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Short communication

Biodeterioration agents dwelling in or on the wall paintings of the Holy Saviour's cave (Vallerano, Italy)

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ABSTRACT

The preservation of the wall paintings in rural habitats may be difficult because of severe damage caused by living organisms that may discolor and degrade substrata as a consequence of their growth and metabolic activity. In order to set proper protocols for conservative interventions, biodeterioration agents dwelling within the wall paintings of the Holy Saviour's Cave, in Vallerano, Italy, were analyzed, taking into account their impact on the substrate and their relation with environmental factors. Pigments, binders, and deterioration products of the paintings were examined by spectroscopic and micro-stratigraphic analysis. A predominant whitish grey crust was due to the massive development of the lichen *Dirina massiliensis* f. *sorediata*, but *Caloplaca xantholyta* and *Lepraria* sp. were also observed, while a brilliant green patina, red powdery granules, and a black biofilm were due to the presence of green algae (*Stichococcus* sp., *Trentepohlia* sp.) and cyanobacteria (*Chroococcus* sp., *Nostoc* sp.). Black fungi, here analyzed by ITS rDNA sequencing, were also present.

1. Introduction

Mural paintings in open sites may be exploited by a large variety of organisms. They may utilize for growth a wide range of organic and inorganic constituents of the substrate, combined with dirt and other environmental contaminants accumulating on the painted surfaces. Specific microbial communities will develop depending on the environmental conditions of the site and the capacity of the substratum to provide different ecological niches (Ciferri, 1999). Biological colonization of mural paintings induces aesthetic and structural damage through mechanical and chemical alteration (Ciferri, 1999; Nugari et al., 2009). Studies on biota involved in cultural heritage decay are relatively recent and essential in designing prevention and restoration strategies; new molecular biology methods have aided these studies greatly, resulting in improvements in the identification of microbes (Gonzáles and Saiz-Jiménez, 2005; Imperi et al., 2007).

The aim of this work is the identification of the biota involved in the biodeterioration phenomena affecting the medieval wall paintings of the Holy Saviour's Cave in Vallerano (Viterbo, Italy) and of the environmental conditions favoring the biological activity, in order to plan appropriate restoration treatments. This study is a part of an interdisciplinary approach that includes biologists, chemists, art historians, and restorers of the University of Tuscia, in Viterbo, Italy.

2. Materials and methods

2.1. Site description and sampling

The studied cave is part of a small Benedictine coenobium carved into a tuffaceous high cliff along the Cannucce stream. The settlement, known as cave of the Holy Saviour, has rooms on two floors that are now visible because of the collapse of a part of the rock that occurred in 1888 due to erosion of the cliff out of which it had been dug. On the upper part there were two rooms, originally meant to be the monks' cells, while, at the lowest level, there were two rooms decorated with wall paintings. The one on the left, the subject of our study, was used as a chapel, and an altar is still visible today.

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The wall paintings, dating back to the 10th century (Piazza, 2006), occupy mostly the upper portion of the cave, while above, where a small niche is also carved in the rock, the surface is apparently not painted. Wall paintings depict, in the western wall, a Communion of Apostles (Fig. 2A) and, in the northern long wall (Fig. 2B), three martyr saints – Agnes, Sophia, and Lucy – as well as the Virgin Mother and infant Jesus, and Saint Benedict and his fellows Mauro and Placid. On the vault there are traces of a jeweled cross that held a medallion with the Pantokrator with the now lost scenes of the Crucifixion, the Incredulity of Saint Thomas and the Nativity with the Adoration of the Magi (Piazza, 1999).

The collapse of the eastern portion of the original benedectine coenobium has changed the environmental conditions of the site; originally in an isolated hypogean condition, it became completely exposed to the external conditions with higher light intensity, winds, and changes in humidity and temperature. The total radiance values measured ranged from 6.0 W m^{-2} (in the upper part of the paintings), to 33.4 W m^{-2} (in the central part of the northern wall).

The cave is located in a rural area north of Vallerano (Viterbo) (42°23′24,22″N 12°16′03.01″E, 356 m above sea level), close to a small stream (the Cannucce stream) and a cultivated hazelnut field. It is carved from tephritic phonolitic ignimbrites, a frequent geological component in the western and eastern sectors of Vico volcanic extrusion; a geological study revealed a risk of landslides and collapses and suggested appropriate interventions (http://www.afs.enea.it/protprev/www/cases/vallerano/vallerano.htm).

Analysis of climatic data, according to a phytoclimatic map of the Latium region (Blasi, 1994), indicates that the studied area is located in the temperate region and belongs to the lower/upper hilly thermotype, upper hyper humid/lower humid ombrotype of the mesaxeric region.

