



Microbial Diversity and Antibiotic Production in Sorcerer's Cave

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Introduction

- **Bacterial resistance is a well documented issue facing medical fields.**
- **Bacteria are becoming resistant to commercial antibiotics faster than they can be produced.**
- **Finding new sources of antibiotics is crucial.**

Introduction

- **Soil bacteria were an early source of antibiotics.**
- **As early as the 1960's soil bacteria had been almost fully mined for culturable antibiotic producers (1).**
- **Microbiologists continue to explore new diverse environments in search of natural antibiotic producers.**

Antibiotic Resistant Cave Bacteria

- **In Lechuguilla Cave in NM, novel antibiotic resistant bacteria were found to be resistant to commercialized antibiotics (2).**
- **The bacteria showed resistance to some of the medically "reserved" antibiotics used to treat MRSA.**

How did these novel bacteria become resistant?



Research Question

- The presence of antibiotic resistant bacteria indicates previous antibiotic exposure.
- Are there novel antibiotic producing bacteria in Sorcerer's cave, the Deepest Cave in Texas?

Cave Microbiology

- **A relatively new field that has become possible with modern lab techniques and exploration equipment.**
- **Caves are already known to possess vast and wild ecosystems that differ from those above ground.**

Goals

- To isolate novel antibiotic producers in Sorcerer's Cave
- Test for antibiotic production
- Sequence 16s DNA for identification
- Determine the type of antibiotic produced
- Determine the antibiotic pathway used to kill bacteria

