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Inactivation of fungal contaminants on Korean traditional cashbox by gamma irradiation

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HIGHLIGHTS

- Fungi were isolated from Korean traditional cashbox stored in museum.
- Radiation sensitivity was measured for the isolated strains.
- A low dose of 5 kGy was successfully applied to decontaminate the cashbox.
- This result supports the application radiation at a low dose for decontamination of cultural relics.

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ABSTRACT

In this study, gamma irradiation was applied to decontaminate a Korean cultural artifact, a wooden cashbox stored in local museum. Fungi isolated from the wooden cashbox were identified by 18S rDNA sequencing methods. It was observed that the isolated fungi exhibited high similarity to *Aspergillus niger*, *Penicillium verruculosum*, and *Trichoderma viride*. Each strain was tested for sensitivity to gamma irradiation, and was inactivated by the irradiation at a dose of 5 kGy. The wooden cashbox was thus gamma-irradiated at this dose (5 kGy), and consequently decontaminated. Two months after the irradiation, when the wooden cashbox was retested to detect biological contamination, no fungi were found. Therefore, these results suggest that gamma irradiation at a low dose of 5 kGy can be applied for successful decontamination of wooden artifacts.

1. Introduction

Wood has been used by humans for thousands of years because of its easy workability, abundant availability, and sufficient stiffness for construction. Wooden relics, including tools, weapons, sculptures, and structures, provide an interesting picture of the skills and the ingenuity of past generations (Nilsson and Rowell, 2012). In Korea, wood has also been used as a material in furniture, agricultural tools, and household items; many wooden relics are conserved in museums or by personal owners. As wood is an organic material, wooden artifacts are subject to biodegradation by insects and fungi (Unger, 2012). In particular, fungi that can grow in diverse and extreme environmental conditions could damage theses relics. Many historical wooden artifacts have undergone chemical and physical changes due to biological contamination, and some have been lost altogether. Wooden artifacts, even if stored in closed, carefully controlled conditions, could still be subject to biodegradation by fungi (Sterflinger, 2010).

Pesticides including dichlorodiphenyltrichloroethane (DDT), pentachlorophenol (PCP), and lindane have been used to protect wooden artifacts from insects and fungi. However, these toxic pesticides, especially DDT, are emitted into the air of the storage rooms, causing potential health risks for staff and visitors (Wörle et al., 2012). In addition, the application of the most extensively used fumigant gases, including methyl bromide and ethylene oxide, has been either phased out or severely restricted (Katušin-Ražem et al., 2009). Therefore, in order to decontaminate the wooden artifacts and avoid potential health risks, different decontamination methods such as gamma irradiation are being developed. Originally, gamma irradiation was used to sterilize and decontaminate food, pharmaceuticals, and medical devices. During irradiation, high-energy photons are emitted from an isotope source (cobalt-60 and caesium-137), producing ionization (electron disruptions) throughout the object being irradiated. In living cells, these disruptions result in damage to the DNA and other cellular structures. At the molecular level, these photon-induced changes cause the death of the organism or render the organism

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incapable of reproduction. The gamma irradiation process does not leave residuals in or impart radioactivity to the processed objects.

Recently, several reports on the decontamination of cultural artifacts by gamma irradiation have been published. One such instance was the disinfestation treatment of the Ramses II's mummy by gamma irradiation, performed by the ARC-Nucleart Laboratory in Grenoble, France (Tassigny and Brouqui, 1978). Another instance was the emergency treatment to arrest mold and insect infestation in a large number of documents damaged by floodwater in the Alan Mason Chesney Medical Archives of the Johns Hopkins Medical Institutions (Sinco, 2000). Recently, Abdel-Haliem et al. (2013) reported that the growth of *Streptomyces* strains isolated from painting and stone surfaces from Egypt tombs were completely inhibited by radiation treatment, which were highly resistant to antibiotics.

The effectiveness of gamma irradiation depends on several factors, including the type of contaminating microorganism, the storage environment, and the tree species of the wooden artifact. Until recently, sufficient information on the microbial contaminants of Korean wooden artifacts was not available, and the effectiveness of gamma irradiation for decontamination has not been tested. Therefore, this study investigated the efficacy of gamma irradiation in decontaminating a wooden cashbox conserved in a local museum. The fungi on the wooden cashbox were isolated and identified for this purpose. The sensitivity of the isolated fungi to gamma irradiation was experimentally measured before the decontamination of the wooden cashbox by gamma irradiation.

2. Materials and methods

2.1. Materials

The cultural artifact used in this study was a Korean traditional wooden cashbox, which has been stored in the warehouse of the Wando Forest Museum (Wando, South Korea) (Fig. 1). The wooden cashbox had been used in the region near Wando Island during the late 19th century.

2.2. Identification of fungi on wooden cashbox

Strains of fungi were isolated from four different locations on the wooden cashbox by using sterilized cotton buds. Sampling was performed by a certain area (about 1 cm^2) by swabbing with the cotton buds. Cotton buds wrapped in gauze were already sterilized in autoclave at 121 °C, and were rubbed with sterile tweezers. The cotton buds with microbes were immersed in 1 mL of Potato dextrose broth (PDB, Difco Laboratories, Sparks, MD) under sterilized condition, and the collected microbial samples were suspended in the medium by vigorous vortexing. Each 200 μL broth was inoculated on a Potato dextrose agar (PDA) medium plates (Difco Laboratories) and subsequently incubated at 28 °C. A method based on DNA sequencing analysis was used to identify the fungi isolated from the wooden cashbox. About 20 strain samples from the cashbox were used for the DNA preparation. Genomic DNA was isolated from the fungi by using a preparation kit (MP Biomedicals LLC, Irvine, CA). To obtain 18S rDNA, polymerase chain reaction (PCR) was performed with the 18S rRNA gene primers of Internal Transcribed Spacers (ITS) 1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) (Korabecna et al., 2003). The gene sequences of the PCR products were analyzed by comparing with the reported 18S rDNA sequences from the National Center for Biotechnology Information (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.3. Radiosensitivity test of isolated fungi

The sensitivity of the isolated fungi to gamma irradiation was measured by irradiating the culture broth of each strain on the agar media with a cobalt-60 irradiator (cylindrical shape with cobalt 60 pencils, AECL, IR-79; Nordion, Canada) in Korean Atomic Energy Research Institute, Jeongeup, South Korea, at various absorbed doses including 0, 0.5, 1, 2, 3, and 4 kGy. The temperature of the irradiation room was controlled at 23 ± 2 °C with a relative humidity of 50 ± 5%. Dosimetry was carried out using Alanine–EPR dosimetry system (Bruker Instruments, Rheinstetten, Germany), which showed that the actual dose was within 2% of the target dose. The colony diameter was measured on the third day after irradiation to verify the fungal growth compared with the non-irradiated control. In detail, the fungi cultured in PDB (100 μL) was inoculated on PDA plates. Immediately after the inoculation, the plates were gamma-irradiated at the specified doses. The time for exposure to irradiation source was 6 min for 1 kGy. The irradiated plates were incubated at 28 °C for 3 days before measuring the colony area. And, to confirm the inactivation of fungi after irradiation, the culture was further incubated for a week. All experiments were performed in triplicate.

Fig. 1. Photograph of the Korean traditional cashbox stored in local museum.