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Assessment of fungi proliferation and diversity in cultural heritage: Reactions to UV-C treatment

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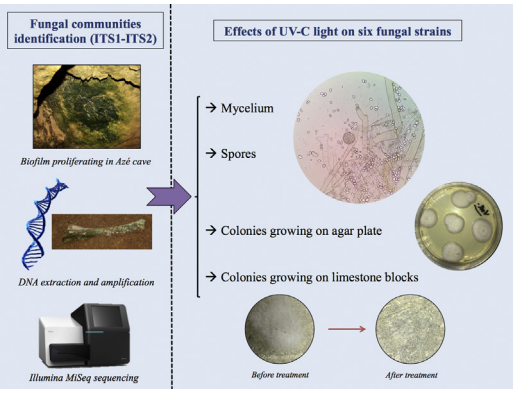
HIGHLIGHTS

- Fungi were sequenced in six French and Swiss show caves.
- UV-C treatment showed high efficiency to eradicate fungal strains.
- Fungal resistances against UV-C light have been identified.
- Several UV-C irradiations are necessary to eradicate fungal proliferation.

GRAPHICAL ABSTRACT



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ABSTRACT

Fungi are present in natural and non-touristic caves due to the presence of organic matter provided mainly by insects or animals such as bats. In show caves, however, tourist infrastructure and the visitors themselves are an important source of organic matter. In addition, photosynthetic biofilms provide a high amount of carbon and nitrogen sources for fungi. This study was conducted to identify the fungal communities present in caves along with the potential use of UV-C treatment against their proliferation. Thus, fungal communities proliferating in biofilms in six French and Swiss show caves were analyzed using high throughput sequencing. The results show 385 species recorded, some of them previously described in cases of fungal outbreak. This preliminary study also aimed to test the use of UV-C light as an environmentally friendly method to treat fungal proliferation. Six fungal strains, from three different sources (Lascaux cave, La Glacière cave, a church in Vicherey, France), were cultivated in an agar dish. Spores, mycelia and the entire colony were irradiated using several UV-C intensities. Results showed that four of the six fungi spores and mycelium died following a low-intensity UV-C treatment (2 kJ m^{-2}, 160 s), though *Ochroconis lascauxensis* and *Penicillium bilaiae* spores showed higher resistance. Finally, it was demonstrated that the fungal colony could resist the UV-C light due to a shadow effect. The

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structure of the fungal colony was affected from the periphery to its inner part. However, after four 30 kJ m^{-2} treatments (39 min irradiation) all strains there definitively eradicated. Further studies will be necessary to examine the potential of UV-C light under cave conditions as a preventive and curative treatment.

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1. Introduction

Caves are oligotrophic environments in which environmental parameters are relatively stable throughout the year (Borderie et al., 2015; Popović et al., 2015). Since the beginning of touristic activities, cave parameters such as temperature, moisture, carbon dioxide concentration, airflow and artificial light regime have been deeply modified, leading to biological contamination. In fact, organic matter imported by tourists (Lamprinou et al., 2014), in addition to both artificial light (Cigna, 2011; Cennamo et al., 2012) and carbon dioxide increases (Baker and Genty, 1998; Dragovich and Grose, 1990), creating favorable conditions for microorganism proliferation (e.g. microalgae, cyanobacteria and fungi). In 1923, Kyrle first studied photosynthetic microalgae, cyanobacteria, diatoms and mosses, followed by Morton and Gams in 1925 (Lamprinou et al., 2014). Since then, these microorganisms, also called "Lampenflora", have been studied extensively (Cennamo et al., 2012, 2016; Borderie et al., 2014; Pfendler et al., 2018a).

Fungi development in caves has frequently been described, especially in the world famous Lascaux cave (Bastian et al., 2009a, 2009b, 2009c) where several fungal outbreaks have taken place. Fungi proliferation is of particular concern to cave managers due to its rapid growth and enormous enzymatic activity and potential to deteriorate stone and prehistoric paintings (Schabereiter-Gurtner et al., 2004; Porca et al., 2011), and to decay paint, textiles, paper, parchment, leather, oil, casein, glue and other materials used in historical art objects (Sterflinger, 2010).

Curators have thus made extensive use of chemical treatments such benzalkonium chloride solutions, streptomycin and polymyxin (Bastian, 2010). However, some chemicals (e.g. sodium hypochlorite) may degrade certain walls and mineral structures (e.g. in natural caves). In addition, it has been reported that certain heterotrophic microorganisms may use chemicals as a source of carbon and nitrogen (Urzì et al., 2016), leading to a second microbial proliferation. Moreover, as reported by Urzì et al. (2000), chemicals become ineffective after a few years of treatment. In recent decades chemical biocides were banned because of toxicity-related hazards to the environment and health (European-Commission-Regulation, 2007; SCENIHR, 2009). It has therefore become urgent to find new treatments that are both effective and environmentally friendly.

