Analysis of prokaryotic communities of microbial mats and organo-sedimentary structures associated with pseudokarst caves in Sierra del Chichinautzin, Mexico.

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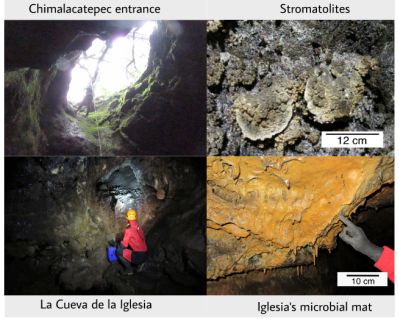
Introduction

Lava tubes are classified as pseudokarstic caves and are considered extreme environments for life due to the aphotic and oligotrophic (< 5mg/L of organic carbon) conditions they present Microorganisms play an important role in the maintenance of subterranean ecosystems through: i) primary productivity, ii)their participation in biogeochemical cycles and iii) the formation of secondary mineral structures.

In subterranean environments, like caves, the organisms who supply carbon are bacteria and archaea. These organisms use some of the seven carbon fixation pathwayas as: i) the pentose phosphate cycle (CBB), ii) the reductive citric acid cycle (rTCA) and iii) the 3-hydroxypropionate/4-hydroxibutirate cycle (3HP/4HB). La Cueva de la Iglesia and the Chimalacatepec Lava Tube system in the Chichinautzin Volcanic Field (Morelos, Mexico) are caves with different geomorphological characteristics whose microbiomes have not been explored. Also, these sites represent an opportunity to develop knowledge about the microorganisms that inhabit these sites and their energetic strategies in order to obtain carbon.



Project goal

To determine the composition, structure and functional potential of the prokaryotic communities of the lava tubes of Chimalacatepec and Iglesia, México.

Methods

Samples were taken from microbial mats, superficial soil and stromatolites from both caves.

Key names
- CE - Chimalacatepec stromatolite
- CM - Chimalacatepec microbial mat
- CS - Chimalacatepec soil
- IM - Iglesia microbial mat
- IS - Iglesia soil

DNA extraction was carried out in triplicates

PCR

Genes for dark carbon fixation

Cycle	Gene	Codified enzime	Primer	Amplicon length (pb)
CBB	*cbb*	RuBisCO IC	*cbbL-R* *cbbL-IC*	~ 818 ~552
3HP/4HB	*hcd*	4-hydroxybutyryl CoA dehydratase	*hcd* *4HBD*	~839 ~1048
rTCA	*acl*	ATP citrate lyase	*aclA*	~564

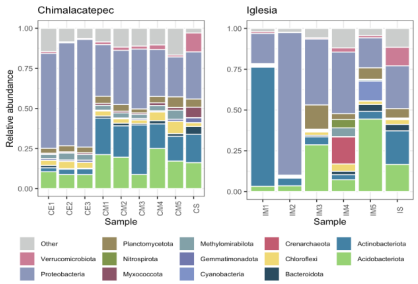
V4 - 16S rRNA

Bioinformatic analysis

Data analysis and visualization

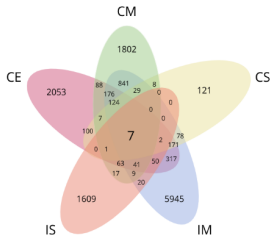
Results and discussion

Taxonomic composition



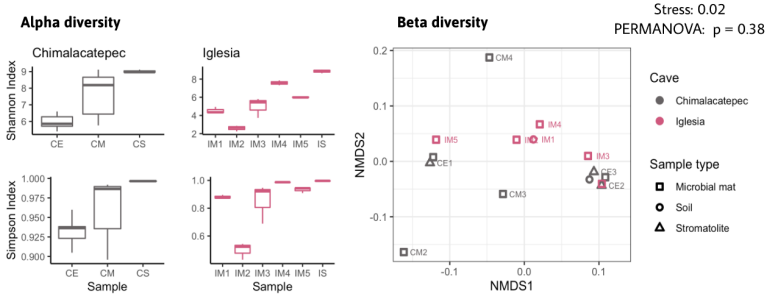
Dominant phyla was Proteobacteria, Actinobacteriota and Acidobacteriota, the same phyla founded in other caves around the world (Gonzalez-Pimentel et al., 2018; Hathaway et al., 2014).

ASVs shared among samples



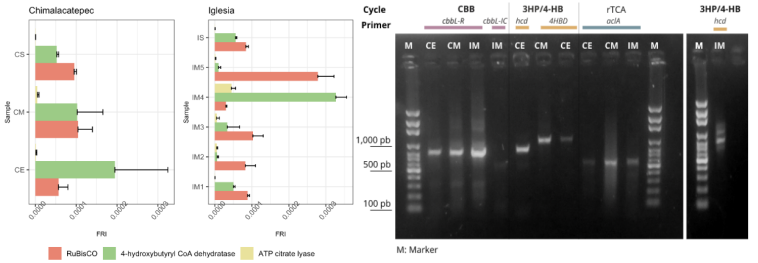
All the samples shared just 7 ASVs, some of them are classified as *Steroidobacter*, *Pedomicrobium* and an uncultered bacteria from Rokubacteriales order. These results agreed with the organisms founded by Zhou et al., 2019.

Diversity



Alpha diversity was high for almost all samples (except IM2). Beta diversity was calculated with weighted UniFrac, NMDS analysis did not show groups in function of the caves or sample type. PERMANOVA analysis indicated that there are not significative difference between communites.

Carbon fixation potential



Functional prediction was performed with `Tax4Fun2`, the figure shows selected key enzimes from the three dark carbon fixation cycles. `Tax4Fun2` results showed that the CBB cycle is dominant in all samples, as Ortiz et al., 2014 found in Kartchner Caves. On the other hand, analysis *in vitro* confirmed the carbon fixation potential of the communites through the three cycles.

Conclusion

This study provides a comprehesive assesment of the prokaryotic communites associated with pseudokarstic caves. The results shows that there are not differences in taxonomic composition or structure between the analyzed prokaryotic communities. These results could imply that microorganisms in caves are specifically adapted to this kind of systems. As for the predicted functional potential, results suggest that the communites aquire carbon through the pentose phosphate cycle, the reductive citric acid cycle, the 3-hydroxypropionate/4-hydroxibutirate cycle.

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