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Fungi in Archive Repositories Environments and the Deterioration of the Graphics Documents

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Abstract

The aims were to characterize the mycobiota in two micro-environments (aeromycobiota of two repositories and fungi isolated from graphics documents surfaces) and to analyze their relation as well their impact on documentary conservation and health. The air samples were made using a biocolletor SAS in two repositories (Maps library -ML-, and Photographic library -PL-) and Malt Extract Agar (MEA) were used. The surfaces of different graphics documents were sampled with sterile and wet swabs and seeded on MEA. The fungal concentrations on indoor air of ML and PL were 38 and 44 CFU/m^3 respectively; hence the environments were classified as not contaminated. The concentrations obtained from the materials ranged between 10 and 10^3 CFU/cm^2. The predominant genera in both ecological micro-environments were *Aspergillus*, and *Penicillium*. Also, the genera *Cladosporium* and *Pestalotia* were in third place of prevalence in the indoor air of PL and ML respectively. The cellulose, gelatin and starch were degraded by the majority of the isolates. Three pathogenic attributes in species of *Aspergillus* and *Penicillium* were analysed. Seven species of *Aspergillus* spp. and eight of *Penicillium* spp. were able to grow at 37°C; some of them produced hemolysins and phospholipases. Near of 40% of fungal species were coincident in the two studied ecosystems. Also, it was demonstrated that all fungal species isolated are risky for the documents and some of them are risky for the workers' health. For the first time in the Cuban archives a study of the interrelationship between the mycobiota existing in the indoor air of the archives and the one obtained from documents as well as their impact on the health is realized.

Keywords: *Aeromycobiota in Archives; Airborne Fungi; Microbiological Quality of the Indoor Environment; Graphics Documents Degradation by Fungi; Pathogenic Fungi*

Introduction

Paper has been the most dominant tool for recording human knowledge and its deterioration is one of the most serious dangers for libraries and archives. Studies have shown that majority of people use printed materials despite of existence and currency of electronic copies. So, the importance of preserving and maintaining printed materials is crucial for librarians, archivists and specialists of information centers.

Documentary heritage materials like books, maps, drawings, films, as well as other paper or parchment documents preserved in indoor environments of libraries, archives and museums are subject to deterioration caused by physical, chemical and biological agents, and can result in heavy economic and cultural losses. Particularly, materials made of organic compounds can be deteriorated for bacteria, fungi, insects and rodent [1].

Multiple studies have been conducted to evaluate the effect of chemical, physical and biological contamination in environments of repository of archives, libraries and museums where documents and artworks are stored [1, 2-11]. Hence, one of the main goals of any preventive approach should be to limit the microbial deterioration of cultural heritage and to prevent diseases in staff and visitors.

During the last decades, a strong correlation between aerobiology and microbial deterioration of artworks has been demonstrated, due to the fact that the air is the main vehicle for the dispersion of microorganisms [12,13]. Generally airborne microorganisms are similar or identical to those isolated from deteriorated artworks and, in many cases; a direct effect was inferred [5,14-16]. Hence surfaces analysis complements microbiological characterization of the air. In fact, in most of the studies on environmental fungal assessment, the results have been achieved only by air sampling. Because mycological contamination can be a result of fungal spores' dispersion in the air and on the surfaces, it is very important to assess it and evaluate a specific environment based on the results of both types of the samples [17].

Filamentous fungi are organisms with an important role in the degradation of organic waste, such as wood and paper [18]. The fungal ability to produce extracellular enzymes is well established. They can produce hydrolytic enzymes such as cellulase, xylanase, pectinase, etc. Also, they spoil valuable documents mechanically, chemically and aesthetically because they form hyphae, produce and excrete pigments and organic acids [19].

The majority of fungi need a high relative humidity and temperature to grow and develop, its development is enhanced in microclimatic environments caused by condensation, but some fungal species are able to live at low water activities for that are classified as xerophilic fungi; they are perfectly adapted to indoor environments and thrive in dusty environments, lack of ventilation or water retention by hygroscopic materials for these materials with a very low water activity can be colonized by xerophilic species [20,21]. They can be found in the indoor air of archives, libraries and museums where much paper exists. Dust is a good source for these fungi to feed and grow; these conditions intensify fungal contamination [22]. Fungal degradation and the documentary materials deterioration is a worldwide problem that causes great damage to especially paper documents stored in the archives, libraries and museums [23,24]. There are few studies about fungal contaminations in libraries and archives [4,14-16,18,22-33].

The environments of archives and libraries provide nutritional requirements for the growth of fungi on aging paper and paper glues. So, according to the geographical location and weather condition, archives and libraries contain more fungi both qualitatively and quantitatively compared to other enclosed spaces. Since paper is made of cellulose and other materials, it can be degraded and deteriorated by fungi. Therefore, protecting documentary materials is necessary since manuscripts, books, magazines, paintings, photographs and made primarily of paper. Other important properties of fungi are related to their pathogenicity for workers involved in collection maintenance [34]. The virulence factors are attributes of a pathogenic microorganism conducive the emergence in the susceptible host of affections or diseases. These vary depending on taxonomic groups or even strains [35]. With the aerobiological studies in archives, libraries and museums fungal taxa of the environment with a greatest potential of degradation and pathogenicity can be determined, important issues for preventive conservation strategies and quality of life of staff.

For these reasons, the aims of this research were to characterize the mycobiota in two micro-environments (aeromycobiota of two repositories and fungi isolated from graphics documents surfaces) and to analyze their relation as well their impact on documentary conservation and health.

Materials and Methods

Repositories Studied

Microbiological studies were conducted in two repositories of the National Archive of the Republic of Cuba. One of them is the Maps library (ML) characterized by large dimensions (15.2 x 6.2 x 5m of length x width x height) and located in first floor while the Photographic library (PL) is a small local (6 x 2 x 3m) located in lower-ground floor of building. These local are acclimatized, the light is artificial and their incidence is very low because these local turned off and the documents are protected by folders and adequate furniture.

Sampling of aeromycobiota

Two samplings were done using a portable volumetric microbiological air sampler (SAS Super 100, Italy). The impactor air sampler was used to collect 100L of air for 1 min at a height of 1.5m approximately. Five different points were analyzed by triplicate in ML whilst only two points by triplicate were sampled in PL taking into account the dimension of the repositories.

The fungal propagules were impacted onto surfaces of Petri dishes with Malt Extract Agar (MEA) (BIOCEN, Cuba) supplemented with NaCl (7.5%) [33,36]. This medium facilitates the growth of halophilic fungi and some xerophilic species; also, it is used to limit the colonies growth of Mucorales and inhibit the growth of the majority bacterial species. Measurements of temperature (T) and relative humidity (RH) were conducted at the moment of the microbiological determination using a digital hygrometer Pen TH 8709 (China).