Sorcerer's Cave

- Isolated cave location



Sorcerer's Cave

- Technically difficult and diverse:
 - Deep vertical pits



Sorcerer's Cave

- Technically difficult and diverse:
- Tight crawl spaces



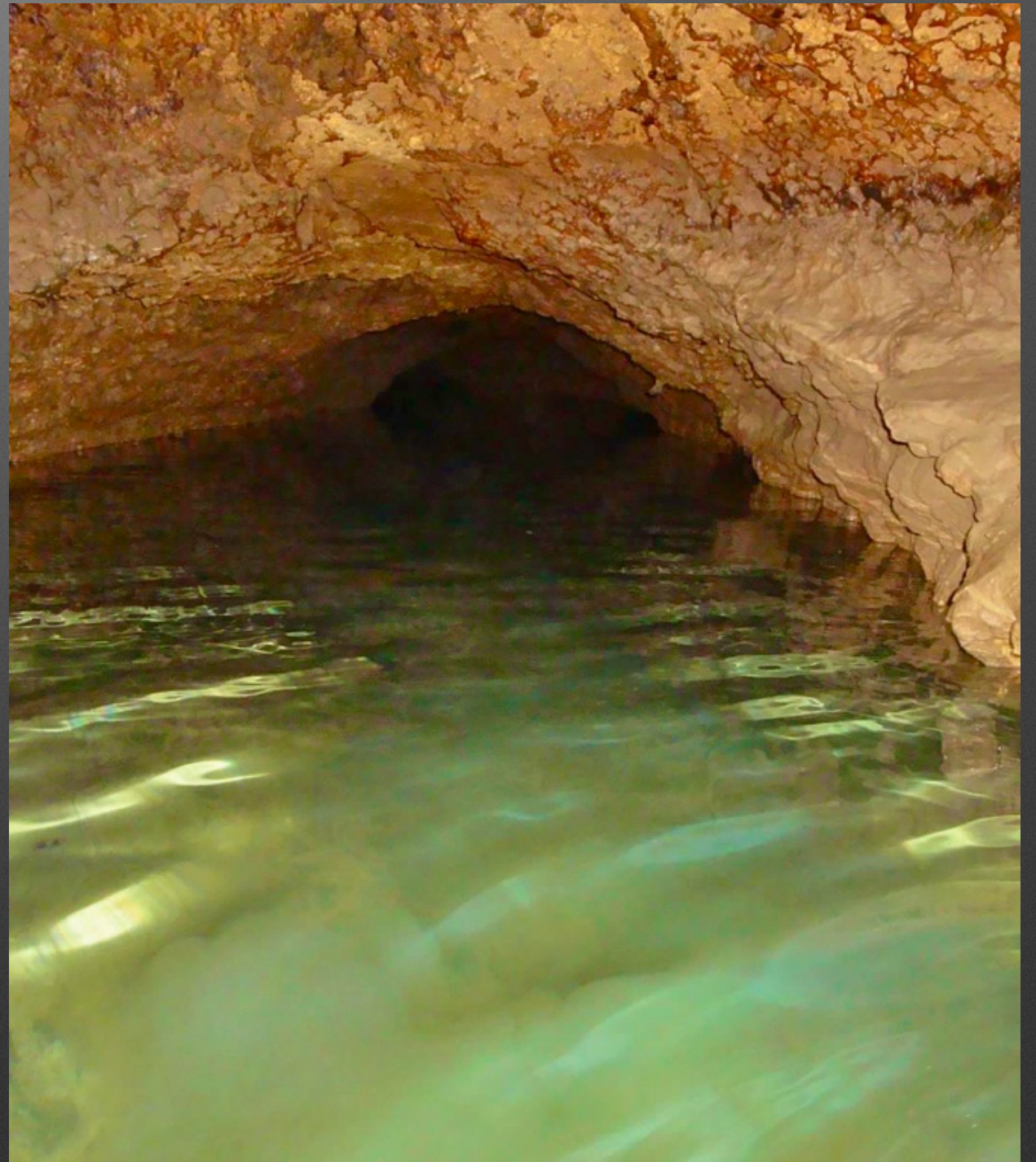
Sorcerer's Cave

- Technically difficult and diverse:
 - Bat Guano



Sorcerer's Cave

- Variety of environments



Samples



Wall deposits



**Bat Guano
Pellets**



**Popcorn
Speleothem**

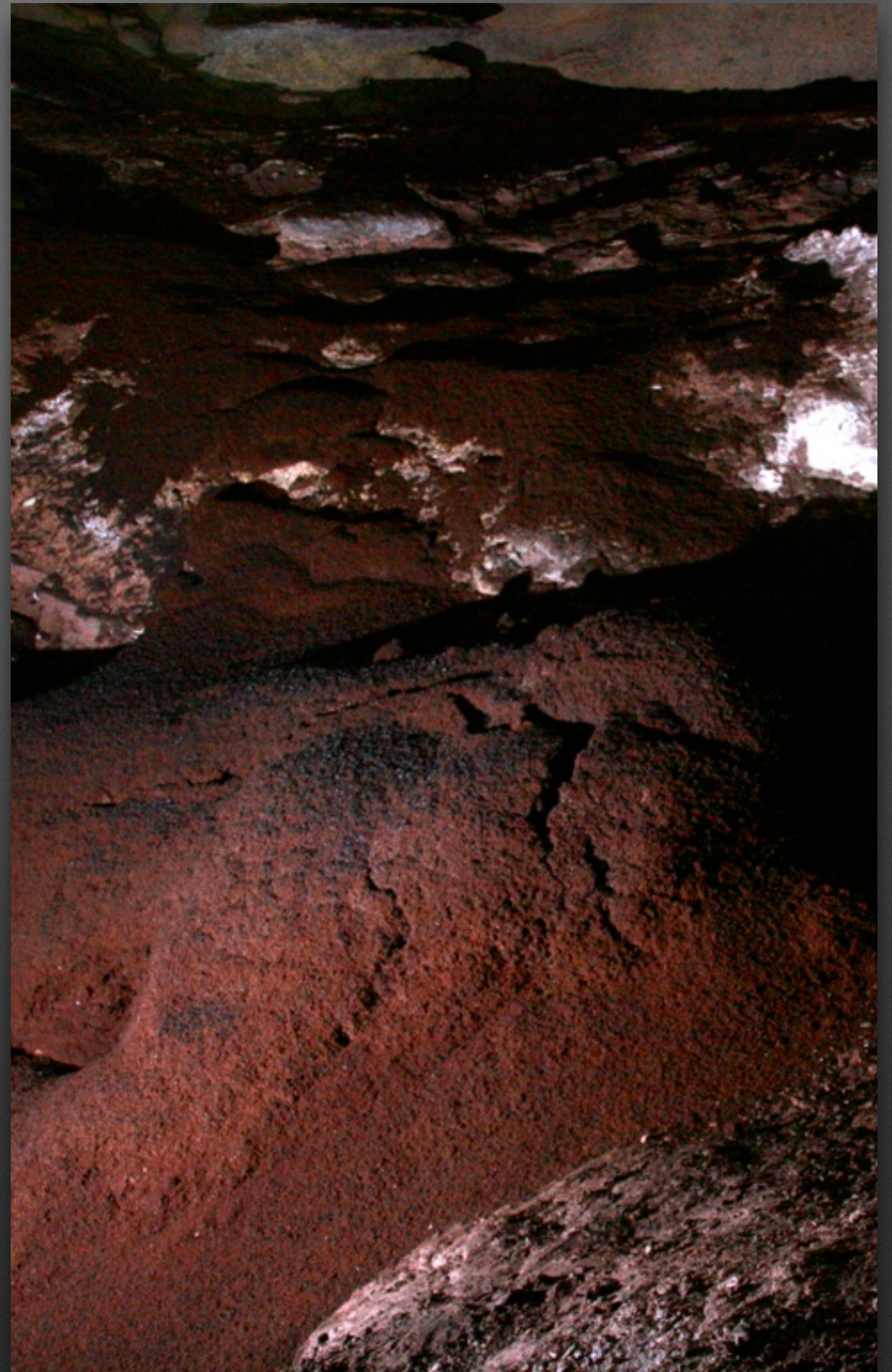
Wall Scrapings

Located 88m underground at a
temperature of 20°C
7 potential producers



Bat Guano Pellets

Located 88m underground at a
temperature of 20°C
5 potential antibiotic producers



