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Editor's Choice

ORIGINAL ARTICLE

Inactivation of foodborne pathogenic and spoilage micro-organisms using ultraviolet-A light in combination with ferulic acid

A. Shirai[1], T. Watanabe[2] and H. Matsuki[1]

1 Department of Bioresource Chemistry and Technology, Graduate School of Bioscience and Bioindustry, Tokushima University, Tokushima, Japan
2 Department of Biological Science and Technology, Faculty of Engineering, Tokushima University, Tokushima, Japan

Significance and Impact of the Study: Microbial contamination is one of the most serious problems for foods, fruit and sugar thick juices. UV light is suitable for the nonthermal decontamination of food products by inactivating the contaminating micro-organisms. However, UV-A exposure is insufficient for disinfection. This study demonstrates that the combination of UV-A LED light (350–385 nm), which is not hazardous to human eyes and skin, and ferulic acid (FA), a known phytochemical and food additive, provides synergistic antimicrobial activity against foodborne pathogenic and spoilage micro-organisms. Therefore, FA addition to UV-A light treatment may be useful for improvement of UV-A disinfection technology to prevent food deterioration.

Keywords
ferulic acid, food spoilers, foodborne pathogens, photoantimicrobial activity, UV-A.

Correspondence
Akihiro Shirai, 2-1, Minamijosanjima-cho, Tokushima 770-8513, Japan.
E-mail: a.shirai@tokushima-u.ac.jp

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Abstract

The low energy of UV-A (315–400 nm) is insufficient for disinfection. To improve UV-A disinfection technology, we evaluated the effect of ferulic acid (FA) addition on disinfection by UV-A light-emitting diode (LED) (350-385 nm) against various food spoilers and pathogens (seven bacteria and four fungi species). Photoantimicrobial assays were performed at FA concentrations below the MIC. The MIC of the isomerized FA, consisting of 93% *cis*-form and 7% *trans*-form, was very similar to that of the commercially available FA (*trans*-form). Irradiation with UV-A (1·0 J cm^{-2}) in the presence of 100 mg l^{-1} FA resulted in enhanced reducing of all of the tested bacterial strains. A combination of UV-A (10 J cm^{-2}) and 1000 mg l^{-1} FA resulted in enhanced reducing of *Saccharomyces cerevisiae* and one of the tested filamentous fungi. These results demonstrated that the combination of a short-term application of UV-A and FA at a low concentration yielded synergistic enhancement of antimicrobial activity, especially against bacteria.

Introduction

UV-bactericidal technology, which produces no residual chemicals and has little influence on the environment, is a convenient method for disinfection of gases, liquids, and solid surfaces. In recent years, a versatile UV source has been provided by the development of light-emitting diodes (LEDs), which yield constant illumination at a specific wavelength and do not contain mercury. Light-emitting diodes are also advantageous because of their durability and low heat generation (Shin *et al.* 2016). A LED illumining UV-C light (266 nm or 275 nm), which is defined as radiation with wavelengths 200–280 nm and has traditionally been used as an effective germicidal disinfectant, exhibited high bactericidal activity (Kim *et al.* 2016; Shin *et al.* 2016). UV-C LED irradiation (266 nm; irradiance 4 μW cm^{-2}) reduced the viability of *Escherichia coli* O157:H7 by 6 log units at 0·5 mJ cm^{-2}. UV-A (315–400 nm) has been also reported to exhibit photobactericidal activity (Hamamoto *et al.* 2007). Notably, exposure to UV-A is not hazardous to human eyes and skin; in contrast, UV-B and UV-C light (i.e. at wavelengths <315 nm) can induce sunburn, cell mutations and cell death (De Gruijl 2002). Moreover, in the food

industry, UV-C exposure is known to inactivate polyphenolic acids (chlorogenic acid and phloridzin) that are abundant in apple juice, thus decreasing the antioxidative activity of this product (Islam *et al.* 2016). These findings suggest that UV-A light may be suitable for nonthermal decontamination of food products by inactivating the contaminating micro-organisms. However, UV-A light, being of lower energy than UV-C, exhibits lower photobactericidal activity. To completely inactivate *E. coli* using UV-A LED irradiation alone (365 nm), a 315-J cm^{-2} fluence (at an irradiance of 70 mW cm^{-2} for 75 min) was required (Hamamoto *et al.* 2007).