The plates were incubated for 7 days at 30°C. Then, the colony count was performed and the necessary calculations of air were made in order to determine the fungal concentration expressed in colony forming units per cubic meter (CFU/m^3).

Ecological criteria to the environmental isolates in each repository

Relative density (RD) of fungal genera isolated from indoor air of each repository was conducted according to Smith (1980) [37] where:
RD = (Number of colonies of the genus or species/Total number of colonies of all genera or species) x 100

The degree of contamination of the indoor environment of two repositories was determined according to Roussel., *et al*. (2012) [7] based on four levels: low (less than 170 CFU/m^3), moderate (between 170 and 560 CFU/m^3), high (between 560 and 1000 CFU/m^3) and very high (greater than 1000 CFU/m^3).

Documentary Analyzed Materials

A total of twenty-three documents of 19th and 20th centuries were analyzed. Three of them were plant leaves carved with the image of Cuban personalities and nine different photographic techniques. Also, eleven maps were studied. Three of them were made on textile as primary support; two have a paper as primary support and textile as second support and six have different kind of paper.

These documents are considered specials materials according to the characteristics of the support and pigments and are maintained in a repository with an appropriate furniture and packaging as well as under conditions of low temperature and relative humidity. Therefore, all documents were clean and showed no visual evidence of fungal affectation.

Sampling of Surface Mycobiota

Sample collections were performed from a 2 cm^2 surface of each graphic material with sterile cotton swabs [1,8]. The swabs were then immersed in 1 ml of sterile physiological solution. The samples were thoroughly shaken and serial dilutions were made. Each dilution was inoculated (0.1 ml) on Petri dishes containing MEA supplemented with NaCl (7.5%). After, the plates were incubated at 30°C for 7 days. Fungal concentration was reported in CFU/cm^2.

Identification of the fungal isolates

Cultural and morphological characteristics of fungal colonies as well as conidiophores and conidia fungal structures were observed (stereomicroscope and microscope Olympus at 14X and 40X respectively) and the identification was performed according to different manuals [38-43]. In identifying yeast, the morphological features (macroscopic and microscopic) as well as nutrient assimilation were taken into account [44].

Determination of the relative frequency (RF) of the fungal genera isolated from graphics documents and their ecological categories in each ecosystem

The relative frequency (RF) determination was made according to Esquivel., *et al*. (2003) [45] to determine the ecological category of the fungi general isolated. It was necessary to use the following formula:
RF = (Times a genus is detected/Total number of sampling realized) x 100

The ecological categories are: Abundant (A) with RF = 100 - 81%; Common (C) with RF = 80 - 61%; Frequent (F) with RF = 60 - 41%; Occasional (O) with RF = 40 - 21%; Rare (R) with RF = 20 - 0%.

Determination of biodegradation potential of the fungal species isolated

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

The isolated fungi were seeded in slants on a saline culture medium of the following composition per liter: sodium nitrate, 2g; dipotassium phosphate, 1g; magnesium sulphate, 0.5g; potassium chloride, 0.5g; ferrous sulphate, 0.01g: agar 20g; and pH 5.5. In one case, a strip of filter paper Whatman No. 1 (4.8 cm of long for 1 cm of wide, equivalent to 50 mg of filter paper) was used as a sole carbon source and in the other, crystalline cellulose (1%) was used. The cultures were incubated at 30°C during 21 days [22,26,31].

Determination of the production of acid

A suspension of spores from each isolated fungus was seeded in a minimal liquid medium of identical composition to the one above, but with glucose at 1% as carbon source, phenol red at 0.03% and the pH was adjusted to 7. The cultures were incubated at 30°C for 3 days. A change of color from red to yellow is indicative of the production of acids and the pH of the culture medium was measured using a pH meter [22].

Qualitative determination of the proteolytic activity

Proteolytic activity was determined using the gelatin hydrolysis assay in a tube test. In this case, each isolate was inoculated by puncture inside gelatin medium in a test tube. The medium composition was identical to that before assays, but gelatin at 120 g/l was added as the carbon source. The inoculated tubes were incubated for 7 days at 30°C. Afterwards they were stored at 4°C and a gelatin hydrolysis reaction was evidenced by medium liquefaction when the tubes were inverted [22,26,31].

Qualitative determination of the amylolytic activity

Each isolated fungal strain was seeded in a Petri dish with a saline composition similar to the one previously used and starch was (5 g/l) employed as the carbon source. After 7 days of incubation at 30°C, 5 ml of Lugol's reagent were added over each culture plate, and the presence of a colorless zone around the colonies was taken as an indication of the positive hydrolysis [22,26,31].

Determination of virulence factors related to the fungal pathogenicity in species belonging to the genera *Aspergillus, Penicillium* and *Candida*

Growth on 37°C

The species were seeded in Petri dishes with Extract Malt agar medium and were incubated for 7 - 10 days at 37°C [46]. A growth of the species was considered as a positive result.

Qualitative determination of hemolytic activity

The hemolytic activity was only evaluated in the strains able to grow at 37°C for the following method. 5 ml of sheep blood defibrinated and chloramphenicol at 0.05% were aseptically added to 95 ml of Czapek agar at 45°C. Each strain was inoculated by puncture in Petri dish and incubated at 37°C by 10 - 14 days. A clear or green halo surrounding the colonies was considered as a positive result [47].

Qualitative determination of phospholipase activity

Strains able to grow at 37°C were seeded on agarized culture medium with a composition for 500 ml: 5g bacteriological peptone, 10g glucose, 29.3g sodium chloride and calcium chloride 2.3g. The pH of the medium was adjusted to 4 and then it was sterilized. When the medium is cooled (45°C approximately), 2 egg yolks aseptically were added. Subsequently the plates were incubated at 37°C for 7 days. The experiment was performed in triplicate in each case. The activity was evidenced by the appearance of a transparent halo around the colony in the light-yellow medium; product precipitated forming salts [47].

Statistical Analysis

The data obtained were analyzed using the statistical program Statgraphics Centurion XV. The Student test was used to determine differences between the fungal propagules concentrations obtained in the repositories studied.

Results

Behavior of aeromycobiota on the indoor air of the studied repositories

The fungal concentration average obtained in the ML air was 50 CFU/m^3 while the average concentration in PL was 44 CFU/m^3. Significant differences were not detected in the fungal concentrations between the repositories according to Student's t test ($P \leq 0.05$) in spite of the differences in the size of them (Table 1). Also, the mean values T and HR at the time of sampling were: Map Library: T = 24°C, RH = 52%, and Photo Library: T = 23°C, HR = 50%.

