Identification of Fungi Resistant to 137Cs Gamma Radiation From an Artwork by Candido Portinari

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Research Article

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Abstract

Gamma radiation is a safe and effective technique for the treatment of art collections being used with high efficiency in reducing microbial loads being obtained by the emission of a radioactive isotope, such as Cesium 137. Portinari's work, from the collection of the National Museum (Brazil), was analyzed and the fungi contained therein were collected, isolated, and further treated with gamma radiation for decontamination. Radiation doses used were 16, 19 and 22 kGy. Results indicated 11 genera and 17 species isolated. *Penicillium* and *Cladosporium* were isolated in air, artwork and its support - emphasizing the predominance they assume in the contamination of works of art under favorable conditions, such as museums. The genera *Penicillium*, *Cladosporium*, *Nigrospora* and *Curvularia* showed high resistances (16 kGy). The most resistant was *Cladosporium*, which showed no growth under 22 kGy. As there are some differences in ionizing radiation resistance among fungi from the same order or species, the results here outlined indicates that the rates of DNA damage and repair were critical, depending on chronic or acute dose irradiated. The biochemical mechanism acting on fungal cells under irradiation was basically the inactivation of specific enzymes and, probably, DNA damage, particularly stimulating double-strand breaks.

Introduction

One of the main problems faced by museum restaurators for the preservation of paper collections and artworks is the damage caused by the action of microorganisms [1, 2].

In general, fungi play a key role in the biodegradation of papers and artworks in museums and archives. Due to their ability to form hyphae, fungi can penetrate into the materials, resulting in losses due to acid corrosion, enzymatic degradation and mechanical attack. The most important genera of fungi found in museums are: *Alternaria*, *Aspergillus*, *Absidia*, *Acremonium*, *Cladosporium*, *Chaetomium*, *Chrysosporium*, *Eurotium*, *Fusarium*, *Geotrichum*, *Penicillium*, *Paecilomyces*, *Epicoccum*, *Phoma*, *Cunninghamella*, *Emericella*, *Scopulariopsis*, *Stachybotrys*, *Trichoderma* and the yeast *Rhodotorula*. For many years, in order to disinfect art collections, they were subjected to fumigation techniques. New alternatives have emerged, such as the use of modified anoxic atmosphere and deep freezing. However, because they do not present a long-term effect, they allow rapid recontamination. Moreover, these procedures are not efficient in eliminating fungi that may be infesting artworks [3, 4].

Within this scenario, studies have been developed to formulate effective conservation strategies in order to prevent biodeterioration of cultural heritage. In this sense, the understanding of the biodeterioration process, the colonization mechanisms developed by microorganisms, the techniques used in the assessment of biodeterioration and strategies that seek to preserve the integrity of the heritage were outlined as relevant objectives in the work of Sarethy and Negi [5]. Therefore, the improvement of methods to detect and characterize microorganisms is being considered in the area of cultural heritage. In the study by Sanmartín *et al.* [6] traditional and modern methods were reviewed in three different approaches – molecular, sensory and and biological control methods – suggesting the use of an

integrated approach regarding the use of such methods in the identification, monitoring and control of microorganisms.

In this context, researchers developed disinfection techniques using gamma-irradiation. Gamma radiation is a safe and effective technique for treating artwork collections as it is used with high efficiency in reducing microbial contaminations without leaving any residual radioactivity [7–9].

Radiation processes were not yet widely accepted by museum restaurators because of the degradation of cellulose caused by radiation. To establish a safe dose of radiation that can cause a reduction in microbial load without damaging the paper structure has been the object of study by Moise *et al.* [10] e Carvalho *et al.* [11]. In the work of Sakr *et al.* [12], 46 *Steptomyces* strains were isolated from paintings in the thumbs of Tell Basta and Tanis and exposed to low and high gamma radiation doses (5 to 25 kGy). The authors concluded that gypsum, pigments and gum arabic were not damaged in the range of gamma radiation studied, indicating a potential technique for microbial decontamination.

For the disinfection of fungi and bacteria radiation doses that cause lethality start from 10 kGy, usually used as a safe dose for disinfecting fungi and bacteria present in food ingredients. This dose has also been used in studies performed on the treatment of paintings contaminated by microorganisms and has been shown to be effective [8]. Silva *et al.* [13] inactivated fungi from paper materials working in the range from 14.5 to 25 kGy using ^{60}Co, emphasizing the presence of resistant fungi 50 inactivated only after 16 kGy.

