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The quality of air at archives and the biodeterioration of photographs

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ABSTRACT

The objectives of the paper were to evaluate the microbial prevalence inside the buildings of the Photographic Library of the National Archive of the Republic of Cuba (PLNARC) and of the Historical Archive of the Museum of La Plata (HAMP) and to estimate the levels of microbial contamination on photos stored in these repositories. We have also examined some of the physiological features of fungal and bacterial isolates in order to evaluate their potential for biodeterioration. A sedimentation method was used for the microbiological sampling of air, while sterile cotton swabs were used for sampling documents. Petri dishes with appropriate selective culture media were used to isolate fungi and bacteria. The cellulolytic, proteolytic, and amylolytic activities and the production of acids and pigments of the fungal isolates were qualitatively determined. The predominant fungal genera in the air of both institutions were *Cladosporium* and *Penicillium*, respectively, for PLNARC and HAMP. At both places, the prevalent bacterial isolates were Gram positive. Among the microbial isolates from photos, various fungal and bacterial isolates were capable of degrading starch and protein and also to excrete acids and pigments, which constitute a high potential risk for the biodeterioration of these documents.

1. Introduction

Knowledge of the microbial contamination inside archive repositories is of great interest because some microorganisms represent a risk for human health (Commission of the European Communities, 1993; Toivola et al., 2002; Scientific Committee on Health and Environmental Risks, 2007) and they are a cause for the deterioration of different stored materials (Nugari and Roccardi, 2001; Shirakawa et al., 2003; Florian, 2003a).

Environmental conditions, such as, air flow, temperature, and staff movement, influence the microbial prevalence inside buildings (Opela, 1992). Climate conditions prevailing in tropical countries favor the increase of dust and the concentration of fungal spores in the air, as well as their sedimentation on the various substrates, facilitating their development. Microorganisms can grow on various materials, causing their biodeterioration. There are different ways in which microorganisms can compromise the structure and function of the substrates (Flemming, 1998). Fungi cause chromatic alterations such as stains of different colours, tonalities, and textures due to mycelial growth and pigment production. When they grow on paper, they degrade all its carbon-containing components such as cellulose, and they excrete organic acids such as oxalic, fumaric, succinic, and acetic acids, which settle over the substrate and acidify it. The highly cellulolytic fungi attack cellulosic fibers and weaken them. Therefore, besides the chromatic alterations they also cause chemical and structural damage (Gallo, 1993; Florian, 2003b; Hidalgo and Borrego, 2006). Other important properties of fungi are related to their pathogenicity for workers involved in collection maintenance (Florian, 2003b; Borrego et al., 2008).

On the other hand, because bacteria require higher humidity conditions, they play a more limited role than fungi do in the biodeterioration of archive and library documents (Guiamet and Gómez de Saravia, 2007; Guiamet et al., 2007). However, they are widely spread in these environments and can cause health disorders (Vaillant, 1996; Valentín et al., 1997). This justifies the need for performing systematic microbiological samplings to estimate the prevalence of microbial contamination, which will help in the management of archive premises in case of water-caused disaster and will provide a better picture of the hazards to which the institutional staff is exposed (Anderson, 2003; Singh, 2003; Florian, 2003b).

In this regard, the objectives of our work were to evaluate the prevalence of microorganisms inside the archive repositories of the

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Photographic Library of the National Archive of the Republic of Cuba (PLNARC) and of the Historical Archive of the Museum of La Plata (HAMP), and to estimate the levels of microbial contamination on photos stored in these repositories. We have also examined some of the physiological features of fungal and bacterial isolates in order to evaluate their potential for biodeterioration.

