Isolating Antibiotic-producing Bacteria From Soil

Michelle Santiago
Augustana College, Rock Island Illinois

Dr. Lori Scott
Augustana College, Rock Island Illinois

Follow this and additional works at: https://digitalcommons.augustana.edu/biolmicro

Augustana Digital Commons Citation
Santiago, Michelle and Scott, Dr. Lori. "Isolating Antibiotic-producing Bacteria From Soil" (2020). Identifying and Characterizing Novel Antibiotic Producing Microbes From the Soil.
https://digitalcommons.augustana.edu/biolmicro/6

This Poster is brought to you for free and open access by the Biology at Augustana Digital Commons. It has been accepted for inclusion in Identifying and Characterizing Novel Antibiotic Producing Microbes From the Soil by an authorized administrator of Augustana Digital Commons. For more information, please contact digitalcommons@augustana.edu.
INTRODUCTION
The misuse of antibiotics, such as persistent use or simply not finishing the prescribed dosage, and nosocomial (medically acquired bacteria) pathogens can be detrimental to the health of an individual as it causes bacteria, specifically the ESKAPE pathogens, to become resistant to such antibiotics. The ESKAPE pathogens (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species) are those that make up most of the antibiotic-resistant infections in medical settings (1). As a way to help fight off those bacteria, emerging strategies have been developed, such as antibiotic combinations, bacteriophage therapy, antimicrobial peptide therapy, and photodynamic light therapy (2). Unfortunately, these ESKAPE pathogens are becoming more resistant and there are simply not enough antibiotic combinations to successfully eliminate the strains (2). Microbes are proliferating and revolutionizing much faster than antibiotics being discovered, further contributing to this crisis. Due to this complication, the Tiny Earth Project was created. The project focuses on educating students about the antibiotic adversity and to find novel antibiotics from soil (1). For this projects, ESKAPE-like strains will be utilized to minimize exposure to pathogens, and the TEPI protocol was followed unless otherwise noted. Contribution to the TEPI project will allow us to discover potential antibiotic-producing bacteria from soil.

Our soil isolates will be tested on two ESKAPE-like strains: Escherichia coli and Bacillus subtilis. E. coli is a safe relative of Klebsiella pneumoniae and is gram-negative, rod-shaped (bacilli), and use flagella for motility. E. coli grown on solid medium will display round, dull colonies that are white in color with entire margins (3). Most E. coli can be harmless and a part of the normal flora in an individual, but some strains can cause harm, such as diarrhea or urinary tract infections (UTI) (3). Bacillus subtilis is not considered pathogenic (unless exposed to immunocompromised individuals) and is commonly used as a fungicide/pesticide in farms because of its spore-forming nature (4). B. subtilis, as given away by its name, is also bacilli in shape. This strain is Gram-positive, therefore, there is a thick layer of peptidoglycan in its cell wall. B. subtilis has an interesting colony morphology as it grows rapidly, and the edges are rough and irregular (5). Our contribution to the TEPI project is vital in order to find potential antibiotic producers from soil. As more antibiotics are discovered, nosocomial infections will hopefully decline and mechanisms to oppose the ESKAPE pathogens will be discovered.

METHODS
Unless described otherwise, the bacterial strains and protocols used in this study were provided by the Tiny Earth Project Initiative (TEPI) (1).
- Obtained rich soil sample from a garden (depth= 4 in.) at 41.4 °N, 90.5° E.
- Dilution of soil sample using PBS
- Created spread plates on different media (LB, 10%, TSA, PDA). Analyzed after 24-72 hours of incubation at 28 °C
- Master plate created and incubated for 24 hours at 28 °C
- Tested for antibiotic production using Bacillus subtilis or Escherichia coli. Incubated at 28 °C
- Analyzed for possible “halos” around the isolates placed on either B. subtilis or E. coli and created streak plate isolates. Isolates were re-streaked again to confirm antibiotic production
- PCR and Gel Electrophoresis (1% agarose) for 30 minutes at 100 V.
- Gel extraction and sequenced at Iowa Institute of Human Genetics, University of Iowa. The primers used were 27F and 1492R.
- Biochemical tests, including Gram stain, and were performed following supplier instructions
- Used NCBI BLAST for genus analysis.

RESULTS
When creating the spread plates of B. subtilis and E. coli to test against the soil isolates, it was observed that E. coli grow on LB agar but did not grow as well on the more selective mediums (10% TSA, PDA). This may be an explanation as to why the isolates did not produce an antibiotic against E. coli. Since E. coli did not grow well, the isolates might not have been able to produce an antibiotic in order to compete for resources. Therefore, the only inhibition zones were observed against B. subtilis. Of those isolates, three were chosen to re-test for antibiotic production. Only two confirmed antibiotic production against B. subtilis (MS-9-LB-B. subtilis, MS-36-TSA- B. subtilis). Strangely enough, MS-36-TSA had originally produced a zone of inhibition on 10% TSA and LB agar but when screened again, it only produced it on LB agar. MS-9-LB-B. subtilis remained consistent as it produced the “halo” on LB agar and 10% TSA.

RESULTS (CONTINUED)
Only MS-9-LB-B. subtilis and MS-36-TSA- B. subtilis were chosen for gel extraction, sequencing, and biochemical tests, such as gram-stains, catalase test, among others. The sequencing results were sent back a couple of days later. This sequence was inserted to the NCBI BLAST program to compare it to known microorganism sequences (6). The bacterial genus that was mostly related to the 16s rRNA sequence of MS-36-TSA- B. subtilis is Pseudomonas. MS-9-LB-B. subtilis was mostly related to the Bacillus genus, which is interesting because it produced an antibiotic to another microorganism of the same genus.

LITERATURE CITED