In 2018, we started a study for the decontamination of an artwork (charcoal on paper), by Candido Portinari, in the process of biodeterioration and part of the collection of the National Museum (Rio de Janeiro, Brazil), unfortunately destroyed by a big fire, in September of the same year. The artwork was analyzed and the fungi contained therein were collected and isolated, so that it could be properly treated and returned to exhibition to the public. The study of gamma radiation for decontamination was then performed. The search for this alternative came from the structural nature of the artwork, made in paper with charcoal drawings, which would prevent any possibility of chemical or aqueous treatment that could destroy its integrity as a work of art. The choice of Candido Portinari's "Índios" (1937), was due to its importance as an object of study because it is one in a series of other artworks painted in the same period (1937–1938), all with the same type of paper and the same technique (coal), thus, serving the present results as a pilot for the preservation of all other artworks with similar characteristics [14]. The work selected as a pilot for the present study belonged to the National Museum's collection in Brazil, which is an autonomous institution, member of the Science and Culture Forum of the Federal University of Rio de Janeiro, linked to the Ministry of Education that completed 200 years in 2018, rettably destroyed by fire in the same year.

The main objective of this work was to irradiate the fungi isolated from Portinari's artwork "Índios" in order to evaluate the ionizing radiation technique for the non-destructive treatment of works of art with

similar characteristics and that are contaminated with fungi. The resistant of fungal species to high levels of radiation were also investigated by classical and molecular biology techniques.

Methods

The charcoal on paper artwork "Índios"

The work depicts the design of three Indian heads and another design of the head, sketched in the background (Fig. 1). The drawings occupy almost the entire area of the bracket. Its size was 99.5 cm height by 119 cm width and does not present signature or date. The work was produced on Kraft paper with charcoal, which excludes any attempt to try aqueous treatment to remove fungi or damage resulting from the biodeterioration or deterioration in the pulp structure of the paper.

The artwork was kept under 30 ^{o}C without a proper control of natural light intensity and humidity.

Samplings

The microbiological characterization of the microbiota in the air of the room where the artwork (charcoal on paper) was made by sedimentation on Petri dishes containing sterile Sabouraud Dextrose Agar supplemented with chloramphenicol solution. The samples were collected every cubic meter of the space, one meter above the floor, for 2 hours.

Some parts of the artwork were monitored, particularly those where fungal contamination was clearly present. On selected parts on the surface of the charcoal on paper, sterile swabs were used to collect the biological material (Fig. 2). The used swabs were placed within 9.0 mL of saline water 0.9 % (m v^{-1}).

Culture and isolation of filamentous and yeast like fungi

After serial dilutions, 0.1 mL of each sample collected in Petri dishes containing Sabouraud Dextrose Agar with 0.05 g L^{-1} of chloramphenicol for growth of fungi were inoculated. The Petri dishes were then placed in a chamber with controlled temperature at 25 °C for 14 days. After growth, the fungi were isolated in Agar media, and incubated for 7 – 14 days in a temperature controlled chamber at 25 °C. The fungi were then isolated with the aid of sterile swabs in glass tubes containing Agar Sabouraud Dextrose culture medium and stored in mineral oil [15,16].

Macroscopic and microscopic identification of fungi

After isolation of the colonies Petri dishes were photographed and macroscopic characteristics such as colony size, coloring, texture, reverse of the dishes, were observed. From the previous isolation, slides were made for microcultivation of the filamentous fungi in Agar Malt Extract culture media for 5 – 14 days. After this period, the staining of the microcultivation slides with Lactophenol Blue Solution was performed. Microscopic observations of the morphological structures for identification at the genera level were then performed.

Fungal identification by Molecular Biology

The extraction of the DNA from the isolated colonies was made with the aid of the Quick DNA Fungal/Bacterial Miniprep Kit, manufactured by ZymoResearch. The PCR was then made to amplify a specific region of the DNA (ITS), which is unique to fungi. PCR results were obtained by electrophoretic migration in agarose gel, and the samples were sent for sequencing. From the results obtained, most fungi were identified.

Phylogenetic identification of fungi

DNA extraction

After growth in specific culture medium, the fungi were removed by scraping the Petri dishes and transferred to a 15 mL Falcon tube. The tube was placed, for 5 min, in a cylinder containing liquid nitrogen, heated in a water bath at 60 ºC for 10 min and macerated with the aid of a pistil. This procedure was repeated three times. Subsequently, DNA extraction was performed with Kit Ultra Clean Soil Isolation (MO BIO Laboratories) According to the instructions provided by the supplier, the DNA was eluted in 30 mL of the solution provided in the kit and quantified by reading on Nano Drop ND-1000 spectrophotometer (Thermo Scientific, Waltham, USA).