2. Materials and methods

2.1. Location

Microbiological studies were conducted at the Archive of the Republic of Cuba (PLNARC) and the Historical Archive of the Museum of La Plata (HAMP). A digital thermohygrometer was used to measure the temperature (T) and relative humidity (RH) inside these buildings at each point of sampling. The median values were, at PLNARC: $T = 24.8$ °C, RH = 78%, sunshine indirect light (protected area), ventilated area using natural air; and at HAMP: $T = 23.3$ °C, RH = 43%, sunshine indirect light (protected area).

2.2. Microbiological sampling of air

Sampling were done by passive sedimentation on petri dishes of 90 mm diameter containing malt agar (Merck) supplemented with NaCl (7.5%) (Rojas et al., 2002) for the isolation of fungi, and nutrient agar for bacteria. Plates were opened at approximately 2 m above the floor and they were exposed for 5 min. Five different locations inside HAMP were sampled in triplicate, while at the PLNARC, taking into account the dimension of the premises, only two different locations were sampled, also in triplicate. The malt agar plates were incubated for 7 days at 28 °C and the nutrient agar plates were incubated at 32 °C for 72 h.

In this work, we have estimated the CFU number per cubit meters of air (CFU m^{-3}) according to Omeliansky (Bogomolova and Kirtsideli, 2009), using the formula:

$$N = 5a \times 10^4 (bt)^{-1},$$

where N = microbial CFU m^{-3} of indoor air, a = number of colonies per petri dish, b = dish surface, in square centimeters, and t = exposure time, in minutes.

Although there are no data available to correlate the Omeliansky method for calculation of microbial prevalence in air with the most frequently pump-operated active sampling methods used in the literature, we have used this method for discussion purposes only, to compare the microbial prevalence inside the two buildings examined; we do not pretend the microbial prevalence we have obtained can be compared to results reported from other studies using active sampling.

Relative microbial density was conducted according to Smith (1980) where:

$$\text{Relative density} = \frac{\text{Number of colonies of the genus or species}}{\text{Total number of colonies of all the genera or species}} \times 100$$

2.3. Photographic techniques analyzed

Two photos printed on paper (FA1 and FA2) and one glass slide (FA3) were selected for the microbiological sampling at HAMP. Six different photographic techniques were chosen for the study at PLNARC, namely, two ferrotypes (FC1 and FC2), one tobacco leaf engraved with the image of a Cuban president from the middle of the 20th century (FC3), one photo with emulsion on glass (FC4), and two photos printed in paper (FC5 and FC6).

The ferrotype is a technique that was used between 1860 and 1930. The process consists of obtaining direct positive copies inside the camera onto a thin enamelled iron layer, called a ferrotype plate, upon which the image-forming substance of dark shades (black and coffee-colored) settles. Though the work of carving images on plant leaves is not precisely a photographic technique, it constitutes an artistic way of preserving important images. There are items at PLNARC in which images of presidents of the 19th century and the first half of the 20th are found carved in tobacco leaves.

2.4. Isolation of microorganisms from photographs

Sample collections were performed from a 2-cm^2 surface of each photograph with sterile cotton swabs (Rempel, 1987). The swabs were then immersed in 1 ml of sterile distilled water. The samples were thoroughly shaken and serial dilutions were made. Each dilution was inoculated (0.1 ml) on petri dishes containing various media: malt agar (Merck, Darmstadt) supplemented with NaCl (7.5%) for fungal growth (Rojas et al., 2002); starch agar to determine amylolytic activity; Frazier gelatin agar for proteolytic activity; broth of total acidifying bacteria (BAT), Cetrimide agar, and nutrient agar for bacterial growth. Some of the media were obtained from Merck (Darmstadt). After inoculation, the plates used for counting fungi were incubated at 28 °C for 7 days while those for bacteria were incubated at 32 °C for 72 h. Likewise, 0.1-ml aliquots were added to 5 ml reinforced clostridial medium (RCM), which is a differential medium for sulfite reducing *Clostridium* sp., and were incubated under anaerobic conditions at 32 °C for 5 days.

