

## COMPOSITION OF BACTERIAL MATS IN EL MALPAIS NATIONAL MONUMENT, NEW MEXICO, USA: COMPARISON AND CONTRASTS WITH BACTERIAL COMMUNITIES IN HAWAII LAVA TUBES

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Cave bacterial mats cover the walls of lava tubes around the world, including the lava tubes in New Mexico, yet little is known about their bacterial composition and role in the ecosystem. We undertook a study of the differently colored bacterial mats found in Pahoehoe, Four Windows, and Roots Galore Caves in El Malpais National Monument (ELMA), located southwest of Grants, New Mexico. To determine the bacterial community composition and the phylogenetic relationships of the bacterial mats found in these three caves, we aseptically sampled bacterial mats found in the twilight and dark zones of each cave. Bacterial DNA was extracted and purified, the 16S rRNA gene was amplified using polymerase chain reaction (PCR), and approximately 1400 bases were sequenced from clone libraries. Bacterial identities of the closest relatives were found using Ribosomal Database Project II and BLAST, while a maximum parsimony phylogenetic tree was constructed using PAUP.

Our results reveal the presence of a diverse bacterial community comprising the differently colored lava tube mats that includes members related to the *Actinobacteria*, *Gammaproteobacteria*, *Alphaproteobacteria*, *Acidobacteria*, *Firmicutes* and *Chloroflexi*. There exists common overlap in bacterial communities across the three cave sites, but most notably within the *Actinobacteria*, the bacterial group that produces many of the antibiotics in use today. In comparison with a parallel study in Hawaiian lava tubes, ELMA microbial mats were less diverse, but overlapped in some phyla present. Our studies show that there is less diversity in yellow bacterial mats than white bacterial mats in both the New Mexican and Hawaiian lava tubes. Putative *Actinomyces* were found among the *Actinobacteria*, which suggests that heterotrophy occurs in these lava tubes. In addition, putative *Nitrosococcus* were found among the *Gammaproteobacteria*, suggesting that ammonia oxidation may also occur. Our studies are shedding light on the nature of these communities and their possible roles in the ecosystem.

### 1. Introduction

The colorful mats that exist in caves and lava tubes all over the world are known to be microbial thanks to culture studies, scanning electron microscopy, and culture-independent molecular phylogenetic techniques. Until recently scientists used only culture-based techniques to study microorganisms in environments such as caves. Researchers have assumed that the microbial mats in lava tubes are primarily composed of actinomycetes. However, our preliminary studies have revealed many new microbial species waiting to be identified in these mats. These unidentified species could have some useful medicinal value as has been shown in actinomycetes. Some types of actinomycetes are medicinally and culturally significant because they excrete antibiotic products to repel invaders (Lazzarini et al. 2000). The antibiotic properties of many bacteria species make them promising biotechnology targets.

Humid lava tube caves contain highly visible mats of

bacteria and other microorganisms, nicknamed "lava wall slime," but they have been studied even less than limestone caves (Northup and Welbourn 1997; Northup et al. 2008). Howarth (1981) has suggested that nutrient recycling (e.g. nitrogen) occurs in the microbial mats. Ashmole et al. (1992) have found microbial mats present in humid caves in the Canary and Azores Islands, but never in caves lacking moisture. Staley and Crawford 1975 have found microbial mats consisting of different species of bacteria, including actinomycetes in the genus *Streptomyces* in research done in lava tubes in Washington

We have had limited success in culturing microorganisms from the environment, including caves, using standard microbiological media (Northup et al. 1994; Amann et al. 1995; Hugenholtz et al. 1998). Molecular phylogeny, using the 16S ribosomal rRNA gene, has revolutionized our understanding of the great diversity and distribution of life present in the environment (Pace 1997). Many

novel bacterial and archaeal species have been detected as a result of this new technology in a variety of environments. Bacteria have been found in some of the most extreme areas including deep-sea hydrothermal vents, kilometers below the surface of the Earth in rock, and in caves. These microorganisms are important participants in the precipitation and dissolution of minerals in caves (Northup and Lavoie 2001; Barton and Northup 2007) and in a variety of surface settings (Ehrlich 1999). However, researchers have barely begun to characterize the microbial diversity of caves and the roles of microorganisms in the subsurface. Additionally, we know little of what abiotic factors control the lava tube microbial diversity. We feel that investigating these mats, using culture-independent techniques, will provide valuable insights into the nature of these communities and what determines their diversity.

