Article

Disinfection of Woodblocks of the Nguyen Dynasty of Vietnam by Low-Energy X-rays

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Received June 25, 2020

Accepted October 5, 2020

The effects of low-energy X-ray irradiation were investigated as an intervention strategy for the disinfection of fungi-contaminated woodblocks. Fungi were isolated from the woodblocks of the Nguyen dynasty of Vietnam and Cladosporium sp. was identified as the most radiation-resistant strain in the woodblock. The dose rates of the F1 (1-mm-aluminum filtered) and F0 (non-filtered) X-rays at the surface of the woodblock were 1.14 and 4.64 kGy/h, respectively. At the middle (8.5 mm from the surface) of the woodblock, the doses of the F1 and F0 X-rays decreased to 76% and 20% of the surface doses, respectively. The F1 X-rays were useful for irradiating the inside of the woodblock; the concentration of the fungi at the middle decreased by more than 4 log fractions at 6.2 kGy and the fungi were eliminated with a surface dose of 8.3 kGy. Furthermore, the contaminated fungi in the woodblock were disinfected by both-side irradiation with F1 X-rays delivering a dose of 10 kGy at a dose uniformity of 1.04.

Key Words: low-energy X-ray, woodblock, fungi, disinfection, both-side irradiation

1. Introduction

Cultural heritage that is inherited from past generations is an integral part of each society and country. Inherited artefacts such as wooden structures, wooden furniture, and woodblocks are a large group of the wooden cultural heritage artefacts. Among them, woodblocks play an important role. Woodblocks, originating in China, were widely used throughout East Asia to print text, images, or patterns. Vietnamese culture is one of the oldest in Southeast Asia, and the many intangible cultural heritage of Vietnam has been presented to the world. There are more than 34,000 plates of woodblocks

from the Nguyen dynasty in Vietnam with engravings in classical Chinese, as well as Chu Nom (the old Vietnamese scripts), that recorded historical and literary works during the Nguyen dynasty from the middle of the 17th century to the beginning of the 20th century. These woodblocks are also evidence of the development of the woodblock carving and printing profession in Vietnam in that period. The woodblocks of the Nguyen dynasty of Vietnam were recognized by UNESCO and registered as a World Heritage Site in 2009. Although these woodblocks have been preserved and displayed at the National Archive Center IV (Dalat, Vietnam) since 2009, they have suffered damage by insects such as termites and

various fungi, which is known to be the main cause of the color change and cellulose biodegradation.¹⁾ Fungi, which can digest wood products, are the most important conspicuous organisms. Some fungi not only affect the quality of the cultural objects but are also hazardous to professionals and users, due to the production of mycotoxins.²⁾

Although lime water or gas fumigation is mainly used to preserve woodblocks,^{3, 4)} these woodblocks remain damaged by insects and fungi. These conventional methods are toxic for humans, and sometimes the disinfection effect is low. Therefore, the disinfection of fungi by an effective method is paramount for the conservation of woodblocks.

The application of radiation technologies for the preservation of cultural heritage has been widely accepted in many countries.⁵⁾ γ -rays from ^{60}Co and electron beams/X-rays with high energy are commonly used for radiation sterilization and food irradiation. The benefits of radiation sterilization are high speed and highly efficient treatments at room temperature. However, these facilities require the installation of radiation shielding to protect personnel from high-energy radiation exposure, which is prohibitively expensive. The use of low-energy X-rays for the sterilization of medical devices and food irradiation has lately attracted more interest. The merit of low-energy X-rays is its low shielding requirements. The irradiation device uses an X-ray tube with low energy, and is reliable, compact, and cost-effective. The compact low-energy X-ray irradiator has been widely used in healthcare for blood irradiation. In addition, compact X-ray machines can be easily transported to storage places such as museums and libraries.

This study aims to investigate the effects of low-energy X-ray irradiation on fungi, and thus determine suitable irradiation conditions for the disinfection of woodblocks.

2. Experimental

2.1 Materials

Woodblock samples were obtained from the National Archive Center IV (Dalat, Vietnam), from which small pieces (ca. 2×3 cm) were used for the irradiation tests.

Standard strains of fungi, *Aspergillus niger*, *Cladosporium cladosporioides*, and *Aureobasidium pullulans*, were purchased from NBRC (Biological Resource Center, NITE).

HD-V2 Gafchromic film⁶⁾ was purchased from Ashland, USA, and used for the dosimetry of X-rays. This film dosimeter is thin (ca. $110\ \mu\text{m}$), comprises an active layer ($12\ \mu\text{m}$) coated on a polyester substrate ($97\ \mu\text{m}$), and operates in a dose range of 10 to 1000 Gy.

