Antibiotics Isolated from Soil

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Background

Antibiotics are drugs that fight infections caused by bacteria in humans and animals. They work by killing bacteria or by making it difficult for them to grow and multiply.

There are two main ways that bacterial cells can acquire antibiotic resistance. One is through mutations that occur in the DNA of the cell during replication. Through the process of cell replication, some bacteria develop mutations that makes them resistant to antibiotics. Bacteria with the resistant mutation have a better chance of survival against antibiotics. The other way that bacteria acquire resistance is through horizontal gene transfer. Horizontal Gene Transfer is when the antibiotic-resistant genetic material is transferred between different bacteria cells. This can happen in three different ways: transformation, transduction, or conjugation.

Although the antibiotic crisis is a phenomenon that can occur naturally, there is an urgent need to change the way antibiotics are prescribed and used, since the current crisis has been caused by the misuse of these drugs in people and in animals, consuming them in excess or inappropriately. If the antibiotic crisis is not addressed correctly, this could lead to increased medical costs, longer hospital stays and increased mortality. Antibiotic resistance is increasing worldwide to dangerous levels. Day after day, new resistance mechanisms are appearing and spreading across the planet, jeopardizing our ability to treat common infectious diseases.

What we are doing in this semester long Project is isolating soil bacteria to find new drugs to treat antibiotic resistance. We are doing this because most microbes that live on land are considered the main source of antibiotics, and this could help combat antibiotic resistance. It is expected that soil bacteria produce antibiotics because soil is the major reservoir of microorganisms that produce antibiotics. Considering that soil is densely packed with microorganisms, it is not a wonder that many bacterial and fungal species have evolved over the years to develop ways of inhibiting their neighbors for the benefit of their own growth. An antibiotic made by a microbe can inhibit many other soil microbes.

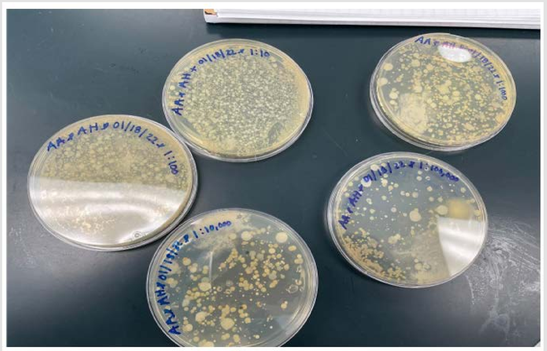


Figure 1: Test for bacteria colonies depicted here shows Plate 1:10,000 & Plate 1:100,000 (the two plates in front) have the most determinable number of colonies

Methods

Sample Collection
- 5g of soil were collected under a tree located at Lindenwood University.

Serial Dilution
- 5g of the soil sample were mixed with 9ml of PBS.
- The dilutions 1:10, 1:100, 1:1000, 1: 10,000, 1: 100,000 were made and were put it on different TSA plates.

Picking and Patching Colonies
- Single colonies of bacteria were picked from a mixed culture of dilutions 1:1000, 1: 10,000, 1: 100,000.
-Using a toothpick, the selected colonies were patched onto a fresh TSA plate.

Screen for Isolate Antibiotic Production
- 2 ESKAPE were used: Escherichia coli and Bacillus subtilis.
- There was 1 TSA plate for Escherichia coli and other TSA plate for Bacillus subtilis.
- Isolates from the master plate were picked and patched around the plates that contained the ESKAPES liquids.

Streak Plate
- Using a toothpick, the colonies from the master plate that produce antibiotics were picked.
- The colonies selected were patched onto a fresh TSA plate in a zigzag form with the toothpick.

Colony PCR Protocol
- Using a toothpick, a single colonies from the streak plate were picked and swirl in PBS.
- Cell suspension were boiled.
- 5µL of cell suspension were added to PCR tube.
- Tubes were transferred to PCR machine.

Gram Stain
- Crystal Violet- A blue or purplish die.
- Iodine- Used to form a complex of crystal violet.
-Alcohol Acetone- Used as a decolorizing agent.
Safarin-A red or pinkish color die.

Results

Upon checking the plates, we realized that there were no colonies, and nothing changed because we didn't put the plates in the incubator to culture. The plates were then put into the incubator to be checked in the following days.

After leaving the plates to incubate for three days, the bacteria cultured and we managed to culture 200 colonies for the 1:10,000 plate and 100 colonies for the 1:100,000 plate. Plates 1:10, 1:100, and 1:1,000 had too many colonies to count thus making it difficult to determine which colonies are not touching.

Bacteria Growth

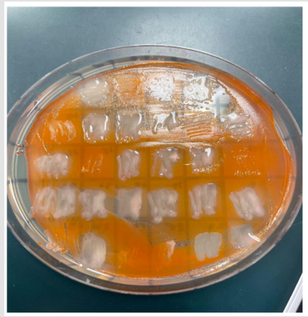
Dilutions	First Soil Sample Growth (CFU)	Second Soil Sample Growth (CFU)
1:10	Too Many To Count	Too Many To Count
1:100	Too Many To Count	Too Many To Count
1:1,000	Too Many To Count	35 colonies = 3.5 x 10^5 CFU
1:10,000	200 colonies =2.0 x 10^7 CFU	55 colonies = 5.5 x 10^6 CFU
1:100,000	100 colonies = 1.0 x 10^9 CFU	17 colonies = 1.7 x 10^7 CFU

As shown above in the table, the first soil sample only produced two plates filled with bacterial colonies and the other three produced too many to count. Our second soil sample produced three plates with bacterial colonies and the other two had too many to count.

