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# Isolated Antibiotic Producing Bacteria in Local Soil Samples Determined to be Bacillus

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# Isolated Antibiotic Producing Bacteria in Local Soil Samples Determined to be *Bacillus*

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## INTRODUCTION

Antibiotics have been crucial in the medical advancements towards decreasing mortality due to microbial infections; however, a group has emerged as multidrug resistant infections arising in healthcare settings, also called nosocomial pathogens[7]. Antibiotics work to eliminate bacterial infections by interrupting protein synthesis or DNA replication, which essentially renders the bacteria inactive[3]. However, pathogenic bacteria are able to adapt and resist antibiotics by random mutations or transference of resistant genetic material from other bacteria[3]. The ESKAPE pathogens, which is an acronym for the six bacterial strains *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp., are resistant to antimicrobial agents and contribute to increased mortality rates among nosocomial infections[4,7]. Identified by World Health Organization (WHO) as dangerous pathogens, ESKAPE bacterial strains pose a public health threat because their antibiotic resistance increases health care costs and decreases the ability to effectively treat these infections[4-5,9]. The health-care field acknowledges the dire need for developing new treatment strategies to reduce ESKAPE pathogens in prevalence and mortality risk with new research studies, like this one.

Further investigative research needs to focus on safe, bacterial related strains that are related to the ESKAPE strains and non-pathogenic. Generally, bacteria are prokaryotic microbes with cell walls that can be beneficial or pathogenic to human beings. They replicate through binary fission on either cultured planktonic or agar plated surfaces[3]. This research study is conducted on *Escherichia coli*, a safe-relative of *Klebsiella pneumoniae*, and on *Bacillus subtilis*. *E. coli* are single or paired rods that are Gram-negative and motile, usually found within the intestines of animal hosts[8]. *B. subtilis*, is rod-shaped, Gram-positive that forms spores under stressful conditions, which contributes to their motile abilities[6]. Various treatment strategies are underway to combat multi-drug resistant bacteria, such as antibiotics in combination, and applying silver nanoparticles or photodynamic light therapy[4]. A new research program, Tiny Earth Project Initiative (TEPI), has emerged to find antibiotic options to stop the ESKAPE pathogens while discovering vital research about lost soil sustainability

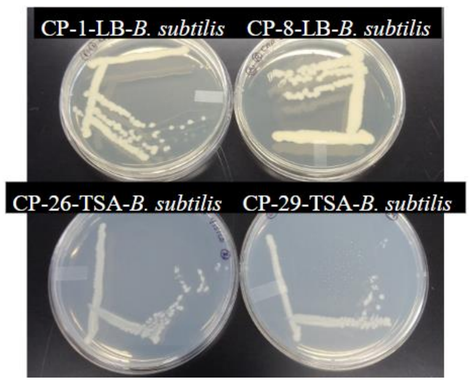


Fig. 1. The four streak plates isolated separate colonies on two types of respective media (LB & 10%TSA). They were streaked to isolate pure colonies.

TEPI is an international research network of students and professors that work to accomplish three goals: student-originated research, identifying novel antibiotic-producing microbes in local soil samples, and understanding how to maintain microbial diversity while combating soil erosion[3]. The purpose of this research project is to identify potential antibiotic-producing bacteria from local soil samples to take the next steps in developing new antibiotics to combat ESKAPE-related strains, such as *Escherichia coli*, and *Bacillus subtilis*.

## METHODS

Unless described otherwise, the bacterial strains and protocols used in this study were provided by the Tiny Earth Project Initiative (TEPI) [3].

- On January 9th, 2020, a soil sample was collected from Duck Creek Park, Bettendorf, IA. (Latitude 41.54; Longitude, -90.53).
- A 1.65 gram dry soil sample was transformed into a slurry mix. From the slurry, serial dilutions of the mix were plated onto three types of media: LB agar, 10% TSA, and PDA. The bacterial cultures were incubated at 28°C-30°C for 24-48 hours.
- Master plates were created by streaking bacterial colonies from the serial dilution plates onto their respective media.
- Then, the same bacterial colonies were streaked onto petri dishes with spread bacterial strains of *Bacillus subtilis* and *Escherichia coli* to test for potential antibiotic producers.
- Once antibiotic producers were identified and confirmed, colony PCR was completed and gel electrophoresis isolated DNA of the bacterial colonies. DNA was removed from the gel and purified for further 16S rRNA sequencing at University of Iowa, Iowa Institute of Human Genetics.
- NCBI BLAST analyzed the data, identifying a genus.

