

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

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Introduction

- Unique environments
- History of Sorcerer's Cave
- Untouched by humankind
- Antibiotic problem



Source: David Sanderson

Materials and Methods

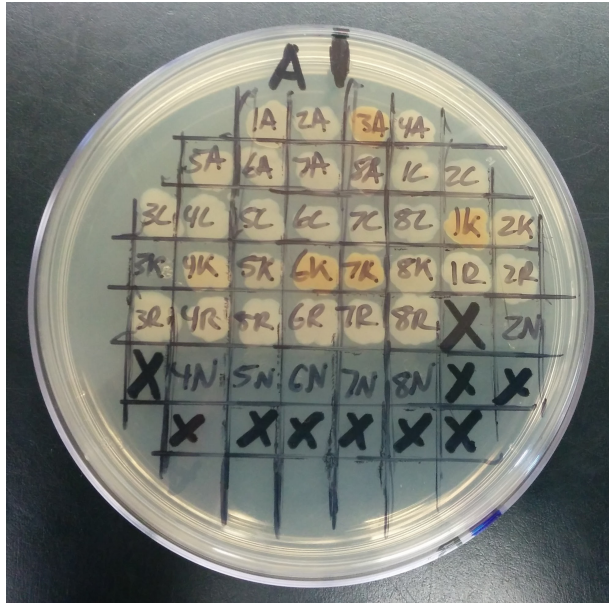
- Sample Collection
- Antibiotic Selection
- Antibiotic and Plate Preparation

List of antibiotics used and their respective source and class.

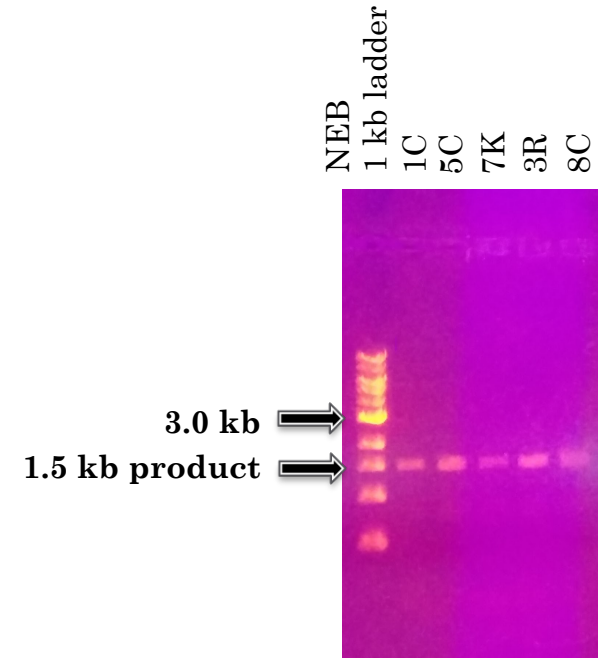
Antibiotic	Source	Class
Kanamycin	Natural	Aminoglycoside Bacteriocidal
Ampicillin	Natural	β -lactam Penicillin
Rifampicin	Natural	RNA Synthesis Inhibitor
Cefotaxime	Semi-synthetic	β -lactam Cephalosporin
Nalidixic Acid	Synthetic	Quinolone
Sulfamethoxazole	Synthetic	Folic Acid Synthesis Inhibitor

Materials and Methods: Sample Testing

sample → antibiotic media → isolate colonies → cross-test on other antibiotic media → amplify 16S rRNA gene → sequence → identify genus



Example of a cross-test plate used in multi-resistance testing.



Amplification of 16S rRNA DNA from selected isolates.

Results

- 17 microbial isolates
- All Gram negative
- 3 were yeast and excluded from further testing

Results of Gram staining performed on microbial isolates from Sorcerer's Cave.

Isolate Code	Gram Staining
1C	Gram Negative Rods
5C	Gram Negative Rods
7K	Gram Negative Rods
6N	Yeast
3N	Yeast
3R	Gram Negative Rods
8C	Gram Negative Filamentous Rods
1N	Yeast
1A	Gram Negative Rods
2A.2	Gram Negative Rods
1C.2	Gram Negative Filamentous Rods
6C.2	Gram Negative Filamentous Rods
2C.2	Gram Negative Filamentous Rods
8K.2	Gram Negative Rods
6R.2	Gram Negative Rods
6A.2	Gram Negative Rods
1K.2	Gram Negative Rods

Results cont.

Results of 16S rRNA gene amplification and BLAST comparative analysis.

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

Results cont.

		Antibiotics					
Isolate Code	Genus	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	<i>Pseudo.</i>	-	R	R	R	R	R
5C	<i>Pseudo.</i>	R	R	R	R	R	R
7K	<i>Sphingo.</i>	R	R	-	-	R	-
3R	<i>Pseudo.</i>	-	R	R	R	R	-
8C	<i>Pseudo.</i>	~	R	R	R	R	R
1A	<i>Pseudo.</i>	-	R	R	R	R	R
2A.2	<i>Pseudo.</i>	-	R	R	R	R	R
1C.2	<i>Pseudo.</i>	-	R	R	R	R	R
6C.2	<i>Pseudo.</i>	-	R	R	R	R	R
2C.2	<i>Pseudo.</i>	-	R	R	R	R	R
8K.2	<i>Sphingo.</i>	R	R	-	-	R	R
6R.2	<i>Pseudo.</i>	-	R	~	R	R	R
6A.2	<i>Pseudo.</i>	-	R	R	R	R	R
1K.2	<i>Chryseo.</i>	R	R	R	-	-	R

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.

Pseudo, Pseudomonas; *Sphingo*, Sphingobacterium; *Chryseo*, Chryseobacterium.

Discussion and Conclusions

- Isolates resistant to multiple antibiotics
- Presence of fluorescent pseudomonads
- Possible industrial applications
- Future directions:
 - More samples
 - Horizontal gene transfer

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Questions?