ARTICLE IN PRESS

International Biodeterioration & Biodegradation xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Bacterial diversity associated with saline efflorescences damaging the walls of a French decorated prehistoric cave registered as a World Cultural Heritage Site

Clémentine Lepinay[a], Agnès Mihajlovski[a,*], Stéphanie Touron[b], Damien Seyer[a], Faisl Bousta[b], Patrick Di Martino[a]

[a] Université de Cergy-Pontoise – ERRMECe-EA1391 – Site de Neuville-sur-Oise, rue Descartes, 95031, Cergy-Pontoise Cedex, France

[b] Laboratoire de Recherche des Monuments Historiques, Ministère de la Culture et de la Communication, Centre de Recherche sur la Conservation (CRC-USR 3224), Muséum national d'Histoire naturelle, CNRS, Sorbonne Universités, 29 Rue de Paris, 77420, Champs-sur-Marne, France

ARTICLE INFO

Keywords:
Cave
Biodeterioration
Saline efflorescences
Biofilm
Bacterial diversity
High-throughput sequencing

ABSTRACT

This study focused on a French prehistoric decorated cave which suffers from the development of saline efflorescences. The goal was to determine the ecology of microorganisms associated with efflorescences, and determine if the sessile microbiota could have a role in the deterioration process. Samples from 2 sampling campaigns were analyzed. Elemental, mineralogical and isotopic analyses showed gypsum containing sulfur originating mainly from the host-rock. Microscopic observations, direct cell counting, culture-dependent assays and high-throughput sequencing (HTS) of bacterial 16S rDNA sequences indicated an abundant sessile microbiota, where Actinobacteria, Alphaprotebacteria, Gammaproteobacteria, Bacteroidetes and Planctomycetes were the main phylogenetic groups. Although a relatively low abundance of genera associated with sulfur cycling were identified, the presence of dense biofilms at the interface rock/efflorescences composed of a high relative abundance of Actinobacteria may reflect previously described indirect biomineralization. An increase of microbial abundance and bacterial richness between October 2014 and April 2015 might be attributed to fluctuations in environmental factors. This study gives insight into origin and bacterial composition of efflorescences that threatened Sorcerer's cave engravings. HTS has rarely been used in the field of cultural heritage and thus represents one of the originalities of this work.

1. Introduction

During prehistoric times, natural cavities, such as rock-shelters and caves, have sometimes been visited by humans who left paintings, sculptures and engravings on cave walls and roofs that represent an inestimable anthropic and artistic heritage. This cultural heritage can be threatened by natural weathering and decay processes resulting from the interaction of biological and/or physicochemical environmental factors that can alter the rock substrate according to its mineralogical composition and structure (Chamley, 2003; Saiz-Jimenez, 2015). Even if natural cavities represent oligotrophic environments, with organic matter content that can be 1000 times less than in ordinary soil (Barton et al., 2007; Lavoie et al., 2010), these environments contain an abundant and diverse sessile biomass that can act as a biodeterioration agent (Barton and Jurado, 2007). The composition of biofilms covering the walls of natural cavities is conditioned by several factors: (i) the micro-environmental conditions, including water availability, pH, climatic conditions and nutrient sources (Gallagher et al., 2012), and (ii) the bioreceptivity of the support defined by Guillitte (1995) as "the aptitude of a material to be colonized by one or several groups of living organisms without necessarily undergoing any biodeterioration". Thus, the mineral composition, the porosity, the rock permeability and the surface roughness influence microbial colonization (Gorbushina, 2007). The conservation state of a material and the accumulation of exogenous deposits, such as soil, dust or organic particles, can also affect its bioreceptivity (Ortega-Calvo et al., 1995; Guillitte, 1995). In regard of tourist caves, the repetitive incursions of tourists, especially in excessive numbers, are responsible for noticeable daily, weekly, seasonal and annual thermo-hydric inputs, variations in luminosity, and introduction of microorganisms and organic matter that can accelerate deterioration processes (Chamley, 2003; Saiz-Jimenez, 2015).