2.5. Identification of the microbial isolates

In the case of fungal colonies, their cultural and morphological characteristics were observed and the identification was performed according to Barnett and Hunter (1987), as well as website published information (Pitt, 2000; Aspergillus Image Bank, 2006). Morphological (macroscopic and microscopic) features as well as nutrient assimilation were taken into account for the identification of yeast (Carrillo, 2003). Bacteria were grouped on the basis of their Gram staining. The biochemical tests described in *Bergey's Manual of Systematic Bacteriology* (Holt, 1984, 1986) were used for the identification of bacteria.

2.6. Qualitative determination of the cellulolytic activity and the production of pigments by fungi

The fungal isolates were seeded in the culture medium of the following composition, per liter: sodium nitrate, 2 g; dipotassium phosphate, 1 g; magnesium sulfate, 0.5 g; potassium chloride, 0.5 g; ferrous sulfate, 0.01 g; agar, 20 g; and pH 5.5. A strip of filter paper 4.8 cm long and 1 cm wide (equivalent to 50 mg of filter paper) was used as a sole carbon source in one case, and in the other, crystalline cellulose (1%) was used. Glucose (1%) was used as a control. The cultures were incubated at 28 °C for 21 days (Rautela and Cowling, 1986).

2.7. Determination of proteolytic activities

Proteolytic activity was determined using two gelatin hydrolysis assays, one on a petri dish and one in a test tube. For the petri dish assay, the fungal strains were inoculated into a culture medium with the same composition as for cellulose degradation, but gelatin at 5 g l^{-1} was added as a carbon source. After 7 days of incubation at 28 °C, 5 ml of Frazier's reagent were poured over each plate, and the presence of a transparent halo around the growth indicated

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a positive hydrolysis of gelatin (Galiotou-Panayotou et al., 1997). The tube assay was only used to confirm the positive assays in petri dish. In this case, each isolate was inoculated by puncture inside gelatin medium in a test tube. The medium composition was identical to that of the petri dish assay but without agar. The inoculated tubes were incubated for 7 days at 28 °C. The tubes were then stored at 4 °C and a gelatin hydrolysis reaction was evidenced by medium liquefaction when the tubes were inverted (Iwatzu, 1984).

2.8. Determination of amylolytic activities

With the fungi isolated from the air, each strain was streaked on a petri dish containing a minimal medium identical to the one described above containing starch (5 g l^{-1}) as sole carbon source. For the fungi isolated from photos, 0.1-ml aliquots of the serial dilutions were seeded on each petri dish. After 7 days' incubation at 28 °C, 5 ml of Lugol's reagent was poured over each culture plate, and the presence of a colourless zone around the microorganisms was taken as an indication of the positive hydrolysis (Galiotou-Panayotou et al., 1997). In the case of the fungi isolated from photos, the colonies with positive hydrolysis were counted.

2.9. Determination of the production of acids by fungi

A suspension of spores from each fungal isolate was seeded in a liquid minimal medium of composition identical to the one described above, but glucose at 1% was the carbon source and the pH was adjusted to 7. Cultures were incubated at the same temperature for three days before measuring the pH of the culture medium.

2.10. Statistical analysis

The Student test was used to evaluate differences in the microbial prevalence of the air of the repositories. Results with $p < 0.05$ were considered statistically significant.

3. Results

3.1. Microbial contamination in air

The fungal and bacterial prevalence in air at the PLNARC and HAMP are shown in Table 1. Data show a higher prevalence for both bacteria and fungi at PLNARC than at HAMP. *Cladosporium* prevailed in the air of PLNARC (87.1%) while *Penicillium* was the prevalent genus in the air of HAMP (48.6%), followed by *Aspergillus* (17.1%). Also, *Geotrichum*, *Curvularia*, *Fusarium*, and *Neurospora* were genera isolated in low percentages (Fig. 1).

