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Looking to Soil Microbes for a Solution to the Antibiotic Crisis

Introduction

The world is quickly approaching an antibiotic crisis where bacteria are adapting faster than we are discovering different types of antibiotics. To slow the nearing crisis we need to discover more varieties of antibiotics to compete with pathogenic bacteria. ESKAPE bacteria are our biggest concern because of their high antibiotic resistance (Hernandez et al., p 58). Soil is full of bacteria with antibiotic properties, but we have yet to discover a fraction of what is available. In this study we investigated soil bacteria for their ability to produce antibiotics against safe relatives of pathogenic ESKAPE strains (nonpathogenic). *Escherichia coli* and *Bacillus subtilis* were used. By discovering new bacteria with antibacterial properties, we may be able to keep up with fast evolving bacteria.

Methods

The complete experimental procedure is outlined in Fig.1. First, we created a soil solution from soil collected from Potawatomi State Park and then made serial dilution 10% tryptic soy agar (TSA) or potato dextrose agar (PDA) plates from 10^{-1} to 10^{-4} (e.g., Fig. 2A). From the serial dilution plates with 30-300 CFU, we created 10 master plates with about 30 colonies on each plate (Fig. 2B). From the master plates, about 80 bacteria colonies, with unique color and/or structure, were selected for a patch plates with safe relatives. Colonies with possible antibiotic producers were defined by a halo on the patch plates (Fig. 2C). 8 possible antibiotic producers were streak plated to produce single colonies (Fig. 2D). Polymerase chain reaction of the 16S rRNA gene was accomplished using universal (Fran et al 2008, Walters et al 2015) or Actinomycete-specific (Stach et al. 2003) primers. Sanger DNA sequencing, biochemical methods, gram staining, and other tests helped to characterize and identify the possible antibiotic producers (Fig. 1).

Experimental Design

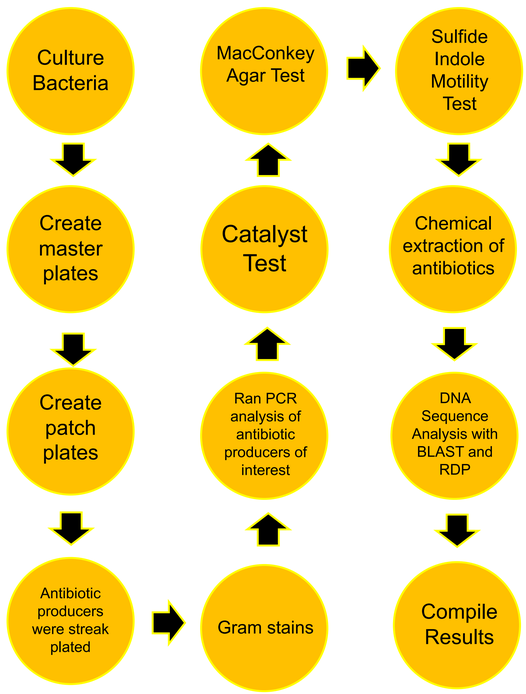

Figure 1: Experimental design showing steps of the experiment from beginning (top left) to end (bottom right) .

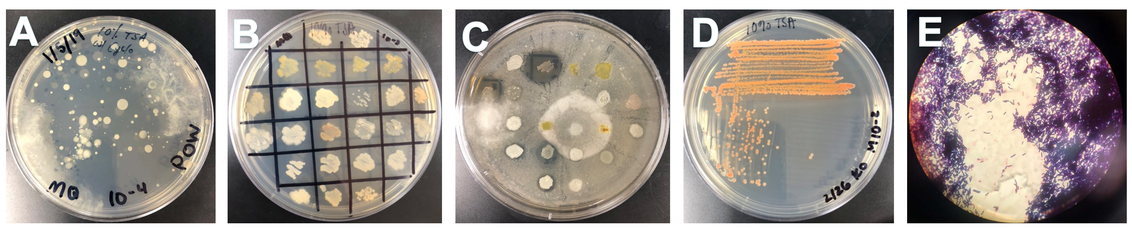

Figure 2: Isolation of antibiotic producing bacteria from soil. A) 10^{-4} serial dilution plate; B) Master plate on 10% TSA; C) Soil-isolated bacteria producing antibiotics (i.e., produce a cleared halo) effective against ESKAPE safe relative *Escherichia coli* and *Bacillus subtilis*; D) Pure culture streak plate of isolate 10-2 on 10% TSA; E) Example of gram positive bacteria isolate 7-25.

Results

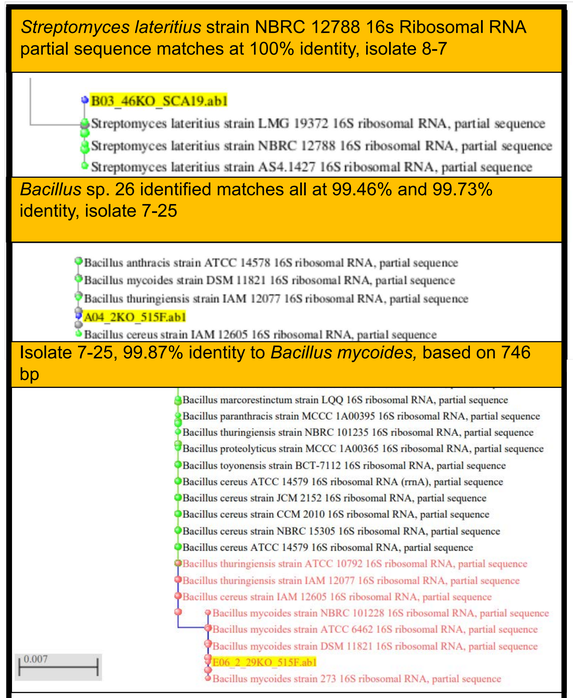

Figure 3: Probable species match based on 16S rRNA Sanger DNA sequencing and BLAST analysis.

Conclusion

The 8 isolates chosen from the patch plates were strong antibiotic producers. Using various tests in lab, we were able to narrow down our interest to a few bacteria.

- Some isolates did not produce single colonies well on the streak plate.
- Gram stains indicated the isolates were all gram positive. Sample 7-25 showed rod morphology, the others showed filamentous morphology.
- 7-25 had a positive reactions in the catalyst test, indicating the isolate was an aerobic bacteria.
- 7-25 did not grow on the MacConkey Media which prevents the growth of gram positive bacteria and fastidious gram negative bacteria.
- 7-25 did not produce sulfide or indole.
- According to our BLAST research, we were able to determine the species of isolate 7-25 to be *Bacillus* sp. With 26 identified matches, most likely *Bacillus mycoides* .
- We are still waiting on results from the chemical extraction of the antibiotics.

We achieved the goals of the tiny earth network and used scientific research to discover antibiotic producing soil bacteria. More research still needs to be done on soil microbes to generate the new antibiotics we need.

Acknowledgements

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