

ORIGINAL**Wavelength-dependent differences in survival of *Cladosporium sphaerospermum* exposed to ultraviolet light**

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Abstract : Fungi are one of the main sources of microbial contamination in food and residential environments. There has been reported that fungi are more resistant to drugs than are viruses and bacteria, so efficient sterilization methods are still required. In this study, we examined the effectiveness of ultraviolet (UV) sterilization against mold by focusing on the wavelength and radiant flux dependence of UV. UVC or UVA irradiation inactivated the mold species *Cladosporium sphaerospermum* in a dose-dependent manner. The energy required to obtain the same inactivation differed significantly between UVC irradiation and UVA irradiation. UVC irradiation induced the formation of substantial amounts of cyclobutane pyrimidine dimers, whereas UVA irradiation did not show any significant difference from the unirradiated group. Furthermore, UVC irradiation induced morphological changes that could be observed by electron microscopy. On the other hand, UVA irradiation suppressed the growth rate. These results suggest that UVC and UVA irradiation have different effects on fungi. The results suggest that both UVC and UVA irradiation may help control fungal contamination. Since the effect of UV differs depending on the wavelength of irradiation, it will be necessary to understand the wavelength-specific effect and use it to control fungal contamination in the living environment. *J. Med. Invest.* 72:260-265, August, 2025

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INTRODUCTION

Fungi are a major source of microbial contamination in food and residential environments (1). Research on indoor environmental fungi has been initiated to search for allergens associated with asthma, but infections caused by fungi living and floating in the indoor environment have become a major problem (2, 3). *Cladosporium* species, commonly found in both indoor and outdoor environments, can pose health risks, particularly to individuals with allergies, asthma, or weakened immune systems. In indoor settings, it can grow on damp surfaces like walls, carpets, and windowsills, producing spores that, when inhaled, may cause respiratory issues, skin irritation, and sinus infections (4, 5). Prolonged exposure to prominent levels of *Cladosporium* spores may exacerbate asthma symptoms and lead to allergic reactions. While it is not harmful to healthy individuals, managing moisture and indoor air quality is important to minimize its growth and reduce health risks.

Recent advances in medical technology have made it possible to extend life expectancies. However, with the advent of an aging society, an increasing number of patients are experiencing opportunistic infections and allergic diseases caused by fungi in the living environment, and to maintain health, it is no longer possible to ignore the microorganisms living around us (6). The same concerns apply not only to the living environment but also to

water and food hygiene, where fungal eradication is important.

Ultraviolet (UV) light irradiation is a method for inactivating pathogenic microorganisms that involves irradiation of objects with electromagnetic waves of 10-400 nm, and was approved by the US Food and Drug Administration in 2000 as an effective method for inactivating pathogens in food, water, and beverages (7).

Inactivation of microorganisms using UV light has been actively studied with mercury lamps such as low-pressure and medium-pressure mercury lamps (8, 9); tunable wavelength lasers (10, 11); and light-emitting diodes (LEDs). In particular, UV-LEDs are expected to be a promising disinfection light source because they have a long lifetime, a radiant flux that can be adjusted by electric current, and wide range of choices of wavelengths; are compact; can be mounted on various devices; and do not emit hazardous waste such as mercury (12-14).

We are focusing on light-based sterilization using UV-LEDs. Among the biological effects of light widely used in the living environment, there are many reports on UV light (15-17). Interestingly, the UVA and UVC bands have different biological effects on viruses and bacteria (16, 18, 19). Furthermore, because UVA and UVC irradiation have different mechanisms of action, it has been reported that simultaneous irradiation can result in additive or synergistic effects (20, 21). These results may lead to new UV irradiation methods.

Most studies on the effects of UV irradiation on fungi have been conducted using 254 nm radiation from mercury lamps for *Aspergillus niger*, *Candida albicans*, and *Saccharomyces cerevisiae*. However, the data found were highly scattered, which could be due to the experimental conditions (22). Although the individual data sets seem large, many important fungi have not been studied extensively to date. For example, no UV irradiation data yet exists for half of the fungi classified as "high" or "medium

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priority" by the World Health Organization (23). Furthermore, there are few reports that have investigated the differences in the effects of UVA and UVC irradiation.

In this study, we investigated the effects of UVA or UVC irradiation on *C. sphaerospermum* spore viability, photoproduct cyclobutane pyrimidine dimer (CPD) production, growth rate, and morphological changes, and discuss about sterilization method used by the UV-LED.

