Inhibition of reproductive sporulation in *Streptomyces viridochromogenes* through specialized metabolite production by environmental *Bacillus* species isolates

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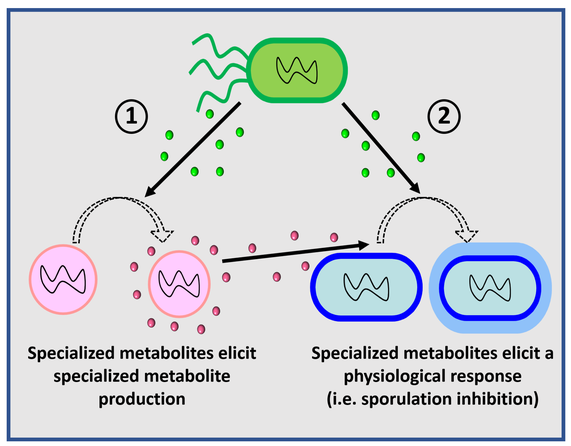
Introduction

Extensive research has been committed to antibiotic discovery when viewing single bacterial colonies[3]. Due to the rise of antibiotic resistance in pathogenic bacteria, new and effective antibiotic compounds are required[1]. Although many chemicals have been discovered through single culture observation, chemicals secreted in coculture have been largely unexplored[3]. Many specialized metabolites are secreted strictly when under competition for nutrients and other growth factors from other bacterial species[2,3]. Studying these coculture interactions open an expansive potential for new antibiotic discovery[3].

Previously in our lab, an environmental *Bacillus* sp. isolate has been observed to secrete a metabolite that negatively effects the reproductive sporulation of the Actinobacteria, *Streptomyces viridochromogenes.* This metabolite doesn't appear consistent with the phenotypic effect shown by previously studied chemicals such as surfactin. This suggests that a new chemical may be being secreted in this interaction that is not found ubiquitously in *Bacillus* cultures.

The purpose of this research is to understand the phylogenetic scope of this interaction. The aim is to determine how prevalent these inhibitory effects are among *Bacillus* species as well as other endospore forming bacteria. These results can aid in categorizing the scope of the production of this unknown secreted compound and determine if there may be others that also effect the reproductive sporulation of *S. viridochromogenes*.



References

[1] 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.

[2] 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

[3] 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

Acknowledgments

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Experiment Methodology

Soil samples were taken from the environment
↓
Soil samples were heat-treated at 80 °C to control for endospore-producers (predominantly *Bacillus* species)
↓
Heat-treated samples underwent a serial dilution and were grown on both 0.5x ISP2 and 0.1x LB media
↓
The resulting colonies were isolated to general morphological differences
↓
Each isolate had its 16S rRNA gene amplified so it could be sequenced effectively
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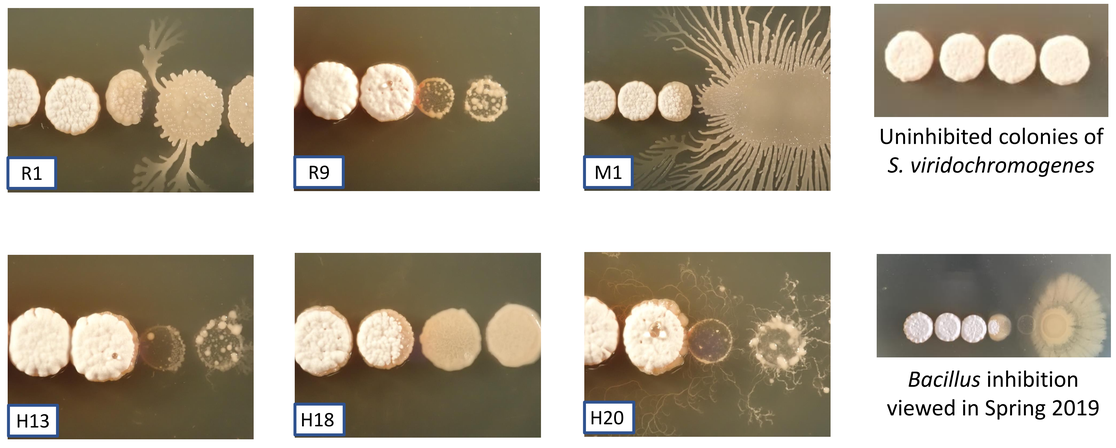
Each isolate was used in a spot-plate assay to determine if inhibitory effects were observable on *S. viridochromogenes* when in proximity.

