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Putative Antibiotic Producer: A Pseudomonas genus with Antibiotic Properties

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INTRODUCTION

There is a serious crisis evolving in the world with catastrophic consequences if action is not taken. Many diseases caused by bacteria are becoming untreatable because of the amount of pathogens resistant to the effect of antibiotics. The Center for Disease Control and Prevention (CDC) reports that over 2.8 million people acquire serious resistant infections, and over 35,000 die as a result from these infections.[1] The most prolific bacteria known to cause these deaths are *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter*, or ESKAPE bacteria. They are nosocomial pathogens that exhibit multidrug resistance and virulence.[2] The mechanisms involved in multidrug resistant pathogens are broadly grouped into three categories: drug inactivation commonly by an irreversible cleavage catalyzed by an enzyme, modification of the target site where the antibiotic may bind, and reduced accumulation of drug either due to reduced permeability or by increased efflux of the drug.[3] There are multiple treatment strategies to combat this issue including antibiotics in combination. This uses multiple antibiotics simultaneously, which can be effective because it is more unlikely for bacteria to be resistant to multiple antibiotics.[2]

The Tiny Earth Project (TEPI) is a network of students and professors collaborating around the world to address, and fix the antibiotic crisis.[4] My project will be working with the TEPI to educate the public about the antibiotic crisis, and obtaining a soil sample to isolate the bacteria in it to discover new antibiotics. Antibiotics are secondary metabolites, naturally found in soil, that inhibit the growth of other microorganisms. Efforts aimed at identifying new antibiotics were once the priority of research and development among pharmaceutical companies, but there is now a long list of microbes that are no longer susceptible to most therapeutic agents.[5]

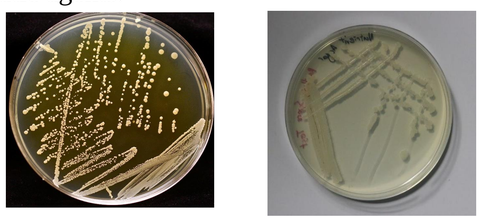

Figure 1. Streak plates of *Escherichia coli* (left) and *Bacillus subtilis* (right). Both samples were grown overnight for 23 hours at 30°C. *B. subtilis* is a spore former which allows for greater mobility on the LB agar plates, and has significant growth compared to *E. coli*. More streaking will continue in the future, but due to the size and amount of growth on both plates both will be grown for 23 hours at 30°C.[7]

Along with the unknown bacteria from the collected soil sample, I will be working with the ESKAPE-related tester strain *Escherichia coli*, and *Bacillus subtilis*. *E. coli* grows in a straight cylindrical rod, typically in pairs, and is Gram-negative. *E. coli* is naturally found in the lower part of the intestine. *B. subtilis* is rod-shaped, growing in chains, and is Gram-positive. It is naturally found in the gastrointestinal tract of humans. [6,7] The ultimate goal of this project is to discover new antibiotics from soil samples collected from around the world. These new antibiotics will help prevent the spread of multidrug resistant strains of bacteria, and we will be able to combat new drug resistant bacteria. The world is in danger, and new antibiotics are all around waiting to be uncovered.

METHODS

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

- We collected soil samples from a place with good vegetation, my sample was from 41.5041269, -90.5516124
- We then isolated a certain amount of bacteria in the soil sample by diluting the sample to 1:1000, 1:5000, and 1:10000, respectively, and grown on LB agar, 10% TSA, and PDA media.
- We used *B. subtilis* and *E. coli* as ESKAPE tester strains to test the soil isolates for antibiotic properties.
- We picked and patched the isolates, if a pick and patch showed antibiotic properties it was restreaked on a fresh LB agar plate.
- Colony PCR was conducted on each colony that showed antibiotic properties, respectively.
- The PCR was ran on an agarose gel to further isolate the 16S rRNA
- Once PCR worked, the PCR samples were shipped to the University of Iowa to be screened, and sequenced.
- Once sequenced, BLAST was used to cross analyzed the 16S rRNA from a national database to better understand what genus the soil isolate comes from.