Amplification of ITS region by PCR

A fragment of 600 pairs of DNA bases corresponding to the ITS gene was ampliwas amplified from the genomic DNA using the ITS5 initiators (senso, 5'- GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (antisense, 5'- TCCTCCGCTTATTGATATGC-3') in the PCR System 9700 ThermoCycler (Applied Biosystems, USA). Each reaction included 25 μL of Top Taq Master Mix Kit (PCR Master Mix, Qiagen, Holland), 0.5 μM of each initiator and 5 μL of extracted DNA added to water, in a total volume of 50 μL. A first denaturation was performed at 94 ºC for 4 min followed by 30 cycles under the following conditions: 94 °C for 30 s, 50 ºC for 30 s and 72 ºC for 30 s. The experiment was fineshed at 72 ºC for 10 min. The amplified fragments were purified using the Kit Wizard® SV Gel and PCR Clean-Up System (Promega, USA), for the removal of nucleotides and initiators not incorporated, followed by proper sequencing [17]. The amplification product was detected through an electrophoretic run in 1% agrose gel in TE 1X buffer.

Sequencing

The DNA fragments of the isolated fungi were submitted to sequencing using the Big Dye Terminator v. 3.1 (Applied Biosystems, USA) kit in an ABI 3130 automatic sequencer (Applied Biosystems, USA) with 4 capillaries of 50 cm. ITS-5 initiators (sense) and ITS-4 (antisense) were used to sequence the region ± 600 Pb and the initiators Sadir (sense) and S17 (antisense) were used to sequence the region 1500 PB. The concentration of primers used was 3.2 pmol. The chromatograms obtained from sequencing were submitted to the Chromas Lite, version 2.01 and Bioedit programs to analyze the quality of the sequences. The sequences validated by the programs were paired to those deposited in the Genbank's DNA database. To validate the sequences, NCBI BLAST (Basic Alignment Search Tool) tool was

performed, to confirm the sequences obtained. Only fragments with similarity above 98% were considered "reliable" and duly annotated.

Irradiation of samples with ^{137}Cs for selection of resistant fungal species

The irradiation time in the ^{137}Cs chamber was increased to establish the radiation intensities capable of eliminating each fungus individually. The irradiations were applied after 14 days of incubation of the isolated fungi at the Institute of Research and Development of the Technological Center of the Army (IPD/CTEx) in Rio de Janeiro. Plates with the fungi grown separately were grouped in the irradiator, in order to occupy the central space of the chamber, according to the pre-established maximum height of 7 cm, in order decrease the uncertainty of the procedure. Then, the irradiation chamber was closed so that the samples were subjected to irradiation with a source of ^{137}Cs at the different times previously calculated. Irradiation time was defined by a program based on recent dosimetric mapping of the irradiator. The average uncertainty in the doses, estimated on the dimensions of the samples and in the dose distribution within the irradiator, was equal to ± 5%. Immediately after irradiation (exposure time directly proportional to the desired dose), samples were replaced in boxes and returned to the laboratory, for analysis of fungal viability, according to the isolation procedure previously described. Thus, irradiations were performed for the time required to reach the doses proposed (14h under16 kGy, 16h under 19 kGy and 19h under 22 kGy). Only doses much higher than those described in the literature were selected, since the main objective of the study was to select fungi that were highly resistant to gamma irradiation.

Gamma Irradiator – IDQBRN/EB

The irradiation chamber of the Chemical, Biological, Radiological and Nuclear Institute (IDQBRN) of the Brazilian Army, located in the Army Technological Center (CTEx), is composed by an irradiator of cavity weighting 19 tons. Currently, its ^{137}Cs sources with 43.2 kCi activity provide a maximum dose rate of 1.45 kGy h^{-1} in two rectangular irradiation chambers 68 cm wide, 137 cm depth and 20 cm height positioned above and 188 below the gamma source. The gamma source consists of 28 spaced parallel plates, doubly encapsulated containing cesium chloride 137. In addition, a pneumatic system allows not only the access port to be moved, but also to move the sources through a control panel. The dosimetric mapping of the chamber, carried out in 2000, indicates a homogeneity in distribution of the dose rate with a variation around ± 3%.

Post irradiation fungal viability

Procedures for post irradiation fungal viability analyses were performed 24h after the irradiations. Fungal species irradiated with ^{137}Cs at 16, 19 and 22 kGy, were inoculated in sterile Petri dishes, containing the culture medium Agar Sabouraud Dextrose, in order to confirm the viability of isolated microorganisms, indicating high resistance to gamma radiation. After inoculation Petri dishes were placed in a germination chamber for 7 days at 25 ºC. After this period, the observation of fungal growth was

performed. In cases where no post-irradiation growth was observed, it was concluded that the irradiation was high enough to destroy the cells.

