Implementation of ATP Bioluminescence Method in the Study of the Fungal Deterioration of Textile Artefacts

Abstract
Two silk artefacts from museum collections in Serbia, a silk icon from Petar Lubarda Legacy and a scroll made with traditional Japanese techniques were analyzed for fungal presence. Fungi isolated from both the silk artefacts belonged to the genera Aspergillus, Chaetomium, Penicillium and Rhizopus. Actively growing colonies of Aspergillus and Penicillium species had caused chromatic alterations on the silk icon and their metabolic activity was demonstrated by high relative light unit values. In this research, measurement of the total microbial activity using the ATP bioluminescence method was used for the first time on silk artefacts and on textile in general. Scanning electron microscopy analysis of the scroll indicated that hyphae of Chaetomium globosum are capable of the mechanical deterioration of silk, as shown by the presence of cracks and gaps in silk fibres. This appears to be the first report of silk fibre degradation caused by C. globosum.

Key words: ATP, biodeterioration, silk fibres, Chaetomium globosum, microfungi, SEM.

Introduction
Nowadays the biodeterioration of textile artefacts in museum collections is a significant problem. Among all natural fibres, of both plant and animal origin, silk is the most resistant to the process of biodeterioration [1]. Silks are fibrous proteins spun into fibres by various insects and spiders [2]. The main producers of commercial silk are lepidoptera: domestic silkworm, Bombyx mori L. and other silkworms of the Bombycidae family [3]. Due to its remarkable properties and availability, the silk produced by silkworms has been used in textile manufacture in the Orient for at least 4000 years and in Western countries after its introduction to Europe around the 4th century AD [4]. Many historically important textile artefacts (tapestry, icons, garments, rugs…) are made of raw silk, which is composed of highly crystalline polypeptide fibres, fibroin, linked to one another by a gum-like protein, sericin [1]. Fibroin is composed mostly of amino-acids with short chain alanine, serine and glycine folded into antiparallel β-sheets as repeated motifs [4]. This amino acid composition results in a very stable β-pleated crystal structure, which essentially makes fibroin totally insoluble in aqueous solvents and very resistant to enzymatic hydrolysis [5]. Nowadays commercial silk production usually involves sericin removal by degumming [4]. Although fungi are the most active microorganisms in textile biodeterioration [6], they are not frequently associated with the deterioration of silk fibres. Micromycetes can induce damage if silk contains a high degree of sericin and is stored under warm and humid conditions [7]. As a result of biodeterioration, most silk textiles and artefacts lose their mechanical strength and become more or less brittle [8]. Hence it is very important to detect the presence of living microorganisms and identify causative agents of textile artefact biodeterioration. In this research we applied the modern ATP bioluminescence method along with traditional culture-based and microscopical analyses to deteriorated silk artefacts. The main goal was the implementation of the ATP bioluminescence method in the study of biodeteriorated textile artefacts and comparison of results obtained, with identified microfungi documented by traditional methods.

Experimental
Description of silk artefacts examined
Two silk artefacts under conservation were chosen for this study. Figure 1.a shows a rectangular silk icon (23.5 × 30 cm) embroidered with metal and silk thread on a linen “canvas” lined with silk. In the central medallion a monk (Benedictine order) is depicted reading a prayer. The icon was found in one of the rooms of the house of the eminent Serbian painter Petar Lubarda. The front and back of the object were covered with dust, cobwebs and rubble (remains of a fallen wall and ceilings). The back of the linen support was completely covered with glue, the thick layer of which acted as a consolidant. There was some smaller mechanical damage on the upper part of the icon, probably a result of rodent activity and some small holes from insects. The velvety surface of the silk was worn out and thinned, and on several spots the warp and weft were torn and missing. Chromatic alterations and visible mould growth are marked in Figure 1.a with a circle. Due to major damage it is difficult to determine the age of the icon without further laboratory analysis. Given the iconography of the medieval monk in prayer and age of materials and fibres used, it can be concluded that the object originates from the 19th century.

The appearance of the silk scroll examined is given in Figure 1.b. The scroll was a painting made using traditional Japanese techniques in the form of a scroll with a narrow wooden slat on its top and bottom. The dimensions of the scroll were 178 × 46.5 cm. There were 14 representations painted on the central (vertical) silk panel, with two frames of silk of different colours and designs. The object was made with a combination of textiles and paper, joined with glue. Due to moisture, in some parts the adhesion of the glue had been lost (especially on the front of the artefact), which had led to the detachment of the silk from the paper support and formed stains from capillary water rising. There were deformations and traces of folding, probably as a result of inadequate storing. Large discolored areas of brown spots are marked on the scroll with a circle in Figure 1.b. There is no information on the age of the scroll, but it is assumed to originate from the 19th century.