This study intends to identify many novel bacterial species that inhabit the walls and ceiling of several El Malpais lava tubes and compare them to the parallel study of the bacterial communities in Hawai'i lava tubes (Garcia et al., this volume). This study will advance our knowledge of the differences and similarities found among lava tubes with varied age flow, surface conditions and different colored bacterial mats.

**2. Methods**

The three lava tubes sampled at El Malpais National Monument, Four Windows, Pahoehoe, and Roots Galore, occur in the Bandera lava flow, which is approximately 10,000 years old. El Malpais National Monument is located southwest of Grants, New Mexico, USA. Age of flow and average area rainfall data for Hawaiian and El Malpais lava tubes were ascertained using a variety of online resources and from previous investigations (Laughlin and Woldegabriel, 1997).

We recorded entrance elevation and GPS coordinates, and cave temperature and humidity were measured using an IMC Digital Thermometer probe. Small samples of wall rock covered with bacterial mats were collected from the three El Malpais Lava tubes under a National Park Service collecting permit. Samples were covered with sucrose lysis buffer to preserve the DNA and transported to the lab where they were stored in a -80°C freezer until DNA extraction. Yellow and white microbial mats were sampled from Pahoehoe and Roots Galore Caves, and white and gold microbial mats from Four Windows Cave, in the El Malpais National Monument.

DNA was extracted and purified using the MoBio PowerSoil™ DNA Isolation Kit using the manufacturer's protocol (MoBio, Carlsbad, CA). Extracted DNA was amplified with universal bacterial primers 46 forward (5'-GCTAAYACATGCAAGTCC-3') and 1409 reverse (5'-GTGACGGGCRGTGTGTRCAA-3')(Vesbach, personal communication). Amplicons were cleaned and

