Biocontrol of the pathogen *Salmonella typhimurium* using Bacteriophages

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Abstract

Salmonella typhimurium is a foodborne pathogen that causes salmonellosis in both humans and animals. An estimated 1.4 million cases of salmonellosis occur among humans in the United States each year. This pathogen is a gram-negative, rod-shaped bacterium that belongs to the Enterobacteriaceae family. The objective of this study is to find the specific bacteriophage that inhibits the bacterium that is *Salmonella typhimurium*. With this bacteriophage, we want to create a type of biocontrol to moderate its growth. The bacteriophage of this bacterium was isolated from two different methods utilizing snake feces and sewer water. With the process utilized in the methods of the research we could find the bacteriophage of the bacterium *Salmonella typhimurium*. This proved that sewer water and snake feces are a viable source of *Salmonella typhimurium* and its bacteriophages. It was evident that bacteriophages exist in every niche where bacterial organisms live. Also, that the bacteriophage found does work as a biocontrol to inhibit the growth of the bacterium.

Objective

This research aims to find the specific bacteriophage that inactivates or inhibits the pathogenic bacterium that is *Salmonella typhimurium*. With this bacteriophage, the aim is to create a type of biocontrol to moderate its growth.

Introduction

Biocontrol refers to when organisms are used to control outbreaks or pests created by other organisms. In this research, the biocontrol would be the bacteriophages when used to inhibit the growth of the bacterium *Salmonella typhimurium*. Bacteriophages, also known as phages, are viruses that infect bacteria and are found in all possible niches where these bacterial organisms reside (Akhwale et al., 2019).

Pathogens are organisms that can produce infectious diseases, these can be bacteria, viruses, fungi, etc. Examples of bacteria that can become foodborne pathogens are *Escherichia coli*, *Salmonella spp*, and *Listeria monocytogenes* (Goodridge & Bisha, 2011).

Salmonella spp is a gram-negative, rod-shaped bacterium that belongs to the Enterobacteriaceae family. This bacterium is the cause of salmonellosis which is a bacterial enteric disease that occurs in both humans and animals. An estimated 1.4 million cases of salmonellosis occur among humans in the United States each year (Brenner et al., 2000). The most common symptoms that people with Salmonellosis experience are: diarrhea, fever, stomach pain, nausea, vomiting, headache, etc. This bacteria can be found in foods such as: raw meat, poultry, fish, raw eggs, and fruits and vegetables. It has been found that the increase in the consumption of fruits and vegetables in recent years is accompanied by an increase in the number of infections and outbreaks in humans, as they can serve as reservoirs of pathogens or opportunistic pathogens (Al-Kharousi et al., 2016).

Methods

Method #1

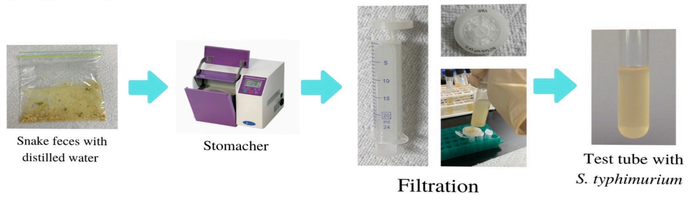

Figure 1. The snake feces with distilled water in the ziploc bag was homogenized in a stomacher for 10 minutes. Then it was filtrated with a 0.45 µm filter and a syringe. The liquid filtrated was then transferred into a test tube that contained *S. typhimurium.*

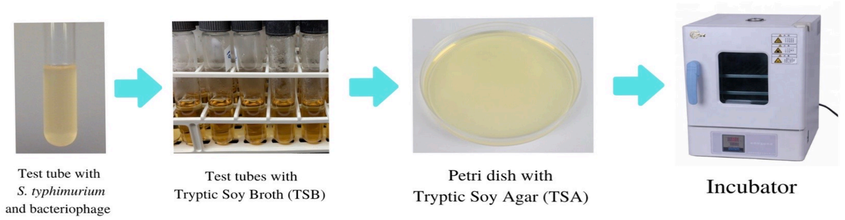

Figure 2. 1000 µL of the test tube containing the bacterium and the phage were extracted and transferred to five test tubes with TSB. The content of these test tubes were poured on five petri dish with TSA. These petri dishes were incubated at 32°C from 24-48 hours.

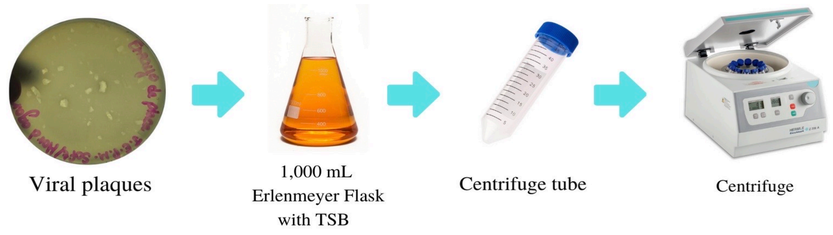

Figure 3. After the incubating time was over, viral plaques appeared. These were transferred to a 1000 mL Erlenmeyer flask with TSB and were transferred again to centrifuge tubes. The plaques were centrifuged for 10 minutes at 4000 RPM.

