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Pseudomonas and Bacillus Soil Isolates Produce Antibiotics

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INTRODUCTION

Antibiotics have been a prominent tool for treating infection for nearly 100 years, but the recent emergence of antibiotic resistant strains of bacteria have prompted scientific inquiry into new methods of fighting infection [8]. With the list of effective medications diminishing and the limited discovery of new antibiotics, antibiotic resistance poses a significant threat to human survival [8]. Of particular importance to the discussion of antibiotic resistance are the ESKAPE pathogens: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp*. These pathogens cause 15.5% of nosocomial infections worldwide, are associated with high risks of mortality, and are becoming increasingly resistant to existing medications, making them one of the most important groups of bacteria to study [2, 6, 9, 10]. While studies of combinations of antibiotics, bacteriophage therapy, antimicrobial peptides, photodynamic light therapy, and silver nanoparticles show potential, the discovery of new antibiotics remains crucial for combating antibiotic resistance [8]. The Tiny Earth Project Initiative (TEPI) is one such global, student oriented research project that confronts the antibiotic resistance crisis by searching for new antibiotics in soil samples [5].

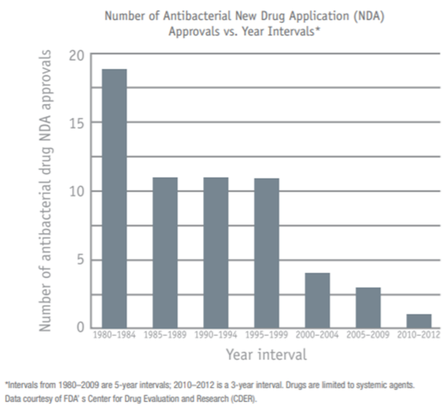

Figure 1. CDC data from 2013 shows a decreasing number of FDA approvals of new antibiotics since the 1980s [4].

On a global level, TEPI aims to educate students and professionals about the antibiotic crisis while amassing soil bacteria data and isolating novel antibiotic producers [5]. This poster details an experiment that contributes to TEPI by isolating antibiotic producing bacteria in a local Iowa soil sample. Soil bacterial isolates are tested against known tester strains *Bacillus subtilis* and *Escherichia coli*. *B. subtilis* is a fast growing, Gram positive, rod shaped bacteria typically found in soil, water, and around plants [7]. It is extensively studied and valued for its protein production abilities [7]. *E. coli* is a fast growing, Gram negative, rod shaped bacteria typically found in the gastrointestinal tract of humans and animals. Most strains of *E. coli* are harmless, but some strains have acquired pathogenicity and are a common foodborne pathogen [11]. By testing unknown soil isolates against *B. subtilis* and *E. coli*, student researchers eliminate the risk of exposure to the dangerous antibiotic resistant ESKAPE strains while still permitting inquiry into new methods of treatment. With antibiotic resistance spreading so quickly between bacterial species, the human population is in dire need of research into new antibiotics. By contributing to TEPI, this project increases the range of potential antibiotic producing bacteria and promotes further research into novel antibiotics to combat antibiotic resistance.

METHODS

Unless described otherwise, the bacterial strains and protocols used in this study were provided by the Tiny Earth Project Initiative (TEPI) [5].

- Soil samples were collected from Duck Creek Park in Davenport, IA on January 9, 2020.
- Using a slurry of the soil sample, serial dilutions were made and then plated onto LB agar, 10% TSA, and PDA petri dishes and cultured at 28ºC for 48 hours.
- Bacteria colonies were streaked and cultured on master plates of corresponding media.
- Bacteria from the master plates were streaked onto spread plates of *Bacillus subtilis* and *Escherichia coli* to observe any zones of inhibition.
- Colony PCR was run on confirmed antibiotic producers. DNA from gel electrophoresis was extracted and analyzed by 16S rRNA genetic sequencing at the Iowa Institute of Human Genetics at the University of Iowa using the primers 27F and 1492R.
- NCBI BLAST was used to identify the genus of the antibiotic producing soil isolates.

RESULTS

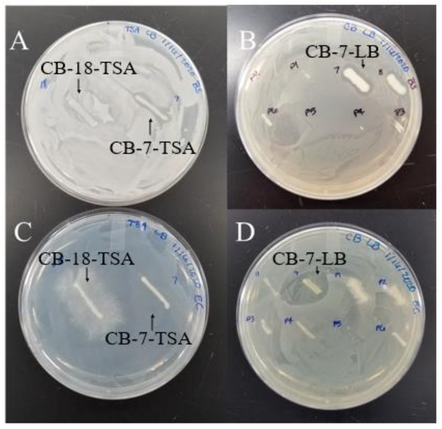

Figure 2. Soil isolates CB-7-TSA, CB-7-LB, and CB-11-LB are confirmed to produce antibiotics. Each of these isolates exhibit a zone of inhibition (ZOI) against *B. subtilis*. Only CB-7-LB exhibits a ZOI against *E. coli*. CB-7-LB and CB-7-TSA show the largest ZOIs so these two isolates were chosen to undergo further genetic testing for identification.