Abbreviations
ATP - adenosine-5’-triphosphate
CzA - Czapek-Dox agar
MEA - malt extract agar
PDA - potato dextrose agar
RLU - relative light units
In situ measurement of the total microbial activity using the ATP bioluminescence method

Living cells present on the sampled areas of the deteriorated silk artefacts were collected using a swab device - Lightning MVP (BioControl Systems). Sampling was carried out by dragging and rotating the ATP swab tip whilst applying firm pressure to break through any microbial growth present on the silk surface. Afterwards activation was carried out by dipping the swab into the kit provided, luciferin/luciferase reagent. Upon activation, the swabs were processed by a portable luminometer and, in only 10 seconds, results were expressed as ZONES OF CLEANLINESS - logarithmic approximated - the amount of light generated from one femtomole of ATP in the chemical reaction [9]:

\[
[\text{ATP} + (\text{Luciferin/Luciferase})] = \text{Light}
\]

The intensity of light emitted is proportional to the ATP concentration in the living cells sampled. Results obtained were then compared to the reference scale given in the manufacturer’s instructions and placed in one of three categories of microbial contamination:

- Clear zone < 2.5, by default,
- Caution zone 2.6 - 3.0, by default,
- Danger zone > 3.0, by default [10].

Isolation and identification of fungi

Sampling for mycological analysis was done using sterile cotton swabs. Samples were taken from those parts of the silk icon and the scroll which had visible alterations. The designated areas of silk to be examined were wiped with cotton swabs. To take samples from the dry silk surface, sterile cotton swabs were first dipped in sterile distilled water. In laboratory conditions the swab samples were diluted in 10 ml sterile distilled water and shaken vigorously for 10 min, after which 1 ml of the resulting suspensions was inoculated on an MEA medium with 500 mg of streptomycin per litre. The inoculated plates were incubated in a thermostat at 25 °C. After incubation for seven days, pure fungal cultures were obtained by single spore transfer from primary isolates onto nutrient media MEA, PDA and CzA. Re-isolated cultures were incubated 7 days at 25 °C. Isolated fungi were identified, based on the macroscopic features of colonies and the micro-morphology of reproductive structures observed by light microscopy using appropriate identification keys [11 - 14].

Microscopic analyses

To confirm the existence of mould growth and identify the type of fungi in the sampling areas, the non-aggressive adhesive tape method was used [15]. Samples were collected by pulling the adhesive tape off the surface with a slow and steady force. For light microscopy, samples were stained with Lactophenol Cotton Blue and put on slides to be analysed using a Zeiss AxioImager M.1 using software AxioVision Release 4.6.

To detect fungal growth and investigate damage in the surface morphology of the deteriorated silk fibres, SEM was performed at the University of Belgrade, Faculty of Mining and Geology, using a JEOL JSM-6610LV (JEOL USA, Lnc) with a W filament gun. Deteriorated silk samples were gold coated (d = 15 nm, \( \rho = 19.2 \text{ g/cm}^3 \)) using a Leica EM SCD005 (Leica Microsystems, USA) sputter coater. Secondary electron and backscattered electron images were obtained at a 20 kV acceleration voltage in the high-vacuum mode (15 - 30 \( \mu \text{Pa} \) in the sample chamber), with magnifications from 150× to 30,000×.

Results

Total microbial activity

The presence of living microbial cells (bacteria, yeasts, moulds) and organic residues on the silk artefacts were shown with ATP analyses. All three samples collected from the silk icon showed Zone levels above 3, which, according to the manufacturer’s guidelines, can be referred to as the “Danger zone”. The scroll showed a lower level of microbial contamination, which was indicated as a “Caution zone”. The results of ATP analyses are given in Table 1.

Identified Fungi

All fungi identified from both silk artefacts examined are presented in Table 1. A total of six fungal species were isolated and identified from the silk artefacts, with four species identified on the scroll, and three found on the silk icon. These fungi belonged to the Aspergillus, Chaeto-

![Figure 1. Silk icon with visible mould growth (a) and the scroll with brown discolouration areas (b). Symptoms are marked with a circle.](image)

Table 1. Total microbial activity and fungi identified from two silk artefacts; ** Danger zone, * Caution zone.