Several kinds of saline efflorescences are found in subterranean environments (Northup and Lavoie, 2001). These microcrystalline mineral aggregates are usually composed of calcite, aragonite,

* Corresponding author.
E-mail address: agnes.mihajlovski@u-cergy.fr (A. Mihajlovski).

https://doi.org/10.1016/j.ibiod.2018.03.016
Received 20 December 2017; Received in revised form 18 March 2018; Accepted 26 March 2018

hydromagnesite and/or gypsum (Northup and Lavoie, 2001; Galdenzi and Maruoka, 2003; Cacchio et al., 2012; Gázquez et al., 2015), and can have a wide variety of colors and shapes (Barton and Northup, 2007; Lavoie et al., 2010). When it comes to art-works, the crystallization of salts may result in a loss of historic or prehistoric information. Indeed, as well as covering art-works, hygroscopic salts occupy variable spaces through dissolution/recrystallization phenomenon. They thus produce an additional pressure that may lead to material loss and destruction due to cracking and detachment of the mineral support (Saiz-Jimenez and Laiz, 2000; Piñar et al., 2009, 2013). Moreover, salt efflorescences favor the proliferation of halotolerant/halophilic microorganisms, which can lead to discoloration caused by pigments released from, or contained within, the microorganisms, such as the rosy coloration of many subsurface monuments (Piñar et al., 2014a, 2014b).

After calcite, calcium sulfates (gypsum) are the most common salts in underground environments (Onac, 2005, Onac et al.,2011). Sulfur can have different origin: air pollution (Camuffo et al., 1983; Bao and Reheis, 2003), pre-existing sulfur-bearing mineral phases (Charola et al., 1990) or biological activities inducing biomineralization (Hose et al., 2000). Biological activities led to an isotopic fractionation of oxygen and sulfur that have allowed determining sulfur origin through measuring $\delta^{18}O$ and $\delta^{34}S$ (Fritz et al., 1989; Philippot et al., 2007; Kloppmann et al., 2011; Onac et al., 2011). Microorganisms can be involved in the formation of these efflorescences as biomineralization processes due to microorganisms result in the precipitation of minerals (Stocks-Fischer et al., 1999; Barkay and Schaefer, 2001; Phillips et al., 2013). In nature, biomineralization is a widespread phenomenon that can lead to the formation of over 60 different biological minerals (Sarikaya, 1999). Three different types of bacterially mediated biomineralization processes have been distinguished (Dupraz et al., 2009): (i) biologically controlled biomineralization, referring to cases in which a specific cellular activity directs the nucleation, growth, morphology and final location of a mineral; (ii) biologically induced biomineralization resulting from indirect modification of chemical conditions, such as a pH shift or redox transformations, in the environment by biological activity; for example, several authors have suggested active participation of Actinobacteria in the bioinduction of calcite deposits in caves (Cañaveras et al., 2006; Cuezva et al., 2009, 2012; Diaz-Herraiz et al., 2013); and (iii) biologically influenced biomineralization, which is defined as passive mineral precipitation in the presence of organic matter such as cell surfaces or extracellular polymeric substances (EPS), whose properties influence crystal morphology and composition. A growing number of reports suggest that the microbial communities, with a diversity equivalent to those found in soil, are important to consider regarding our understanding of mineral precipitation. Nevertheless, the role of bacteria in the formation of salt efflorescences remains unclear (Barton et al., 2007; Lavoie et al., 2010; Cacchio et al., 2012; Marvasi et al., 2012; Farias et al., 2014; Marnocha and Dixon, 2014).

