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Diversity and biodeteriorative potential of fungal dwellers on ancient stone stela

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ABSTRACT

Biodeterioration caused by fungal colonizers on an ancient stone stela, excavated from the former Roman settlement (Eastern Serbia) was investigated. According to selected biodeterioration elements, average deterioration index was assessed (0.8), prompting the need for conservation. Fungal somatic and reproductive structures, along with lichen thalli and moss fragments, were detected on the surface using different microscopy and cultivation methods. *In situ* microscopy on the site was implemented, for the first time, in the study of stone monuments. Biodeteriorative potential of 5 selected isolates was tested using qualitative biochemical tests, SEM-EDS and XRPD analyses. *Fusarium proliferatum* and *Penicillium crustosum* altered the pH value in broth minimal medium. Pigment production was demonstrated for *F. proliferatum*, while *P. crustosum* showed potential for calcite dissolution. All isolates induced biomineralization on solid medium with calcium acetate, where weddellite, calcite and subordinate whewellite crystals were confirmed via SEM-EDS and XRPD. Weddellite and calcite production was documented for *P. crustosum* in solid medium with calcium carbonate. Conservation treatment was carried out with benzalkonium chloride-based biocide, in addition to mechanical treatment. After conservation, *in situ* microscopy showed deteriorated stone surface covered with residual lichen thalli fragments, while mycelium and reproductive structures of micromycetes were not detected.

1. Introduction

Significant percent of the world's cultural heritage is represented by archeological antiquities, including architectural monuments, statues, tombstones, stelae etc., made of stone material. Stone antiquities are generally slowly degrading due to weathering caused by both environmental factors and biological agents. For example, it was estimated that only 1.5–3 mm of a limestone surface erodes in 100 years period in temperate climates (Scheerer et al., 2009). Microorganisms (bacteria, archaea, fungi, algae and lichens) are often involved in stone deterioration (Ortega-Calvo et al., 1995; Albertano and Urzì, 1999; Scheerer et al., 2009; Dakal and Cameotra, 2012; Sterflinger and Piñar, 2013; Bartoli et al., 2014). Among them, special attention is given to micromycetes, since they are significant biodeteriogens of stone and other cultural heritage object materials. Fungal spores are carried by air, atmospheric water, insects, and other biotic vectors. Therefore, fungi can easily colonize various microenvironments and proliferate if optimal growth conditions (humidity, temperature, nutrients, etc.) are met (Florian, 2002).

Fungi degrade stone both mechanically and chemically. Mechanical deterioration is achieved via hyphal penetration which causes breakup and fragmentation of stone. On the other hand, production of numerous inorganic and organic acids, as an important physiological activity, also has influence on stone degradation. Organic acids are chelating agents and can demineralize substrata by forming stable complexes with metal cations (Griffin et al., 1991; Gadd et al., 2014). Although many

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microorganisms are capable of producing these acids, fungi are considered to be the most significant organisms in nature to biodegrade rocks and minerals (Warscheid and Braams, 2000). Organic acid production by fungi is one of the factors important in calcite dissolution mechanism which is also achieved through oxidation and reduction processes (Hou et al., 2013). Production of fungal pigments in/on substrate leads to various discolorations which depend not only on pigment and substrate chemical composition but also on biotic interactions and environmental conditions (Garg et al., 1995; Florian, 2002).

Since it is well known that fungal induced deterioration can cause damage to cultural heritage objects, mycological investigations, as a precondition for efficient conservation of cultural heritage objects, are becoming mandatory in recent years. Biodeterioration assessment should include application of traditional cultivation methods, different types of microscopic, physiological, biochemical, molecular, as well as mineralogical and petrographical analyses which provide adequate characterization of both fungal metabolic activities and stone material. It is necessary to use different microscopy approach, especially in preliminary investigations, in order to assess biodeterioration level, to examine microbial community and to develop efficient conservation strategies (Rosado et al., 2015). Conservation can be efficiently implemented only through a multidisciplinary approach which involves a profound understanding of fungal biology and implementation of new, contemporary and practical methods.

