

Abstract

Our Goal was to investigate the presence of antibiotic-resistant bacteria (ARB) in the soil at Maritime Heritage Park in Bellingham, WA. This site served as a garbage dump from the 1920s to the 1940s and connects to Whatcom Creek, which flows into Bellingham Bay. Given this history and the potential for pollution to spread through water, we aimed to determine whether harmful, resistant bacteria could still be found in the area. To conduct our study, we collected soil samples from the estuary and grew bacteria on agar plates with various concentrations of tetracycline. We utilized PCR and DNA sequencing to help identify the bacterial species present. Ultimately, our research successfully identified multiple bacterial species, including some resistant to antibiotics, which supports our hypothesis that ARB are present in this environment.

Introduction

Maritime Heritage Park in Bellingham, WA, has a history of pollution. From the 1920s to the 1940s, this area was utilized as a garbage dump, which may still contaminate the soil and water today. The park is connected to a stream that flows into Bellingham Bay, meaning that any pollutants in the water could impact the larger environment.

One concern is the presence of ARB. These bacteria have developed the ability to survive antibiotics, which poses a significant threat. When ARB are present in the environment, they can harm animals, transfer resistance to other bacteria, and make it more difficult to treat infections in both people and wildlife (Baquero et al., 2008). These bacteria can spread through water and infect fish, birds, and other animals, leading to problems within the ecosystem (Taylor et al., 2011). Additionally, pollution, such as outdated waste or urban runoff, can create conditions that allow these bacteria to thrive and multiply (Berendonk et al., 2015).

Methods

Materials & Location

Bacteria were sampled from Whatcom Creek, Bellingham, WA (48.75509 N, 122.48218 W) and cultured on agarose plates with tetracycline (0, 3, and 30 µg/ml). A *HotShot* solution lysed the bacteria, followed by neutralization. PCR was conducted using primers 320F and 817R on a BIO-RAD thermocycler, with a loading dye for gel electrophoresis performed using an Edvotek chamber and BIO-RAD PowerPac Basic

Antibiotic Resistance Found in Soil Samples from Bellingham’s Estuary Site

Kelsey Alcraz, Komal Kaur, Logan Donofrio
BIOL 222: Majors Cell Biology/Molecular

Procedure

We diluted soil samples in a series from 1:10 to 1:100,000 and plated them on various agar types, including those with and without Tetracycline (3 µg/mL and 30 µg/mL). After incubating at 28°C for 72 hours, we counted the colonies to calculate colony-forming units (CFUs) per gram of soil and assess antibiotic resistance.

Subsequently, we performed PCR amplification of the 16S rRNA gene, verified the products using gel electrophoresis, and sent successful samples for sequencing. The DNA sequences were then uploaded to the BLAST database for bacterial identification based on genetic similarity.

Concentration of ARB

Antibiotic Resistant Bacteria (Tetracycline 3ug/ml)		Non-Antibiotic Resistant Bacteria (Agarose)	
Number of Colonies	151	Number of Colonies	141
Volume Plated	0.2ml	Volume Plated	0.2ml
Dilution Factor	10	Dilution Factor	10^3
Total CFU Per Gram of Soil	1,510	Total CFU Per Gram of Soil	141,000

Percentage of Bacteria that are Antibiotic Resistant	1.1%
--	------

Table 1. This table provides a quantitative overview of our results

Maritime Heritage Park



Figure.1 This image depicts the location of the estuary.



Figure.2 This image provides a visual overview of the sampling site, illustrating the environmental context in which the soil sample was collected.

Discussion

In this study, we identified three different species of ARB: *Rahnella victoria*, a symbiotic plant bacterium commonly found in root systems; *Aeromonas salmonicida*, which is known to inhabit fish from the family Salmonidae and can cause lesions, often leading to fatal outcomes in these fish; and *Aeromonas sobria*, a bacterium recognized for causing gastroenteritis in humans.

The presence of antibiotic-resistant strains in our samples suggests that resistance genes are present and may be spreading in the local environment. These genes can be transferred between bacteria, increasing the risk of widespread resistance. This creates a selective environment where resistant strains can thrive and outcompete non-resistant ones.

While our study provided valuable insights, it also had some limitations. For example, the sterility of our PCR processes may have been compromised. Our negative control during gel electrophoresis showed contamination with DNA. Additionally, we only tested for resistance to a limited number of antibiotics and did not identify the exact sources of contamination. Therefore, we cannot definitively conclude whether the resistant bacteria originated from animal waste, agricultural chemicals, or another source.

Acknowledgements

We would like to express our gratitude to Professor Lauren Maniatis for her unwavering support and guidance throughout our bacteria lab project. Her generosity in providing the necessary materials and her encouragement at every stage of our research truly made a difference. We are deeply appreciative of her dedication to our success.

References/ Work Cited

- Baquero, F., Martínez, J. L., & Cantón, R. (2008). Antibiotics and antibiotic resistance in water environments. *Current Opinion in Biotechnology*, 19(3), 260–265. <https://doi.org/10.1016/j.copbio.2008.05.006>
- Taylor, N. G. H., Verner-Jeffreys, D. W., & Baker-Austin, C. (2011). Aquatic systems: Maintaining, mixing and mobilising antimicrobial resistance? *Trends in Ecology & Evolution*, 26(6), 278–284. <https://doi.org/10.1016/j.tree.2011.03.005>
- Berendonk, T. U., Manaia, C. M., Merlin, C., Fatta-Kassinos, D., Cytryn, E., Walsh, F., ... & Martinez, J. L. (2015). Tackling antibiotic resistance: The environmental framework. *Nature Reviews Microbiology*, 13(5), 310–317. <https://doi.org/10.1038/nrmicro3439>
- European Society of Clinical Microbiology and Infectious Diseases. (n.d.). *The negative impact of antibiotic resistance*. Clinical Microbiology and Infection. [https://www.clinicalmicrobiologyandinfection.com/article/S1198-743X\(20\)30629-2/fulltext](https://www.clinicalmicrobiologyandinfection.com/article/S1198-743X(20)30629-2/fulltext)
- Bullock, G. L. (1983). Furunculosis and other diseases caused by *Aeromonas salmonicida*. *Fish Disease Leaflet*, (66), 0–29. <http://articles.extension.org/sites/default/files/w/b/b7/Furunculosis.pdf>