Antibiotic Resistance and Biosynthetic Potential of Microbes Isolated from Sorcerer's Cave, Texas

Biology Research

Jeffrey Wooliscroft and Dr. Jennifer Huddleston

Biology Department, Abilene Christian University, Abilene Texas 79699

Abstract

Unexplored cave environments provide a valuable opportunity for novel microorganisms and compounds. In total, fourteen culturable microbial organisms were isolated from Sorcerer's Cave, Texas. Antibiotic resistances were studied, showing levels of resistance in all organisms. Composition of studied organisms was largely dominated by the genus *Pseudomonas* (~79%). Our results addressing antibiotic resistances were not bizarre in regards to pseudomonad multiple antibiotic resistance properties. However, this study provides an early first view of this unique cave environment and will pursue the biochemical opportunities these organisms may hold in the future.

Introduction

Cave environments exhibit an environment consisting of nutrient poor surfaces accompanied by low temperatures and high humidity levels. Although they display challenging living conditions, caves provide unique opportunities for microorganisms that favor cooler environments (2).

Unique biofilm inhibition compounds and mechanisms have been linked to microorganisms present in cave environments (6).

At this point in time, many caves throughout the world remain vastly undiscovered and untouched by humankind. At the same time, humans have struggled to continually develop new and emerging antimicrobial agents to deter infection (3). Humans are challenged to find and develop these compounds as we fight the resistances developed every time we use them (4). As these issues have become progressively more prevalent, caves have become an increasingly viable option to provide novel and relevant antimicrobial compounds. Several studies have been successful in pursuit of these compounds, but cave environments still stand to be explored much more extensively. In combination with limited knowledge regarding cave microbial diversity, caves provide a possible new frontier for novel microorganisms that could provide these compounds (5).

Located in Terrel County, Sorcerer's Cave is the deepest cave in Texas at 558 feet (13). Although the shallower portion was previously inhabited by Native Americans, Sorcerer's Cave has been largely untainted by humankind. It was rediscovered in 1941 and remained unmapped and unexplored until 1978 (13). Since this time, a limited number of expeditions have been executed with varied purposes ranging from archaeological to narrow biological work concerning bats (11,12).

Access has been heavily limited for the past 10 years due to private ownership of the cave. In combination with the scarce biological work and absence of microbial analysis, Sorcerer's Cave provides an excellent opportunity for the discovery of novel microorganisms. In this study, culturable microbes from various sources within the cave were isolated and analyzed in order to assess their innate antimicrobial resistances.

Acknowledgements

This project was supported by funds from an ACU Pursuit Grant (2015), a summer stipend from the ACU Office of Undergraduate Research, and an ACU Math-Science Grant.

NCBI BLAST Database

Isolate Code	Genus & Species	Strength (E-value)	Identification
1C	*Pseudomonas azotoformans* IHBB 9881	0.0	100%
5C	*Pseudomonas* HP10C	0.0	100%
7K	*Sphingobacterium* FLX-7	0.0	100%
3R	*Pseudomonas* TK30	0.0	100%
8C	*Pseudomonas* HP10C	0.0	100%
1A	*Pseudomonas* LHYT-8	0.0	100%
2A.2	*Pseudomonas* HP10G	0.0	100%
1C.2	*Pseudomonas* HP10C	0.0	100%
6C.2	*Pseudomonas azotoformans* IHBB 9881	0.0	100%
2C.2	*Pseudomonas* HP10C	0.0	100%
8K.2	*Sphingobacterium* LCB40	0.0	100%
6R.2	*Pseudomonas* DSM 29140	0.0	100%
6A.2	*Pseudomonas jessenii* IHBB 11152	0.0	100%
1K.2	*Chryseobacterium* A1-652	0.0	100%

Table 1. Results of 16s rRNA DNA gene amplification and BLAST comparative analysis.

Antibiotics

Isolate Code*	Kanamycin (64 mg/ mL)	Ampicillin (32 mg/ mL)	Cefotaxime (32 mg/ mL)	Rifampicin (4 mg/ mL)	Nalidixic Acid (32 mg/ mL)	Sulfamethoxazole (197 mg/ mL)
1C	-	R	R	R	R	R
5C	R	R	R	R	R	R
7K	R	R	-	-	R	-
3R	-	R	R	R	R	-
8C	~	R	R	R	R	R
1A	-	R	R	R	R	R
2A.2	-	R	R	R	R	R
1C.2	-	R	R	R	R	R
6C.2	-	R	R	R	R	R
2C.2	-	R	R	R	R	R
8K.2	R	R	-	-	R	R
6R.2	-	R	~	R	R	R
6A.2	-	R	R	R	R	R
1K.2	R	R	R	-	-	R

Table 2. Antimicrobial activity of bacterial isolates from Sorcerer's cave.
R, resistant. -, sensitive. ~, partial or indeterminate growth. *Isolate codes are indicative of the antibiotic in the initial isolation medium: C, cefotaxime. K, kanamycin. R, rifampicin. A, ampicillin.

Materials and Methods

Samples were aseptically collected in January 2015 and then refrigerated until use. Six antibiotics (ampicillin, kanamycin, rifampicin, cefotaxime, nalidixic acid, and sulfamethoxazole) were used for testing. Antibiotics were sterilely incorporated into tryptic soy agar. 500 mg or 500 µL of each sample was placed into 4.5 mL of 0.85% NaCl solution and diluted five times. Each dilution level was then tested by placing 100 mL of each level onto an antibiotic plate as well as a control for the lowest dilution levels. All plates were then incubated at 25 ℃ and monitored at 24 h intervals over the course of 2-3 days. Plates were then evaluated for levels of growth and colony counts were performed. Eight colonies were randomly chosen from each set of antibiotic plates. These isolates were then subcultured on their respective antibiotic and then tested for multiple resistances. Fourteen isolates in total were selected for further testing and subcultured. DNA was extracted using a Zymo DNA extraction kit and their 16S rRNA DNA gene amplified, purified, and sent to Yale University DNA Analysis Facility on Science Hill for sequencing.