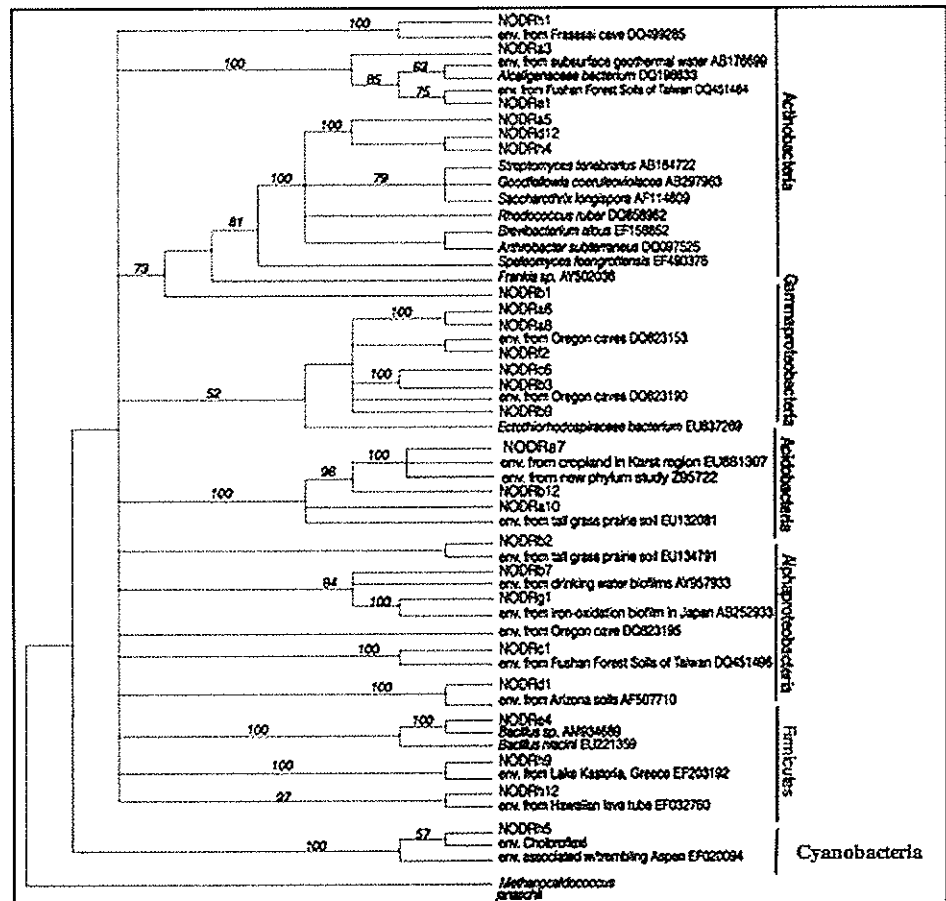


Figure 1: Parsimony tree of bacterial clone sequences from a yellow microbial mat from Pahoehoe Cave in El Malpais National Monument. Numbers on the branches indicate bootstrap values from 1000 re-samplings and indicate the degree of support for this tree topology.

purified using the Qiagen PCR cleanup kit (Qiagen, Germantown, Maryland) and were cloned using the TOPO TA Cloning kit (Invitrogen, Carlsbad, CA) and sent to Washington University Genome Sequencing Facility for sequencing with primers M13F and M13R. Once received, sequences were edited and contiged with Sequencher 4.8. (Gene Codes, Ann Arbor, Michigan). To check the orientation of our sequences and to convert from antisense to sense, OrientationChecker ([www.cardiff.ac.uk/bioinformatics/biosoft/](http://www.cardiff.ac.uk/bioinformatics/biosoft/)) was used. Chimeras were detected using the Mallard software (<http://www.bioinformatics-toolkit.org/Mallard/>). Rarefaction curves were generated using Dotur (<http://schloss.micro.umass.edu/software/dotur.html>) to ascertain whether sequencing had detected a comprehensive set of community members.

Sequences were analyzed using BLAST (NCBI; Altschul et al. 1997) to identify closest relatives. Initial alignment was completed with Greengenes ([greengenes.lbl.gov/](http://greengenes.lbl.gov/)) and manually corrected using BioEdit editor (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>), guided by 16S primary and secondary structure considerations. Parsimony analysis was performed using PAUP (version 4.0b10, distributed by Sinauer; <http://paup.csit.fsu.edu/>) and bootstrap analyses were conducted on 1000 re-sampled datasets using PAUP.

Samples of the lava tube wall rock covered with microbial colonies were examined on a JEOL 5800 scanning electron microscope (SEM) equipped with an Oxford (Link) Isis energy dispersive x-ray analyzer (EDX). Rock samples with adherent bacterial colonies were mounted directly on an SEM sample stub while in the cave and then coated by evaporation with Au-Pd in the lab prior to imaging.

