International Biodeterioration & Biodegradation 122 (2017) 116–127

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

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

Biofilm communities survey at the areas of salt crystallization on the walls of a decorated shelter listed at UNESCO World cultural Heritage

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

[a] Laboratoire ERRMECe-EA1391, Université de Cergy-Pontoise, Cergy-Pontoise, France
[b] Laboratoire de Recherche des Monuments Historiques, Ministère de la Culture et de la Communication, Champ-sur-Marne, Sorbonne Universités, Centre de Recherche sur la Conservation (CRC, USR 3224), Muséum national d'Histoire naturelle, Ministère de la Culture et de la Communication, CNRS, Paris, France

ARTICLE INFO

Article history:
Received 2 March 2017
Received in revised form
28 April 2017
Accepted 4 May 2017

Keywords:
Biodeterioration
Biofilm
Microbial diversity
Shelter
Gypsum

ABSTRACT

The microbial diversity on the walls of the UNESCO World cultural Heritage site Laugerie-Haute east shelter, France, was surveyed in a context of degradation by formation of salt efflorescences. Samples of salt efflorescence and underlying rock were collected in autumn and spring. Sessile microorganisms were studied by cultivation, microscopy, and molecular methods. Concentrations of the order of 10^3 fungal and 10^7 bacterial cells per gram of sample were quantified by culturing at both seasons. Higher concentrations (10^7 to 10^9 cells per gram) of sessile microorganisms were determined by microscopic counting after DAPI staining with a significant increase in sessile cells in spring. The presence of biofilms was confirmed by electron microscopy with the observation of microorganisms embedded in an abundant matrix in association with rock. Members of Firmicutes, Actinobacteria, and Ascomycota were identified among the cultivable sessile microflora. *Bacillus herbersteinensis* and *Engyodontium album* were the only microorganisms to be isolated in both seasons. Sequence analysis of 16S rRNA genes and internal transcribed spacer clone libraries showed dominance of Bacteroidetes, Proteobacteria, and Ascomycota. Bacteroidetes and Actinobacteria were more abundant in autumn, and in spring, respectively. Cyanobacteria were present at both seasons. Bacterial richness increased during spring. Whatever the season, clones affiliated to the 4 bacterial genera *Aureimonas, Sphingopyxis*, *Halomonas*, and *Crossiella*, and to the two fungal genera *Engyodontium* and *Teratosphaeria* were present. In conclusion, we demonstrated the presence of abundant biofilm at efflorescence salt on the walls of the shelter; that was increased in quantity and diversity in spring.

1. Introduction

Prehistoric decorated shelters are natural environments directly exposed to daylight and seasonal climatic conditions. Energy and carbon sources favourable to microbial growth are diverse and more abundant than in caves. These environmental conditions are favourable for the development of autotrophic and heterotrophic microorganisms; they might also promote degradation and biodeterioration of the rock. Culture-dependent and -independent methods have been widely used to describe microbial communities colonizing the walls of decorated caves (Stomeo et al., 2008; Adetutu et al., 2012; Borsodi et al., 2012) while very little data on prehistoric shelters ecosystems are available in the literature (Gonzalez et al., 1999; Portillo et al., 2009; Mas et al., 2013). Located in karst areas and therefore mainly composed of carbonate rocks, decorated shelters can suffer damages comparable to those of limestone built monuments (Watchman, 1990). Thus, rock alterations are thought to be the result of physical, chemical and biological processes (Papida et al., 2000; Di Martino, 2016). Such processes lead to flaking, peeling, material losses, salt efflorescence and biofilm development both on the walls and on the ground, creating major conservation problems such as covering and loss of archaeological information. Sulphates can play a major role in the alteration of shelters. As on buildings, sulphates are frequently encountered salts since decorated shelters are chemically

* Corresponding author. Laboratoire ERRMECe-EA1391, Université de Cergy-Pontoise, 5 mail Gay-Lussac, CS 20601, Neuville 95031, Cergy-Pontoise cedex, France.
E-mail address: patrick.di-martino@u-cergy.fr (P. Di Martino).

http://dx.doi.org/10.1016/j.ibiod.2017.05.007
0964-8305/

favourable to the crystallization of sulphur carbonates in the form of gypsum. Thus, sulphates are the second largest group of minerals in caves (Onac, 2005). Sulphates observed in shelters are white and appear as indurated or efflorescence. Salts present on walls can have a significant impact on microbial biodiversity. Thus, areas of high salt concentrations can host biomineralizing microorganisms involved in the precipitation of salts and/ or halotolerant and halophilic microorganisms adapted to that particular environment (Hose et al., 2000; Saiz-Jimenez and Laiz, 2000; Piñar et al., 2009).