2.2 Microbiological analysis

The swab method was used to collect the fungi from the woodblocks. The sterile swabs were directly rubbed on the woodblock to collect fungal cells and transferred to potato dextrose agar (PDA) medium and M40Y medium (for xerophilic fungi). Chloramphenicol, a broad-spectrum antibiotic, was used in all the culture media for the inhibition of bacterial growth. Isolated fungi were identified by the morphological method.⁷⁾

The isolated fungi were incubated on PDA for 7 days at 25°C . The conidia were harvested using phosphate-buffered saline (PBS, pH 7.0) with 0.05% Tween 80. The conidia density was adjusted to ca. 10^7 CFU/mL by using a disposable Optical Plastic Plankton Counter. Japanese paper ($1\ \text{cm} \times 1\ \text{cm}$, $0.1\ \text{mm}$) was inoculated with $10\ \mu\text{L}$ of the conidia suspension (10^5 CFU/sheet) and dried overnight in a biological safety cabinet at room temperature. The paper samples with fungi were inserted and sealed within a gas-permeable pouch for autoclave or EO sterilization (Hogy medical, HM1301) and subjected

to irradiation. After irradiation, a paper sample with fungi was put in 1 mL PBS (pH 7.0) with 0.05% Tween 80 and vortex 20 minutes. The viability of irradiated conidia was determined using the dilution plating method with PDA. The viable cell count after the irradiation is expressed as an average log₁₀ value with a standard deviation determined from triplicate experiments.

2.3 Irradiation

X-ray irradiation system model MBR-1618R-BE (Hitachi Power Solutions, Japan) installed at the Faculty of Physics and Nuclear Engineering (Dalat University, Dalat, Vietnam) was used in this research. The irradiator can change the tube voltage (35 to 160 kV), tube current (1 to 30 mA), and irradiation time or irradiation dose to emit X-rays. The irradiator was equipped with five types of filters to cut the very low energy part of the X-rays. X-rays at 160 kV, 18.6 mA (3 kW) with no filters (F0), and a 1 mm aluminum filter (F1) were used in this experiment. The distance from the X-ray focal point to the sample was 150 mm. HD-V2 film dosimeter calibrated with a Fricke dosimeter⁸⁾ was used to measure the dose rates of the X-rays. The pouches containing the paper samples with fungi were put on the surface and bottom of the woodblock samples (density: 0.71 g/cm³) as well as inserting between the samples cut into half, and irradiated for the predetermined time calculated from the dose rate.

In the case of γ irradiation, ⁶⁰Co sources were used (dose rate: 1.87 kGy/h) in the irradiation pool at the Radiation Research Center of Osaka Prefecture University, Sakai City, Japan. The dose was determined with an ion chamber (Applied Engineering Inc.) and PMMA dosimetry.⁹⁾

3. Results and discussion

3.1 Isolation of fungi from woodblock samples

Fungi in the woodblock samples were detected

Fig. 1 Radiation sensitivity of the fungi isolated from the woodblocks. The isolated strains were labeled from VN1 to VN14. The conidia suspension (10^7 CFU/mL) of the 14 strains were irradiated at 3, 4, and 5 kGy by γ rays and 100 μ L irradiated sample was inoculated on PDA plate. * n.d., not detected (<10 CFU/mL).

before radiation disinfection. The deteriorating spots on the woodblocks, identified as areas of color change, were randomly chosen by the naked eye. A total of 14 fungal strains (hyphomycetes) were isolated from the woodblocks and labeled from VN1 to VN14. The isolated fungi were incubated on PDA for 7–10 days at 25°C, and the conidia were harvested. The conidia of these 14 strains were irradiated with γ rays at a dose range of 3 to 5 kGy to select the strains with the highest radiation resistance. Eight strains survived at 3 kGy irradiation, while only three strains (VN1, VN3, and VN7) survived at 5 kGy irradiation, as shown in Fig. 1. All of these 3 strains belonged to the genus *Cladosporium*, based on morphology identification. The highest radiation resistant strain, VN3 (*Cladosporium* sp.), was selected for subsequent experiments.

