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Full Length Research Paper

Moulds on paintings in Serbian fine art museums

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The main reasons of fungal expansion in museums are inadequate relative humidity and temperature and the spore's germination can be controlled by regulation of these two factors. Numerous paintings, originated from 11 Serbian museums, were analyzed for presence of moulds. Samples from canvas, dyes and wooden frames with visual changes were subjected to the analysis. Species of genera *Alternaria*, *Aspergillus*, *Aureobasidium*, *Cladosporium*, *Drechslera*, *Epicoccum*, *Penicillium*, *Rhizopus*, *Trichoderma*, *Ulocladium* and *Wardomyces* were isolated and identified. Some of the found species are known as potential producers of lignocellulolytic enzymes and other destructive metabolites, as well as causative agents of human diseases.

Key words: Micromycetes, paintings, dyes, fungal deterioration.

INTRODUCTION

Microorganisms usually attack materials such as paper, textile, wood, dyes and leather, forming well known symptoms on the surface. Dust and other air components can be potentional natural sources of fungi and bacteria. Relative humidity over 70%, temperature over 15°C, a neutral to acid pH, and presence of organic nutritive sources are the optimal conditions for fast growth and reproduction of mould species, which attack museum objects (Gorbushina et al., 2004). However, the spore can lie dormant at lower temperature and humidity, for a long time and the infected objects stay more or less under control.

Presence of fungi in exhibition and storage spaces of museums, aesthetic and structural changes, as well as physical degradation of the painted surface are serious problems in the conservation of cultural heritage (Bussjaeger et al., 1999; Milanesi et al., 2006). Garg et al. (1995) summarized the data about role of fungi in the deterioration of wall paintings. Indoor wall paintings are widely recognized as a favorable environment for biofilms, in general, which part is numerous microscopic fungal species. Dominant fungal species can vary depending on climate and surface conditions (Gorbushina et al., 2006). The aim of this study was the isolation and determination of mould species in paintings which can cause significant damages, both in storage spaces and galleries in Serbia as well as on health of employees.

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MATERIALS AND METHODS

Samples for mycological analysis were obtained from 11 museums and galleries in Serbia. They were collected from paintings which were either deposited in museum storage spaces or exhibited in galleries. About one hundred samples from canvas, dyes and wooden frames with visual changes were taken by sterile cotton swab for mycological analysis.

Samples were inoculated on malt streptomycin agar (MSA) medium (malt extract agar with 500 mg streptomycin per liter) in three replications. The inoculated plates were incubated at 25°C and fungal growth was daily observed during ten days and 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 (Booth, 1971). Reisolated cultures were also incubated at 25°C. Identification of obtained isolates to species level was done by macroscopic and microscopic examination. Microscopic preparates were dyed with lacto phenol or fuchsin acid, observed by light microscopy and determined by appropriate keys (Raper and Fennell, 1965; Ramirez, 1982; Watanabe, 2002).

Table 1. List of isolated moulds species from deteriorated paintings.

Isolated moulds species	
canvas and dyes	**wood frames**
Alternaria alternata	*Alternaria alternata*
Alternaria tenuissima	*Alternaria* sp.
Alternaria sp.	*Aspergillus flavus*
Aspergillus candidus	*Aspergillus fumigatus*
Aspergillus clavatus	*Aspergillus niger*
Aspergillus fumigatus	*Cladosporium cladosporioides*
Aspergillus niger	*Cladosporium tenuisimum*
Aspergillus versicolor	*Cladosporium* sp.
Aspergillus wentii	*Drechslera dematoidea*
Aspergillus sp.	*Epicoccum purpurascens*
Aureobasidium pullulans	*Mycelia sterilia*
Chaetomum globosum	*Mycotypha microspora*
Cladosporium cladosporioides	*Penicillium* sp.
Cladosporiun herbarum	*Rhizopus stolonifer*
Cladosporium macrocarpum	*Trichoderma viride*
Cladosporium sp.	*Ulocladium chartarum*
Drechslera dematoidea	*Ulocladium* sp.
Drechslera sp.	
Epicoccum purpurascens	
Fusarium sp.	
Geotrichum candidum	
Graphium putrendis	
Mycelia sterilia	
Mucor sp.	
Paecilomyces variotii	
Penicillium verrucosum var. *cyclopium*	
Penicillium sp.	
Rhizopus stolonifer	
Stachybotrys chartarum	
Trichoderma viride	
Ulocladium chartarum	
Ulocladium oedemansii	
Ulocladium sp.	
Wardomyces sp.	

RESULTS AND DISCUSSIONS

A large diversity of fungal isolates was obtained from all investigated painting, 36 taxa were isolated and identified in total, representing 19 genera. The fungal species were more numerous on canvas and dyes. Twenty four species, belonging to 18 genera, were isolated from canvas and dyes and 12 species from 10 genera from wooden frames (Table 1). Slight differences in species diversity in painting surface and frame were noticed. Thus, considering the genera distribution, the genus *Mycotypha* was presented only on the frames, not in painting surfaces. *Mycotypha microspora* comprised a very small proportion of the fungal biota on frames, *Cladosporium* was the most abundant isolated genus, generally (Figures 1 and 2). However, according to the number of identified species, genus *Aspergillus* showed the highest diversity, represented with 7 species on canvas, dyes and frames. *Aspergillus niger* was the most frequent species, detected in almost all samples. The species of the genera *Penicillium*, *Alternaria*, *Epicoccum* and *Ulocladium* were also isolated frequently from paintings (Figures 1 and 2). Other detected genera were only occasionally isolated and presented with one species, a few isolates were not identified to the species level and several non-sporulating isolates were reported as *Mycelia sterilia* (Table 1). Generally, members of subdivisions Ascomycotina and Deuteromycotina are