Results

A total of 17 resistant microbes were isolated from a bat guano lake within Sorcerer's Cave. After Gram staining and analyzing isolates, three were recognized as yeast. Among the 14 other isolates, all were Gram negative and four out of 14 appeared as filamentous rods. The 16S rRNA DNA genes were amplified and analyzed from all but the three identified as yeast. Of these, 11 isolates were identified as a species of *Pseudomonas*, two isolates as a species of *Sphingobacterium*, and one isolate as a species of *Chryseobacterium* (Table 1). Approximately 29% of the sequenced isolates were resistant to kanamycin at the tested concentration. Isolates were resistant for ampicillin (100%), nalidixic acid (~93%), and sulfamethoxazole (~86%). Isolates also displayed resistance to cefotaxime (~79%) and rifampicin (~79%) (Table 2). Among isolates, 5C (*Pseudomonas azotoformans* IHBB 9881) displayed the most resistances, surviving all antibiotics at tested levels. Every isolate tested exhibited resistance to at least three of the six antibiotics used. The majority of isolates (10 of 14) showed resistances to at least five antibiotics. Organisms originally isolated off of the medium containing cefotaxime tended to be resistant to multiple antibiotics as well (Table 2).

Discussion and Conclusion

This study demonstrates the level of innate antibiotic resistance present in many microbial cave organisms. Using TSA medium, we isolated numerous culturable microbial organisms from Sorcerer's Cave ranging from yeast to various strains of *Pseudomonas*. Only the most antibiotic-resistant bacterial organisms were further tested and their resistances were determined. *Pseudomonas* isolates were the most resistant bacteria tested. Innate resistances present in pseudomonads came as no surprise as they have been known to present a wide array of multidrug resistances due to integrons on plasmids (1). *Pseudomonas azotoformans* and *Pseudomonas jessinii* were particularly interesting isolates as they have both been found to be in the fluorescent group of pseudomonads (8,9). Fluorescent pseudomonads are most commonly associated with the soil and rhizosphere (10). Interestingly enough, there is only a single study loosely linking fluorescent pseudomonads to bat guano (7).

In conclusion, our study provides a valuable first report on the innate microbial resistances and diversity of microbial life present in Sorcerer's Cave. Our results show that these organisms can exhibit multiple resistances to antimicrobial compounds in different classes. As this study progresses, the association between fluorescent microorganisms in the genus *Pseudomonas* will be explored in more depth. Additionally, in future studies these organisms, as well as organisms isolated from our other samples, can be assessed for their abilities in producing novel compounds relevant to the medical and biochemical industries.

Citations

1. Adesoji, A. T., Ogunjobi, A. A. & Olatoye, I. O. Molecular characterization of selected multidrug resistant *Pseudomonas* from water distribution systems in southwestern Nigeria. *Ann Clin Microbiol Antimicrob* **14,** (2015).
2. Barton, H. A. & Northup, D. E. Geomicrobiology in cave environments: past, current and future perspectives. *Journal of Cave and Karst Studies* 163–178 (2007).
3. Brandenburg, K. & Schürholz, T. Lack of new antiinfective agents: Passing into the pre-antibiotic age? *World J Biol Chem* **6,** 71–77 (2015).
4. Davies, J. & Davies, D. Origins and Evolution of Antibiotic Resistance. *Microbiol Mol Biol Rev* **74,** 417–433 (2010).
5. Engel, A. S. Microbial Diversity of Cave Ecosystems. *Geomicrobiology: Molecular and Environmental Perspective* 219–238 (2010). doi:10.1007/978-90-481-9204-5_101.
6. Farmer, J. T. *et al.* Environmental bacteria produce abundant and diverse antibiofilm compounds. *J. Appl. Microbiol.* **117,** 1663–1673 (2014).
7. Iva Tomova, I. L. Diversity and biosynthetic potential of culturable aerobic heterotrophic bacteria isolated from Magura Cave, Bulgaria. *International Journal of Speleology* **42,** 65–76 (2013).
8. Iizuka, H, & Komogata, K . New species of *Pseudomonas* belonged to fluorescent group (Studies on the microorganisms of cereal grains. Part V). *Journal of the Agricultural Chemical Society of Japan* 37: 137–141 (1963).
9. Kosina, M. *et al.* Description *of Pseudomonas jessenii* subsp. pseudoputida subsp. nov., amended description of *Pseudomonas jessenii* and description of *Pseudomonas jessenii* subsp. jessenii subsp. nov. *Folia Microbiol* **58,** 631–639 (2013).
10. Scales, B. S., Dickson, R. P., LiPuma, J. J. & Huffnagle, G. B. Microbiology, Genomics, and Clinical Significance of the Pseudomonas fluorescens Species Complex, an Unappreciated Colonizer of Humans. *Clin. Microbiol. Rev.* **27,** 927–948 (2014).
11. Steele, D. G. Human and Non-Human Skeletal Remains Recovered from Sorcerer's Cave, Terrell County, Texas. *The Texas Journal of Science* **36,** 169–184 (1984).
12. Veni, G. Thanksgiving 2001 – Sorcerer's Cave. *The Texas Caver* **48,** 12–14 (2002).
13. Veni, G. Sorcerer' s Cave – It's Story. *The Texas Caver* **25,** 2–21 (1980).