Understanding underground biodiversity in the Azores - a perspective

CÂMARA, S., BAPTISTA, I., VARELA, A. R., DAPKEVICIUS, M. L. N. E., AMORIM, I., PEREIRA, F. & NORTHUP, D.

Centro de Investigação e Tecnologia Agrária dos Açores

Universidade dos Açores

Introduction

Volcanic caves with organic matter input and constant levels of humidity and temperature such as the ones in Terceira Island are starting to be investigated worldwide with respect to their biology resulting in the discovery of new taxonomic groups of Archaea and Bacteria and a great diversity of Actynomicetes [1, 2, 3]. This latter group of microorganisms is the source of most of the antibiotics presently in use and although the antibacterial activity of actinomycete metabolites is well established, it is still an important issue due to the development of resistant strains of pathogens [4]. Recently, our work team has observed that Azorean caves are very rich in biofilms, which are yet to be fully characterized. We found that some of the bacteria present in these biofilms are active against human pathogens and may be promising sources of antibiotics for the treatment of infectious diseases in humans. These bacteria were isolated from areas with less human impact. The number of visitors of the caves has increased in recent years, which may alter the air quality and bring new microorganisms into the cavities, changing the natural balance that allows the biofilms to proliferate and ultimately leading to a loss of biodiversity.

Methods

Sampling for isolation of microorganisms was carried out by swabbing spots colonized by microbial mats in 2 chosen lava tubes, namely Gruta das Agulhas and Gruta Branca Opala. Swabs were plated on R2A culture media made with water retrieved from the caves. The plates were incubated both in laboratory and *in situ* for several days, after which microbial growth was analyzed. Isolated colonies were picked from the initial samples for isolation on R2A Agar. Morphological and cultural characteristics (cultural characteristics on agar media, Gram, presence of catalase and oxidase and cell morphology) were recorded. The antimicrobial spectrum of the isolated microorganisms was evaluated by the streak assay, using the human pathogenic microorganisms *Proteus* spp., *Salmonella* spp., *Escherichia coli, Staphylococcus aureus* ATCC 9144 and *Staphylococcus aureus* 2 LAQ as target strains.

Results and discussion

Mats were classified as yellow, tan or white according to their aspect (Figure 1). Gruta das Agulhas and Gruta Branca Opala showed a high culturable microbial biodiversity, with 8 different morpho-physiological groups (Figure 2). Gruta das Agulhas had the higher number of isolates. G+C+Ox+ bacteria were found only in this cave.
A higher number of isolates was obtained from yellow and white mats. G-C+Ox+ bacteria were only found in yellow mats. One strain inhibited all of pathogens, showing greater antimicrobial activity on *Staphylococcus aureus*, as presented in Figure 3. This was G-C+Ox+, isolated from a yellow mat.

Figure 1 – Microbial mats classified according to their color: a) tan, b) yellow and c) white.