	Map library (ML)			Photo library (PL)		
Concentrations	**Fungi (CFU/m^3)**	**T (^{0}C)**	**HR (%)**	**Fungi (CFU/m^3)**	**T (^{0}C)**	**HR (%)**
Maxim	84	25	53	99	24	51
Minimum	21	22	51	10	22	49
Average	50 ± 29	24 ± 1	52 ±1	44 ± 31	23 ± 1	50 ± 1

Table 1: *Concentrations of fungal propagules in the indoor air of two repositories which conserve graphic documents.*

The microbial determination was made in 5 (ML) and 2 points (PL) by triplicate, respectively and the data averaged (n = 15 in ML, n = 6 in PL).

As no specific microbiological international standard exists for indoor quality in the field of cultural heritage preservation, microbiological contamination was then determined by referring to the classification of Roussel., *et al*. (2012) [7]. According to this both repositories are not contaminated.

Ten fungal genera and one pigmented septate mycelium were detected in the ML indoor environment while six genera were detected in PL (Figure 1). *Aspergillus* spp. was the predominant one in the air of ML (30.7%, equivalent to 12 CFU/m^3) followed by *Penicillium* spp. (22.2%, equivalent to 8 CFU/m^3) whilst in PL the opposite occurred because *Penicillium* was the predominant genus with 35% (equivalent to 15 CFU/m^3) followed by *Aspergillus* spp. (30%, equivalent to 13 CFU/m^3). The third place of prevalence was occupied by genus *Pestalotia* (10.1%, equivalent to 4 CFU/m^3 approximately) on the ML whilst in PL was the genus *Cladosporium* (17%, equivalent to 7 CFU/m^3). Other genera isolated were *Curvularia, Alternaria, Fusarium, Cephalosporiun, Epicoccum, Trichoderma* and *Bipolaris* in a percentage lower than 10%.

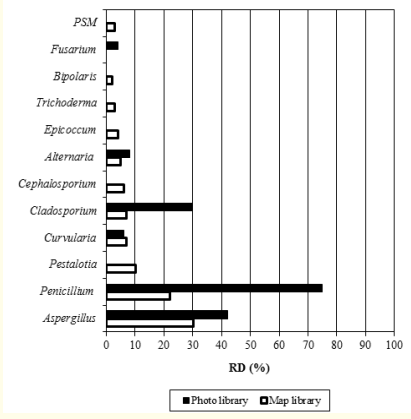


Figure 1: *Relative density (RD) of the fungal genera isolated from indoor air of two repositories.*

PMS: Pigmented and septate mycelium.

As *Aspergillus, Penicillium* and *Cladosporium* were the predominant genera, the RD of all species isolated was determined in both repositories (Table 2). The most species were isolated in the two locals, but among the species with RD higher than 20% in ML were detected *Aspergillus flavus, A. niger, A. ostianus, Cladosporium cladosporioides, Penicillium chrysogenum* and *P. citrinum*. In PL, the species with RD higher than 20% were *A. niger, C. cladosporioides, P. chrysogenum* and *P. janthinellum,* but the species *Aspergillus alliaceus* and *A. unilateralis* were detected with an RD of 20%. Of all isolated species, *A. niger, C. cladosporioides* and *P. chrysogenum* were the only detected in both repositories with high RD. Other species were isolated in lower concentration such is the case of *Cladosporium elatum* detected in PL and ML (with 17.3% and 15.4% respectively), and *Penicillium simplicissimum* (15.8%) detected only in ML.

Species	Map library	Photo library
	RD (%)	
Aspergillus alliaceus Thom and Church	6.0	20.0
A. auricomus (Guegen) Saito	6.0	8.0
A. candidus Link	7.0	5.0
A. flavus Link	23.0	9.0
A. japonicus Saito	5.0	5.0
A. niger van Tieghem	25.0	23.0
A. oryzae Cohn	7.0	0
A. ostianus Wehmer	21.0	0
A. penicillioides Speg.	0	4.0
A. ungüis (Weill & L. Gaudin) Dodge	0	6.0
A. unilateralis Thrower *	0	20.0
Cladosporium cladosporioides (Fres.) de Vries. (complex)	31.8	35.5
C. herbarum (Pres.) Link ex Gray	14.0	12.8
C. sphaerospermum Penz	13.7	11.5
C. oxysporum Berk & Curt	14.0	9.7
C. fulvum Cooke	11.1	13.2
C. elatum (Harz) Nannf	15.4	17.3
Penicillium canescens Sopp	10.0	5.8
P. chysogenum Thom	27.0	25.4
P. citrinum Thom.	25.0	4.6
P. griseofulvum Dierckx	0	12.0
P. janczewskii K.M. Zalessky	0	8.0
P. janthinellum Biourge	0	23.0
P. simplicissimum (Oudem.) Thom	15.8	3.5
P. waksmanii K.M. Zalessky	10.6	6.1
P. wortmannii Klöcker	0	2.5
Penicillium sp. Link. Ex Fries	11.6	9.1

Table 2: *Relative density (RD) of the fungal species corresponding to the dominant genera detected in the indoor air of the two studied repositories.*

****:*** *Indicates that this specie corresponds to the Aspergillus fumigatus complex.*

Behavior of the fungal isolates from graphics documents

Table 3 shows the concentrations of fungi isolated from the different graphics documents which are preserved in studied repositories in furniture and wrappers appropriate. The concentrations of the fungi on photos and plant leaves carved ranged between 10 - 10^3 CFU/cm^2 whilst on maps the concentrations ranged from 0 - 10^2 CFU/cm^2.

Code	Material analyzed	Characteristics of the support	Fungal concentration (CFU/cm^2)
P1	Different photographic processes and plant leaves carved	Tobacco leaf carved 1	2.0 x 10^2
P2		Tobacco leaf carved 2	5.0 x 10^2
P3		Poplar leaf carved	1.0 x 10^3
P4		Ferrotype (iron support) 1	5.0 x 10
P5		Ferrotype 2	0.2 x 10
P6		Negative on glass plate with wet collodion emulsion 1	3.0 x 10
P7		Negative on glass plate with wet collodion emulsion 2	2.0 x 10
P8		Photo on paper with albumen emulsion	5.0 x 10^2
P9		Photo on paper with gelatin emulsion 1	1.2 x 10^2
P10		Photo on paper with gelatin emulsion 2	5.0 x 10^2
P11		Photo on paper with gelatin emulsion 3	3.2 x 10^2
P12		Photo on silk with gelatin emulsion	2.5 x 10^3
M1	Maps on different supports	On paper 1	0.8 x 10
M2		On paper 2	0.5 x 10
M3		On paper 3	1.8 x 10
M4		On paper (cyanotype)	4.5 x 10^2
M5		On textile (cotton)	8.0 x 10^2
M6		On textile (silk) 1	0
M7		On textile (silk) 2	7.0 x 10
M8		Paper on textile (silk) 1	4.2 x 10
M9		Paper on textile (cotton) 2	5.0 x 10^2
M10		On transparent paper 1	1.5 x 10
M11		On transparent paper 2	0.5 x 10

Table 3: *Fungal concentration detected on the graphics documents conserved in archival quality wrappings.*

Among the different photographic techniques, the ferrotypes (P4, P5) were the less contaminated followed by the negative on glass (P6, P7). Fungal concentrations obtained from the leaves carved of plants (P1 - P3) ranged between the orders 10^2 and 10^3 CFU/cm^2. Similar behaviors showed the paper photographs with emulsions of albumin and gelatin (P8 - P11), and the photo on silk with gelatin emulsion.

