Inactivation of fungi from deteriorated paper materials by radiation

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Abstract

Books and documents are subject to decay by a variety of organisms, including mould. Experiments were conducted to find the lowest gamma radiation dose needed to inactivate fungi. <i>Aspergillus versicolor</i> and <i>Eurotium chevalieri</i>, previously isolated from naturally contaminated book from a Brazilian public library and from the environment, respectively, were cultivated and treated in a Co<sup>60</sup> irradiation unit with doses ranging from 14.5 to 25 kGy. The minimum dose required to kill these fungi was 16 kGy. Thus, this dose was applied directly to a severely attacked book and deteriorated old documents. Pieces of damaged paper from these materials were incubated in culture media before and after the treatment. Several fungi were isolated and identified, including representatives of <i>Acremonium</i>, <i>Aspergillus</i>, <i>Cladosporium</i>, <i>Fusarium</i>, <i>Penicillium</i> and <i>Trichosporon</i>. After the treatment, no living fungi were detected from the irradiated material. The book was maintained in favorable conditions for new fungal attack for 2 months and no fungal growth was detected. These results are very promising and demonstrate the effectiveness of gamma-ray radiation for the recovery of severely damaged books and old documents, leading to the preservation of our cultural heritage and prevention of diseases caused by moulds in libraries and archives.

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1. Introduction

Libraries and archives sometimes have suitable conditions for fungal growth, which is highly dependent of temperature and humidity. Tropical countries, like Brazil, where high humidity and temperature are frequent, have environmental conditions that encourage the development of these microorganisms. This situation can bring risks to human health by mould contamination of books and documents, besides causing the decay of these publications, which sometimes are antique and rare (Gonzalez et al., 2002). Libraries and museums are responsible for preserving our heritage; they have the challenge to preserve millions of books printed on fragile paper manufactured through processes from the 19th century (Egan et al., 1995).

Fungi can <i>hydrolyse</i> a wide variety of polymers, including cellulose, as a result of their efficient degradative enzymes (Bennett and Faison, 1997). Cellulolytic fungi, which use cellulose as substratum, when growing in favorable environmental conditions, can destroy paper material in a short time (Adamo et al., 2003). Several techniques have been developed for book and document conservation reducing the threat of biodeteriorating agents, such as fungi. Some of these techniques involve the use of very toxic chemicals, including ethylene oxide, which has carcinogenic properties and is banned in a number of countries, besides being expensive (Flieder et al., 1994; Adamo et al., 2001; Gonzalez et al., 2002). An alternative is the use of gamma-ray radiation, a promising treatment in the preservation field.
Gamma radiation as sterilizing treatment causes direct damage to cell DNA through ionization inducing mutation and killing the cell. It also has an indirect effect as a result of radiolysis of cellular water and formation of active oxygen species, free radicals and peroxides causing single and double strand DNA breakages (McNamara et al., 2003).

Gamma rays, electromagnetic waves with high penetrating power, pass through materials without leaving any residue, an advantage comparing to other disinfection treatments, since the handling of books and documents may be done safely just after irradiation (Adamo et al., 1998, 2001). Studies demonstrated that the damage in mechanical–physical properties caused by gamma rays on paper was not significant (Adamo et al., 1998, 2001; Gonzalez et al., 2002). These studies were extended to the color of printing inks on paper and the result was that they are resistant to gamma radiation (Rocchetti et al., 2002).

Fungi have been successfully inactivated from different materials, such as paper, wood and soil with radiation doses ranging from 6 to 15 kGy (Hanus, 1985; Jörg et al., 1992; Pointing et al., 1998; McNamara et al., 2003). However, in a Brazilian study some fungi from books could not be completely eliminated after irradiation with doses of 20 kGy (Tomazello and Wiendl, 1995). Thus, new studies on the irradiation dose required to inactivate fungi were conducted on axenic cultures and on naturally contaminated book and documents, taking into account the tropical climatic conditions present in Brazil and its rich fungal diversity, resulting in many different fungal species as potential biodeteriorating agents.

2. Materials and methods

2.1. Irradiation

Gamma radiation was conducted in an irradiation facility at COPPE/UFRJ (Federal University of Rio de Janeiro), Rio de Janeiro, Brazil, using a research unit, Gamma Cell 220 (MDS Nordion, Ottawa, Ontario, Canada), equipped with a Co-60 source. The gamma-ray dose rate was 1.56 Mrad/min. Dose monitoring was conducted using alanine electron paramagnetic resonance (EPR) dosimetry (Ikeya, 1993).

