Discovering Antibiotic Producing Bacteria from Soil

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Introduction

Antibiotic resistance is one of the biggest public health concerns worldwide. The six ESKAPE bacterial pathogens are highly virulent and increasingly resistant to antibiotics. These six species are responsible for most hospitalizations and deaths related to pathogenic bacteria[1]. Bacterial species found in soil have yielded antibiotics that can kill pathogens resistant to many drugs. The majority of novel antibiotics have been discovered by screening bacteria obtained from soil and other natural habitats for antibiotic producing ability. In this study, soil was collected from the lawn of DeSales University campus and bacteria were extracted. These bacteria were tested for antibiotic activity against several non-pathogenic relatives of the ESKAPE pathogens. We have isolated and are characterizing a bacterium which demonstrates antibiotic activity against two ESKAPE relatives.

ESKAPE Pathogen	SAFE Relative
Enterococcus faecium	*Bacillus subtilis*
Staphylococcus aureus	*Staphylococcus epidermidis*
Klebsiella pneumoniae	*Escherichia coli*
Acinetobacter baumannii	*Acinetobacter baylyi*
Pseudomonas aeruginosa	*Pseudomonas putida*
Enterobacter species	*Enterobacter aerogenes*

Figure 1: The six ESKAPE bacterial pathogens and their corresponding SAFE relatives.

Methods

- A sample of soil was collected from a lawn located on the DeSales University campus.
- A serial dilution was performed using 1g of collected soil to create stocks of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} concentrations.
- On LB + 10 mg/L of cycloheximide plates, we spread the serial dilutions using the hockey stick method and incubated them at 37°C overnight.
- A master streak plate was created from isolated individual colonies grown on the serial dilution plates and incubated at 37°C overnight.

Figure 2: The location on the DeSales Campus where the soil sample was taken.

- LB broth was inoculated with *S. epidermidis, E. raffinosus, M. smegmatis, B. subtilis,* and *L. antibioticus* and incubated at 37°C overnight without shaking.
- LB+cycloheximide plates were plated with lawns of the 5 tester strains.
- A sterile velvet was used to replica plate the soil-derived bacteria from the master streak plate to the tester strain plates.
- These were incubated at 37°C overnight before inspection for zones of inhibition.

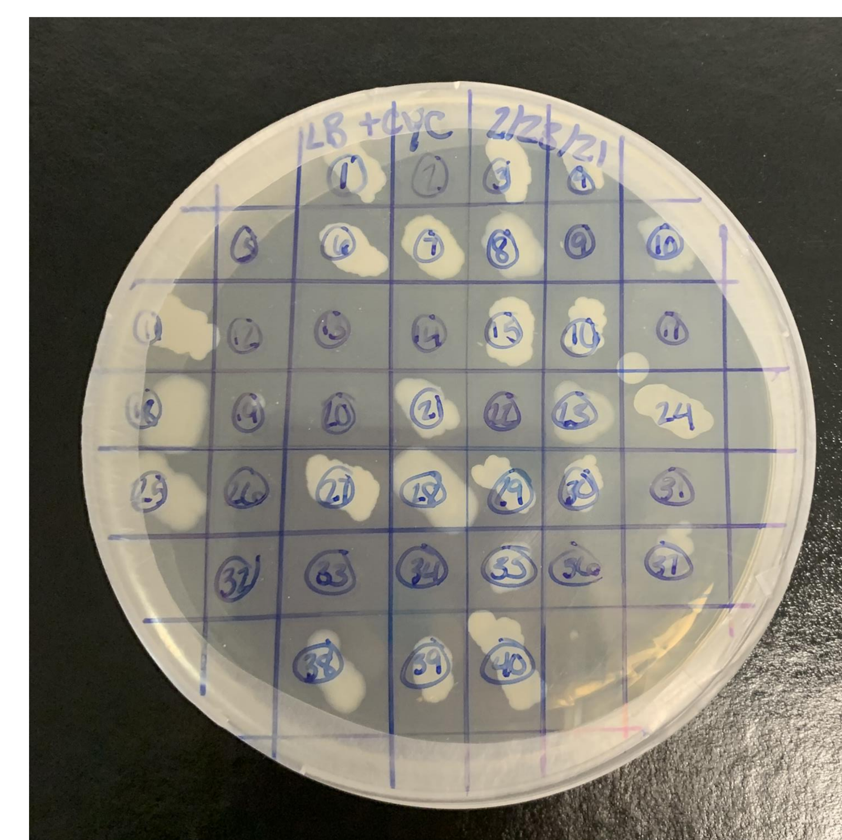

Figure 3: The masterplate obtained from individual colonies of the soil bacteria after incubation