About the maps, M5 (on cotton) was the most contaminated whilst those on paper (M1, M2, M3, M10 and M11) were lest contaminated, but M6 (on silk) was not contaminated. In paper and silk maps the values of contamination detected were lower than those detected in the maps on textiles (cotton) as primary support or paper over textile (cotton).

From the photos P5, P10 and maps M3 and M9 were isolated only one genus in each one whilst from the other graphics material were isolated between two until five genera with the exception of M6 from which no fungus was isolated. Also, it was observed that the predominant genera were *Aspergillus* and *Penicillium* with an ecological category of abundant and frequent, respectively (Table 4). However, other genera as *Emiricella, Eurotium, Cladosporium, Talaromyces, Candida* and *Rhodotorula* were detected but ecologically were less important because are considered as rare genera.

	Different photographic processes and plant leaves carved														Maps on different supports												
Genera	**P 1**	**P 2**	**P 3**	**P 4**	**P 5**	**P 6**	**P 7**	**P 8**	**P 9**	**P 10**	**P 11**	**P 12**	**RF (%)**	**EC**	**M 1**	**M 2**	**M 3**	**M 4**	**M 5**	**M 6**	**M 7**	**M 8**	**M 9**	**M 10**	**M 11**	**RF (%)**	**EC**
Aspergillus	X	X	X	X	-	X	X	X	X	X	X	X	91.7	A	X	X	X	X	-	-	X	X	X	X	X	81.8	A
Penicillium	X	-	X	X	-	-	X	X	X	-	-	-	50.0	F	X	X	-	-	X	-	-	X	-	X	X	54.5	F
Emiricella *	-	-	-	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-	X	-	-	-	-	9.1	R
Eurotium *	-	-	X	-	-	X	-	-	-	-	-	-	8.3	R	-	-	-	-	-	-	-	-	-	-	-	0	-
Cladosporium	-	-	-	-	-	X	-	-	-	-	X	-	16.7	R	-	-	-	X	-	-	-	-	-	-	X	18.2	R
Talaromyces §	-	-	-	-	-	-	-	-	-	-	-	X	8.3	R	-	-	-	-	-	-	-	-	-	-	-	0	-
Candida	-	X	-	-	-	-	X	-	-	-	-	-	16.7	R	-	-	-	-	X	-	-	-	-	-	-	9.1	R
Rhodotorula	-	-	-	-	-	-	X	-	-	-	-	-	8.3	R	-	-	-	-	-	-	-	-	-	-	-	0	-
WNSM	-	-	-	-	X	-	X	-	-	-	-	-	16.7	R	-	-	-	-	-	-	-	-	-	-	-	0	-

Table 4: *Fungal genera detected on graphics documents, their relative frequency (RF) and ecological category (EC).*

**: Indicates a telemorph of Aspergillus genus. §: Indicate a teleomorph of Penicillium genus. WNSM: White Non-sporing Septate Mycelia. According to Esquivel et al. (2003) [45] when the RF is between: 100 - 81 % the genus is considered ecologically as Abundant (A); 80 - 61 % as Common (C); 60 - 41 % as Frequent (F); 40 - 21 % as Occasional (O); 20 - 0.01 % as Rare (R).*

In relation with *Emiricella* genus (a teleomorph of *Aspergillus*), was only isolated from a map (M7), *Eurotium* spp. (other teleomorph of *Aspergillus*) was isolated from a poplar leaf carved (P3) and glass plate with wet collodion emulsion 1 (P6), *Cladosporium* spp. was detected in two photographic techniques (P6 and P11) and two maps (M4 and M11), *Talaromyces* genus (a teleomorph of *Penicillium*) was only isolated from a photo (P12). About yeasts, *Candida* genus was detected in three documents (P2, P7 and M5) whilst *Rhodotorula* spp. was only detected in one of them (P7).

Notably that from negative on glass plate with wet collodion emulsion 2 (P7) a great variability of fungal genera (four genera and one white non-sporing septate mycelia) were isolated despite having a low concentration fungal (20 CFU/cm^2).

Relation of the mycobiota detected on indoor air and the documentary supports

Indoor air of the repositories and the graphics documentaries can be considered as two different micro-environments. From these ecosystems were isolated different species but only five of them were common in both (Table 5). It is highlight that the specie *Penicillium janczewskii* was only isolated from indoor air of PL and from one photo whilst *P. janthinellum* was detected only in the ML environment and one map. However, the specie *Aspergillus candidus* was isolated from indoor air of both repositories and only from a map.

Specie	Air	Maps	Photos and plant leaves carved
A. candidus	X *	X	-
A. flavus	X	X	X
A. niger	X	X	X
Cladosporium cladosporioides	X	X	X
P. chrysogenum	X	X	X
P. citrinum	X	X	X
P. janczewskii	X **	-	X
P. janthinellum	X †	X	-

Table 5: *Similar fungal species detected in the indoor air of two repositories as well as on the analysed graphics documents.*

**: This specie was isolated from the indoor air of the two repositories but it was only detected in one map; **: This specie was detected only in the PL indoor air; †: This specie was detected only in the ML indoor air.*

A total of 16 species were detected in the indoor air of PL but from these only 6 species were also detected on photos and plant leaves carved, this represents almost the 38% of coincidence. On the other hand, from ML indoor air 18 species were isolated and 7 species detected in maps were similar to those isolated from air, this represents almost the 39% of coincidence. These results indicate that the coincident species between these two micro-environments are very next to 40%.

Qualitative physiological characteristics of the isolates

Physiological characterization of filamentous fungal isolates revealed that all of them grew at the expense of filter paper as sole carbon source (this paper is composed by pure cellulose, but is a mixture of α and β cellulose) and that all species were capable of degrading the cellulose of paper with varying intensity as well as the crystalline cellulose (α-cellulose) (Table 6). Likewise, all of them produced acids; the 84% of them degrading starch and the 80% of them degrading gelatin, but only a few species excreted pigments on paper (44%). It is noteworthy that only one strain was able to degrade cellulose and to excrete acids (lowering the pH of the culture medium), but not degraded or starch or gelatin and nor was excreted pigments (*Aspergillus japonicus* 2 isolated from PL) whilst another species (*Pestalotia* sp. 2) was not able to degrade the cellulose and to excrete pigments but the acids were excreted as well as the starch and gelatin were degraded. Also, it can see that the different behaviors of enzymatic activities even among strains of the same species, it is indicates that the metabolic variability among the strains.