Physiological characterization of fungal isolates revealed that most of them grew at the expense of filter paper as sole carbon source, whereas nearly 20% grew abundantly on crystalline cellulose (α-cellulose) (Table 2). Likewise, most of the isolates showed amylolytic activity and approximately 50% of them showed proteolytic activity and all of them produced acids, but only few strains excreted pigments on paper.

Table 1
Microbial prevalence in the air at PLNARC and at HAMP.

Repository	Fungi (CFU m^{-3})	Bacteria (CFU m^{-3})
PLNARC	492.8[a]	1010.4[a]
HAMP	229.9[b,c]	176.9[b,c]

[a] Represents the median value of the measurements carried out in two different points by triplicate.
[b] Represents the median value of the measurements carried out in five different points by triplicate.
[c] Indicates significant differences according to the Student test ($P < 0.05$) on comparing the microbial concentration between the repositories studied. The microbial determination was made by triplicate and the data averaged ($n = 3$).

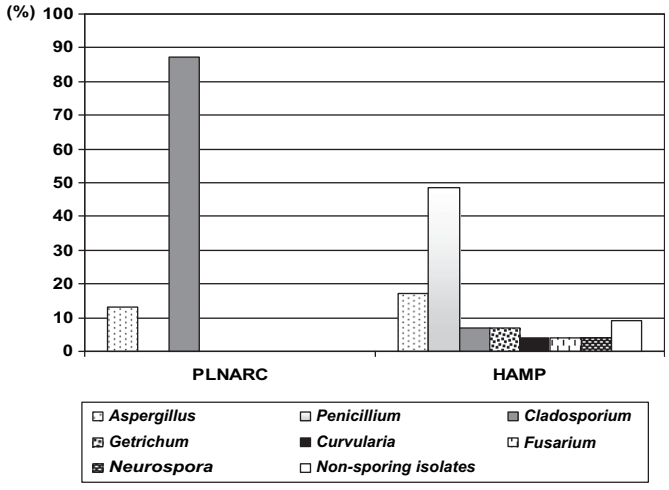

Fig. 1. Relative density of the fungal genera isolated from the air of PLNARC and HAMP.

With respect to the bacterial isolates from air, Gram-positive bacteria were the most abundant at both repositories. At HAMP only Gram-positive bacteria were detected while at PLNARC they represented about 74% of total bacterial isolates (Table 3).

On the basis of biochemical tests, *Staphylococcus*, *Streptococcus*, *Micrococcus*, *Streptomyces*, and *Bacillus* genera were isolated among Gram-positive bacteria. Among Gram-negative bacteria, we have detected strains of *Serratia marcenscens*, *Serratia* sp., *Enterobacter agglomerans*, *Acinetobacter* sp., *Azotobacter* sp., *Beijerinckia* sp., and *Pseudomonas* sp.

3.2. Microbial contamination on photographs

The occurrence of microorganisms isolated from photos randomly selected is shown in Table 4. As can be seen, bacteria were the most prevalent organisms, but a high number of fungi were only found in three cases, and of yeasts in two cases from the photos of PLNARC.

The prevalence of bacteria isolated from photos at HAMP is higher than for the photos at PLNARC (Table 4). This result is not consistent with the bacterial prevalence we have observed in air sampling of these buildings (Table 1).

The physiological features shown by the bacterial strains isolated were various (Table 5), which evidences the variability of the population colonizing these documents, though there was a preponderance of amylolytic and proteolytic bacteria. In the case of fungal strains, a high prevalence of proteolytic and amylolytic isolates was also observed (Table 6).

Table 7 shows the different types of microorganisms isolated from the photos. As can be seen within fungi, *Aspergillus* and *Penicillium* were the only genera isolated from three of them. Different strains of yeast were also isolated from an engraving on a tobacco leaf and a glass plate that were stored at PLNARC. Moreover, principally Gram-positive but also Gram-negative bacteria were isolated from a majority of samples. Different groups of bacteria were determined and *Clostridium*, *Bacillus*, and *Pseudomonas* were some of the genera identified.

