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MOLD ATTACK ON FRESCOES AND STONE WALLS OF GRADAC MONASTERY

ABSTRACT: Microfungi can colonize stone surfaces and form sub-aerial biofilms which can lead to biodeterioration of historic monuments. In this investigation samples for mycological analyses were collected from stone material with visible alteration on stone walls of Gradac monastery exterior. The prevailing fungi found on stone walls were dematiaceous hyphomycetes with melanized hyphae and reproductive structures (*Alternaria*, *Aureobasidium*, *Cladosporium* and *Epicoccum* species). The frescoes inside the monastery building were also analyzed for the presence of mycobiota. The predominant fungi found on frescoes were osmophilic species from genera *Aspergillus* and *Penicillium*. The significant result is identification of human pathogen species *Aspergillus fumigatus* on frescoes.

KEY WORDS: *Aspergillus fumigatus*, biodeterioration, biofilm, cultural heritage, mycobiota

INTRODUCTION

During the recent decades there has been a general concern regarding the deterioration of historic and art buildings. The deterioration problem of works of art is significantly relevant in countries that are rich in cultural heritage, such as Serbia. Therefore, it is important to protect the cultural heritage objects from damages caused by air pollution, physical and chemical agents and biodegrading activity of microorganisms. Microorganisms can colonize monuments' surface and form microbial sub-aerial community, biofilm. All biofilm-forming microorganisms may cause biodeterioration and degrade monuments mechanically, chemically and aesthetically through the metabolic activities and biomineralization process in these biofilms (S u i h k o et al., 2007). Filamentous fungi are also part of sub-aerial biofilms and they can cause biodeterioration of historic monuments resulting in weathering of monuments. There are many publications focused on fungal deterioration of stone monuments, historical

buildings, frescoes and art objects (G a y l a r d e and M o r t o n, 2005). The two main mechanisms of fungal biodeterioration activity is hyphal penetration through the substratum and biocorrosive activity as a result of production of organic acids and pigments (W a r s c h e i d and B r a m s, 2000).

Object of this research was the Gradac monastery, which was founded in 13th century by Helen of Anjou, and it was declared as Monument of Culture of Exceptional Importance in 1979, due to its architecture, wall paintings, and role in the history. As a part of the conservation process of cultural heritage of the Republic of Serbia, aim of this study was to isolate and identify biodegrading filamentous fungi from stone walls and frescoes.

MATERIAL AND METHOD

Samples for mycological analyses were collected from stone walls and frescoes with visible alterations of Gradac monastery. Thirty six samples from stone walls and 20 samples from frescoes were taken by swabbing with sterile cotton swabs. In the laboratory, swab samples were shaken mechanically for 10 minutes in 10 ml sterile distilled water and 1 ml aliquots of the resulting suspension were used for inoculation of spread plates on Malt agar medium containing 500 mg streptomycin per litre (MSA) in three replications. The inoculated plates were incubated at 25°C. Fungal growth was observed daily, for the period of 10 days, and they were submitted to the routine laboratory procedure to obtain pure fungal culture. Reisolations were done successively to the selective nutrient media: Potato dextrose agar (PDA), Czapek's agar (CzA) and Malt extract agar (MA) using standard mycological methods (B o o t h, 1971). Reisolated cultures were also incubated at 25°C. Identification of the obtained isolates to species level was done by macroscopic and microscopic examination. Microscopic preparates were dyed with lactophenol or fuchsine acid, observed by light microscopy (Zeiss AxioImager M.1, with software AxioVision Release 4.6) and determined by appropriate keys (A i n s w o r t h et al., 1973; E l l i s and E l l i s, 1997; P i t t, 1979; R a p p e r and F e n n e l, 1965).

RESULTS AND DISCUSSION

Out of a total of 56 samples, 17 fungal taxa were identified (Table 1). All isolates were obtained in pure culture by single conidial transfer. Mycological analyses showed that mycobiota was specific to different substrata (stone walls and frescoes).