To increase the inactivation efficiency of UV-A, the synergistic bactericidal activity of the combination of UV-A light and organic substrates, a quaternary ammonium salt (Shirai *et al.* 2014) and natural compounds (coumaric acid and ferulic acid (FA)) and their derivatives (Shirai *et al.* 2015a,b) has been investigated in our laboratory. In these reports, the addition of FA enhanced the photobactericidal activity of UV-A such that the combination of approximately 20 mg l^{-1} FA with UV-A (irradiance 4·09 mW cm^{-2}, 30 min) resulted in a > 5-log decrease in the survival of *E. coli*.

Ferulic acid, one of the most abundant phenolic acids in plants such as rice, wheat, barley, citrus fruits and tomatoes, occurs as esters conjugated covalently with mono-, di- and polysaccharides and with lignin of cell walls (Graf 1992; De Paiva *et al.* 2013). It is a potential therapeutic agent with demonstrated antioxidant, antimicrobial, hepatoprotective and UV protective activities (De Paiva *et al.* 2013). In Japan, FA is approved for use as an antioxidant food additive (JFCRF 1996).

Microbial contamination is one of the most serious problems for foods, fruit and sugar thick juices (Braun *et al.* 1999; Fleet 2007; Justé *et al.* 2008; Tribst *et al.* 2009). In these reports, *Salmonella* are described as typical pathogenic bacteria associated with fruit juices. *Alicyclobacillus* and *Sporolactobacillus* have been isolated from spoiled fruit juices and can survive heat treatments by forming heat-tolerant spores. Yeasts negatively affect the flavour, turbidity and odour of the juice. The micro-organisms that we tested included two heat-tolerant filamentous fungi (*Byssochlamys fulva* and *Eupenicillium lapidosum*) and a third filamentous fungus, *Cladosporium cladosporioides*, found in canned or bottled fruit. *Escherichia coli* is commonly used as an indicator organism. As an UV-A disinfection technology for applications in the food industry, the goal of this study was to investigate the synergistic effect of FA on the photoantimicrobial activity of UV-A (wavelength range 350–385 nm produced by a LED source) using those multiple food spoilers and pathogens and to reveal enhancement of photoantimicrobial activity by FA addition.

Results and discussion

Antimicrobial activity of FA isomers

Ferulic acid (*trans*-form) and isomerized FA, consisting of 93% *cis*-form and 7% *trans*-form, were evaluated for antimicrobial activity on the basis of MIC assays against various micro-organisms (Table 1). The MICs for both FAs were 125 to 1000 mg l^{-1} or >1000 mg l^{-1} for the tested micro-organisms. The MICs of isomerized FA and *trans*-FA were very similar and no significant differences were observed between the isomers.

Other work has shown that *cis*-form phenyl compounds substituted with propenoic acid moieties have notable biological properties. For instance, the antimicrobial activity and the anti-invasive activity (against adenocarcinoma cells) of cinnamic acid depend on its isomerization (Chen *et al.* 2011; Yen *et al.* 2011). The *cis*-isomer of cinnamic acid has a strong bactericidal effect against a multidrug-resistant *Mycobacterium tuberculosis* at a concentration two orders of magnitude lower than that observed for the *trans*-isomer (Chen *et al.* 2011). Cinnamic acid acts by causing a disruption in the cell wall of the bacterium, resulting in a wrinkled and rough colony phenotype in micrographs. Similarly, other phenolic acid compounds (caffeic acid and gallic acid) have been shown to bind to (or be incorporated into) the cytoplasmic membrane of *E. coli* and *Staphylococcus aureus* (Nakamura *et al.* 2015). Ferulic acid is isomerized from the *trans*-form to the *cis*-form through phenoxy radical formation during UV exposure (Graf 1992).

