

Microbial Diversity and Antibiotic Production in Sorcerer's Cave

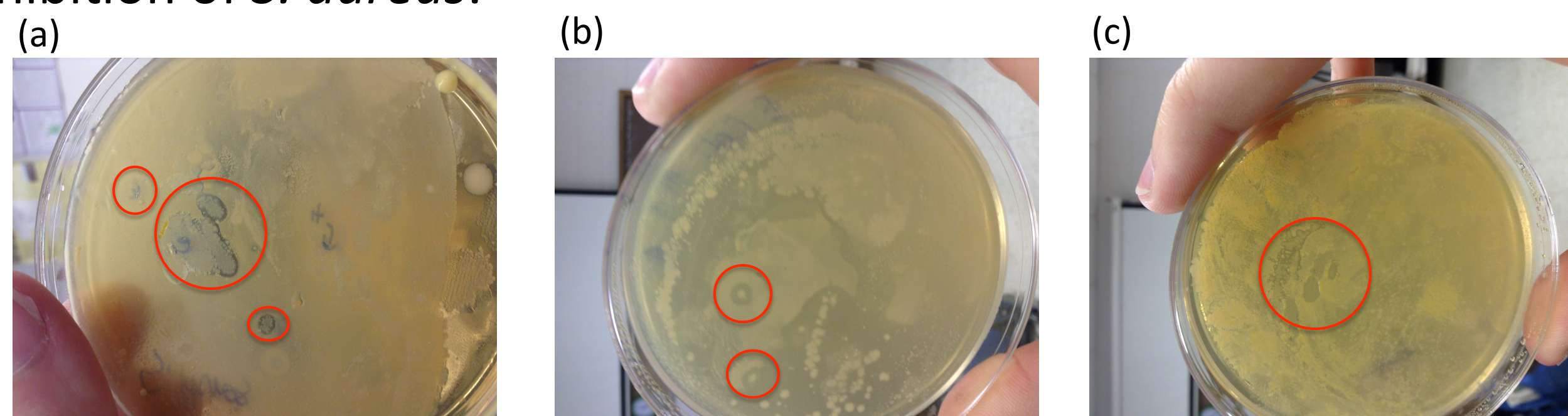
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Abstract

Access was granted with express permission from the landowner to a deep private cave that previously had limited exploration and no conducted microbial research. Cave microbiology is a relatively new field that possesses many environments previously unknown to contain microbes and has the potential for new drug discovery. The goals of this study where to find new species of bacteria as well as antibiotic-producing bacteria. The samples where aseptically taken from deposits on speleothems (cave formations), pools of water deep in the cave, and from bat guano. The samples were pulverized, diluted in a 0.85% NaCl solution, and plated onto various types of growth media, including 0.01X, 0.1X and 1X tryptic soy agar. The colonies were then transferred by replica-plating onto fresh plates of media and then overlaid with *E. coli* and *S. aureus* in 0.7% soft agar to screen for the presence of antibiotic production from the cave microbes. A preliminary investigation indicated the presence of antibiotic-producing bacteria.

Figure 1. Inhibition of growth and clearing of overlaid inoculum. (a) colonies from the wall scrapings shows clearing and inhibition of *S. aureus*, (b) colonies from the wall scrapings shows clearing and inhibition of *E. coli*. and (c) samples from Bat Guano shows clearing and inhibition of *S. aureus*.



Introduction

Bacterial resistance to antibiotics is a well documented problem facing the medical and pharmaceutical fields. With bacteria becoming resistant faster than new antibiotics are produced, finding new sources of antibiotics is critical. By as early as the 1960's culturable soil bacteria had been almost fully mined for any antibiotic producers (1) causing microbiologists to explore diverse environments in search of different types of antibiotic producers. Bacteria obtained from an isolated cave have been found to be highly resistant to modern antibiotics but have had no possible contact with humans (2). This implies that where there is antibiotic resistance, they must have been in contact with antibiotic producers. Cave microbiology is a relatively new field that looks differently into caves that are already known to have vast and wild ecosystems that greatly differ from those above ground and between other caves. Because many caves are isolated, they have very little to no contact with humans and other animals. Sorcerer's Cave is one of the most isolated and technically difficult caves in Texas. These characteristics prevent considerable human contact. Furthermore, most, if not all, trips have been documented. Samples were taken aseptically at depths of greater than 85m, past very tight crawl ways, squeezes, lakes of chest deep bat guano and vertical drops. The equipment and skills required have protected the cave and left many interested persons at the surface looking in.