<table>
<thead>
<tr>
<th>Alterations</th>
<th>Zone</th>
<th>Identified fungi</th>
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<tbody>
<tr>
<td>Silk icon</td>
<td></td>
<td></td>
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<tr>
<td>Visible black mould growth</td>
<td>5.4**</td>
<td>Aspergillus niger Tiegh</td>
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<tr>
<td>Visible yellow-green mould growth</td>
<td>6.1**</td>
<td>Aspergillus flavus Link</td>
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<tr>
<td>Visible blue mould growth</td>
<td>5.5**</td>
<td>Penicillium Link sp.</td>
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<tr>
<td>Silk scroll</td>
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mium, Penicillium and Rhizopus genera. Aspergillus and Penicillium species were documented on both silk artefacts, while Chaetomium and Rhizopus were found only on the scroll.

**Light Microscopy and SEM observations**

Microscopic observation of silk samples taken with adhesive tape from the icon showed superficial growth of fungi belonging to the Aspergillus and Penicillium genera. From the areas with visible blue mould growth Penicillium sp. was identified. Branched conidiophores with abundant bluish conidia are shown in **Figure 2.b**. In addition, from the areas with visible yellow-green mould growth Aspergillus flavus was detected, while A. niger was identified from visible black mould growth. These species formed hyphal mats with a dense felt of erect conidiophores, and abundant conidia were observed during microscopic examination of altered areas of the silk icon. **Figure 2.a** shows the conidiogenous apparatus of A. niger with a mass of black conidia, while **Figure 2.c** shows a rough conidiophore with yellow-green conidia forming chains.

**Chaetomium globosum** perithecia and ascospores were abundant on brown spots within the areas of the silk scroll sampled. Perithecia and a mass of lemon-shaped ascospores of C. globosum were observed during microscopic analysis of adhesive tape samples from the scroll. **Figure 2.d** indicates that hyphae of C. globosum were intertwined with the silk fibres.

**Figure 2.** Light microscopy of adhesive tape samples. Fungi detected on the silk: Aspergillus niger (a), Penicillium sp. (b) and Aspergillus flavus (c). Chaetomium globosum hyphae intertwined with silk fibres on the Japanese scroll.