In our previous studies, we have demonstrated that ultraviolet light (UV-C, 245 nm) is an efficient tool to combat photosynthetic organisms (Borderie et al., 2014; Pfendler et al., 2017b). Its highly non-selective action on organic compounds such as proteins and DNA, lipids (Pfendler et al., 2018b) is successful in eradicating large amounts of microorganisms forming biofilms (Pfendler et al., 2018a). Accordingly, UV-C germicide properties are used in hospitals and industry for water, air and instrument decontamination (Begum et al., 2009). It has been reported in the literature that the validation of the UV-C method consists of irradiating fungal spores (Valero et al., 2007; Begum et al., 2009). The spores are then cultured on a specific medium in agar plates to determine their viability. However, as far as we know, no study has demonstrated the UV-C effects on fungal colonies and this method's potential in treating fungal outbreaks in show caves.

In this study, the first objective was to investigate fungal communities, in five show caves in France and one in Switzerland, using high throughput sequencing. The second objective was to determine whether UV-C treatment can be an efficient tool to combat fungal outbreak. To achieve this objective, we studied UV-C efficiency on fungal spores, on cell suspensions and in colonies using six fungal species, previously sampled in the two French cultural heritage caves of La Glacière and Lascaux and also in a church in Vicherey, France. Finally, the effects of UV-C on fungal colony structure were studied using *Rhizomucor* sp., a fungal strain frequently recorded in show caves.

2. Materials and methods

2.1. High throughput sequencing

2.1.1. Caves description

Both Azé and Blanot caves are installed in the limestone of the Middle Jurassic period from Aalenian to Bathonian ages. Soyons caves are located in the South part of a huge anticline with Upper Jurassic limestone from Kimmeridgian ages. Réclère cave is located on the top of the Lomont anticline and is a tectonic cave installed in the limestone of the upper Jurassic period from Kimmeridgian age. On the floor of Saint-Marcel cave, we can observe a calcite crust, 5 to 20 cm of thickness over the clay sediments.

2.1.2. Sampling

Twenty-three samples were collected in different parts of six caves (Blanot, Azé-préhistorique, Azé-rivière, Soyons-renard, Saint-Marcel and Réclère) according to the protocol described in our previous study (Pfendler et al., 2018a). Fresh matter (100 to 200 mg) was taken from each biofilm. In order to avoid unwanted contamination, samples were directly collected in 2-ml tubes (MicroStynth AG) containing balls for mechanical lysis, and subsequently kept on dry ice (−78 °C) for the 3 days of sampling. Samples were conserved by MicroSynthAG (Swiss) at −20 °C until total DNA extraction, amplification steps and sequencing.

2.1.3. Molecular methods and sequencing

A PowerBiofilm DNA Isolation Kit was used by MicroSynth AG following the manufacturer's instructions (MoBio Laboratories, Inc., Carlsbad, CA, USA). The polymerase chain reaction (PCR) amplification was performed following a two-step PCR protocol using a state-of the-art high fidelity polymerase. This two-step PCR was applied in order to increase reproducibility and to improve the production of high-quality multiplex amplicon libraries. PCR amplifications were performed with the primers ITS1 f (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2 r (5′-GCTGCGTTCTTCATCGATGC-3′) (Durand et al., 2017) as follows: denaturation at 95 °C/3 min, 20 cycles of 98 °C/20 s, 56 °C/30 s, 72 °C/30 s and final elongation 72 °C/5 min. PCR products were purified, quantified with fluorescence spectroscopy using Picogreen (Quant-iT™ PicoGreen™ dsDNA Assay Kit, Thermo Fisher) and then pooled in equimolar amounts. DNA sequencing was performed on one MiSeq run with 2 × 250v2 according to standard protocols (capacity per run: app. 10 million reads, Illumina passed filter data). Bioinformatics data analyses were performed as described in Pfendler et al. (2018a, 2018b, 2018c).

2.2. UV-C effect on six fungal strains

2.2.1. Site description and sampling

The Lascaux cave was discovered in 1940 and is famous for its prehistoric paintings, now considered among the finest known examples of rock art paintings (Bastian, 2010). In 2001 and 2004, two major fungal outbreaks occurred. The first was treated with chemicals and the

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