Table 1 MICs of *trans*-FA and isomerized ferulic acid (FA)

	MIC (mg l^{-1})*	
Micro-organism	*trans*-FA	Isomerized FA†
Escherichia coli NBRC12713	>1000	>1000
Salmonella enterica NBRC13245	1000	1000
Staphylococcus aureus NBRC12732	500	1000
Bacillus cereus NBRC15305	1000	1000
Bacillus subtilis ATCC6633	1000	670 ± 290
Kocuria rhizophila NBRC12708	500	500
Alicyclobacillus acidoterrestris NBRC108913	125	125
Sporolactobacillus inulinus NBRC13595	500	500
Saccharomyces cerevisiae NBRC1136	>1000	>1000
Cladosporium cladosporioides IFM63149	>1000	>1000
Byssochlamys fulva NBRC31767	>1000	>1000
Eupenicillium lapidosum NBRC6100	>1000	>1000

*Values are the mean ± SD obtained from three independent experiments. Values without SDs were identical in each of the three independent experiments.

†Composed of 93% *cis*-form and 7% *trans*-form.

Photoisomerization of FA to the *cis*-isomer could directly injure the cell membrane (as *cis*-cinnamic acid does). By analogy to cinnamic acid, we expected that the antimicrobial activity of FA would exhibit isomeric specificity, such that the inhibitory activity of the *cis*-form of FA would be much higher than that of the *trans*-form. However, the MICs of isomerized FA and *trans*-FA were very similar in our experiments, suggesting that the antimicrobial activity of FA does not depend on isomerization. Therefore, the photo-induced conversion of FA would have no effect on its photoantimicrobial activity when combined with UV-A irradiation.

Photoantimicrobial activity

Photoantimicrobial assays were performed at FA concentrations below its MIC. Bactericidal activity against *E. coli* was investigated by treating this organism with a combination of FA and UV-A irradiation. A total UV-A fluence at 30 J cm^{-2} (58·3-min irradiation) in the absence of FA resulted in a 2·82-log unit reduction in the viable cell count (Fig. 1a). The addition of FA to the suspension before UV-A irradiation enhanced bactericidal activity in a manner that was dependent on FA dose and UV-A fluence. The combination of FA (at 50, 100, and 150 mg l^{-1}) with UV-A light (at 1·5 J cm^{-2} (2·92-min irradiation), 1·0 J cm^{-2} (1·95-min irradiation) and 0·75 J cm^{-2} (1·46-min irradiation) respectively) yielded viable cell counts of <10 CFU ml^{-1} (Fig. 1b). Compared with the bactericidal activity of UV-A at 3·0 J cm^{-2} fluence in the absence of FA, the combination of FA with UV-A yielded significant ($P < 0·01$) decreases in the viable cell count. Exposure to 150 mg l^{-1} FA for an equivalent time interval (5·83 min, the incubation time used above to provide a fluence of 3·0 J cm^{-2} UV-A) in the absence of UV-A (i.e. in the dark) had a much smaller antibacterial effect (0·37-log reduction in *E. coli* cell density).

The efficacy of the combination of FA (100 mg l^{-1}) and UV-A (1·0 J cm^{-2}) was tested with six other bacterial strains (Fig. 2). Under these conditions, cell counts of *Salmonella enterica*, *Staph. aureus* and *Sporolactobacillus inulinus* decreased below the detection limit of 10 CFU ml^{-1}. Cell counts of *Bacillus cereus*, *Kocuria rhizophila* and *Alicyclobacillus acidoterrestris* decreased by 4·04-logs, 3·75-logs and 2·20-logs respectively. In contrast, no significant decrease in viability was observed for any of these strains (except for *Sporolact. inulinus*) when exposed to FA in the absence of UV-A (for 1·95 min, the incubation time used above to provide a fluence of 1·0 J cm^{-2} UV-A), or to the UV-A fluence in the absence of FA. For *Sporolact. inulinus*, a decrease in viable cells (3·15-logs) was observed upon treatment with FA in the dark. Notably, the combined treatments with UV-A and FA provided significant ($P < 0·01$) increases in bactericidal activity compared with those with FA addition alone or UV-A irradiation alone.

