Overview of fungal isolates on heritage collections of photographic materials and their biological potency

Janez Kosel[a]* and Polonca Ropret[a]

[a]Institute for the Protection of Cultural Heritage of Slovenia, Restoration Center, Poljanska 40, 1000

*Corresponding author: janez.kosel@zvkds.si

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Abstract

Despite controlled relative humidity in archives and private collections, fungi are a widespread cause of biodeterioration of cinematographic films and historic photographs, which represent a significant cultural and historic loss to society. Photographic emulsions and coatings are organic and hygroscopic in nature and represent a good and easily accessible source of nutrients. Because archives hold whole stacks of these materials, they subsequently contain more fungi in comparison to other enclosed spaces**.** This in turn generates a need for a systematic microbiological evaluation of fungi isolated from photographic documents in order to pinpoint the potentially biodeteriorative fungal species and increase awareness and control readiness when these species are encountered. With this aim, we have decided to collect data regarding fungal isolates and their biological potency from the following originating materials: gelatin cellulose triacetate or cellulose nitrate films, albumen or gelatin paper photographs, cellulose nitrate negative films, gelatin glass plate negatives and positive paper prints. In addition, the most efficient biotic degraders of gelatin binder are presented and the degradation of cellulose based supports as well as the occurring microbial interactions and the impact of inhibitory silver salts are reviewed. Lastly, based on the origins of fungal contamination and the occurrence of fungi related to allergenic and toxicogenic diseases, prevention and control measures are suggested.

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1. Introduction

Photographs and films, popularly referred to as "the seventh art", represent an ethnographic, social and artistic [1] narrative of life events [2] as part of humanity's historic and cultural heritage and, as such, need to be conserved for future generations. In the digital era, taking a picture is trivial with a modern digital camera, but until the last decade of the 20th century, it required considerable skill, and photographs had to be developed using a special light-sensitive emulsion [3].

Moreover, photographs and celluloidic material, which is used to make cinematographic films, are very sensitive and are susceptible to various factors, in particular to temperature, moisture, chemical compounds and biological contaminants [4]. Their deterioration is common and represents a significant economic and cultural loss [5]. Chemical compounds (sulphur dioxide, nitric acid, nitrogen peroxide, ozone, formaldehyde and formic acid) can originate from atmospheric pollution, the building and furniture materials and the collections themselves. In general, chemical aspects of film degradation had been thoroughly described [6,7]. However, microbiological aspects of mould growth are considerably less studied [4].

Biodeterioration can be caused by fungi (lichenized fungi or lichens), bacteria and microalgae [8]. There are different ways in which microorganisms can compromise the structure and function of polymeric materials. These can be classified as mechanical, chemical and aesthetical [9]. For instance, they can produce pigmentation or degrade one or more compounds with extracellular enzymes and secreted organic acids [10]. In some cases, they can hydrate (cause corrosion of materials) and cause fouling; or they may even penetrate into the polymeric material [11]. Chromatic alterations involve the formation of different colours, tonalities, and textures [12]. Furthermore, microbial growth may obscure the image, and irreversible damage to the binder affects the image quality and consequently devalue the heritage status of the photographic content [2]. Fungi are a particular problem, because they can tolerate lower relative humidity (RH) and a wide range of temperatures, can easily be transported by air, and because of their versatile extracellular enzymatic machinery can utilise any available organic material.

2. Research aims

Our aim was to review fungi isolated from historic photographical material and pinpoint their biological biodeteriorative potency for binder and support degradation. We also focused on the occurring microbial interactions and their impact on biodeterioration and on the effect of silver

salts and pigments used for black and white and color films. The origins of fungal contamination and as well as the reasons for their development are discussed. The readers are warned of the potential human allergenic and toxicogenic diseases that some of these isolates can inflict in immunocompromised personnel. Finally, prevention and control measures for museums and film archives are suggested, which help to prevent contamination, biodeterioration and fungi-related diseases.

3. Composition of photographic materials

Cinematographic film is composed of three generic components: a flexible plastic support, an image-forming material and a gelatin binder. Throughout history, several supports have been used, including cellulose nitrate (CN) (from 1889 to 1950), cellulose triacetate (CTA) (from 1948 to 2000), and polyethylene terephthalate (from the 1990s to the present). The first CN supports were chemically instable, in addition to being highly flammable. Since 1948 and until 2000, an enormous amount of cinematographic film based on CTA material has been stored in archives [13] and these proved to be non-flammable and met all the technical and safety requirements [2]. To reduce rigidness, they are treated with various plasticizers, such as triphenylphosphate [14,15]. In 1970, a serious decomposition of CTA films was discovered, with the loss of many film images as well as entire films. The release of acetic acid caused the so called "vinegar syndrome" resulting in the fading of colour and in the deformation and stiffening of the material [16]. The manufacture of CTA support involves the acetylation of the hydroxyl groups on the poly(anhydroglucose) structural repeat unit of cellulose. The degree of substitution (DS) of cellulose acetate, i.e., the average number of acetyl groups per anhydroglucose unit, ranges from 0 in the case of cellulose to 3 in the case of CTA (DS of films is around 2.7) [17]. Recently, CTA supports and plasticizers have been replaced by polyethylene terephthalate supports, due to their exceptional physical properties [17].

Photographs are composed of at least three components: a rigid metal-free paper support, an image-forming material and a binder that in 20th century photographs is mainly based on gelatin, whereas in the late 19^{th} and early 20^{th} cent. albumen prints were also used [1]. A layer of barium sulphate (baryta paper, $BaSO_4$) is placed between the gelatin and paper layers in order to increase the light reflection coefficient [18,19]. After the image is taken, image processing proceeds in the following order: printing in the darkroom; chemical development of the latent image using solutions that reduce silver halides in the presence of free silver atoms; immersion in the stop bath (undeveloped silver salts must be removed by fixing in ammonium thiosulphate); and thorough rinsing in water to remove the fixer [19]. Albumen photographs were invented around 1850 by a French photographer, Louis Désiré Blanquart-Evrard. The albumen, derived from the clear white of fresh eggs, was mixed with NaCl or NH_4Cl [3]

Film or image support is coated with one or more successive layers of light sensitive photographic emulsion. These photosensitive emulsions are mostly made up of a gelatine binder and, in the case of black and white (B&W) materials, silver halide salts (chlorides or bromides) [20]. The process that leads to image formation is based on the light induced selective trapping of metallic silver particles (average size of ca. 0.5 nm) in gelatin emulsion [19,21]. When small crystals/grains of silver salts (silver chloride) are exposed to light, a reduction of silver takes place, which frees/detaches a certain amount of atoms from the free metallic silver grains. These free silver atoms form the latent image by darkening the film [1]. On colour grade material, the photosensitive layer is more complicated and is composed of green, red and blue sensitive emulsion layers separated by clear gelatine interlayers [22]. Within this layer structure, antihalation layers coat the support in order to avoid reduction in the sharpness of the image and light scattering, called halation, around images of bright objects. For this purpose, in B&W negative films, a neutral density grey dye is added to a layer of gelatin [23].

For films, mostly type-B gelatin is used which is produced from alkaline-pretreated bovine bones (liming process) [1,24]. Gelatin, as a hydrocolloid and polyelectrolyte, plays a role in controlling the growth of silver halide crystals and in preventing coagulation [1,19]. It contains substances which influence the photographic properties of the light-sensitive material and stabilises hydrophobic additives in the formulation [25]. Many efforts have been made to replace gelatine with synthetic polymers but have failed to produce a fully satisfactory material [26].