Results

Several fungi were found in the atmosphere of the room where the artwork was located, on the artwork itself and in the fabric support of the piece. A total of 17 fungi were isolated, corresponding to 11 different genera and 17 different species. Table 1 presents the results of the distribution of the species and fungal genera obtained from each sampling region. Given the presence of so many isolated species, some repeated in several points, the use of gamma radiation was used to eliminate these fungi. It is known that gamma radiation destroys the structure of the cells' DNA by inhibiting fungal growth completely once they lose their functions. Incomplete inhibition can cause only a slight damage to cells. High-energy irradiation rays directly impact the DNA of living organisms, inducing cross-linking and other changes that make an organism incapable of growing or reproducing. When these rays interact with water molecules in an organism, they generate transient free radicals that can cause additional indirect damage to the DNA. Most microorganisms did not present viability from the first radiation dose to which they were submitted, that is, 16 kGy, being classified, then, as sensitive to gamma radiation. This viability was verified by the existence or not of microbial growth after irradiation. Of all the isolated fungi that were subjected to gamma radiation, only those listed in Table 2 were resistant to 16 kGy. Thus, higher radiation doses were tested, i.e., 19 kGy and 22 kGy (Table 3).

Table 1 Fungal genera and species isolated from the atmosphere, engraving and fabrics of the artwork "Índios" from Candido Portinari

Atmosphere	Artwork	Support (fabrics) of the artwork
Aspergillus aculeatus	*Cladosporium xanthochromaticum*	*Cladosporium perangustum;*
Rhizopus sp.	*Cladosporium cladosporioides*	*Penicillium* sp.
Fusarium equiseti	*Penicillium* sp.	*Cladosporium halotolerans;*
Penicillium sp.	*Pestalotiopsis* sp.	*Curvularia lunata;*
Cladosporium sp.	*Arthrinium marii*	*Cladosporium tenuissimum;*
	Daldinia eschscholtzii	*Cladosporium xanthochromaticum*
	Nigrospora sp.	*Rhizopus* sp.
	Curvularia luneta	*Periconia macrospinosa;*
	Cladosporium sp.	*Penicillium raistrickii*
		Cladosporium sp.

Table 2 Macroscopic and microscopic aspects of isolated fungi resistant to 16 kGy

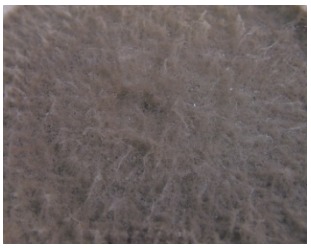
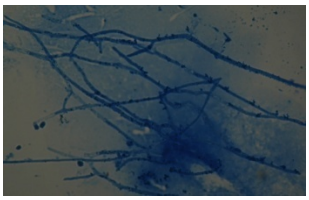
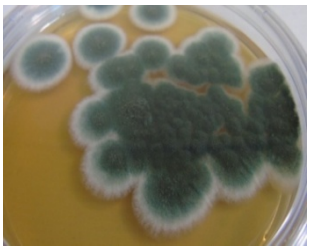
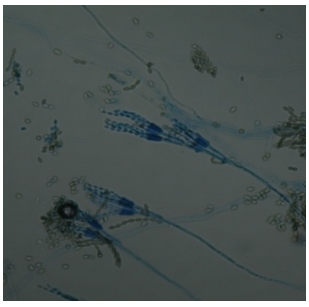
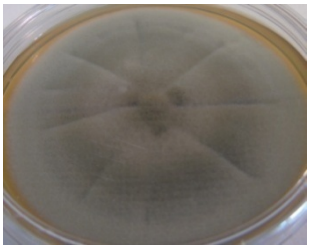
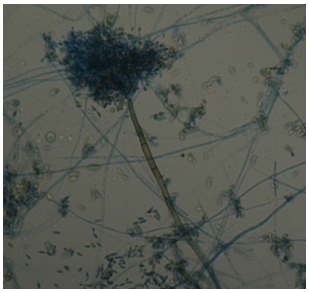
		*Nigrospora* sp.
		*Curvularia Luneta*
		*Cladosporium Halotolerans*
		*Penicillium* sp.
		*Cladosporium* sp.

Table 3 Fungal viability after high doses of gamma irradiation

Fungus	16 kGy	19 kGy	22 kGy
***Nigrospora* sp.**	✓	X	X
Curvularia luneta	✓	X	X
Cladosporium halotolerans	✓	✓	X
***Penicillium* sp.**	✓	X	X
***Cladosporium* sp.**	✓	✓	X

✓ Active growth (viable); X: innactivated

Discussion

Some microbial genera and species found in the artwork of Portinari (Table 1) are common in museums and libraries. Under moderate or humid climate conditions, fungal communities are dominated by *Alternaria, Cladosporium, Epicoccus, Aureobasidium* and *Phoma* species. *Penicillium, Aspergillus* and *Fusarium*, among others, are also commonly found in books and paintings [18]. The presence of certain species of contaminating fungi can represent, by itself, a threat to human health and to the conservation of cultural heritage, since many of them may present some degree of pathogenicity, in addition to being, mostly, potential cellulolytic agents. In these cases fungi of the genera *Cladosporium* and *Penicilium* are included, both found in the present artwork and considered potential pathogenic agents [11].