3.2 Radiation sensitivity of fungi

The radiation sensitivities of the *Cladosporium* sp. isolated from the woodblock were examined by using X-rays and γ rays. Fig. 2 shows the sur-

Fig. 2 Radiation sensitivity of *Cladosporium* sp. □; F0 X-rays (non-filtered), ○; F1 X-rays (1-mm-aluminum filtered), △; γ rays. The conidia were dried on paper and irradiated.

vival curves of *Cladosporium* sp. subjected to the F0 (non-filtered) X-rays, F1 (1-mm-aluminum filtered) X-rays, and γ rays. The dose rates for the F1 X-rays, F0 X-rays, and γ rays were 1.10, 4.65, and 1.87 kGy/h, respectively. All 3 survival curves of *Cladosporium* sp. exhibited an exponential trend. The D_{10} values corresponding to the F1 X-rays, F0 X-rays, and γ rays were 0.79 kGy, 0.57 kGy, and 0.89 kGy, respectively. The radiation sensitivity of *Cladosporium* sp. to the F1 X-rays and γ rays was similar, although it was higher in the case of the F0 X-rays. Ha et al.¹⁰⁾ reported a greater reduction in *Bacillus pumilus* spores by X-ray irradiation with a cut-off energy of 50–150 keV. Miura et al.¹¹⁾ also reported that the low energy component of the X-rays was remarkably removed by using the filter, and that the thinner filter exerted a greater effect on the cell. Further studies are necessary to clarify the effects of the low energy parts of the X-rays by using different energy and cut filters.

The radiation sensitivities of the *Cladosporium* sp. isolated from the woodblocks to γ rays were compared with those of three NBRC strains (*A. pullulans*, *C. cladosporioides*, and *A. niger*) to better understand the radiation resistance of the isolated strains. The dry

Fig. 3 Survival curves of fungi by γ irradiation. □; *A. pullulans*, ○; *C. cladosporioides*, △; *A. niger*, ▽; *Cladosporium* sp. (VN3). The conidia were dried on paper and irradiated.

conidia on paper were irradiated with γ rays, and the survival curves of the 4 strains are shown in Fig. 3. Both the survival curves of *Cladosporium* sp. and *C. cladosporioides* show an exponential trend, and the D_{10} values were almost the same. *A. pullulans* was radioresistant with a large shoulder. Tolerance to γ rays decreased as follows: *A. pullulans* > *C. cladosporioides* (*Cladosporium* sp.) > *A. niger*. The D_{10} value of *A. pullulans* was approximately 5 and 11 times as high as those of *Cladosporium* genus and *A. niger*, respectively. *A. pullulans*, a ubiquitous fungus that can be found in environments with background radioactivity, is one of the most radioresistant species because of their stress protector trehalose and melanin production.¹²⁾ These results suggest that the *Cladosporium* strain belongs to the medium level of radioresistant fungal species.

3.3 Decontamination of woodblock by X-ray irradiation

Woodblock samples (ca. 2 cm × 3 cm, thickness 17 mm) were cut in half and used as a model to investigate the necessary dosage for fungal disinfection in woodblock. Table 1 shows the dose rates for the F1 X-rays and F0 X-rays at 3 positions, which are

Table 1 Dose rates in the woodblocks of the F1 and F0 X-rays

Position	Depth (mm) from top to bottom	Dose rate (kGy/h)	
		F1	F0
Top	0	1.14 (100%)	4.64 (100%)
Middle	8.5 ± 0.8	0.87 (76%)	0.94 (20%)
Bottom	17 ± 2.1	0.65 (57%)	0.69 (015)

F1: with 1 mm aluminum filter, F0: no filter. The percentage of the dose relative to that at the top are shown in parentheses.

Table 2 Dose distribution in the woodblocks of the F1 and F0 X-rays

	Depth from top (mm)	Dose (kGy)					
		F0			F1		
Top	0	4.0	7.9	18.5	4.1	6.2	8.3
Middle	8.5	0.8	1.6	3.7	3.2	4.7	6.3
Bottom	17	0.6	1.2	2.7	2.4	3.6	4.7

F1: with 1 mm aluminum filter, F0: no filter

the top, middle (8.5 mm depth), and bottom (17 mm depth) of each woodblock. The dose rates of the F1 and F0 X-rays at the surface of the woodblock were 1.14 and 4.64 kGy/h, respectively. The result indicates that approximately 75% of the low energy part of the X-rays was cut by a 1 mm aluminum filter. At the middle position of the woodblock, the doses of the F1 and F0 X-rays decreased to 76% and 20% of the surface doses, respectively.

Paper sheets contaminated with 10^7 CFU of *Cladosporium sp.* were placed at 3 positions on the woodblock and irradiated with F1 X-rays and F0 X-rays at various doses. The paper sheet samples (thickness ca. 0.1 mm) were covered by a plastic bag (thickness ca. 0.05 mm) and used for irradiation in the woodblock. The doses at each position are shown in Table 2.

