Screening *Tetragenococcus halophilus* for Bacteriophage in Smokeless Tobacco Production Streams Fermentation Process

by

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A thesis presented to the Honors College of Middle Tennessee State University in partial fulfillment of the requirements for graduation from the University Honors College

Spring 2024

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Dedication

I would like to dedicate this thesis to my little brother, Noah Hatcher, who has leukoencephalopathy with vanishing white matter. Leukoencephalopathy is an incredibly rare, autosomal recessive, and progressive central nervous system disorder. Noah, you are a symbol of strength and perseverance, and you teach me every day to strive to be a better person. You are loved beyond measure, and you inspire me to continue in the biology field to learn miraculous ways to improve the world around us. God has a great plan for you, and I am so thankful He allowed me to be your big sister.

Acknowledgments

First, I would like to thank MTSU and the Honor's College for the opportunity, support, and resources they provided me with to complete this thesis.

I would like to show my gratefulness to my thesis advisor Dr. Robertson for the time that he committed to my project, as his insight built not only this paper but my confidence in the subject matter as well. His advice was encouraging, wise, and complete; I am thankful to have a professor who cares so much about his students.

I would also like to thank my second reader Dr. Wright, who provided me with boundless knowledge about microorganisms, viruses, and lab techniques. His intelligence has pushed me to become a better student, and I am so appreciative of the classes I was able to take to learn from him.

I would like to acknowledge my thesis committee chair Dr. Elrod-Erickson and his inspiring and dedicated personality. His guidance throughout the completion of my thesis project has made this process much easier than I could ever imagine and has helped me stay motivated throughout difficult times.

Lastly, I would like to express my gratitude towards my parents, Tommy and Cynthia Hatcher, and my fiancé, Jordan Johnson. They have always supported me and my educational goals, and they have encouraged and provided resources for me in countless ways to reach them. They are all huge role models, and I am appreciative that they are so influential in my life.

Abstract

Smokeless tobacco is created through a heavily monitored fermentation process but is prone to bacteriophage contamination. As a preventative measure, using samples from our industry provider, we screened for bacteriophages capable of infecting *Tetragenococcus halophilus*, a critical, lactic-acid-producing microorganism in the smokeless tobacco fermentation process. Samples were enriched with *T. halophilus*, filtered, and tested on nutritious Petri plates for the presence of these bacteriophages. After testing over 100 samples, no bacteriophages capable of infecting *T. halophilus* were discovered. These results reassured our industry partner that there is not currently bacteriophage capable of infecting *T. halophilus* contaminating their fermentation process. Some options to further this research could be to screen for bacteriophages that are capable of infecting other bacteria present in the fermentation process of smokeless tobacco (or the fermentation processes of other industries) and to use the screening protocol to search for bacteriophages capable of infecting antibiotic-resistant bacteria.

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List of Symbols and Abbreviations

°C: degrees Celsius

µL: microliters

CFU: colony forming unit

CO_2: carbon dioxide

E. coli: *Escherichia coli*

MRS: De Man–Rogosa–Sharpe agar

NaCl: sodium chloride; table salt

T. halophilus: *Tetragenococcus halophilus*

TSA: tryptic soy agar

TSB: tryptic soy broth

List of Terms

Anaerobic: living optimally in the absence of free oxygen.

Bacteriophage (phage): a virus that infects only bacteria.

Facultative Anaerobe: an organism that grows optimally in the presence of oxygen but can survive and grow under anaerobic conditions.

Gram Stain: a method of staining used to classify if a bacterium is gram-positive or gram-negative. Gram-positive bacteria stain purple and indicate the presence of a thick cell wall, while gram-negative bacteria stain pink and indicate the presence of a thin cell wall.

Lactic Acid: an organic acid ($C_3H_6O_3$) normally present in muscle tissue as a by-product of anaerobic glycolysis, produced in bacterial fermentation, and used especially in industry, food, and medicine.

Plaque: a clear area on a field of bacteria that indicates the inhibition of bacterial cells, typically by a virus or an antibiotic.

T4: name of a bacteriophage that infects *E. coli*.

Introduction

Fermentation

Fermentation is a process used in industries to create thousands of commercial goods, such as beer, cheeses, sauces, and tobacco products. During this process, microorganisms (often bacteria or fungi) break down chemicals such as sugars, polysaccharides, lipids, metabolites, and proteins. In doing so, the microbes produce several enzymes, acids, and alcohols (Haile, 2019). These chemical products created through fermentation are necessary to provide flavor and texture to the food, as well as increase its shelf life, so the fermentation process is rigorously controlled to optimize the growth and survival of these essential microorganisms (Waites, 2007).

The creation of smokeless tobacco is a long process that involves the harvesting, curing, and fermenting of the leaves of the tobacco plant. The fermentation process starts with making 100-pound stacks of tobacco leaves and relying on the self-heating actions of tobacco (Bunn, 2013). Once the internal temperature of the stack reaches 55-65°C, the stack is taken apart to release tar, nicotine, and ammonia (Geiss, 2007). This process is repeated 2 to 5 times and can last for weeks (Li, 2020). The fermentation steps of tobacco products can vary slightly depending on the type of smokeless tobacco, the intended flavor, and the tobacco harvesters' preferences (Wen, 2022).

Tetragenococcus halophilus

An organism that can be used in the tobacco fermentation process is *Tetragenococcus halophilus* (Han, 2016). *T. halophilus* is a facultatively anaerobic,

coccus-shaped, lactic acid-producing bacterium (Guindo, 2022) that is used in the fermentation of several salty foods, such as soy sauce, soybean paste, jeotgal, and fish sauce (Udomsil, 2011). The optimal growth conditions for *T. halophilus* are a pH of 7-8, an incubation temperature ranging from 25-35°C, and a high salt concentration of up to 26% (Vyas, 2022). *T. halophilus* is a gram-positive bacterium, so it stains purple after a Gram stain and appears in tetrads when examined under a microscope (Jeong, 2017). A heat-fixed slide of *T. halophilus* examined under a microscope is shown in Figure 1.