4. Discussion

Our analysis shows a higher microbial prevalence at PLNARC than at HAMP. This may be due to the fact that HAMP is surrounded

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Table 2
Qualitative cellulolytic, proteolytic and amylolytic activities, production of pigments and acids by the fungi isolated from air.

Location	Strain	Degradation of cellulose			Production of acids	Amylolytic activity	Proteolytic activity
		Growth on filter paper	Growth on crystalline cellulose	Pigment production[a]	pH	Starch degradation	Degradation of gelatin
PLNARC	*Cladosporium* sp. 1	+++	+	+	3.93	−	+
	Cladosporium sp. 2	±	±	−	6.42	+	+
	Cladosporium sp. 3	+	±	−	6.47	+	+
	Cladosporium sp. 4	++	++	+	6.90	−	−
	Cladosporium sp. 5	++++	+++	+	6.87	−	+
	Cladosporium sp. 6	++++	+++	+	6.70	+	−
	Cladosporium sp. 7	++	+++	+	6.86	−	−
	Aspergillus flavus	++	±	−	5.99	+	+
HAMP	*Penicillium* sp. 1	++	−	+	5.10	+	+
	Penicillium sp. 2	−	−	−	5.26	−	+
	Penicillium sp. 3	++	−−	+	5.83	+	+
	Penicillium sp. 4	−	−	+	5.73	+	+
	Aspergillus sp. 1	+++	±	+	3.90	+	+
	Aspergillus sp. 2	±	−	−	5.20	+	+
	Aspergillus sp. 3	++	+	+	6.10	+	+
	Aspergillus sp. 4	++	±	−	4.46	+	+
	Fusarium sp. 1	+++	++	+	5.95	+	−
	Fusarium sp. 2	+++	−	−	5.32	+	+
	Curvularia sp. 1	+++	±	−	6.77	+	−
	Alternaria sp. 1	+++	+++	+	6.54	+	−
	Geotrichum sp. 1	+++	+	−	5.70	+	+
	Geotrichum sp. 2	++	+	−	6.37	+	+

+++: Indicates abundant growth, ++: Indicates moderate growth, +: Indicates poor growth, it is also indicative of the presence of pigment, ±: Indicates very poor growth or production of pigment, −: Indicates NO growth and NO production of pigment.
[a] The production of pigments was evidenced on the filter paper strip.

by a forest, whereas PLNARC is located in a zone with few trees, very close to the harbour and factories, and in the vicinity of a very busy street, conditions likely to favor the inflow of large amounts of dust. Our data are consistent with those obtained by Guiamet et al. (2007). In our investigation air sampling was done by passive sedimentation and the microbial prevalence was determined using the Omeliansky formula. Both of these approaches are not frequently reported for estimation of microbial prevalence in air. Nevertheless, the microbial prevalence in the air of PLNARC is low in comparison to studies previously performed (particularly for bacteria) and using the same approach (Borrego et al., 2008).

Despite the fact that there is no international standard to determine whether an indoor environment as contaminated or not, it has been suggested that environments with a microbial prevalence above 1000 CFU m^{-3} should be considered contaminated (Wonder Makers Environmental, Inc., 2001). Similarly, Eagle Industrial Hygiene Associates (2004) suggests that indoor microbial concentration should be lower than 1000 CFU m^{-3}. Other authors consider that microbial prevalence should not exceed 750 CFU m^{-3}—above this level the environment is regarded as contaminated (Radler de Aquino and de Góes, 2000)—and still other authors consider that 300 CFU m^{-3} should be the lower limit for fungi (Kolwzan et al., 2006).

Table 3
Relative density of the different bacterial groups isolated from the air.