Fig. 4 shows the number of survivors of *Cladosporium sp.* in the woodblock irradiated with the F1 X-rays. At a surface dose of 4.1 kGy, 1.8×10^2 at the

Fig. 4 Effect of 1-mm-aluminum-filtered (F1) X-rays on the *Cladosporium sp.* conidia in the woodblock. The effect was examined at 3 positions of the woodblock (top, middle, and bottom). Dose of each position are shown in Table 2.

top, 4.4×10^3 at the middle, and 1.6×10^4 CFU at the bottom were detected. More than 2 log reduction at 4.1 kGy and 4 log reduction at 6.2 kGy in the middle

Fig. 5 Effect of the non-filtered (F0) X-rays on the *Cladosporium* sp. conidia in the woodblock. The effect was examined at 3 positions of the woodblock (top, middle, and bottom). Dose of each position are shown in Table 2.

of the woodblock were observed, whereas no survival conidia could be found at 8.3 kGy.

In the case of the F0 X-rays, no conidia were detected at all doses at the top of the woodblocks (Fig. 5). More than 10⁴ CFU were detected at the middle and bottom positions of the woodblock at 4.0 kGy and 7.9 kGy surface irradiation. At the dose of 18.5 kGy, the number of conidia was below detection limits at the middle position.

For the disinfection of fungi in the woodblock by low-energy X-rays, both-side irradiation is necessary. In the case of 6.2 kGy irradiation by the F1 X-rays shown in Fig. 4, more than 4 log reduction was obtained in the middle of the woodblock. The doses at the surface and middle for both-side irradiation were calculated as 9.8 (6.2+3.6) kGy and 9.4 (4.7×2) kGy. The results show that more than 8 log reduction can be obtained by both-side irradiation of the F1 X-rays at 10 kGy with a dose uniformity 1.04 in the depth direction of woodblock.

The radiation dose required to disinfect the fungi-contaminated woodblocks varied according to the fungal species, the population of the contaminants, the surface area, and the thickness of the objects.¹³⁾

The sterilization assurance level, SAL (10⁻⁶), is commonly used for the sterilization of medical devices, and the IAEA recommended a dose of 10 kGy for safe sterilization of wooden artifact against fungi.¹⁴⁾ Moreover, the European Standard EN 113¹⁵⁾ recommended doses between 25 and 50 kGy for wood sterilization in laboratory testing procedures. In this study, the sterilization dose of the woodblock can be calculated as 9.5 kGy by 0.79 kGy (D_{10} value obtained in Fig. 2) × 12 log reduction. In addition, more than 8 log fractions can be reduced with both-side irradiation of 10 kGy. Considering that the contamination by fungi at the middle position of the woodblock is usually lower, it can be concluded that the necessary dose for the disinfection of fungi in the woodblock is less than 10 kGy.

Wooden cultural heritage objects are treated for insect eradication with a dose of 2 kGy.⁵⁾ Therefore, insects would be eradicated while the woodblock was exposed to 10 kGy irradiation for fungal disinfection. The disinfection dose of 10 kGy obtained in this study could also protect woodblocks from the negative effects of high dose irradiation.¹⁶⁾ The use of a cut-off filter for the low energy parts of the X-rays is essential to avoid high surface doses by the X-rays (F0).

Recently, Haff et al.¹⁷⁾ designed an inline system of X-ray tube-based irradiators for fruit irradiation. The merit of low-energy X-rays is its low shielding requirements and thus the design of the mobile system is recommended for the irradiation of cultural heritage objects.

Acknowledgments

We thank Mr. Nguyen Xuan Hung, Director of the National Archive Center IV (Vietnam), for providing the woodblock samples. We also thank Ms. Pham Thi Ngoc Ha, Mr. Nguyen Dang Trong Phuc, and Mr. Cao Van Hai of the Faculty of Physics and Nuclear Engineering, Dalat University, Vietnam for

their support on X-ray irradiation and dosimetry.

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要 旨

低エネルギー X 線によるベトナム阮朝の版木の殺菌

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2020 年 6 月 25 日 受付

2020 年 10 月 5 日 受理

文化財である版木の汚染カビについて、低エネルギー X 線による殺菌効果を検討した。ベトナム阮王朝の版木から分離されたカビの中で、最も放射線抵抗性の株は *Cladosporium* sp. であった。用いた X 線 F1 (1mm アルミニウム フィルター装着) と F0 (フィルターなし) の版木表面における線量率は、1.14 及び 4.64 kGy/h であった。版木の中心部 (表面からの深さ 8.5 mm) の線量は、表面線量に対し F1 で 76%, F0 で 20% に減少した。F1 は深部の照射に適しており、版木中心部のカビは表面 6.2 kGy の照射で 4 桁減少し、8.3 kGy で検出されなくなった。これらの結果から、版木中の汚染カビは X 線 (F1) を用いた両面照射により、線量 10 kGy、線量均一度 1.04 で効果的な殺菌が可能であることが示された。