The 86% of the isolates of the graphics materials showed proteolytic activity, aspect worth noting to be an important part of the studied photographic documents. The albumin or gelatin emulsion is the area where the image representing the information provided by the document. It is therefore proteolytic activity, a key attribute when defining the main biodeteriogenic agents by this collection. While the test performed to determine gelatinases was qualitative, good activity was observed in *Candida* sp. evidenced by a most liquid gelatin to the rest of the strains.

Given that the existence of several biodegradation attributes in a single fungal strain is an important factor that determines its ability to cause severe damage to archival collections, this analysis was conducted.

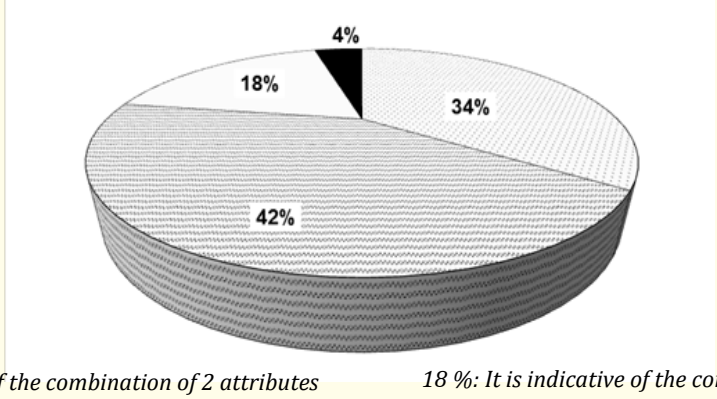
In this study, all strains tested showed at least 2 biodegradation attributes. The combinations of different biodegradation attributes are high, but it was possible to highlight four combinations (Figure 2). The sum of the strains with five (36%) and four (14%) attributes indicates that 50% of them showed a high capacity to degrade organic substances which they are part of the documentary support in archives, libraries and museums. This evidences that the isolated fungi have an important biodeteriorative potential.

Specie	Place of the isolation	Cellulolytic Activity		Amylolytic Activity	Proteolytic Activity		
		Filter paper degradation	Crystalline cellulose degradation	Starch degradation	Gelatin degradation	Acids production (pH)	Pigment production *
Alternaria sp.	ML air	+ + +	+	-	-	3.9	+
Aspergillus alliaceus 1	ML air	+ +	+ +	-	-	5.9	+
Aspergillus alliaceus 2	PL air	+ +	+ +	+	+	7.4	-
Aspergillus auricomus	ML air	+	±	+	+	5.4	-
Aspergillus candidus 1	ML air	+ + +	+ + +	+	-	5.7	+
Aspergillus candidus 2	M9	+ + +	+ +	+	+	6.6	-
Aspergillus flavus 1	ML air	+ + +	+ +	+	+	4.5	+
Aspergillus flavus 2	M2	+ + +	±	+	+	4.4	-
Aspergillus japonicus 1	ML air	+ + +	+ +	+	+	5.9	+
Aspergillus japonicus 2	PL air	+ + +	+ +	-	-	7.0	-
Aspergillus niger 1	ML air	+ + +	+	+	+	5.7	-
Aspergillus niger 2	P7	+ +	+ +	+	+	5.5	+
Aspergillus niger 3	M4	+ + +	+ +	+	+	4.0	+
Aspergillus oryzae 1	ML air	+ + +	+ + +	+	+	6.0	+
Aspergillus oryzae 2	PL air	+ + +	+ +	+	+	4.9	-
Aspergillus ostianus 1	ML air	+ + +	+ +	+	+	5.3	+
Aspergillus ostianus 2	PL air	+ + +	+	+	-	6.7	-
Aspergillus penicillioides	PL air	+ +	+ +	+	+	6.6	-
Aspergillus unguis	PL air	+ + +	+ +	+	+	7.1	-
Aspergillus unilateralis	PL air	+ + +	+ +	+	+	6.4	+
Bipolaris australiensis	ML air	+ + +	+ +	+	+	6.2	+
Cephalosporium sp.	ML air	+ + +	+ +	+	+	4.4	-
Cladosporium cladosporioides 1	ML air	+ + +	+ +	+	+	5.6	+
Cladosporium cladosporioides 2	M11	+ +	+	+	+	4.5	+
Cladosporium cladosporioides 3	P6	+ + +	+ +	+	+	6.6	+ +
Curvularia pallecens 1	ML air	+ +	+	-	+	5.2	+
Curvularia pallecens 2	PL air	±	+ +	+	-	6.4	-
Curvularia pallecens 3	PL air	+ + +	+ + +	+	+	6.7	-
Curvularia eragrostidis	ML air	+ + +	+ +	+	+	6.0	+
Epicoccum sp.	ML air	+ + +	+ +	+	+	5.8	-
Eurotium chevalieri	P6	±	+	+	+	5.0	-
Fusarium sp.	PL air	+ + +	+ +	+	+	4.5	-

Pigmented and septate mycelium	ML air	+	+	-	-	5.5	+
Penicillium canescens	ML air	+	±	+	+	4.5	+
Penicillium chrysogenum 1	ML air	+ +	+	+	-	5.2	+
Penicillium chrysogenum 2	PL air	+ + +	+ +	+	+	6.0	-
Penicillium chrysogenum 3	P7	+ + +	+ +	+	+	6.5	-
Penicillium citrinum 1	ML air	+ + +	+ +	+	+	4.3	+
Penicillium citrinum 2	P3	+ + +	+	+	+	6.1	-
Penicillium griseofulvum	PL air	+ + +	+ +	+	+	5.8	-
Penicillium janczewskii 1	PL air	+ +	+ +	-	+	6.3	-
Penicillium janczewskii 2	P8	+ +	+ +	+	+	5.5	+
Penicillium janthinellum 1	ML air	+ + +	+	+	+	6.4	-
Penicillium janthinellum 2	M5	+ + +	+ + +	+	+	5.6	+
Penicillium simplicissimum	ML air	+ + +	+ + +	+	+	6.6	-
Penicillium waksmanii	PL air	+ +	+ +	-	+	6.5	-
Penicillium wortmannii	PL air	+ + +	+	-	-	6.7	-
Penicillium restrictus	M1	+ + +	+ +	+	-	5.4	-
Pestalotia sp. 1	ML air	+ + +	+ +	+	+	4.5	-
Pestalotia sp. 2	ML air	-	-	+	+	5.3	-

Table 6: *Qualitative physiological characteristics of the fungal strains isolated from the indoor air of the repositories and the graphics documents.*

**: The production of pigments was evidenced on the filter paper strip. + + +: Indicates abundant growth (100% of the saline culture medium slant and on the paper); + +: Indicates moderate growth (70 - 50% of the slant and on the paper); +: Indicates poor growth (> 50% of the slant and on the paper), it is also indicative of presence of pigment; ±: Indicates very poor growth or production of pigment; -: Indicates NO growth and NO production of pigment.*



4 %: It is indicative of the combination of 2 attributes

18 %: It is indicative of the combination of 3 attributes

34 %: It is indicative of the combination of 4 attributes

42 %: It is indicative of the combination of 5 attributes

Figure 2: *Behavior of the combination of different biodegradation attributes related to the physiological characteristics of the analysed fungal strains which were detected in the indoor air of the two repositories as well as on different graphics documents.*

By these results the strains tested classify as high-risk agents for document preservation, if their propagules are present in high concentration on the documentary surfaces and favorable conditions for its development are given.

