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Fungal biodeterioration of color cinematographic films of the cultural heritage of Cuba

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ABSTRACT

Until recently, cinematographic film was largely cellulose-triacetate-based. However, this material is highly susceptible to biodeterioration, thus placing historic film collections, an important part of the cultural heritage of many countries, at risk. In the present study, samples taken from several biodeteriorated color cinematographic films belonging to the collection of the Cuban Institute for Cinematographic Industry and Arts (ICAIC) were investigated. Infrared spectroscopy showed that all films were of the same composition, i.e., a gelatin emulsion coating one side of a cellulose-triacetate-based film support. The films were analyzed by environmental scanning electron microscopy and scanning electron microscopy to determine the degree of biodeterioration and the type of colonizing microorganisms. Significant fungal colonization was found on both sides of the films in all samples, with a higher concentration of fungi on the gelatin emulsion side. Epifluorescence microscopy of fluorochrome-dyed films demonstrated that some of the fungi were still active, indicating that the films under study, and probably others at the ICAIC, are at risk of further deterioration. Fungi were identified by molecular biology techniques. The fungi mainly responsible for the observed biodeterioration were those belonging to the genera *Aspergillus* and *Cladosporium*, although other genera, such as *Microascus* and *Penicillium*, were identified as well. In accordance with the findings described herein, the existing guidelines for the prevention and control of film biodeterioration are discussed.

1. Introduction

Cinema, popularly referred to as "the seventh art," is part of humanity's cultural heritage and, as such, should be conserved for future generations. However, celluloid, the material used to make cinematographic films, is vulnerable to both chemical and biological deterioration. An understanding of these processes is essential to the development of tools to aid us in the task of maintaining or recovering these culturally and historically significant icons.

A cinematographic film is composed of a flexible plastic support coated with a layer of photosensitive emulsion. Throughout cinematographic history, several flexible plastic supports have been used to manufacture professional motion-picture films, including cellulose nitrate (from 1889 to 1950), cellulose triacetate (from 1948 to 2000), and polyethylene terephthalate (from the 1990s to the present). The first nitrate-based supports were of very poor chemical stability, in addition to being a fire hazard. Triacetate supports met all the technical and safety requirements for professional motion-picture films and, since the early 1950s, have completely replaced cellulose nitrate. All cellulose-based supports are highly rigid; thus, in order to provide the required flexibility they are treated with various plasticizers, such as triphenylphosphate (Wypych, 2004).

Around the early 1970s, film archivists became aware of the serious decomposition of cellulose-triacetate-based cinematographic film, with the loss of many film images as well as entire films. This problem is immediately recognized when the film container is opened, by the release of acetic acid. Its characteristic odor accounts for what is known in the film world as the "vinegar syndrome." Acetic-acid-type degradation is largely responsible for the fading color in chromogenic emulsions, which are currently universally employed in cinematography, and for the deformation and stiffening of the material (del Amo, 2006). In addition, over time the plasticizer migrates, leaving obvious viscous liquid or crystalline deposits on the film's surface (Allen et al., 1988b).

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To address the problems of the "vinegar syndrome," cellulose triacetate (CTA) supports have recently been replaced by the polyester PET (polyethylene terephthalate), due to its exceptional physical properties as a safe cinematographic film support (Abrusci et al., 2004). Polyester-based films are less biosusceptible than those with CTA as the support. In fact, due to the inherent strength and flexibility of polyester, plasticization is no longer required. This is an important advantage, as these additives, in addition to their migration, have been identified as substrates for microbial colonization, including in the cellulose plastic materials used as film supports (Lourenço and Sampaio, 2009). However, CTA-based films constitute the bulk of historical film collections throughout the world (Abrusci et al., 2004).

The manufacture of CTA 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. Although the objective is to obtain a cinematographic film that is completely acetylated, in general, the DS of cinematographic CTA is around 2.7 (Abrusci et al., 2004). Various authors have reported that biodegradation is highly dependent on the DS, and in all cases the rate of biodegradation was shown to increase as the degree of the cellulose acetylation decreased (Buchanan et al., 1993; Samios et al., 1997).

The photosensitive emulsion that coats the cellulose support is made of gelatin, a polypeptide that forms a biodegradable substrate. In the case of black and white films, the emulsion consists of silver salts and other chemical products. The photosensitive layer of color films is somewhat more complicated, as it is composed of green, red and blue sensitive emulsion layers sometimes separated by clear gelatin interlayers. The gelatins used in film are distinguished by their production process and the nature of the raw materials. Conventionally, type A gelatins are produced from acid-pretreated pig skins, type B gelatins from alkaline-pretreated bovine bones, type C gelatins from alkaline-pretreated bovine and cattle hides, and type AB from acid-pretreated bovine bones (van den Bosch and Gielens, 2003). For cinematographic films mostly type-B gelatins are used (Abrusci et al., 2004).