2.2. Radiosensitivity tests of isolated fungi

From chemically treated books from a Brazilian Public Library (Biblioteca de Manguinhos, FIOCRUZ, Rio de Janeiro, Brazil) some resistant fungi were isolated, including Aspergillus versicolor. This fungus and Eurotium chevalieri, which was isolated from the environment, were used to determine the required dose needed to inactivate fungi on axenic cultures. A. versicolor was inoculated on plastic Petri dishes with MA2% (Malt Extract Agar 2%; Difco Laboratories, Sparks, MD, USA) and incubated at 25 °C for 4 days. Following the incubation period the cultures were irradiated with doses of 15, 20 and 25 kGy. After irradiation the plates were incubated at 25 °C for 10 days. The controls were cultivated on MA2% and were maintained in the same conditions as the treated fungi. The colony diameter was measured on the first, sixth and tenth day following irradiation to verify fungal growth comparing to the colony diameter measured just before irradiation and to the controls. The fungal inactivation was confirmed with the inoculation of the irradiated fungi into Yeast Malt Extract Broth (Difco Laboratories, Detroit, MI, USA) and the survival was recorded over a 10-day period at 25 °C. All assays were conducted in duplicates, and the means and standard deviations were calculated. Following the same procedure described above, other doses were tested, 14.5, 15, 15.5 and 16 kGy, on E. chevalieri.

2.3. Gamma irradiation of book and documents

Pages of paper (around 5 mm square) from an extremely deteriorated book from a private library and two old damaged archive documents (Documents 1 and 2), dated from the 1960s, from a Mental Health Hospital (Instituto Municipal Nise da Silveira, Rio de Janeiro, Brazil) were plated on MA2% containing the antibiotic chloramphenicol in a concentration of 400 μg/mL−1 and incubated at 25 °C. Each 2 days after the inoculation fungal colonies were isolated over a period of 7 days and purified. The isolated fungi were examined macroscopically by colony observation and microscopically by squash mounts stained with Lactophenol and Cotton Blue, which were examined through light microscopes (Olympus CBA-K, Micronal, Japan and Axiphot, Zeiss, West Germany). Based on these observations the fungi were identified. The book and the documents were individually sealed in polyethylene bags and then irradiated with 16 kGy. After irradiation the book and the documents were brought to the laboratory and under aseptic conditions pieces of paper of these materials were inoculated following the same procedure already described and fungal growth was observed for 10 days during incubation at 25 °C.

In order to verify a new fungal attack, following the radiation treatment, the book was taken to a warm (around 25 °C) and humid environment (75% RH) and left resting on a shelf with other books for a natural re-contamination. After 2 months the book was returned to the laboratory and the same tests conducted just after irradiation were repeated.

3. Results and discussion

The anamorphic Ascomycetes A. versicolor initially isolated from a chemically treated book, hence resistant to toxic chemicals, was used for radiosensitivity tests. Among fungi found on deteriorated paper and wood, representatives of the genus Aspergillus are ubiquitous (Hanus, 1985; Tomazello and Wiendl, 1995; Pointing et al., 1998). Pointing et al. (1998) studied the gamma irradiation dose required for inhibiting wood biodeteriogens and used several axenic fungal cultures, including Aspergillus niger, Penicillium notatum and Trichoderma viride, to determine 15 kGy as the required dose needed for fungal inactivation. In the present study A. versicolor was resistant to 15 kGy although it was inactivated with doses of 20 kGy (Fig. 1). The dose of 15 kGy has initially inactivated the colonies, which had a mean diameter of 1.9 ± 0.0 cm. After 1-day incubation the resistant cells started to develop again and resumed the colony growth reaching 4.8 ± 0.3 cm diameter after 10-day incubation, while the control colonies had 6.5 ± 0.05 cm diameter after the same period. Fig. 2 shows the irradiation effect on A. versicolor with doses of 15 and 20 kGy compared to the control.

Following these experiments other doses around 15 kGy were tested, beginning with 14.5 and reaching 16 kGy. E. chevalieri, the sexual phase of the asexual Aspergillus chevalieri, was inactivated with doses of 14.5 kGy (Fig. 3).

Since 15 kGy was not sufficient to kill A. versicolor, yet was
enough to inhibit *E. chevalieri*, the dose elected for book and document irradiation was 16 kGy. Inactivation of fungi on axenic culture and on ligninocellulose substrate was previously achieved with the same irradiation dose (Pointing et al., 1998).