However, based on the results obtained and presented in Table 1, the presence unusual fungal species was observed, such as *Cladosporium halotolerans*, *Cladosporium xantocromaticum*, *Daldinia eschscholtzzi*. However, they all contribute to the biodeterioration process. A similar observation was confirmed in a work by Ortiz *et al.* [19] who identified wood rot fungal species in eight historic churches in Chile and found several groups of fungi of various genera and species not previously reported in Chile, also demonstrating the great decomposition power of this varied microflora.

The proliferation of fungi in museums is closely determined by the interior climate conditions and the available nutrients, which is directly related to the amount of impurities brought by the air conditioners or the air quality in the external environment. The internal conditions, as indicated by temperature, relative and specific humidities is the most important factor responsible for fungal growth. In spaces where humidity is higher que70% for a period of many weeks or months, a large fungal diversity can be expected being 55% the limit for fungal growth, considered as a standard for countries with cold weather. Thus, climate control should be adjusted to values below this limit, since water availability seems to be adequate for uncontrolled proliferation. Institutions such as museums, galleries, libraries and archives are strongly advised to control temperature and humidity conditions in order to maintain their collections free from mechanical damage and biodeterioration [18].

Regarding "Índios" official records for the city of Rio de Janeiro indicate that the relative humidity of the air at the date of sample collection was around 64%. However, inside the room there was no control of relative humidity, temperature or light intensity. Such conditions may have worsened the contamination and the disorderly and unpredictable fungal proliferation. As has been reported, even when the relative humidity is maintained around 60%, the works of art or collections of books are not free from contamination and fungal proliferation. The condensation of water occurs on cold surfaces even when they are under low relative humidity, and this represents an opportunity for fungal growth, since they are not very demanding for specific substrates, being able to grow in a wide variety of substrates [11]. The most precious documents of mankind are made of paper, fabrics, papyri and scrolls, as well as artworks of high artistic value. Due to the great diversity of exoenzymes produced by fungi – cellulases, glucanases, lacases, phenolases, keratinases, mono oxygenases, among others – and its remarkable ability to grow in low aw values, the preservation of museum objects is inevitably connected with prevention, monitoring and treatment of the occurrence of fungi in contaminated pieces [18].

<[>In the present work, the isolation of several species of *Penicillium* and *Cladosporium* was made in the three points - air, the artwork itself and edge of the artwork - evidencing the predominance these fungi assume in fungal contamination and proliferation in works of art. Studies show that the genus Penicillium, among most agents that promote biodeterioration, is a ubiquitous organism responsible for modifications of paper support. Being a cellulolytic fungus, it attacks the support of artworks when living in a favorable environment, that is, with favorable temperature and humidity and availability of other essential nutritional elements. Such conditions are very common in libraries and collections that store historical pieces since many of them do not have a rigid control of these conditions, so there may be presence of dirt, humidities from infiltrations and even floods, demolitions [20]. Because they produce acidic pigments, fungi end up generating particular local conditions that modify the physicochemical properties of materials, as seen in the work of Lavin *et al.* [21]. In this work, they observed that biofilm formation by *Scopularriopsis* sp. and *Fusarium* sp., isolated from paper document collections, produced reddish-brown stains, attacked paper structure and produced a pH reduction in the magnitude of one unit, accelerating paper's biodeterioration processes.

The present work also revealed high contamination levels inside the room due to the presence of some species of fungi. The danger of this contamination lies in the fact that spores suspended in the air represent a risk for both human health and constitution of the materials. Its biodeterioration capabilities can potentially cause irreversible damage to artworks and other objects of cultural heritage. It has been reported by other authors that air contamination is the main vehicle for spore's dissemination. In addition, the co occurrence of the same fungal species in works of art and in air samples is by itself a demonstration of this cross-contamination [11].

Irradiation of fungal samples at three different doses, 16, 19 and 22 kGy revealed different levels of tolerance between microorganisms. Figures 3 and 4 show the growth on Petri dishes of radiation tolerant species. It can be observed that most of them are dark colored.