CFU Calculations #1:
- **Plate 1:10,000:** (200*10,000) / 0.1 mL = **2.0 x 10^7 CFU**
- **Plate 1:100,000:** (100* 100,000) / 0.1 mL = **1.0 x 10^9 CFU**

CFU Calculations #2:
- **Plate 1:100,000:** (17*100,000) / 0.1 mL = **1.7 x 10^7 CFU**
- **Plate 1:10,000:** (55*10,000) / 0.1 mL = **5.5 x 10^6 CFU**
- **Plate 1:1,000:** (35*1,000) / 0.1 mL = **3.5 x 10^5 CFU**

Master Plates:
- **TSA Master Plates #1:** 1:100,000 Dilution
- **TSA Master Plates #2:** 1:10,000 Dilution
- **TSA Master Plates #3:** 1: 1,000 Dilution



There were bacteria present on the plates when we checked them. The LB Plates cultured the bacteria, but the PDA Plates grew an unknown specimen we could not use.

The ***Bacillus Subtills*** helped cultivate our bacteria, but the soil cannot produce any colonies. We will have to start over with new soil. We have 17 single colonies on the 1:100,000 plates, 35 colonies on the 1:1,000 plates, 55 colonies for the 1:10,000 Plates.

Figure 2: Master Plate shows antibiotics but no zones of inhibition and changed to a bright orange color

Plates 1:10 & 1:100 had too many colonies to count. The TSA Plates cultured the bacteria on plates 1:100,000, 1:10,000, and 1:1,000 and we will make master plates and check them in a few days. Our master plates cultured the bacteria, and we will also make extra TSA master plates for backups and check them in the following days.

The backup master plates cultured and made antibiotics but most of them did not have zones of inhibition and one turned orange but was not viable due to the lack of zones of inhibition. Master Plate #3 was the plate that produced antibiotics and zones on streaks 4, 10, & 20. We used the bacteria from the different isolates to make streak plates. The streak plates we made from Master Plate #3 cultured the bacteria. The streak plate for isolate #4 produced 5 colonies of bacteria, plate #10 produced 1 colony of bacteria, and plate #20 produced no bacteria colonies. Due to spring break, we made our streak plate for isolate #10 again and the plate produced no bacteria at first, but we left it to incubate for a few days. Our streak plate for isolate #10 produced 4 colonies of bacteria which we will use for the PCR gel experiment

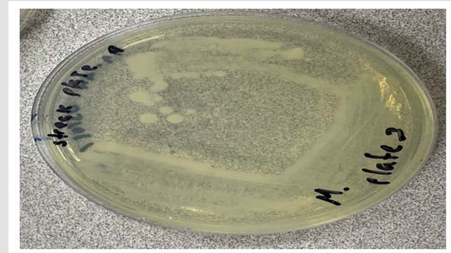


Figure 3: Streak plate from colony 4

Discussion

Antibiotics are chemicals that kill or inhibit the growth of bacteria. Some antibiotics are bactericidal, which means that they work by killing bacteria. Some antibiotics are bacteriostatic, which means that they stop bacteria from multiplying. Certain bacteria produce antibiotics to give them an advantage when competing for food and water and other limited resources in a particular habitat because the antibiotic kills off their competition. The experiment we performed works by creating dilutions of a soil sample and creating spread plates with them. From the soil sample, 5 serial dilutions were made. The dilutions 1:10 and 1:100 had too many colonies of bacteria to count. The dilution 1:1000 obtained 350000 CFU, dilution 1:10,000 obtained 5.5\times{10}^6 CFU and dilution 1:1000,000 obtained 1.7\times{10}^8 CFU. From the dilution 1:1000 a master plate was made with 24 different colonies.

Escherichia coli and Bacillus subtilis were the indicators organisms and they were used to test for the presence of antibiotics produced from the bacteria in the soil. The plates were observed to identify colonies that produced antibiotics. A zone of inhibition surrounds colonies that produce antibiotics, which is an area where the E. coli and B. sub does not grow. Our isolate number 4 was the one which produce a zone of inhibition. Zones of inhibition only extend so far from the colony because the bacterial antibiotic is only strong enough to kill some surrounding bacteria, but not all of it. A bigger area of bacteria-free media surrounding an antibiotic means the bacterium is more sensitive to the antibiotic. Some interesting facts about antibiotics were that antibiotics cannot distinguish between the "good" and the "bad" bacteria in our bodies. Antibiotics are not completely metabolized in the body, and they are released as active compounds in the environment. Antibiotic resistance is becoming one of the world's public health problems. Antibiotic resistance occurs when bacteria change in a way that reduces or eliminates the effectiveness of an antibiotic designed to cure or prevent infections (Johnston).

Acknowledgements

Johnston, B. "Interesting Facts About Antibiotics." Interesting Facts About Antibiotics. N.p., 2009. Web. 30 Mar. 2016.
“Aseptic Technique, Dilution, Streaking, and Spread Plates.” Boundless, 21 July 2015.
“Dilution Theory and Techniques.” World of Microbiology and Immunology, 2003.