Investigation to Discover Antibiotic Producing Soil Bacteria

Small World Initiative
crowdsourcing antibiotic discovery

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Purpose

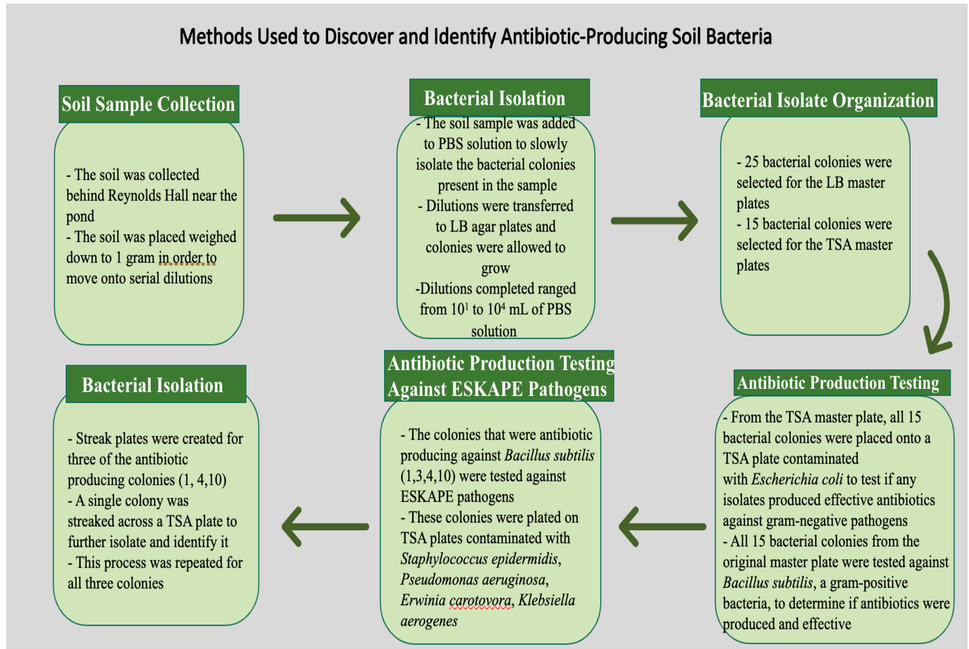
In this lab we will be utilizing a variety of biological concepts as well as scientific methods to isolate and observe bacterial microbes from soil samples collected around the greater St. Louis area. We will follow lab procedures at our own pace according to the unique test results we obtain. By doing this we will be given the chance to see what a true lab environment is like while simultaneously learning a lot about microbiology through real world experiences. We will upload our findings to the Small World Initiative, an international information bank that is working to obtain a better understanding of our environment.

Antibiotics are bacterial microbes that are effective at fighting off pathogenic bacteria, the kind of bacteria that makes you sick. Antibiotics treat disease by using a variety of methods to either kill bacterial cells or slow the spread of bacterial cells. To kill bacterial cells, antibiotics attack the cell walls of the bacteria and or prevent them from creating a cell wall. Antibiotics slow the growth and spread of bacterial cells by blocking processes necessary for the bacterial cells to reproduce. Antibiotics target the cell cycle, specifically processes such as DNA replication, enzyme activity, and protein production. Antibiotics help fight against bacterial cells and lower the amount of pathogenic bacterial cells in the body, helping the ill person to overcome sickness.

Today, antibiotics are widely used in medicine. Antibiotics are given to treat a variety of diseases with varying severities. Bacterial pathogens are beginning to become immune to antibiotics as antibiotic medicine is being overprescribed, taken incorrectly, and no new antibiotics are being discovered. The bacteria start to learn more about the antibiotics as their usage is abused. Mutations form that helps the bacteria become resistant to antibiotic attacks. These genetic mutations are able to be passed between bacterial generations and species, creating superbugs, and the antibiotic crisis. This is a “crisis” as antibiotic resistance is ever growing, and antibiotic discovery is not happening at a fast enough rate to provide new treatments to combat the increasingly dangerous bacteria. If the antibiotic crisis isn't addressed worldwide health will likely decline as mutant bacteria will evolve and create superbug diseases, making getting sick more dangerous than ever before. It will make routine surgeries, broken skin and bones, and petty infections become deadly as the medicine that used to be effective at keeping us healthy and safe during such minor medical issues will now have no way of combatting disease. The doctors will fail to have treatment methods to provide, and many patients will die.

