Isolation of Antibiotic Producing Bacteria from Local Soil

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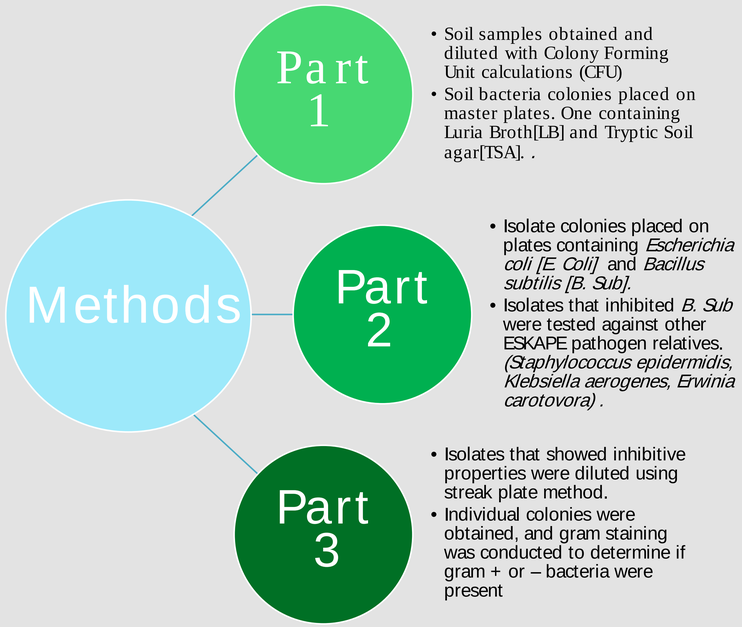
Purpose

Antibiotic resistance has become a global health crisis. There has been a vast production of antibiotics in the last few decades which has led to antibiotic resistance. Many antibiotic-resistant infections are a result of six pathogens known as "EKSKAPE pathogens." ESKAPE pathogens are bacteria that are known for developing multidrug resistance and virulence (Pendleton, et al.). ESKAPE pathogens are important to antibiotic discovery because they help in ensuring that the bacterial isolates are effective against multidrug-resistant bacteria, and hence can further be tested for possibilities of antibiotic production (Mulani et al.). Many antibiotics that are used today for commercial and clinical use are derived from soil bacteria. This research utilized soil bacteria, collecting and isolating bacteria that can be further tested for inhibition zones. Inhibition zones are perimeters surrounding a soil isolate where no pathogenic bacteria grow (Hernandez et al.). Testing these isolates against safe relatives of ESKAPE pathogens could be used to determine whether the isolate can indeed inhibit the growth of tester strains. To safely determine the structure of the cell wall of the bacteria, gram staining is used. Gram staining is a process used to identify whether a bacteria isolate is gram-positive or gram-negative. Gram-positive is identifiable when crystal violet dye is added, their thick peptidoglycan layer clings to the dye, making the purple color visible under a microscope. Gram-negative bacteria have a thin peptidoglycan layer, so they will not cling to the dye and will appear pink or red under a microscope (Tripathi). Testing against safe relatives of ESKAPE pathogens and conducting gram staining ensured that the process of screening for new bacteria was successful. This process contributes to the search for a new bacterial species that can produce antibiotic substances.

Procedure



Results

Figure 1: Serial Dilutions

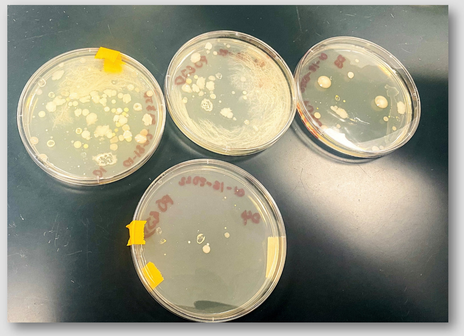


Figure 3: Gram Stain Results for Isolate 5 LB (A), Isolate 7 TSA (B), and Isolate 4 LB (C)