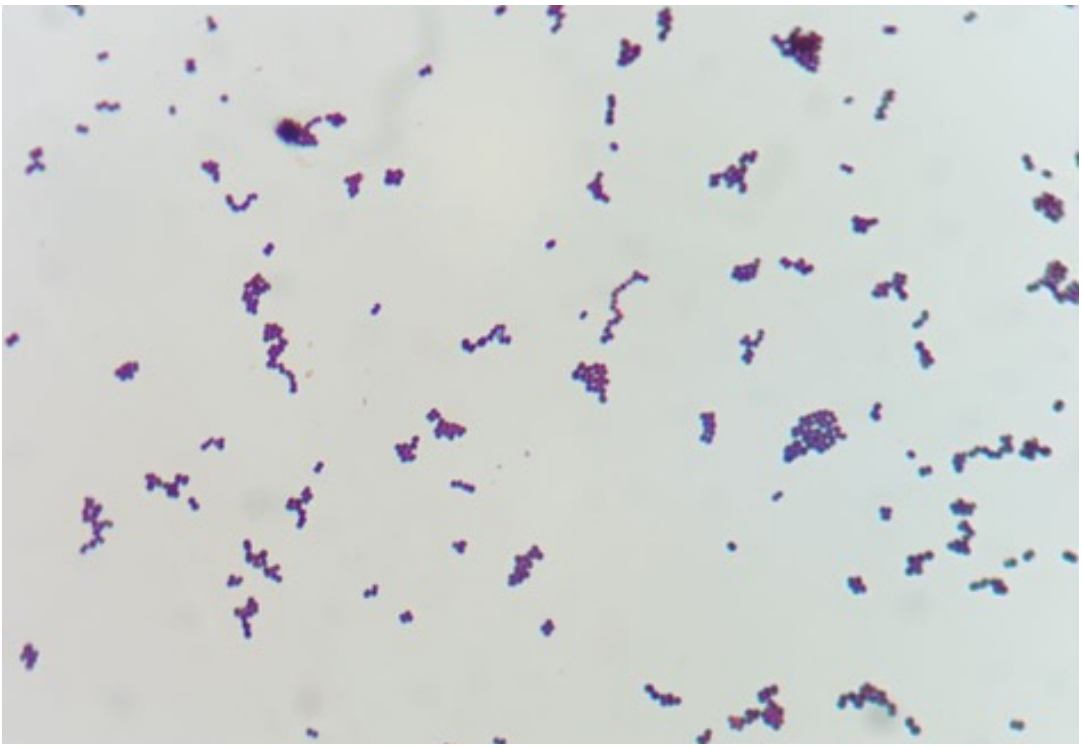

Figure 1: Gram's stain of *T. halophilus*. The figure depicts a slide of *T. halophilus*, taken from a colony on a TSA plate, stained, and examined under a microscope at 1000x magnification. *T. halophilus* is a Gram-positive organism that is coccus-shaped and commonly found in tetrads.

Bacteriophages

A bacteriophage is a virus that infects and replicates inside bacteria. Bacteriophages are considered the most abundant biological agent on Earth and are diverse in size, morphology, and genetic organization (Kasman, 2022). Bacteriophages are the most common contaminant of fermentation, as they can destroy the culture of essential microorganisms (Marcó, 2012). The results of infection during the fermentation process can range from decreased lactic acid production to entire batches being destroyed (Ranveer, 2024).

Because of this infection potential, bacteriophage screening is common in fermentation processes (Zaki, 2023). Although bacteriophages have not caused a reported economic impact, screening is warranted to avoid a severe impact on fermented products' quality and safety (Wu, 2023). Through bacteriophage screening, fermentation delays and improper production can be avoided (Marcó, 2012). If a bacteriophage is discovered that proposes a risk to the fermentation process, then only a few options are available to the organization conducting the fermentation. The organization could stop fermentation and sterilize all equipment (Sommer, 2019), find a naturally resistant microorganism, begin using a different organism that creates the same end products (Zou, 2022), or genetically engineer bacteria that are resistant to the phage (Geis, 2006).

There have not been many reported bacteriophages capable of infecting *T. halophilus*, likely because of its diverse structure of capsular polysaccharides (Wakinaka, 2023). Due to the discovery of CRISPR loci and accumulation/deletion effects of spacer sequences found on some strains, it has been determined that *T. halophilus* genomes may have acquired strain-specific bacteriophage resistance (Matsutani, 2021). However, a few

bacteriophages capable of infecting *T. halophilus* have been discovered, including Φ7116, ΦD-86, and ΦD10 (Wakinaka, 2022; Uchida, 1993).

Project Feasibility

Optimal growth conditions for *T. halophilus* were determined by a prior research group to be on a TSA medium with 7% NaCl concentration. Incubation at 30°C with increased CO_2 production encouraged growth for the facultative anaerobe *T. halophilus*. The data from this research is shown in Figure 2. Verification of the bacteriophage isolation protocol was also completed by a prior research group with the use of *Escherichia coli B* and T4, a common bacteria and bacteriophage pairing. The results are shown in Figure 3.

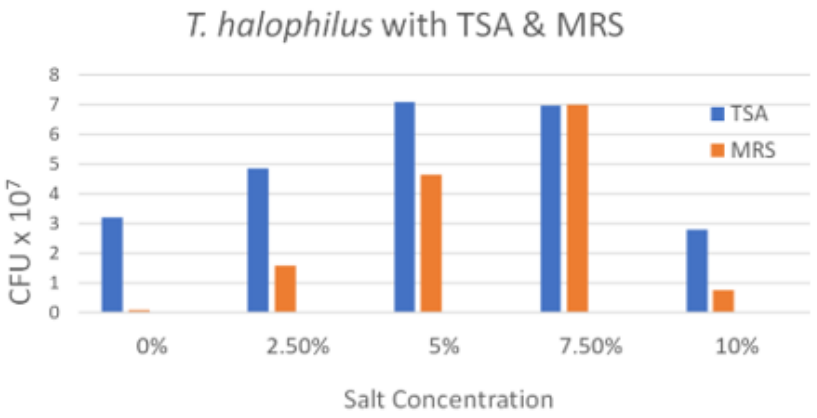

Figure 2: Optimal *T. halophilus* Growth Conditions in NaCl Testing Results. The x-axis shows 0%, 2.5%, 5%, 7.5%, and 10% NaCl within the media composition. The y-axis shows the number of CFUs in tens of millions. The media tested were TSA (depicted in blue) and MRS (depicted in orange). Across most salt concentrations, *T. halophilus* had a higher number of CFU on TSA than on MRS. TSA plates showed the highest growth between 5% and 7.5% NaCl. Because of this, optimal growth conditions were determined to be on TSA plates at 7% NaCl concentration.

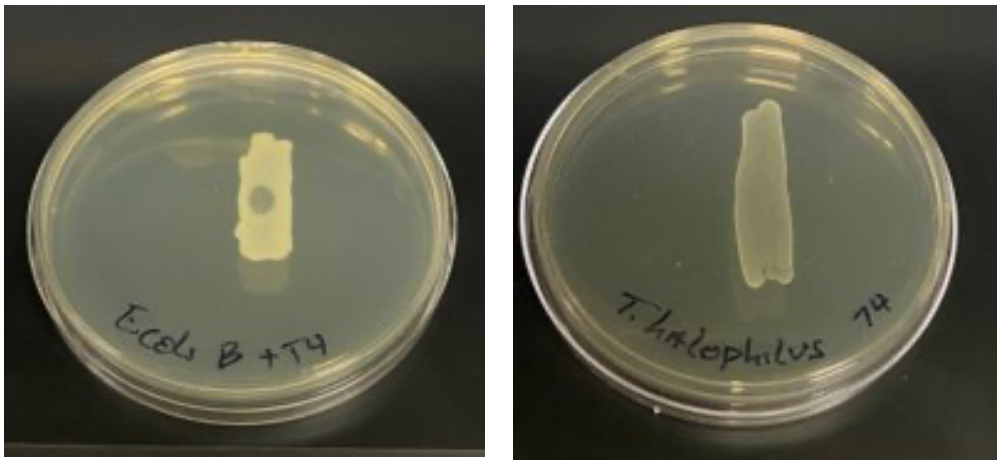

Figure 3: Comparison of Positive and Negative Bacteriophage Isolation. The line of *E. coli* on the left plate shows a clearing: this is where T4 lysis of *E. coli* has occurred, and bacteriophage is present. This shows a positive result. The line of *T. halophilus* on the right plate shows no clearing: T4 is incapable of infecting *T. halophilus*, so a clearing is not present. This shows a negative result.

Thesis Statement

This research aims to test samples provided by our industry partner and collaborators' smokeless tobacco production stream for the presence of bacteriophage capable of infecting their strain of *T. halophilus*. *T. halophilus* is a bacterium that can be used in the smokeless tobacco fermentation process to enhance the flavor of tobacco products. If bacteriophages capable of infection are present in the fermentation process, the *T. halophilus* bacteria will be killed, and the tobacco products will not ferment correctly. This could lead to devastating supply drops or distasteful tobacco products for consumers.