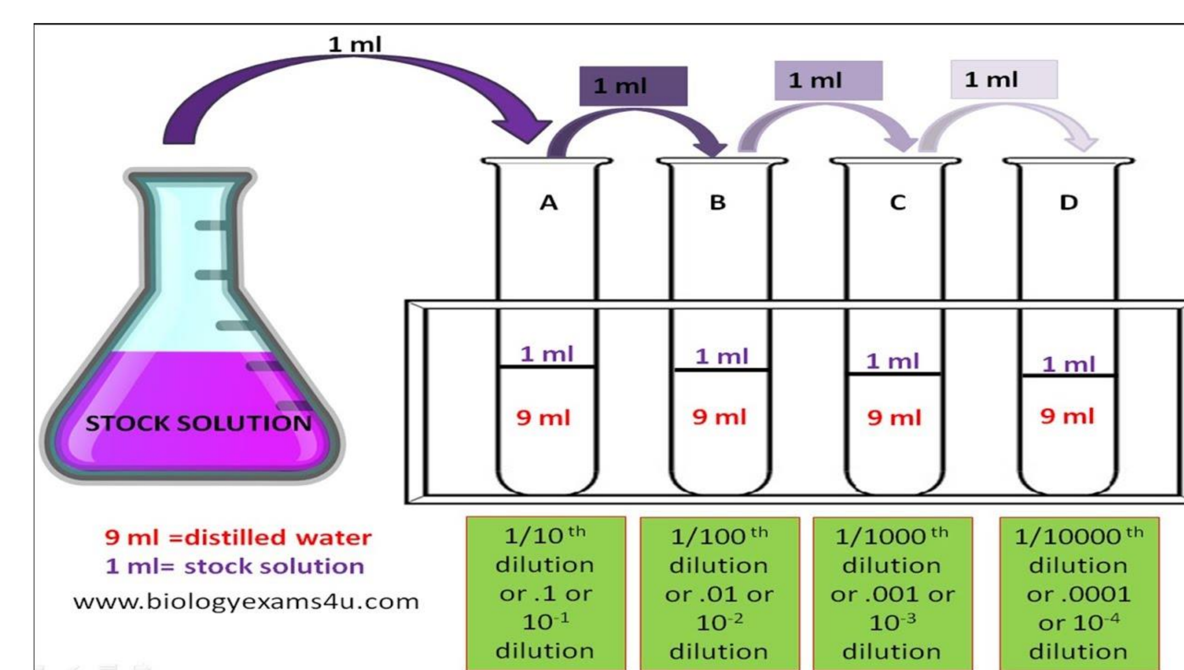

Figure 4: Serial dilution performed on the collected soil
https://www.biologyexams4u.com/2013/12/serial-dilution-protocol-pdf.html

Results

We discovered a zone of inhibition around one of the isolated soil colonies on the replica plates inoculated with *B. subtilis* and *S. epidermidis.* Using the master plate as a guide, we determined that isolate #35 was the bacterium producing the antibiotic.

Figure 5: The zone of inhibition on the *B. subtilis* plate produced by bacterium #35

Figure 6: The zone of inhibition produced by bacterium #35 on the *S. epidermidis* plate

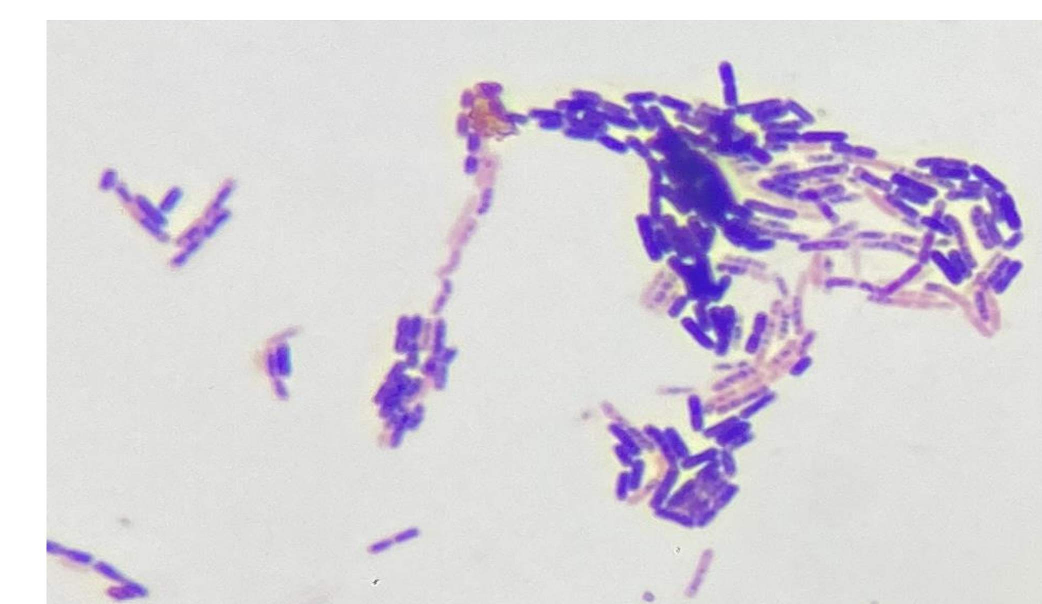

Figure 7: Gram stain of bacterium #35

We performed a Gram stain and ascertained that #35 is a gram-positive bacillus. We also determined that #35 is catalase positive which means it produces an enzyme that breaks down toxic reactive oxygen species into oxygen gas and water.