Pathogenic attributes of the isolates

In this study, the hemolysins and phospholipases production at 37°C were evaluated using common solid test media because these analyses count among the virulence factors of many human pathogenic fungi (Table 7). Of the 34 strains tested belonging to the genera *Aspergillus* and *Penicillium*, only 19 grew at 37°C which represented 55.9% of the total. Of those 19 strains, 7 strains corresponded to the genus *Aspergillus* (36.8%) and 12 strains corresponded to the genus *Penicillium* (63.2%).

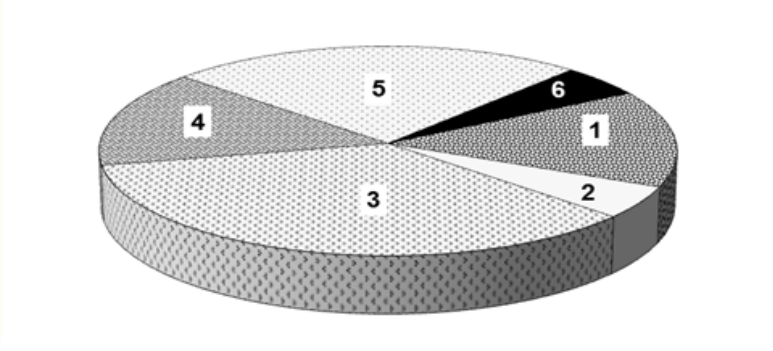
Specie	Hemolytic activity (hemolysis type)	Phospholipase activity
A. candidus 1	γ	+
A. flavus	β	+
A. niger 2	β	+
A. oryzae 1	α	-
A. ostianus 2	α	+
A. unguis	β	+
A. unilateralis	γ	+
P. chrysogenum 2	β	+
P. chrysogenum 3	β	-
P. citrinum 1	γ	+
P. citrinum 2	α	+
P. griseofulvum	β	+
P. janczewskii 1	β	-
P. janczewskii 2	α	+
P. janthinellum 1	γ	+
P. janthinellum 2	γ	+
P. simplicissimum	β	+
P. waksmanii	β	+
P. wortmannii	γ	-
Candida sp.	β	-

Table 7: *Results of another virulence factors detected in the species of Aspergillus, Penicillium and Candida grew at 37°C as prerequisite.*

β*: Indicates total hemolysis;* α*: Indicates partial hemolysis;* γ*: Indicates no hemolysis.*

The 42.9% of the *Aspergillus* species and 33.3 % of the *Penicillium* species were able to excrete hemolysins and phospholipase. Highlighted among them the species *A. niger, A. flavus* and *P. chrysogenum* as the most dangerous to the health of staff therefore should pay special attention.

Figure 3 shows the behavior of three virulence factors analyzed in the isolated species. As the saprophytic fungal species can have a similar behavior, their occurrence in the isolated fungi can only provide a rough assessment of the potential fungal health risk.



1: α hemolysis and phospholipase activity (+) = 15 %
2: α hemolysis and phospholipase activity (-) = 5 %
3: β hemolysis and phospholipase activity (+) = 35 %
4: β hemolysis and phospholipase activity (-) = 15 %
5: γ hemolysis and phospholipase activity (+) = 25 %
6: γ hemolysis and phospholipase activity (-) = 5 %

Figure 3: *Behavior of the two virulence factors related to the pathogenicity of the Aspergillus, Penicillium and Candida species grew in 37°C previously. These species were detected in indoor air of the two repositories as well as on different graphics documents.*

Discussion

The environmental study in the two repositories showed not significantly differences in the fungal concentrations. The obtained fungal concentrations in both repositories were lower than 50 CFU/m^3; hence both were classified as not contaminated and they have a good quality environmental. These repositories are characterized by a good constructive and hygienic conditions as well as they have acclimated environments where the T and RH are keeping constantly all year; therefore, these repositories don't have the so-called "amplification sites" according to Pinzari (2011) [48].

Despite that there is no international standard to determine whether an indoor environment of the heritage institution is contaminated or not, the Italian Official Document for Conservation of Indoor Cultural Heritage the fixed limit for viable fungal contamination of museum environments is 150 CFU/m^3 [19,34,48]; also French author have reported permissible microbial concentrations by archives and established four air quality levels when the concentration lower than 170 CFU/m^3 is indicative of the environments no contaminated [7].

In Cuba, there is still no standard to assess the microbiological quality of indoor environments in archives, libraries and museums, so it is essential to make comparisons with reports of foreign authors. But if our results are compared with this Italian standard or the results reported previously by French authors the environments of the two repositories would be classified as not contaminated; although, it should be highlighted that environmental conditions in Cuba and Italy or France are not equal because in Cuba the relative humidity and temperature are high during all year.

Aspergillus and *Penicillium* were the predominant genera in the indoor air of both repositories with a relative density of 30% or more and their concentrations ranged between 12 - 13 CFU/m^3 for *Aspergillus* spp. and 8 - 15 CFU/m^3 for *Penicillium* spp. These concentrations are considered very low according to Harkawy., *et al*. (2011) [5], who suggested that the levels of *Aspergillus* spp. should be lower than 50 CFU/m^3 to avoid excessive exposure to these bioaerosols and their consequent effects on health.

Other genera such as *Cladosporium, Curvularia* and *Alternaria* were detected in both repositories too; *Fusarium* was only genus isolated in very low concentrations in PL as well as the genera *Pestalotia*, *Cephalosporiun, Epicoccum, Trichoderma* and *Bipolaris* were only detected in the ML indoor environment. In the case of *Cladosporium* spp. and *Alternaria* spp. the concentrations detected in both repositories were lower than 50 CFU/m^3 which is the permissible concentration for these fungal bioaerosols [49].