Materials and Methods

As a preventative measure to avoid bacteriophage contamination in their production stream, our industry partner provided products to be screened. The screening was completed through filtration, phage enrichment, and spot testing. To accomplish this, TSA plates containing 7% NaCl, TSB containing 7% NaCl, and *T. halophilus* cultures were made frequently. The creation of these materials is outlined below.

Material Production

TSA Plates Containing 7% NaCl

In a 500 mL Erlenmeyer glass flask, the following was added: 200 mL of deionized water, 14 grams of Fisher sodium chloride, and 8 grams of BD Difco Culture Medium Tryptic Soy Agar. A stir bar was added to the flask, and the top of the flask was covered with aluminum foil. The flask was then placed on a hotplate stirrer and allowed to mix while heating. Once the NaCl and TSA had dissolved, the flask was removed from the hotplate stirrer and the stir bar was taken out of the flask. Then, with aluminum foil covering the top of the flask, the flask was autoclaved for 15 minutes at 121°C.

After the TSA mixture had been autoclaved, 10 plastic Petri dishes (100mm x 15mm) were placed underneath the Biological Safety Cabinet. From the flask, 20 mL of TSA mixture was added to each petri dish. The 7% NaCl TSA plates were allowed to solidify under the safety cabinet and were stored and labeled in the laboratory refrigerator.

TSB Containing 7% NaCl

In a 500 mL Erlenmeyer glass flask, the following was added: 200 mL of deionized water, 14 grams of Fisher sodium chloride, and 6 grams of BD Bacto Tryptic Soy Broth Soybean-Casein Digest Medium. A stir bar was added to the flask, and the flask was put on a hotplate stirrer to mix – heat was not required. Once the NaCl and TSB had dissolved, the flask was removed from the hotplate stirrer. Sixteen glass test tubes received 5 mL of the TSB mixture and were covered with caps. The top of the flask containing the TSB mixture was covered with aluminum foil, and the covered flask and test tubes were autoclaved for 15 minutes at 121°C.

After the TSB mixture had been autoclaved, ten 50 mL sterile centrifuge tubes were placed under the safety cabinet. While working under the safety cabinet, each centrifuge tube received 12 mL of the TSB mixture. Both the centrifuge tubes and the test tubes containing 7% NaCl TSB were stored and labeled in the laboratory refrigerator.

T. halophilus Culture

Our industry partner provided us with their strain of freeze-dried *T. halophilus*, which was put into a glass test tube containing 5 mL of 7% NaCl TSB as described above by using a sterile loop. The test tube was incubated at 30°C for 48 hours and streaked for isolation to ensure that the test tube contained a pure culture. To culture more *T. halophilus*, 0.1 mL of the current sample was placed into 5 mL of 7% NaCl TSB in a glass test tube twice a week; the test tubes were incubated for at least 48 hours before use. These cultures were streaked on a plate once a week to ensure that pure cultures were being made and were not contaminated.

Increased CO_2 Conditions

Our spot test plates needed to be incubated under increased CO_2 conditions, which was accomplished using a CO_2 chamber. The plates were set in a Becton Dickinson BBL GasPak 100 Jar, where a Thermo Fisher Scientific CO_2 Gen 2.5L Atmosphere Generation System package was placed inside. The GasPak jar was closed, and the plates were incubated at 30°C.

Sample Preparation

Our industry partner and collaborators provided samples of soil and plant material from various stages of the fermentation process, potentially containing bacteriophage. For each sample to be tested, about 4 grams of provided soil or plant material were added to a sterile 50 mL centrifuge tube containing 12 mL of 7% NaCl TSB, made as described previously. This tube was labeled "Original Sample" and mixed by inversion every 5 minutes for half an hour. The tube contained some water-insoluble material, so it had to be passed through a funnel containing a Whatman #4 filter paper into a sterile 15 mL centrifuge tube. After filtration, this tube contained only water-soluble material and microbes and was labeled "Whatman Filtered Sample". Streak plates were made from the filtrate in this tube on TSA plates. These plates were incubated overnight at 30°C and were examined through gram-staining to determine the morphology of the bacteria in the sample, as well as the potential presence of *T. halophilus.*

Using filtrate from the "Whatman Filtered Sample", 2-3 mL were passed through a 0.45-micron syringe filter into a snap cap tube using a 3 mL syringe. This process separated the bacteria in the sample from potential bacteriophages, as bacterial cells are too large to pass through the 0.45-micron filter, while bacteriophages are small enough to

pass through. The snap-cap tube, potentially containing bacteriophage, was labeled “Non-enriched, Filtered Sample”.

To a sterile glass tube, 800 µL of filtrate from the non-enriched sample, 1 mL of 7% NaCl TSB made as described, and 200 µL of *T. halophilus* culture as previously described were added. This *T. halophilus* culture was concentrated before addition to the glass tube; in other words, 2.5-3 mL of broth was removed from the top of the settled culture, and the tube was vortexed. The sterile glass tube was labeled “Enriched Sample” and was incubated for 24 hours at 30°C. The addition of *T. halophilus* and overnight incubation allowed for any potential bacteriophages to infect the bacteria and replicate, enriching the sample.

Bacteriophage Screening

Following incubation, 1.5 mL of the enriched sample was passed through a 0.45-micron filter into a 15 mL centrifuge tube using a 3 mL syringe. This process removed the added *T. halophilus* from the tube, as the enrichment process was completed by this stage and only the potential bacteriophages were needed. This tube was labeled “Enriched Filtered Sample”.

Then, a spot test was conducted to determine the presence of bacteriophage in the filtrates. Several lines of *T. halophilus* from an active culture were prepared on a 7% NaCl TSA plate using a sterile loop. Once the *T. halophilus* lines were dry, the plates and tubes containing the filtrates and *T. halophilus* culture were moved to work under the safety cabinet to prevent contamination. Each line on the plate received a 4 µL drop of the non-enriched filtrate, a 4 µL drop of the enriched filtrate, and a 4 µL drop of *T. halophilus* culture. The application of *T. halophilus* culture served as a control to

determine if the added filtrates created a clearing on the lines of the plate, as the addition of filtrates can sometimes be confused with a plaque. These additions were labeled “NE” for the non-enriched filtrate, “E” for the enriched filtrate, and “C” for the control *T. halophilus* culture. The layout of these plates is shown in Figure 4.

The plate was incubated at 30°C under increased CO_2 and checked daily. Once confluent *T. halophilus* growth had occurred on the lines, the plates were removed from the incubator and examined for plaques. Clearing indicated bacteriophage lysis of *T. halophilus*. This process is visually outlined in Figure 5.

