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Identification of Uncommon Antibiotic-Producing Illinois Soil Isolates

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INTRODUCTION

The discovery of penicillin transformed the medical field in 1928 by using these antibiotics to combat infectious bacteria. Infectious microbes from hospitals known as nosocomial bacteria have become more responsive through natural selection and have been able to resist antibiotics leaving researchers to continue this ongoing battle with each passing year.[6,12] 15.5% of nosocomial infections result in an increase in mortality rate and the cost of health care.[6] Microbes have formed a part of the soil crisis by acts of erosion that can result to be harmful to the human body directly or indirectly. Although we live to coincide with microbes, the Tiny Earth Project Initiative strives to educate and equip students and instructors about the soil and antibiotic crisis to discover new antibiotics in the soil. The TEPI project has benefited students to carry on original antibiotic research in tester strains of the ESKAPE pathogens. These ESKAPE bugs are known to be multidrug-resistant, extensively resistant, and virulence: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp*.[6] The tester strains used in this study, guided by the TEPI project, include *Bacillus subtilis* and *Escherichia coli*, which are much safer strains compared to the ESKAPE microbes.

Fig 1. Location of collected soil sample provided rich and moist soil. The site was near a cemetery by Riverside Park in Moline, IL (geographical coordinates: latitude 41.51˚N and longitude -90.5 ˚W. The soil collected was from under 2.5 inches from decomposing leaves. On January 9th of 2020, the temperature of the air was 49 degrees Fahrenheit and cloudy with 18 mph winds.

Bacillus subtilis and *Escherichia coli*, the safe relative of Klebsiella species, are involved with cases of food poisoning while oddly enough *E. coli* is a very common bacterium in the human gastrointestinal tract.[8,13] Despite similar signs of infection, the characteristics and biochemical tests of the colonies may be distinct to differentiate them from each other. According to Bergey's Manual of Systematics of Archaea and Bacteria, both strains are rod-shaped, but the *B. subtilis* will arrange in chains while the *E. coli* will have no specific type of arrangement.[11] After conducting a gram strain, *B. subtilis* will stain purple testing positive, and *E. coli* will stain pink testing negative. Other distinct characteristics of *B. subtilis* include tests negative for Oxidase, tests positive for Catalase and endospores, and will form irregularly shaped colonies with undulated edges. Other distinct characteristics of *E. coli* include tests negative for endospores, Oxidase and Amylase, tests positive for Catalase, and will colonize circularly with the entire margin and convex elevation.[2,13]

Using this information about the tester strains *Bacillus subtilis* and *Escherichia coli*, we will conduct antibiotic research following but not limited to the TEPI, which consists of lab protocols with bacteria. If our biochemical tests and colony morphology match the descriptions of *Bacillus* and *Streptomyces*, then we will identify our unknowns as such.

METHODS

Unless described otherwise, the bacterial strains and protocols used in this study were provided by the Tiny Earth Project Initiative (TEPI).[6]

- Soil samples were collected from the environment to make serial dilutions.
- We spread plated three different media of three different dilutions. We pick and patched colonies on their assigned media.
- We pick and patched colonies chosen on each tester strain on their designated media.
- We streak plated the isolates of antibiotic-producing colonies until there were individual colonies. Then, were confirmed those antibiotic producers on the respected tester strain.
- We calculated the frequency of antibiotic producers.
- Using those individual colonies, we could have run PCR samples on 1% agarose gel, at 100v, for about 30 minutes. The PCR samples would then be used and proceeded to extract DNA.
- The DNA sequencing sample would be sequenced to analyze 16s rRNA. The 16s rRNA sequences would have then been used by BLAST for further identification.[2]
- We conducted a Gram stain and a series of biochemical tests to confirm the identity of the antibiotic producer found using colony morphology.