4. Analysis of biodeteriorated photographic material

4.1 Microscopic analysis

For cinematographic films Vivar et al. [4] noticed that microbial growth can be observed mostly along the edge of the cinematographic film and in the vicinity of the film transport, rather than in the centre of the film, possibly because contamination is easier and a greater amount of humidity is absorbed. However, clear infestation was also detected on individual frames between loose coils at the beginning of rolls of film and also on the margins in the appressed coils [27]. Epiflourescent microscopy performed by the same team [8] showed that most of the fungi colonizing the cinematographic films were strongly adhered and were still active. This finding exposed biodeterioration as an agent of inevitable gradual degeneration. Additionally, SEM analysis revealed that fungi contribute to the fragility and breakage of affected film support, since in some films the fungal hyphae penetrated the support, causing mechanical damage. Bučková et al. [28] also reported “deep” tracks and tunnelling on the surface of the photographic material, and in some places producing holes, presumably to get access to the inner parts of the substrate. They suggested that the tracks were excavated by eso-enzymes secreted by fungal hyphae, but other authors claim that organic acids, such as oxalic, fumaric, citric, itaconic, succinic, lactic or acetic acid, are responsible [29–35].

4.2 Infra-red spectrum analysis

FTIR spectroscopy analysis studies indicate that the relative intensity of the hydroxyl band compared to that of the carbonyl stretching vibration correlated with the DS value of the CTA material, which in turn related to the number of acetate groups [36]. When CTA deteriorates, a decrease in the bands associated with the loss of acetate groups and a corresponding increase and shift in the broad OH stretching vibration at high frequency, can be observed [37,38]. Interestingly, the changes in relative intensities corresponding to OH/C=O can be interpreted as being a rough indication of the age of the films (5-10 years older films increase the ratio by ~1) [4].

According to Oberle-Kilic et al. [39–41] bands in the range of 1736– 960 cm^{-1}; most probably arise from microbiological contaminations and Zotti et al. [42] showed that the presence of active fungi on paper can be easily detected on the basis of the amide I and amide II bands around 1635 and 1540 cm^{-1} and a plateau between 1500 and 1200 cm^{-1}. Interestingly, Vivar et al. [4] observed a broad band centered at around 1040 cm^{-1} which appeared only in surface zones that were visibly contaminated with fungi, which, as reported by other authors, can be ascribed to polysaccharides in the cell walls of fungi [43–45]. Nevertheless, Puškárová et al. [3] reported difficulties in fungi detection on albumen photographs, because the albumen spectrum was very similar to that of microfungi [46].

5. Source of contamination

Given favourable conditions for growth, fungi can colonise almost everything, and their spores can remain viable for hundreds of years [47]. The environments of film archives, which contain stacks of highly susceptible photographic emulsion material (more hygroscopic than paper) [28], consequently contain more fungi than other enclosed spaces. The main limiting factor that determines the development of fungi on photographic materials is water. The majority of fungi need a high RH to develop ($a_w \approx 1$) and its development is enhanced in microclimatic environments due to condensation, although some fungal species are able to survive at low water activities. The latter are collectively classified as xerophilic fungi [48,49]. In fact, some xerophilic/osmophilic fungal species of the *Aspergillus* and *Penicillium* are considered to be primary colonizers since they are capable of growing at $a_w < 0.8$. Species of other genera (*Alternaria*, *Cladosporium*, *Phoma*, etc.) are secondary colonizers, (a_w 0.8 - 0.9) [50]. When settling on the surface, primary colonizers create micro-ecosystems that stop the normal flux of air on them. This situation conditions the surfaces to absorb humidity that helps the microbial adherence and the subsequent biofilm formation [51,52]. Then the fungi with the most efficient enzymatic machinery will prevail, resulting in a decline in biodiversity on the surface [11,53].

Although it had been established that the indoor fungal contamination comes from outdoor air [54,55] in addition to being transported inside by visitors, personnel and insects [56], this is not the case for professional archive vaults which are relatively well protected from any exterior

airborne contamination. These repositories are characterized by good construction and hygienic conditions and maintain an acclimated environment where the T and RH are kept constant [57]. However, problems arise in cases of HVAC (Heating, ventilation, and air conditioning) air conditioning system failure, resulting in both the temperature and the humidity inside buildings being directly influenced by climatic conditions outside. When frequent, such disturbances can lead to temperature fluctuations between 9 and 14 ◦C in buildings, which can peak at up to 25 ◦C in summer. Additionally, moisture in the rooms can rise above 60 % RH, causing fungal development on walls and ceilings (highly hygroscopic) [58]. Moreover, such fluctuations impact the microclimate inside plastic or metal (rusty) film cans and promote the growth of moulds on film rolls [2]. Moreover, the actual temperature and humidity readings of the ambient air frequently do not match the detected temperature and humidity set points values, and humidity can vary from one storage room to another. So, condensation may be formed on cooled surfaces due to a sudden drop in temperature.

All archives measure temperature and humidity by means of a modern data logger, data writers or simple hygro- and thermometers. However, climate conditions cannot simply be measured by a single data logger in the middle of a room [59]. The influence of air stream through doors, warming by sunlight and daily changes of temperature gradients as well as the isolation and exposition of the building envelope have to be considered. Additionally, microniches are created by wrapping objects into plastic foils or into small boxes which prevent air exchange [60]. Air flow is crucial for combating mould development as it directly impacts air humidity and facilitates the movement of particles in the air and makes their settlement more difficult. This is especially the case for fungal spores that do not settle easily (*Aspergillus fumigatus* having an equinulated spore shape) [61]. Fungi are able to degrade different types of organic pollutants in the aerosol including polycyclic aromatic hydrocarbons (PAHs) [62]. As a consequence, the fungal diversity in an urban environment was found to be much higher than that of rural places. For example, the Historical Archive of the Museum of La Plata (HAMP), which is surrounded by a forest, contains less dust in comparison to the Photographic Library of the National Archive of the Republic of Cuba (PLNARC), which is located close to the city's factories [12]. In the dust, arthropods and pollen can act as fungal vectors and represent an additional source of nutrients [63].

6. Fungi on photographic material

Articles regarding the occurrence of fungal species on the surfaces of photographic materials are reviewed in Table 1. The overall diversity of detected fungal species is presented in a descending order: *Aspergillus* (11 species), *Penicillium* (9 species), *Trichoderma* (4 species), *Cladosporium* (3 species) and *Chaetomium* spp. (2 species). The rest of the genera, belonging to the ascomycetes, are represented by only one identified species each: *Alternaria*, *Geotrichum*, *Microascus*, *Phoma*, *Pleosporales*, *Gnomonia* and *Nectria* spp.

Of the zygomycetes, *Mucor* spp. predominated (3 different species), and *Rhizopus microsporus* was also identified (RF of 8 % on B&W gelatin CN negative film). Basidiomycete species are

usually not detectable by culture-based methods because of their nutritional requirements [64]. Therefore, Bučková *et al.* [28], applied high-throughput sequencing methods and Puškárová et al. [3], used the DGGE-cloning approach, which resulted in the detection of *Ceriporiopsis gilvescens* (RF of 25 % on B&W gelatine positive paper photo), *Bjerkandera adusta* [65], *Phlebia* spp., *Pleurotus pulmonarius*, *Malassezia* spp. (10 % on B&W albumen paper photo) and *Trichosporon aquatile* (10 % on B&W albumen paper photo).

Penicillium, *Aspergillus*, *Trichoderma* and *Cladosporium* spp. are usually found as contaminants or biodeterioration agents in many different habitats and materials, including those considered as representative of historical and cultural heritage [66–70]. *Penicillium, Fusarium, Aspergillus, Microascus* and *Trichoderma* spp. are producers of cellulases (especially *Penicillium chrysogenum*) [71–73], while *Aspergillus* and *Penicillium* spp. are known for their protease activity [74], produce diffusive pigments and secrete malic and citric acids [75]. These acids form calcium salts or act as chelating agents of mineral cations, favouring the biodeterioration process [47]. *Chaetomium* spp. are known to produce cellulases, laccases, lipases, proteases and chitinases [76]. *Cladosporium* spp. produce cellulases and *Geotrichum* spp. produce proteases; both are frequent in photographic archives [77].
Of the basidiomycetes, species of genus *Nectria* are plant pathogens with a lignocellulolytic activity [78,79]; *Malassezia* strains are normally isolated from epidermises or skin scalp [80]; and *Pleurotus* spp. have strong proteolytic activities [3]. *Mucor* spp., the predominant zygomycota, exhibit proteolytic (*Mucor racemosus*) and cellulolitic activities (*Mucor plumbeus*) [3,27].