In other work, Nakamura *et al.* (2015) showed that irradiation with short wavelength light (380–420 nm; irradiance 0·26 W cm^{-2}) in combination with caffeic acid (a polyphenol similar to FA) killed each of four bacterial strains; notably, however, these effects required a fluence of 78 J cm^{-2} and a caffeic acid concentration of 1000 mg l^{-1}. In contrast, inactivation of bacteria by the methods described in this study required much lower light fluence and reagent concentration. Potent inactivation (to microbial densities below 10 CFU ml^{-1}, the lower limit of detection) of *E. coli* was achieved at total fluences of 1·0 J cm^{-2} in combination with a FA concentration of 100 mg l^{-1}. Similar enhancement of UV-A

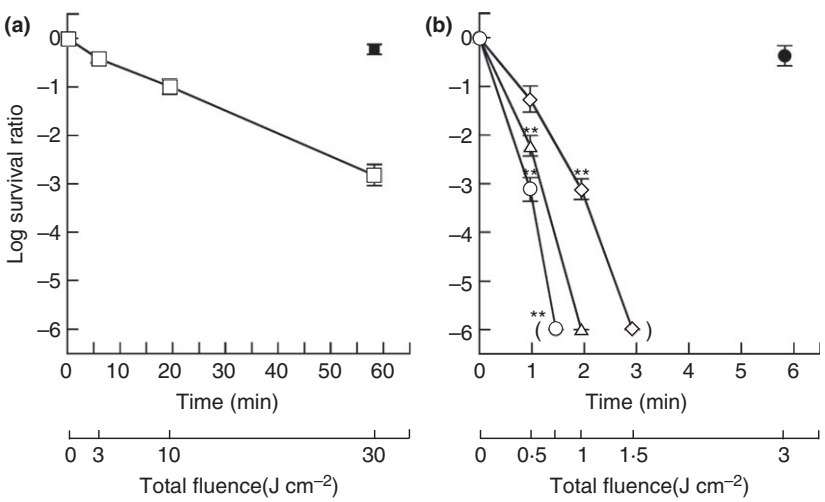

Figure 1 UV-A fluence-dependent changes in *Escherichia coli* survival following irradiation in the absence (a) or presence (b) of ferulic acid (FA). Filled (black) symbols, no UV-A exposure; unfilled (white) symbols, UV-A exposure. Cell suspensions were treated with FA at 50 mg l^{-1} (diamond), 100 mg l^{-1} (triangle) or 150 mg l^{-1} (circle). Data are presented as means ± SD ($n = 3$). Significant differences (**$P < 0·01$) were calculated based on comparison to samples irradiated with UV-A (3·0 J cm^{-2}) in the absence of FA. Samples for which survival was <10 CFU ml^{-1} (lower limit of detection) are noted in parentheses as 6-log unit reductions.

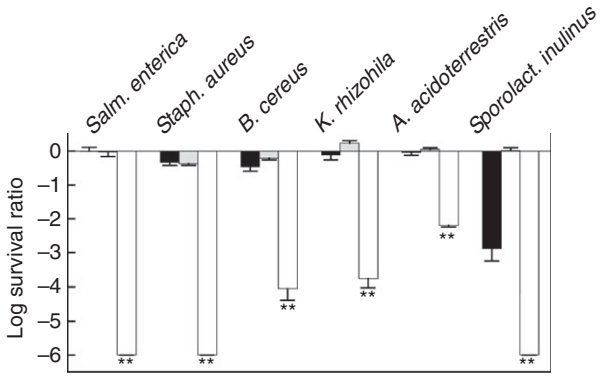

Figure 2 Photobactericidal activity against the six indicated bacterial species of UV-A irradiation (1·0 J cm^{-2}) in the absence (light-grey bars) and presence (unshaded bars) of 100 mg l^{-1} ferulic acid (FA). Black bars indicate 100 mg l^{-1} FA treatment in the absence of UV-A (for 1·95 min, the incubation time used to provide a fluence of 1·0 J cm^{-2} UV-A). Data are presented as means ± SD (n = 3). Significant differences (**P < 0·01) were calculated based on comparison to viability of the respective bacterium exposed to FA alone and to UV-A exposure without FA. Samples for which survival was <10 CFU ml^{-1} (lower limit of detection) are represented as 6-log reductions.

