MICROORGANISMS INVOLVED IN THE FORMATION AND DISSOLUTION OF CARBONATE DEPOSITS IN SPANISH CAVES

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Microorganisms play critical roles in biogeochemical processes. In caves, carbonate deposition constitutes a common process. Although microorganisms able to induce these deposits have been previously reported, little information is available on the microorganisms involved, their activity, and the conditions required for the process to occur. This study presents results on the induction of carbonate precipitation and dissolution by microorganisms. RNA-based molecular fingerprinting procedures were used for profiling the metabolically active bacterial communities involved in the preliminary stages of moonmilk deposits. Bacteria were cultured and isolated to analyze the required conditions and nutrients inducing these depositions. Bacteria were able to differentially induce carbonate precipitation depending on the nutrients available for growth. In addition, similar microorganisms can be involved in precipitation and dissolution of carbonates mainly depending on the available nutrient sources. These results suggest the existence of an active carbon cycling process in caves dominated by microorganisms.

1. Introduction

The role of microorganisms in shaping the geology of our planet has been a topic of investigation for many years. Biomineralization in cave environments is one of the scenarios where microorganisms have been reported to actively participate (Cañaveras et al. 2006; Barton & Northup, 2007). The case of moonmilk formation, a soft, wet, fine-grained secondary cave deposit constituted mainly of carbonates, has been suggested to be induced by microbial metabolism (Barton & Northup, 2007). Recent reports point to the active role of bacteria as the most common microbial type participating in these depositions (Cañaveras et al., 2006).

While bacteria are considered as the major inducers of carbonate deposits, little information is available concerning the conditions and mechanisms leading to the accumulation of these precipitates in caves. Carbonates precipitate at alkaline pH (pH > 8) according to the well studied CO_2-carbonate equilibrium in natural systems. Consequently, any increase of pH (above pH 8) generated as a result of bacterial metabolism could lead to carbonate precipitates. In a similar way, pH decreases as a result of bacterial metabolism might lead to carbonate dissolution since then CO_2-carbonate equilibrium is altered towards the CO_2-carbonic acid soluble forms. Thus, microorganisms can directly influence both precipitation and dissolution of carbonates.

The aim of this study is to provide some evidence leading to an understanding of the role microorganisms on carbonate deposition and dissolution and the conditions required for each of these two processes to occur.

2. Materials and Methods

Moonmilk deposits were sampled from different spots along Altamira Cave (Cantabria, North Spain). Samples were collected aseptically using sterilized instruments, maintained on ice during transportation and processed as soon as possible upon arrival at the laboratory.

Bacterial communities were analyzed by RNA-based molecular methods as previously described (Gonzalez et al. 2003; Portillo & Gonzalez, 2008). Bacteria were isolated using Nutrient Agar at 28° C. The effect on pH as a function of different supplied nutrients was studied in a medium base composed of 0.4 g l^{-1} peptone per liter. Base liquid medium was supplemented with 1.6 g l^{-1} of one of the following: glucose, calcium or sodium acetate, sodium oxalate, casein, and ammonium chloride. Visual inspection of the formation of carbonate precipitation or dissolution during bacterial growth on solid media (15 g l^{-1} agar) was carried out at 10x using a stereomicroscope.

The semiquantitative mineral composition of crystals formed during bacterial growth was determined by X-ray diffraction (XRD) with a Philips PW-1710 and examined using a Philips Quanta 200 environmental scanning electron microscope (ESEM).

3. Results and Discussion

Bacterial isolates belonging to the major bacterial groups constituting the bacterial communities in the studied cave environment were obtained. These communities were studied through RNA-based molecular survey and DGGE analyses. Results showed that most bacterial isolates tested induced the highest pH conditions by consuming acetate while the lowest pH conditions were induced when highly rich nutrients were consumed (i.e., glucose) (Fig. 1). Growth on protein-rich nutrients (peptone, casein) resulted in moderate pH increases suggesting that ammonia production from protein consumption could be an alternative route inducing carbonate precipitation. The source of calcium to form calcium carbonate deposits is irrelevant since either calcium acetate or calcium chloride leads to similar amounts of precipitates. Lower temperature resulted in a slowing down of the metabolic rate, but similar effects of nutrients on pH were observed at different temperatures. Low molecular weight organic acids, such as acetate, are the final metabolic products of heterotrophic bacterial metabolism (Madigan et al., 2003). These are produced during the degradation of complex organic compounds usually available in cave environments. Drastic pH changes provided by bacterial metabolism can easily result in values above pH 8 which is adequate for carbonate precipitation.