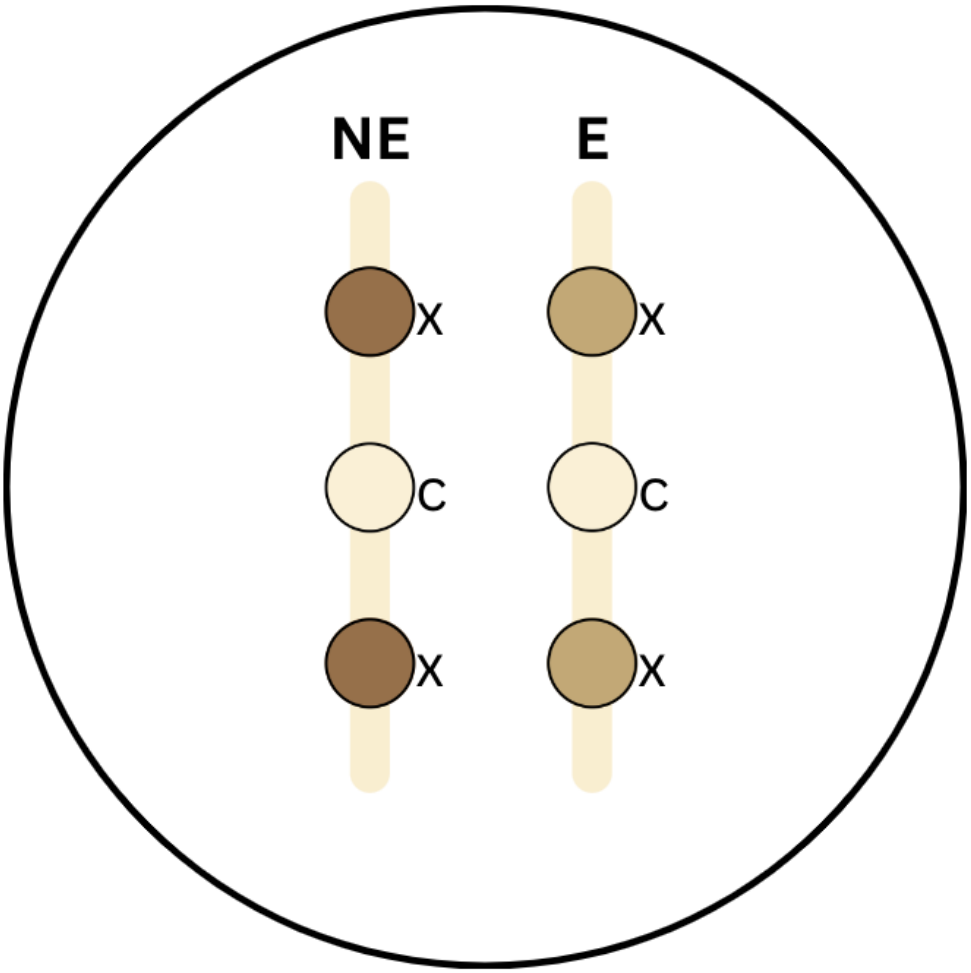

Figure 4: Plate Layout for Bacteriophage Screening. NE labels lines of *T. halophilus* that received drops of non-enriched filtrate, while E labels lines that received drops of enriched filtrate. The "x" markings to the side of the line of *T. halophilus* serve as a guide for the addition of the drop of filtrate while the "c" markings serve as a guide for the addition of *T. halophilus* as a control.

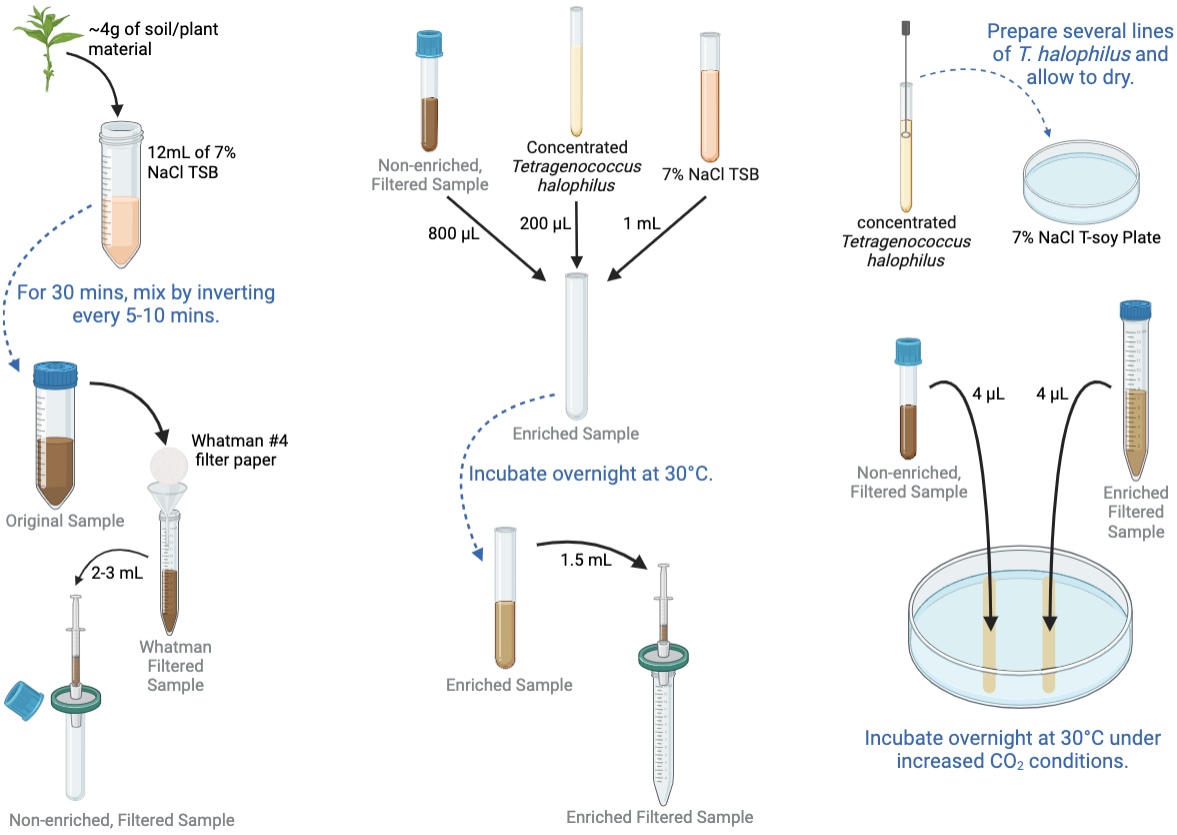

Figure 5: Methodology of Bacteriophage Screening on Provided Samples. Figure created using BioRender. The left side and the middle of the figure depict the steps of sample preparation. The right side of the figure depicts the bacteriophage screening protocol.

Results

Each of the created streak plates were stained and examined under the microscope to estimate the type of microorganisms present in each sample. Within the dirt, GCB, semi-finished, trash, and 2X samples, several Gram-positive bacilli were found, estimated to be from the environmental genus *Bacillus*. Within the retain and ship samples, isolates were Gram-positive cocci, estimated to be *T. halophilus*. Lower stalk samples did not have colony growth on the streak plates.

Over 100 bacteriophage screenings were run on samples provided, and each one was negative for bacteriophage capable of infecting the industry stain of *T. halophilus*. There were some instances of contamination on the filtrate spot tests, which could be from the 0.45-micron filter being broken due to too much pressure during the sample preparation.

Pictures were taken of several samples' streak plates and screening plates, which are provided in the appendix. The results of these findings (including the test date, incubation date of *T. halophilus*, figure numbers in the appendix, and bacteriophage screening result) are summarized in Tables 1-5, separated by the date that the samples were received.

Table 1: Results Summary of Samples Received on November 9th, 2022.

Sample Name	**Date Received (DR) and Date Tested (DT)**	***T. halophilus* Incubation Date**	**Bacteriophage Screening Result**	**Streak Figure and Bacteriophage Screening Figure**
Dirt dated 4/18/23	Received: 11/9/22 Tested: 4/18/23* 10/23/23	10/20/23 (3 days prior)	Negative	Streak: A1 Screening: A2
Stem/Dirt dated 4/27/23	Received: 11/9/22 Tested: 4/27/23* 10/23/23	10/20/23 (3 days prior)	Negative	Streak: B1 Screening: B2
Lower Stalk Dark Fired Samples 1-5	Received: 11/9/22 Tested: 2/1/23* 11/6/23	11/3/23 (3 days prior)	Negative	Streak: C1-3 Screening: C4-6

* Samples tested previously but retested for streak plate and bacteriophage screening plate figures.

Table 2: Results Summary of Samples Received on December 12th, 2022.

