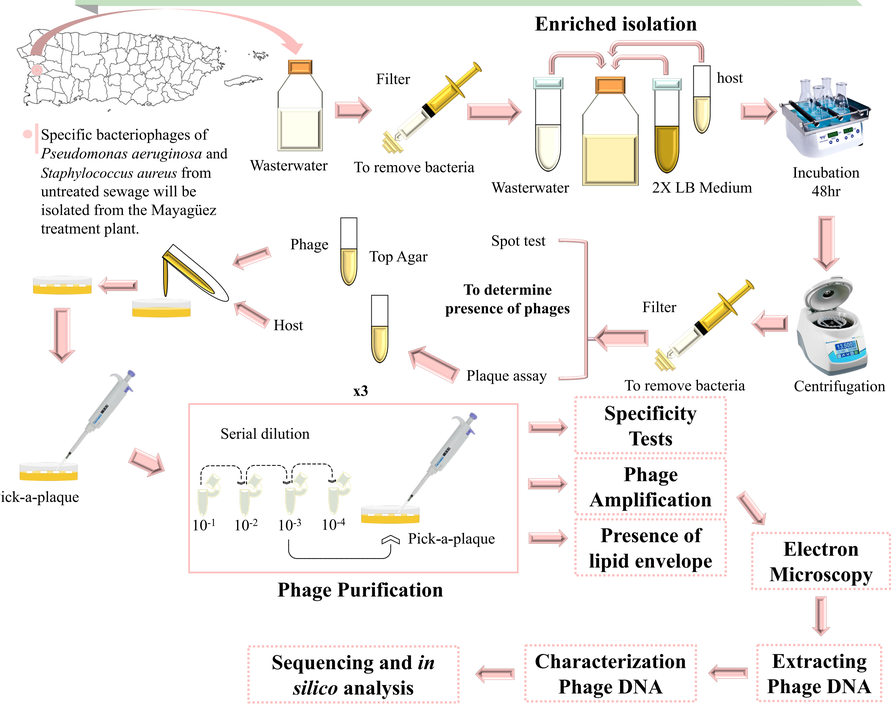


Figure 1: Isolation of specific bacteriophages of *Pseudomonas aeruginosa* and *Staphylococcus aureus*

RESULTS

Phages of *Pseudomonas aeruginosa*

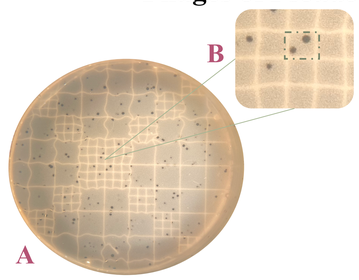


Figure 2: Plaques Produced by Phage A, After 24 Hours of Infection. A) Shows the plaques produced by the infection of phage A in a *Pseudomonas aeruginosa* grass. B) Amplified image of a plaque in figure A, the up arrow indicates a length of 0.8 mm.

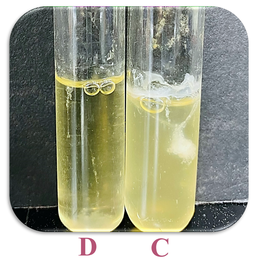


Figure 3: Ampification of *P. aeruginosa* phages. C) negative control, growth of *P. aeruginosa* without the presence of phage. D) positive control, phage infection against *P. aeruginosa.*

Phages of *Staphylococcus aureus*

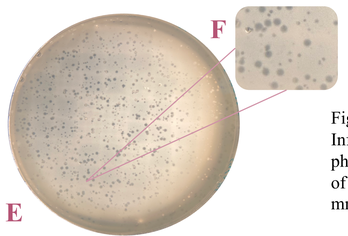


Figure 4: Plaques Produced by Phage B, After 24 Hours of Infection. E) Shows the plaques produced by the infection of phage B in a *Staphylococcus aureus* grass. F) Amplified image of a plaque in figure E, the up arrow indicates a length of 0.4 mm.

RESULTS

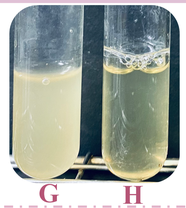


Figure 5: Ampification of *S. aureus* phages. G) negative control, growth of *S. aureus* without the presence of phage. H) positive control, phage infection against *S. aureus.*

Table #1: Specificity Test of the isolate phage of *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Bacteria vs phage	A	B
Staphylococcus aureus (ATCC 6538)	-	+
Escherichia coli (ATCC 259225)	-	-
Klebsiella pneumoniae (ATCC 13883)	-	-
Salmonella diarizonae (ATCC 107496)	-	-
Bacillus cereus (ATCC 10876)	-	-
Pseudomonas aeruginosa (ATCC 19660)	+	-
Klebsiella aerogenes (ATCC 13048)	-	-
Enterococcus faecalis (ATCC 29212)	TBD	TBD

Phage A (of *P. aeruginosa*) and phage B (of *S. aureus*) are inoculated with various bacteria to detect host specificity. Specificity testing will be ongoing with other bacterial strains; Enterococcus faecalis (29212). (TO BE DETERMINED)

SUMMARY

- A virus particles that infect *P. aeruginosa* and *S. aureus* was isolated. Enrichment was performed by inoculating filtrated wastewaters with its respective host.
- Bacteriophages of *P. aeruginosa* and *S. aureus* were successfully isolated, being necessary to dilute the sample to 10^{-6}-10^{-12} to avoid concurrent lysis in the plaque assay. The estimated bacteriophages in the lysate of *P. aeruginosa* was 2.5 x 10^{12} pfu/mL, and the average diameter of the plaques ranged from 0.8 to 1.0 mm. However, the estimated bacteriophages in the lysate of *S. aureus* was 1.87 x 10^{6} pfu/mL, and the diameter of the plaques was 0.4mm
- The specificity test of bacteriophages isolated from *P. aeruginosa* and *S. aureus* was tested with a bacterial group that included seven certified strains, suggesting that these phages are specific to their respective host.
- The presence of lipid envelope of *P. aeruginosa* and *S. aureus* bacteriophages was detected.
- The *P. aeruginosa* bacteriophage genetic material have been successfully extracted to further perform molecular analysis, including genome sequencing, restriction and in silico analysis

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REFERENCES

1. Reed, C., Bryant, R., Ibrahim, A. S., Edwards Jr, J., Filler, S. G., Goldberg, R., & Spellberg, B. (2008). Combination polyene-caspofungin treatment of rhino-orbital-cerebral mucormycosis. *Clinical Infectious Diseases*, *47*(3), 364-371..
2. Pendleton, J. N., Gorman, S. P., & Gilmore, B. F. (2013). Clinical relevance of the ESKAPE pathogens. *Expert review of anti-infective therapy*, *11*(3), 297-308.
3. Clark, J. R., & March, J. B. (2006). Bacteriophages and biotechnology: vaccines, gene therapy and antibacterials. *Trends in biotechnology*, *24*(5), 212-218.
4. Lin, D. M., Koskella, B., & Lin, H. C. (2017). Phage therapy: An alternative to antibiotics in the age of multi-drug resistance. *World journal of gastrointestinal pharmacology and therapeutics*, *8*(3), 162.
5. Furfaro, L. L., Payne, M. S., & Chang, B. J. (2018). Bacteriophage therapy: clinical trials and regulatory hurdles. *Frontiers in cellular and infection microbiology*, *8*, 376.
6. Kadri, S. S. (2020). Key Takeaways From the US CDC's 2019 Antibiotic Resistance Threats Report for Frontline Providers. *Critical Care Medicine*.