Identification and Comparison of Microbial Diversity in Azorean Lava Tubes according to Human Impact

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Introduction

Lava tubes exhibit stunning microbial mats worldwide, contributing significantly to their attractiveness to human visitors. Very little attention has been given to microbial biodiversity and the role that microbial members might play in this subsurface environment. Considering the large number of caves found in the Azores archipelago – 271 caves under four categories of Rank A, B, C and D, the Government of Azores has initiated a proposal for submission of a few selected caves to UNESCO for inscribing under the UNESCO World Heritage List. The selection criteria for inclusion of the caves in the list was based on the scientific value, tourism prospects, approach conditions, perceived threats, data base and conservation standing. Caves and volcanic pits are key touristic attractions in the Azores and ecosystem alterations are expected due to human visitation (Northup, 2009).There are five show caves in the archipelago (Gruta do Natal, Gruta das Torres, Algar do Carvão, Gruta do Carvão e Furna do Enxofre), three of them located in Terceira and Pico islands, where more than 70% of the Azorean caves are located. Gruta das Torres is located on the western flanks of the island of Pico and has been open to the public since 2005. The first 450 meters of Gruta das Torres are accessible to visitors accompanied by a guide. Visitors are allowed in groups of 15 at a time and each visitor is provided with a protective gear of a helmet fitted with lamps to see the dark interior. Algar do Carvão, in Terceira, is the most visited volcanic cave in the Azores with 22,000 people visiting in 2009. In 1968 was first opened to visitors, illuminated by hand or helmets lamps. In 1987 a first lighting system fixed in the interior was assembled, powered by a generator until nowadays. Gruta de Natal's firstly opened in 1969 for a mass celebration. Show cave managements include artificial lighting. In order to determine the diversity within these lava tubes, and to infer whether human impact can influence the microbial diversity, 16S rRNA gene clone libraries were constructed and sequence analysis and comparison of communities was analyzed from three show caves and four low human usage ones. Conserving microbial diversity is fundamental to maintenance and conservation of global genetic resources. Measures must be taken to describe, estimate, record, and conserve microbial diversity, not only to sustain human health but also to enrich the human condition globally through wise use and conservation of genetic resources of the microbial world.

Methods

Cave samples: Small samples of wall rock covered with microbial mats were collected aseptically from each lava tube. Samples were covered with sucrose lysis buffer (Giovannoni *et al.*, 1990) to preserve the DNA, and transported to the laboratory where they were stored in a -80° C freezer until DNA extraction. Samples were collected from Gruta dos Balcões, Gruta da Achada, Gruta do Natal, Algar do Carvão in Terceira island and Gruta dos Montanheiros, Gruta das Torres and Gruta Ribeira do Fundo in Pico island. At each site, entrance elevation, cave temperature and humidity (wet bulb/dry bulb) were recorded, the latter two measured with an IMC Digital Thermometer probe. Average area rainfall was researched and recorded.

DNA Extraction, Amplification, and Sequencing: 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'-GCYTAAYACATGCAAGTCG-3') and 1409 reverse (5'-GTGACGGGCRGTGTGTRCAA-3') (Snider et al., 2009a). Amplicons were cleaned and 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.

Molecular Phylogenetics: Sequences were edited and assembled with Sequencher 4.8. (Gene Codes, Ann Arbor, Michigan). Orientation was checked with OrientationChecker (www.cardiff.ac.uk/biosi/research/biosoft/). Chimeras were detected using the Mallard/Pintail software (http://www.bioinformatics-toolkit.org / /). Initial alignment was completed with greengenes (greengenes.lbl.gov/) and manually corrected using BioEdit editor (www.mbio.ncsu.edu/BioEdit/bioedit.html), guided by 16S primary structure considerations. Sequences were then classified at the phylum level using RDP classifier (rdp.cme.msu.edu; Maidak et al. 2001). Mothur was used to conduct community analyses to determine if community structure was different among the samples. (Schloss *et al.*, 2009; Chao, 1984)

Results

A total of 1690 full-length non-chimeric sequences were obtained from seven caves, 4 from Terceira Island and 3 from Pico Island, after screening for quality and the presence of chimeric sequences. Eighteen phyla were identified across the 25 clone libraries using the Ribosomal Database Project analysis tools after Operation Taxonomic Units (OTU) were defined at 97% sequence similarity. The largest percentage of sequences was identified as Acidobacteria (15%), followed by Actinobacteria (14%). Alpha and Gamma-proteobacteria represented approximately 12% of the sequences. Twelve percent of Azorean sequences could not be assigned to the phylum level. Out of the 18 phyla found, 8 were identified in all the studied lava tubes, i.e. Actinobacteria, Proteobacteria (Alpha, Beta, Gamma and Delta), Acidobacteria, Nitrospira and Bacteroidetes. Comparing sequences from show caves and non visited caves at the phylum level, Actinobacteria and GammaProteobacteria showed a higher number of sequences in show caves related to non-visited ones. Nearest neighbors for the most cosmopolitan OTUs found in Azorean lava tubes belonged to the Phyla Actinobacteria, GammaProteobacteria, AlphaProteobacteria and Nitrospira. The proportion of OTUs occurring in only one cave is much larger (33%) than the proportion of OTUs appearing in more than two caves (4%). Show caves present high levels of cosmopolitan OTUs (58%) when compared to non visited caves (42%).

