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Tutorial

Biogenesis of secondary mycogenic minerals related to wall paintings deterioration process

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ABSTRACT

Present study addresses potential of fungal strains, isolated from deteriorated mural paintings and surrounding air environment of the Church of the Holy Ascension in Veliki Krčimir (Serbia), to precipitate mycogenic minerals, when cultivated on agarized B4 medium. Utilizing culture-based isolation methods, 38 filamentous fungi were obtained in total, 23 from mural paintings and 15 from air, respectively, mainly ascomycetes, while *Bjerkandera adusta* and *Thanatephorus cucumeris* were only basidiomycetes. A total of 31 of 38 fungal isolates, more than 80%, were able to form minerals of different morphologies and variable size, determined via SEM-EDS and XRPD, to be either calcite or calcite and weddellite association. Among screened fungi, all *Penicillium*, *Chaetomium* and *Cladosporium* isolates, as well as most of the *Aspergillus* isolates (8/11) precipitated minerals, whereas cultures of *Bionectria, Bjerkandera,* and *Seimatosporium* isolates lacked any observable crystal forms. With the exception of two *Alternaria alternata* strains, no apparent disparity in potential to precipitate minerals in general, or form particular crystal phase was documented among the air and mural paintings isolates. Possible mechanisms of fungal mineralization of calcite and weddellite are further proposed. In addition to providing experimental evidence for fungal induced precipitation of oxalate and carbonate minerals, presented data suggest that fungal activity could be an important factor in a weathering process affecting cultural heritage exhibited and stored in inadequate conditions. Implementation of B4 plate assay for screening of mineralization potential of the isolated fungi could be used to assess biodegradative risk mycobiota pose to the mural paintings, so appropriate conservation measures may be utilized.

1. Introduction

Fungal induced mineralization is still an insufficiently studied phenomenon, notably in regards to origin and mechanisms of oxalate formation. Biogenesis of oxalate minerals constitutes a normal physiological process in plants and a range of free-living and symbiotic fungi (Thomas, 2009). Occurrence of microbially induced oxalates, on the other hand, is known to have a wide range of devastating effects on cultural heritage. Calcium oxalate, most abundantly found oxalate ubiquitously associated with fungi, is well-known to crystallize on hyphae, strands, cords and rhizomorphs, as well as fruiting bodies, lichen thalli, and mycorrhizal systems in soil. Likewise, it can be formed on rocks and built environment, stone and mineral-based cultural heritage exposed to the open air (Gadd et al., 2014). Calcium oxalate patina of uniform thickness can thus be commonly observed on fungal infested surfaces of marble and limestone monuments, in which case they are called "*scialbatura*", as well as on sandstone, granite, plasters, sculptures, cave and mural paintings (Del Monte et al., 1987; Lazzarini and Salvadori, 1989). Albeit, there is a lengthy and yet unresolved debate within microbiological community in regards to origin, and especially impact of oxalate patina formation on structural and aesthetic properties of works of art, their formation is generally regarded as the most important weathering process affecting historic monuments and other cultural heritage objects (Del Monte et al., 1987; Gadd et al., 2014). And while hypotheses on mechanisms of oxalate formation on murals and other works of art ranged from lichen metabolism and atmosphere pollution, to oxidative decomposition of coatings applied for protective and colouring purposes (Lluveras et al.,

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Fig. 1. The Old Church of The Holy Ascension: exonarthex with surrounding environment (a); deteriorated mural paintings on frontal façade (b, c); details of deteriorated mural paintings decorating nave (d–f).

2008), fungi were never given proper consideration as possible main source of oxalate patinas. This is especially puzzling given their role in soil oxalate-carbonate pathway and fact that fungi are widely considered as the main biodeteriogens of cultural heritage (Sterflinger, 2000, 2010).

It's not frequently implemented in practice, that data regarding fungal diversity on wall paintings be complemented with research on physiological properties of isolated fungi. Only by fully understanding the total spectrum of physiological features of fungal isolates can a precise assessment of the amount of risk to the wall paintings be made. Therefore, the principal purposes of this study were to establish diversity of fungi present in the air and on the wall paintings of the old Church of the Holy Ascension in Veliki Krčimir (Serbia), provide experimental evidence for fungal-mediated biogenesis of mycogenic oxalate minerals, and determine the potential of air and wall painting isolates able to precipitate oxalates. Thus, constituents of fungal community thriving in air and on/within painted layers of murals and potentially capable of inducing oxalate-related deterioration symptoms will be known and appropriate conservation measure considered and implemented.

2. Materials and methods

2.1. Isolation of fungi

2.1.1. Sampling of surface mycobiota

For the isolation of fungi, colonizing painted layers of nave and exonarthex mural paintings, dip slide method with Dipslide w/TSA/Rose Bengal CAF (3M Microbiology, USA) was used. Sampling was conducted on 6 indoor wall paintings, and 2 wall paintings that decorate frontal façade of the investigated church (Fig. 1). Areas of wall paintings with abundant surface efflorescence and/or evident deterioration symptoms were targeted during sampling. In each sampling point, sampling was conducted in triplicate. Inoculated dip slides were incubated at 25 ± 2 °C for 7 days (UE 500, Memmert, Schwabach, Germany).

2.1.2. Sampling of airborne fungi

Viable fungal propagules, present in the air of the nave and exonarthex, were sampled using air sampler MAS-100 Eco (Merck Eurolab, Darmstadt, Germany). Flow of air through the perforated head sampler (400 × 0.7 mm) was set to 100 l min^{-1}. Streptomycin-enriched Malt extract agar (MEA) was used as a nutrient medium of choice for the isolation of fungi. Inoculated plates were incubated at 25 ± 2 °C for 7 days. Sampling in both church areas were performed in triplicate.

2.2. Fungal identification

Isolated fungi, re-inoculated from dip slides and streptomycin-enriched MEA onto Czapek dox agar, Potato dextrose agar and MEA were identified based on macroscopic features of formed colonies, observed under stereomicroscope (Stemi DV4, Carl Zeiss, Oberkochen, Germany), and micromorphological features of asexual and sexual reproductive structures, analyzed via microscope Axio Imager M1 (Carl Zeiss, Oberkochen, Germany), with AxioVision Release 4.6 software. Several dichotomous keys were used for identification (Raper and Fennel, 1965; Pitt, 1979; Ellis and Ellis, 1997; Watanabe, 2002; Samson et al., 2010; Bensch et al., 2012). These identifications were also confirmed by DNA sequencing. For this purpose, fungal isolates were grown on MEA for 7 days at 25 °C and after cultivation period, 70 mg of marginal mycelia was harvested for total genomic DNA extraction with the ZR Fungal/Bacterial DNA Mini Prep KIT, per manufacturer protocol of Zymo Research (USA). Prior to DNA extraction, mycelia were suspended in 200 µl of sterile deionized water. Two gene regions were amplified: ITS 1 (partial 5.8S + internal transcribed spacer 2 + partial