MATERIALS AND METHODS

Fungal culture and preparation

Cladosporium sphaerospermum (NBRC6348) was purchased from the National Institute of Technology and Evaluation (NBRC). *C. sphaerospermum* was stored as a glycerol stock (final concentration of glycerol, 10%) at -80°C. When preparing experimental samples, thawed stock was cultured at 25°C and incubated for 100 h on a Potato Dextrose Agar plate (PDA plate; Shimadzu Diagnostics Corporation; SDC). After incubation, the fungal body was transferred to a 50 mL tube with the entire medium and washed with PBS. The resulting suspension was filtered (Cell Strainer 70 µm, AS ONE CORPORATION, Osaka, Japan) to remove agar debris. The filtered suspension was further filtered through a fine filter (Cell Strainer 10 µm, Funakoshi Co, Ltd. Tokyo, Japan) to separate the hyphae from the prepared spore suspension. The concentration of each spore suspension was then adjusted to a final concentration of 4.1×10^5 spores/mL based on counts using a hemocytometer.

UV-LED irradiation

UV irradiation was conducted using the UV-LED standardized irradiation system reported previously (18). The temperature of the LED was controlled at $25^\circ\text{C} \pm 3^\circ\text{C}$ by connecting the cooling water flow path in the heat sink to a chiller. A heat sink was also connected to the stage on which the fungus sample was placed, and the sample temperature during UV irradiation was kept at $25^\circ\text{C} \pm 3^\circ\text{C}$ (15). First, each fungal sample was prepared with sterile PBS to prepare a spore suspension of a predetermined concentration, of which 1 mL was dispensed into a 35 mm dish (Sarstedt, Nümbrecht, Germany). Samples were then UV irradiated using the device and two wavelength ranked LEDs, U280 LED for UV-C irradiation and U365 LED for UV-A irradiation (Nichia Corporation, Tokushima, Japan). Irradiance at the sample surface for each wavelength of UV light was shown in a previous study (18). All UV irradiation was performed in the dark to reduce the influence of ambient light, such as light recovery by photolysis.

Measurement of fungal inactivation

After irradiation, the fungal samples were again collected in 1.5 mL tubes and diluted with D-PBS. Next, 100 µL aliquots of the samples were plated on PDA plates and were incubated at 25°C for 3 days. The numbers of the fungal cells were estimated using colony-forming unit (CFU) assays. The inactivating effects of UV-LED irradiation were evaluated using the \log_{10} CFU reduction compared with an unirradiated sample. This value was defined as "log survival rate" and was calculated using the following equation: log survival rate = $\log_{10}(N_t/N_0)$, where N_t is the CFU/mL of the UV-irradiated sample and N_0 is the CFU/mL of the sample without UV irradiation.

Fungal DNA extraction

An aliquot of 1 mL of the fungal suspension was centrifuged at 12000 rpm for 5 minutes to remove the supernatant. The pellet was dissolved in 600 µL of sorbitol buffer (1 M sorbitol, 0.1 M

sodium EDTA, 14 mM β -mercaptoethanol (Fujifilm Wako Pure Chem. Co., Osaka, Japan) and then 200 units (50 µL) of Lyticase (Zymolyase®, NACALAI TESQUE, INC., Kyoto, Japan) were added, and the resulting suspension was incubated at 30°C for 30 minutes. Again, centrifugation was performed at 7500 rpm for 10 minutes, the supernatant was removed, and fungal DNA was extracted using DNeasy Blood & Tissue Kit (50) (QIAGEN, Germany). After DNA extraction, DNA concentration was measured using UV-Vis spectroscopy (Beckman Coulter DU-730, Germany).

Electron microscopy

The fungal suspension was placed in a 35-mm dish, irradiated with UVA/UVC, and pre-fixed with 2% glutaraldehyde for 1 hour. The fungal cells were then trapped by suction filtration through a membrane (WHA110606, Merck, Germany) with a filter holder for decompression filtration and an absorption filtration bottle. The fungal sample was then washed three times with sterile filtered PBS and post-fixed with 2% osmium (Nissin-EM, Co., Ltd, Tokyo, Japan) for 20 min. Next, the fungal sample was dehydrated in steps of 5 min each with ethanol at concentrations of 50%, 70%, 90%, and 100%, and then the ethanol was removed with T-butyl alcohol (06104-25, NACALAI TESQUE, INC., Kyoto, Japan). Next, the fungal samples were lyophilized and sublimated to remove the T-butyl alcohol and then examined by field emission scanning electron microscopy (FE-SEM) (S-4700 FE-SEM, Hitachi, Ltd., Tokyo, Japan).