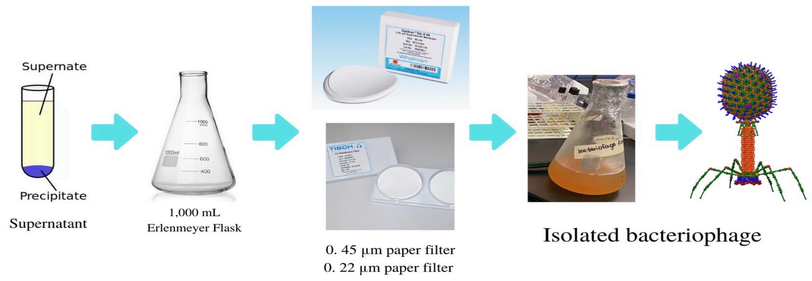

Figure 4. The supernatant was extracted from the centrifuge tubes and transferred to a sterile 1000 mL Erlenmeyer flask. The supernatant was filtered first by a 0.45 µm paper filter, and then by a 0.22 µm paper filter. The liquid filtrated contained the isolated bacteriophage.

Method #2

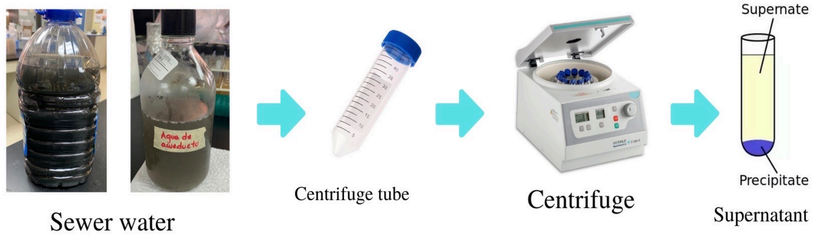

Figure 5. 500 mL from the sewer water were extracted and transferred to centrifuge tubes. This water was centrifuged for 5 minutes at 4000 RPM. After centrifugation, the supernatant was drawn out for filtration.

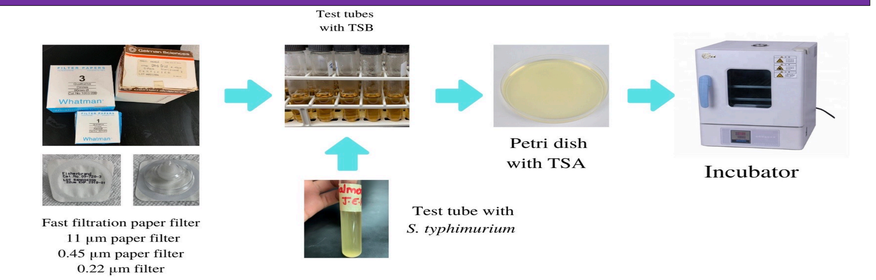

Figure 6. The supernatant was filtered using the different filters mentioned in the figure above. The liquid filtered was then introduced into test tubes with TSB as well as the bacterium *S. typhimurium*. These test tubes were poured in petri dish with TSA and left to incubate at 32°C from 24-48 hours.

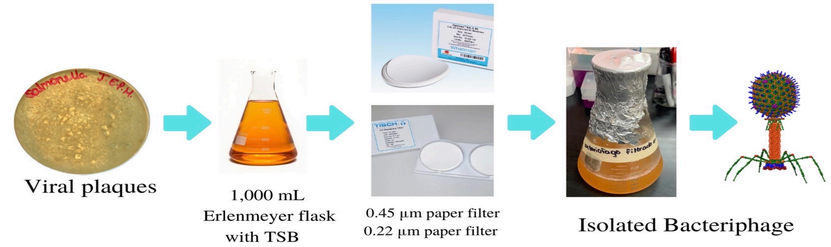

Figure 7. After the incubating time was over, viral plaques appeared. These were transferred into a 1000 mL Erlenmeyer flask with TSB, and filtered again with 0.45 µm and 0.22 µm paper filters. The filtrate obtained contained the isolated bacteriophage.

Results

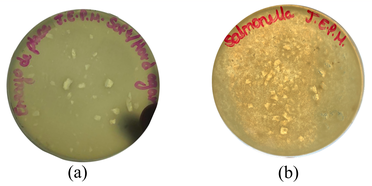

Figure 8. Viral plaques that show the result of the inhibition of the bacterium by the bacteriophage. (a)Viral plaques that resulted from the bacteriophage of the snake feces. (b)Viral plaques that resulted from the bacteriophage of the sewer water.

Summary and Conclusions

- Sewer water and snake feces are a viable source of *Salmonella typhimurium* and its bacteriophages.
- Bacteriophages exist in every niche where bacterial organisms live.
- The bacteriophage found does work as a biocontrol to inhibit the growth of the bacterium.

Future Directions

- Characterize the bacteriophage.
- Utilize the bacteriophage in edible sheets or essential oils as a source of biocontrol in food.

References

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Acknowledgements

Symbiosis Laboratory members and the department of Biology at UPRM.