In this semester long project, we will collect samples of soil from around the greater St. Louis area. We will then use a variety of experimental procedures to extract bacteria from the soil, taking it down to the molecular and cellular levels to identify its unique characteristics. Most importantly we are trying to identify antibiotics as we assess the biodiversity and bioactivity of the soil around us. We will follow the procedures outlined in our research guide and research protocol books. General deadlines will be set that will help us guide our research, but we will also be given a great deal of freedom to repeat processes or backtrack if any experiments fail or require repetition. We will learn a great deal through real-world, hands-on application and use of the scientific method. We will upload our findings to the Small World Initiative database which aims to amass information from around the world to help us form a greater understanding of the world beneath our feet.

This is related to the antibiotic crisis as we aid the search for new antibiotics through sharing our research. There is a potential that we will be able to find antibiotics within our soil! Throughout this entire experiment we will be learning about the biodiversity of the soil we collected, which tells us a lot about our environment and the soil around us. The findings we obtain through research and share will hopefully provide enough insight to help medical professionals find and produce more antibiotics quicker and help combat the antibiotic resistance from the cellular angle. Just like space and the ocean, there is so much we still do not know about the dirt beneath our feet as it is so vast and unique. By doing this experiment we will be able to add to the understanding of the biodiversity of the soil around the greater St. Louis area which will in turn help the Small World Initiative gain knowledge about Missouri soil, American soil, and international soil. By collecting this information, researchers and healthcare professionals will hopefully be provided with a better understanding of the soil, its antibiotic potential, and hopefully be able to discover and produce new antibiotics that will be more effective as the bacterial pathogens will not be mutated and immune against them.



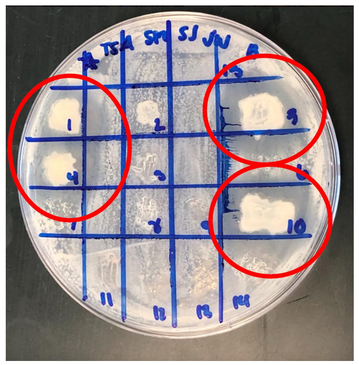


Figure 1: Test of isolates for antibiotic production against *Bacillus subtilis.* Zones of inhibition are visible for isolates 1, 3, 4, and 10.

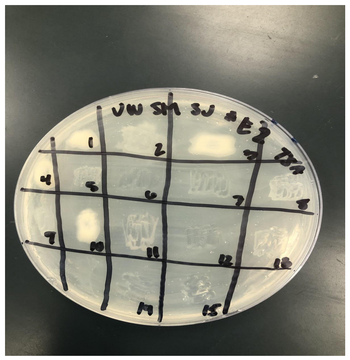


Figure 2: Test of isolates for antibiotic production against *Escherichia coli*. No zones of inhibition are present.

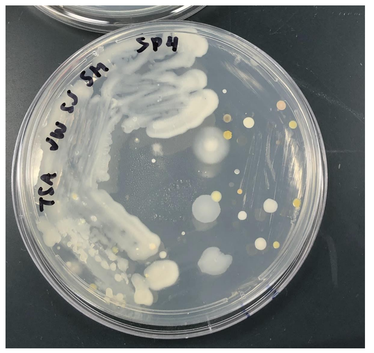


Figure 3: Streak Plate of Isolate Number 4. Other colonies seem to be present on the plate.

Table 1: Number of Isolated Colonies From Dilutions

Dilution Plate Number	Number of Colonies Observed
D1	Too many to count
D2	Too many to count
D3	88
D4	15

Table 2: Master Plate Isolates and Their Antibiotic Production

	Isolate 1	Isolate 3	Isolate 4	Isolate 10
Bacillus subtilis	✓	✓	✓	✓
Escherichia coli	✗	✗	✗	✗
Staphylococcus epidermidis	✗	✗	✗	✗
Pseudomonas aeruginosa	✗	✗	✗	✗
Erwinia carotovora	✗	✗	✗	✗
Klebsiella aerogenes	✗	✗	✗	✗

Isolates that were effective in limiting the growth of pathogenic bacteria are indicated with a checkmark and those that were ineffective are indicated with an “X” mark.