Both the cellulose support and the gelatin emulsion in cinematographic films are susceptible to microbial attack, leading to biodeterioration. The biodeterioration of cinematographic films is the loss of images due to the biodegradation of photosensitive emulsion and/or the biodegradation of the plastic support. While the microbial contamination of black and white cinematographic films collected from the archives of the Czech Republic and from Spain has been studied (Opela, 1992; Abrusci et al., 2005), the biodeterioration of archived film is still relatively poorly understood. Moreover, chromogenic photographic materials appear to be more susceptible to fungal colonization than black and white materials (Lourenço and Sampaio, 2009), based on the observations of several professionals working with photographic collections, who found that color images are frequently more contaminated than black and white ones. The same general observation has been made for color cinematographic films.

In the present work, the biodeterioration of different color cinematographic films belonging to the Cuban Institute for Cinematographic Industry and Arts (ICAIC) was studied as part of the long-term effort to preserve these culturally valuable materials.

2. Materials and methods

2.1. Sampling

Biodeterioration was grossly observed on cinematographic films stored at the ICAIC and considered to be part of the Cuban cultural heritage. On first examination, it was clear that the films were contaminated by filamentous fungi, as colonies were observed between the loose coils at the beginning of the film spools as well as along the edges of the tightly pressed inner coils. From among the many color films, six were randomly chosen, representing different storage zones of the archive (Table 1). Films were filmed between 1890 and 1991. The archive did not have the right conditions for the preservation of the cinematographic films. Temperature and RH values were 31 °C and 65%, respectively. In September 2010, samples taken under aseptic conditions from the outermost ends of these films were transferred to the Biodeterioration Laboratory at the Technical University of Madrid (UPM), Spain. The samples were preliminarily observed using a stereomicroscope (SZX12, Olympus) to gain an initial impression of the state of the biodeterioration. Areas of interest chosen for further analysis were cut into small fragments using sterile scissors.

2.2. Analysis of the film composition

The composition of representative film fragments was studied by infrared (IR) spectroscopy using a Perkin Elmer *i*-Series IMAGE IR microscope equipped with a 16× Cassegrain objective, incorporating a germanium ATR crystal with a 100-μm diameter contact surface, and an HgCdTe detector, coupled to a Spectrum GX FTIR spectrometer. Spectra were recorded from various positions on both sides of the films at a spectral resolution of 4 cm^{-1} in the range of 4000–580 cm^{-1}, accumulating 20 scans at each sampling point.

2.3. Analysis of biofouling on films

2.3.1. Electron microscopy

Without previous preparation, the film samples were observed by environmental scanning electron microscopy (ESEM) using an INSPECT, QUANTA 200 scanning electron microscope operated at an accelerating voltage of 20–25 kV, in order to assess the degree of deterioration of the support and the type of colonizing microorganisms. Film samples were also observed with SEM. For the latter, the specimens were washed twice with Milli-Q sterile water, fixed with 2.5% glutaraldehyde in sodium cacodylate 0.01 M at 4 °C for 2.5 h, dehydrated with a series of alcohol–water rinses (20, 40, 60, and 80%), and then submerged in these solutions for 30 min at 4 °C. The fixed samples were maintained in a 100% alcohol solution at 4 °C. They were further processed in a critical-point procedure (CPD 030, BAL-TEC) followed by gold sputtering (SCD 005, BAL-TEC) and then observed under a scanning electron microscope (DSM 960, Zeiss) operated at an accelerating voltage of 15 kV.

2.3.2. Study of fungal viability with epifluorescence microscopy

The viability of the microorganisms colonizing the cinematographic films was determined based on epifluorescence microscopy observation of the samples. These were washed twice with Milli-Q

Table 1
Color cinematographic films under study from "The Cuban Institute for Cinematographic Industry and Arts".

Sample	Film title/code	Year
Film 1	*Papeles Secundarios*. Roll #4-3	1989
Film 3	*1425*.	1981
Film 4	*Leyenda*. Key – 21–1698 – II – A	1981
Film 5	*Nueve entradas de pelota*. Key – 21–198 – I – A	1985
Film 6	*Llamada al Sol*. Key – 21–1158 – II	1980
Film 7	*Mascaró, el cazador americano*. Key – 21–1866 – XII – A	1991