Popcorn Speleothem

Located 85m underground at a
temperature of 20°C
No potential antibiotic producers



Methods

- 0.01g of each sample was ground and dilutions performed in 0.85% NaCl solution
- 1.0x, 0.1x and 0.01x TSA incubated at 20°C
- Master plates were created from these colonies and replica plated, incubated and overlaid with *S. aureus* and *E. Coli*

Results

SAMPLE	1.0 X TSA	0.1 X TSA	0.01 X TSA
BAT GUANO PELLETS	$>3.0 \times 10^7$	1.8×10^6	1.4×10^5
POPCORN SPELEOTHEM	3.0×10^3	$<3.0 \times 10^2$	$<3.0 \times 10^2$
WALL DEPOSITS	7.3×10^3	$<3.0 \times 10^2$	6.2×10^3

Total culturable CFU/g from the samples

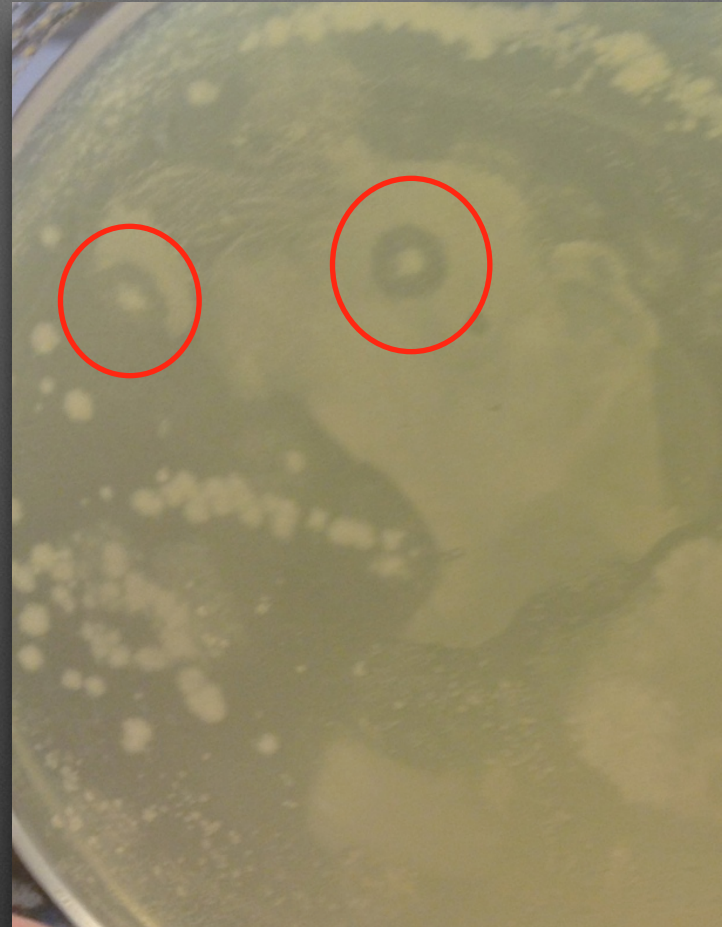
Results

SAMPLE	DEPTH	TEMPERATURE	POTENTIAL PRODUCERS
BAT GUANO PELLETS	90 m	20°C	5
POPCORN SPELEOTHEM	58 m	20°C	0
WALL DEPOSITS	88 m	20°C	7

Results



Colonies from
Bat Guano
overlaid with *S.*
aureus



Colonies from
Wall Deposits
overlaid with *E.*
coli



Colonies from
Wall Deposits
overlaid with *S.*
aureus

Discussion

- **This Study indicates potential antibiotic production in isolated cave bacteria.**
- **Assists in the assumption that if there are antibiotic resistant bacteria, there are antibiotic producers as well**

Next Steps

- **Sequence 16s DNA for identification**
- **Determine type of antibiotic produced**
- **Determine antibiotic pathway used to kill bacteria**

Acknowledgments

- Property owners of Sorcerer's Cave
- Images were taken by David Sanderson unless otherwise noted
- Exploration team
 - From Left: Alice Sanderson, David Sanderson, Hadley Robinson, and Jason Tilley



Citations

1. 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
2. Bhullar K, Waglechner 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
3. <http://www.rocketswag.com/medicine/anatomy/nervous-system/human-nervous-system/How-Does-Central-Nervous-System-Affect-Human-Behavior.html>
4. http://commons.wikimedia.org/wiki/File:Map_of_Texas_highlighting_Terrell_County.svg



Questions?