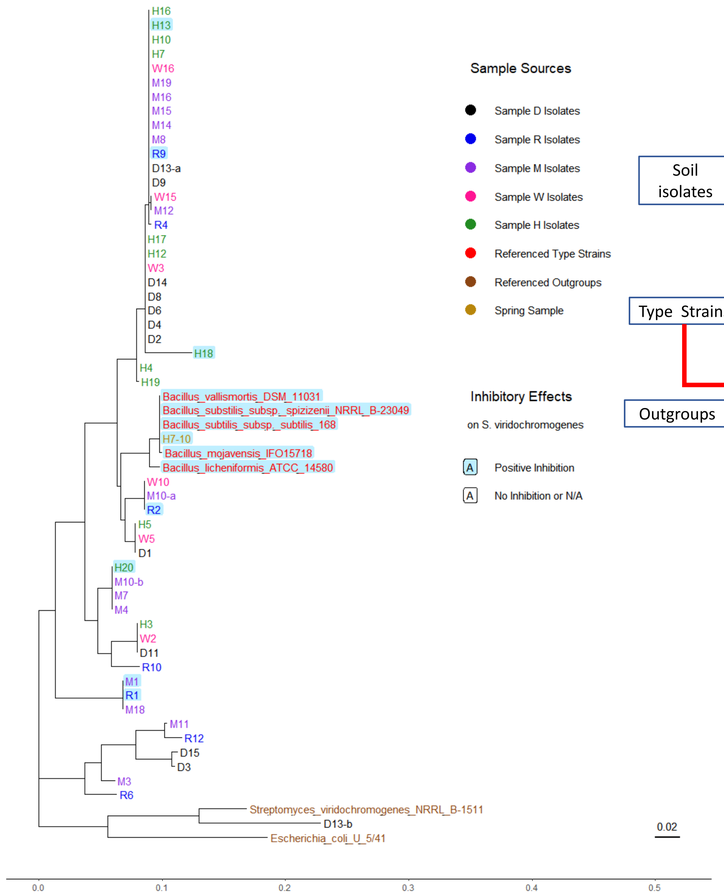
Inhibitory isolates

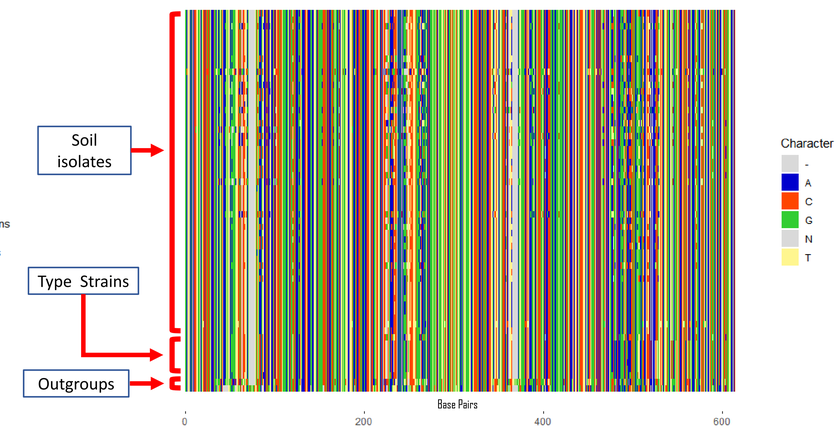


Uninhibited colonies of *S. viridochromogenes*

Bacillus inhibition viewed in Spring 2019

Sanger sequencing and phylogenetic analysis





From the resulting 60 bacterial isolates, the overwhelming majority were identified as species within the *Bacillus* genus. The alignment of the sequencing output along with the constructed phylogenetic tree illustrates the genetic relationship between the species, other reference samples, and two outgroups.

Conclusions

Certain *Bacillus sp.* can reliably inhibit the reproductive sporulation in *S. viridochromogenes.* A variety of different chemical compounds are likely responsible for the inhibition reactions seen in *S. viridochromogenes.* This can be safely assumed due to the phylogenetic distance between the species and the variety of inhibition phenotypes seen. Further analysis must be performed on these competition reactions with *S. viridochromogoenes* and various *Bacillus* or Bacillus-related species.

In the future…

The chemicals responsible for each inhibiting interaction must be purified and identified. Further analysis can be done to determine whether multiple chemicals are responsible for the observed phenotype. These inhibiting chemicals can then undergo further analysis to determine the specificity of its antimicrobial behavior.