Sample Name	**Date Received (DR) and Date Tested (DT)**	***T. halophilus* Incubation Date**	**Bacteriophage Screening Result**	**Streak Figure and Bacteriophage Screening Figure**
Trash 5A/5B	Received: 12/12/22 Tested: 3/17/23* 10/9/23	10/6/23 (3 days prior)	Negative	Streak: D1 Screening: D2
Semi-Finished Samples 1A-C	Received: 12/12/22 Tested: 1/25/23* 11/13/23	11/10/23 (3 days prior)	Negative	Streak: E1 Screening: E2
Semi-Finished Samples 2A-C	Received: 12/12/22 Tested: 1/31/23* 11/13/23	11/10/23 (3 days prior)	Negative	Streak: F1 Screening: F2
Semi-Finished Samples 3A-C	Received: 12/12/22 Tested: 2/21/23* 11/13/23	11/10/23 (3 days prior)	Negative	Streak: G1 Screening: G2
Semi-Finished Samples 4A-C	Received: 12/12/22 Tested: 2/1/23* 11/13/23	11/10/23 (3 days prior)	Negative	Streak: H1 Screening: H2
Semi-Finished Samples 5A-C	Received: 12/12/22 Tested: 2/8/23* 11/13/23	11/10/23 (3 days prior)	Negative	Streak: I1 Screening: I2
Semi-Finished Final Target	Received: 12/12/22 Tested: 4/18/23* 11/13/23	11/10/23 (3 days prior)	Negative	Streak: C3 Screening: C6
Trash Samples 1A-C, 2 A-C, 3 A-C, 4, 5 A-C	Received: 12/12/22 Tested: 1/25/23* 11/20/23	11/17/23 (3 days prior)	Negative	Streak: J1-3 Screening: J4-6

* Samples tested previously but retested for streak plate and bacteriophage screening plate figures.

Table 3: Results Summary of Samples Received on January 25th, 2023.

Sample Name	**Date Received (DR) and Date Tested (DT)**	***T. halophilus* Incubation Date**	**Bacteriophage Screening Result**	**Streak Figure and Bacteriophage Screening Figure**
2X 5588	Received: 1/25/23 Tested: 10/30/23	10/16/23 (14 days prior)	Negative	Streak: K1 Screening: K2
2X 4654	Received: 1/25/23 Tested: 3/17/23* 10/30/23	10/16/23 (14 days prior)	Negative	Streak: K1 Screening: K2
2X 5503A-B	Received: 1/25/23 Tested: 3/8/23* 10/30/23	10/16/23 (14 days prior)	Negative	Streak: L1 Screening: L2
2X 5490	Received: 1/25/23 Tested: 2/28/23* 10/30/23	10/16/23 (14 days prior)	Negative	Streak: M1 Screening: M2
2X 5483	Received: 1/25/23 Tested: 10/30/23	10/16/23 (14 days prior)	Negative	Streak: M1 Screening: M2

* Samples tested previously but retested for streak plate and bacteriophage screening plate figures.

Table 4: Results Summary of Samples Received on March 13th, 2023.

Sample Name	**Date Received (DR) and Date Tested (DT)**	***T. halophilus* Incubation Date**	**Bacteriophage Screening Result**	**Streak Figure and Bacteriophage Screening Figure**
GCB 80/20 dated 3/9/23 Samples A-E	Received: 3/13/23 Tested: 10/2/23	9/28/23 (4 days prior)	Negative	Streak: N1-3 Screening: N4-5
4654	Received: 3/13/23 Tested: 3/17/23* 10/9/23	10/6/23 (3 days prior)	Negative	Streak: O1 Screening: O2
Cure	Received: 3/13/23 Tested: 3/17/23* 10/9/23	10/6/23 (3 days prior)	Negative	Streak: O1 Screening: O2
INT B/C	Received: 3/13/23 Tested: 4/27/23* 10/9/23	10/6/23 (3 days prior)	Negative	Streak: P1 Screening: P2
GCB 80/20 dated 3/2/23 Samples A-D	Received: 3/13/23 Tested: 3/29/23* 10/23/23	10/20/23 (3 days prior)	Negative	Streak: Q1-2 Screening: Q3-4
GCB 80/20 dated 3/22/23 Samples B-C	Received: 3/13/23 Tested: 10/23/23	10/20/23 (3 days prior)	Negative	Streak: R1 Screening: R2
GCB 80/20 dated 2/17/23	Received: 3/13/23 Tested: 10/23/23	10/20/23 (3 days prior)	Negative	Streak: A1 Screening: A2

* Samples tested previously but retested for streak plate and bacteriophage screening plate figures.

Table 5: Results Summary of Samples Received on May 21st, 2023.

Sample Name	**Date Received (DR) and Date Tested (DT)**	***T. halophilus* Incubation Date**	**Bacteriophage Screening Result**	**Streak Figure and Bacteriophage Screening Figure**
NFC Retain/Ship	Received: 5/21/23 Tested: 9/11/23	8/31/23 (12 days prior)	Negative	Streak: S1 Screening: S2
XFC Retain/Ship	Received: 5/21/23 Tested: 9/18/23	9/8/23 (10 days prior)	Negative	Streak: T1 Screening: T2
B-2 Retain/Ship	Received: 5/21/23 Tested: 9/25/23	9/20/23 (5 days prior)	Negative	Streak: U1 Screening: U2
NIS Retain/Ship	Received: 5/21/23 Tested: 10/2/23	9/28/23 (4 days prior)	Negative	Streak: V1 Screening: V2

Discussion

The goal of this research was to determine whether bacteriophages capable of infecting industry strain *T. halophilus* were present in smokeless tobacco industry-provided samples. From over 100 samples tested, no bacteriophages capable of lysing *T. halophilus* were discovered. While this could be expected from the low reported number of bacteriophages that have been determined to infect *T. halophilus*, our findings reassure our industry partner that there are no bacteriophages currently present in their samples that could harm their *T. halophilus* strain, an essential microorganism in their fermentation process.

To continue this research, it could be beneficial to screen other bacteria prevalent in the provided samples to determine if there are bacteriophages present that could interrupt the industry's fermentation process through infection of a different microorganism. This could be accomplished by using the streak plates to determine a common bacteria present across several provided samples, as several plates contained gram-positive rods. Another option to continue this research would be to screen foods known to contain *T. halophilus*, such as soy sauce, fish sauce, and fermented fish, for bacteriophage. If a bacteriophage is found, screening could be performed with the industry strain of *T. halophilus* to determine if it can infect the bacteria. If bacteriophages are found in either of the described screenings above, genetically engineering the bacteria or the *T. halophilus* strain to resist the bacteriophage could be taken as a preventative measure to avoid potential fermentation delays.

Despite the absence of bacteriophages in our samples, we verified that our screening protocol would be successful by using *E. coli* and T4. Our methods could be repeated in other fermentation industries to search for bacteriophages capable of infecting different critical microorganisms. This would decrease the threat of one of the largest contaminants in the fermentation process, reducing the risk of harmful products for consumers. On a larger scale, this method could be used for phage therapy by discovering bacteriophages that could infect antibiotic-resistant bacteria. By taking samples from areas known to contain antibiotic-resistant bacteria, the enrichment process we used could identify environmental bacteriophages that lyse the resistant bacteria if they are present in the area. With more research to ensure safety, these bacteriophages have the potential to be used as a substitute for antibiotics, providing an alternative route to treating persistent infections. Bacteriophages are incredibly diverse biological agents, and they could provide countless advances in the industrial, clinical, and research fields.

