Analysis of Slime Deposits on Fiberglass Surfaces in Kartchner Caverns

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Abstract

Kartchner Caverns, discovered in November 1974 in the Whetstone Mountains of southern Arizona, is a wet "living" cave. Carbonate features continue to grow due to percolating waters from the surface. In an effort to maintain Kartchner Caverns as a living cave, it has been carefully developed and access to the cave, which was opened to the public in 1999, is restricted and supervised. Fiberglass surfaces installed to partition sections of the cave, including construction and maintenance work areas, have developed a slimy growth that returns even after washing of the fiberglass with bleach solution. Samples of the slime were collected aseptically by swabbing, and a variety of bacteria were subsequently isolated on a minimal heterotrophic medium (R2A). Repetitive Extragenic Palindromic Sequence (REPS) and 16s rDNA PCR methods were performed to characterize the organism(s) responsible for the slime production. Sequencing and BLAST analyses of the 16s rDNA PCR products have identified nine of the eleven isolates.

Figure 1. Installed fiberglass barriers

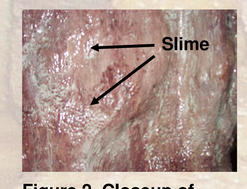


Figure 2. Closeup of painted fiberglass surface

Background Studies

- Microbial processes and ecology in cave environments are unique. **(5, 7, 10)**
- A majority of indigenous cave microbes are chemoautotrophs. Heterotrophs are opportunistic and are transported from the surface by air or water flow, or via animal activity. **(4, 9)**
- Normal culture methods obtain approximately 1% of viable, indigenous cave microbes in the environment. **(1)**
- Development of molecular-phylogenetic techniques has aided in demonstrating the variety of novel organisms to be found in caves. **(2)**
- Biofilms are found on cave surfaces as they are on all other surfaces. **(3, 8)**
- Human activity appears to encourage biofilm growth in caves as evidenced in Kartchner (slime on fiberglass) and other caves (ancient rock-art paintings Atlanterra shelter, southern Spain). **(6)**

Objectives

- Identify the microbial populations responsible for the slime production
- Determine whether population(s) are indigenous or anthropogenic in origin

Methods

1. Copious amounts of slime visible on the painted fiberglass surface(s) called for use of culture methods. Sample swabs were taken of painted fiberglass and true rock surfaces (control). Sterile R2B served as the transport medium.
2. Sample tubes were vortexed to allow for physical release and subsequent dilution plating was done on R2A, with incubation at 25^{O}C. Unique colonies were isolated as they appeared, with a total incubation time of 12 weeks for slow-growing microbes. Isolates were restreaked to obtain pure cultures and Gram -stained.
3. REPS-PCR was performed to differentiate unique isolates.
4. Isolates were regrown in R2B from 24-48 hours, and lysed. 16s rDNA PCR was performed using the following protocol:

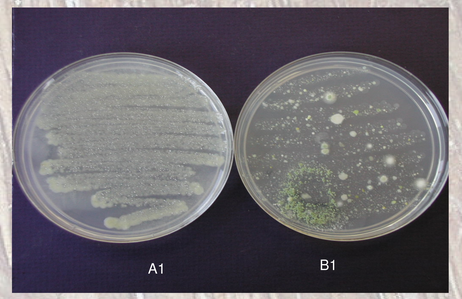
Reagent	Per 25 uL RxN (uL)
Buffer B	2.5
Primers (27f and 1492r)	2.5
dNTP's	2.0
DMSO	1.25
Taq reg.	0.1
Molecular-grade H_2O	14.15

Cycling times (min)	Temp (^{O}C)	Purpose
5	95	initial denaturation
1*	**94**	**denaturation**
1*	**63**	**annealing**
1.25*	**72**	**extension**
10	72	final extension

* 30 total cycles were run.*

5. 2% agarose gels were run to determine successful amplification of the 1500 base pair product. The product was further purified using a Quiagen PCR purification kit, and dsDNA density analysis was performed using a spectrometer.
6. If A260:A280 was in the 1.8-2.0 range, then samples were sent to the University of Arizona DNA sequencing facility for sequencing. BLAST analysis served to identify the isolates.

- Slime-producing bacteria predominated on painted fiberglass surfaces ((Figure 1, plate A1) compared to true cave rock surfaces (Figure 1, plate B1) .



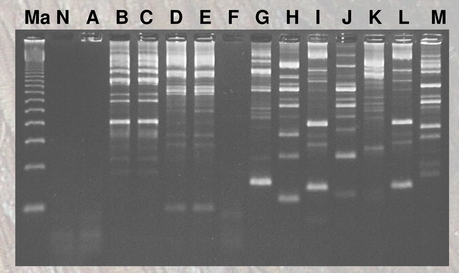
- Sequencing of the 16s rDNA region of each bacterium and subsequent BLAST analyses have allowed for identification of nine of the eleven isolates. (See table below.)

ISOLATE	IDENTIFICATION	SLIME PRODUCER
A	***Bacillus sp.***	Y
B, C	***Dyadobacter fermentens***	Y
D, E	***Sphingomonas yanoikuyae***	N
F	***Staphylococcus sp.***	N
G	***Brevibacillus sp.***	N
H	***N/A***	Y
I, L	***Sphingomonas sp.***	Y
J	***N/A***	Y
K	***Sphingobium chlorophenolica***	N
M, N	***Rhizobium sp.***	N
O	***Sinorhizobium sp. S009***	N

Results

- REPS - PCR differentiated eleven total bacterial isolates

(See sample REPS gel below)



Conclusions

- Preliminary evidence suggests the causative agents of the slimy biofilms were not anthropogenically transported into the cave.
- A number of the slime-producers are common soil isolates that may have arrived via dripping waters that percolated down through overlying surface soils and rock material.

Future Objectives

- Field experimentation (in-cave) will be conducted in order to determine whether a single isolate or a consortium of bacteria is causing the slimy-biofilm growth
- Both painted and non-painted fiberglass pieces will be tested to assess the role of the paint as growth substrate.

Acknowledements

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