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Identification of Antibiotic Producing Soil Bacteria Against Bacillus subtilis

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INTRODUCTION

Antibiotic resistant bacteria are becoming a serious threat to people in the United States, especially in hospital settings. These infections are called nosocomial infections because they are caught while in a hospital and are potentially resistant to antibiotics *(10)*. According to the Centers for Disease Control and Prevention (CDC), at least 2.8 million people obtain an antibiotic-resistant infection in the U.S. each year, and more than 35,000 people die from these infections (*3*). The Infectious Diseases Society of America refers to these nosocomial bacteria as ESKAPE pathogens, which is an acronym that stands for *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, & *Enterobacter* species *(10)*. When a bacteria is resistant to an antibiotic, there are usually other antibiotics that can be used as they target different areas of the cell. However, bacteria are becoming multi-drug resistant creating a need for new antibiotics to be discovered.

In order to study this antibiotic crisis, the Tiny Earth Project Initiative (TEPI) is collecting data from students and instructors to discover new antibiotics from soil *(5)*. Soil contains an abundance of diverse microbes that have evolved within their competitive environment *(4)*. Some bacteria have adapted to produce secondary metabolites, which are organic compounds released in order to enhance the bacteria's chance of survival. An antibiotic is a type of secondary metabolite that inhibits the growth of surrounding microorganisms. Therefore, it is possible to discover new antibiotics from soil bacteria.

The current research tests for antibiotics produced against the ESKAPE-like microbes *Bacillus subtilis* and *Escherichia coli*.

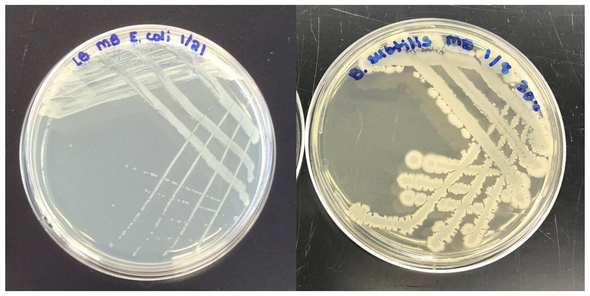

Fig. 1. Isolated colonies developed on streak plates of LB agar with *E. coli* (left) and *B. subtilis* (right). *E. coli* colonies appear beige, moist, circular with entire margins, and flat in elevation. *B. subtilis* colonies appear cream, matte, irregular shaped with undulate margins, and flat in elevation. These plates were incubated at 28ºC for 24 hours.

B. subtilis is a common, fast-growing decomposer found in soil *(7)*. Its cells are rod-shaped, gram-positive, and often arranged in short chains. The resistance of its spores to heat, radiation, and disinfectants results in the bacteria being difficult to completely remove from unwanted locations. *E. coli* is a gram-negative, rod-shaped bacteria commonly found in a single cell arrangement *(8)*. It can beneficial to humans as it is an important species in the normal intestinal microflora, but it has also shown to be pathogenic (*6*).

It is important to study these ESKAPE-like bacteria in order to prevent increased antibiotic resistance and discover resolutions to the antibiotic-resistance crisis. The purpose of this research is to contribute to the TEPI by assessing ESKAPE-like pathogens to find possible antibiotics produced from soil bacteria.

METHODS

Unless described otherwise, the bacterial strains and protocols used in this study were provided by TEPI (*5*).

- Collected soil sample: damp soil in a forested area with fallen leaves; Temperature of air = 13ºC
- Performed serial dilutions of soil sample on LB agar, 10% TSA, and PDA
- Created master plates of each media
- Used two techniques to isolate antibiotic production on *B. subtilis* and *E. coli*
- Created streak plates of four isolates that seemed to produce antibiotic
- Used technique #2 to confirm antibiotic producers
- Performed colony PCR and agarose gel electrophoresis on four isolates; Used 16S rRNA primers 27F and 1492R
- Performed DNA extraction on isolates #25 and #29 using IBI extraction kit
- Prepared DNA sequencing sample and sent it to Iowa Institute of Human Genetics at Univ. of Iowa; Used 16S rRNA sequence to identify isolates using BLAST analysis (*2*)
- Performed Biochemical tests and Gram stains

RESULTS

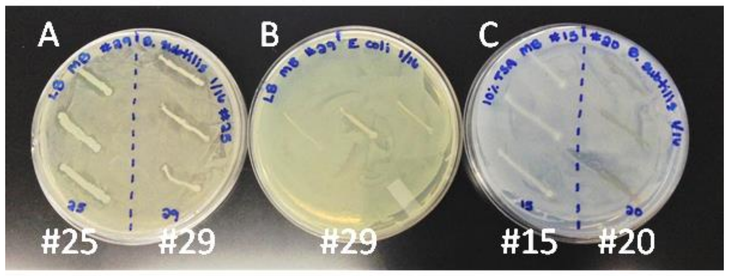

Fig. 2. Confirmation of antibiotic producing isolates on LB and 10% TSA agar against *B. subtilis* and *E. coli*. Plate A contains 3 streaks of isolates #25 (left) and #29 (right) on *B. subtilis* and LB agar. All streaks on plate A produced clear halos revealing antibiotic production. Plate B contains 3 streaks of isolate #29 on *E. coli* and LB agar, which did not produce clear halos. Plate C contains 3 streaks of isolates #15 (left) and #20 (right) on *B. subtilis* and 10% TSA. Streaks from isolate #15 produced slight halos, and streaks from #20 produced irregular shaped halos. Plates were incubated at 28ºC for 24 hours.

