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Screening for antibiotic-producers in soil from a garden

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Screening for antibiotic-producers in soil from a garden

Long Tran and Dr. Lori Scott

INTRODUCTION

The antibiotic resistance is a naturally occurred process of the bacteria, which has been accelerated by the widespread use of antimicrobial agents (1). More than 30,000 people in the United States die each year due to multidrug-resistant infection (2). Although many bacteria are still vulnerable to antimicrobial agents, a small group, referred to as "ESKAPE bugs", can avoid the lethal effect of those drugs (3). ESKAPE bugs are nosocomial pathogens, which means they are caught in a hospital. They include *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* spp.

Tiny Earth initiated a worldwide project called Tiny Earth Project Initiative (TEPI) to convey the antibiotic resistance crisis, intrigue students' interest in science via original research, and discover new antibiotics producing bacteria in the soil (4). Soil microbes are important in terms of breaking down the complex materials and cycling the nutrients into the ecosystem. They are living a harsh and competitive environment so it's likely that they produce antibiotics as tools for eliminating or suppressing other bacteria. Most of the natural antibiotics in use today are isolated from *Streptomyces,* a soil microbe. As a matter of fact, the soil becomes the source of potential antibiotic-producers in this project. Due to the danger of multidrug-resistant bacteria, safe relatives of ESKAPE pathogens were utilized for student's research purposes: *Bacillus subtilis* and *Escherichia coli* (5).

B.subtilis are rod-shaped, Gram-positive, and endospore producing germs that occur singly, in pairs, or in chains (6). *B.subtilis* can be found in soil, water, and food. The colony morphology on the LB broth agar plate is circular, cloudy, slightly yellow, and spiky edges (7). The biochemical tests for *B.subtilis* include negative indole formation, negative methyl red, and positive Voges Proskauer test.

E.coli are motile, bacillus, and Gram-negative bacteria (8). They are naturally be found in the intestine of humans and animals, singly or in pairs. Most strains of *E.coli* are benign but few detrimental strains are able to cause severe stomach cramp, bloody diarrhea, and nausea (9)

Soil microbes are thriving in A Horizon, where organic matters are rich (4). To screen for the antibiotic producer, a soil sample from the Augie Acres, student-run garden, was collected at a depth of 3.5 inches.

Figure 1. *E.coli* normally live in the intestine of warm-blooded animals. https://www.inquirer.com/health/ecoli-explained-outbreak-romaine-fda-cdc-20191125.html

The project goals are to comprehend the biodiversity in a student-run garden's soil and collect data regarding the novel antibiotics-producers that can potentially inhibit the growth of safe relatives of ESKAPE strains.

METHOD

The material and method was primarily based on TEPI Protocol (4):

1) Soil sample collected at Augie Acres.

2) Separation of individual colonies.
- Serial dilution plate
- Incubated at 28°C for 24hrs to 72hrs

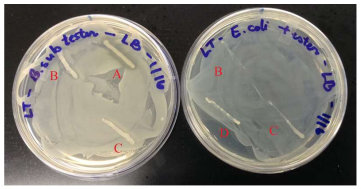
3) Generation of master plates by pick-and-patch
- LB; 10% TSA; and PDA media
- Incubated at 28°C for 24hrs

4) Antibiotic-producer test:
- Technique 1 and 2
- *B.subtilis* and *E.coli*
- Incubated at 28°C for 24hrs

5) 16S rRNA sequencing
- Sequencing facility: Iowa Institute of Human Genetics, U. of Iowa
- 16S rRNA primers are the 27F and 1492R

6) Biochemical tests

RESULTS



A: isolate 8 on LB master plate C: isolate 10 on PDA master plate
B: isolate 9 on PDA master plate D: isolate 9 on LB master plate

Figure 2. Isolate 8 on the LB master plate produces antibiotics against *B.subtilis*, which was indicated by the appearance of a halo. Potential antibiotic producers from the soil were tested against two tester strains: *E.coli* (right) and *B.subtilis* (left). A tester strain was spread on LB plate, and the isolates were patched onto the plates. All plates were incubated at 28°C for 24 hours. On the *B.subtilis* tester plate, only A showed a zone of inhibition. B and C, previously showed halo on PDA media, did not show any zone of inhibition in this trial. On the *E.coli* tester plate, no zone of inhibition was observed in this trial either.

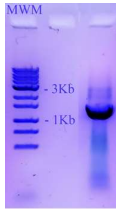


Figure 3. Gel electrophoresis confirms the successful colony PCR of the 16S rRNA sequence of the potential antibiotic-producing isolate A (isolate 8 on LB master plate) with the molecular weight of about 1.5Kb. 5ul of the molecular marker from Bullseye and 10ul of DNA were added. The gel ran for 30 minutes. MWM=molecular weight marker.

RESULTS (CONTINUED)

Description	Query Cover	E value	Per. Ident
Pseudomonas mosselii strain CFML 90-83 16S ribosomal RNA, partial sequence	98%	0.0	99.72%
Pseudomonas entomophila L48 16S ribosomal RNA, partial sequence	98%	0.0	99.43%
Pseudomonas taiwanensis DSM 21245 strain BCRC 17751 16S ribosomal RNA, partial sequence	98%	0.0	99.36%
Pseudomonas entomophila L48 16S ribosomal RNA, partial sequence	98%	0.0	99.15%
Pseudomonas plecoglossicida strain NBRC 103162 16S ribosomal RNA, partial sequence	98%	0.0	99.22%
Pseudomonas monteilii strain CIP 104883 16S ribosomal RNA, partial sequence	98%	0.0	99.22%

Figure 4. The isolate 8 on LB master plate is most closely related to *Pseudomonas,* according to the 16S rRNA sequence data. BLAST analysis was utilized to compare the 16S rRNA sequence of the unknown bacteria with the sequence from the NCBI database (10). Query Cover is the percentage of the sample that was compared to the sequence in the database; E value is the likelihood that the sequence in the database matched these tested sequences by chance. Per. Ident is the percentage identical between the sequences.

DISCUSSION

Pseudomonas is the potential antibiotic producers against the *B.subtilis* on LB media. *Pseudomonas* is one of the most dominant genera in the soil and up to 23% of this strain has antibiotic-producing genes (11 – 12). As the identity of the isolate was revealed, the next step for this project will be searching for the active antibiotic compound that it produces, developing a plan to purify the substance, and studying the mechanism which this strain of *Pseudomonas* uses to generate a zone of inhibition. This project was successful because an antibiotic-producer was identified, which contributed to the TEPI's common database and the understanding of soil microbes in this region.

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