Results

In the experiment, various tests were conducted to determine if the soil sample, which was collected behind Reynolds Hall near the pond , contained antibiotic-producing bacteria. At the start of the experiment, the soil was isolated and grown by dilutions. During the dilutions stage of experimentation, the soil sample was further isolated, creating visible colonies on the LB agar plates. The number of isolated visible colonies was recorded in colony-forming units, also referred to as CFU, and can be summarized in table 1. Cell density can be calculated through colony-forming units, as they provide an approximation of the number of viable cells per gram or millimeter of the sample. There were enough colonies to create master plates and twenty-four individual bacterial colonies were plated. The colonies demonstrate many visible differences. Of the twenty-four colonies, all of them were introduced to *Escherichia coli* and *Bacillus subtilis* bacteria. Isolates 1, 3, 4, and 10 showed zones of inhibition when introduced to the *Bacillus subtilis* bacteria, but no zones of inhibition with the *Escherichia coli*. Refer to figures 1 and 2 and table 2. The isolates that were antibiotic-producing were further explored and introduced to various ESKAPE pathogens. Colonies 1, 3, 4, and 10 were plated on plates contaminated with *Erwinia carotovora*, *Staphylococcus epidermidis*, *Klebsiella aerogenes*, and *Pseudomonas aeruginosa*. None of the isolates were antibiotic-producing against any of the ESKAPE pathogens and there were no apparent zones of inhibition. Streak plates were created for isolates 1, 4, and 10, and isolate number four can be seen in figure 3. Further exploration is required, as other colonies seem to be present on the streak plates.

Discussion

As observed in figure 1 and table 2, when the bacterial isolates were exposed to *Bacillus subtilis*, a relative of a pathogenic bacteria. Bacterial colonies 1, 3, 4, and 10 were the only isolates that showed zones of inhibition on the plates. The zones of inhibition indicate that these bacterial colonies are producing antibiotics that are effective in eliminating *Bacillus subtilis* pathogenic bacteria. However, table 2 and figure 2 illustrate that these, as well as the other bacterial colonies on the tested plates, were not effective against all kinds of pathogenic bacteria. When exposed to *Escherichia coli* and four other ESKAPE pathogens, *Staphylococcus epidermidis, Pseudomonas aeruginosa, Erwinia carotovora,* and *Klebsiella aerogenes*, the bacterial isolates showed no zones of inhibition. This indicates that while these bacteria may be producing antibodies, they are not effective at and fighting against the ESKAPE pathogens. Due to differences in colony morphology and genetic makeup, some antibiotics are more effective at penetrating the cell wall of pathogenic bacteria and slowing the essential reproductive cell processes. Thus indicating that the antibodies present in the bacterial isolates 1, 3, 4, and 10 specifically had the properties to penetrate the cell wall of the *Bacillus subtilis* but not the cell walls of the other ESKAPE pathogens. However, showing strong and repeated evidence of effective antibiotic production against *Bacillus subtilis* proved our bacterial isolates expressed the desired properties to move forward with further testing.
Streak plating followed the pathogen testing. Streak plates were done for bacterial isolates 1, 4, and 10. The initial streak plates proved unsuccessful. When removed from the refrigerator following a two week lab hiatus it could be observed that mold began growing on the plates alongside the isolated bacteria. Mold may have grown on the plate as a result of mold spores that are present within the air. In streaking the plates, these spores may have contaminated the agar plate causing mold growth to occur. Agar plates are specifically formulated to grow bacteria and fungi, creating a superior environment for mold growth. As seen in Figure 3, the bacteria growing on the TSA agar plate had many different colored bacteria, showing that they were not of the same species as the first quadrant of the streak plate. To resolve this error, a second and third set of streak plates were done on the same bacterial colonies. This error may have occurred as a result of individual isolates on the master plate being too close in proximity and other bacteria circulating in the room. With the isolates being too close together, there may have been some contamination. Bacteria that were present within the environment when the streak plates were created are also a potential reason why there were different types of bacteria present on the plate. The second set of streak plates used the bacteria from the first quadrant on the first set. The third set of streak plates used the bacteria from the master plates. These sets were placed in the incubator for three days and were left to sit in the refrigerator for four days. Streak plate #1, taken from the original streak plate, had shown the most promise in creating single isolated colonies. Too many single isolated colonies grew too closely to one another, meaning had they been pulled from the incubator sooner, they would have produced isolated colonies. The same can be said about streak plate #1, taken from the master plate. Had it been pulled from the incubator sooner, there may have been more well isolated colonies to select from. Streak plate #4, taken from the original streak plate, had a lawn of bacteria in the first quadrant but showed no other signs of bacterial growth on the other quadrants. Streak plate #4, taken from the master plate, had similar results, however, had a larger isolated colony in the second quadrant. Streak plate #10, taken from both the master and original streak plates, did grow smaller clusters of bacteria, but not enough that they would have produced single colonies if they were removed from the incubator sooner. A fourth set of streak plates is currently being experimented with. The six plates of the new streak plate set will be removed one day after being placed into the incubator and will be left in the refrigerator for six days.

References

Hernandez S, Tsang T, Bascom-Slack C, and Handelsman. *Small World Initiative.* 4th ed. XanEdu Press. 2016. 2vols.