Variable Antibiotic Production among Soil Bacteria Populations in the presence of Differing Plant Habitation

David Jakab
Carroll College, 100 N. East Ave., Waukesha, WI 53186

Abstract

In this study, soil samples representing four different areas of plant habitation obtained from the Greene Property in Waukesha County will be examined for the prevalence of antibiotic production in the soil bacteria population. Frequency of antibiotic production at each site will be recorded to identify trends between plant type and amount of antibiotic production. It is hoped that this will shed more light on the selective pressures that these microbial communities encounter.

Introduction

In a study conducted by Davelos et al., antibiotic interactions of bacteria and their spatial variations within the soil were examined (1). At this time, little is known about antibiotic interactions within soil bacteria, so the authors wanted to find out how antibiotic producing bacteria were grouped within a prairie soil ecosystem in hopes to learn more about the selective pressures that these bacteria encounter. They found highly variable amounts of antibiotic production in the varying depths that were examined. Most importantly, no clear trend regarding spatial distribution and antibiotic production was observed. Perhaps if this study was conducted with a greater variation of soil samples, a trend within bacterial distribution would be observed and might provide some insight as to what selective pressures microbial communities face in a soil ecosystem. The focus of this study will be to see if there is a correlation between the amount of antibiotic production within soil and the type of vegetation growing on the soil.

Methods

- A preliminary survey of the Greene property was taken and 4 sites with different plant vegetation were selected.
- Three 5" soil cores were obtained from each site.
- Cores were serially diluted in water to obtain a final dilution factor of 10^{-5}.
- Soil Dilutions 10^{-3}, 10^{-4}, and 10^{-5} were pour plated with Glycerol Yeast Extract Agar and incubated for a week at 25°C.
- 16 colonies were selected from each site and streaked on a GYEA plate against an evenly distributed culture of *Escherichia coli* or *Staphylococcus aureus.*
- After several days of incubation, plates were examined for zones of inhibition and observations were recorded.

Acknowledgments

I would first like to thank Dr. John Bennett for all of his guidance during this project. Special thanks also to Dr. Susan Lewis for providing numerous books that aided in my plant identification.

Site 1

Marshy wetland area

Site 2

Small forested area

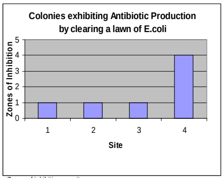
Site 3

More densely wooded area near pond

Site 4

Grassy path near pond

Figure 1



Zones of inhibition per site

Figure 2

	Site 1	Site 2	Site 3	Site 4
Physical Characteristics	- Very wet - Black soil	- Wet sample - Black soil	- Moist sample - Sandy soil	-Moist sample - Sandy soil with Clay
Presence of Roots	Small and Stringy	Small and Stringy	Small Roots	Larger Roots, but Fewer in #
Plants Identified	Sweet Goldenrod	Steeplebrush Meadowsweet	Flat-Topped White Aster	Lawn Grass

Soil core observations and plant identification at each site

ZOI in suspected fungus

ZOI in plated soil dilution

16 colony grid (Control)

Soil Isolate

Soil Isolate

Results

In considering the nature of this project, a quick glance at Figure 1 reveals that the results obtained are not statistically significant. Despite the lack of statistical support in correlating antibiotic production with any one site, several interesting results were observed. The observation of more ZOIs at the fourth site suggests that perhaps antibiotic producing bacteria are more prevalent in drier soil conditions (figure 2). Finally, a rather unexpected but intriguing observation was made from several of the control plates that contained only the 16 colonies chosen from a given site. One of these colonies grew much quicker than the rest and it spread across much of the plate, growing near many other selected colonies. Indeed what was observed looked very much like a ZOI. It appeared that some of the selected colonies were secreting an antibiotic that prevented the rapidly growing colony from growing any closer to them. Several tests conducted suggested that perhaps this was not a bacterium, but a fungus instead. Additionally, after the soil dilutions were pour plated it was observed in several instances that where two colonies grew close to each other, one formed a ZOI in the other. When these organisms were tested against E.coli or S.aureus however, no ZOIs were formed.

Discussion

As already mentioned in the results section, it appeared that the soil bacteria might be secreting molecules that can prevent fungal growth. While this project was concerned with interactions between bacteria, this observation certainly is intriguing. It is reasonable to expect that bacteria may have several different mechanisms to use against other competing organisms.

As I conclude my contribution to this work I recognize several directions in which this project may lead. Perhaps using an organism other than E.coli or S.aureus may provide a better means for detecting antibiotic production within soil bacteria which may yield statistically significant results. Thus using another soil microbe may be a better indicator of antibiotic production. Another way in which statistically significant results may be obtained is to conduct this project on a much larger scale. Taking such measures would also aid in determining the frequency of antibiotic production per gram of soil. If one desired to take this work even further, after antibiotic producing colonies are isolated, one could extract the antibiotic from the bacteria in order to subject it to further testing to understand its mode of action and how it may vary with external pressures.

References

1) **Davelos, A. Kinkel, L. Samac, D**. 2004. Spatial Variation in Frequency and Intensity of Antibiotic Interactions among Streptomycetes from Prairie Soil. *Applied and Environmental Microbiology.* 70: 1051-1058.