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References

Barton, H.A. and D.E. Northup (2007). Geomicrobiology in cave environments: past, current and future perspectives. *Journal of Cave and Karst Studies* **69**: 163–178.

Cañaveras J.C., S. Cuezva, S. Sanchez-Moral, J. Lario, L. Laiz, J.M. Gonzalez, and C. Saiz-Jimenez (2006). On the origin of fiber calcite crystals in moonmilk deposits. *Naturwissenschaften* **93**: 27–32.

Gonzalez, J.M., A. Ortiz-Martinez, M.A. Gonzalez-delValle, L.C. Laiz, and C. Saiz-Jimenez, (2003). An efficient strategy for screening large cloned libraries of amplified 16S rDNA sequences from complex environmental communities. *Journal of Microbiological Methods* **55**: 459–463.

Madigan M., J. Martinko and J. Parker (2003). Brock Biology of Microorganisms. Prentice Hall, Inc. New Jersey, USA.

Portillo M.C. and J.M. Gonzalez (2008). Statistical differences between molecular fingerprints from microbial communities. *Antonie van Leeuwenhoek* **94**: 157–163.

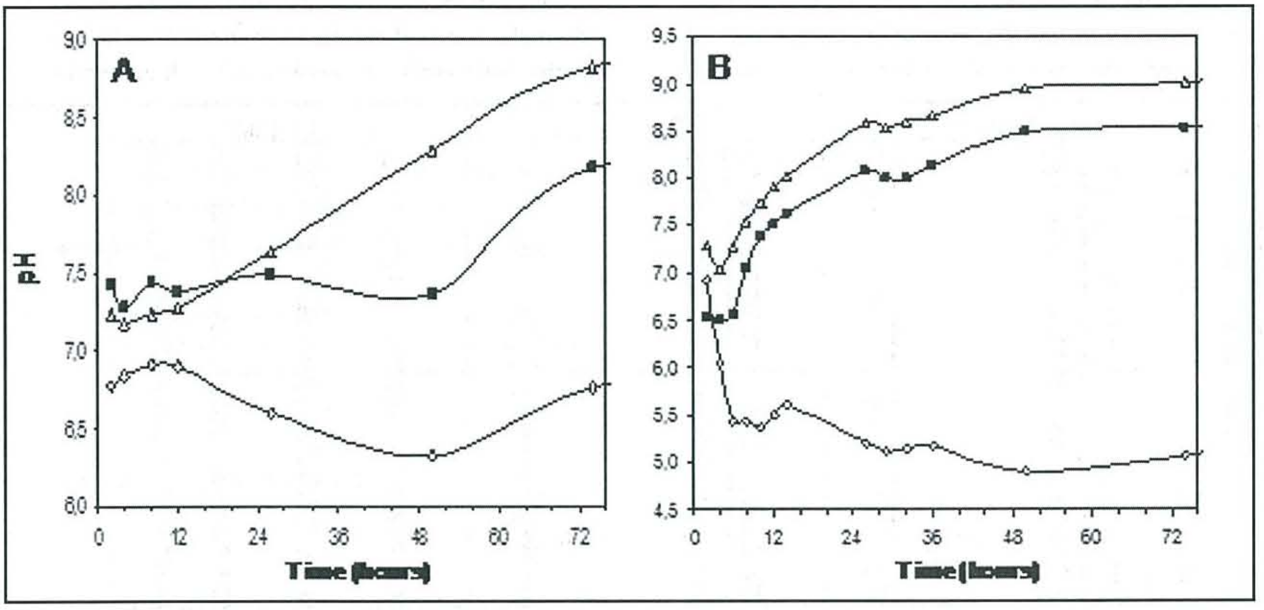

Figure 1: Changes in pH during growth on different nutrients of two bacterial isolates from Altamira Cave. A, an Alphaproteobacterium, Sphingomonas; B, a Firmicutes, Bacillus. Dark squares correspond to the base medium containing peptone as the only carbon source; open triangles correspond to base medium supplemented with acetate; open diamonds correspond to base medium supplemented with glucose.