Gram staining	PLNARC	HAMP
	Relative density (%)	
Gram-positive cocci	59.0	69.6
Non-sporing Gram-positive bacilli	10.3[a]	8.7
Sporulated Gram-positive bacilli[b]	5.1	21.7[c]
Gram negative	25.6	−

[a] It indicates that 2% correspond to *Streptomyces* sp.
[b] It indicates that they correspond to *Bacillus* sp.
[c] It indicates significant differences according to the Student test ($P < 0.05$) upon comparing the sporulated Gram-positive bacilli obtained in both repositories.

The fact that the fungal genus *Cladosporium* prevailed (87.1%) at PLNARC is worrying since this fungus is known for its allergenicity (Indoor Air Quality Association Inc., 1995). In the case of HAMP, *Penicillium* was the prevailing fungus. This genus is most frequently found in indoor air and buildings in cold and temperate climate regions (Frisvad and Gravesen, 1994; Hyvärinen et al., 2002). *Penicillium*, like *Aspergillus*, is considered to be a primary colonizer, since both can grow at low water activity (a_w) values ($a_w < 0.8$). Other fungal genera (e.g., *Alternaria*, *Cladosporium*, and *Phoma*) require a_w values at least between 0.8 and 0.9 (Nielsen, 2003). At the time this experiment was performed the RH at PLNARC was very high (78%), which could explain the high prevalence of *Cladosporium*. Other fungal genera were not detected despite the existence of a high RH due to the natural ventilation of the repository, which facilitates the movement of particles in the air and makes their settlement difficult. This is particularly so for fungal spores that do not settle easily, as in the case of *Aspergillus*

Table 4
Prevalence of microorganisms on photos of different supports.

Code[a]	Type of document	Location	Bacteria (CFU cm^{-2})	Fungi (CFU cm^{-2})	Yeasts (CFU cm^{-2})
FA1	Photo on paper support 1	HAMP	2.4×10^6	2.0×10^5	−
FA2	Photo on paper support 2	HAMP	2.0×10^5	−	−
FA3	Glass slide 3	HAMP	4.5×10^5	1.0×10^4	−
FC1	Ferrotype 3	PLNARC	2.5×10^3	−	−
FC2	Ferrotype 4	PLNARC	3.5×10^3	−	−
FC3	Engraving on a tobacco leaf	PLNARC	2.5×10^4	2.8×10^4	1.0×10^4
FC4	Glass plate	PLNARC	5.0×10^4	−	3.6×10^6
FC5	Photo on paper support 1	PLNARC	3.8×10^2	−	−
FC6	Photo on paper support 2	PLNARC	2.0×10^3	−	−

[a] Code: FA documents from HAMP, FC documents from PLNARC.

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Table 5
Prevalence of bacterial isolates from photos exhibiting different physiological characteristics.

Code[a]	Total aerobic bacteria[b]	Amylolytic bacteria	Proteolytic bacteria[c]	Acidifying bacteria[d]	*Pseudomonas* sp.[e]	Sulphite reducing bacteria[f]
	CFU cm^{-2}					
FA1	2.4×10^6	–	3.8×10^5	–	–	+
FA2	2.0×10^5	–	5.0×10^5	–	–	+
FA3	4.5×10^5	2.5×10^5	8.8×10^4	–	+	+
FC1	2.5×10^3	1.9×10^2	–	–	–	–
FC2	3.5×10^3	–	2.3×10^2	–	–	–
FC3	2.5×10^4	–	1.5×10^2	–	–	–
FC4	5.0×10^4	–	5.6×10^2	+	–	–
FC5	3.8×10^2	2.8×10^2	1.0×10^2	–	–	–
FC6	2.0×10^3	2.0×10^2	1.9×10^2	–	–	–

[a] Codes are same as for Table 4.
[b] Determined on Nutrient Agar.
[c] Determined on Frazier Gelatin Agar.
[d] Determined on BAT.
[e] Determined on Cetrimide Agar.
[f] Determined on DRCM.

