A Search for Antibacterial Agents through Soil Sample Screening

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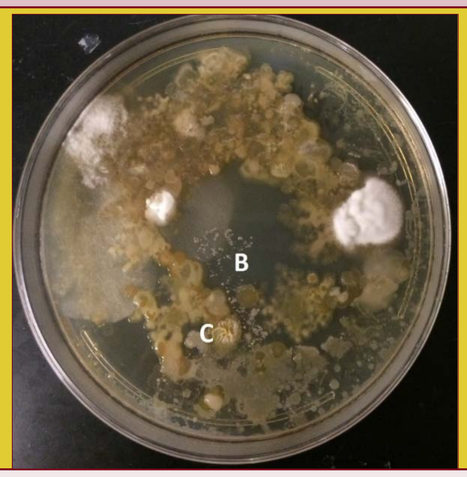
Background

With antibiotic-resistant infections at an **all-time high**, the lack of effective medications for treatment is concerning. The rapid replication time of bacteria and the overuse of antibiotics in medicine and agriculture have contributed to this growing problem. Because of this, the need for new antibiotics is **critical** to modern medicine. This project works to discover novel antibacterial compounds through the examination of microbial natural products and their secondary metabolites. As bacterial and fungal natural products make up almost 50% of commercially available antibiotics, their continued study is a viable novel compound discovery. [1,2]

Organism Selection and Library Development

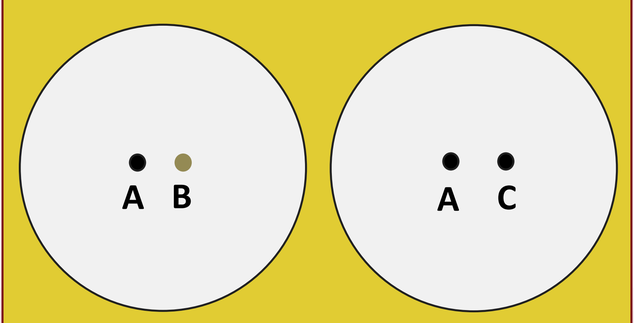
1. Collection of Soil Samples: Nearly 100 samples were collected from diverse ecological systems. These samples contained organisms that would form the basis of the microbial library.

2. Crude Sample: Soil samples were plated on ISP2 media and incubated at 30°C for 10 days. Organisms with interesting phenotypes or unusual growth patterns were selected for inclusion in the library.



Organism B

Organism C



(1) Crude Extract

(2) Co-Culture

3. Isolated Organisms: The selected organisms were re-plated multiple times to ensure purity, then stored as glycerol stocks for long-term use.

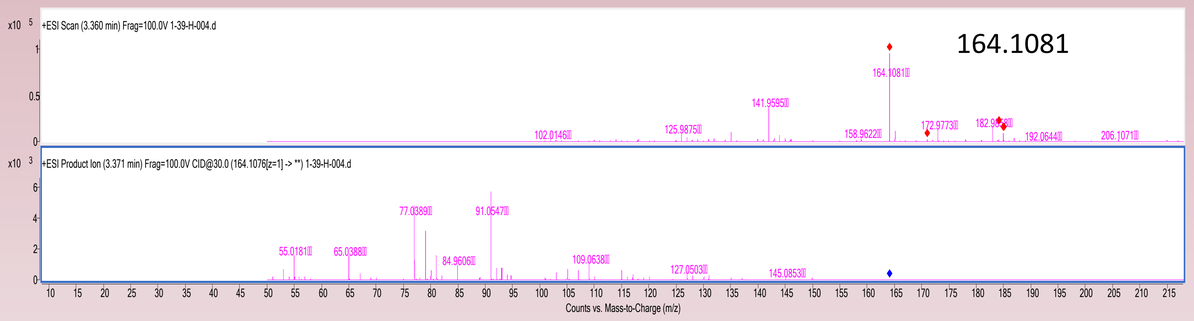
4. Plating Method: A common OOI was plated against each library organism as either an extract **(1)** or in co-culture **(2)**.

- In **(1),** extract from the OOI was loaded onto a sterile disk **(B),** alongside a 2µl culture of each library organism **(A).**
- In **(2)**, **(A)** was plated against a 2µl culture of the OOI **(C).**

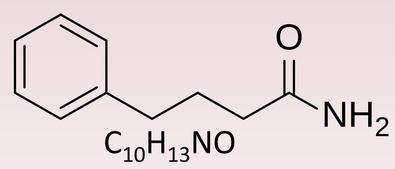
Preliminary Results

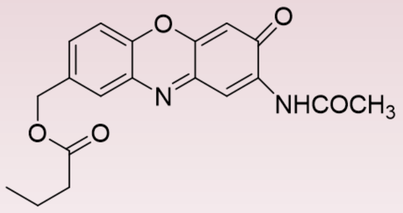
Preliminary results show that this is a viable strategy for natural product lead development. When a co-culture shows signs of inhibition but its crude extract counterpart did not, we hypothesize that a natural product gene cluster has been activated. The compounds corresponding to these activated gene clusters will form the basis of further bioactivity studies.

Mass Spectrometry Analysis



77.0389 & 91.0547





Mass spectrometry indicates a structure similar to above. This structure can be produced by a known venezueline seen below. Venezuelines are known compounds produced by *S. venezuelae*

Future Work

1. Repeat procedure to confirm compound presence.
2. Use DNA sequencing to determine genus and/or species.
3. Repeat co-culture process with other known *Streptomyces.*

References & Acknowledgments

References:

1. Anderson, D. and Hughes, D. Microbiological Effects of Sub lethal Levels of Antibiotics. *Nature Reviews Microbiology.* July **2014**. 23:465-475.
2. Spellberg, B. Guidos, R. Gilbert, D. et al. The Epidemic of Antibiotic-Resistant Infections: A Call to Action for the Medical Community from the Infectious Diseases Society of America. *Clinical Infectious Diseases.* **2008.** 46 (2):155-164.
3. Van Wezel, G. and McKenzie, N. Chapter 5 Applying the Genetics of Secondary Metabolism in Model Actinomycetes to the Discovery of New Antibiotics. *Methods in Enzymology.* **2009.** Vol 458.

Acknowledgements:

- Nikki Niewold, Stephanie Mitchell, Andy Johnson, Dr. Erin E. Carlson, and the entire Carlson lab for their continued help and guidance
- Undergraduate Research Program and the University of Minnesota Chemistry Department
- Additional funding provided by:

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