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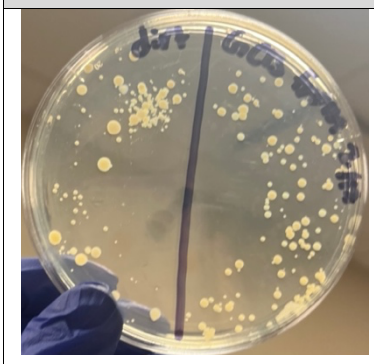
Appendix

I. Streak Plates and Bacteriophage Screenings



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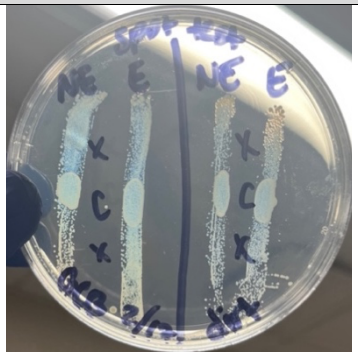

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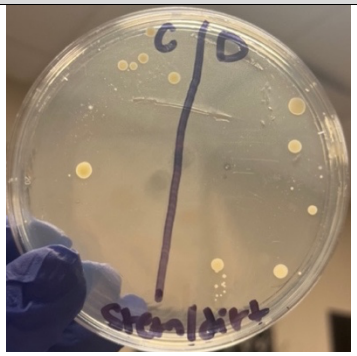

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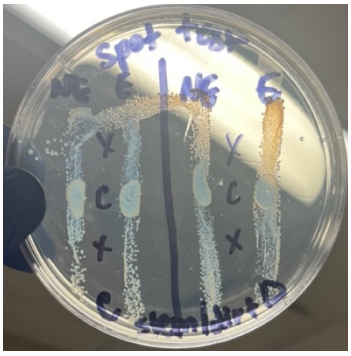

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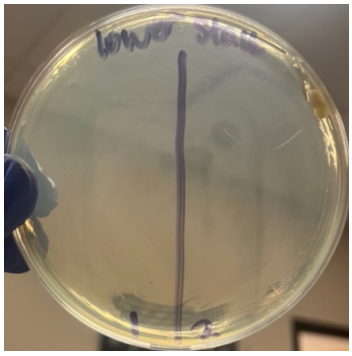

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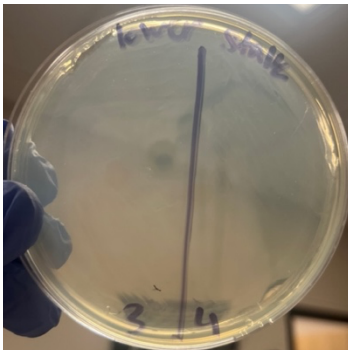

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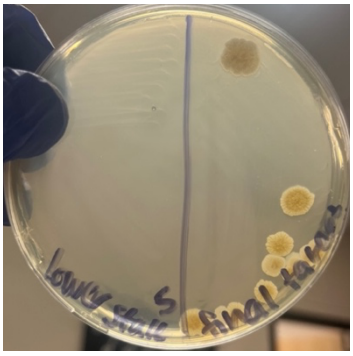

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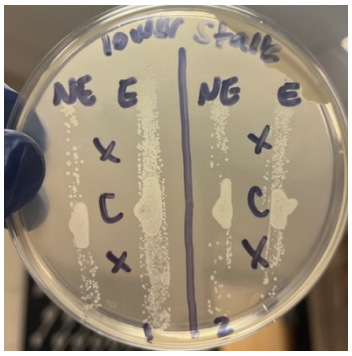
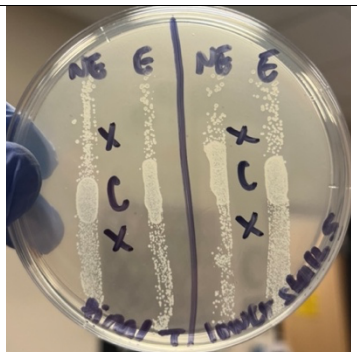


Figure C4

Figure C5



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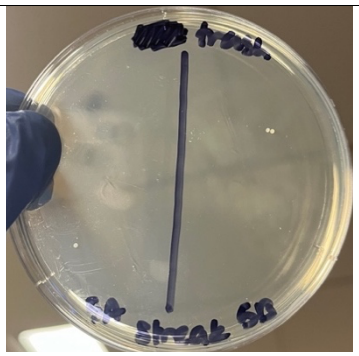

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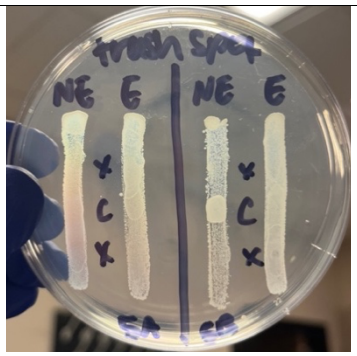

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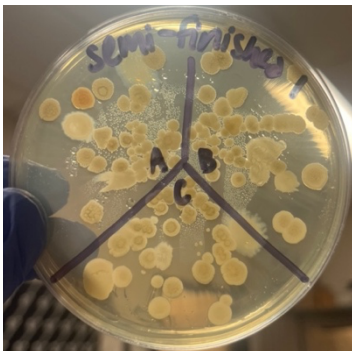

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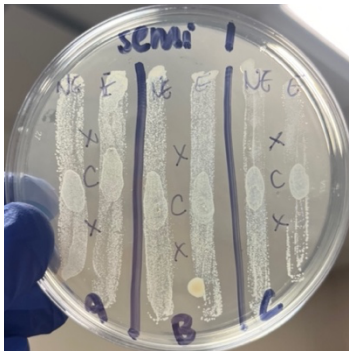

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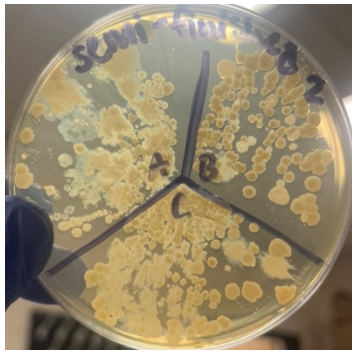

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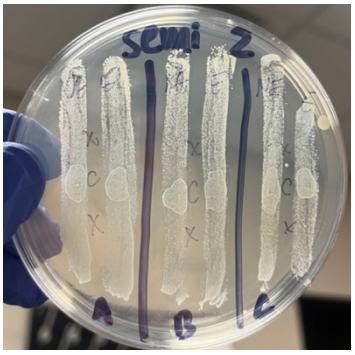

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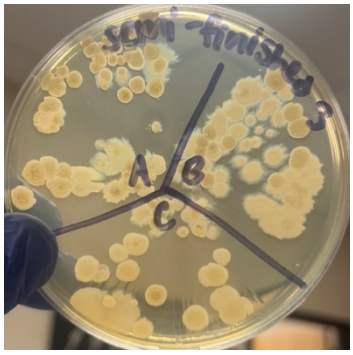

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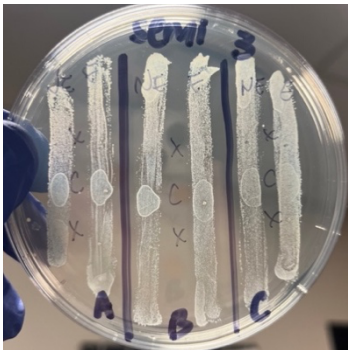

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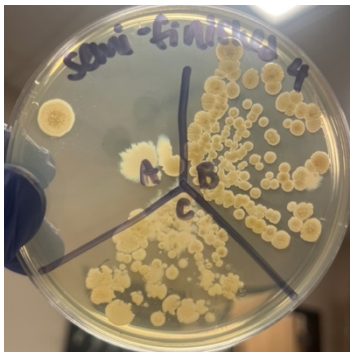