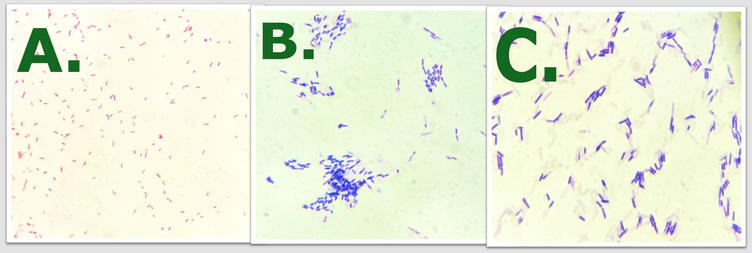
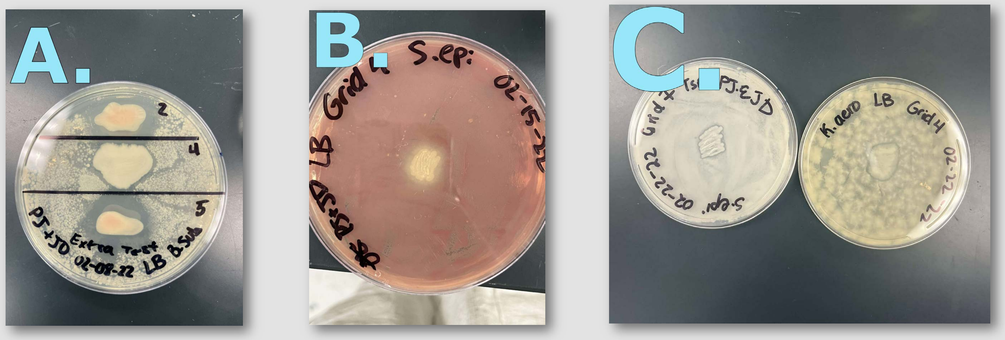


Figure 2: Zones of inhibition observed on Isolate 2,4,5 LB (A), isolate 4 LB (B) and Isolate 7 TSA alongside Isolate 4 LB (C)



Results cont'd

- Colonies were counted to determine the colony forming unit values for each dilution plate. The average CFUs on the TSA agar appeared to be higher than the average CFUs calculated on the LB agar.
- Isolate 2, 4 & 5 (LB) exhibited antibiotic activity against *B. Subtilis.* (Figure 2, A)
- Isolate 4 also exhibited antibiotic activity against *S. epidermidis* (Figure 2, B).
- Isolate 4 (LB) also exhibited antibiotic activity against *K. aerogenes*. (Figure 2, C)
- Through further testing, isolate 4 was also identified as gram positive with a streptobacillus shape (Figure 3, C) .
- Isolate 5 (LB) did not show antibiotic activity against other ESKAPE pathogen relatives. Through further testing, isolate 5 was found to be gram negative with a bacillus shape (Figure 3, A) .
- Isolate 7 (TSA) exhibited antibiotic activity against S.*epidermidis*. (Figure 2, C)
- Through further testing, isolate 7 was identified as gram positive with diploid bacilli shapes. (Figure 3, B)

Discussion

It was possible to successfully obtain antibiotic producing isolates from soil microbes. Serial dilutions were done to isolate bacteria from the soil sample. The dilutions were then placed on agar media in order to grow colonies of the soil bacteria. More colony forming units were observed on the Tryptic Soy agar [TSA] in comparison to Luria Broth [LB] agar. Specific isolates were placed on master plates and were then tested against pathogens, *Escheria Coli [E.coli] and Bacillus Subtilis [B.subtilis],* for zones of inhibition. Zones of inhibition were observed on three isolates (Grid 4 and 5 [LB] and Grid 7 [TSA]) on the plates containing *[B.subtilis],* while no zones of inhibition were observed on the plate containing *[E.coli].* The isolates that were found to inhibit the growth of *[B. Subtilis]* were then tested against ESKAPE pathogen relatives, *Staphylococcus epidermidis [S. epidermidis], Klebsiella aerogenes [K. aerogenes], and Erwinia carotovora [E. carotovora].* The grid 4 LB isolate showed inhibition of *[S. epidermidis]* and *[K. aerogenes].* The grid 7 TSA isolate showed inhibition of *[S. epidermidis].* In order to further identify the types of bacteria that were growing, gram staining was conducted. Gram staining revealed that the grid 7 TSA isolate, and the grid 4 LB isolate were both gram positive. Grid 7 appeared to have a diploid bacilli shape while grid 4 appeared to have a streptobacillus shape. Grid 5 LB appeared to be gram-negativenegative with a bacillus shape. Unfortunately, the bacteria could not be specifically identified due to a failure in the PCR and gel electrophoresis procedure. The three isolates that were found appear to be different strains of bacteria due to their different shapes, along with their different gram staining results. All three strands exhibit antibiotic qualities. The Small World Initiative is making strides in antibiotic discovery from soil microbes, and further testing can be conducted on these isolates to aid in the fight against multidrug-resistant bacteria.

Acknowledgements

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