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 (Prokopio 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 production of bacterial metabolites.
2. Specifically, I will test metabolites that will combat ESKAPE pathogens (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, 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.

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°C 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°C 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).

Methods Continued

3) Cell-free supernatants (CFS) will be obtained from cultured Streptomyces sp. in broth media (saproaurad dextrose broth, yeast dextrose broth, potato dextrose broth) at 25, 30, and 35°C (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).

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.

Literature Cited

Bell et al., 2006). Isolation and biochemical studies. I. Mechanism of action of an antibiotic produced by a soil isolate of Streptomyces spp., I

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