The rise of antibiotic-resistant bacteria is of increasing concern due to the lack of effective drugs for treatment. One potential source of new leads is from bacteria which produce more than 50% of commercial used antibiotics. These microorganisms were the primary sources used during a major period of antibiotic development from the 1950s and 1960s and their examination is now being revived due to the potential of integrating analytical tools like mass spectrometry and NMR alongside more traditional methods to analyze the secreted natural products.

Organism Selection and Isolation
In this project, I developed a microbial library of organisms found in soil samples from the upper Midwest. These organisms are the basis of future natural product discovery and characterization efforts.

Crude Soil
Step 1. Crude Soil: Soil samples were thawed, plated on agar media and incubated at 30°C. Every 3 days, organisms with variable or interesting phenotypes or unusual participation in inter-organismal interactions were selected for purification.

Semi-Purified
Step 2. Semi-Purified: Organisms of interest were taken from the crude sample and re-plated onto new plates until purified species were obtained.

Purified Organisms
Step 3. Purified Organisms: Isolated organisms were re-plated to ensure purity and stored as glycerol stocks.

Compound Characterization
Two of the unknown Actinomycetes exhibited significant inhibitory growth against fungal microbes. The compounds secreted by these organisms were extracted and analyzed using mass spectrometry.

Figure 1. Whole Plate Extract:
Unknown Actinomycetes (A) and (B) against unknown fungus (F) collected from soil samples. Plated on International Streptomycetes Project Media #2, 7 days of growth.

Figure 2. Mass Spectrometry Analysis.
Agar plates of B, F, and B vs. F were grown for 7 days prior to compound extraction. The samples were run using LC-ESI-MS and the spectra were compared. Compounds that appeared in B vs. F but not the other two plates are considered leads for future bioactivity analysis.

References: