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Fungal contamination of textile objects preserved in Slovene museums and religious institutions

Katja Kavkler [a, *], Nina Gunde-Cimerman [b, c], Polona Zalar [b], Andrej Demšar [d]

[a] Gorenjska predilnica d.d., Škofja Loka, Slovenia
[b] Department of Biology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia
[c] Centre of Excellence for Integrated Approaches in Chemistry and Biology of Proteins (CIPKeBiP), Jamova 39, SI-1000, Ljubljana, Slovenia
[d] Faculty of Natural Sciences and Engineering, University of Ljubljana, Ljubljana, Slovenia

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ABSTRACT

This investigation into fungal growth on historical textiles, including the canvases of easel paintings stored in museums and religious institutions (churches and cloisters) in Slovenia, initially indicated relatively widespread fungal contamination. Closer investigation revealed that only 21 objects out of 38 were positive for fungal contamination, with the other objects being discoloured or stained due to other factors. On the objects that were stored at low humidity and temperature, fungal growth remained restricted for several years, even if the objects were contaminated before storage. Although most of the textile specimens contaminated by fungi were from those institutions without any control of internal environmental conditions, the rate of textile degradation due to fungal growth was generally low. The dominant contaminant fungal species, detected by culture-dependent techniques and identified by the use of current molecular genus-specific barcodes, belonged to the genus *Penicillium*, followed by *Aspergillus* and *Cladosporium*. Microscopy analyses of the fungal growth revealed that on most of these objects fungal growth was limited to the surface. The enzymatic profile of selected isolates was determined. Most of the fungi were isolated from the flax of the linen objects, confirmed also by their enzyme activities, particularly by strong beta-glucosidase activity. Amylase activity of selected isolates was also evident; this is important since starch can be added as filling or glue to textile materials. Examination of the structural and physical changes to the fibres on contaminated and non-contaminated objects showed the most pronounced structural changes on flax and other cellulosic fibres, while proteinaceous fibres (e.g., wool and silk) were generally not affected.

Introduction

One of the main aims of museums is the preservation of historical objects. During long-term storage of objects of historical value, the materials can change structurally due to exposure to different deteriorative conditions, e.g., UV irradiation, high humidity, and changing temperatures. Ultraviolet light causes oxidation of the polymers that constitute the natural fibres, which results in the breaking of intermolecular bonds and facilitates penetration of microbial enzymes (Tomšič et al., 2007; Zotti et al., 2008). High humidity accelerates microbial attack and the consequential degradation processes.

Textile objects are an important part of our cultural heritage objects, but unfortunately, since they are made of natural fibres, they are a target of microbial attack and degradation, resulting as discoloration, staining, and loss of structural strength (Tiano, 2002). In addition, textiles can also act as carriers of microorganisms such as pathogenic bacteria, odor-generating bacteria, and fungi. To forestall biodeterioration, textile manufacturers have for a long time been interested in the degree of fabric processing (e.g., Burgess, 1928) or in antimicrobial protection of textiles (Simončič and Tomšič, 2010). Currently research is also oriented toward the protection of historical textiles stored in museums against microbes (Ilec et al., 2012).

Since almost any material can be attacked by microbes, including those made of synthetic polymers (Gu et al., 1998, 2011; Gu, 2003; Singh and Sharma, 2008), historical textiles, which consist of mostly organic materials, are at special risk. The control of physical conditions in museums, especially the establishment

* Corresponding author. Present address: Institute for the Protection of Cultural Heritage of Slovenia, Metelkova 6, SI-1000 Ljubljana, Slovenia. Tel.: +386 (0)1 400 7927; fax: +386 (0)1 400 7986.
E-mail address: katja.kavkler@rescen.si (K. Kavkler).

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and maintenance of appropriate temperature and relative humidity (16–18 °C and 40–65% RH, respectively) in storage and showrooms, are currently the accepted and effective ways to deal with fungal contamination and reduce consequent biodeterioration. Fungal contamination of stored objects has other undesirable effects as well, such as lessened air quality due to the production of volatile metabolites, which has an impact on environmental health. A problem appears also due to the fact that natural fibres have different rates of absorption and desorption of moisture (Fusek, 1985), and thus local, uncontrollable microclimatic conditions might occur that can affect the possibility of fungal contamination of natural fibers.

The degree and speed of degradation depend on the chemical and physical properties of the substrate, in terms of the chemical structure, molecular weight, and crystallinity, along with the environmental conditions, the dominant microbial contaminants, and the synergy of the infecting microbial community (Warscheid, 2000; Tomšič et al., 2007). Infecting microorganisms can change the structure and stability of stored materials by enzymatic reactions and excretion of metabolic products, such as organic acids, oligopeptides, secondary metabolites, dyes, and volatile organic compounds (Warscheid, 2000). Deterioration can result in discoloration, staining, and loss of structural strength (Tiano, 2002). In summary, inappropriate storage and microbial contamination can often badly disfigure objects of art.

Although both bacteria (Seves et al., 1998; Szostak-Kotowa, 2004; Capodicasa et al., 2010) and fungi (Abdel-Kareem, 2010) can be isolated from historical textiles, fungi in particular have been reported as the main deteriorative agents. The prerequisites that facilitate the contamination of objects by fungi are high humidity and initial oxidative degradation of hygroscopic natural fibres (Valentin, 2003). The main targets of fungal enzymatic degradation are cellulosic natural fibres, although fungi can also degrade natural proteinaceous materials, as well as non-organic materials (Caneva et al., 2005), and even synthetic ones (Breuker et al., 2003; Gu, 2003; Cappitelli et al., 2005). Different fungal species can cause staining of various colours and sizes; this can be difficult to remove, as the hyphae can grow not only on the surface, but also within the fibres (Strzelczyk, 2004).

The most common genera of fungi that are known to occur on modern natural textiles are *Alternaria*, *Aspergillus*, *Chaetomium*, *Cladosporium*, *Curvularia*, *Fusarium*, *Memnoniella, Myrothecium, Penicillium*, *Pestolotia*, *Pullularia*, *Rhizopus, Stachybotrys*, *Trichoderma*, and *Verticillium* (Marsh and Bollenbacher, 1949; Marsh et al., 1949; White et al., 1950; Raschle, 1989; Szostak-Kotowa, 2004; Kaese et al., 2008). However, a slightly narrower range of fungal genera has been reported to grow on historical textiles: *Alternaria*, *Aspergillus*, *Chaetomium*, *Ctenomyces*, *Fusarium*, *Memnoniella*, *Myrothecium*, *Neurospora*, *Penicillium, Scopulariopsis*, and *Stachybotrys* (Tiano, 2002; Cybulska et al., 2008; Kvavadze and Gagoshidze, 2008; Abdel-Kareem, 2010). Most of these fungi were isolated from historical cellulosic fibres, while *Aspergillus*, *Chrysosporium*, *Ctenomyces*, *Fusarium, Penicillium*, and *Trichoderma* have also been reported from historical proteinaceous fibres (Tiano, 2002; Cybulska et al., 2008). Interestingly, the dermatophytic fungal genera *Trichophyton* and *Microsporum* have been reported to grow on historical wool (Tiano, 2002).

In contrast to the relatively well-documented fungal biodeterioration of stone, wood, paintings, and paper cultural heritage objects (Zyska et al., 1997; Montemartini Corte et al., 2003; Strzelczyk, 2004; Caneva et al., 2005; Sterflinger, 2006; Zotti et al., 2008), the above listed genera of fungi isolated from historical textiles have been reported in only a few studies (Tiano, 2002; Cybulska et al., 2008; Kvavadze and Gagoshidze, 2008; Abdel-Kareem, 2010). These studies have been limited to the identification of the fungal species in the framework of general investigations into degraded archaeological textiles (Kvavadze and Gagoshidze, 2008), general introductions to the research of historical textiles (Cybulska et al., 2008), studies about microflora-promoted deterioration of historical textiles in Egyptian museums (Abdel-Kareem, 2010), and a case report of fungal deterioration of a 16th century painting (Capodicasa et al., 2010).

Along with the development of microbiological methods, identification of fungi from historical textiles no longer involves culturing techniques only, but also employs non-culture methods based on the isolation of total DNA (Di Bonaventura et al., 2003). Nonetheless, the documentation of an active culture together with its biodegradation potential, e.g., enzyme profiling, can give us important information before a restoration procedure is conducted.

The aim of this study was to determine the extent of damage and degradation of historical textiles stored under varying conditions in six museums and six religious buildings in Slovenia, and which were potentially contaminated by fungi. The oldest stored textile sample was from the Roman period, while the most recent was from the 21st century. All 38 objects that were sampled were woven exclusively of natural fibres. The fungi were isolated from the textile objects using classic culturing techniques, and were identified to the species level through established, classic, and molecular methods. The aim was to find any connections between storage conditions, type of material, presence of fungi, and the rate of degradation of these different materials, and to identify the "typical museum mycobiota."

Materials and methods

Selection of samples

The samples originated from six museums (the National Gallery of Slovenia, National Museum of Slovenia, Slovene Ethnographic Museum, Museum of Christianity in Slovenia, Provincial Museum of Ptuj, and the Provincial Museum of Murska Sobota) and six religious buildings (Ursuline Convent; Franciscan Monastery; subsidiary Church of St. Radegunda, near Šenčur; Provost Church in Novo Mesto; Parish Church of St. Jacob, in Ljubljana; St. Nicolas Cathedral of Ljubljana), all in Slovenia. They were taken either from storage rooms in the museums or from the conservation workshops in the Slovenian Restoration Centre. In all, 35 different textile and three leather objects were obtained from three national museums (two, six, and eight objects), one national gallery (one object), one provincial museum (seven objects), and the Restoration Centre (14 objects), with these last originally stored in four churches, a provincial museum, and two cloisters. The choice of the numbers of objects examined from each institution was made at random, and was based on the selection of the curators and restorers. The selection criterion was the presence of stains appearing to be fungal contamination, or the presence of fungal mycelia on the surface of the objects examined. Where several different types of stains appeared, more than one smear sample was taken, which resulted in 60 sampled sites on these above-listed objects.

Storage conditions

Different types of museums that had different storage conditions for their historical objects were chosen. With the exception of the National Gallery, the conditions in the museum storage rooms were not controlled, and thus changed according to the outdoor conditions (Table 1). The conditions were measured on various occasions.

Sampling of fabric and identification of textile fibres

With the objects that would not be additionally damaged by sampling, pieces of fabric, loose fibres, or threads were taken for

Table 1
Storage conditions in the institutions from which the samples investigated were obtained.

Institution	Temperature [°C][a]	Relative humidity [%]	Number of objects investigated
National Gallery of Slovenia	Controlled; from 19 to 19.5	Controlled; 48 to 55	1
Slovene Ethnographic Museum	Not controlled	Until 2006 not controlled; then 40 to 60	6
National Museum of Slovenia	Not controlled until 2008	Not controlled until 2008	2
Ptuj Provincial Museum	Not controlled; range: 5 to 25[b]	Not controlled; range: 50 to 80[b]	7
Museum of Christianity in Slovenia	Not controlled; range: 2 to 25[b]	Not controlled; range in winter, 70 to 85; range in summer, 55 to 60[b]	8
Churches and cloisters	Not controlled	Not controlled	14

[a] All data obtained from curators or conservers.
[b] According to the occasional measurements available. Controlled: held at constant conditions; Not controlled: variable – not held at constant conditions.

determination of the fibre origin and for the examination of eventual damage, using forceps. Sample size varied according to the properties of each object, from loose threads of a few millimetres in length in more precious objects, up to 5 cm^2 in canvas linings, which were detached during conservation processes. The number of selected samples was determined according to the number of different textiles types of which an object was composed.

For the visual analysis, an Olympus BX60 transmission optical microscope was used (magnification, 400×). Polarisers and a compensator (lambda plate 530 nm) were applied for the confirmation of bast fibres.

Isolation of fungi

The sampling for the fungi was performed with areas of the material that showed unusual surface changes, such as discoloration or black staining, or from areas where mycelium-like structures were visibly overgrowing the surface of the objects (Table 2).

The fungi were sampled from the selected objects by rubbing with a sterile cotton swab that was either soaked in physiological solution (0.9% [w/v] NaCl), or was dry, in the cases of the more sensitive objects. The fungi were isolated from these swabs by subsequent inoculation onto malt extract agar in petri dishes, with the added antibiotic chloramphenicol (50 mg l^{-1}), to prevent overgrowth with bacteria. In several cases, a roughly 1 cm^2 piece of the infested object was cut off the sample, soaked with sterile physiological solution, and plated directly onto the malt extract agar culture medium. They were incubated at 25 °C for up to 21 days. Pure cultures of the fungi were obtained from the primary isolation plates by the further culturing of selected colonies with different morphologies. The isolated fungal strains are maintained in the Ex Culture Collection of the Department of Biology, Biotechnical Faculty, University of Ljubljana (Infrastructural Centre Mycosmo, MRIC UL, Slovenia). The strains identified to the species level are listed in Table 3.

Identification of fungi

The obtained fungal isolates were identified according to their macromorphology and micromorphological characteristics, and using genus/species-specific molecular markers, according to current taxonomic standards.

For the fungal DNA isolation, the strains were grown on malt extract agar for 7 days. Their DNA was extracted according to Gerrits van den Ende (1999), by mechanical lysis of about 1 cm^2 of their mycelia.

The fragment of rDNA that included internal transcribed region (ITS)1, 5.8S rDNA, and ITS2 was amplified and sequenced using the ITS5 and ITS4 primers (White et al., 1990). The large subunit of the rDNA (domains D1/D2 of the 28S rDNA) was amplified and sequenced with the NL1 and NL4 primers (Boekhout et al., 1995). For amplification and sequencing of the β-tubulin (*TUB*) gene, primers Bt2a and Bt2b were used (Glass and Donaldson, 1995). For amplification and sequencing of the partial actin gene (*ACT*), primers ACT-512F and ACT-783R were used, according to Carbone and Kohn (1999). The reactions were run in a PCR Mastercycler Ep Gradient machine (Eppendorf), with the protocol of: initial denaturation for 2 min at 95 °C, followed by 30 cycles of 45 s at 95 °C, 30 s at 54 °C, and 2 min at 72 °C, with a final elongation for 4 min at 72 °C. BigDye terminator cycle sequencing kits were used in the sequence reactions (Applied Biosystems, Foster City, CA, USA). The sequences were obtained with an ABI Prism 3700 sequencer (Applied Biosystems). These were compared with sequences deposited in the public databases of the National Center for Biotechnology Information (NCBI). The newly generated sequences were deposited with GenBank (www.ncbi.nlm.nih.gov), and their accession numbers are accessible under the numbers KJ468760-KJ468784, and are also listed in Table 3. The strains were identified on the basis of amplicons, using the BLAST tool on the NCBI web page and taxonomically relevant databases (e.g., CBS-KNAW Fungal Biodiversity Centre [Centraalbureau voor Schimmelcultures]).

Testing for extracellular fungal enzymatic activity

The preliminary general screening for enzymatic activity of the tested fungal strains was done using the API ZYM semi-quantitative micromethod (BioMerieux), which allows systematic and rapid screening of 19 enzymes, using small quantities of sample. Initially, pure cultures of the selected fungi were grown under submerged fermentation in Erlenmeyer flasks on a rotary shaker, for 7 days at 28 °C at 180 rpm. The complex medium was composed of: whey (10 g), yeast extract (1 g), olive oil (5 ml), glucose (10 g), peptone (5 g), glycerol (1 ml), and soluble starch (5 g), all dissolved in minimal salts solution: KH_2PO_4 (2.0 g), $(NH_4)_2SO_4$ (1.2 g), urea (0.3 g), $CaCl_2$ (0.3 g); all of these ingredients were dissolved in a solution of $FeSO_4 \cdot 7H_2O$ (0.1 ml: 5 mg in 0.1 ml distilled H_2O), $ZnSO_4 \cdot H_2O$ (1 ml: 1.4 mg in 1 ml distilled H_2O), $MnSO_4 \cdot H_2O$ (1 ml: 1.56 mg in 1 ml distilled H_2O), $CoCl_2 \cdot 6H_2O$ (1 ml: 2.0 mg in 1 ml distilled H_2O) in 1 l of distilled water. The pH of the medium was set at 6.5 prior to autoclaving. After the fermentation, the fungal broth was centrifuged and the supernatant was used for the API ZYM assays. The readings were performed using the API ZYM colour scale, ranging between 0 (negative) and 5 (maximum), depending on the amount of substrate metabolised: 1, <5 nmol; 2, 5 nmol–10 nmol; 3, 10 nmol–20 nmol; 4, 20 nmol–30 nmol; and 5, >30 nmol.

The activities of additional hydrolytic enzymes were detected using various solid media, according to Paterson and Bridge (1994). The cellulolytic activities were determined by hydrolysis of ground cellulose (Merck, Darmstadt, Germany), the esterase activities with a mixture of elaidic, linoleic, and palmitic fatty acids in Tween 80

Table 2
Descriptions of the objects investigated and the damage seen according to age and material, as determined by macromorphological observations and with transmission optical microscopy.

Location	Description of object	Predicted composition of textile material	Dating	Number of samples	Description of damage	Growth of fungi
Museum of Christianity in Slovenia	Wooden statue in textile dress	Cotton	1850	1	Grey spots	+
	Painting on canvas with a paper patch on the back	Paper or cotton fibres	End 19th/beginning 20th century	1	Greenish spots	+
		Flax		1	Green-greyish spots	+
	Embroidery, with metal threads	Cotton	Unknown	3	Light grey spots	–
	Painting on canvas	Flax	18th century	1	Light grey spots	+
	Textile pad in the museum showcase, in contact with infected books	Silk and cotton	21st century	1	No observable changes on textile, but on the books exposed on it	–
	Velvet chasuble, embroidered with metal threads	Silk	Unknown	2	Tiny white dots	–
	Prayer book bound in leather, with brass relief	leather[a]	1856	1	Visible mycelium	+
	Travelling suitcase with interior canvas lining	Cotton	Mid-20th century	4	Grey and brown spots	–
National Gallery of Slovenia	Painting on canvas	Hemp	Mid-20th century	1	Visible mycelium	+
National Museum of Slovenia	Archaeological textile, supp. Roman hair cover	Flax	1st to 2nd century AD	1	Grey artefacts	+
Provincial Museum of Ptuj	Underskirt	Cotton	Mid-20th century	4	Brown spots	–
	Embroidered tablecloth	Flax (tablecloth), Cotton (embroidery)	Mid-20th century	5	Yellow spots	–
	Drawers (underwear)	Cotton	Unknown	2	Yellow spots	+
	National costume of Montenegro	Silk degummed	Unknown	2	White dots	+
	Leather belt with textile lining	leather[a]	20th century	2	Visible mycelium	+
		Cotton		2	Greyish veil	–
	Jacket of a uniform	Wool	20th century	2	White spots	+
	Military coat	Wool	20th century	2	Grey spots	+
Slovene Ethnographic Museum	Embroidery	Silk (?)[a]	End of 19th or beginning of 20th century	2	White powder	–
	Embroidery	Silk (?)[a]	End of 19th or beginning of 20th century	2	White powder	
	Embroidery	Silk (?)[a]	End of 19th or beginning of 20th century	1	White spots	+
	Embroidered napkin	Cotton	1906	1	Grey spots	+
	Clogs	leather	Mid-20th century	1	Visible mycelium	+
	Female handbag (leather and textile)	Cotton	1950–1960	1	White spots	–
Succursal Church of St. Radegunda	Painting on canvas (original canvas)	Flax	Mid-20th century	3	Visible mycelium	+
Provincial Museum of Murska Sobota	Painting on canvas (original canvas)	Flax	End of 17th century	1	Grey veil	–
Provost Church, Novo Mesto	Painting on canvas (lining)	Cotton (warp), Flax (weft)	Unknown	1	White stains	+
	Painting on canvas (original canvas)	Flax	1582–1584	2	White veil	–
Ursuline Convent	Painting on canvas (original canvas)	Flax (warp), Hemp (weft)	End of 17th century	1	White downy stains	–
	Painting on canvas (original canvas)	Flax	1st decade of 18th century	1	White powder	–
	Painting on canvas (original canvas)	Hemp (warp), Flax (weft)	Unknown	1	Grey veil	–
	Painting on canvas (lining)	Flax	Unknown	1	Grey veil	+
	Painting on canvas (lining)	Flax	Unknown	1	Grey stains	–
Franciscan Monastery	Painting on canvas (original canvas)	Flax	1729	1	Grey veil	+
Cathedral of St. Nicolas	Painting on canvas (original canvas)	Flax	17th century	1	Greyish powder	+
Parish Church of St. Jacob	Painting on canvas (original canvas)	Flax	1821	1	White veil	+
	Painting on canvas (lining)	Flax	Unknown	1	White veil	+
	Painting on canvas (lining)	Flax	Unknown	1	Grey stains	–

[a] Object allowed no sampling, as no loose fibres or threads were present and sampling would cause visible damage to it.

(Biolife, Milan, Italy), the beta-glucosidase activities with aesculin (Sigma Chemical Co., St. Louis, MO, USA), the protease activities with casein (Sigma–Aldrich Chemie, Steinheim, Germany), the amylase activities with soluble starch (Merck, Darmstadt, Germany), and the urease activities with urea (Merck, Darmstadt, Germany). Depending on the enzymatic activity, the readings were evaluated as: +, weak activity; ++, moderate activity; and +++, strong activity. The proteolytic and lipolytic activities were tested using protease fluorescent detection kits (Sigma Aldrich, PF0100) and Quanticrom™ lipase assay kits, respectively.

Results and discussion

Controlled storage conditions limit fungal growth

Fungi are known for their wide degradation abilities. They can hydrolyse a great variety of natural materials, as well as many resilient man-made compounds (Gu, 2003; Cappitelli et al., 2005). Although fungal growth and the consequential degradation of materials are most enhanced under conditions of high humidity and room temperature, some xerophilic fungi can also degrade

Table 3
Objects sampled and fungi identified following the sampling.

Object	Identified fungal species	EXF[a]-	Gene bank accession number (ITS/LSU/BTUB/ACT)
Flax			
Linen; painting on canvas (?)	*Penicillium crustosum*	5888	–
Linen; painting on canvas (18th century)	*Alternaria* sp.	5881	KJ468770/–/–/–
	Penicillium corylophilum	5897	–/–/KJ468774/–
Linen; archaeological textile, supp. Roman hair cover (1st to 2nd century AD)	*Aspergillus clavatus*	5900	–/–/KJ468760/–
	Penicillium chrysogenum	5901	–/–/KJ468776/–
Linen; painting on canvas (original canvas) (mid-20th century)	*Penicillium corylophilum*	5896	–/–/KJ468775/–
	Penicillium bialowiezense	5894	–/–/KJ468783/–
	Aspergillus proliferans	5885	–/–/KJ468765/–
	Cladosporium cf. *tenuissimum*	5887	–
	Penicillium crustosum	5891	–/–/KJ468784/–
Linen; painting on canvas (lining) (unknown)	*Penicillium chrysogenum*	5912	–/–/KJ468777/–
Linen; painting on canvas (original canvas) (1729)	*Penicillium* cf. *corylophilum*	5910	–
Linen; painting on canvas (original canvas) (17th century)	*Chaetomium globosum*	5911	–/–/KJ468771/–
Linen; painting on canvas (lining) (unknown)	*Penicillium chrysogenum*	5913	–/–/KJ468778/–
Cotton			
Wooden statue in textile dress (1850)	*Penicillium chrysogenum*	5890	–/–/KJ468779/–
Paper patch on the back of the canvas (unknown)	*Cladosporium* cf. *tenuissimum*	5883	–/–/–/KJ468767
Drawers, underwear (mid-20th century)	*Aspergillus clavatus*	5892	–/–/KJ468761/–
		5895	–/–/KJ468762/–
Embroidered napkin (1906)	*Aspergillus* sp.	5893	–
Silk			
National costume of Montenegro (unknown)	Yeast	–	–
Embroidery (end of 19th to beginning of 20th century)	*Aspergillus versicolor*	5902	–
Embroidery (end of 19th to beginning of 20th century)	*Fomes fomentarius*	5903	–/KJ468773/–/–
	Penicillium chrysogenum	5904	–/–/KJ468780/–
Leather			
Leather; prayer book bound in leather, with brass relief (1856)	*Cladosporium* cf. *tenuissimum*	5886	–/–/–/KJ468768
	Penicillium chrysogenum	5889	–/–/KJ468781/–
	Hypoxylon fragiforme	5882	KJ468769/–/–/–
Leather; clogs (mid-20th century)	*Eutypa consobrina*	5905	–/KJ468772/–/–
Wool			
Jacket of a uniform (20th century)	*Cladosporium* cf. *tenuissimum*	5884	KJ468766/–/–/–
Hemp			
Painting on canvas (mid-20th century)	*Cladosporium* cf. *tenuissimum*	–	–
Mixed textiles			
Cotton and linen; painting on canvas (lining) (1582–1584)	*Penicillium chrysogenum*	5906	–
	Aspergillus jensenii	5907	–/–/KJ468764/–
	Aspergillus clavatus	5908	–/–/KJ468763/–
	Penicillium chrysogenum	5909	–/–/KJ468782/–
Painting on canvas (lining) (unknown)	*Penicillium crustosum*	5898	–

[a] EXF – designation of fungal strains in Ex Culture Collection of the Department of Biology, Biotechnical Faculty, University of Ljubljana (Infrastructural Centre Mycosmo, MRIC UL, Slovenia).

substrates at low water activity and optimal temperatures, while others can grow at unfavourable temperatures under optimal humidity (Gu et al., 1998; Strzelczyk, 2004). In nature, these fungal processes facilitate the turnover of high-molecular-weight compounds, and they can cause unwanted damage to stored objects. This study focused on visibly changed cultural historic objects, which were assumed to be affected by fungi.

Fungal damage of textiles can result in discolouration, staining, and/or a "mouldy" smell due to the production of volatile compounds, and the enzymatic and mechanical degradation of the materials through the active growth of the fungi (Tiano, 2002). These were therefore the selection criteria for the 38 objects under study here, as described in Table 2. The selected historical textile objects differed in age and materials, and were kept under different storage conditions. In total, 60 samples were taken from suspected objects. On objects with several stains that resembled fungal growth, multiple samples were taken from a single object. For 22 of the objects (58%) and 28 of the samples (47%), infestation by fungi was confirmed, while in the remaining 16 objects (42%) and 32 samples (53%) the stains were of different origins. The negative samples are shown in Table 4, and the positive samples in Table 3.

To diminish the damage caused by environmental impact, museums need to control the conditions that can lead to the destruction of materials and objects and to provide stable environmental parameters. As moisture is the primary environmental condition that facilitates fungal growth, control of the relative humidity is very important if destruction of materials and objects is to be prevented (Vukojević and Ljaljević-Grbić, 2005; Sterflinger, 2010). Sterflinger and Piñar (2013) have also stated the importance of a simple cleaning, since dust layers on objects carry high numbers of fungal spores and bacteria, and also serve as a nutrient source for those organisms. However, surprisingly, in the present study, regardless of the storage conditions, approximately half of the objects stored in different institutions were contaminated. Indeed, as only a few of the institutions investigated actively controlled the atmospheric conditions (Table 1), the fungal contamination rate cannot simply be correlated with inappropriate environmental control.

Surprisingly, even with the least appropriate environmental conditions, in our study represented by the Museum of Christianity, where some storage rooms were even open to the outdoors and thus exposed to drastic environmental changes, only half of the investigated objects were showing biodeterioration. Approximately two thirds of the objects were affected in the provincial museum, where the humidity was high, and in one national museum, which had previously had inappropriate storage

Table 4
Visibly damaged objects that were not infected by fungi, according to museum origin, age, and material.

Location	Object	Dating	Substrate
Museum of Christianity in Slovenia	Cloth, partly embroidered with metal threads	Unknown	Cotton
	Textile pad in the museum showcase, in contact with infected objects	Contemporary	Silk and cotton
	Velvet chasuble, embroidered with metal threads	Unknown	Silk
	Travelling suitcase, the interior protected with canvas	Mid-20th century	Cotton
National Museum of Slovenia	Archaeological textile, supposing Roman hair cover	Roman period	Flax
Provincial Museum of Ptuj	Underskirt	Mid-20th century	Cotton
	Embroidered tablecloth	Mid-20th century	Flax (tablecloth), Cotton (embroidery)
	Textile lining of leather belt	20th century	Cotton
Slovene Ethnographic Museum	Embroidery	End of 19th or beginning of 20th century	Silk (?)*
	Embroidery	End of 19th or beginning of 20th century	Silk (?)*
	Female handbag (leather and textile)	1950–1960	Cotton
Provincial Museum of Murska Sobota	Painting on canvas (original canvas)	End of 17th century	Flax
Provost Church, Novo Mesto	Painting on canvas (original canvas)	1582–1584	Flax
Ursuline Convent	Painting on canvas (original canvas)	End of 17th century	Flax (warp), HA (weft)
	Painting on canvas (original canvas)	1st decade of 18th century	Flax
	Painting on canvas (original canvas)	Unknown	HA (warp), Flax (weft)
	Painting on canvas (lining)	Unknown	Flax
Parish Church of St. Jacob	Painting on canvas (lining)	Unknown	Flax

conditions. One reason for the high fungal infestation rate might also be the past removal of surface hyphae by mechanical brushing or by the use of a mixture of water and ethanol (1:1 ratio). After these treatments, some of the fungal propagules probably remained viable and were sampled in our study.

The situation differed in the Provincial Museum, where the conditions in storage rooms were not controlled, and thus the temperature and humidity changed with changing outdoor conditions. The storage room for the ethnological objects was drier and airy, whereas the storage rooms for the historical department, where uniforms and similar objects were kept, were more humid and changeable. Inappropriate conditions resulted in a relatively high proportion of affected objects (5 out of 7).

There was also a contaminated sample in the National Gallery, with controlled climatic conditions in the storage rooms (Table 2). A closer look at the photographs of the object at the time of acquisition (eight years ago) revealed recent fungal growth. It appears that the fungal growth in this period neither progressed (Fig. 1) nor spread to the neighbouring objects. This indicates that the controlled conditions in the storage rooms prevented fungal expansion and further contamination.

The two samples containing metal embroidery were not affected, probably due to the biocidal effects of metals (Tiano, 2002). Surprisingly, among the other objects, there was also no infestation remaining on padding cloth that had been laid for several years under infested books in a showcase in the religious museums. The reason for this might be the antimicrobial finishing of the contemporary textiles (Gu and Mitchell, 2001; Szostak-Kotowa, 2004; Tomšič et al., 2008).

Although our data indicate that the relative humidity and the temperature are crucial environmental conditions that can facilitate fungal growth, unfortunately this type of data cannot be evaluated statistically, as the objects were chosen due to their uncommon surface appearances and assumed contamination. However, our data confirm that the control of the environmental conditions can limit fungal growth, although not prevent it completely, as shown in the case of the object from the National Gallery. Under extremely dry conditions and at low temperatures, hyphae can eventually die out; however, most fungal spores remain viable due to their high resistance to dryness and low temperatures, and this can cause further contamination, even when some of the conditions are sub-optimal for fungal growth (Strzelczyk, 2004).

Influence of textile fibre composition on the degree and rate of fungal biodeterioration

The degree and rate of biodegradation of textile fibres is affected by their chemical and structural composition, such as the degree of polymerisation, fibre crystallinity, and orientation (Tiano, 2002). The biodegradation of fibres is also affected by the properties of fabrics (e.g., yarn and weave structure) and by the materials that are in contact with the textiles. Organic materials (e.g., animal glues) increase the susceptibility to microbial degradation, whereas metals (e.g., copper) and some dyes and mordants decrease it (Chen and Jakes, 2001; Tiano, 2002).

The results of the microscopic identification of the textile fibres are listed in Table 2. In the present study 27 samples (75% of all investigated) were of cellulose origin, five were silk, two were wool,

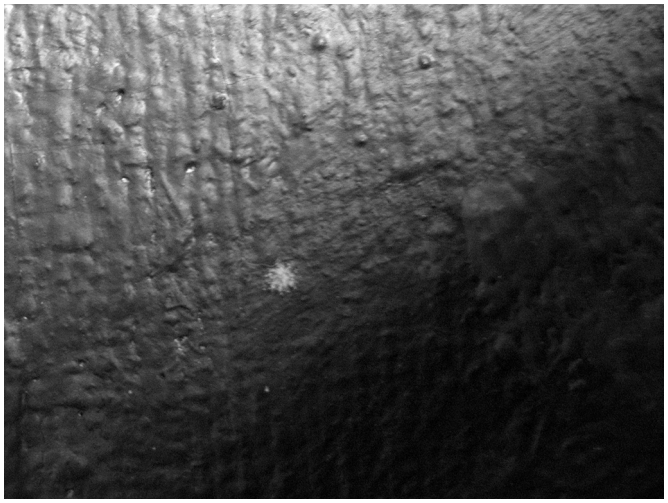

Fig. 1. Colony of a fungus on the surface of a painting from the National Gallery. The appearance of the fungal colony did not change during the eight years of storage.

and two were mixtures of proteinaceous and cellulosic materials. Most of the fibres investigated were not dyed. Many of the objects were composed of several different materials (e.g., paintings, lined paintings, embroidery). Among the organic materials, protein, acrylic, and calcium organic salts were identified, while among the inorganic materials, calcite, gypsum, and silicate fillers were confirmed. The various additional materials, like the animal glue in the paintings, might have contributed to the fungal growth and acted as additional nutrition sources.

In the present study 52% of cellulose samples were contaminated by fungi. In the cases in which no fungi were isolated, closer examination revealed colour impurities closely bound to cellulose fibres, which can be mistaken for fungal growth. Fungal contamination of the cellulose materials did not show any correlation with the age of the object or with the storage conditions.

Crystalline cellulose is least susceptible to attack by fungal cellulolytic enzymes (Chen and Jakes, 2001). Lignin and waxes in cellulose fibres decrease the susceptibility to fungal attack, whereas other carbohydrates, such as hemicelluloses, pectins, and pentosans, increase the susceptibility (Szostak-Kotowa, 2004). Cellulose is thus decomposed only after more accessible incrusting materials, such as pectin, hemicellulose, and waxes, are degraded, so the more easily accessible free sugars are used (Tomšič et al., 2007). The mechanism of fungal degradation of cellulose fibres is still under debate (Zotti et al., 2008). Most probably, after spore germination, fungal hyphae penetrate into the lumen of the fibres through cracks in the wall or at the cut ends, heading inwards, which results in the production of small fragmented fibres (Szostak-Kotowa, 2004).

Natural cotton contains less non-cellulosic substances (about 5%) than does flax (about 15%), so it is less susceptible to biodeterioration. In the present study, the rate of degradation of the flax was slightly higher in comparison with cotton objects. Fourier transform infrared spectroscopy revealed different structural changes in flax fibres, and fewer changes in cotton fibres (Kavkler, 2011).

According to the literature (Raschle, 1989; Cybulska et al., 2008) the fungi that most often degrade cellulose fibres belong to the genera *Aspergillus, Chaetomium*, *Fusarium*, *Memnionella, Myrothecium*, *Penicillium*, *Stachybotris*, and *Trichoderma*. Of these genera, the present study identified different species of *Aspergillus* and *Penicillium* (Table 3).

Proteinaceous objects showed a high degree of contamination, with both wool and half of the silk objects infested by fungi. Although protein fibres are less susceptible to fungal deterioration than are cellulosic fibres, a high degree of impurities (e.g., sericin in silk, and suint in wool) can increase their susceptibility to fungal attack (Tiano, 2002). Keratinolysis of wool depends on the chemical composition, molecular structure, degree of polymerisation, and, to a lesser extent, the histological structure of the keratin molecules. This starts by denaturation (fission) of disulphide bridges, which are the main reason for the natural resistance of keratin. This is achieved by extracellular microbial proteolytic enzyme activity, which results in damage of the cortical cells and decreased fibre strength (Szostak-Kotowa, 2004).

According to the literature, wool can be degraded by *Aspergillus*, *Chaetomium, Chrysosporium, Ctenomyces*, *Fusarium, Penicillium*, and *Rhizopus*, and by dermatophytic genera such as *Microsporum* and *Trichophyton.* Although *Aspergillus* and *Penicillium* can occur on wool (Sommro, 2000), they are not considered to be keratinophylic fungi. In the present study the *Cladosporium cladosporioides* species complex member has been isolated from wool. To the best of our knowledge, there have been no previous reports of the isolation of these species from wool or any other historical textiles.

Silk is the natural fibre that is most resistant to biodeterioration (Szostak-Kotowa, 2004). Sericin and fibroin from silk can be used as a source of carbon and nitrogen for microbial growth. Sericin, which protects the fibres against light damage, is more susceptible than fibroin. Removal of sericin from silk fibres increases their resistance; however, exposure of silk to increased heat and light renders fibroin more susceptible to colonisation by fungi (Seves et al., 1998). In the present study, three fungal species were isolated from silk embroidery samples: *Aspergillus protuberus*, *Fomes fomentarius*, and *Penicillium chrysogenum*, while in the literature to date, only *Aspergillus niger* has been indicated as a silk-degrading agent (Seves et al., 1998; Cybulska et al., 2008).

Detected species of "museum mycobiota"

Most of the isolated filamentous fungi were identified to the species level. They are presented in Table 3, according to the fibre composition of the contaminated material of the historical object. A few yeast isolates were not identified.

The species isolated most frequently was *P. chrysogenum*. This species was isolated from seven different objects made of both cellulosic (i.e., cotton, flax) and proteinaceous (i.e., leather, silk) materials; these were dated from Roman times to the 20th century, and were stored in three different museums (Museum of Christianity in Slovenia, National Museum of Slovenia, and Slovene Ethnographic Museum), and in a provost church and a convent.

The second most frequently found group of species was the *C. cladosporioides* species complex (*C. tenuissimum*, *C. cladosporioides*); these were isolated from five objects, again from both cellulosic (i.e., cotton, flax, hemp), and proteinaceous (i.e., leather, wool) materials. These objects dated from the 19th and 20th centuries and were stored in a religious museum, a state gallery, a provincial museum, and a church. Species of the *C. cladosporioides* species complex, *C. tenuissimum* and *C. cladosporioides* are ubiquitous melanized species. Although they are often isolated from plants, soil, air, textiles, and paint, they have only rarely been isolated from cultural heritage textile objects (Vukojević and Ljaljević-Grbić, 2005). They have generally low cellulolytic activity, and thus they cause textile degradation mainly due to their abundant excretion of succinic acid (Gutarowska and Czyżowska, 2009).

Aspergillus clavatus and *Penicillium corylophilum* both occurred on three different objects. *A. clavatus* was isolated from the linen and cotton objects in three different institutions (two museums and a church), whereas *P. corylophilum* was only on the linen objects, which were stored in a museum, a church, and a monastery. According to data in the literature, both of these fungi have intensive cellulolytic activity (Elnaggar et al., 2010), although this was not confirmed by enzymatic tests performed in this study (Table 5).

All of the other species were isolated only occasionally, from single objects. One of these was *Chaetomium globosum,* an important cellulolytic species frequently associated with paper spoilage (Zyska, 1997; Pinzari et al., 2006) and also known to damage cotton fibres (Pekhtasheva et al., 2012), and deteriorate archaeological textiles, mainly linen objects (Abdel-Kareem, 2010). This species is often found indoors, for instance on wall paper, but it is also present in soil and on plant materials (Samson et al., 2004). The genus *Alternaria* has been reported from Egyptian museum storage rooms (Abdel-Kareem, 2010).

All three of the most frequently encountered genera, i.e., *Aspergillus, Penicillium*, and *Cladosporium*, occurred in the museums as well as in the churches and convents. We expected the frequent isolation of *Aspergillus* and *Penicillium*, as these two genera are present in the air and are adaptable to different environmental conditions (Moraes, 2000). However, it should be noted that in the churches and cloisters, where there were generally lower average

Table 5
Enzymatic activities seen for the six selected fungi.

Enzyme	*Aspergillus clavatus EXF-5895*	*Penicillium chrysogenum EXF-5913*	*Penicillium corylophilum EXF-5897*	*Hypoxylon fragiforme EXF-5882*	*Fomes fomentarius EXF-5903*	*Cladosporium tenuissimum EXF-5883*
Alkaline phosphatase	1[a]	4	1	4	5	0.5
Esterase (C4)	3	1	1	1	1	1
Esterase, Lipase (C8)	2	0.5	1	1	0	1
Lipase (C14)	0	0	0.5	0	0	0
Leucine arylamidase	1	0.5	0	0	0	1
Valine arylamidase	1	0	0	0	0	0
Cysteine arylamidase	1	0	0	0	0	0.5
Trypsin	0.5	0	0	0	0	0
Alpha-chymotrypsin	1	0	0	0	0	0
Acid phosphatase	5	5	5	5	5	3
Napthol-AS-BI-phosphohydrolase	1	3	1	5	1	1
Alpha-galactosidase	0	2	0	2	2	0
Beta-galactosidase	0	5	0	0.5	2	1
Beta-glucoronidase	0	0	0	0	2	0
Alpha-glucosidase	0.5	0	0	0.5	0	0
Beta-glucosidase	0.5	0	0	3	1	0
N-acetyl-beta-glucosamidase	0.5	4	1	3.5	0.5	1
Alpha-mannosidase	0	0	0	0	0	0
Alpha-fucosidase	0.5	0	0	0	0	0
Amylase[b]	++	+++	−	+	++	++
Beta-glucosidase (aesculin)[b]	+++	+++	+++	++	+	+++
Cellulase[b]	+	+	+		+	+
Esterase[b]	+++	+++	+	+	−	+++
Protease (casein)[b]	+++	+	−	−	++	−
Protease (units)[c]	0.099	0.241	0.034	0.200	0.666	0.010
Urease[b]	−	+++	−		+	+++
Lipase[b]	−/+	−	−	+	−	−

[a] Activity evaluated as 0 (negative activity) to 5 (maximum activity) in API ZYM tests, or as + weak, ++ moderate, and +++ strong activity in other tests.
[b] Paterson and Bridge (1994).
[c] Protease Fluorescent Detection kits (Sigma Aldrich, PF0100).

temperatures, *Penicillium* species were the ones mainly present, whereas in the museums, all three of these genera occurred with similar frequencies. The same genera were also detected as being dominant in a study by Zyska on library textiles (Zyska, 1997). The genera *Penicillium* and *Aspergillus* are considered xerophilic, and thus they can degrade materials under conditions of very low relative humidity. Such conditions can cause additional problems for the museums, as at extremely low humidity, textiles become brittle and susceptible to mechanical influence (Montegut et al., 1991). During the degradation processes, xerophilic fungi can accumulate water, thus raising the moisture level of the invaded materials and facilitating the invasion of highly degrading, but less xerophilic, species (Szostak-Kotowa, 2004). The only species that were observed in the present study that have been seen in other studies were *Aspergillus sydowi* (Srivastava, 1980) and *P. chrysogenum* (Abdel-Kareem, 2010), both of which are classified as non-cellulolytic species. All of the other isolated *Penicillium* and *Aspergillus* species have not been reported previously as being in historical textiles.

Extracellular enzymatic activities of selected fungal strains

Six representatives of different species were selected for further analysis, to determine their extracellular enzymatic activities, using the API ZYM semi-quantitative micromethod and various enzyme-inducing solid media (Table 5). The selection criterion for four of these species (*A. clavatus*, *C. cladosporioides*, *P. chrysogenum*, and *P. corylophilum*) was based on their frequency of isolation, while the wood-degrading *H. fragiforme* was selected to analyse its influence on cellulosic textiles, and *F. fomentarius* was selected as the only representative of Basidiomycota.

A. clavatus, *C. cladosporioides*, *P. chrysogenum*, and *P. corylophilum* showed a broad range of enzymatic activities, with particularly strong beta-glucosidase activity, which is important for the decomposition of cellulose fibres, as well as strong phosphatase, esterase, and amylase activities. Although starches are not present in cellulosic or proteinaceous fibres, they can be added as filling or glues to textile materials, which will accelerate the degradation of the objects investigated. These species also showed different degrees of proteolytic activity, which is important for the degradation of silk and leather materials. Lipolytic activity is particularly important for the degradation of lipids present in leather (from the leather production processes), and this was detected to a lesser extent.

Conclusions

This extensive investigation of fungal contamination of historical textile objects stored in 12 institutions in Slovenia initially indicated relatively widespread fungal contamination. Closer investigation, however, reveals that only about one third of the objects were positive for fungal growth, whereas the others appeared to be damaged for other reasons. If the temperature and relative humidity are kept low, the growth of fungi can remain restricted, even if the contamination begins before the storage period. Although most fungal contaminations were detected in institutions that lack any control of the environmental conditions, the rates of degradation were generally relatively low. The two dominant fungal species detected were *P. chrysogenum*, followed by *C. cladosporioides* species group members. All of these species were isolated mainly from the linen objects. As the same fungal genera and species were identified across these very different environments, we can define these fungi as typical “museum mycoflora.” This identification and assessment of the extent of damage caused by these individual species should allow more rapid detection of the most frequent deteriorating fungal agents in similar museums in central Europe in the future.

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