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Gymnoascus arxii's potential in deteriorating woollen textiles dyed with natural and synthetic dyes

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ABSTRACT

So far, very little is known about microbiological deterioration of dyed woollen textiles. In this paper, the influence of the *Gymnoascus arxii* fungus on woollen textiles dyed with natural and synthetic dyes was studied. What is more, it was analysed whether the enrichment of the culture medium with additional nutrients has any impact on the deterioration of dyed woollen fabrics caused by a strongly keratinolytic strain. The study was carried out by means of a pure culture method over three different time periods, i.e. 1, 2 and 4 weeks. Within a week, the pure *Gymnoascus arxii* strain led to a severe deterioration in the mechanical strength of the examined woollen textiles, with the raw fabric being the most severely damaged. After the two-week incubation period, only the fabrics coloured in yellow, i.e. the fabric dyed with natural dye weld, and the synthetic yellow textile as well as the textile dyed with natural dye indigo survived, exclusively on the enriched medium. Solely the weld dyed textile withstood the four-week culture on the nutrient-enriched medium. The conducted studies demonstrated a strong influence of *Gymnoascus arxii* on dyed fabrics leading to their irreversible destruction. It has been also shown that the presence of nutrients in the substrate that are readily available to microorganism may hinder the development of the *Gymnoascus arxii* strain and thus, prevent textile deterioration.

1. Introduction

There are few scientific institutions in the world that deal with the problem of microbiological deterioration of woollen textiles. Among natural fabrics, woollen fabrics happen to be the least investigated. Biological deterioration of these textiles is an important problem in conservation of works of art since museums contain rich collections of woollen textile products such as: tapestries, kilims, carpets, embroideries and woollen applications, banners, flags, uniforms, ethnographic costumes etc. Woollen fabrics are also found in archaeological excavations.

From among many factors that affect the rate and extent of microbiological destruction of woollen textile products, the presence of dyestuffs and various contaminants in the material, which provide nutrients readily available to microorganisms, is of the greatest importance. The research on the effect of pure microbe strains on the deterioration of dyed textiles is scarce in the literature. Also, the question of how the susceptibility to biodegradation of woollen fabrics changes depending on the group of used dyes, remains unanswered. It should be emphasised that this issue is of great significance, particularly in the conservation of antique textiles. In addition, woollen fabrics belong to the easiest to dye, and thus the knowledge of the relationship between the type of dyestuff used and the degree of dyed fabric deterioration is of the utmost importance, also for modern textiles. Similarly, it is essential to understand the effect which the readily available nutrients exert on the process of dyed textile decay. The fungal growth often begins in a readily available nutrition source that may constitute organic compounds in dust, such as textile filament fragments, cuticle cell debris, starch grains or inorganic compounds of various types. These substances together with absorbed water may create a favourable microenvironment on the surface of the material. The started growth may expand into the textile fibres from which microorganisms draw nutrients in order to continue their life cycles and thus, the product undergoes deterioration. Therefore, the presence of additional food for microorganisms in the form of various contaminants that are often present on fabrics may have a considerable effect on the process of microbiological textile destruction (Błyskal, 2005, 2012).

The main aims of this paper were: to assess the effect of *Gymnoascus arxii* on the extent of biodeterioration of woollen textiles dyed with natural and synthetic dyes and to verify whether the

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enrichment of culture medium with additional nutrients has an effect on woollen fabric destruction caused by a strongly keratinolytic strain. The impact of the process of textile dyeing and fabric disinfection on its strength parameters was also analysed.

2. Material and methods

2.1. Fungus

Gymnoascus arxii Cano & Guarro (strain number in author's own collection BBW 100 kept in Microbank™, strain number in CBS collection: CBS 116064) was isolated by the author from a contemporary woollen textile. This fabric did not demonstrate any signs of biodeterioration, but when placed (after prior washing and ironing) on a culture medium favourable for growth, it proved to be a source of many microorganisms (Fig. 1). The fungus was identified by means of classical and molecular techniques. Due to the time required for this species to achieve the maturity and in order to trace the changes occurring with culture ageing, the study of morphogenesis and other structural features important to the identification process was started on day 3, and continued for the next 6 weeks of culture. Information contained in the references was used in this study (Van Oorschot, 1980; Cano and Guarro, 1989; Kane et al., 1997; Guarro et al., 2002). Molecular identification methods were also applied. This enabled to recognise the sequences of selected ribosomal DNA (rDNA) fragments of the microorganism, i.e. ITS1 and ITS2. This part of the research was carried out at Centraalbureau voor Schimmelcultures (CBS), Fungal Biodiversity Center, Institute of the Royal Netherlands Academy of Arts and Sciences in Utrecht and was performed in accordance with appropriate protocols (Möller et al., 1992; Sambrook and Russell, 2001). A detailed comparative analysis of the obtained fungal rDNA sequences with the homologous ones available in the NCBI and with the database of fungal rDNA sequences available in the CBS was performed with the use of the BioNumerics program.

