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Counts of fungal spores released during inspection of mouldy cinematographic film and determination of the gelatinolytic activity of predominant isolates

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ABSTRACT

Film archivists have expressed concern regarding the release of aerial spores during inspection of mouldy cinematographic film. This study investigated the release of fungal spores during a simulated inspection procedure, and identified the key contaminants, the aim being to make recommendations to archivists regarding safe handling of such film. Eighteen films (black and white or colour) donated to the North West Film Archive, in Manchester, UK, were examined. During simulated inspection, spore release ranged from zero to several thousand spores per m^3, with the films showing the most visible mould colonisation usually yielding the highest numbers of colonies. There was no significant difference between the number of spores released from black and white film in contrast with colour film reels. Major contaminants were of the genera *Aspergillus* or *Penicillium*. *A. versicolor* was the most common species isolated. Gelatinase assays were performed on predominant isolates, to investigate whether fungal species which released the most spores, were also the most gelatinolytic, thus posing the greatest threat to film preservation. However, this doesn't prove to be the case. Some films released spores in numbers greater than are deemed 'safe' levels; therefore caution is advised when dealing with very mouldy film reels.

1. Introduction

National and regional archive material encompassing books, papers and film, provides an invaluable record of social history, personal achievement and historic occasions for the population served (Gallo, 1993; Florian, 2002). On a personal level, material such as home movies represents a treasured narrative of life events. Deterioration of cinematographic film devalues its heritage status, and can irreversibly damage the content of the film, thus storage under appropriate conditions is of paramount importance, coupled with an effective means of monitoring the quality of the film. Film consists of several layers: an outer protective layer, image forming materials consisting of dyes in colour film and silver particles in black and white and an anti-halation layer to prevent the scattering of light around bright objects (http://motion.kodak.com/motion/uploadedFiles/US_plugins_acrobat_en_motion_newsletters_filmEss_04_How-film-makes-image.pdf). The image forming layers and anti-halation layer are held onto a base support by a gelatin layer and together these constitute the emulsion layer (Lourenco and Sampaio, 2009). Different materials have been used for the base support since the invention of cinematographic film, and some of these are susceptible to chemical deterioration. Cellulose nitrate (1900s–1960s) is highly flammable and releases nitric acid when decomposing, cellulose diacetate and later cellulose triacetate ('safety film') (1960s–1990s) was susceptible to vinegar syndrome i.e., the breakdown of acetate to form acetic acid; and polyethylene terephthalate (1990's-present) which has been shown to be very chemically stable. Chemical deterioration has been the focus of several publications, but the biological deterioration has received less attention (Abrusci et al., 2004b). The key substrate for microbial growth in cinematographic film is the gelatin binder. Gelatin is used to stabilise the film and can be cross-linked with other compounds to increase strength and tolerance towards higher temperatures, and due to these properties, gelatin has been used in all materials in photographic history (Abrusci et al., 2004b). Gelatin can be hydrolysed by extracellular enzymes produced by microorganisms. If the environmental conditions are appropriate, then microorganisms contaminating the film can multiply, using the gelatin as a growth substrate, and colonise the film. Fungi are a particular problem, because they can tolerate conditions of lower relative humidity than bacteria. The use of the gelatin layer as an energy source will

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enable fungal growth to occur on the outside of the reel, as well as on the film frames. Mycelial growth obscures the image, and damage to the gelatin binder also affects the image quality.

Previous studies have shown that a range of fungal species can colonise and deteriorate cinematographic film (Zyska, 1997; Abrusci et al., 2004a, 2004b, 2005, 2006, 2007, 2009; Lourenco and Sampaio, 2009). Studies by Zyska (1997) and Abrusci et al. (2004b) revealed the predominance of *Aspergillus* and *Penicillium* species in the library environment and their ability to colonise and contaminate a wide range of library materials. In a limited study by Lourenco and Sampaio (2009), it was found that colour film reels were more affected by the fungal deterioration by *Aspergillus* and *Penicillium* sp. than black and white reels, assumed to be due to the inhibition of growth caused by the silver particles present in black and white film. Genera found on cinematographic film are generally typical of those found in the outdoor and indoor environment (Florian, 2002), but it is interesting to note the predominance of certain species, such as *A. versicolor*. In one study, up to 50% of the isolates from cinematographic film were identified as this particular species (Opela, 1992) – which indicates that there might be some predilection for the particular substrate and/or enhanced gelatinolytic activity in this species. In addition, although the range of contaminants has been explored, no attempts have been made to quantify the contamination, other than descriptively.

Films are typically stored in-can. In the domestic environment, storage conditions provided by the ambient environment of cupboards, attics, trunk or sheds, may be particularly favourable for fungal growth – moisture and warmth being the key prerequisites. In film archives, storage conditions are carefully controlled, thus minimising the growth of fungal deteriogens.