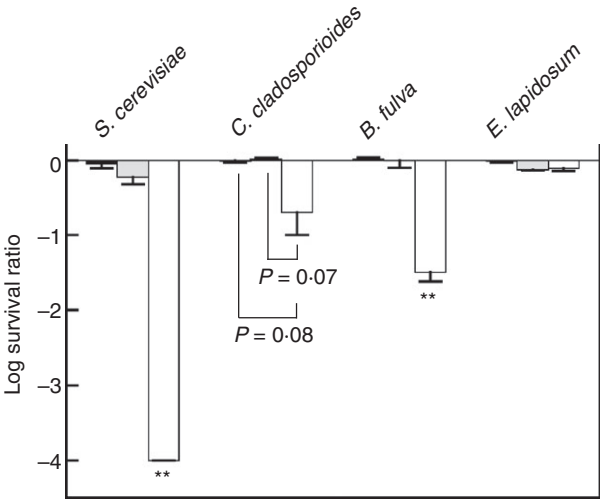

Figure 3 Photoantifungal activity against four fungal species of UV-A irradiation (10 J cm^{-2}) in the absence (light-grey bars) and presence (unshaded bars) of 1000 mg l^{-1} ferulic acid (FA). Black bars indicate 1000 mg l^{-1} FA treatment in the absence of UV-A (for 19·4 min, the incubation time used to provide a fluence of 10 J cm^{-2} UV-A). Significant differences (**P < 0·01) were calculated based on comparison to viability for the respective fungus exposed to FA addition alone and UV-A without FA. Samples for which survival was <10 CFU ml^{-1} (lower limit of detection) are noted in parentheses as 4-log unit reductions.

bactericidal efficacy was also demonstrated for the other six tested bacterial strains. The bactericidal synergy of UV-A and FA against some Gram-positive bacteria, including some *Bacillus*, *Kocuria* and *Alicyclobacillus*, was lower than against Gram-negative bacteria. As shown in a previous report by Nakamura *et al.* (2015), the affinity of FA, which is an analogue of caffeic acid, to Gram-positive bacteria should be lower than that to Gram-negative bacteria. These results demonstrated that the combination of UV-A and FA yielded synergistic enhancement of bactericidal activity while using a short-term application of UV-A light (1·0 J cm^{-2}) in combination with 100 mg l^{-1} FA. We infer that the high synergism of UV-A light with FA reflects the production of phenoxy radicals that in turn leads to the production of hydrogen peroxide as supported by the fact that photobactericidal activity is quenched by the addition of catalase (Shirai *et al.* 2015b). In this work, microbial inactivation by the FA + UV-A regime was assayed against vegetative cells. Future work on the synergistic efficacy will be needed to investigate activity against spores of organisms like *Bacillus subtilis*, *B. cereus*, *A. acidoterrestris* and *Sporolact. inulinus*.

We investigated antifungal activity by testing the effect of the combination of FA (1000 mg l^{-1}) and UV-A (10 J cm^{-2} fluence) on *Saccharomyces cerevisiae* and three filamentous fungi.

UV-A irradiation alone, at fluences of up to 10 J cm^{-2}, yielded little decrease in viable yeast cell counts (Fig. 3). Exposure to 1000 mg l^{-1} FA for an equivalent time interval (19·4 min, the incubation time used above to provide a fluence of 10 J cm^{-2} UV-A) in the absence of UV-A (i.e. in the dark) resulted in a very small amount of anti-yeast activity (0·05-log reduction in yeast cell density). The anti-yeast activity of UV-A was enhanced in the presence of 1000 mg l^{-1} FA; at a fluence of 10 J cm^{-2} in the presence of 1000 mg l^{-1} FA, yeast viability fell below the detection limit of 10 CFU ml^{-1}.

The photo-antifungal activity against *B. fulva* was significantly increased in the presence of FA, with survival ratios without and with FA of 0·01-logs and 1·49-logs respectively (P < 0·01). In contrast, FA addition did not significantly enhance photoinactivation of *C. cladosporioides* by UV-A irradiation (P > 0·05), although the treatment yielded a nominal decrease in viability. Similarly, FA addition did not significantly enhance photoinactivation of *E. lapidosum*. For the three filamentous fungi, FA addition alone and UV-A irradiation alone had a low fungicidal activity. Additional investigations with various combinations of irradiance and FA concentration may reveal conditions suitable for the inactivation of those fungi; such efficacy would be of great value, given that these organisms are often resistant to heating and UV-C exposure (254 nm) (Hamanaka *et al.* 2010).