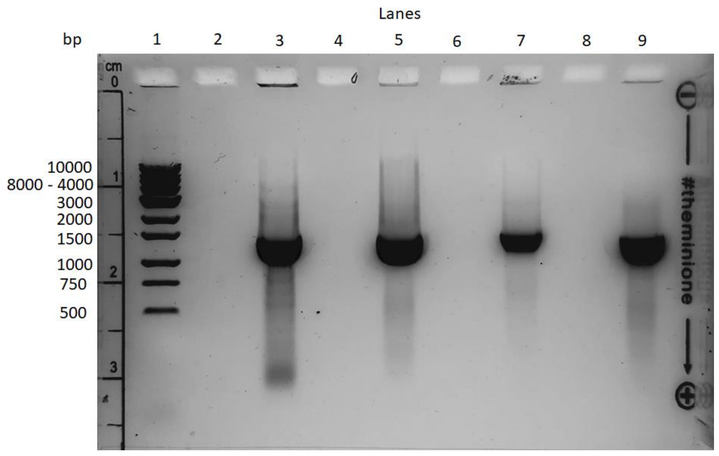

Fig. 3. Agarose gel electrophoresis revealing isolated 16S rRNA from soil bacterial isolates with evidence of producing antibiotics. Lane 1 = molecular weight marker; Lane 3 = sample #25 grown on LB agar; Lane 5 = sample #29 grown on LB agar; Lane 7 = sample #15 grown on TSA; Lane 9 = sample #20 grown on TSA. All isolates previously showed evidence of producing antibiotic against *B. subtilis*. A 1% agarose gel was run at 100V for 25 minutes.

RESULTS (CONTINUED)

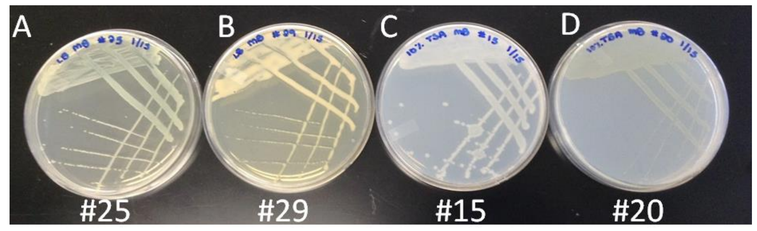

Fig. 4. Isolated colonies developed on streak plates of potential antibiotic producers on LB (plates A and B) and 10% TSA agar (plates C and D). Sample #25 has moist, cream, circular colonies with entire margins and a flat elevation. Sample #29 has shiny, beige, circular colonies with entire margins and a convex elevation. Sample #15 has matte, white, irregular colonies with undulate margins and a flat elevation. Sample #20 has moist, orange, opaque, circular colonies with entire margins and a flat elevation. Plates were incubated at 28ºC for 24 hours.

	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
☑	Pseudomonas plecoglossicida strain NBRC 103162 16S ribosomal RNA, partial sequence	2560	2560	99%	0.0	99.79%	NR_114226.1
☑	Pseudomonas plecoglossicida strain FPC951 16S ribosomal RNA, partial sequence	2560	2560	99%	0.0	99.79%	NR_024662.1
☑	Pseudomonas taiwanensis DSM 21245 strain BCRC 17751 16S ribosomal RNA, partial sequence	2549	2549	99%	0.0	99.64%	NR_116172.1
☑	Pseudomonas monteilii strain CIP 104883 16S ribosomal RNA, partial sequence	2549	2549	99%	0.0	99.64%	NR_024910.1
☑	Pseudomonas monteilii strain NBRC 103158 16S ribosomal RNA, partial sequence	2545	2545	99%	0.0	99.57%	NR_114224.1

	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
☑	Pseudomonas silesiensis strain A3 16S ribosomal RNA, complete sequence	2556	2556	99%	0.0	99.50%	NR_156815.1
☑	Pseudomonas mandelii strain NBRC 103147 16S ribosomal RNA, partial sequence	2551	2551	99%	0.0	99.43%	NR_114216.1
☑	Pseudomonas mandelii strain CIP 105273 16S ribosomal RNA, partial sequence	2551	2551	99%	0.0	99.43%	NR_024902.1
☑	Pseudomonas frederiksbergensis strain DSM 13022 16S ribosomal RNA, partial sequence	2545	2545	99%	0.0	99.36%	NR_117177.1
☑	Pseudomonas caspiana strain FBF102 16S ribosomal RNA, partial sequence	2523	2523	99%	0.0	98.87%	NR_152639.1

Fig. 5. 16S rRNA sequence data for isolates #25 (top) and #29 (bottom) are most related to the genus *Pseudomonas*, as determined by BLAST analysis (*2*).

DISCUSSION

Four antibiotic producing bacteria were isolated from a soil sample, and two of these isolates were sequenced using the 16S rRNA gene. The sequencing data revealed that the isolates are most related to the genus *Pseudomonas*, which is a common type of soil bacteria (*1*). Many species of *Pseudomonas* are known antibiotic producers, further confirming these isolated samples as antibiotic producing soil bacteria (*9*). Future research should test these isolates on different tester strains to possibly identify the mode of action of the antibiotic. The specific gene controlling the enzyme that produces the antibiotic should also be identified and researched.

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