Other factors may be involved in the viability of fungi or tolerance to gamma radiation. Multicellular or bicellular spores are more tolerant to gamma radiation than unicellular spores. In addition, the number or density of mycelia in the inoculum exposed to radiation may affect the radiation dose required for the inactivation of the microorganism. Generally, a high density of mycelium in the inoculum requires an elevation of the radiation dose [22]. Shuryak *et al.* [23] also studied fungi and yeasts to resist exposure to chronic and acute radiation up to 36 kGy. They concluded that resistance leves were different among organisms from the same order and even species sharing a large core of genes. Basidiomycetes and Ascomycetes constitute a relatively resistant grou with marked differences in acute and chronic radiation doses. Levels of DNA damage production and repair are critical to chronic resistance in replicating cells. On the other hand, DNA damage (particularly double-strand breaks) prevent survival during acute irradiation, as observed in the present work. As previously mentioned, three radiation doses were adopted: 16, 19 and 22 kGy. These values were based on previous studies already conducted and published in the literature that reveal that some species of fungi may be resistant to gamma radiation or may develop some mechanisms of tolerance being therefore difficult to have a complete metabolic inhibition. The results showed that the fungi of the genus *Penicillium*, *Cladosporium*, *Nigrospora* and *Curvularia* presented a resistance to radiation when the dose of 16 kGy was tested, since, after irradiated, they still presented growth. However, the most resistant of all was the genus *Cladosporium*, which only presented lack of growth in the dose of 22 kGy (Fig. 5). The results obtained in this study are in agreement with those already presented by other authors who conducted studies with *C. cladosporioides*. According to Boniek *et al.* [24], this was the only species resistant to exposure of gamma radiation and this characteristic may be related to the fact that some strains have the metabolic capacity to produce a dark coloured pigment (the biopolymer melanin) that accumulates within the mycelium and protects against UV rays and ionizing radiation. Other published studies have shown that radiotrophic fungal species use melanin to convert beta and gamma radiation to chemical energy for growth [24]. In fact, according to the results presented in Figures 3 e 4, it was found that the tolerant fungi exhibited a dark color, evidencing the presence of the melanin pigment that may be associated with this resistance mechanism.

The resistance of some fungi common to gamma radiation was also studied by Saleh *et al.* [25]. Ten species of fungi representing the genera *Alternaria*, *Aspergillus*, *Cladosporium*, *Curvularia*, *Fusarium* and *Penicillium* were examined for their resistance to gamma radiation from a source of ^{137}Cs. In this study, it was found that fungi with melanin hyphae such as *Alternaria alternata*, *Cladosporium cladosporioides*, *Curvularia lunata* and *Curvularia geniculata* survived after high doses of gamma radiation. The macroconidia of *Curvularia* and *Alternaria* sp., which are of a thick wall and contain the pigment melanin, showed resistance and, these two characteristics can probably contribute to the increasing resistance of these species. In another study, it was observed that among all fungi isolated from documents with parchment as support, the most frequent (and more resistant to gamma radiation) genera were *Penicillium* and *Aspergillus* [26]. These results corroborate those already presented in this study, where there a high resistance to radiation of the genus *Penicillium* was observed.

Microbial resistance to gamma radiation was investigated by Múčka *et al.* [27] and Neuzilová *et al*. [28], using ^{60}Co in order to determine DNA stability and effects on cell membranes. Other authors also studied the effect of ^{60}Co, confirming that the negative effect of the radiation was not linearly dependent on the radiation dose [29].

It is important to emphasize that there is a concern about the consequences of high radiation doses used in relation to the integrity of the support of artworks. Although no studies have been conducted in the present work, there is a common sense about this matter that only very high doses could affect the integrity of the support. In our work, this was not a concern as the isolated fungi were irradiated on Petri dishes. The literature reports that gamma radiation has already been used in other opportunities to decontaminate paper, showing that doses between 3 and 10 kGy were effective in fungal decontamination, without causing significant damage to the materials. The authors also identified that high doses (around 15 kGy) were tested for disinfection of the paper, without also causing any injury to the support. Even so, there is almost nothing in the literature about the effects of gamma radiation on parchment documents. The authors suggested 5 kGy as a minimum dose to be used for decontamination of parchment documents when the main objective is to decontaminate instead of sterilize [27]. A work carried out by Rizzo *et al.* and Tomazello [30,31] in the restoration of 17th. Century paintings, considered that the appropriate radiation dose to eliminate microorganisms is 6 kGy, being 25 kGy the standard dose for sterilization [30]. In other studies, it was found that the maximum dose already used on parchment support was 30 kGy since there was no damage to the support. However, the study was not conclusive, as there are many types of supports that need to be tested [26]. Negut *et al.* [32] conducted a study on the defects induced by gamma radiation with a ^{60}Co source in historical pigments. The results revealed that the radiation-induced changes in 22 historical pigments. Even after three months after irradiation changes were not significant either because there was in fact no pigment color change or because the changes were reversible. Thus, it was concluded that gamma radiation presented itself as a reliable decontamination treatment. Decontamination techniques using other radiation sources have been studied in search of decontamination techniques that have less harmful effects on the object of cultural heritage to be disinfected.

It was concluded that cosmopolitan fungi, isolated from the air, from a charcoal on paper artwork and fabrics of the artwork can be highly resistant to gamma radiation. The same cosmopolitan fungi that proved to be resistant to radiation are worldwide spread in museums, libraries and archives: *Nigrospora*, *Curvularia*, *Cladosporium* and *Penicillium*. Even though, the literature reports lower levels of radiation, some species are only eliminated under radiation up to 22 kGy. The mechanism involved in the elimination of fungi is probably associated to DNA damages due to acute irradiation, differently from what is observed under chronic irradiation. The results here observed are useful for similar Portinari's artworks exposed in museums all over the world, if a decision for a non-destructive decontamination technique is taken.