Cyclobutane pyrimidine dimer measurement

The concentration of CPD was measured by high-sensitivity ELISA, High Sensitivity CPDs ELISA Kit (Cosmo Bio Co., Ltd., Tokyo, Japan). Genomic DNA extracted from the fungi was diluted to 0.4 µg/mL, and CPD was detected by the CPD-specific monoclonal antibody clone TDM-2 (CAC-NM-DND-001, Cosmo Bio Co., Ltd., Tokyo, Japan). Subsequently, the DNA samples were treated with a biotinylated secondary antibody, streptavidin-peroxidase, and o-phenylenediamine (OPD) to determine the amount of the complex formed by TDM-2 antibody and CPD. The reaction of peroxidase, H₂O₂, and OPD creates orange color whose strength is generally proportional to the amount of CPD. The absorbance at 492 nm was measured with a microplate reader (SpectraMax i3, Molecular Devices, USA).

Spore germination rate and hyphae elongation speed

To assess spore germination rate, 100 µL of *C. sphaerospermum* spore suspension, adjusted with PBS to a concentration of 2.0×10^4 spores/mL, was applied to PDA medium and the number of colonies observed after 3 days was counted. To assess hyphae elongation speed, 10 µL of spore suspension, adjusted with PBS to a concentration of 2.0×10^4 spores/mL, was dropped into the center of a Φ90 mm petri dish, and the diameter of the colonies formed thereafter was measured.

STATISTICAL ANALYSIS

The data are presented as the mean \pm SEM for all experiments. We calculated p-values using a Student's t-test with the threshold for significance set at $p < 0.05$.

RESULTS

Dose-dependent inactivation of *C. sphaerospermum* with UVC or UVA irradiation

The UV sensitivity of *C. sphaerospermum* was estimated with

UVC or UVA irradiation. UVC or UVA inactivation levels were dose dependent (Fig. 1). The log survival ratio was $-2.95 \log_{10} \pm 0.25$ with 150 mJ/cm^2 UVC irradiation. On the other hand, at 365 nm irradiation, the log survival ratio was $-2.79 \log_{10} \pm 0.31$ with 1180 J/cm^2 UVA irradiation. The energy required to achieve the same inactivation was significantly different between UVC and UVA band irradiation, with UVC irradiation showing higher efficiency.

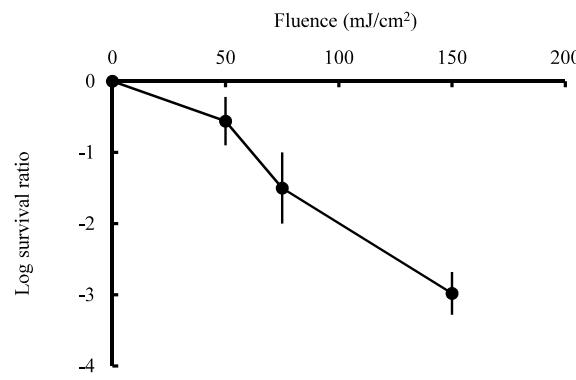
To analyze the effects of UVA and UVC irradiation on biological functions, in future experiments, UVA irradiation was set at 576 J/cm^2 and UVC irradiation at 60 mJ/cm^2 to obtain a log

survival rate of -1.

Morphological change induced by irradiation of UVC or UVA

To further investigate the inactivation mechanism, morphological changes were examined. The morphological changes of spores irradiated with UV were observed using an electron microscope. Under conditions where -1 log was obtained, spore destruction was observed with UVC irradiation. Less spore destruction was observed with UVA irradiation compared with UVC irradiation (Fig. 2).

(A) UVC irradiation



(B) UVA irradiation

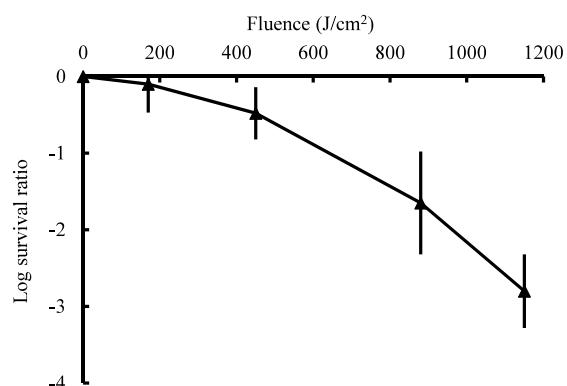


Fig 1. Wavelength dependence of *C. sphaerospermum* sterilization efficiency.
 (A) *C. sphaerospermum* was irradiated with UVC.
 (B) *C. sphaerospermum* was irradiated with UVA.
 All experiments were repeated four times and performed on four separate days.
 The data are shown as mean \pm SD. SD, standard deviation.