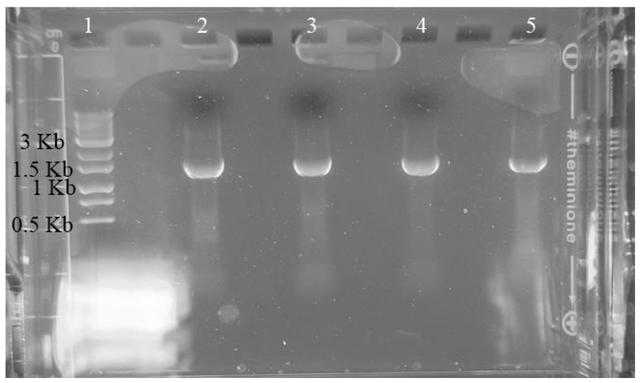

Figure 3. Gel electrophoresis of CB-7-LB (lanes 2&3) and CB-7-TSA (lanes 4&5) shows discrete DNA bands at approximately 1.5 Kb. The electrophoresis was performed in a 1% agarose gel run at 100V for thirty minutes. The lanes are as follows: 1: molecular weight ladder, 2&3: CB-7-LB and 4&5: CB-7-TSA. The DNA from these bands were excised and purified to be sent to the University of Iowa for 16S rRNA genetic sequencing.

RESULTS (CONTINUED)

CB-7-LB Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Bacillus pacificus strain MCCC 1A06182 16S ribosomal RNA, partial sequence	2615	2615	99%	0.0	99.72%	NR_157733.1
Bacillus paranthracis strain MCCC 1A00395 16S ribosomal RNA, partial sequence	2615	2615	99%	0.0	99.72%	NR_157728.1
Bacillus toyonensis strain BCT-7112 16S ribosomal RNA, partial sequence	2610	2610	99%	0.0	99.65%	NR_121761.1
Bacillus thuringiensis strain ATCC 10792 16S ribosomal RNA, partial sequence	2610	2610	99%	0.0	99.65%	NR_114581.1
Bacillus thuringiensis strain IAM 12077 16S ribosomal RNA, partial sequence	2610	2610	99%	0.0	99.65%	NR_043403.1

CB-7-TSA Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Pseudomonas frederiksbergensis strain DSM 13022 16S ribosomal RNA, partial sequence	2562	2562	99%	0.0	99.50%	NR_117177.1
Pseudomonas silesiensis strain A3 16S ribosomal RNA, complete sequence	2556	2556	99%	0.0	99.43%	NR_156815.1
Pseudomonas mandelii strain NBRC 103147 16S ribosomal RNA, partial sequence	2553	2553	99%	0.0	99.36%	NR_114216.1
Pseudomonas mandelii strain CIP 105273 16S ribosomal RNA, partial sequence	2551	2551	99%	0.0	99.36%	NR_024902.1
Pseudomonas frederiksbergensis strain JAJ28 16S ribosomal RNA, partial sequence	2540	2540	99%	0.0	99.22%	NR_028906.1

Figure 4. NCBI BLAST results show that CB-7-LB is of the genus is *Bacillus* and CB-7-TSA is of the genus *Pseudomonas* [3]. Query coverage indicates the percentage of the gene that was sequenced in the database. The percent identity indicates the percentage of base pairs from the experimental sample that are identical to the hit from the database. The E value indicates the probability that the hits would have been pulled out of the database at random. Both samples show a query coverage at 99%, percent identity above 99%, and E values at zero which indicates the validity of these results. For both samples, the genus shows no variability in the top ten hits while the species varies without significant differences in the statistical values. Therefore, the identity of the genus can be confirmed while the species cannot be and it is concluded that CB-7-LB is *Bacillus* and CB-7-TSA is *Pseudomonas*.

DISCUSSION

Antibiotic producing bacteria were isolated from an Iowa soil sample and identified as *Bacillus* and *Pseudomonas*, which are both consistent with common bacteria found in soil [1, 3]. Data from this project was sent to TEPI to be recorded in the national database and used for further research. In future studies, the bioactive molecule produced by the samples should be identified and isolated using a biological assay and chemical techniques for isolation and purification [5]. The identity of the molecules should be compared with known antibiotics to determine whether a novel antibiotic was produced and can be developed into new antibiotics. Additionally, CB-18-TSA should be taken through the processes of16S rRNA gene sequencing and identification of bioactive molecules as it was also a confirmed antibiotic producer.

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