There is no doubt that the technique used presents a key role in the applicability for artworks with similar characteristics, but it still needs to be studied in collaboration with other control techniques, as some level

of damage to the support is expected in some cases. Unfortunately, the artwork studied in the present paper was destroyed in a huge fire in 2018 in the Museu Nacional. However, Brazil still have some artworks by Candido Portinari with the same type of support and technique; this way, results from the present work can be useful to solve biodeterioration processes, probably produced by similar fungi here detected.

Declarations

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Conflicts of interest/Competing interest

We would like to inform that there is no conflict of interest by the authors.

Ethics approval

Not applicable.

Consent to participate

The authors inform that the present manuscript is not being considered for publication in any other journal, the results here presented are original and no texts from any other papers/documents were here included as part of the present paper (plagiarism). We also inform that all authors agree with the present submission to Microbial Ecology.

Consent for publication

Authors agree (in case of acceptance) to publish all data exclusively in Microbial Ecology.

Availability of data and material

Data here presented, basically culture cells isolates, are stored in the cell bank of National Institute of Technology. Code availability is not applicable.

Code availability

Not applicable.

Authors' contributions

Prof. Renata Nascimento and Dr. Fernanda Corrêa were responsible for sample collections, preparations and microbial analysis; Prof. Ana Oliveira was responsible for fungal identification by conventional techniques and Dr. Márcia Lutterbach responsible for molecular biology analysis. Dr. Ana Ferreira conducted gamma radiation experiments and Prof. Márcia Souza was responsible for the conservation and safeguard of the artwork at the National Museum. Prof. Antonio Costa was dedicated to collect all sets of data, from microbiological to irradiation, and write the present manuscript connecting all the results.