The strain isolated and identified by the author was included in the CBS collection where it is the only available representative of such species (CBS ID: 116064). Additional information on the *Gymnoascus arxii* fungus may be found in Błyskal (2005, 2009).

The inoculum was prepared from the 14-day *Gymnoascus arxii* culture grown on PDA medium. The culture was washed with sterile 0.9% NaCl solution and picked up from the substrate surface with an inoculating needle. The suspension was shaken for 20 min and subsequently, filtered through a sterile gauze filter. The density of the spore suspension per 10^6 CFU ml^{-1} was determined. Such premade inoculum was used to inoculate textile samples.

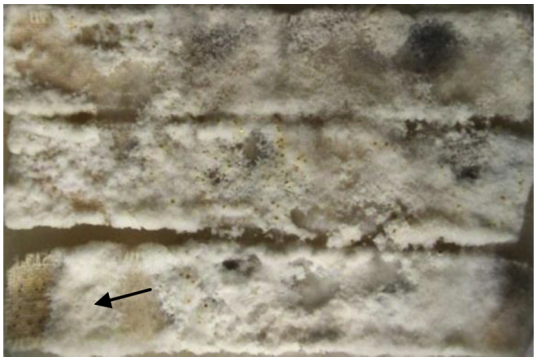

Fig. 1. The presence of microorganisms in the raw textile revealed after placing it on a mineral medium. An arrow indicates the growth of *Gymnoascus arxii*.

2.2. Textile

The textile used was made of 100% Australian merino wool, plain weave, of weight per unit area of 162 g/m^2 and average fibre diameter of 19–20 μm, chemically untreated. The raw fabric was dyed with natural dyestuffs and corresponding in terms of colour synthetic dyes that are currently used for wool dyeing. The selection criteria for natural dyes were as follows: their prevalence over the centuries in dyeing woollen fabrics (Böhmer, 2002; Hofenk de Graaff, 2004; Balfour-Paul, 2006; Cardon, 2007) and the frequency of occurrence of microbiological damage to antique woollen products (staining, fading, thinning) in the sites coloured in navy blue, yellow, crimson and coral (author's personal observation).

Dyeing with natural dyes was carried out based on guidelines found in the literature (Adrosko, 1971; Robertson, 1973; Weigle, 1974; Dalby, 1985; Liles, 1990; Cannon and Cannon, 1994; Alatrache and Ayed, 2001; Ayed and Alatrache, 2001; Böhmer, 2002) and information obtained during personal conversations with dyers. Dyeing with synthetic dyes was performed in accordance with recipes created by the computer program based on spectrophotometric measurements of fabrics dyed with natural dyestuffs. The detailed information on the natural and synthetic dyes used in this study may be found in Tables 1 and 2, respectively. The composition of mordant and dye baths is provided in Table 3. The fabric mordanting and dyeing process was carried out by means of the Benz laboratory jigger dyeing machine. Textile dyeing with natural indigo dye was performed in a dyeing vat.

Prior to microbiological examination, the textile specimens, cut warp-wise into strips of 15 × 120 mm, were disinfected in 70% ethyl alcohol for 4 h. Such a procedure is a necessary requirement to conduct studies using the pure culture method. The optimal disinfection method was selected from among several methods verified in a separate experiment on raw woollen textile (data not shown).

2.3. The pure culture method

The microbiological testing was conducted by means of the pure culture method. Two kinds of microbiological media were used, one of which was a mineral medium (Weary and Canby, WiC-), i.e. containing only essential salts without any source of carbon and nitrogen. Thus, to start its growth, *Gymnoascus arxii* had to derive these missing elements from the textiles (raw or dyed) which in this research variant constituted their only source accessible to the microorganism. The mineral medium contained (g l^{-1}): K_2HPO_4, 1.5; $MgSO_4 \times 7H_20$, 0.025; $FeSO_4 \times 7H_20$, 0.015; $ZnSO_4 \times 7H_20$, 0.005; $CaCl_2$, 0.025; chloramphenicol, 0.07; agar, 15. The pH was adjusted to 7.5 (Safranek and Goos, 1982; modified).

The enriched medium (Weary and Canby, WiC+) was used as the second variant of the studies on microbiological decay of dyed woollen textiles to simulate the widespread phenomenon of fabrics contamination with various compounds. The WiC+ medium was supplemented with a source of carbon and nitrogen, i.e. glucose, 2 g l^{-1} and yeast extract, 3 g l^{-1}. This substrate is the original author's modification of the WiC- medium developed on the basis of fungal physiology and customized to its specific application. It was selected from among several analysed combinations which had been examined in a separate experiment (Błyskal, 2012).

The textile samples prepared as described above were placed in Petri dishes that contained media (WiC- and WiC+) and were inoculated with the *Gymnoascus arxii* spore suspension (0.2 ml) which was evenly spread over the surface with a glass spreader. The dishes containing fabric samples were sealed with a Sigma-*PARAFILM*® M and placed in the incubator at a temperature of 28 ± 2 °C and humidity of 65 ± 2%. The culturing lasted for 1, 2 and 4 weeks.