The results of identification by morpho-physiological methods showed the abundance of dematiaceous hyphomycetes with melanized hyphae and reproductive structures on exterior stone walls of the monastery, while the prevailing taxa isolated from frescoes were osmophilic *Aspergillus* and *Penicillium* species. Non-sporulating isolates that could not be assigned to any

Tab. 1 – Molds isolated from stone walls and frescoes of Gradac monastery

Micromicetes	stone walls	frescoes
<i>Alternaria alternata</i>	+	
<i>Alternaria</i> sp	+	
<i>Aspergillus fumigatus</i>		+
<i>Aspergillus nidulans</i>		+
<i>Aspergillus versicolor</i>		+
<i>Aureobasidium pullulans</i>	+	
<i>Cladosporium cladosporoides</i>	+	
<i>Epicoccum purpurascens</i>	+	
<i>Fusarium</i> sp.	+	
<i>Mycelia sterilia</i>	+	
<i>Mycelia sterilia</i> (melanized)	+	
<i>Nigrospora nigra</i>	+	
<i>Paecilomyces</i> sp.		+
<i>Penicillium</i> sp.	+	
<i>Penicillium</i> sp. 2		+
<i>Sporobolomyces roseus</i>	+	
<i>Ulocladium</i> sp.		+

taxonomic group were referred to as *Mycelia sterilia*. The production of dark conidia and pigments was recorded in culture media during the cultivation of melanized fungi isolated from stone walls. According to Milaneš et al. (2005), species from genera *Alternaria*, *Aureobasidium*, *Cladosporium*, *Epicoccum*, *Nigrospora*, and *Ulocladium*, which were found during our study, cause discoloration, as well as the mechanical exfoliation of building stone material that was analyzed through mechanical hyphae penetration and the production of different pigments and organic acids. *Aureobasidium pullulans* is a stain fungus that usually causes staining of different stone surfaces which decreases the aesthetic qualities of historic monuments. *Penicillium* and *Aspergillus* species are ubiquitous taxa that can produce numerous conidia and are widespread in the environment and easily dispersed by air. These fungi are usually found as contaminants or biodeterioration agents in many different habitats and materials, including those considered as representative of historical and cultural heritage. The significant result is the presence of *A. fumigatus* on frescoes inside the monastery. This mold can cause opportunistic infections in humans including aspergillosis which can sometimes be fatal. Indoor mold presence, including the interior of monasteries and other culture heritage buildings, can lead to sick building syndrome. This is why the fungal presence in indoor environment should not be neglected.

In conclusion, deterioration and alteration of stone walls and frescoes can be related to growth of filamentous fungi. The most frequent fungal contaminants of the historical and cultural monuments are air-borne and soil-borne micromycetes with cosmopolitan distribution that can colonize many kinds of surfaces.

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ПЛЕСНИ НА ФРЕСКАМА И КАМЕНИМ ЗИДОВИМА МАНАСТИРА „ГРАДАЦ”

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Резиме

Плесни могу да колонизују камени супстрат и да са другим микроорганизмима формирају специфичну микробијалну заједницу, биофилм. Присуство биофилма на каменим површинама доводи до процеса биодетериоризације који нарушава естетски изглед објеката од културно историјског значаја. Објекат истраживања, манастир Градац, који је основала Јелена Анжујска у 13 веку, је због посебног архитектонског значаја, зидног сликарства и његове улоге у историји 1979. године проглашен за културни споменик од изузетног значаја за Републику Србију. Као део конзервационог процеса, у оквиру програма очувања културне баштине, постављен је циљ – изолације и идентификације филаментозних гљива са камених зидова и фресака. Узорковање је извршено са 56 места са видљивим променама и оштећењима и стандардним миколошким методама изоловане чисте културе. Највећи број идентификованих плесни са спољашњих зидина манастира су из групе *Dematiaceae* са карактеристичних меланизованим хифама и конидијама (врсте родова *Alternaria*, *Aureobasidium*, *Cladosporium* и *Epicoccum*). Међутим, у узорцима са фресака доминирале су врсте родова *Aspergillus* и *Penicillium*. Значајно је истаћи да је на фрескама идентификована врста *A. fumigatus*, опортунистички хумани патоген, изазивач респираторне аспергилозе, и њено пренамножавање у затвореном простору је опасно по здравље људи.