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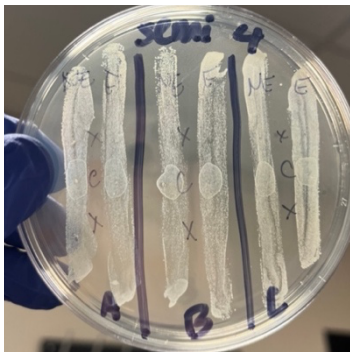

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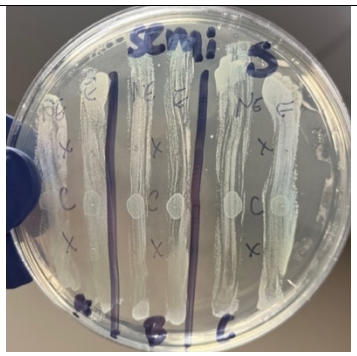

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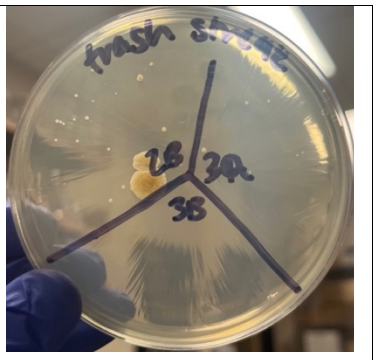

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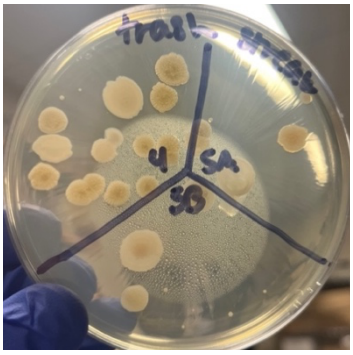

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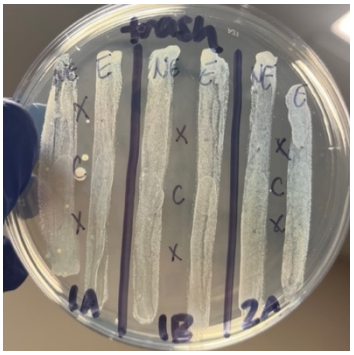

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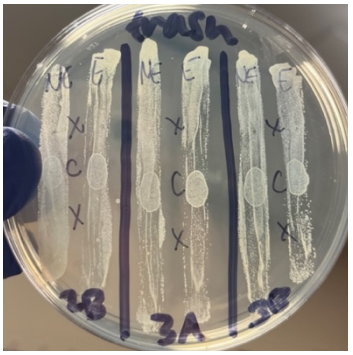

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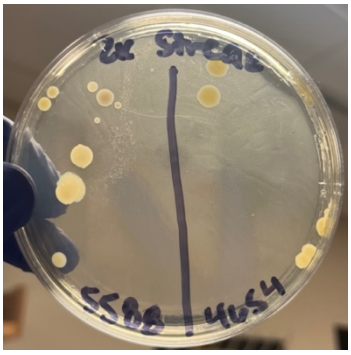

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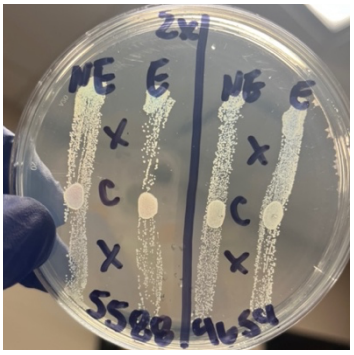

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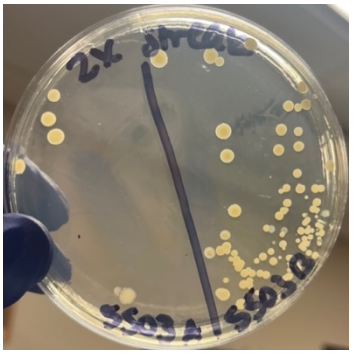

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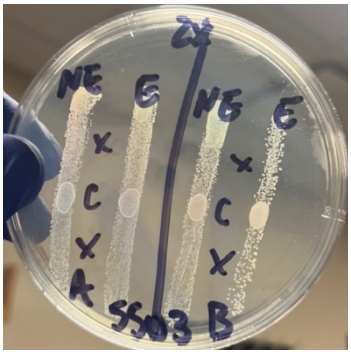

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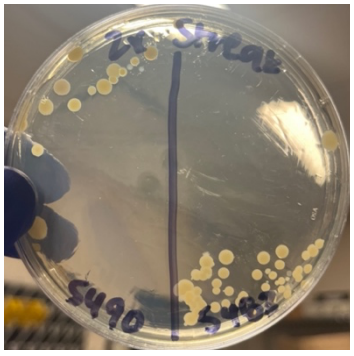

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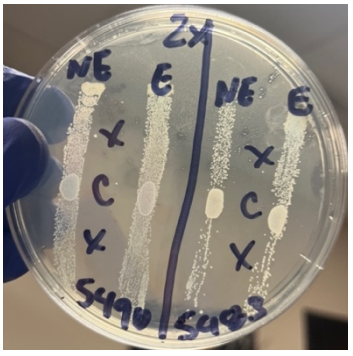

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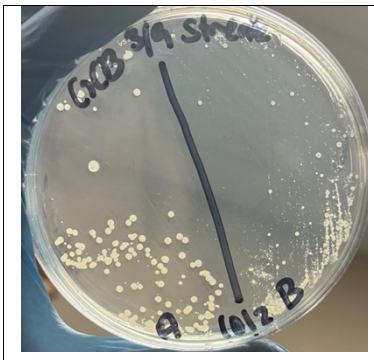

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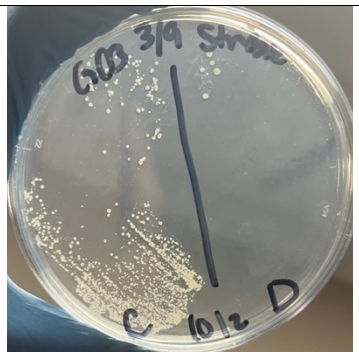

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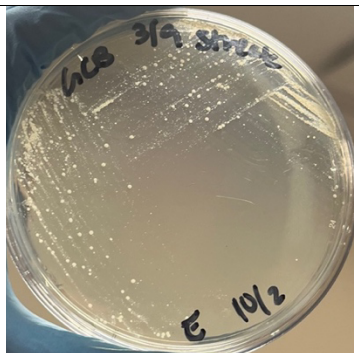

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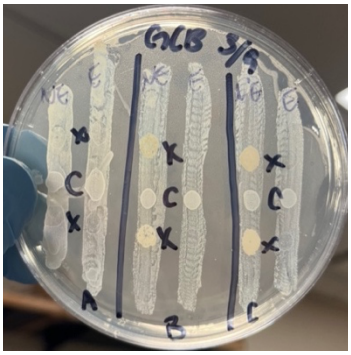

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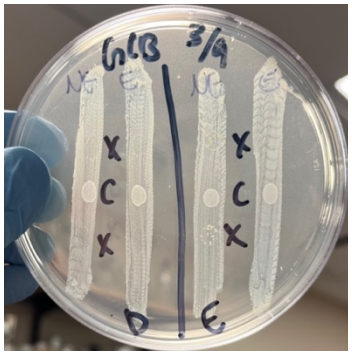

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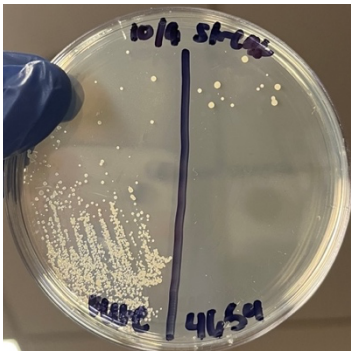