fumigatus, which, due to their equinulated shape, are stable in the air (Levetin, 2002). On the other hand, even though the outdoor environment was not analyzed, it is possible that *Cladosporium* and *Aspergillus* were the prevailing genera at the time of the sampling and that they might have been introduced inside the building. However, the fungal genera prevailing in the air of both archives (*Cladosporium*, *Aspergillus*, and *Penicillium*) are commonly found in indoor environments of houses, archives, libraries, and museums (Valentín et al., 1997; Maggi et al., 2000; Rojas et al., 2002; Gorny et al., 2002; Hidalgo and Borrego, 2006; Borrego et al., 2008).

It is known that the majority of the fungal strains isolated from the air of archives, libraries, and museums exhibit cellulolytic, proteolytic, and/or amylolytic activities; produce acids; excrete different pigments; and contribute to the formation of biofilms, which accelerate the deterioration of the different document substrates (Vaillant, 1996; Hidalgo and Borrego, 2006; Guiamet and Gómez de Saravia, 2007; Guiamet et al., 2007; Abrusci et al., 2007; Borrego et al., 2008). Our results are consistent with these findings.

Our observation that Gram-positive bacteria prevailed among bacterial isolates from both buildings is consistent with other studies performed in the same type of buildings (Valentín et al., 1997; Guiamet et al., 2007; Borrego et al., 2008). *Staphylococcus*, *Streptococcus*, *Micrococcus*, *Streptomyces*, and *Bacillus* genera were identified at PLNARC. These genera have been isolated from archive environments (Vaillant, 1996; Valentín et al., 1997; Borrego et al., 2008).

Staphylococcus and *Streptococcus* genera are widespread in nature. They are major components of the microbial flora of skin and mucous membranes of animals (Kloos et al., 1992). It could explain why they prevailed in archive environments.

Some species of *Staphylococcus*, *Streptococcus*, and *Bacillus* are human pathogens (Isenberg and D'Amato, 1991; Santini et al., 1995; Miller et al., 2004). Furthermore, it has been reported that some species of *Streptomyces* are noxious to the human respiratory system (Dutkiewiecz et al., 1988; Hirvönen et al., 1997). In addition, *Bacillus* and *Streptomyces* constitute a potential risk for photos on paper support, because they can produce cellulases (Ramírez and Coha, 2003).

Gram-negative bacteria such as *S. marcenscens*, *Serratia* sp., *Pseudomonas* sp., and *E. agglomerans* have been detected previously in indoor archive or library environments (Valentín et al., 1997; Borrego et al., 2008). Gram-negative bacteria produce endotoxins composed of lipopolysaccharides, which are related to the bacterial membrane, and upon being inhaled they can cause irritation of the respiratory system, resulting in fever, shivers, malaise, and headache. Continued exposure to this type of bacteria may lead to bronchitis and asthma. Within this group of bacteria, it is reported that *S. marcenscens* and *Enterobacter* sp. are associated with respiratory diseases (America's Most Wanted Biological Agents, 2003).

A high prevalence of microorganisms was isolated from all the different photographic substrates analyzed. Their physiological characteristics were varied, but, notably, there was a high prevalence of bacteria and fungi exhibiting proteolytic and amylolytic activities. With respect to proteolytic activity, it has recently been demonstrated that many bacteria can colonize gelatin during photographic

Table 6
Prevalence of fungi isolates from photos exhibiting different physiological characteristics.

Code[a]	Total fungi[b]	Amylolytic fungi[c]	Proteolytic fungi[d]
	CFU cm^{-2}		
FA1	2.0×10^5	1.9×10^2	1.5×10^4
FA3	1.0×10^4	1.2×10^2	0.6×10^3
FC3	2.8×10^4	0.5×10	–

[a] Codes are same as for Table 4.
[b] Determined on Malt Agar.
[c] Determined on Starch Agar.
[d] Determined on Frazier Gelatin Agar.