Several laboratories have shown that UV-A LED light exhibits much lower photobactericidal activity

(Hamamoto *et al.* 2007; Shirai *et al.* 2014) than UV-C LED light (Kim *et al.* 2016). Inactivation with UV-A light alone requires high irradiation and long exposure times. In the present work, to increase the inactivation efficiency of UV-A, we investigated the FA + UV-A combination with respect to its photoantimicrobial activity against various foodborne pathogenic and spoilage micro-organisms related to foods, fruit and sugar thick juices.

In conclusion, a combination of FA with UV-A irradiation resulted in a significant enhancement in their individual efficacy towards the tested organisms. The high synergistic activity against bacteria was observed when UV-A at low fluence and short time exposure was combined with FA at a low concentration. Reducing of yeast and at least one filamentous fungus was observed upon treatment with longer UV-A exposure and higher FA concentration. Therefore, FA combined with UV-A light treatment may be useful for the improvement of UV-A disinfection technology. Also, in future work, surface decontamination of fruits will be investigated using the combination of UV-A light and FA for applications in postharvest disinfection.

Materials and methods

Microbial strains

Micro-organisms used in the determination of antimicrobial activity were purchased from NITE Biological Resource Center (NBRC), American Type Culture Collection (ATCC) and Institute of Food Microbiology (IFM). Those strains are listed in Table 1. Endospore-forming bacteria (*Bacillus*, *A. acidoterrestris* and *Sporolact. inulinus*) were tested in their vegetative state.

UV-A source and irradiation

A device equipped with a UV-A LED (NCSU033B; Nichia Corp., Anan, Japan), as previously described, was used in all photoexperiments (Shirai *et al.* 2015b). The LED has a radiation angle of about 120° as the full width at half maximum. The peak wavelength was 365 nm and the wavelength range was 350–385 nm, which was measured with a cumulative UV meter (MCPD-3700A; Otsuka Electronics Co. Ltd., Hirakata, Japan) (Fig. S1).

Chemistry

Ferulic acid (PubChem CID: 445858) was purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). Except as noted, FA used for testing consisted of the *trans*-form obtained as commercially available FA.

MIC assay

Details regarding preparation, separation and purity of isomerized FA are summarized in the Supporting Information (Data S1). The antimicrobial activity of FA and the isomerized FA, consisting of 93% *cis*-form and 7% *trans*-form, against the organisms listed above was evaluated by determining MICs using the broth dilution method (Shirai *et al.* 2005). Preculture of organisms was performed with the conditions described in Table S1. Cell density was determined from OD_{660} using a UV-1700 spectrophotometer (Shimadzu Ltd., Kyoto, Japan) for bacteria and yeast, and using a haemocytometer (Burker-Turk; depth 0·1 mm, 1/400 qmm; Erma Inc., Tokyo, Japan) for filamentous fungi.

Ferulic acid solutions for testing against bacteria (except *A. acidoterrestris* and *Sporolact. inulinus*) were generated by diluting the FA stock solution (100 g l^{-1} in 80% dimethylsulfoxide (DMSO)) with nutrient broth (Becton, Dickinson and Company, Franklin Lakes, NJ) to generate the highest testing concentration of 1000 mg l^{-1}; this solution was then subjected to a two-fold serial dilution using nutrient broth. To generate FA solutions for testing against *A. acidoterrestris* and *Sporolact. inulinus*, all dilutions (starting from the stock solution) were performed as above but using a specific broth (No. 323), which is recommended on the NBRC Website (NBRC 2016), and GYP broth (glucose 2% (w/v), yeast extract 0·5% (w/v), Bacto peptone 0·5% (w/v)) (Kitahara and Suzuki 1963) respectively. To generate FA solutions for testing against fungi, all dilutions (starting from the stock solution) were performed as above but using Sabouraud broth (polypeptone 1% (w/v) and glucose 4% (w/v)). The final cell densities were approx. 1×10^5 CFU ml^{-1} for bacteria and yeast, and approx. 1×10^4 conidia per ml for filamentous fungi in a transparent 96-well culture plate (Corning Inc., Corning, NY). MICs for *A. acidoterrestris* and *Sporolact. inulinus* were determined after 24-h incubation at 45°C and 48-h incubation at 37°C in an anaerobic chamber with an AnaeroPack Kenki that can reduce the oxygen percentage to <0·1% within 2 h (Mitsubishi Gas Chemical Company, Inc., Tokyo, Japan) respectively. MICs for the other bacteria, yeast, and filamentous fungi were determined after 24-h incubation in ambient air at 37, 28 and 25°C respectively.