The relative frequencies (RF), calculated from CFU/m^2 (superscripted a in Table 1) or from the fraction of isolates per total number of isolates in a given study (superscripted b in Table 1), are presented in Figure 1. *Aspergillus versicolor* predominated the surfaces of colour gelatin CTA films, having an RF which ranged between 30 % [4] and 99.5 % [2]. *Penicillium chrysogenum* followed with RFs of up to 78 % [2]. *Penicillium citreonigrum* reached 21 % [2] and *Cladosporium phaenocomae* 14 % [4]. Lower RFs for colour gelatin films were calculated for *Aspergillus penicillioides* (8 %), *Aspergillus sydowii* (5 %) *Aspergillus unguis* (5 %), *Aspergillus vitricola* (3 %), *Microascus* spp. (5 %) [4] and *Penicillium brevicompactum* (1 %) [2].
On the surface of a B&W gelatin CN negative film Bučková et al. [28] identified *Geotrichum* as the predominant fungal species (21 %) (Figure 1B). The presence of *Alternaria* spp. (12 %) and *Cladosporium cladosporioides* (6 %) was also considerable. Furthermore, on the surface of B&W albumen paper photographs Puškárová et al. [3] recognised *Nectria* spp. as the predominant genus (31 %) (Figure 1B), followed by *Eurotium halophilicum* (22 %), *A. penicillioides* (19 %), *Geotrichum* spp. (16 %) and *Cladosporium ramotenellum* (16 %). Also represented were *Gnomonia setacea* (12 %), *Alternaria* spp. (10 %), *Chaetomium globosum* (10%) and *Cladosporium* spp. (8 %). Lastly, 28 % and 12 % of the B&W gelatin positive print was covered by *Cladosporium* spp. and *Geotrichum* spp., respectively [28].

In comparison to the B&W photographs and CN films of the same gelatin binder, CTA films were significantly more overgrown by *A. versicolor* and *P. chrysogenum* (higher RF) (Figure 1). They seems to be more susceptible to biodeterioration, as was also concluded by Rakotonirainy and Lavédrine [64].

The conditions at which the reviewed photographic material was kept differed significantly among studies. Most of the archives had unsatisfactory storage conditions, especially materials originating from Gran Canaria, Havana and the Historical Archive of the Museum of La Plata (HAMLP), which had critically high RH and T values (Figure 2). Figure 2 elucidates that visible mould growth was present only on materials stored at a RH higher than 55 %. However, when RH values surpassed this boundary, mould growth manifested even at temperatures below 10 °C (Prague) [53]. Interestingly, even if materials were seemingly clean and were stored in satisfactory conservation conditions (RH of 50 %) [5] fungal spores on the surfaces were still present and a sudden increase in humidity would result in structural damage [54,81].

For Table 1, we have counted all reported isolates (from photographic material) of a given fungal genus and have potted these values against the temperatures and relative humidity values described above (Figure 3). Figure 3A shows that, in general *Penicillum* spp. favour lower temperatures, whereas *Apergillus* spp. favour higher temperatures (28-31 ^{o}C). Nevertheless, at low temperatures, *Apergillus* are still almost as numerous as *Penicillum* spp. It is well established that *Aspergillus* spp. grow well in temperate and tropical climates (min. of 9 ^{o}C, opt. 25–37 ^{o}C) and are tolerant of both elevated temperatures (max. 47 ^{o}C) and reduced water activities. Moreover, the ability of *Penicillium* spp. to grow at lower temperatures (below 15 ^{o}C) can select for these species [84]. Interestingly, as the temperatures increase from ~22 ^{o}C to ~29 ^{o}C, the diversity decreases. This is most likely a consequence of *Aspergillus* spp. surface overgrowth, which can occur at optimum temperatures. Among the reviewed photographic material, *Cladosporiumn* spp. also occurred in all temperature ranges (Figure 3A), which means that this genus can cause problems in elevated humidity. *Alternata* spp. and *Trichoderma* spp. were present at lower temperatures, as they are well adapted to cold conditions, with a minimum growth temperature ranging from -5 to 0 ^{o}C [85].

According to figure 3B, primarily *Aspergillus* spp. but also *Penicillum* spp. are the only genera that occur at RHs which are lower than 60 %. The genus *Aspergillus* contains the most vigorous xerophiles, *Eurotium halophilicum*, a telemorphic form of *Aspergillus* being highly xerophilic [10,86]. However, only a few *Penicillium* spp. are capable of growth below 0.80 a_w (*P. chrysogenum* (a_w of 0.78), *P. citrinum* (0.8) and *P. janczewskii* (0.78)) [87]. As expected, the greatest fungal diversity is reached when RH is between 62 – 75 % (Figure 3B).

6.1 Binder degradation

Gelatin and albumin are readily utilised by fungi that produce extracellular hydrolysing gelatinases and alkaline serine proteases [88]. Gelatin is even used within a standard microbiological assay to study the proteolytic activity and most of the isolates from albumin photographs also proved positive in these assays [3]. Luminescence measurements by Abrusci et al. [26] showed that gelatine was oxidised faster in presence of fungi than in the presence of bacteria. They further found that out of 17 gelatinase positive strains, isolated from biodeteriorated B&W films [27], *P. chrysogenum* was the more efficient fungus, yielding approximately a 30 % of gelatin mineralisation in 3 weeks. They additionally observed that *A. versicolor* and *P. glomerata* were capable of biodeteriorating 25 % of the gelatin emulsion. The rest of the fungal species (*M. racemosus, Alternaria alternata, Apergillus ustus, Trichoderma longibrachiatum, C. cladosporioides* and *Apergillus nidulans*) exhibited variable biodeterioration rates (from 5% to 15%) [89]. According to Bingley and Verran [2] *A. versicolor* isolates from donated films exhibited a higher gelatinolytic activity to that of the isolated *Penicillium* spp., which could explain their frequent isolation from mouldy photographic material (up to 50 % of isolates) [53].
In accordance to Bingley and Verran [2], Kwiatkowska et al. [90] also observed a slower ability of *Penicilium* spp. to degrade gelatin. They also identified *T. longibrachiatum* as the most efficient fungal species among isolates obtained from historic Polish photographs (1864-1909). This species rapidly degraded the gelatine binder (21 days) even when the emulsion was protected by sandarac and dammar varnishes. Nevertheless, the varnish layers did significantly hinder its growth. Similarly, rapid degradation due to *Hypocrea lixii* (teleomorph of *Trichoderma harzianum*) was also partially inhibited by the sandarac varnish.

6.2 Cellulose based support degradation

Filamentous fungi are efficient producers of extracellular cellulolytic enzymes [91], reflecting the fact that the first steps in depolymerisation take place outside microbial cells, followed by the cellular uptake of the resulting oligomers for final mineralization [92]. It is well established that fungi are the primary cause of degradation of cellulose based material (photographic archives and book libraries). The biodeterioration process of CTA in films requires a cooperative action of esterases (deacetylation), lipases and cellulases (cleavage of the C–C backbone) [6,36,93–95]. Interestingly, Vivar et al. [4] observed that during the degradation of CTA both the molecular weight and the DS decrease, suggesting that deacetylation and decomposition of the polymer backbone proceed simultaneously. Moreover, in the usual dry environment, oxidoreductive enzymes (catalase and peroxidase) are also present during the biodeterioration of cellulose materials [96,97], suggesting that oxidoreductases are also involved in biodeterioration and aid in the substrate utilization process. Hence, the biodeterioration of CTA is a slow and complex process, which means that the resistance of CTA is much greater than that of the binder [27]. Vivar et al. [4] noted that the natural deacetylation of CTA, causing the “vinegar syndrome”, accelerates the biodeterioration process because a pH value of slightly below 7 favours fungal growth.