Table 7
Types of microorganisms isolated from the photos.

Type of microorganism and/or morphological characteristics	FA1	FA2	FA3	FC1	FC2	FC3	FC4	FC5	FC6
Fungi									
Aspergillus sp.	+	–	–	–	–	–	–	–	–
Penicillium sp.	–	–	+	–	–	+	–	–	–
Yeasts									
Small, white colony, mucous.	–	–	–	–	–	–	+[a]	–	–
Small colony of intense golden yellow colour, mucous	–	–	–	–	–	+[b]	–	–	–
Small, pale, creamy, orange colored colony	–	–	–	–	–	+[c]	–	–	–
Bacteria									
Sporulated Gram-positive bacilli	+[d]	+[d]	+[d]	–	–	+[e]	–	–	–
Non-sporing Gram-positive bacilli	–	–	–	+	–	+	–	–	–
Gram-positive cocci	–	–	–	–	–	+	–	–	–
Gram-negative bacilli	–	–	+[f]	–	–	–	+	–	–
Gram-negative cocci	–	–	–	–	–	–	–	–	–

[a] It indicates the presence of *Candida parapsilosis*.
[b] It indicates the presence of *Cryptococcus* sp.
[c] It indicates the presence of *Rhodotorula* sp.
[d] It indicates the presence of *Clostridium* sp.
[e] It indicates the presence of *Bacillus* sp.
[f] It indicates the presence of *Pseudomonas* sp.

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manufacturing processes. In a previous investigation *Bacillus* strains were among the contaminating organisms (Stickley, 1986). In addition to *Bacillus*, which is a predominant bacterium among our isolates, different non-sporulating Gram-positive bacterial strains such as *Staphylococcus* and *Streptococcus* are known to have the ability to liquefy gelatin (De Clerck and De Vos, 2002).

De Clerck et al. (2004) reported that in semi-final gelatin extracts almost all the bacterial contaminants belonged to the genus *Bacillus* or other related endospore formers (*Brevibacillus*, *Geobacillus*, and *Anoxybacillus*), although different *Enterobacter*, *Staphylococcus*, and *Streptococcus* species were also identified. These authors concluded that at least some spores or vegetative cells were able to resist the final treatment used to prevent the potential microbial contamination during the gelatin manufacturing process. Similarly, Abrusci et al. (2005) demonstrated that the gelatin was degraded by some species of *Bacillus* and *Staphylococcus*. In this work, only one Gram-negative genus, *Pseudomonas*, was detected on a photograph (FA3); this is also a bacterial genus containing species able to contaminate gelatin during the photographic manufacturing process (Stickley, 1986).

It is known that many fungal genera exhibit proteolytic activity and they are able to biodegrade the emulsion of different photographic techniques. *Aspergillus* and *Penicillium* are among the genera in this group (Abrusci et al., 2005; Bogomolova et al., 2007). Similarly, filamentous fungi are very important microbial agents involved in biodeterioration and they can degrade many kinds of substrates (Abrusci et al., 2007; Bogomolova et al., 2007).

The cellulolytic activity of the microbial isolates from photos was not measured in the current study, but it is known that many strains of the *Clostridium* genus can produce cellulases (Ágoston-Szabó et al., 2006).

Finally, with respect to yeasts, it was only possible to detect them on two substrates (FC3 and FC4), and not in the air. Of the genera identified it is known that *Candida* (*Candida parapsilosis*), *Rhodotorula*, and *Cryptococcus* contain species that are harmful to human health (Solla et al., 2008; Castañón et al., 2000; Singh, 2005; Comarú et al., 2005). Of these, *Cryptococcus* sp. had never previously been isolated in the National Archive of Cuba (Valentín et al., 1997; Borrego et al., 2008). In conclusion, data from this work show the prevalence in ambient air and on document substrates in archives of many fungal and bacterial strains that are likely to cause damage to these documents.

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