The Vézère Valley in Dordogne, France, is a region with numerous caves and shelters with rock art. Understanding cave and shelter wall alterations is essential for rock art preservation (Lacanette et al., 2013). In this report we present a case study of the Laugerie-Haute east (Eyzies de Tayac-Sireuil, Dordogne, France). This shelter suffers from extensive salt crystallization on the surface of the walls: white efflorescence indicates that gypsum is widely present. This phenomenon causing material losses threatens to destroy original Palaeolithic engravings. The objectives of this study were i) to describe the bacterial, and fungal communities inhabiting the salt efflorescence present on the walls of the shelter using cultivation, microscopy, and molecular methods, ii) to analyse the seasonal fluctuations in the biofilms microbial ecology among these communities, and iii) to analyse the origin of salt precipitation using geochemical markers such as sulfur stable isotopes.

2. Materials and methods

2.1. Sampling

The prehistoric shelter chosen for the study is the UNESCO World Heritage site from Laugerie-Haute East in the city of Eyzies de Tayac-Sireuil, Dordogne, France. The Laugerie-Haute shelter is located on the right bank of the Vézère river upstream Eyzies de Tayac-Sireuil and is carved into a Coniacian limestone. Archaeological studies have separated the shelter of 160 m long in two parts known as Laugerie-Haute west and Laugerie-Haute east sites (Demars, 1995). The latter of 20 m long, is characterized by the presence of engravings representing animals (Fig. 1). The wall of the shelter is not protected by a roof and is therefore subject to weathering. White efflorescences of salts are visible all over the walls (Fig. 1). Salt efflorescences are not homogeneously distributed on walls because the shelter is submitted to large fluctuations in water circulation, like successive periods of desiccation and wetting. Samples were collected from the surface of rock at the level of three areas showing salt crystallization: the efflorescence and a layer of the rock below the efflorescence from a depth of 2 mm were collected. For elemental analyses and scanning electron microscopy, efflorescences and rock samples were analysed separately. For all other analyses, salt efflorescences were mixed with the underlying rock. Sampling was carried out in October 2014 and April 2015. The samples were taken with sterile scalpel and stored in sterile boxes at 4 °C for cultural and microscopic analyses and at −20 °C for molecular biology analyses.

2.2. Elemental and isotopic analyses

Sulphur, nitrogen and carbon contents of salt efflorescences were performed on an elemental analyser (Elementar Micro vario) at the “Laboratoire des transferts lithosphériques“, UMR6524, at Jean Monnet University, Saint-Etienne. The combustion of samples was realized using a helium-gas continuous-flow. A thermal conductivity and an infrared detector were used to detect carbon/nitrogen and sulphur, respectively. Calibration was performed with sulphanilic acid. A pure gypsum sample ($CaSO_4$) containing 23.5% of sulphur and 47% of oxygen and a pure carbonate sample ($CaCO_3$) containing 12% of carbon were chosen as reference. 3 aliquots per sample were realized. Isotopic analyses were carried out on the gases obtained by combustion of the samples (elemental analyser (Elementar Micro Vario) coupled to mass spectrometer (ISOPRIME)). The isotopic composition is expressed as a per mil (‰) deviation of the heavy-to-light isotope abundance ratio ($^{34}S/^{32}S$) in the sample from a standard, sulphur isotopes as $\delta^{34}S$ with respect to the CDT standard.

Fig. 1. Picture showing the walls of the Laugerie-Haute East shelter, Eyzies de Tayac-Sireuil, Dordogne, France. Efflorescences of white salts distributed heterogeneously are visible on the wall (upper part of the image, yellow arrow). Engravings are present below this area efflorescence (black arrow). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.3. Scanning electron microscopy

Scanning electron microscopy was used to examine the morphological structure of the microbial biomass covering efflorescence and rock samples in high resolution. Samples were fixed in a 2% glutaraldehyde, 0.1 M cacodylate buffer (pH 7.4) for 30 min and rinsed (3 × 10 min) in 0.2 M cacodylate buffer (pH 7.4). Samples were then dehydrated by passing them through the following ethanol series: 30%, 50%, 80%, each for 10 min; 100% ethanol, 2 × 10 min. Support samples were then dried at 37 °C for 24 h. Once coated with gold-palladium, samples were examined under a scanning electron microscope (Leica S430i). For elemental imaging a BSD detector was employed (Quantax Synergie 4, Bruker).

2.4. Total microbial counts

Total microbial counts were determined by fluorescence microscopy observation after DAPI staining of microbial cells. 100 mg of each sample (efflorescence and rock) were suspended in 5 ml of