However, Rakotonirainy and Lavédrine [64] showed that films affected by this syndrome show no signs of mould growth suggesting that the acetic acid is fungistatic. A high DS value of the CTA polymer decreases its solubility, and it has been demonstrated by X-ray and ^{13}C NMR spectra that CTA with a low DS value was amorphous [36], suggesting that the predominant factor decreasing biodeterioration of cellulose was the DS value alone [23,36,98]. Nevertheless, Vivar et al. [4] showed that biodeterioration can still occur at higher DS values, ranging from 2.51 to 2.81, although the process was considerably slower compared to that of CTA with a DS of 0.82.

6.3 Silver salts and fungal adaptation

Chromogenic photographic materials appear to be more susceptible to fungal colonization than B&W materials [18], and consequently, colour images are frequently more contaminated than B&W ones [4,89]. This is congruent with the well-established biocidal effect of metallic silver, which reacts with thiol groups in enzymes and proteins [99].

In contrast, Bingley and Verran [2] found no significant differences between the numbers of fungal spores released from B&W films in comparison to colour film reels. This could be due to the occurrence of a microbial resistance to silver salts, akin to microbial resistance to silver salt based biocides commonly reported from hospitals [100]. Szulc et al. [20] observed that a local fungal biodeterioration of gelatin has led to the agglomeration and merging of silver grains. In effect, degraded gelatin sank into the deeper layers of print (weak baryta layer) and the grains stripped away from the binder. Furthermore, *Trichoderma*, *Penicillium* spp., *Aspergillus niger*, *Aspergillus flavus*, *A. fumigatus* [101] and *Geotrichum* spp. [12] may secrete proteins that are capable of reducing metal ions to form nanoparticles of silver, either in the solution where they are stabilised by organic acids, or on the surface of the cell wall where they are bound to cell wall proteins [102–106]. Lastly, we need to consider that for B&W films a fixing bath usually contains fungicides, but since these agents are not compatible with the dyes in colour prints, they are omitted when processing colour materials [107]. Therefore, fungicide residues on materials containing silver could facilitate a permanent preservation effect.

6.4 Microbial interactions

Stickley et al. [108] reported the synergy of bacterial microbiota (*Bacillus* and *Pseudomonas* spp.) in deteriorating a gelatine-silver positive print. A similar cooperation was observed by Abrusci et al. [109] between bacterial isolates of *Bacillus amyloliquefaciens* (B3BA) and *Bacillus subtilis* (B3BS) within the biofilm on a photographic film. Interestingly, the observed gelatin biodeterioration activity of the microbial mixture was greater than expected from the additivity law of the separate viscosity decay efficiencies for each individual isolate.

However, antagonistic effects were also documented, for example Vivar et al. [8] reported the presence of small holes in the fungal structures present on a cinematographic film, which resulted from the lytic activity of bacteria [66]. In accordance with this Borrego et al. [83] observed a marked decrease in fungi concentration on a silk photograph dominated by bacteria from the

Bacillus and *Streptomyces* spp. These bacteria are able to excrete hydrolytic enzymes such as proteases and chitinases [110,111] that can degrade proteins and chitin of the fungal cell wall [112]. However, fungi can also reside other microorganisms and Borrego et al. [12] reported an abundant presence of the fungus *Zygosporium gibbum* on the photographic materials which has a hyperparasitic saprobic lifestyle and can attack other fungal or bacterial species [48]. According to the authors, its growth manifested only after the establishment of other microorganisms and its activities were directed towards those rather than to the deterioration of the material.

7 Impact on health

People working regularly with historic photographic material should be aware of the potential toxicogenic and allergenic properties of moulds from the *Alternaria*, *Aspergillus*, *Fusarium* and *Penicillium* spp. [113,114]. They are known to cause immunotoxic diseases such as the sick building syndrome [115] and at elevated spore counts are linked to respiratory diseases such as asthma and sinusitis [20,116,117]. Wiszniewska et al. [118] found that 30 % of Polish National Museum employees were sensitized to at least one of the fungal allergens and recommend protective gloves and respiratory protection halfmasks. Common isolates from photographic material such as *Cladosporium* spp. [119], *Stachybotrys* spp. [120,121] and *A. niger* [82,83] are known for their allergenicity, the latter producing allergens Asp n14 and Asp n18 [122]. *Stachybotrys* spp. can cause symptoms of ill health in workers exposed to damp indoor places [120,121].

Most fungi are not able to grow at temperatures above 30°C, although certain species (mainly *Aspergillus*) can grow at 37°C and represent opportunistic pathogens capable of colonising immunocompromised individuals [123,124]. *Aspergillus unilateralis* and *A. niger*, commonly isolated from the air of a photo library [5] are correlated to the *A. fumigatus* complex [125,126] causing aspergilloma and invasive aspergillosis [127]. Moreover, film archive isolates of *A. niger, A. flavus* [82,83] and *P. chrysogenum* [128,129] exhibit strong hemolytic (rupture of erythrocytes) and phospholipase activities (damage to cell membranes) [5,130–132].
Microascus spp. (teleomorph of *Scopulariopsis*), which were isolated from colour films [4], can cause infections in toenails and eyes, skin lesions, respiratory disorders, and brain abscesses [133–135], while *Talaromyces* spp., which were found in the air of a photographic library [20], may cause fungaemia, rib osteomyelitis (*T. piceus*) [136–138] and onychomycosis with skin lesions (*T. indigoticus*) [139,140]. *Fusarium* spp., also common in photographic library air [20], are reported to be involved in infections, especially superficial (keratitis and onychomycosis), locally invasive or disseminated infections [141].

Most critically, *Aspergillus* spp. (especially *A. flavus*), known for their mycotoxin production, represent the most serious threat in film archives [82]. Using LARESI MSI scanning, Szulc et al.

[20] detected the presence of hazardous toxins, ochratoxin B, T2-toxin and aflatoxins B1 and B2 on surfaces of historic photographs. Concerningly, absorption of these lipid soluble mycotoxins can occur via inhalation or via dermal exposure (occupational skin penetration). This can lead to cancers, aflatoxins B1 and T-2 having the highest reported skin tumour initiating properties [142,143].

8 Prevention and control

If a film or a photograph is contaminated by moulds, the damage is not immediate. In carefully controlled conditions, the airborne microbiota can coexist with collections and staff without causing any damage. Even if visible microbial growth is discovered in time it is still possible to remove it and to prevent its regrowth without damaging the preserved material. However, continued growth will cause irreversible damage and the destruction of gelatin [27,107,109], and to date, no satisfactory method to inhibit its onset is available [4] due to an insufficient availability of effective preservation procedures as well as of substances restricting the spread of contamination [90].

When photographic material is donated to archives or when it is returned from a foreign exhibition, it is firstly quarantined in a separate room, inspected, transferred to a digital medium for ready access and viewing, copied onto a new base, while the original, of considerably better image quality, is archived in air-conditioned vaults [2,64]. The general recommendations for storage of the International Federation of Film Archives (FIAF) are very strict (e.g. for B&W CTA film: 21 ^{o}C and 30% RH; and 2 ^{o}C and 30% if in colour) [13] often barely correspond to the actual storage conditions of photographic material (Figure 2). Metal boxes should be used for storage, as wooden or cardboard boxes tend to absorb and hold moisture [107]. A dehumidifier can be used to control the RH, since desiccants such as silica gel can cause the material to dry out. Films should occasionally be projected to increase dryness and cleanness [4].