Materials and Methods

We collected samples from a trip down into Sorcerer's Cave during January 1-4, 2015. Samples were taken aseptically and transported in sterile 50-mL conical tubes from specific locations and sample types in the cave (Table 1). Upon exit from the cave samples were refrigerated until return to the lab for analysis. 0.1g of each sample was ground using a sterile mortar and pestle and dilutions were performed using 0.85% sodium chloride and plated to isolate colonies. 1.0x, 0.1x and 0.01x TSA was inoculated in order to isolate microbes that could lyse in high nutrient environments and incubated at 20°C (the temperature of Sorcerer's Cave). This allowed us to find the Colony Forming Units per mL (CFU/mL) at different nutrient levels and prevent overgrowth of fast growing bacteria to allow unique bacteria to grow (Table 2). Master plates were created from these colonies, replica plated, incubated, and overlaid with *S. aureus* and *E. coli* cultures in 0.7% soft agar. *S. aureus* and *E. coli* were cultured in liquid medium from stock plates. Colonies that inhibited growth of *S. aureus* or *E. coli* were then isolated by streak plating for further testing.

Results

Preliminary results seem to provide evidence of antibiotic producing bacteria isolated from the obtained samples. So far, 12 potential producers were obtained from the samples (Table 1, Table 2). Potential producers from the wall scrapings inhibited *S. aureus* and *E. coli*, and potential producers from Guano pellets inhibited only *S. aureus* (Figure 1). The isolates from the popcorn speleothem did not show any signs of antibiotic producers.

Sample Source	Depth	Temperature	Potential Producers
Guano Pellets	90 m	20°C	5
Popcorn Speleothem	85 m	20°C	0
Wall Deposits	88 m	20°C	7

Table 1. Sampling site, depth, temperature and potential antibiotic producers isolated from these sites.

Sample Source	1.0X TSA	0.1X TSA	0.01X TSA
Guano Pellets	>3.0 x 10 ⁷	1.8 x 10 ⁶	1.4 x 10 ⁵
Popcorn Speleothem	3.0 x 10 ³	<3.0 x 10 ²	<3.0 x 10 ²
Wall Deposits	7.3 x10 ³	<3.0 x 10 ²	6.2x10 ³

Table 2. Total culturable CFU/g from the samples.

Acknowledgements

We would like to thank the property owners of Sorcerer's Cave for permission to enter the cave and collect samples. Travel was supported by ACU Office of Undergraduate Research. All images were taken by D. Sanderson.