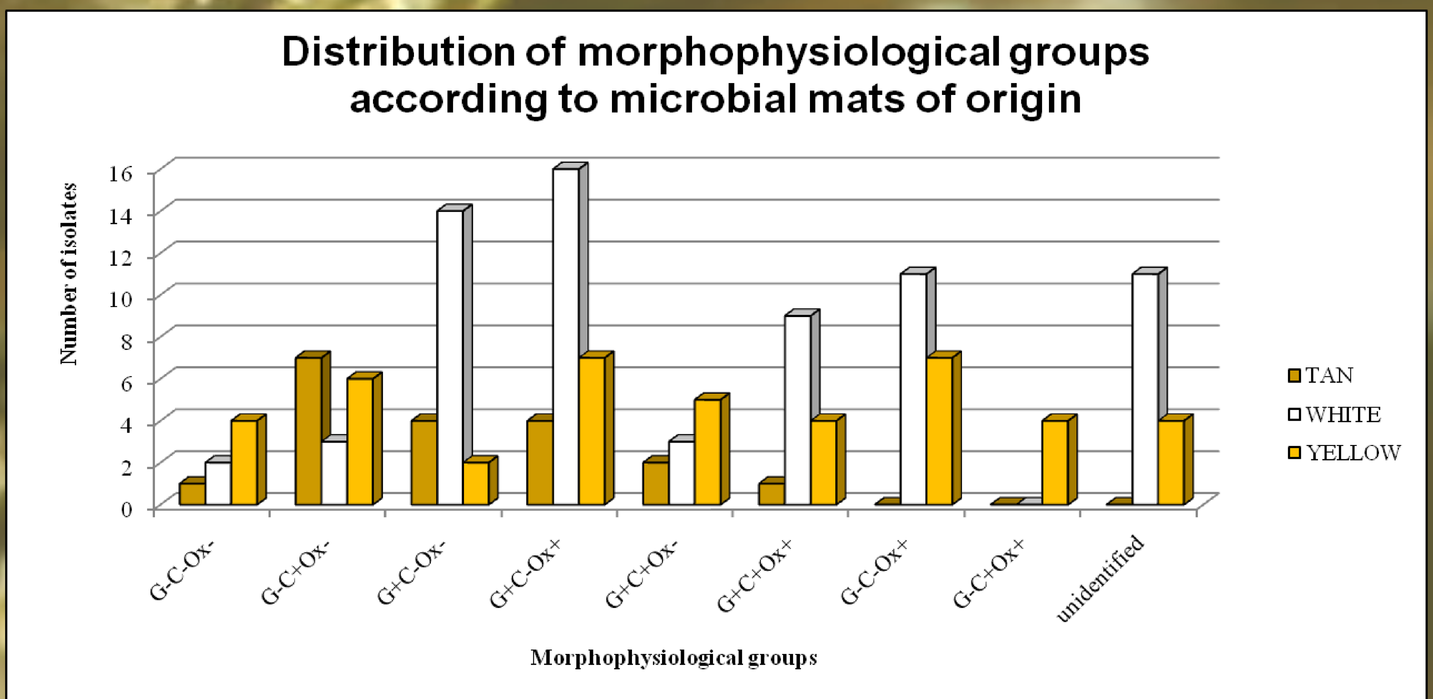

Figure 2 – Distribution of morpho-physiological bacterial types according to microbial mat of origin. G – Gram; C – presence of catalase; Ox – presence of oxidase; +/- stands for positive or negative, respectively.

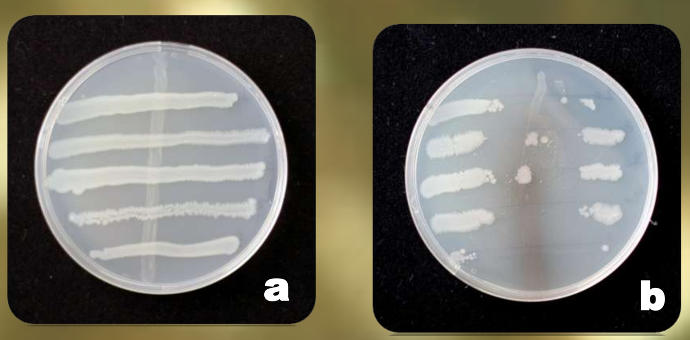

Figure 3 – a) example of a cross-steak test and b) aspect of antimicrobial activity of a G-C+Ox+ bacteria towards the selected pathogens.

Contact: mariaenes@uac.pt

Aknowledgments: We wish to thank Berta Borges and Guida Pires for their collaboration in the laboratory, and to Kenneth Ingham and Pedro Cardoso for allowing us to use their photographies.

References

1. Northup, D. E. & Welbourn, W. C. (1997). Life in the twilight zone: Lava tube ecology. New Mexico Bureau of Mines & Mineral Resources Bulletin, 156: 69-82.
2. Northup, D. E. & Lavoie, K. H. (2001). Geomicrobiology of Caves: A Review. Geomicrobiology Journal, 18(3): 199-222.
3. Northup, D. E. & Lavoie, K. H. (2004). Microbiology in caves. pp.: 506-509. In: Gunn, J. (ed.) Encyclopedia of Cave and Karst Science. New York. Fitzroy Dearborn Publishers.
4. Busti, E., Monciardini, P., Cavaletti, L., Bamonte, R., Lazzarini, A., Sosio, M. & Donadio, S. (2006). Antibiotic-producing ability by representatives of a newly discovered lineage of actinomycetes. Microbiology SGM, 152: 675-683.