Due to frequent disturbances in RH, systematic and periodical microbiological samplings, are needed to estimate the prevalence of microbial contamination [144]. These can help in the management of the photographic archive in the case of a water leak or flood and will provide a better picture of the hazards to which the workers are exposed [52,145]. There is currently no international standard to determine whether an indoor air of a heritage institution is considered contaminated or not. The strictest limit of 150 CFU/m^3 was suggested by the Italian Official Document for Conservation of Indoor Cultural Heritage [10,57,146], however, limits of 300 CFU/m^3 [147]; 750 CFU/m^3 [148] and 1000 CFU/m^3 [149] have also been proposed.

If mould growth on the original material is discovered, the material may be physically treated to remove visible parts (gentle suction) [2]. However, biodeterioration may not always be apparent to the naked eye, but can still affect the internal structure of the substrate [150]. Colour changes,

perhaps the most common manifestation of fungal contamination, may be a harbinger of eventual structural damage [52]. Moreover, Bingley and Verran [2] advise the measurement of microbial volatile compounds as indicators of fungal growth [151,152]. On the other hand, some photographic material which appears to be very mouldy, sometimes harbours spores which lost their viability in the more distant past. Nevertheless, these may still be allergenic and therefore Bingley and Verran [2] propose for the establishment of an arbitrary ranking of photographic material in terms of visible mould growth (spore count) with consequent adjustment of handling of any affected material.

9 Future perspectives

Some of the work that could improve in the future is to standardise the sampling procedures, especially air sampling procedures as, there are no widely accepted methodologies for its measurement. Therefore, there is a need to investigate the potential of passive (based on spore sedimentation; expressed in CFU/m^2/h) and active (based on vacuum pumps sucking air; expressed in CFU/m^3) air sampling strategies to explain visible mold, and understand how room characteristics of a museum or a film archive correlate with the obtained readings [161–165]. During air sampling in any indoor environment, outdoor bioaerosol sampling should be performed for comparison and indoor and outdoor microbial sources should therefore be clearly defined (indoor/outdoor ratio) [166]. The established culture-based PCR identification methods usually do not detect Basidiomycete species, which can harm the supporting cellulose material (strong cellulolytic activity), and therefore next generation sequencing (NGS) should be employed [3,28,167–170]. Furthermore, museums and film archives should aim towards an automated control of humidity within the exhibition halls. Sensors based on “Internet of Thing” (IoT) modules have been developed which can monitor relative humidity within a compartment and can automatically update the status to the museum employees. Based on the measurement, this sensor system can automatically control the system*'s HVAC* and humidifier networks, resulting in constant relative humidity throughout the entire building which is independent of any environmental fluctuations [171–175]. Lastly, for the most fragile photographic materials, visits should be regulated and these materials should be digitalized and exhibited within virtual tours [176–180].

10 Conclusions

Even professional archives with controlled climates experience frequent fluctuations in RH and T during which the contaminated photographic material can be overgrown with fungi causing mechanical, chemical and aesthetic deterioration. *A. versicolor* was identified by 6 and *P. chrysogenum* by 10 studies that examined the surface of different photographic materials, confirming their widespread occurrence. Both species exhibit a very strong ability to hydrolyse gelatin, the xerophilic *P. chrysogenum* also being very efficient in cellulose degradation. In general *Penicillum* spp. favour lower temperatures, whereas *Apergillus* spp. favour higher temperatures, but are also present below 10 °C. Moreover, *Aspergillus* and *Penicillum* spp. are the only genera that occurred at RHs which were below 60 %. On some photographic materials, gelatin-degrading *Geotrichum* spp. and *P. citreonigrum*; lignocellulose degrading *Nectria* spp. and highly xerophilic A. *penicillioides* and *E. halophilicum* were the dominant species. Airborne spores may cause allergies, and the prevalence of sensitisation of archive workers to mould spore allergens can be considerable (30 %). Additionally, potent tumour inducing aflatoxins present on stored photographs can be absorbed via inhalation or through dermal exposure. When faced with these problems, restorers should consult microbiologists and employ their expertise. Currently, only the World Federation of Culture Collections, i.e. the Austrian Center of Biological Resources and Applied Mycology, offers such services.

Highlights:

1. Fungal biodeterioration of photographic materials is reviewed.
2. Fungal isolates from biodeteriorated photographic materials are analyzed.
3. Origins of fungal contamination, prevention and control measures, and binder and support material deterioration is discussed.

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Figures and tables embedded in text

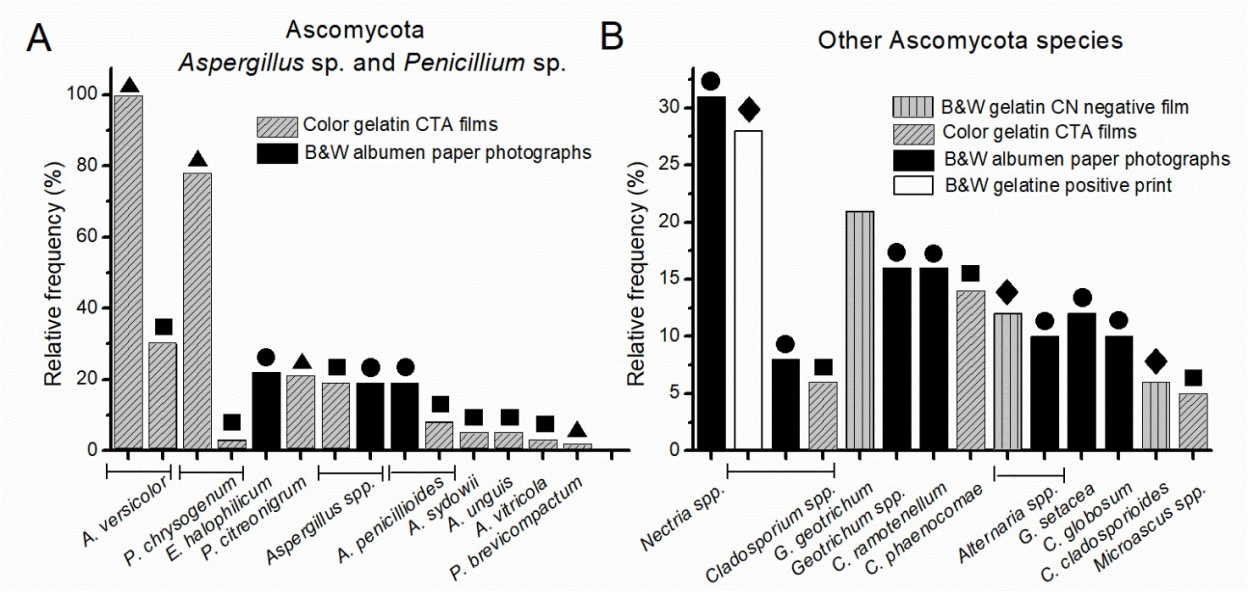

Figure 1: Relative frequencies of fungal species isolated and numerated from reviewed photographic material. Only studies from which relative frequencies could be calculated (from CFU/m^2 or from the number of isolates) were considered. Graph A represents species belonging to the *Aspergillus* and *Penicillium* genera and graph B represents species from other ascomycota genera. Letters depict the following studies: square [4]; circle [3]; triangle [2]; and diamond [28].