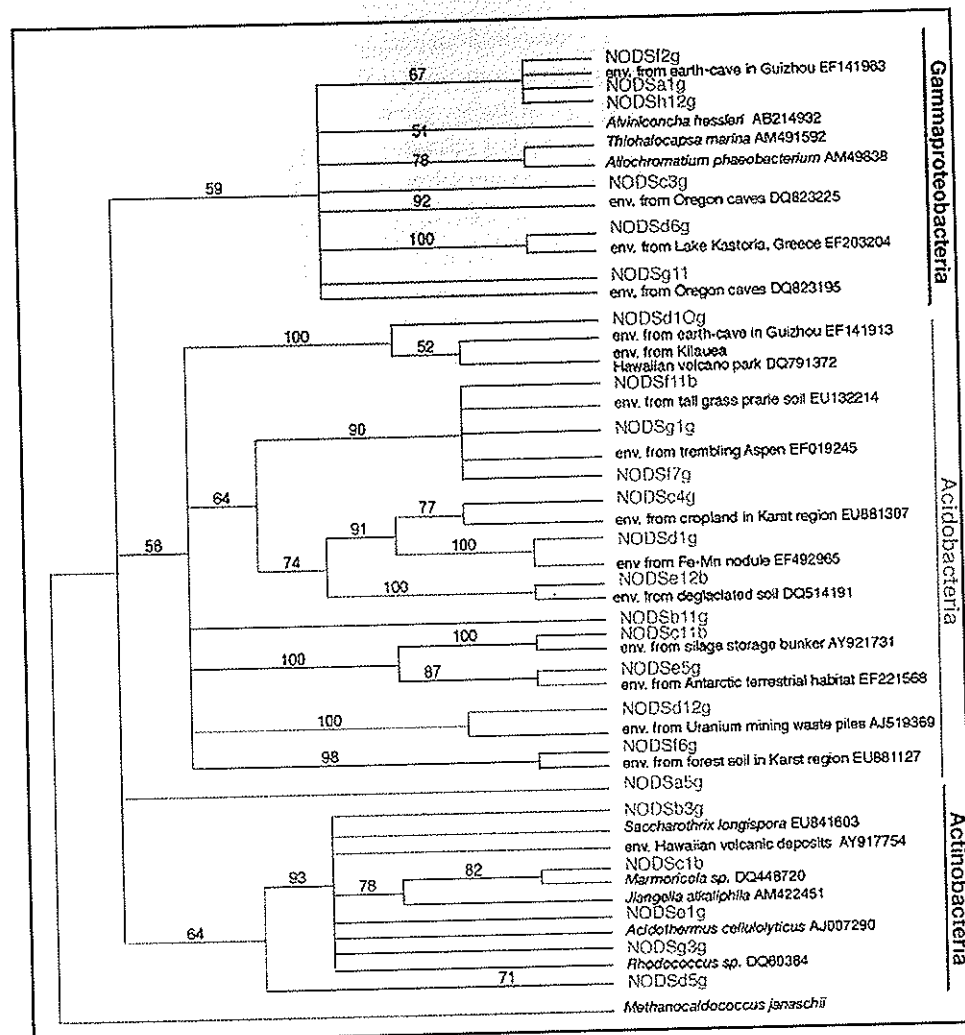


Figure 2: Parsimony tree of bacterial clone sequences from a yellow microbial mat in Roots Galore Cave in El Malpais National Monument. Numbers on the branches indicate bootstrap values from 1000 re-samplings and indicate the degree of support for this tree topology.

### 3. Results and Discussion

The phylogenetic analysis of yellow microbial mats from Pahoe Cave and Roots Galore Caves revealed that the sequences group within six phyla: *Actinobacteria*, *Gammaproteobacteria*, *Alphaproteobacteria*, *Acidobacteria*, *Firmicutes* and *Chloroflexi*. Pahoe Cave yellow bacterial mats are more diverse than the Roots Galore mats, which only contacted sequences from the *Actinobacteria*, *Gammaproteobacteria*, and *Acidobacteria* phyla. Interestingly the Pahoe Cave phylogenetic tree (Fig. 1) and the Roots Galore tree (Fig. 2) overlap in the *Gammaproteobacteria*, *Acidobacteria* and *Actinobacteria*. Some of the close relatives that group with our sequences were isolated from other caves around the world, such as Frasassi cave (*Actinobacteria*) and Oregon caves (*Gammaproteobacteria*). Other closest relatives were environmental isolates from a variety of soils.

In Figure 2 the *Actinobacteria* clone sequences from our study have mainly cultured closest relatives, something that is rarely encountered in our environmental studies where novel species are common. Also, there are close relatives that came from Oregon caves and Hawaiian lava tubes. In addition, there is a close relative that came from an Fe-Mn nodule, suggesting the presence of possible iron and manganese oxidizing microbes in this lava tube. Because of the iron in the basalt of the El Malpais lava tubes, this is not a surprising finding and reinforces the idea of using various iron media to isolate iron bacteria for further physiological and biochemical study.

