Spring 5-27-2020

Analysis and Exploration of Novel Antibiotic-Producing Streptomyces spp. in Spokane County, Washington

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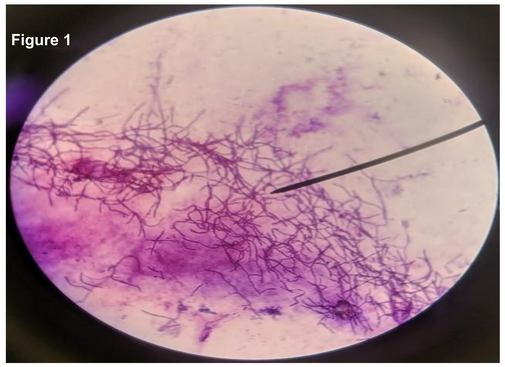
Introduction

- The genus *Streptomyces* accounts for producing 80% of antibiotics in use today (Procopio et al., 2012). The discovery and production of antibiotics is imperative to keep up with the ever-growing strains of drug-resistant pathogens.
- According to the Centers for Disease Control and Prevention, a US citizen is infected by an antibiotic-resistant pathogen every 11 seconds, every 15 minutes a patient dies as a result (CDC, 2019).
- With this project, I intend to explore and analyze antibiotic-producing *Streptomyces* species from soil in Spokane County, WA.

Objectives

1. To test the efficacy of secondary metabolites from local *Streptomyces* species, I will adjust variables to determine what environmental conditions (e.g. temperature) and media types will influence the best metabolite production.
2. Specifically, I will test metabolites that will combat ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanni, Pseudomonas aeruginosa,* and *Enterobacter* species) and *Candida albicans*, which are common nosocomial pathogenic bacteria and fungi with drug-resistant strains.
3. After the efficacy of secondary metabolites have been examined, future projects will intend to identify the species of *Streptomyces*.



K.Kramer, preliminary data.

Figure 1.
Gram-stained *Streptomyces* hyphae from Medical Lake, WA.

Hypothesis

1) *Streptomyces* spp. capable of combating pathogenic microbes exist in Spokane County soil.

2) Modifying laboratory variables such as incubation time, temperature, and media type will influence the production of bacterial metabolites.

Methods

1) Obtain soil sample and determine colony-forming units (CFUs) by suspending the sample in phosphate buffered saline (PBS) (Reynolds, 2005). Dilutions of 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} will be incubated on potato dextrose agar (PDA) serial dilution plates at 30℃ for at least 48 hours.

2) CFUs will be examined and colonies of interest will be selected and placed on a master plate (thick-poured PDA) and incubated again at 30℃ for at least 48 hours.Colonies will be selected based on macroscopic observations of known *Streptomyces* spp. morphology. Additional identification will include Gram-staining acid-fastness, and microscopic morphology from isolated colonies (Taddei et al., 2006).

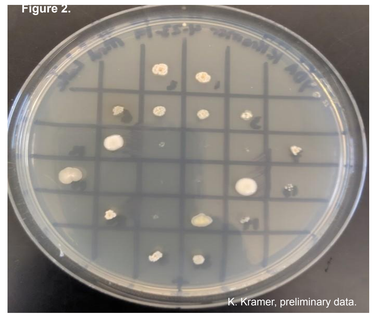


Figure 2.
An example of a completed master plate.

Methods Continued

3) Cell-free supernatants (CFS) will be obtained from cultured *Streptomyces* sp. in broth media (sabouraud dextrose broth, yeast dextrose broth, potato dextrose broth) at 25, 30, and 35℃ (Bell et al., 2015). Flow-through containing CFS will be used to saturate disks using the Kirby-Bauer technique and zones of inhibition will be analyzed against pathogens.

4) CFS will be used again to fill 96-well plate assays. Fifty ul of CFS will be combined with 50ul of one of each of the selected ESKAPE pathogens (*E. coli* for gram negative, *S. aureus* for gram positive and *C. albicans* for the fungi).

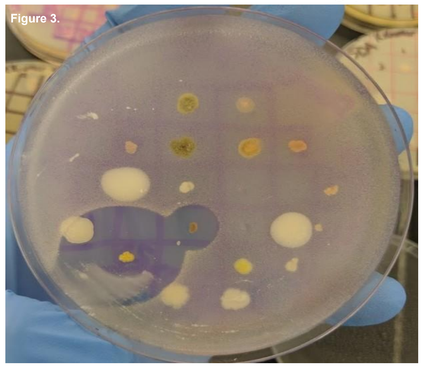


Figure 3.
Using the Top-Layer Overlay assay (not included) one can visualize the inhibitory capabilities of an isolated colony of *Streptomyces* sp.. In this assay, selected microbes were grown on a medium and an overlay of agar inoculated with *S. aureus* was poured, and zones of inhibition measured (K. Kramer, preliminary data).

Methods Continued

Fifteen replicate wells for each treatment will be included with each assay and positive controls will be present as well as media-only control. Microplates will be checked every 24 hours for three days and absorbance measured at 600 nm using a spectrophotometer.

5) To identify the *Streptomyces* spp., I will amplify the 16S rRNA gene using polymerase chain reaction (PCR) and verified using gel electrophoresis, followed by Sanger sequencing of the 16S rRNA gene and BLAST analysis with the National Center for Biotechnology Information (NCBI) database.

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Acknowledgements

Special thanks to Dr. Jenifer Walke, Erin Bruns M.S., Eastern Washington University Biology Department, Eastern Washington University Biology Department's Indirect Cost Committee, Spokane Community College Biology Department, and the Tiny Earth Project.