RESULTS

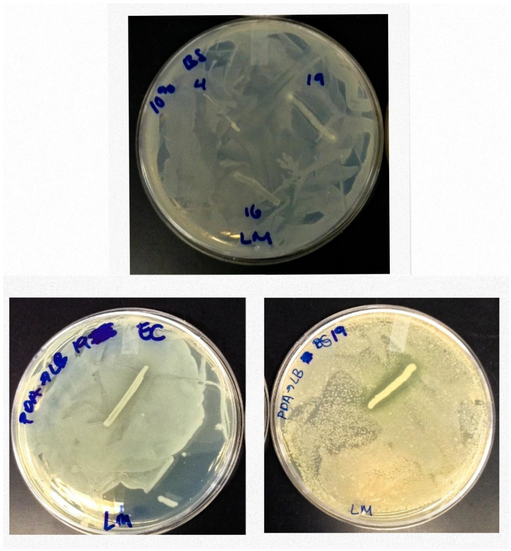

Fig 2. Conformation of antibiotic producers. The top plate consists of three different tests on 10% TSA against *B. subtilis*. ID 19 from TSA (Unknown A) was further analyzed based on a clearer halo. The bottom plates were ID 19 from an original PDA media (Unknown B) that was transferred onto LB/agar. The left plate's tester strain was *E. coli* and the right plate had *B. subtilis.* All plates were incubated at 28˚ C for 24 hours.

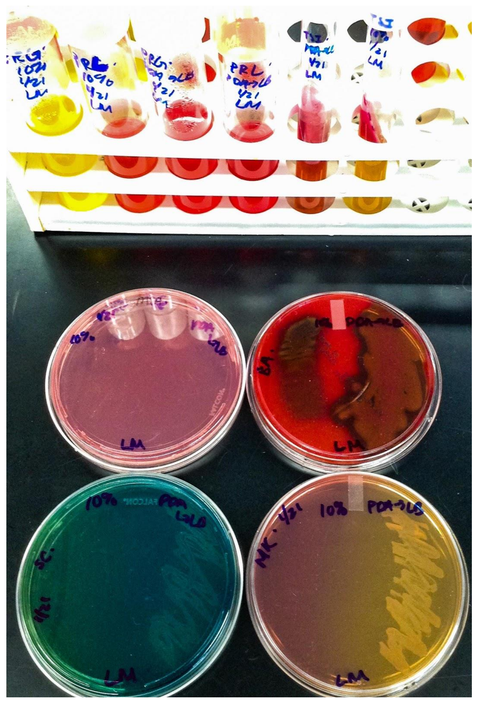

Fig 3. Diverse biochemical results from Unknown A (TSA-19-BS) and Unknown B (PDA/LB-19-BS/EC). The biochemical tests performed in test tubes were for glucose, lactose, and triple sugar iron agar, and the plates tested on MSA, blood agar, Simmon citrate, and MacConkey's agar. All tubes were differential, and there were no signs of gas or dihydrogen sulfide for the TSPI. Most plates were selective and differential. There might have been some discrepancies with the tests because of unknown variables. Plates were incubated at 37˚C and tubes were incubated at 28˚C, which were both incubated for 24 hours.

RESULTS (CONTINUED)

Biochemical Results for Unknowns and Antibiotic Strains

	Unknown A	Unknown B	*Bacillus*[3,9]	*Streptomyces*[4,5,7,10]
Catalase (Release of O2)	+	+	+	+
MacConkey's Agar	+ (yellow)	– (red)	–	–
Triple Sugar Iron Agar	+, (K/A)	–, (K/K)	No gas	–, Black for H2S production
Blood Agar	+, β	+, β	+	–
Simmons Citrate	–	+	+	+
Phenol Red with Glucose	+	–	+	–, gas
Phenol Red with Lactose	–	–	Varies	–
Gram Stain	+	–	+	+

K/K for TSI indicate no carbohydrate fermentation. K/A for TSI had results that it was able to ferment dextrose only. TSI did not show signs of gas production which shows the test tube partially empty. Unknown A had a negative result for only lactose, and the rest were positive. Unknown B had positive results only for blood agar and Simmon citrate. Various results of *Bacillus* and *Streptomyces* did not concur with either unknowns.

DISCUSSION

We were able to accomplish finding antibiotic-producing isolates from the soil and were not able to confidently confirm the identity. The most common phyla of soil bacteria are Proteobacteria, yet most antibiotic producers were Firmicutes and Actinobacteria.[1] The identification that compared the most the unknowns were not from the antibiotic producing phyla. Ironically, Unknown B was more closely identifiable to a pathogenic bacteria, which could mean the antibiotic-producers found were attempting to outcompete their own. Without being able to properly identify the unknowns' identities are still not clear, but proper isolation of the 16s rRNA would help identify the genus using BLAST.[2] Afterward, we would able to purify the samples and obtain the proper chemical agent being produced.[1] The hope is to understand the biochemical pathways to be able to recreate them synthetically for antibiotics against the ESKAPE microbes: *Klebsiella* and *Bacillus subtilis*.

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