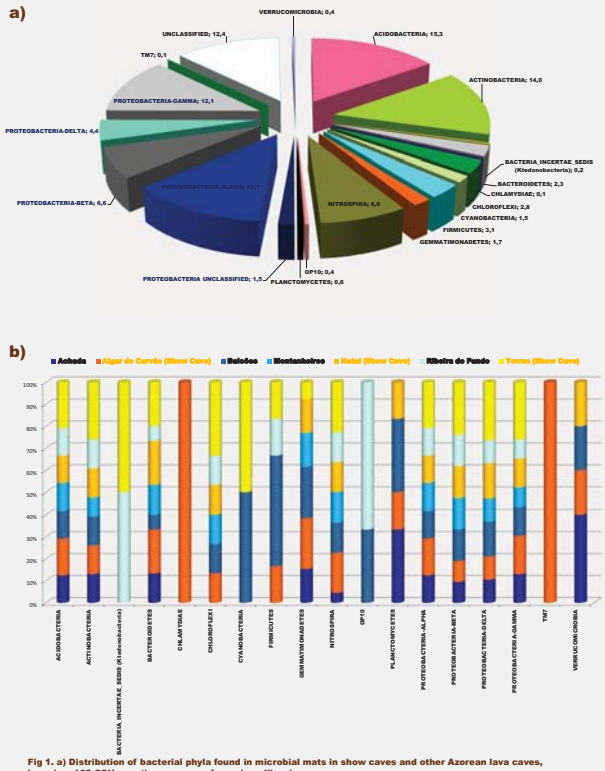

Fig 1. a) Distribution of bacterial phyla found in microbial mats in show caves and other Azorean lava caves, based on 16S SSU genetic sequences from clone libraries.
b) Presence of Phyla recovered along the different caves sampled in two islands (Pico and Terceira – Azores).

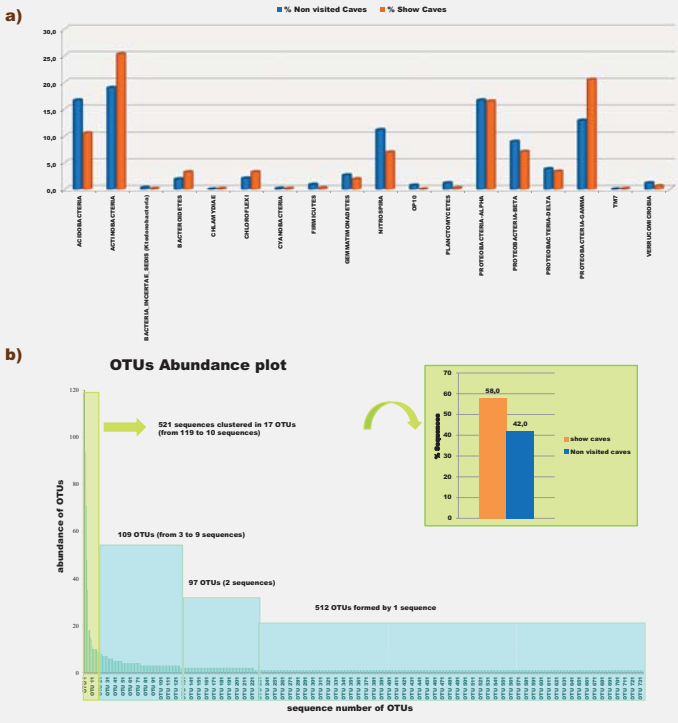

Fig 2. a) Comparison of percentages of bacterial Phyla found in show caves and non visited caves
b) The sequence numbers of each OTU are sorted in descending order of abundance. (N_{OTU}=735)

Conclusions

- Lava tubes contain considerable microbial diversity. Based on our data, each cave is unique in its bacterial diversity. Conservation and management of natural ecosystems focused on microbiodiversity is pointed to preserve the uniqueness of each cave and the useful genetic diversity present there for biotechnology.
- Preliminary results suggest that show caves harbour a larger amount of cosmopolitan OTUs compared to non visited caves. Due to the large number of microbial diversity, further studies are needed to provide a deeper knowledge of bacterial communities composition.

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