The main goal of conducted study was to assess fungal community, deterioration potential of selected isolates and efficacy of conservation treatment via biological, geological and conservation science approach. Research was carried out on an ancient stone stela, excavated from the former location of the pristine Roman settlement Viminacium (present day Kostolac, Eastern Serbia). To assess deterioration potential of micromycetes, colonizing stone surface, a variety of microscopy techniques, as well as numerous traditional and contemporary microbiological, mineralogical and petrographical methods were applied. In addition, direct *in situ* optical microscopy, was carried out for the first time on stone-made archeological object, at the site. Intently implemented set of adequate techniques, as an integral part of the applied methodology is to provide us with better insight into main causes and flows of microbial induced weathering of stone monuments.

2. Material and methods

In this multidisciplinary research, all applied methods were done within three main research areas: (1) biological – with the aim of fungal isolation, identification and biodeterioration assays, as well as assessment of fungal growth and colonization of stone stela surface (2) geological – in order to characterize mineralogical and petrographical features of stone material and mineral products of fungal activities and (3) conservation science – with a goal to apply conservation procedures and preserve the treated stela.

2.1. Description of the studied stone stela

The studied stone stela belongs to the Roman collection of the National Museum in Požarevac, Eastern Serbia. The stela was found at the Kostolac site (44°43′58.4″N 21°13′49.9″E) and is estimated to the late Roman period (end of 3rd, beginning of 4th century AD; according to the Museum documentation, unpublished). It is exhibited in the lapidarium of the Museum's garden. It is placed directly on the ground and is exposed to different environmental agents.

The stela has cuboid form, with dimensions of 66 × 67 × 28 cm. Front is divided onto two niches, both with carved male figures in deep relief. The figures are represented like reflections in the mirror, each wearing pointed hat and holding long cane, slightly bent at the bottom part. The relief is much eroded; left side is more damaged then the right. The stela is overgrown with biological mat, especially abundant on the top and in the upper part on the front, above the niches. In the upper half of the niches, thick black crust developed, changing the appearance of the stone surface. Stone disaggregation occurred below detached zones of the crust-covered surface. Lower parts of the stela were soiled with dirt deposits. The appearance of the examined stela, prior to and after the applied conservation treatment, is presented in Fig. 1.

2.2. Petrographical analyses

Due to necessity of applying only nondestructive methods in characterization of rock type from which the stela is made of, only set of macroscopical tests could be done. The rock was analyzed by: (a) both naked eye and lenses (magnification of 50×) in order to define color, texture, fabric and macroscopically visible constituents such as minerals, fossils and rock fragments, (b) set of tests to observe relative rock hardness (scratching by nail, metal object, glass) and (c) reaction of rock constituents with cold, diluted HCl for indication of presence of specific mineral phases within a rock.

2.3. Deterioration index

In order to determine deterioration level of the examined stela, visual evaluation of 4 selected elements of deterioration (pitting, discoloration, moss and lichen thalli colonization and hyaline mycelia coverage) was carried out. Based on intensity of observed macroscopic signs of deterioration, a 4-point scale was utilized, according to Piotrowska et al. (2014): 0 – no changes, 1 – small changes (up to 20% of the examined area), 2 – moderate changes (from 20% to 50% of the area), 3 – intensive changes (over 50% of the area). Deterioration index was estimated for each examined side of the stone stela (front, back, top and lateral – left/right) according to equation (1):

$$DI = \frac{a_1 + a_2 + \ldots + a_n}{n * a_{max}} \quad (1)$$

n – number of examined elements (n = 5); a_1, a_2 … a_n – score of evaluation for each element ($a_{max} = 3$).

Total DI for the entire stela was estimated as an average value of deterioration indices for each examined side of the stela.

2.4. In situ optical microscopy

In order to study biocolonization on the stone surface, the stela was investigated at the site, as well as in laboratory, after applied conservation treatment, using portable digital microscope (ShuttlePix P-400R; Nikon). *In situ* microscopy was carried out directly on selected areas of the stela surface with visible deterioration signs. Image processing and measurements were acquired via ShuttlePix Editor v3.4.0.2 software.

2.5. Sampling methods for mycological analyses

Non-invasive adhesive tape (Urzi and de Leo, 2001) was adhered to the stone surface and removed with a steady force. Tape samples were attached to microscope slides and preserved in sterile container for microscopic analyses.

Sterile cotton swabs and dip slides (Dipslide w/TSA/Rose Bengal CAF, 3M Microbiology) were used for sampling of viable fungal