Several anamorphic fungi were isolated from the deteriorated book and the two archive documents (Documents 1 and 2), including members of the Moniliaceae and Dematiaceae families, *Acremonium* spp., *Aspergillus* spp., *Cladosporium* spp., *Fusarium* sp., *Penicillium* spp. and *Trichosporon* spp. (Table 1). Representatives of *Aspergillus*, *Cladosporium*, *Fusarium* and *Penicillium* were previously isolated from contaminated books (Tomazello and Wiendl, 1995) and *Penicillium* spp. were isolated from a photograph infested by fungi (Adamo et al., 2001). *Trichosporon* spp. and *Acremonium* spp. were isolated from air from a Brazilian University library (Gambale et al., 1989). These fungi are commonly found on paper material and in library environment in Brazil (Tomazello and Wiendl, 1995; Gambale et al., 1989).

The book and the documents were irradiated with 16 kGy and following the incubation of paper samples from these materials no fungal growth was detected. In the studies of Tomazello and Wiendl (1995), *Aspergillus* spp. were resistant to 20 kGy, *Penicillium* spp. to 17.5 kGy and *Cladosporium cladosporioides* were previously isolated from contaminated books (Tomazello and Wiendl, 1995) and *Penicillium* spp. were isolated from a photograph infested by fungi (Adamo et al., 2001). *Trichosporon* spp. and *Acremonium* spp. were isolated from air from a Brazilian University library (Gambale et al., 1989). These fungi are commonly found on paper material and in library environment in Brazil (Tomazello and Wiendl, 1995; Gambale et al., 1989).

Our results demonstrated that there was no need of applying higher doses such as 20 kGy to completely inactivate *A. niger* and *C. cladosporioides*, fungi studied in the present and in the previous Brazilian investigation (Tomazello and Wiendl, 1995). Sixteen kGy was the needed dose to inactivate different fungal species on paper material, even the more resistant fungi, such as *Cladosporium* spp. (Dematiaceae), whose dark pigments, including melanin, produced and accumulated in their mycelium, might protect them against environmental extremes (Saleh et al., 1988; Freitag and Morrell, 1998).

Adamo et al. (1998) demonstrated that doses of 10 kGy did not cause any negative effect on the mechanical–physical properties of paper, even after an accelerate ageing of 12 days. Gonzalez et al. (2002) confirmed these results using doses of 14.4 kGy. They also observed that there were no changes in the paper color. However, there was a reduction of the degree of cellulose fiber polymerization when the dose rate was low (2.8 Gy/h), that is because the irradiation time was long enough to allow oxidative degradation (Adamo et al., 1998). Thus, the longer is the
irradiation period because of lower dose-rate, the greater possibility oxygen has to interact by chemical modification on cellulose polymers and the greater will be the indirect damage (Magaudda, 2004). Even though, the level of depolymerization did not change appreciably the mechanical properties of the paper (Adamo et al., 1998; Gonzalez et al., 2002). These tests demonstrate that in regard to the cost–benefit analysis, the benefits are greater than the costs (Magaudda, 2004).

The book left for natural re-contamination was still sterile after 2 months following irradiation, no fungi was isolated from the paper samples plated on media culture. Adamo et al. (1998) believed that the depolymerization resultant from the irradiation would lead to an increased susceptibility of the cellulose to new attacks by fungi. Yet, on paper irradiated with doses of 3 and 10 kGy, followed by fungal inoculation, the fungal growth was comparable to the control, while higher doses, 100 and 200 kGy, caused a significant inhibition of *Penicillium chrysogenum* growth. The high irradiation dose induced some structure alteration that may affect fungal growth (Adamo et al., 2003). This structure alteration on the paper might have happened in the irradiated book using 16 kGy, avoiding fungal growth even in favorable environmental conditions.

These results confirmed that radiation treatment of books and documents is extremely efficient. The gamma radiation preservation technology has brought a powerful way to save ancient books, archives documents and other paper materials from being damaged by moulds, besides guaranteeing a good quality of life for the library and archive employees and users.

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### References


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### Table 1

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<thead>
<tr>
<th>Fungi isolated from deteriorated book and documents before irradiation treatment</th>
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<tr>
<td><strong>Book</strong></td>
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<tr>
<td>(a) Moniliaceae</td>
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<tr>
<td><em>Acremonium</em> spp. Link</td>
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<td><em>Fusarium</em> sp. Link</td>
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<td><em>Penicillium</em> spp. Link</td>
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<td><em>P. arenicola</em> Chalabuda</td>
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<td>(b) Dematiaceae</td>
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<td><em>Cladosporium cladosporioides</em> (Fresen.) de Vries</td>
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<tr>
<td><em>C. herbarum</em> (Pers.) Link ex S.F. Gray</td>
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