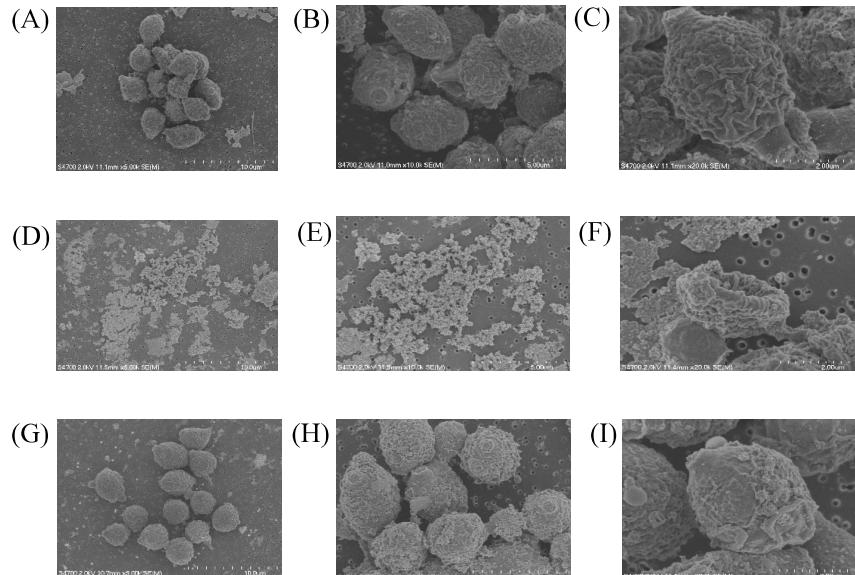


Fig 2. Electron microscope images of *C. sphaerospermum* irradiated with UVC and UVA.
 Suspensions of *C. sphaerospermum* were irradiated with UVA and UVC and observed by field emission scanning electron microscopy. (A), (B), (C) As a control, images without UV irradiation. (D), (E), (F) UVC irradiation (-1 log condition), and (G), (H), (I) UVA irradiation (-1 log condition) are shown at different magnifications. The magnifications are shown in the photographs.
 Suspensions of *C. sphaerospermum* cells were irradiated with UVA or UVC and observed with field emission transmission electron microscopy. As a control, images without UV irradiation, UVC irradiation (-1 log condition), and UVA irradiation (-1 log condition) are shown at different magnifications. The magnification is shown in each photograph.

CPD production

To investigate the inactivation mechanism in more detail, the amount of CPD produced by UV irradiation was measured. The amount of CPD produced was significantly greater with UVC irradiation than with UVA irradiation (Fig. 3).

Fungal growth after UV irradiation

To evaluate the growth potential after UV irradiation, we measured the diameters of the colonies. We found that the growth rates of the colony diameters decreased after UV irradiation (Fig. 4). Furthermore, we found that the growth inhibitory effect of UVA irradiation was greater than that of UVC irradiation.

DISCUSSION

In this experiment, we evaluated the UV sensitivity of fungi, which has rarely been reported, and investigated the mechanism of UV inactivation. It became clear that UV irradiation is effective in sterilization of fungi and inhibiting fungal growth, and that the inactivation mechanism is likely to differ depending on the irradiation wavelength, UVC and UVA.

The UV energy required to inactivate fungi is greater than that required for bacteria and viruses (15-20). Fungi have more elaborate response mechanisms to stresses such as osmotic pressure, temperature, and UV radiation (24). In addition, the cell wall of a spore has a five-layer structure (from the inside, the spore membrane, the spore wall, the spore outer wall, the peripore wall, and the hectorporium) (25). These characteristics lead to the concern that more energy is required to damage a fungal spore than a general bacterium or virus.

The fungi can also show strong resistance to chemical disinfectants due to their complex cell structures and defense mechanisms (26). Many fungi have their own intrinsic resistance that helps them adapt to chemical disinfectants. For example, yeasts

and molds have a unique cell wall composition that prevents certain chemicals from entering their cells, which allows them to develop resistance to disinfectants (27).