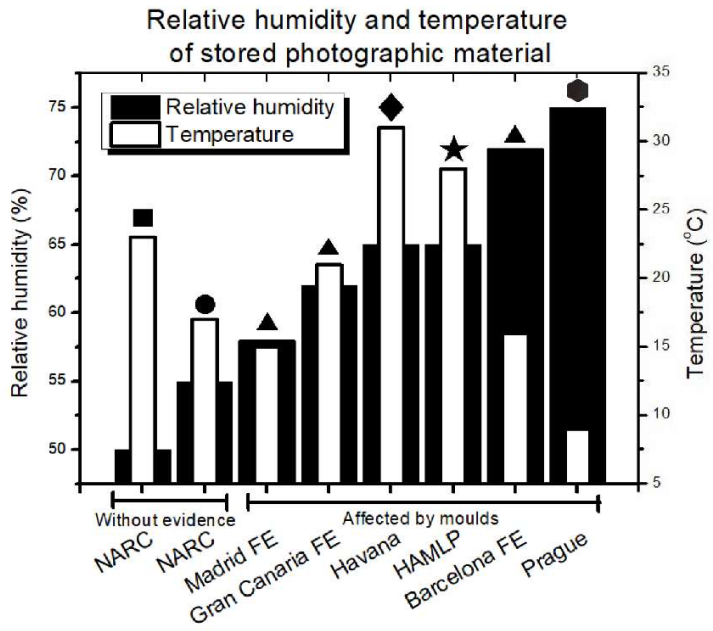

Figure 2: Conditions at which the reviewed photographic material was kept. Only studies with reported RH and T values were considered. Letters signify the following studies: square [5]; circle [82]; triangle [27]; diamond [4]; star [83]; and hexagon [53]. Abbervations: *National Archive of the Republic of Cuba (NARC); Historical Archive of Museum of La Plata (HAMLP); and Filmoteca Espanola (FE).*

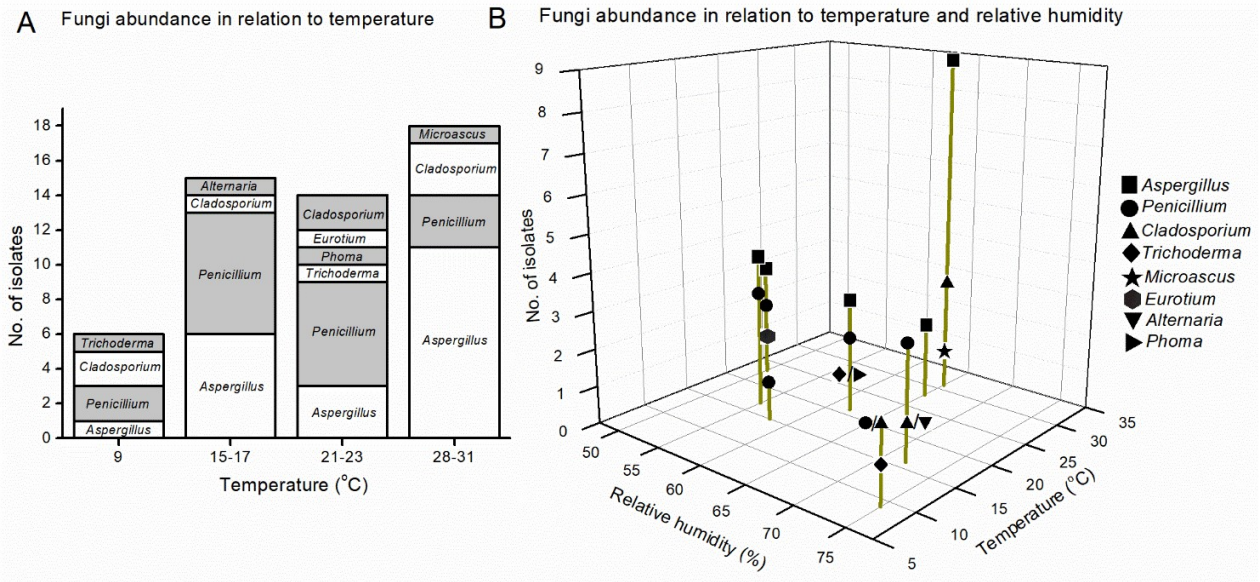

Figure 3: Number of isolates for a given Ascomycota genus plotted against temperature (graphs A and B) and relative humidity (graph B).

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609 Table 1: List of fungi isolated from the surfaces of various photographic cultural heritage items.

Species	Identification method	Photographic sample	RH and T	Documented symptoms	Name of strain/ isolate	Biological potency	RF (%)
			Ascomycota				
Aspergillus sp.	ITS1 and ITS2 5.8S rRNA [153]	Color gelatin CTA films 1980-1991; Havana NARC [4]	65 % and 31 ºC	Brown stains	1L9, 1R4 and 1L3		19[a]
	DGGE communal fingerprinting profiles	B&W albumen paper photograph of the TSNA [3]	Unknown	Foxing stains	/		19[b]
Aspergillus versicolor	ITS1 and ITS2 5.8S rRNA [153]	Color gelatin CTA films 1980-1991; Havana NARC [4]	65 % and 31 ºC	Brown stains	1R8 and 1R10		30[a]
	ITS (ITS1/ITS4)	B&W albumen paper photograph of the TSNA [3]	Unknown	Foxing stains	PA2_6_Fu and PA2_3_Fu	Cellulolytic, catalase and peroxidase activities	
	ITS1 and ITS2 and the 5.8S	20th cent. B&W gelatin CTA films from Gran Canaria (FE) [11,27,89]	62 % and 21 ºC	Affected by moulds	HGC3	Strong gelatin hydrolysis (HGC3)	
	/	20th-cent. B&W gelatin CTA films, (Prague) [53]	75 % and 9 ºC	Affected by moulds			
	ITS (ITS1/ITS4)	Colour gelatin CTA films (NWFA) [2]	Unknown	Mouldy films	Isolates RR1549 I2, RR1549 I3, RR1399 I1, I2, I3, I4, I6 and RR1470 I1	Gelatin hydrolysis (RR1549 I2, RR1549 I3, RR1399 I1, I3, I6 and RR1470 I1) and strong gelatin hydrolysis (RR1399 I2 and I4)	99.5[b]
	ITS (ITS1/ITS4)	19th cent. B&W gelatine glass plate negatives by *Łukasz Dobrzański* (FCRWA at JMAFAK) [90]	Probably flooded	Visible stain marks	KX232646	Poor degradation of gelatin and acid production (pH 4)	
Aspergillus flavus	Morphological characteristics [154–156]	19th cent. B&W albumen paper photograph (NARC) [82]	55 % and 17°C	Without evidence of mould	Isolates 1 and 2	Aflatoxin production, strong cellulose degradation, gelatin degradation, acid production and hemolytic activity (37°C) (only isolate no. 2)	
	Identification key [154]	19th cent. paper gelatin photos (HAMLP) [83]	65 % and 28 ºC	Affected by moulds	/	Aflatoxin production, cellulose and gelatin degradation, reddish stains and acid production (pH 5.0)	
Aspergillus niger	Morphological characteristics [154–156]	19th cent. B&W albumen paper photograph (NARC) [82]	55 % and 17 ºC	Without evidence of mould	Isolates 1 and 2	Allergen production (Asp n14 and Asp n18), strong cellulose degradation, gelatin degradation, acid production, growth at 37°C	
	Identification key [154]	19th Cent. paper gelatin photos (HAMLP) [83]	65 % and 28 ºC	Affected by moulds	/	Strong cellulose degradation, gelatin degradation, yellow stains and acid production (pH 3.2)	
	Manuals [157]	19th and 20th cent. B&W gelatin negatives on glass plate (NARC) [5]	50 % and 23 ºC	Without evidence of mould	Isolate number 2	Cellulose and gelatin degradation, stains and acid production (pH 5.5), hemolytic (37°C) and phospholipase activities	
Aspergillus penicillioides	ITS1 and ITS2 5.8S rRNA [153]	Color gelatin CTA films 1980-1991; Havana NARC [4]	65 % and 31 ºC	Brown stains	7L1	Highly xerophilic	8[a]
	DGGE communal fingerprinting profiles	B&W albumen paper photograph of the TSNA [3]	Unknown	Foxing stains	/	Highly xerophilic	19[b]
Aspergillus ustus	ITS1 and ITS2 and the 5.8S	20th cent. B&W gelatin CTA films from Barcelona (FE) [11,27]	72 % and 16 ºC	Affected by moulds	HB2	Strong gelatin hydrolysis	
		20th cent. B&W gelatin CTA films from Gran Canaria (FE) [27]	62 % and 21 ºC	Affected by moulds	HGC2B	Gelatin hydrolysis	
Aspergillus sydowii	ITS1 and ITS2 5.8S rRNA [153]	Color gelatin CTA films 1980-1991; Havana NARC [4]	65 % and 31 ºC	Brown stains	1R7		5[a]