## RESULTS

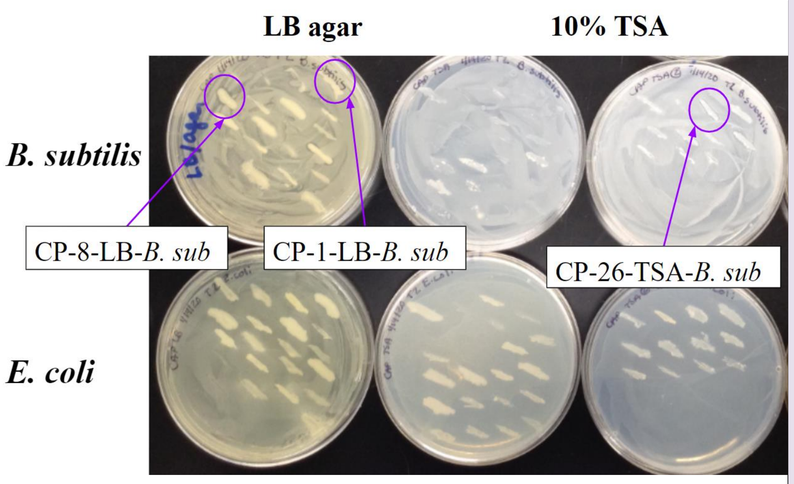


Fig. 2. Three soil isolates produced zones of inhibition (halos) when tested for antibiotic producing abilities against *B. subtilis* and *E. coli*: CP-1-LB-*B.subtilis*, CP-8-LB-*B. subtilis*, and CP-26-LB-*B. subtilis*. Soil isolate 8 on LB and 26 on TSA produced bigger halos than 1 on LB, which indicates further testing confirmation on soil isolate 1 is necessary. Both CP-8-LB-*B. subtilis* and CP-26-TSA-*B. subtilis* were effective against *Bacillus subtilis*, yet showed no halo effects or zones of inhibition against *E. coli*.

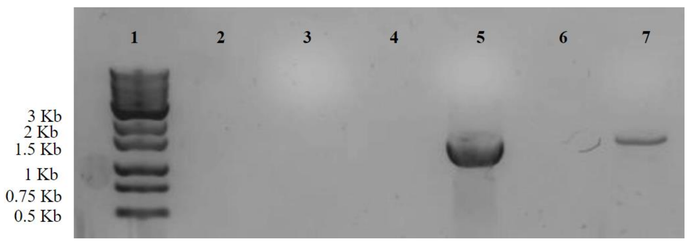


Fig. 3. The agarose gel electrophoresis isolated the DNA of confirmed antibiotic-producing soil isolates, CP-8-LB-*B. subtilis* and CP-26-TSA-*B. subtilis*, which were removed and purified for further 16S rRNA sequencing. Lane 5 contained CP-8-LB-*B. subtilis* and maintained a length of between 1 and 1.5 Kb. Lane 7 contained CP-26-TSA-*B. subtilis* and maintained a length of about 1.5 to 2 Kb. Lanes 2, 4, and 6 were skipped to provide room for DNA purification. The DNA from lanes 5 and 7 were sent for 16S rRNA sequencing at University of Iowa, Iowa Institute of Human Genetics.

## RESULTS (CONTINUED)

Table 1. NCBI Blast Program 16S rRNA Genetic Sequencing Analysis of Soil Isolate **CP-8-LB-*B. subtilis***

| | Query cover | E value | Percent Identity |
|---|---|---|---|
| *Bacillus mobilis* strain MCCC 1A05942 | 99% | 0 | 99.72% |
| *Bacillus thuringiensis* strain NBRC 101235 | 99% | 0 | 99.65% |
| *Bacillus toyonensis* strain BCT-7112 | 99% | 0 | 99.65% |
| *Bacillus thuringiensis* strain ATCC 10792 | 99% | 0 | 99.65% |
| *Bacillus thuringiensis* strain IAM 120777 | 99% | 0 | 99.65% |

Table 2. NCBI Blast Program 16S rRNA Genetic Sequencing Analysis of Soil Isolate **CP-26-TSA-*B. subtilis***

| | Query cover | E value | Percent Identity |
|---|---|---|---|
| *Bacillus mobilis* strain MCCC 1A05942 | 98% | 0 | 99.14% |
| *Bacillus thuringiensis* strain NBRC 101235 | 98% | 0 | 98.93% |
| *Bacillus toyonensis* strain BCT-7112 | 98% | 0 | 98.93% |
| *Bacillus thuringiensis* strain ATCC 10792 | 98% | 0 | 98.93% |
| *Bacillus thuringiensis* strain IAM 120777 | 98% | 0 | 98.93% |

The tables above show the NCBI BLAST program analysis for the 16S rRNA sequence after the primers 27F and 1492R for both soil isolates, CP-8-LB-*B. subtilis* and CP-26-TSA-*B.* subtilis, and determined them to be of the *Bacillus* genus[2]. Query coverage indicates the percent of the inputted sample compared against other known bacteria. Percent identity indicates the percentage of identical base pairs of the inputted sample against the matched database bacteria. The probability that the hits in the database are pulled out by random are found with any numerical value in the E value. Both soil isolates show high validity indicated by above 98% on QC and PI, and 0 in the E value.

## DISCUSSION

NCBI BLAST analysis determined the bacterial strains were likely from the genus of *Bacillus*, which makes sense as an antibiotic producer pulled from the soil[2]. Both soil isolates, CP-8-LB-*B. subtilis* and CP-26-TSA-*B. subtilis*, demonstrated antibiotic abilities when halos or zones of inhibition were produced against *B. subtilis*. *Bacillus* lies within the phyla of Firmicutes and ranges 0-7% in being found in soil samples with a mean contribution of 2%[1].

Future direction will look into understanding the organic molecular structure of *Bacillus* using NMR spectrometry[3]. Also, researchers will analyze other soil samples in the area of the current soil sample to obtain further understanding of local soil bacteria.

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