RESULTS

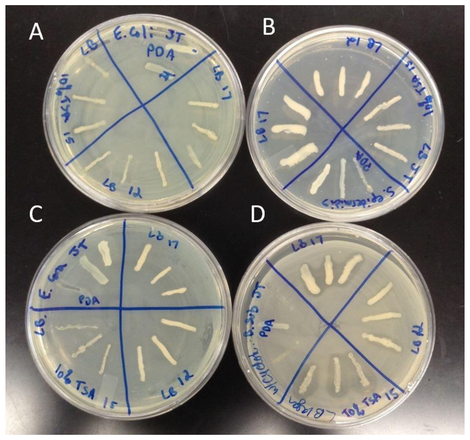

Figure 2. Retesting possible antibiotic producers on LB agar plates confirms soil isolates 17, 15, and 12 show a distinct halo. All of the plates are LB agar media grown overnight at 28°C. Each possible antibiotic producing soil isolate was patched three times on each plate to further understand if the halo is a mistake, or if there is more than one type of bacteria in each patch. PDA was also repatched to see if the colonies would grow on LB and if there would be different results of antibiotic producing if grown on LB. All plates used Technique 2. Plate A is testing against *E. coli*. Plate B is testing against *S. epidermidis* which did not grow on LB particularly well. Plate C is testing against *E. carotovora*, which did not grow on LB particularly well. Plate D is testing against *B. subtilis*. The only plates to show signs of antibiotic producers were Plate A and D. As seen, there are distinct halos on Plate D for soil isolate 17, 15 and 12. While on Plate A there is a little sign of a possible halo for soil isolate 15. Soil isolates 17 and 15 will be used for PCR and DNA purification to be sequenced.

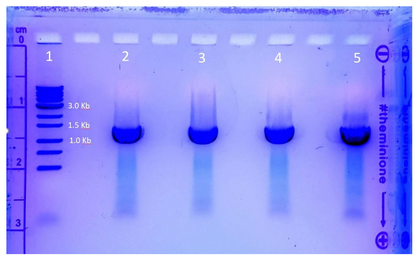

Figure 3. Amplification of 16S ribosomal DNA was detected in the agarose gel, amplification occurred for soil isolates 15 and 17. Lane one is the 10Kb molecular weight standard. Lane 2 and 3 are soil isolate 17 from the LB master plate, and lanes 4 and 5 are soil isolate 15 from the 10% TSA master plate. All of the bands are the 16S rDNA and are the correct size of Kb. These samples were used for DNA sequencing, will be cut out from the gel, and purified.

RESULTS (CONTINUED)

	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
☑	Pseudomonas frederiksbergensis strain DSM 13022 16S ribosomal RNA, partial sequence	2571	2571	99%	0.0	99.57%	NR_117177.1
☑	Pseudomonas silesiensis strain A3 16S ribosomal RNA, complete sequence	2566	2566	99%	0.0	99.50%	NR_156815.1
☑	Pseudomonas mandelii strain NBRC 103147 16S ribosomal RNA, partial sequence	2562	2562	99%	0.0	99.43%	NR_114216.1
☑	Pseudomonas mandelii strain CIP 105273 16S ribosomal RNA, partial sequence	2560	2560	99%	0.0	99.43%	NR_024902.1
☑	Pseudomonas frederiksbergensis strain DSM 13022 16S ribosomal RNA, partial sequence	2560	2560	99%	0.0	99.43%	NR_117177.1
☑	Pseudomonas silesiensis strain A3 16S ribosomal RNA, complete sequence	2555	2555	99%	0.0	99.36%	NR_156815.1
☑	Pseudomonas mandelii strain NBRC 103147 16S ribosomal RNA, partial sequence	2549	2549	99%	0.0	99.29%	NR_114216.1

Figure 4. DNA sequencing BLAST data shows that the soil isolate JT-17-LB-*B. subtilis*, the first 4 entries, and *JT-15-LB-B. subtilis*, the last 3 entries as potential antibiotic producer is under the genus *Pseudomonas*. This makes sense because *Pseudomonas* is one of the most common bacteria found in soil. As seen in the Percent Identification column, there is a 99.57% match for 15 and a 99.43% match for 17 to another 16S rRNA sequence meaning it is very likely the soil isolate is of this genus. The E value is 0.0, meaning that there is no chance that this is random, if it was any number above 0 it would mean there is a possibility that the match is by chance. [8]

DISCUSSION

The DNA sequencing data suggests that the soil isolates JT-15-LB-*B. subtilis* and JT-17-LB- *B. subtilis* are from the same genus *Pseudomonas*. The BLAST data suggests this because there is a 99.43% and a 99.57% match to another bacterial isolate's 16S rRNA sequence. The future direction of the project will be to isolate the antibiotic within the bacteria by means of separation. Typically separated by size, polarity, solubility, or affinity.[4] Finally, once complete separation and isolation is complete, NMR will be done to analyze the exact chemical structure of the possible antibiotic producer. If a new antibiotic is found, further assays will be conducted to determine if it is viable in *vivo*.

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