Aspergillus unguis	ITS1 and ITS2 5.8S rRNA [153]	Color gelatin CTA films 1980-1991; Havana NARC [4]	65 % and 31 ºC	Brown stains	1L12		5[a]
Aspergillus nidulans var. nidulans	ITS1 and ITS2 and the 5.8S	20th cent. B&W gelatin CTA films from Madrid (FE) [27]	58 % and 15 ºC	Affected by moulds	HM3	Gelatin hydrolysis	
Aspergillus vitricola	ITS1 and ITS2 5.8S rRNA [153]	Color gelatin CTA films 1980-1991; Havana NARC [4]	65 % and 31 ºC	Brown stains	7.1.1		3[a]
Eurotium halophilicum (telemorph of Aspergillus)	DGGE communal fingerprinting profiles	B&W albumen paper photograph of the TSNA [3]	Unknown	Foxing stains	/	Highly xerophilic	22[b]
Eurotium chevalieri (telemorph of Aspergillus)	Manuals [157]	19th cent. B&W gelatin glass plate negative (NARC) [5]	50 % and 23 ºC	Without evidence of mould	One isolate	Poor cellulose degradation, gelatin degradation and acids production (pH 5.0)	
Penicillium sp.	ITS (ITS1/ITS4)	1889 B&W albumen paper photograph by Max Stern (TSNA) [3]	Unknown	Foxing stains	P1_3_Fu, P1_4_Fu, and P1_10_Fu	Cellulolytic and catalase activities (P1_4_Fu for all)	
	ITS (ITS1/ITS4)	19th cent. B&W gelatine glass plate negatives by *Łukasz Dobrzański* (FCRWA at JMAFAK) [90]	Probably flooded	Visible stain marks	KX232645	Poor degradation of gelatin and acid production (pH 3)	
Penicillium chrysogenum	ITS1 and ITS2 5.8S rRNA [153]	Color gelatin CTA films 1980-1991; Havana NARC [4]	65 % and 31 ºC	Brown stains	6R8		3[a]
	ITS (ITS1/ITS4)	1889 B&W albumen paper photograph by Max Stern (TSNA) [3]	Unknown	Foxing stains	P1_18_Fu		
	ITS (ITS1/ITS4)	B&W albumen paper photograph of the TSNA [3]	Unknown	Foxing stains	PA2_4_Fu		
	ITS1 and ITS2 and the 5.8S	20th cent. B&W gelatin CTA films from Barcelona (FE) [27,89,158]	72 % and 16 ºC	Affected by moulds	HB41B and HB6	Gelatin hydrolysis	
	ITS1 and ITS2 and the 5.8S	20th cent. B&W gelatin CN films from Barcelona (FE) [27,89,158]	72 % and 16 ºC	Affected by moulds	HB7	Strong gelatin hydrolysis	
	ITS1 and ITS2 and the 5.8S	20th cent. B&W gelatin CTA films from Gran Canaria (FE) [27]	62 % and 21 ºC	Affected by moulds	HGC1, HGC3B and HLV1	Gelatin hydrolysis	
	ITS1 and ITS2 and the 5.8S	20th cent. B&W gelatin CTA films from Madrid (FE) [27]	58 % and 15 ºC	Affected by moulds	HMI	Gelatin hydrolysis	
	ITS (ITS1/ITS4)	Colour gelatin CTA films (NWFA) [2]	Unknown	Mouldy films	Isolates RR1093 I4 and RR1093 I1	Gelatin hydrolysis	78[b]
	Morphological characteristics [154–156]	19th cent. B&W albumen paper photograph NARC [82]	55% and 17°C	Without evidence of mould	One isolate	Cellulose and gelatin degradation, acid production and hemolytic activity (37°C)	
	Manuals [157]	19th cent. B&W gelatin glass plate negative (NARC) [5]	50 % and 23°C	Without evidence of mould	isolate number 3	Strong cellulose degradation, gelatin degradation, acids production (pH 6.5) and hemolytic activity (37°C)	
Penicillium citrinum	Morphological characteristics [154–156]	19th cent. B&W albumen paper photograph NARC [82]	55% and 17°C	Without evidence of mould	Isolates 1 and 2	Strong cellulose degradation, gelatin degradation (isolate 1), yellow stain (isolate 1) and acid production, and hemolytic activity (37°C)	
	Identification key [154]	19th cent. paper gelatin photos (HAMLP) [83]	65% and 28 °C	Affected by moulds	/	Strong cellulose degradation, gelatin degradation and acid production (pH 3.1)	
Penicillium janczewskii	Morphological characteristics [154–156]	19th cent. B&W albumen paper photograph NARC [82]	55% and 17°C	Without evidence of mould	One isolate	Cellulose and gelatin (isolate 1) degradation, yellow stain and acid production, growth at 37°C	
	Manuals [157]	19th cent. B&W albumen paper photograph (NARC) [5]	50 % and 23 °C	Without evidence of mould	Isolate number 2	Cellulose and gelatin degradation, stains and acids production (pH 5.5), partial hemolytic (37°C) and phospholipase activities	
Penicillium brevicompactum	ITS (ITS1/ITS4)	Colour gelatin CTA films (NWFA) [2]	Unknown	Mouldy films	Isolate RR1093 I2	Gelatin hydrolysis	1[b]