The site was totally abandoned, with the walls and the vault showing a diffuse biological attack, more compact in the lower portion where paintings were absent, and more scattered above. Table 1 summarizes the biodeterioration patterns observed, defined according to the ISCS (International Scientific Committee for Stone) glossary of ICOMOS (the International Council on Monuments and Sites) (ICOMOS-ISCS, 2008). Pattern locations on the paintings are shown in Fig. 1. Samples from each type of biological alteration were collected by non-destructive techniques and submitted to different analyses.

2.2. Lichen identification

Lichen species, responsible for alteration patterns 1–3, were identified by in-situ observation and laboratory analyses according to current methods, with flora as described by Clauzade and Roux (1985), Purvis (2000), and Wirth (1995).

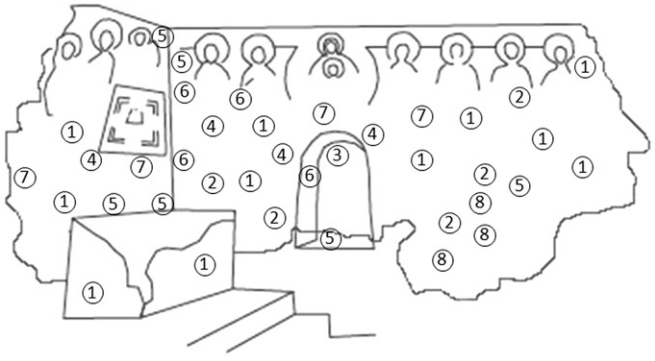

Fig. 1. Location of the different types of biodeterioration patterns.

2.3. Cultivation, isolation, and identification of algae and cyanobacteria

Phototrophic microorganisms were identified both by direct observation using an optical microscope and by cultivation methods. A small amount of the green patina (5.2 mg) was aseptically collected in a sterile 1.5 ml micro test tube using a lancet, split into two portions, and suspended in 50 ml of Cyanobacteria BG-11 freshwater medium (BG11, Sigma Aldrich) and Bold Basal liquid Medium (BBM, Sigma Aldrich), developed for cyanobacteria and microalgae, respectively. Cultures were maintained for 2–3 wk under cool-white fluorescent illumination (Osram Dulux L 36W/840 Lumilux, 2900 lumens), with a 12-h photoperiod, at 20 ± 2 °C.

Identifications were carried out using the monographs of Bourelly (1966) and John et al. (2002).

2.4. Isolation and cultivation of microfungi

Samples from deterioration pattern 4 were collected with non-destructive adhesive tape strips sampling method and observed with a light microscope. Black colonies (alteration pattern 7) were aseptically sampled with a sterile needle, plated on Dichloran Rose-Bengal (DRBC, Oxoid Ltd, Basingstoke, Hampshire, UK) and malt extract agar (MEA, Applichem Gmbh.), and incubated at 15 °C for 15 days. Isolation of pure cultures was performed on MEA. All strains isolated were included in the Culture Collection of Fungi from Extreme Environments (CCFEE, Viterbo, Italy).

2.5. Molecular analyses of fungi: DNA extraction, sequencing alignment, and tree reconstruction

Due to the scarce differentiation of black meristematic fungi, morphological approaches for identification were not applicable and they were studied using molecular techniques based on ITS rDNA sequencing. After 30 days incubation, 100–130 mg of the biomass was taken for DNA extraction. Fungal genomic DNAs were extracted using the Nucleospin Plant kit (Macherey–Nagel, Düren, Germany) following the protocol optimized for fungi.

The amplifications of the ITS rDNA portion for fungi were performed using BioMix (BioLine GmbH, Luckenwalde, Germany), employing 5 pmol of each primer ITS1, ITS4, ITS5, and ITS4a and 20 ng of template DNA in a total volume of 25 μl (Selbmann et al., 2005). The amplifications were carried out using MyCycler™ (Bio-Rad, Hercules, CA). The program for amplification was as follows: A first denaturation step at 95 °C for 5 min was followed by denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 32 s. The last three steps were repeated 35 times, with a last extension at 72 °C for 5 min.

The PCR products were visualized on agarose gel electrophoresis and quantified by comparison with the Ladder GeneRulerTM 1 kb DNA (Fermentas). The products were purified using the Nucleospin Extract kit (Macherey–Nagel, Düren, Germany). Sequencing reactions were performed according to the dideoxynucleotide method using the TF Big Dye Terminator 1.1 RR kit (Applied Biosystems). Fragments were analyzed using an ABI 310 Genetic Analyser (Applied Biosystems). Sequence assembly was done using the software Chromas (version 1.45 1996–1998, Conor McCarthy School of Health Science, Griffith University, Southport, Queensland, Australia). Sequences with high similarity available in the NCBI Genbank were identified using BLASTn search (Altschul et al., 1990; http://www.ncbu.nml.nih.gov/blast/).

ITS sequences were aligned using the Mega5 package (Tamura et al., 2011) adding sequences of black yeasts of the CCFEE data base. Due to gaps necessary for alignment, the sequences spanned 582 positions. The initial and the final parts were cut off to compare