Figure 1. Sampling sites



Guano Pellets



Popcorn Speleothem

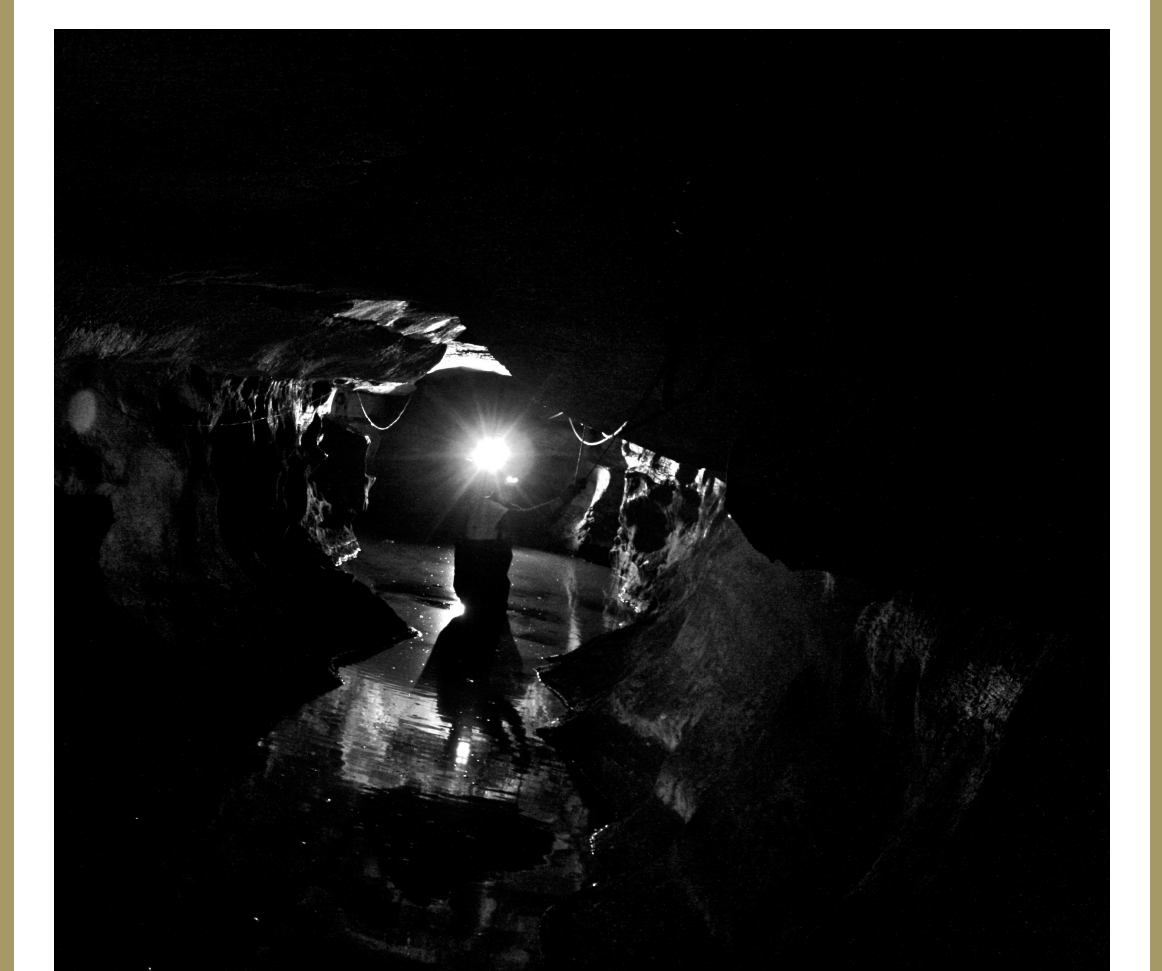


Wall Deposits

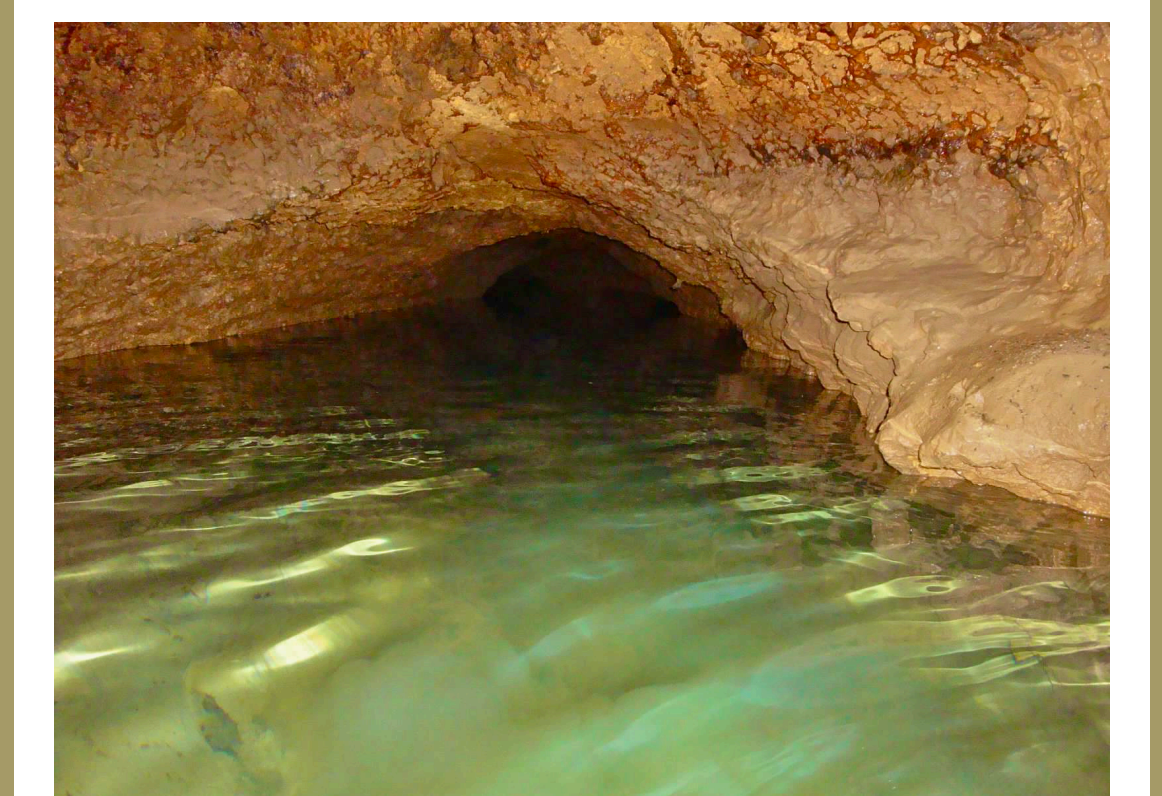
Images of Sorcerer's Cave



Entrance to Guano Lake



Guano Lake



Sirion River

Disussion and Conclusions

Although results are limited and preliminary, this study indicates potential antibiotic production in isolated cave bacteria assisting in the assumption that if there are antibiotic resistant bacteria, there should be producers as well. This preliminary study has shown exciting results in the area of cave microbiology and has potential to find new antibiotics and pharmaceuticals in the near future.

With these promising results, further research will be done to isolate more colonies, test for antibiotic production, and sequence 16s DNA for identification.



Small speleothems in Sorcerer's Cave.

Citations

- Ling L, Schneider T, Peoples A, Spoering A, Engels I, et al. (2015) A new antibiotic kills pathogens without detectable resistance. Nature 517. doi:10.1038/nature14098
- Bhullar K, Wagliechner N, Pawlowski A, Koteva K, Banks ED, et al. (2012) Antibiotic Resistance is prevalent in an Isolated Cave Microbiome. PLoS ONE 7(4):e34953. doi:10.1371/journal.pone.0034953