The fungal prevalence behavior obtained in this study is similar to that in other studies carried out on indoor environments of libraries, archives and museum in other countries [5,6,8,9,11,24,30,50] as well as in Cuban heritage institutions [22,31-33,51-56].

Given the predominance of the genera *Aspergillus, Penicillium* and *Cladosporium*, a taxonomic identification until species was made because it is known that some species of these genera can severely affect the documents and human health. Some species were detected with percentages above 20% but these values not represent concentrations of 50 CFU/m^3 for any species.

It is important highlighting that *A. unilateralis* was detected in PL among the species of *Aspergillus* spp. at RD of 20% (equivalent to 3 CFU/m^3 approximately) and this species corresponds to *A. fumigatus* complex [56], therefore, it is a potentially dangerous species for human health. However, the species *A. fumigatus* was reported in a previous study in the same repository with a value of RD lower (10.2%) [51].

In relation to behavior of the fungal isolates from graphics documents was possible to detect that of all the carved leaves, poplar was the most contaminated (P3 with 1000 CFU/cm^2). Among photographic techniques the photos in paper with emulsions were the most contaminated with ranged between 120 - 2500 CFU/cm^2. About the maps, those on paper (cyanotype), cotton and paper on cotton were the most contaminated (450, 800 and 500 CFU/cm^2 respectively). These results are similar to other previously reported in Cuba [22,32,55].

The fact that there are viable fungi on most graphic documents analyzed despite being thoroughly clean and properly wrapped is a logical and expected result as some processes are composed of organic materials with high biological receptivity such as the plants leaves which provide storage conditions that maintain viable fungal spores. For its delicacy, this type of natural material is unusual for the realization of graphic works. During preparation for draft, the leaf is not subjected to invasive chemical processes, so fungal spores may prevail in the piece and proliferate under appropriate conditions for its development. That is, since its own creation the piece brings its own microbiota.

The photographic emulsion is more hygroscopic than paper and textiles and moreover its protean nature makes it highly receptive to the fungal agents [58]. This contributes significantly to the maintenance of viable fungal propagules. It is a fact that fungal propagules are in all ecosystems, such as is the case of the indoor air of document repositories, dust and on the collections materials. Although it is possible to maintain the propagules in low concentrations (through regular cleaning and establishing physical barriers that help eliminate or prevent the arrival of dust) it is impossible to eliminate them entirely. All microorganisms have certain nutritional and environmental requirements (maximum, minimum and optimal) that favor its growth and colonization. It is therefore important to pay special attention to environmental conditions that can foster their development, which unlike the presence of their propagules can be controlled. Fungi are mostly mesophilic (live at temperatures between 22 - 30°C), acidophilus (pH 4 - 6) and grow well at relative humidity above 70%. Only if the temperature, humidity conditions (water activity) and acidity in the substrate are favorable, the fungal spores can germinate and grow abundantly. The main limiting factor that determines the development of fungi on photographic materials is the water although some xerophilic/halophilic fungi have been associated with these materials [58].

Each graphic document evaluated represents one microbial ecosystem composed by a fungal community which is formed by one or more fungal species. For this reason, different genera could be detected in one only material. In the majority of the graphics documents two or three fungal genera were detected with exception to P7 (negative on glass plate with wet collodion emulsion 2) where five genera were isolated despite has a low fungal concentration (only 20 CFU/cm^2). This result showed that not always the fungal variability is direct associated with the detected concentration because each substrate can support different fungal communities, depending on their specific water activity, carbon to nitrogen ratio, and the presence of small nutritive molecules such as sugars and peptides, which can assist in a spore's reactivation [48].

In the photos and maps the genus *Aspergillus* proved to be ecologically classified as abundant (91.7% in the photos and 81.8% in the maps) whilst *Penicillium* was classified as frequent (50% in the photos and 54.5% in the maps). That were the predominant genera, consistent with that reported in the scientific literature [22,32,58-61].

The *Penicillium* and *Aspergillus* genera are the major constituents of the aeromycobiota indoor, the fact that the main pollutants of surfaces come of air indoor of the repositories justifies its prevalence on the supports. The prevalence of genus *Candida* in the repository can be an indication that it is characteristic to this environment and which initially could have been introduced from the outside environment or have come from contaminated materials after their production. Moreover, certain species of *Candida* are typical body biota such as *C. albicans* [62], which might suggest anthropogenic pollution by mishandling of the collection. There may be cross-contamination between documents (especially photographs) and people. If precautions are not taken when handling photographic collections, this phenomenon can lead to both infestation and biodeterioration of materials as serious affectations of health staff.

However other genera were detected and classified as rare. Teleomorph of *Penicillium* (*Talaromyces* sp.) and *Aspergillus* genera (*Emericella nidulans* and *Eurotium chevalieri*) were detected again on the supports studied. These results are in concordance with other previous [4,22,32,58,61]. Some species of these teleomorph are xerophilic and osmophilic fungi with a high tolerance to water stress. The occurrences of these fungi are associated with air-dust [58] in association with mites and *Aspergillus penicilloides* recently reported its occurrence in library materials [58,63].

Similar results to other previous were obtained with respect to species fungus of the levaduriform *Candida* detected in a plant leaf carved (tobacco), a negative glass plate with collodion and in a map over textile (cotton) as well as species of the genus *Rhodotorula* which they were also detected in photographs [22,32,61].

Also, these results demonstrated that fungi are present on the documentaries materials even if they are clean and in good conservation conditions, so if the temperature and relative humidity increase sharply and remain high for several days the chemical and structural damage culminates in the formation of biofilms and in the degradation of these documents [11,19].

In relation to the mycobiota detected in indoor air and on the documentary surfaces it is important highlight that eight species were coincident. Among them seven were detected on indoor air of both repositories and over maps and photos (*Aspergillus flavus, A. niger, Cladosporium cladosporioides, Penicillium chrysogenum* and *P. citrinum*). The specie *A. candidus* was isolated from indoor air of both repositories but only from maps, the fungus *P. janczewskii* was detected only in the PL indoor air and over photos whilst *P. janthinellum* was a fungus isolated only from the ML indoor air and over maps.

This coincidence of species can be due for three reasons: first, to the deposition in different moments of some of their propagules on the documentary surfaces which can be isolated later on; second, to the detachment of some of these propagules from the polluted documentary surfaces which can be aerosolized and pass to be part of the bioparticles of the indoor air (indoor ecosystems); and third to that the two phenomena before described they happened at the same time. However, as the documentaries materials are very clean and in good conservation conditions this coincidence is low.

In an indoor environment, there is a close relationship between air and surface microbiota [17,64]; these results are evidence of this. The fact that the agreement between the two ecosystems is not greater than 50% (≤ 40%) is due to the limitations imposed for mobility of staff working in the file, the furniture and the packaging used to protect documents. These elements as well as the archive building should function as an effective barrier between environmental contaminants and stored materials.

