Snail Shell Cave Research Summary
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January 28th, 2018

The work undertaken at Snail Shell Cave is part of a discovery program at Vanderbilt University. The goal of the research is to explore the microbial ecology of Snail Shell cave as a resource for novel biochemistry and potentially new pharmaceutical leads in the form of natural products.

Natural products are small molecules that can be isolated from microorganisms and are the basis for the majority of drugs found in the clinic. Because targets of drugs in the clinic develop resistance, new natural products are necessary to replenish our nation's medical arsenal.

Some groups of microorganisms are 'gifted' in producing natural products. When peering into the genomes of gifted microorganisms, they often have the capacity to produce over a dozen families of natural products. In contrast, many microorganisms only have the capacity to produce 1-3 families.

However, a major problem in natural product research is that few of the potential natural products are produced under laboratory conditions. Over the last few years, laboratories have found that various stimuli activate natural products in the laboratory. These stimuli include stressors and increase the potential for novel natural product discovery.

To accomplish our goal, we first aimed to isolate gifted microorganisms from Snail Shell Cave. The most common group of gifted microorganisms organisms are Actinobacteria. This group of microorganisms is responsible for producing common antibiotics such as penicillin. However, we focused on a different group of gifted microorganisms called myxobacteria. Myxobacteria are a group of predatory, social microorganisms that are known for their vast biosynthetic potential, making them ideal model organisms for novel biochemistry and pharmaceutical leads. They possess a similar biosynthetic capacity as Actinobacteria but have not been studied in as great of detail, making them an ideal group of organisms to explore for novel natural products.

We were able to successfully cultivate 5 myxobacteria strains from Snail Shell Cave. In total our laboratory now has 16 strains of myxobacteria that we are exploring for their biosynthetic potential. Along these ends, we have begun to explore ways to expand the metabolome of these stains in order to isolate novel natural products.

The first method we have used is called stimulus liquid cultures. Other studies that have used Actinobacteria have found that growing them with stimuli such as sub-lethal antibiotics, rare earth metals, and competing strains have increased the production of natural products. However, this method had not been previously used in myxobacteria.

Toward this end, we explored the utility of stimulus culture as a method to expand metabolite and natural product production in myxobacteria. We used rare earth metals, sub-lethal antibiotics, and competitor cultures. Our results suggest that different suites of metabolites and natural products are emitted in myxobacteria when grown with these stressors. This suggests that the potential to discover new natural products is increased when growing with these stressors. Thus, our initial results have provided a roadmap to use these stimuli as a means to isolate natural products from myxobacteria.

The second method that we have used is desorption electrospray ionization-imaging mass spectrometry (DESI-IMS). This method allows us to localize metabolites in space that are responsible for myxobacteria predatory behavior. Like stimulus cultures, this method uses a competitive organism that affects the metabolite production of the myxobacteria. Unlike stimulus cultures, the metabolites are extracted at room temperature without the need for costly and timely liquid extraction. Furthermore, DESI-IMS allows us to localize metabolites in space, linking their putative function (predation) to natural product chemistry. We hypothesize that metabolites localized to the predation front are good starting points new antibiotics.

Thus far, using this method we have been able to prioritize molecules involved in myxobacterial predation. We have also created a database that contains over 300 known myxobacterial natural products in which we can compare our results to. Our results suggest the presence of previously known myxobacterial natural products and potential unknown natural products involved in predation. Future work will be focused on isolating some of these natural products involved in predation and assessing their utility as general antimicrobial agents.

In conclusion, the strains collected from Snail Shell Cave have been a vital part of the work in the Dr. Bachmann's laboratory at Vanderbilt University. Thus far, initial work has established several feasible methods for expanding the metabolomes of myxobacteria, and thus increases the potential for natural product discovery. Future work will be focused on applying the methods to the cave strains, isolating natural products from these caves strains, and determining their functional activity, including their potential in killing pathogenic microorganisms.