Photoantimicrobial assay

Photoantimicrobial activity against bacteria and fungi was determined by plating and counting the colony-forming units remaining after treatment of a microbial suspension as reported previously (Shirai *et al.* 2014). Cells of organisms, precultured according to Table S1, were prepared as

described in the same report. Conidial suspensions were prepared as described previously (Shirai *et al.* 2005). Cell density was determined as described in the section above.

All antimicrobial assays with UV-A irradiation or with no irradiation (in the dark) were performed in an incubator box maintained at 30°C, using suspensions of organisms placed in the individual wells of a transparent 48-well culture plate (AGC Tecno Glass Co. Ltd., Tokyo, Japan). An aliquot of microbial suspension (0·1 ml, approx. 2×10^7 CFU ml^{-1} for bacteria or approx. 2×10^5 CFU ml^{-1} for fungi) was added to each well of a 48-well culture plate already containing 0·01 ml of test compound at concentrations of 5, 10, 15 and 100 g l^{-1} in 80% DMSO and 0·89 ml of sterile water per well (volume of the final tested suspensions 1 ml; depth 13 mm). For UV-A irradiation alone (no test compound), DMSO was added to a concentration of 0·8% to each well of the 48-well plate. The single UV-A LED was placed face-up to permit upward irradiation into the bottom of the 48-well culture plate. The device was set 30 mm (height) from the middle of the tested suspensions. The intensity of was 8·58 mW cm^{-2} at the bottom of the well, which was measured with a laser power and energy meter (Nova II; Ophir Optronics Solutions Ltd., Saitama, Japan) equipped with a photodiode sensor (PD-300-UV; Ophir Optronics Solutions Ltd.). Total fluence tested (0·5, 0·75, 1·0, 1·5, 3·0, 10 and 30 J cm^{-2}) was calculated based on the irradiance (8·58 mW cm^{-2}) and exposure time (0·97 to 58·3 min). After treatments with or without UV-A irradiation for bacteria, aliquots (0·15 ml) of each suspension were diluted 10-fold with SCDLP broth (Nihon Pharmaceutical Co., Ltd., Tokyo, Japan) followed by serial 10-fold dilutions with 0·8% (w/v) physiological saline containing 0·7% (w/w) Tween 80 (Kanto Chemical Co., Inc., Tokyo, Japan). Viable cell counts (CFU ml^{-1}) after 10-fold serial dilutions were determined by plating on suitable agar plates and incubating them (Table S1). For fungi, 10-fold serial dilutions were performed with Sabouraud broth containing 0·1% (w/w) Tween 80.

Antimicrobial activity was expressed as the log survival ratio (log S) according to the equation: $\log S = \log(N_t/N_0)$, where N_0 represents the number of CFUs before bactericidal treatment and N_t represents the number of CFUs after treatment for time t.

Statistical analysis

All antimicrobial experiments were performed as three independent procedures, and results are presented as the mean and SD. Inferential analysis was performed using a two-tailed, unpaired Student's t-test. P-values of <0.05 were considered significant.

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Conflict of Interest

No conflict of interest declared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Emission spectrum of the UV-A LED as used in this study; the spectrum exhibited a maximum at 365 nm, which was measured at a distance of 30 mm between the illumination source and the UV meter.

Figure S2 HPLC chromatogram of FA prepared by UV-A exposure: before separation (A), and after separation and purification of the *cis*-form (B).

Figure S3 UV-visible absorption spectra of FA (*trans*-form, solid line) and isomerized FA (mixture of 93% *cis*-form and 7% *trans*-form, dashed line). Samples were dissolved at 50 μmol l^{-1} in 0·4% DMSO.

Table S1 Conditions for preculture and viability assay of micro-organisms tested.

Data S1 Preparation, separation and purity of *cis*-FA.