Biodegradation of materials by moulds can affect the material integrity of the same because the fungus's hyphae penetrate the substrate. Damage can also occur as a result of enzymatic action. Moulds can produce a wide range of enzymes (proteinases, gelatinase, cellulases, etc.) which are able to destroy the component materials of items included in collections [4,10,22,48,61].

Cellulose is the most abundant organic compound found on Earth (30 - 50% of plant dry weight) and, in natural environments, represents a major source of energy for microorganisms. The storage of books and archival materials inside buildings devoted to their preservation has created unique environments for cellulolytic fungal and microbial species to inhabit. As cellulolytic fungal species require available water in order to be metabolically active [48] but the conservation conditions of the studied documents are characterized by a low water values the degradation of the cellulose and the other substances (starch and proteins) is not activated. For this reason, to know the biodegradation potentiality of the fungal strain isolated from the two ecological niches (indoor air and graphics documents surfaces) is an important requirement to establish a good preservation strategy.

According the obtained results from the determination of biodegradation potential of the fungal species isolated it can see that the majority of the isolates degraded all substrates. The enzymatic degradation of cellulose and starch by almost all strains tested endanger photographic paper supports and textiles. The cellulose fibers are crucial for the stability of paper and textile, degradation favors the break and mechanical fatigue of these materials. Moreover, cellulolytic fungal strains can also affect collodion photographic emulsions, consisting of nitrocellulose. Starch, as well as being constituent of paper can be found as part of the adhesives used in the production of maps. The *Aspergillus flavus, A. candidus, Penicillium citrinum, P. jantinellum* and *P. janczewskii* species showed higher capacity to secrete the cellulases and amylases in this study. Acidification of the medium through the excretion of organic acids favors the acid hydrolysis of the aforementioned polymers and promotes the colonization of the material by other fungal species that can act as secondary colonizers [48]. Among the various acids secreted by fungi are acetic, fumaric, citric, and oxalic acids [26].

A microorganism can be considered risky for documental preservation if it has at least one biodegradation attribute that compromises the status of the collections [65], however combining them in a single strain, can mean greater potential to colonize and deteriorate the materials in different ways accelerating its deterioration. From the ecological point of view the fact that the same fungi present more than one biodegradation attribute means that can be developed on different substrates, which greatly facilitates its prevalence in the ecosystem over other microorganisms with less possibilities [35].

According to these results the strains tested classify as high-risk agents for document preservation. Hence if their propagules are in high concentration over documentary support and the conditions of T and RH are favorable for its development, the biodegradation process will occur.

The most of the environmental fungi are not able to be developed to temperatures superior to 32°C [62]. Sánchez and Almaguer (2014) [66] reported that in tropical countries the most temperature in the year varies between 25°C and 30°C, condition that favors the fungal development and the formation and liberation of spores. Nevertheless, some species have optimal growth temperatures that include the 37°C. Such are the cases of *Aspergillus niger* (35 - 37°C) and *A. fumigatus* (37°C) [67]. It is probable that these fungal species unleash their full physiologic potential at the corporal temperature, aspect that transforms them into opportunists' pathogens with potentialities invasive more dangerous.

Hedayati., *et al*. (2007) [68] reported that 37°C is the optimal temperature to the growth of *A. flavus*, and this specie can grow in a range of temperature among 12°C at 48°C. *Aspergillus fumigatus, A. flavus* and *A. niger* are the main causal agents to the aspergilloma and invasive aspergillosis, aspects that are related to the potential growth at 37°C.

Individual ability of a microorganism to cause diseases in other organisms depends on a number of mechanisms known as pathogenic virulence factors or pathogenic attributes [46]. Such attributes will determine the degree of pathogenicity of certain microbial agent.

The growth of fungi at 37°C is a virulence factor necessary and essential to consider a potentially pathogenic fungal strain, because this is the body temperature of humans and other warm-blooded animals [46]. The development under this condition allows the invasion of epithelial, endothelial and blood vessels surfaces [69]. Furthermore, according to Rementería., *et al*. (2005) [70] the expression of some genes involved in invasive mechanisms occur at that temperature. For these reason, this parameter was evaluated as a principal condition in the fungal strains of genera *Aspergillus, Penicillium* and *Candida*.

Hemolysins are toxins produced by certain microorganisms and are secreted into the extracellular medium producing erythrocytes rupture [71]. The imbalance in the osmotic pressure of the cell due to the pores formed by these proteins has been suggested as the mechanism of hemolysis [72]. This phenomenon compromises the transport of oxygen to tissues, triggers episodes of anemia and facilitates invasion of blood vessels by the pathogen because the iron is a compound very necessary for the metabolic processes as enzymatic cofactor [71].

All cells are surrounded by a membrane, which in turn is composed of lipids, phospholipids, among other biomolecules. Its functions include nutrient transport and power generation, in addition to receiving signals, etc. It is therefore the integrity of the cell membrane is vital to the cell [73]. Phospholipases are a group of enzymes secreted by various pathogenic microorganisms have the ability to damage cell membranes of the host. The ability to produce and secrete enzymes with phospholipase activity of certain fungi has been considered by several authors as a potential virulence factor that contributes significantly to their pathogenicity [74], as they are involved in important physiological processes such as fungal dissemination in the host and the defense or evasion to mechanisms of the immune system [75].

From the obtained isolates, some strains were able to grow at 37°C and the enzymes hemolysins and phospholipases were secreted. These evidence the high risk that they represent to human health. Among the species the most risk highlighted *Aspergillus niger, Aspergillus flavus* and *Penicillium chrysogenum* which were reported previously as opportunistic pathogens [62,76].

These results are agree with those reported by other authors, who detected hemolysin production in strains of *Aspergillus niger* and *A. flavus* using a methodology similar to what was used in this research [77,78]. Moreover, it has been reported the detection of more than one type of hemolysin in strains of *Aspergillus flavus* and *A. niger*, producing species par excellence such mycotoxins [71] and clinical importance widely cited [63,79]. Bomogolova and Kirtsideli (2009) [47] studied the phospholipase activity in fungi isolated from outdoor environments of the city of St. Petersburg. They found that representatives of the genus *Penicillium* including *P. chrysogenum*, showed positive enzymatic activity, which is consistent with this study.

In filamentous fungi the virulence factors are diverse and vary by group, genus or species [80]. Unlike biodegradation attributes, the union in the same microorganism of several virulence factors is a necessary condition to classify it as potentially pathogenic [62]. Although in the infection process can be displayed other pathogenic attributes that determine the potential and extent of infection, the pathogenic attributes analysed in this study are of vital importance in fungi with multi-host capacity [80].

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Conflict of Interest

The authors declare that there is no conflict of interests or financial interests.

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