	ITS (ITS1/ITS4)	Colour gelatin CTA films (NWFA) [2]	Unknown	Mouldy films	Isolate RR1399 I5	Gelatin hydrolysis	0.5[b]
Penicillium decumbens	Identification key [154]	19th cent. paper gelatin photos (HAMLP) [83]	65% and 28°C	Affected by moulds	/	Strong cellulose degradation, gelatin degradation and acid production (pH 6.2)	
Penicillium frequentens		20th-cent. B&W gelatin CTA films, (Prague) [53]	75 % and 9 ºC	Affected by moulds			
Penicillium thomii	ITS (ITS1/ITS4)	B&W albumen paper photograph of the TSNA [3]	Unknown	Foxing stains	PA2_5_Fu and PA2_2_Fu	Cellulolytic activity, catalase and peroxidase activity	
Penicillium lanosum		20th-cent. B&W gelatin CTA films, (Prague) [53]	75 % and 9 ºC	Affected by moulds			
Penicillium citreonigrum	ITS (ITS1/ITS4)	Colour gelatin CTA films (NWFA) [2]	Unknown	Mouldy films	Isolate RR1093 I3	Gelatin hydrolysis	21[b]
Cladosporium sp.		Color gelatin CTA films 1980-1991; Havana NARC [4]	65 % and 31 ºC	Brown stains	6R1 and 6R6		6[a]
	ITS (ITS1/ITS4)	B&W gelatin CN negative film and B&W gelatine positive print, 1938-1940 (ACS, Italy) [28]	No regulation	Affected by moulds	GU395509 and KF367501		27[a] (neg.); 28[a] (pos.)
	DGGE communal fingerprinting profiles	B&W albumen paper photograph of the TSNA [3]	Unknown	Foxing stains	/		8[b]
Cladosporium cladosporioides	ITS1 and ITS2 and the 5.8S	20th cent. B&W gelatin CTA films from Barcelona (FE) [11,27,158]	72 % and 16 ºC	Affected by moulds	HB3A	Strong gelatin hydrolysis	
		20th-cent. B&W gelatin CTA films, (Prague) [53]	75 % and 9 ºC	Affected by moulds			
	ITS (ITS1/ITS4)	B&W gelatin CN negative film, 1938-1940 (ACS, Italy) [28]	No regulation	Affected by moulds	KC113301		6[a]
	Manuals [157]	19th and 20th cent. B&W gelatin negatives on glass plate (NARC) [5]	50 % and 23 °C	Without evidence of mould	isolate number 3	Strong cellulose degradation, gelatin degradation, strong staining and acids production (pH 6.6)	
Cladosporium phaenocomae	ITS1 and ITS2 5.8S rRNA [153]	Color gelatin CTA films 1980-1991; Havana NARC [4]	65 % and 31 ºC	Brown stains	6R5		14[a]
Cladosporium ramotenellum	DGGE communal fingerprinting profiles	1889 B&W albumen paper photograph by Max Stern (TSNA) [3]	Unknown	Foxing stains	/		16[b]
Trichoderma longibrachiatum	ITS1 and ITS2 and the 5.8S	20th cent. B&W gelatin CTA films from Gran Canaria (FE) [11,27,158]	62 % and 21 ºC	Affected by moulds	HGC2	Strong gelatin hydrolysis	
	ITS (ITS1/ITS4)	19th cent. B&W gelatine glass plate negatives by *Łukasz Dobrzański* (FCRWA at JMAFAK) [90]	Probably flooded	Visible stain marks	KX232648	Moderate degradation of gelatin (also on Sandarac and dammar varnishes) and acid production (pH 5)	
Trichoderma viridiae		20th-cent. B&W gelatin CTA films, (Prague) [53]	75 % and 9 ºC	Affected by moulds		Producers of cellulases	
	Identification key [159]	Paper gelatin photographs by Aleksandar Rafajlović (MCAB) [160]	flooded	visible colonies	/	Producers of cellulases	
Trichoderma harzianum	ITS (ITS1/ITS4)	Colour gelatin CTA films (NWFA) [2]	Unknown	Mouldy films	Isolate S1	Strong gelatin hydrolysis	
Hypocrea lixii (teleomorph of *Trichoderma harzianum*)	ITS (ITS1/ITS4)	19th cent. B&W gelatine glass plate negatives by *Łukasz Dobrzański* (FCRWA at JMAFAK) [90]	Probably flooded	Visible stain marks	KX232647	Extensive degradation of gelatin	
Alternaria sp.	ITS (ITS1/ITS4)	B&W gelatine positive paper photo, 1938-1940; (ACS, Italy) [28]	No regulation	Affected by moulds	KF193517		12[a]
	DGGE communal fingerprinting profiles	B&W albumen paper photograph of the TSNA [3]	Unknown	Foxing stains	/		10[b]

Alternaria alternata	ITS1 and ITS2 and the 5.8S	20th cent. B&W gelatin CTA films from Barcelona (FE) [27]	72 % and 16 ºC	Affected by moulds	HB1 and HB41N	Gelatin hydrolysis (adapted to cold)	
Chaetomium globosum	DGGE communal fingerprinting profiles	1889 B&W albumen paper photograph by Max Stern (TSNA) [3]	Unknown	Foxing stains	/	Cellulase, laccase, lipase, protease and chitinase	10[b]
Chaetomium elatum	ITS (ITS1/ITS4)	1889 B&W albumen paper photograph by Max Stern (TSNA) [3]	Unknown	Foxing stains	P1_13_Fu and P1_1_20_Fu	Proteolytic, cellulolytic, catalase and peroxidase activities (P1_13_Fu for all)	
Geotrichum sp.	DGGE communal fingerprinting profiles	1889 B&W albumen paper photograph by Max Stern (TSNA) [3]	Unknown	Foxing stains	/	Resistant to the toxicity of silver	16[b]
	ITS (ITS1/ITS4)	B&W gelatin CN negative film and B&W gelatine positive paper print, 1938-1940 (ACS, Italy) [28]	No regulation	Affected by moulds	JQ668738		21[a] (neg.); 12[a] (pos.)
Microascus sp.	ITS1 and ITS2 5.8S rRNA [153]	Color gelatin CTA films 1980-1991; Havana NARC [4]	65 % and 31 ºC	Brown stains	4L1	Dermal and eye infections and respiratory disorders	5[a]
Phoma glomerata	ITS1 and ITS2 and the 5.8S	20th cent. B&W gelatin CTA films from Gran Canaria (FE) [27,89]	62 % and 21 ºC	Affected by moulds	HGC3N	Strong gelatin hydrolysis	
Pleosporales sp.	ITS (ITS1/ITS4)	1889 B&W albumen paper photograph by Max Stern (TSNA) [3]	Unknown	Foxing stains	P1_Na_21_Fu	Cellulolytic activity, strong catalase and peroxidase activities	
Gnomonia setacea	DGGE communal fingerprinting profiles	B&W albumen paper photograph of the TSNA [3]	Unknown	Foxing stains	/		12[b]
Nectria sp.	DGGE communal fingerprinting profiles	1889 B&W albumen paper photograph by Max Stern (TSNA) [3]	Unknown	Foxing stains	/	Plant pathogen (lignocellulolytic activity)	31[b]
			Basidiomycota				
Ceriporiopsis gilvescens	ITS (ITS1/ITS4)	B&W gelatine positive paper photo, 1938-1940 (ACS, Italy) [28]	No regulation	Affected by moulds	HQ659222		25[a]
Bjerkandera adusta	ITS (ITS1/ITS4)	1889 B&W albumen paper photograph by Max Stern (TSNA) [3]	Unknown	Foxing stains	P1_1_Fu and P1_6_Fu	Strong cellulolytic activity, catalase and peroxidase activities	
Phlebia sp.	ITS (ITS1/ITS4)	1889 B&W albumen paper photograph by Max Stern (TSNA) [3]	Unknown	Foxing stains	P1_2_Fu and P1_2_3_Fu	Cellulolytic, catalase and peroxidase activities	
Pleurotus pulmonarius	ITS (ITS1/ITS4)	1889 B&W albumen paper photograph by Max Stern (TSNA) [3]	Unknown	Foxing stains	P1_15_Fu	Strong proteolytic and peroxidase activities; cellulolytic and catalase activities	
Malassezia sp.	DGGE communal fingerprinting profiles	1889 B&W albumen paper photograph by Max Stern (TSNA) [3]	Unknown	Foxing stains	/	From human skin	10[b]
Trichosporon aquatile	DGGE communal fingerprinting profiles	1889 B&W albumen paper photograph by Max Stern (TSNA) [3]	Unknown	Foxing stains	/		10[b]
			Zygomycota				
Mucor sp.		20th-cent. B&W gelatin CTA films, (Prague) [53]	75 % and 9 ºC	Affected by moulds			
Mucor racemosus	ITS1 and ITS2 and the 5.8S	20th cent. B&W gelatin CTA films from Madrid (FE) [27]	58 % and 15 ºC	Affected by moulds	HM4	Gelatin hydrolysis	
Mucor plumbeus	ITS (ITS1/ITS4)	1889 B&W albumen paper photograph by Max Stern (TSNA) [3]	Unknown	Foxing stains	P1_Na_18_Fu	Cellulolytic activity, strong catalase and peroxidase activities	
Rhizopus microsporus	ITS (ITS1/ITS4)	B&W gelatin CN negative film, 1938-1940 (ACS, Italy) [28]	No regulation	Affected by moulds	KC206538		8[a]

Abbervations: *Trenčin, Slovak National Archives (TSNA); National Archive of the Republic of Cuba (NARC); Historical Archive of Museum of La Plata (HAMLP); Filmoteca Espanola (FE); North*

West Film Archive (NWFA); Faculty of Conservation and Restoration of Works of Arts (FCRWA), at Jan Matejko Academy of Fine Arts in Krakow (JMAFAK); Italy Archivio Ente EUR e Archivio Centrale dello Stato (ACS); and Museum of Contemporary Art in Belgrade (MCAB). [a]RFs calculated from CFU/m^2 or [b] from the number of isolates per total number of isolates in a given study.

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