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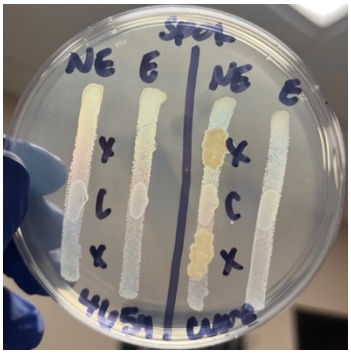

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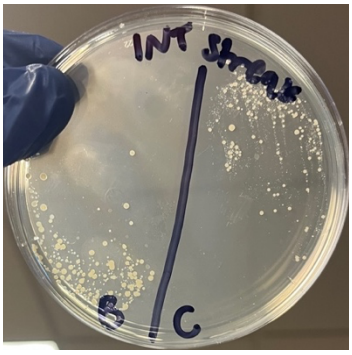

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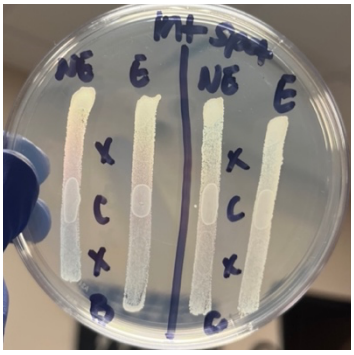

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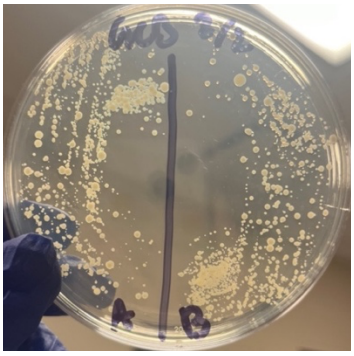

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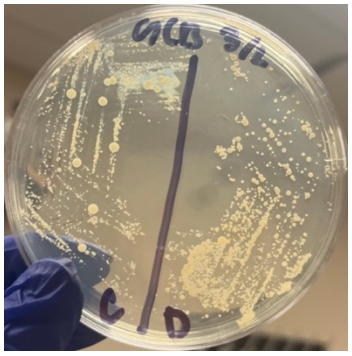

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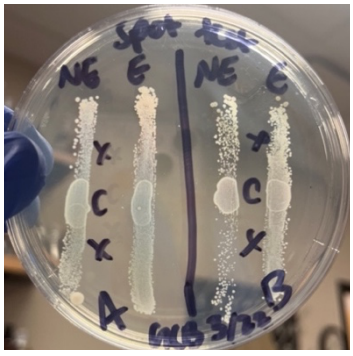

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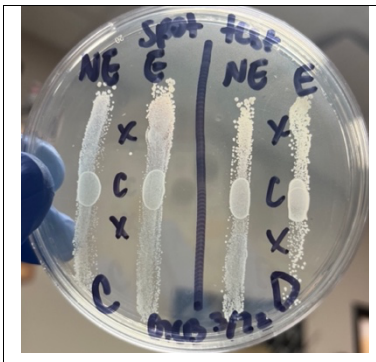

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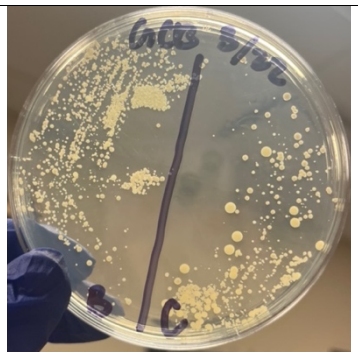

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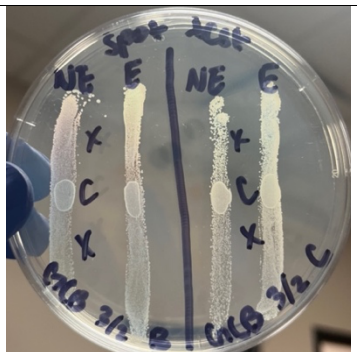

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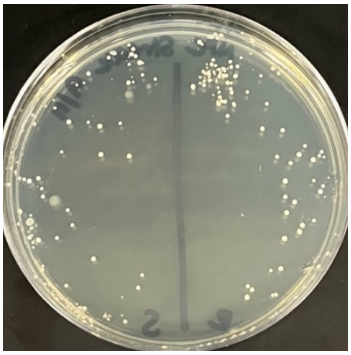

Figure S1

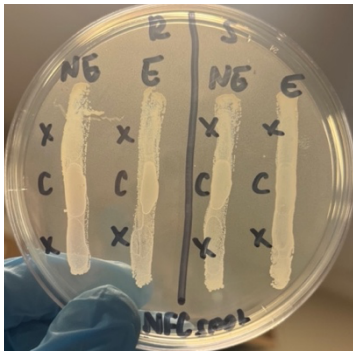

Figure S2

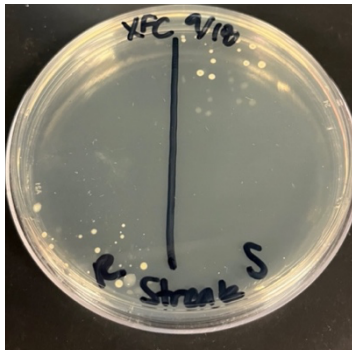

Figure T1

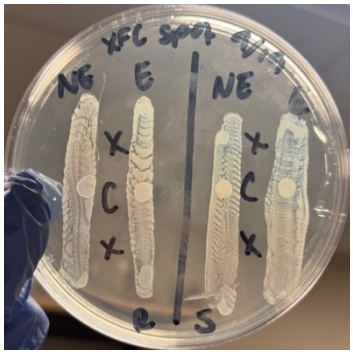

Figure T2

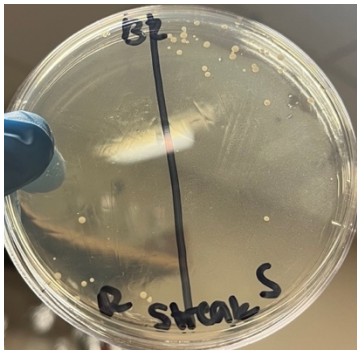

Figure U1

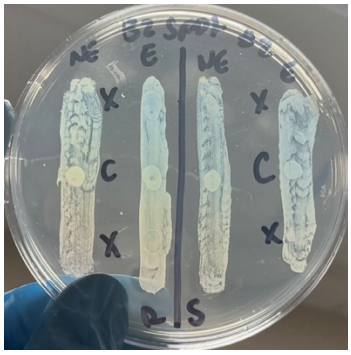

Figure U2

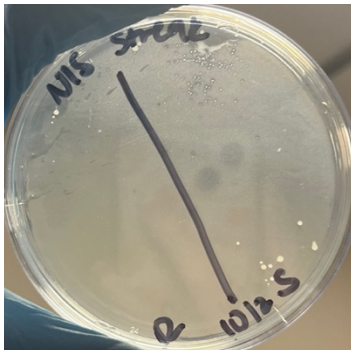

Figure V1

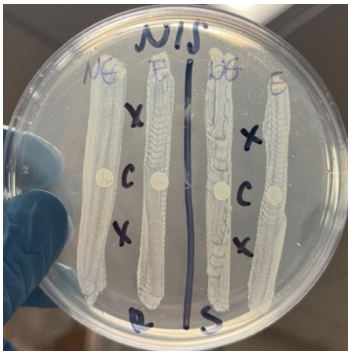

Figure V2