Describing the diversity of micro-organisms is an interesting first step in understanding the functioning of an ecosystem, and in particular the role of bacteria in salt efflorescence formation processes associated with alteration of artwork on cave walls. High-throughput sequencing (HTS) methods are powerful tools in carrying out these studies. These methods are widely used to describe the microbial diversity of many ecosystems, such as soil, compost, oilfield environments, animal and human gut, marine and freshwater habitats (Ju and Zhang, 2012). However, when the field of cultural heritage is considered, only a few works using HTS are found in literature e.g., brick and wood (Gutarowska et al., 2015), mural painting (Rosado et al., 2014), sandstone buildings (Cutler et al., 2013), decorated siliceous stone (Ogawa et al., 2017) and more recently on tombs and show caves (Huang et al., 2017; Pfendler et al., 2018). Most earlier works are based on other less exhaustive capillary sequencing and non-sequence-based molecular methods, as well as cultural approaches (González and Saiz-Jiménez, 2005; Dakal and Arora, 2012; Otlewska et al., 2014; Mihajlovski et al., 2015; Lepinay et al., 2017). However, compared to capillary sequencing and non-sequence-based methods, HTS provides an unparalleled insight into community structures. Whatever the molecular approach used, however, extraction of nucleic acids (DNA or RNA) from deteriorated surfaces does not necessarily provide insight into the active microorganisms most involved in biodeterioration, and cultivation approaches should not be put aside (Stomeo et al., 2008; Portillo et al., 2009a).

In this study, we have focused on a French prehistoric cave, the Sorcerer's cave ("La grotte du Sorcier"). The walls of this cave have saline efflorescences that damage the engravings (Pigeaud et al., 2012). To characterize these saline efflorescences more precisely and their origin, a combination of several techniques was used. The elemental and mineralogical compositions of the saline efflorescences were analyzed using several geochemical approaches. Microscopic observations allowed the analysis of the structure and abundance of the microbial biomass associated with these saline efflorescences. The bacterial diversity associated with areas of saline efflorescences was analyzed with high-throughput 454 GS FLX + + sequencing technology and microbial cultivation. Finally, to examine a potential seasonal impact, samples from two sampling campaigns were analyzed, one in October 2014 (SCOct14) and another in April 2015 (SCApr15).

2. Materials and methods

2.1. Site description and sampling

The prehistoric Sorcerer's cave is located at Saint-Cirq-du-Bugue (Dordogne, France; Fig. 1A). Its engravings were discovered in 1952 and have been described by numerous archeologists (Delluc et al., 1987; Pigeaud et al., 2012). Its entrance has been used as a troglodyte habitat in the past. The site is dug in Turonian siliceous limestone (Karnay et al., 1999). The cave is partially filled with sediments and is about 5–6 m wide and 13 m long. Developments of gypsum efflorescences that can damage engravings are present (Pigeaud et al., 2012). As it is impossible to collect samples from engravings, they were

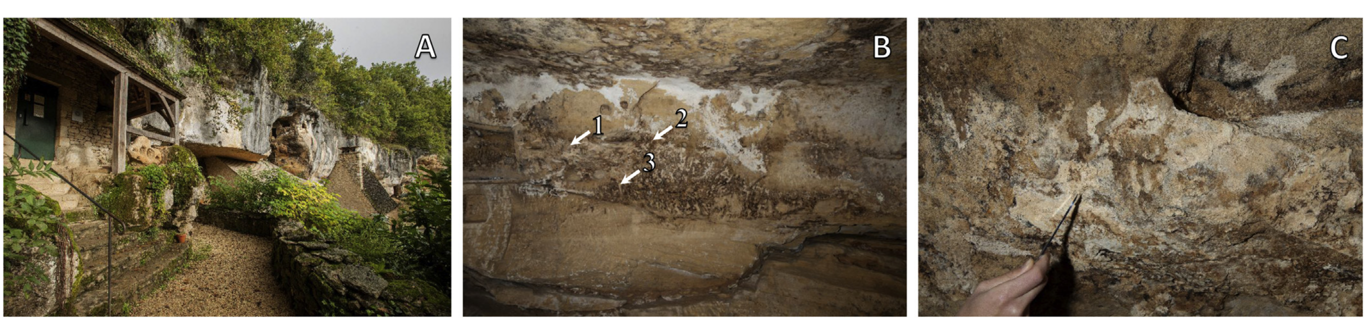

Fig. 1. The Sorcerer's cave and the sampling areas: (A) The entrance of the Sorcerer's cave (B) General view of the three sampling areas and (C) Enlarged view of the sampling area 1.