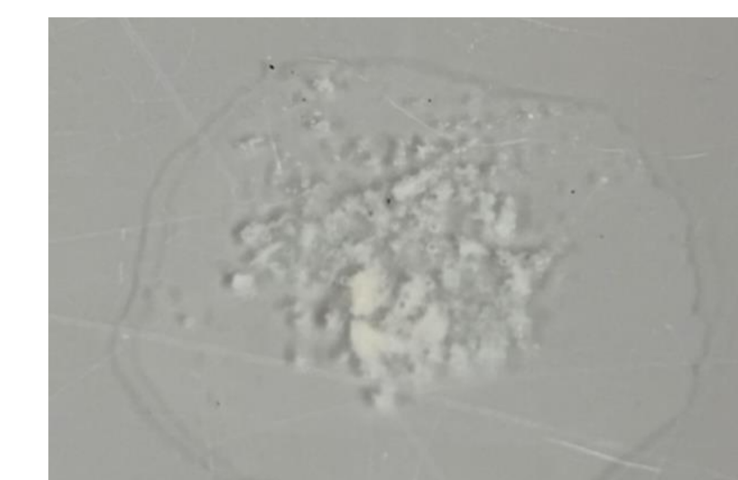

Figure 8: Bubbles produced by bacterium #35 in the presence of hydrogen peroxide indicating that it produces catalase

Discussion

- The next step is to identify and characterize the bacterium isolate genetically.
- We have PCR-amplified a portion of the 16S ribosomal RNA gene and are outsourcing our product for DNA sequencing.
- The resulting DNA sequence will be compared to a bacterial genomic database to narrow down #35's identity to the species level.
- We will continue to test #35 against more ESKAPE relatives to assay for zones of inhibition.
- We will also attempt to extract and purify the antibiotic for biochemical analysis.
- Finally, we will continue testing the ESKAPE bacteria relatives against soil bacteria in hopes of finding more novel antibiotic-producing bacteria.

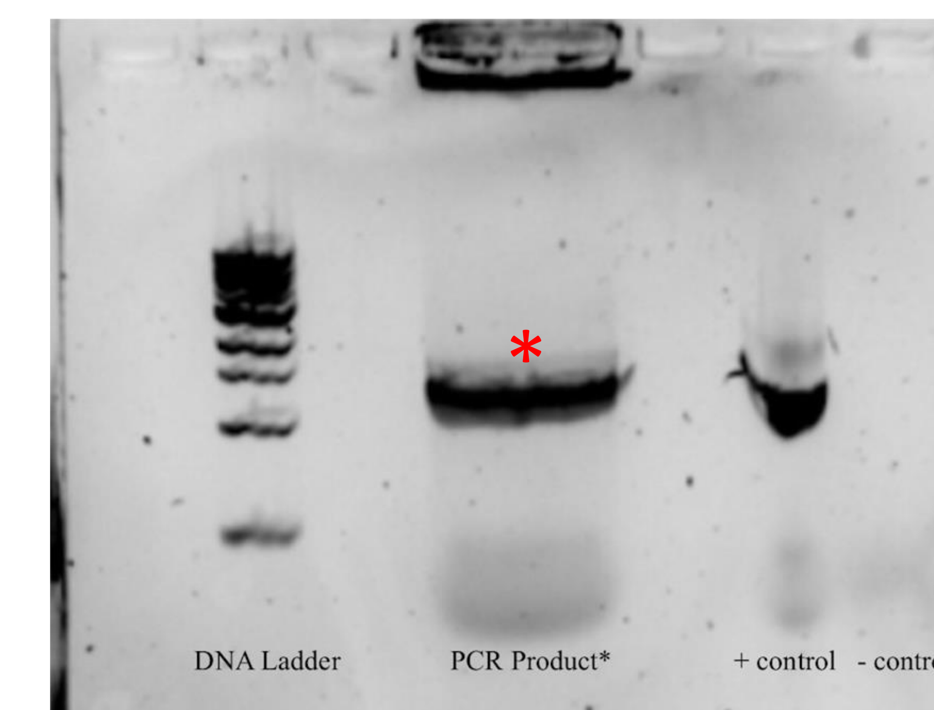

Figure 9: Colony PCR amplification of the 16S rRNA gene from bacterium #35 using primers 27F and 1492R.

*PCR product of expected molecular weight

1. CDC. Antibiotic Resistance Threats in the United States, 2019. Atlanta, GA: U.S. Department of Health and Human Services, CDC; 2019.