CPD production was higher in the UVC-irradiated group. This is because DNA does not absorb much energy in the UVA wavelength range, so UVA cannot directly damage DNA (28). However, cells contain dye cluster molecules, such as NAD, FAD, and porphyrin compounds, which absorb energy in the UVA wavelength range. These molecules were originally found to play an important role as coenzymes involved in molecular and energy metabolism in cells. Excitation of these coenzymes has been observed in UVA-irradiated cells, and the resulting energy transfer generates reactive oxygen species (ROS) (29). In addition, we observed that UVC irradiation damages the fungal cell shape, leading to changes such as cell wall damage and depression of spores. This damage may inactivates the fungal cells through the leaching out of water-soluble substances and organelles in the cytoplasm. These observations indicate that the inactivation mechanisms differ between UVC and UVA irradiation.

We observed that the fungal growth rate differed between UVC and UVA irradiation. Upon UV irradiation, the cell elicits a DNA damage response to perform repairs or, in case of excessive damage, to initiate programmed cell death (30-32). The growth rate after UV irradiation is determined by the balance between the damage and repair. Because the damage is different between UVC and UVA irradiation, the activation of these repair mechanisms will likely be different. Analysis of the repair mechanisms of the damage will also be necessary in the future.

While UVA light is significantly less bactericidal compared to UVC light, the sublethal effects of UVA may be more relevant for potential synergies than its germicidal action (33). The inclusion of UVA light may prolong the effects of UVC disinfection by impeding DNA damage repair and protein synthesis, which would also limit the growth of surviving cells (34). Consequently, the emergence of ultraviolet light-emitting diodes (UV LEDs), which offer a range of wavelengths, provides an opportunity to combine UVC and UVA wavelengths in compact devices suitable

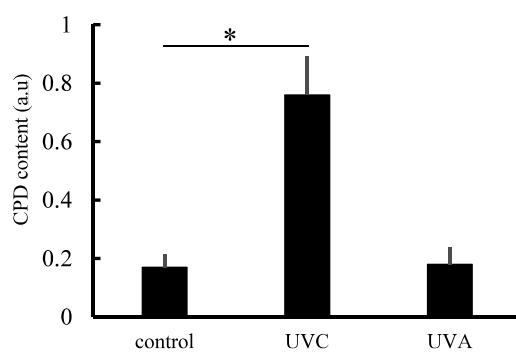


Fig. 3. CPD production with UVC or UVA irradiation of *C. sphaerospermum*.

UVA and UVC irradiation of *C. sphaerospermum* were performed under conditions that yielded a survival rate of $\log -1$, and then CPD production was measured. All experiments were repeated four times and performed on four separate days. The mean \pm SD is shown in the Figure. The asterisk denotes p -values <0.05 (*). CPD, cyclobutane pyrimidine dimer

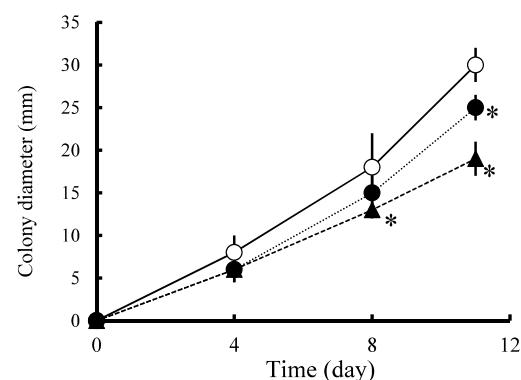


Fig. 4. Effect on *C. sphaerospermum* growth after UVA or UVC irradiation.

Incubation time-dependent *C. sphaerospermum* colony diameters were measured after UVA or UVC irradiation. ○: control, ●: UVC irradiation, ▲: UVA irradiation. UVA or UVC irradiations were each irradiated under conditions that yielded a survival rate of $\log -1$, and then incubated for 11 days and measured. The horizontal axis represents the number of days of incubation, and the vertical axis represents the colony diameter. All experiments were repeated four times and performed on four separate days. The mean \pm SD is shown in the Figure. The asterisk denotes p -values <0.05 (*). SD, standard deviation

for point-of-use applications. By integrating these two wavelengths in disinfection systems, overall costs could potentially be reduced as fewer UVC LEDs would be required in the design, considering that UVA LEDs are more efficient and currently easier and cheaper to manufacture than the UVC LEDs (35).

These results indicate that UV sterilization is effective in suppressing fungal contamination. However, the wavelength dependence of the observed inactivation and the difference in the mechanism of action depending on the wavelength suggest that the UV wavelength and exposure dose have to be selected according to the purpose and situation of the fungicide.

Based on the results of this study, it will be necessary in the future to clarify the dynamics of fungi in actual living environments related to the lifestyles of residents and to design the antifungal defensive measures accordingly.

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