Travis LaGree
Class of 2020
Biology, Forensic Science

Inhibition of Reproductive Sporulation in *Streptomyces viridochromogenes* through specialized metabolite production by environmental *Bacillus* species isolates

Mentor: Dr. Nikolas Stasulli, Biology and Environmental Science

As bacterial pathogens continue to evolve and develop additional resistances to antibiotics, the collection of effective treatments continues to shrink (Andersson, et al., 2011). Using single-cultured bacterial cultures has produced a limited specialized metabolite diversity, therefore, new methods using co-culture metabolite screens are required for further discovery (Straight, et al., 2006). There are groups of bacteria, such as Actinobacteria and Bacillus, that contain many gene clusters, corresponding to specialized metabolites that have yet to be fully analyzed (Straight, et al., 2006). These largely ignored specialized metabolite clusters, many of which are only produced in situations of bacterial competition or stress, can be used to expand the current collection of antibiotic compounds (Straight, et al., 2006).

Before experimentation began, appropriate bacteria were selected for interaction due to their possessed traits. *Streptomyces viridochromogenes* was chosen as an indicator to detect specialized metabolite secretion based on the inhibition of reproductive sporulation. This bacteria's aerial mycelium and spore layers are commonly impacted by chemical compounds which can be easily observed during inhibition. The bacteria also has a high volume of biosynthetic gene clusters (BGC's), which can elicit chemical responses from other bacteria (Straight, et al., 2006 and de Lima Procópio, et al., 2012) . Preliminary research performed prior to SURF has shown that certain *Bacillus* species are responsible for inhibiting the reproductive sporulation *S. viridochromogenes* through specialized metabolite secretion. The aim of the project was to isolate a large quantity of *Bacillus,* or other closely related, endospore-forming, species isolates, and to identify the breadth of spore inhibition with *Bacillus* species. This clade of bacteria also has a high number of unidentified BGC's, which make it appropriate for experimentation (Grubbs, et al., 2017). This can help to establish relationships of chemical signaling between these two groups of bacteria and can assist in uncovering novel antibiotic compounds.

To begin experimentation, soil samples were heat-treated, which controls for *Bacillus* and other endospore-producing bacteria. From these samples, approximately 50 isolates that exhibited unique colony morphology were narrowed and picked for experimentation. The isolates were successfully sequenced using PCR amplification of the 16S rRNA gene. Almost all the isolates were identified as a *Bacillus* or belonging to a closely related *Bacillus* subclass. Each isolate was spotted, using a competition assay with *S. viridochromogenes* on 0.5x ISP2 plates. The plates were observed mainly at 2 and 4 days in order to visualize potential inhibitory effects on *S. viridochromogenes* colonies.

Through repetitive experimentation, six different isolates appeared to cause a significant inhibition response in the *S, viridochromogenes* spore layer, although the intensity and appearance of the inhibition varied greatly between these isolates. The experimental isolates were mapped using a maximum-likelihood phylogenetic tree. Also included were typed *Bacillus*

strains, typed outgroups and the *Bacillus* strain that showed inhibitory effects previously. This technique showed that these six inhibitory isolates are disparate in phylogeny. This conclusion, coupled with the variance in the inhibition phenotype, indicates the presence of multiple specialized metabolites that elicit an inhibitory effect on sporulation in *S. viridochromogenes*. Along with the further isolation and co-culture analysis of additional *Bacillus* species, future research will purify and identify the chemicals responsible for these inhibition reactions using chemical techniques such as solid-phase extraction and high-performance liquid chromatography - mass spectrometry.

References:

Andersson, D. I., and Hughes, D. (2011). Persistence of antibiotic resistance in bacterial populations. *FEMS Microbiology Reviews*, Vol: 35 (5): 901–911. doi: 10.1111/j.1574-6976.2011.00289.x.

de Lima Procópio, R. D., da Silva, I. R., Martins, M. K., et al. (2012). Antibiotics produced by Streptomyces. *Brazilian Journal of Infectious Diseases*, Vol: 16 (5): 466-471. doi: 10.1016/j.bjid.2012.08.014

Grubbs, K. J., Bleich, R. M., Santa Maria, K. C., et al. (2017). Large-Scale Bioinformatics Analysis of Bacillus Genomes Uncovers Conserved Roles of Natural Products in Bacterial Physiology. *mSystems,* Vol: 2 (6). doi: 10.1128/mSystems.00040-17.

Straight, P.D., Willey, J. M., and Kolter, R. (2006). Interactions between Streptomyces coelicolor and Bacillus subtilis: Role of Surfactants in Raising Aerial Structures. *Journal of Bacteriology*, Vol: 188 (13): 4918-4925. doi:10.1128/JB.00162-06