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Figures

Figure 1

“Índios” from Candido Portinari, artwork from 1937

Figure 2

Sample collection with sterile swabs

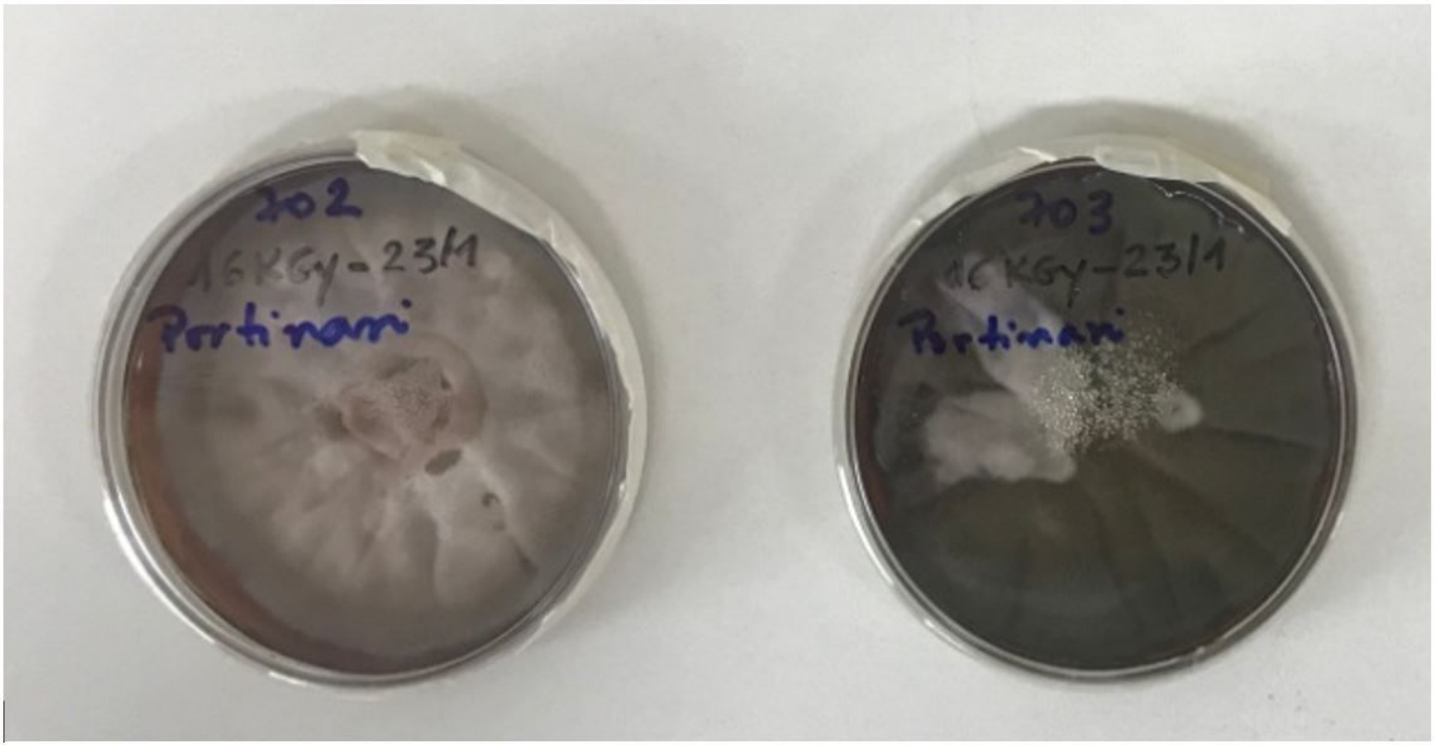

Figure 3

Viability of the fungus Nigrospora sp. (left) and Curvularia lunata (right) after irradiation of 16 kGy

Figure 4

Viability of the fungus Cladosporium halotolerans after irradiation of 16 kGy

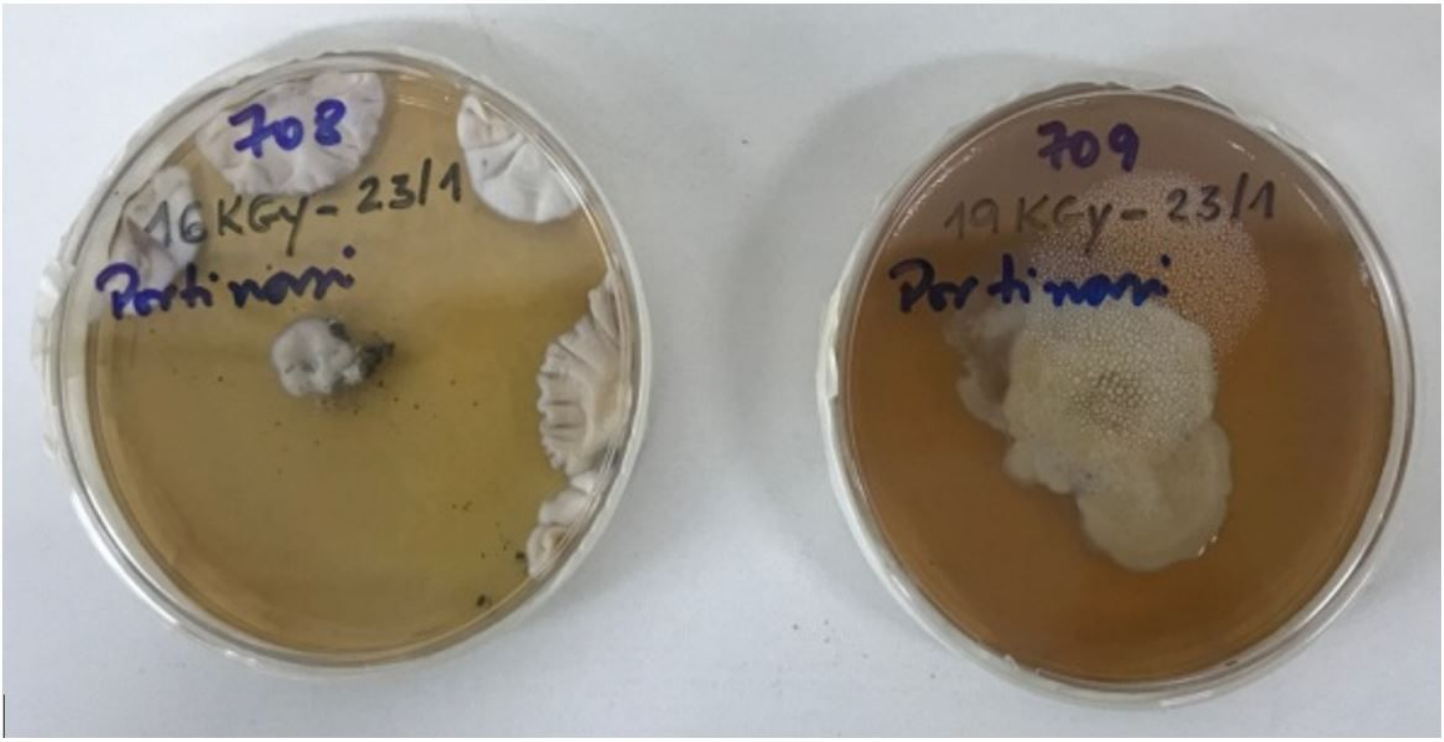

Figure 5

Viability of the fungus Penicillium sp (left) and Cladosporium sp. (right) after irradiation of 16 kGy and 19 kGy, respectively