**Discussion**

Portable ATP bioluminescent-based tests are not intended to give precise quantitative determinations of the ATP level on substrata, but they are widely recognised methods for rapid hygiene testing. However, this method is seldom used on cultural heritage objects [16]. In the case presented here, the rapid ATP bio-
luminescence method (Lightning MVP, BioControl) was used for the first time on textile artefacts to determine the level of microbial contamination. Microorganisms can deteriorate silk, causing exfoliation and abrasion on its surface as well as chromatic variations and the loosening of the strength of warps and wefts [4]. There are many reports regarding the bacterial deterioration of silk, but the role of fungi in the process of silk biodeterioration has been neglected. Both silk artefacts examined in this research showed clear signs of biodeterioration, such as heavily damaged fibres and various types of discoloration. Chromatic alterations documented on the silk icon as black, yellow-green and blue discolorations represent the visible mould growth of Aspergillus niger, A. flavus and Penicillium sp., respectively. ATP analyses together with direct light microscopy observations showed that silk icons were heavily contaminated with moulds. The high Zone values are due to the metabolic activity of two Aspergillus species and Penicillium sp. Most likely, air-borne conidia present in the storage-room where the silk icon was deposited settled on the icon's surface. Due to environmental conditions such as nutrients, humidity, temperature and pH [17], conidia germinated and formed a mycelial network on the silk surface. Conidiogenous apparatus and mass production of conidia on the surface were documented on samples using the adhesive tape method. Heyes and Holden (1926) reported that species of the genus Aspergillus are able to grow on silk [18], while Sato (1976) reported that A. niger, via acidic proteases, and its cell-free extracts degrade fibroin, causing alterations in the fibre structure [19]. According to Seves et al. (1998) fungal colonisation of silk can only occur after it has been partially degraded by bacterial activity [4]. Additional nutrients for fungi on the silk surface could be organic material and particles deposited from the air. Also the glue used as a consolidant on the icon could be utilised by some fungi as a nutrient source. Brown discoloured areas observed on the scroll were due to Chaetomium globosum growth and perithecia formation. Although four fungal species were isolated from the scroll, using cultural analyses, the Zone values were significantly lower compared to those for the silk icon, suggesting that fungal propagules which settled on the scroll surface showed a lower metabolic activity. "Caution zone" was probably due to bacterial activity. This result is supported by the finding that C. globosum was documented only on adhesive tape samples, but was not growing on the culture media used. Dark perithecia and ascospores of C. globosum sampled with adhesive tape and documented with light microscopy and SEM were probably from old colonies with very low metabolic activity, suggesting that contamination had occurred long before sampling. C. globosum is a soft-rot fungus, thus more often associated with cellulose substrata, and is often isolated from cellulose fibres; but information regarding association Chaetomium species with protein fibres is very scarce [20]. As the scroll was made of a combination of textiles and paper, the presence of cellulose fibres on the scroll would have facilitated colonisation by C. globosum. Abdel-Kareem (2010) isolated C. globosum from protein fibres of various biodeteriorated textile artefacts stored and displayed in the Museum of Jordanian Heritage [6]. Seves et al. (1998) isolated C. globosum from Bombyx mori cocoons and demonstrated its proteolytic activity, but also its failure to degrade fibroin [4]. Silk deterioration documented via SEM is likely to be due to C. globosum hyphal penetration through the silk fibres. This finding shows that fungal hyphae are capable of deteriorating the mechanical strength of silk fibres, causing cracks and transversal gaps within the fibre. Our finding will be the first report of silk fibre deterioration caused by C. globosum. According to Seves et al. (1998) the biodeterioration of silk is due to bacterial activity only, and fungi can only subsequently colonise silk after partial degradation of fibroin [4]. Kuruppillai et al. (1989) suggested that fungi can utilise fibroin as a nutrient source after treatment with a xenon arc lamp, which decreases the tensile strength and results in an increase in the content of amino groups [21]. In this research, it was not possible by SEM analysis to determine whether the silk was already deteriorated by bacterial activity prior to C. globosum colonisation. Other fungal genera associated with the deterioration of silk artefacts and protein fibres are Alternaria [6], Fusarium [7], Penicillium [22] and Trichoderma [7].

Conclusions

In the research presented herein, the ATP bioluminescence method was successfully applied to deteriorated silk artefacts. We highly recommend the use of this bioluminescent method to assess the level of microbial contamination of textile artefacts together with traditional cultural-based microbiological methods for isolation of fungi and bacteria. Results of the ATP test can be very helpful for restorer and conservators to preserve deteriorated textile artefacts.

In the case presented herein, a highly-contaminated silk icon, but with only superficial mould growth, was subjected to conservation treatment and was successfully restored. On the other hand, the first time recorded devastation of the scroll by Chaetomium globosum was unrestorable due to irreversible, structural alterations of silk fibres.

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INSTITUTE OF BIOPOLYMERS AND CHEMICAL FIBRES
LABORATORY OF BIODEGRADATION

The Laboratory of Biodegradation operates within the structure of the Institute of Biopolymers and Chemical Fibres. It is a modern laboratory with a certificate of accreditation according to Standard PN-EN/ISO/IEC-17025: 2005 (a quality system) bestowed by the Polish Accreditation Centre (PCA). The laboratory works at a global level and can cooperate with many institutions that produce, process and investigate polymeric materials. Thanks to its modern equipment, the Laboratory of Biodegradation can maintain cooperation with Polish and foreign research centers as well as manufacturers and be helpful in assessing the biodegradability of polymeric materials and textiles.

The Laboratory of Biodegradation assesses the susceptibility of polymeric and textile materials to biological degradation caused by microorganisms occurring in the natural environment (soil, compost and water medium). The testing of biodegradation is carried out in oxygen using innovative methods like respirometric testing with the continuous reading of the CO₂ delivered. The laboratory’s modern MICRO-OXYMAX RESPIROMETER is used for carrying out tests in accordance with International Standards.

The methodology of biodegradability testing has been prepared on the basis of the following standards:

- **testing in aqueous medium**: "Determination of the ultimate aerobic biodegradability of plastic materials and textiles in an aqueous medium. A method of analyzing the carbon dioxide evolved" (PN-EN ISO 14 852: 2007, and PN-EN ISO 8192: 2007)


The following methods are applied in the assessment of biodegradation: gel chromatography (GPC), infrared spectroscopy (IR), thermogravimetric analysis (TGA) and scanning electron microscopy (SEM).