Problems may arise when films are donated to archives. Typically, films are inspected, and transferred to a digital medium for ready access and viewing, whilst the film itself, which is of considerably better image quality, is archived. In some instances, the film may be treated to physically remove visible mould, for example by gentle suction. Conversely, films which present evidence of fungal growth may not be handled or inspected by archivists due to concerns regarding health and safety. It is then not possible to inspect the film, to describe its content, determine its condition or transfer the film to a more accessible medium. Such films are stored separately, in the hope that future technologies might enable decontamination, inspection, conservation and archiving (http://cool.conservation-us.org/byorg/abbey/an/an21/an21-7/an21-709.html). The authors could find no specific health and safety recommendations regarding handling of these items.

This paper describes a survey of cinematographic film donated to, and deemed 'mouldy' by, a regional archive. Efforts were made to identify the fungal contaminants, quantify the release of fungal spores into the air during a simulated inspection to see whether they pose a health risk to archivists, and determine whether predominant species were capable of degrading gelatin, thus posing a danger to the preservation of film stock.

2. Material and methods

2.1. Film reels

The North West Film Archive (NWFA), based at the Manchester Metropolitan University (MMU), is the largest UK archive outside London (www.nwfa.mmu.ac.uk). Set up in 1977, the NWFA has a collection of over 33,000 items, which include black and white film, colour film, photographs and paper. Most have been donated from sources across the North West of England. Films are stored under controlled conditions (black and white archived at 10 °C and 35% relative humidity; colour at 4 °C and 30% relative humidity). NWFA donated eighteen cellulose films for this study, having obtained prior permission from the film donors to do so. After use in our laboratory, the films were returned to the archive.

The film reels had been identified as 'mouldy' by the archivists and were mouldy upon donation to the archive (by members of the public). However, storage condition of films prior to donation is unknown. Film reels are given reference numbers by the archive and films with the same reference number have the same donor. Of the eighteen reels studied, three belonged to one donor (RR1491), four belonged to a second donor (RR1494), two belonged to a third donor (RR1514), three belonged to a fourth donor (RR1440) and the remaining six reels belonged to six individual donors (RR1470, RR1399, RR1549, RR1093, RR1533 and RR1511). Six films were colour and 12 were black and white.

2.2. Simulated film inspection, spore capture and cultivation

All inspections were performed in a polymer isolation glove box (Wolf Laboratories, York, UK (Model number: 8307030 (3 ft))) approximately 2.3 m^3 volume. This had been customised by removing the gloves at the wrists, so that manipulations in the box could be carried out with bare hands, but fungal spores released during inspection would be contained. This was chosen, rather than a biological safety cabinet level 2 (BSL2), because archivists have access to similar apparatus, thus making the simulation more accurate.

All fungal culture media (malt extract agar (MEA)) (Oxoid, Basingstoke, UK), decontamination fluid (Trigene, Medichem, Kent, UK), and film, in-can, were placed in the glove box. For the 'simulated inspection' each film was mounted on spools at either end of a manual film spool winder so that 50 cm of film was exposed and could be transferred from spool to spool under controlled conditions. An air sampler (Desaga Germ Sampler gs 100: Oklahoma City, US) was also placed in the glove box, so that the number of fungal spores released per unit volume over time during inspection could be assessed. This was set to measure 100 L air, over 60 s with a 5 s delay. When the air sampling began, with 50 cm of film exposed, the film was wound forwards for 60 s, at approximately 90 turns per minute, after which the agar plate on the air sampler was removed, and replaced by a second plate (to give counts of an average of two plates ($n = 2$)) during a repeated winding process where the film was re-wound back to its original spool. These two plates were taken as duplicates, although this is not strictly the case; on occasion the process was repeated, enabling four counts to be made. The film was then replaced in its can, the glove box sanitised with Trigene spray and left for 10 min before the next film was processed. Plates were incubated at 25 °C for 5–7 days. Colonies were counted, and calculated as counts per cubic metre (m^3) of air (Test 1). Films were re-sampled again after 7 (Test 2) and 14 days (Test 3), to determine the effect of repeated inspection on spore release.

After total colony counts were obtained via air sampling for Tests 1, 2 and 3, differential colony counts were also made for two representative heavily contaminated reels (RR1093 (colour), RR1399 (black and white)) and two representative lightly contaminated reels (RR1470 (black and white) and RR1549 (colour)). The aim of this procedure was to determine whether spores of certain species were released on first, second or third handling ('inspection').

2.3. Identification of fungal isolates and subsequent selection for gelatinase assays

Thirteen out of twenty one isolates from reels RR1093, RR1399, RR1470 and RR1549 were identified by CABI Microbial Services