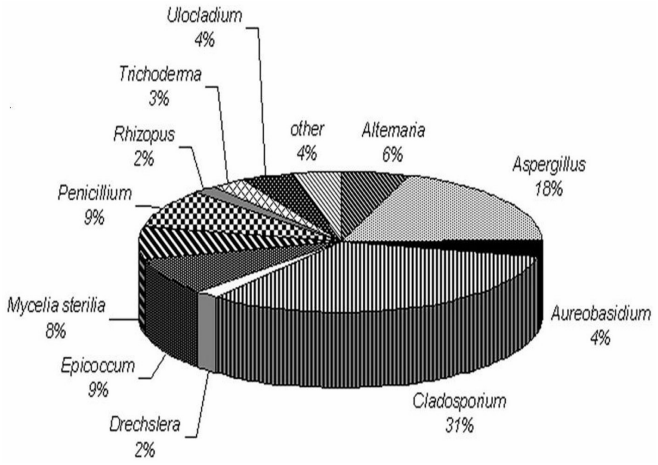

Figure 1. Proportion of fungal genera isolated from paintings.

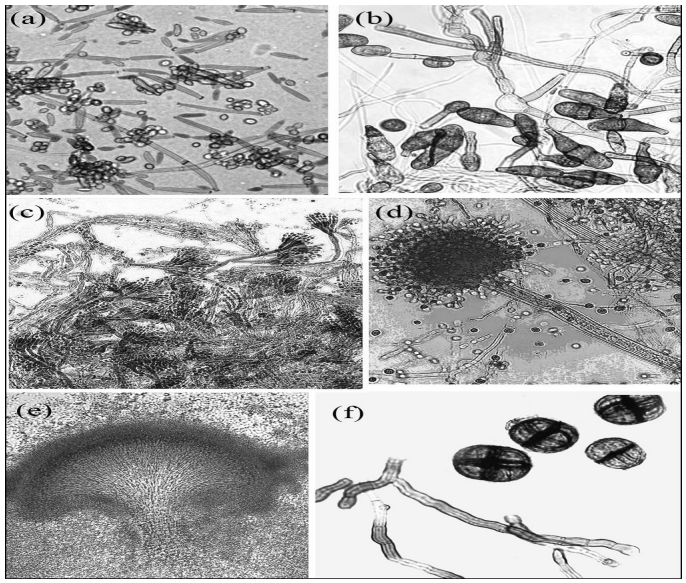

Figure 2. Selected fungal species isolated from deteriorated paintings: a. *Cladosporium cladosporioides*; b. *Alternaria alternata*; c. *Penicillium* sp.; d. *Aspergillus flavus*; e. *Graphium putrendis*; f. *Ulocladium chartarum.*

very aggressive agents of biodeterioration and present the most common problem of preservation and protection of artworks.

Obtained results are in accordance with literature data, which noted that the most common moulds in paintings belong to the genera *Aspergillus* and *Penicillium* (Berner et al., 1997; Guglielminetti et al., 1994). Milanesi et al. (2006) showed that environmental factors triggering degradation of paintings by moulds include relative humidity and temperature. Decolorisation of dyes and wood degradation by mentioned mould species are based on production of various metabolites, among which lignocellulolytic enzymes and different acids have main role. *Alternaria* and *Trichoderma* species have good developed lignocellulolytic enzyme system and present very destructive moulds in museums, especially for wooden frames (Garg et al., 1995). *Aureobasidium pullulans* is known as potential dye and polish degrader, while species of the genus *Drechslera* are causative agents of the mouldness in museum storage spaces (Dix and Webster, 1995).

Majority of isolated species are common allergens and some of them are potential mycotoxin producers (Avila and Lacey, 1974). *Cladosporium* and *Penicillium* species, which were the most abundant, are known as causal agents of asthma. Some authors noted that exposure to *Alternaria alternata* spores presented a risk factor for asthma and caused significant respiratory problems (O'Hollaren et al., 1991; Salo et al., 2006). The members of the genus *Aspergillus* are causative agents of large spectrum of diseases known as aspergillosis. Thus, *Aspergillus fumigatus*, found in paintings in this research, is an extremely angioinvasive species, particularly in the immuno-compromised patients and the most common agent of aspergillosis in human (de Hoog et al., 2000). The infamous toxic mould *Stachybotrys chartarum* is also a species in the indoor environment and causes building-related illness (Sudakin, 2000).

Nowadays, mechanical cleaning of contaminated museum objects with moulds and treatment with appropriate commercial fungicides are used with the aim of their prevention and protection. However, fungal resistance to fungicides is developed with their application for long time and that is why numerous studies deal with finding new natural antifungal agents. It was reported that various plant essential oils could find practical application in the prevention and protection from fungal infections (Soković et al., 2009). More of them have better antifungal activity than commercial fungicides. With regards to the fact that essential oils are high fungi-toxic and non-toxic for human, they could by effective agents for fine arts protections. Special attention should be pay to finding new fungicides of natural origin, which will be the topic of many further studies.

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