In comparing results from this study and a parallel study by Garcia et al. (this volume) of microbial mats in lava tubes on Hawai`i, we see a combined diversity that spans 13 phyla, including four of the subdivisions of the *Proteobacteria*. The greatest overlap amongst New Mexican and Hawaiian lava tubes occurred among the *Actinobacteria* and *Acidobacteria* phyla (Table 1), with all but one lava tube with closest relatives found within those phyla. The second most abundant group found was *Gammaproteobacteria*, which had eight lava tubes with closest relatives among this phylum. There is a slight trend for yellow microbial mats to be more diverse than white mats, but more sequencing and analyses are needed before a definitive assessment can be made.

Scanning electron micrographs revealed the presence of many different possible microbial cell shapes, including cells in the shape of rods (e.g. Fig. 3), filaments, and spores. The fuzzy rods seen in Figure 3 are one of the more common

morphologies seen and may indicate the widespread presence of *Actinobacteria* in these mats.

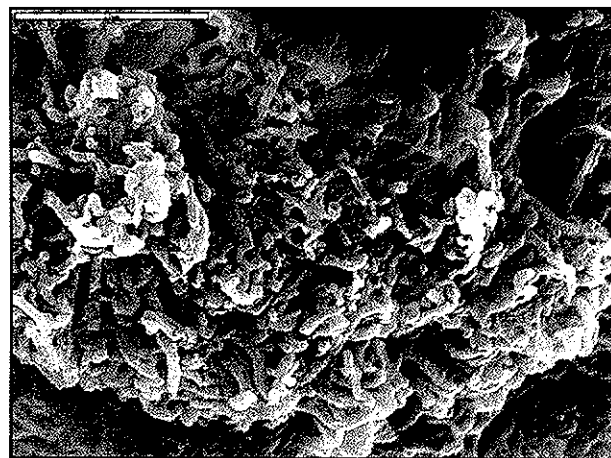


Figure 3: Scanning electron micrograph of a yellow microbial mat in Pahoe cave. The presence of the rods suggests a microbial presence. Scale bar is 10 microns.

#### 4. Conclusions

We conclude that the Hawai`i lava tube microbial mats are quite diverse, overall containing 13 phyla of bacterial life as opposed to seven phyla for the New Mexico lava tubes. Hawai`i receives considerable more moisture, especially on the eastern side of the island, than does the region of New Mexico where El Malpais National Monument is located. Additional moisture will infiltrate the lava tubes and may bring additional nutrients to fuel some microbial metabolic lifestyles. The Hawai`i lava tubes samples are several thousand years younger than are those of El Malpais, but whether this is a factor in the decreased diversity

Phyla Cave	Actn	aP	βP	γP	δP	Acid	Clfx	Cyan	Nit	Ver	Gem	Planc	Bact	Dein	OP11	Firm
4W	§		§				§			§						
Pahoc	#	#		#		#	#									#
RG	#§	§		#§		#§					§					
Bcals	#/§	#/§	#/§	#/§	#/§	#/§		#/§	#/§		#/§	#/§	#/§		#/§	
BP	§	§		§	§	§	§		§							
Epper	><	><	><			><	><		><					><		
Kau	∧			∧	∧	∧										
Kula	#			#		#							#			
Maels		*	*	*	*	*		*	*		*					*

Table 1: Comparison and contrast of Hawai`i and El Malpais caves by bacterial phyla. Actn- Actinobacteria, aP- Alphaproteobacteria, βP- Betaproteobacteria, γP- Gammaproteobacteria, δP- Deltaproteobacteria, Acid- Acidobacteria, Clfx- Chloroflexi, Cyan- Cyanobacteria, Nit- Nitrospirae, Ver- Verrucomicrobia, Gem- Gemmatimonadetes, Planc- Planctomycetes, Bact- Bacteroidetes/Chlorobi Group, Dein- Deinococcus-Thermus, OP11 and Firm- Firmicutes. The symbols represent a different color of microbial mat as follows: \*- blue/green ooze, #- yellow, §- white, #/§- yellow and white, ><- orange and ∧- purple.

will need to be determined by additional sampling and sequencing of older lava tubes on Hawai'i. Many novel species were detected in the clone libraries and many of the sequences from this study are from other volcanic or cave environments. Our knowledge of lava tube microbial mats is increasing as a result of these studies and we know that the microbial mats can be quite diverse in their phylogenetic makeup.

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