2012

Blue light (470 nm) effectively inhibits bacterial and fungal growth

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Letters in Applied Microbiology

Letters in Applied Microbiology ISSN 0266-8254

ORIGINAL ARTICLE

Blue light (470 nm) effectively inhibits bacterial and fungal growth

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Significance and Impact of Study: Light from two arrays of different blue LEDs significantly reduced bacterial (*Leuconostoc mesenteroides*, *Bacillus atrophaeus* and *Pseudomonas aeruginosa*) viabilities. Significant *in vitro* viability loss was observed for the filamentous fungi, *Penicillium digitatum* and *Fusarium graminearum* when exposed to pure blue light only plus a photosensitizer. *F. graminearum* viability was significantly reduced by blue light alone. Results suggest that (i) the amount of significant loss in bacterial viability observed for blue light that is pure or with traces of other wavelengths is genus dependent and (ii) depending on fungal genera, pure blue light is fungicidal with or without a photosensitizer.

Keywords
antibacterial, antifungal, visible blue light.

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2012/1233: received 9 July 2012, revised 18 September 2012 and accepted 18 September 2012

doi:10.1111/lam.12002

Abstract

Blue light (470 nm) LED antimicrobial properties were studied alone against bacteria and with or without the food grade photosensitizer, erythrosine (ERY) against filamentous fungi. *Leuconostoc mesenteroides* (LM), *Bacillus atrophaeus* (BA) or *Pseudomonas aeruginosa* (PA) aliquots were exposed on nutrient agar plates to Array 1 (AR1, 0·2 mW cm^{-2}) or Array 2 (AR2, 80 mW cm^{-2}), which emitted impure or pure blue light (0–300 J cm^{-2}), respectively. Inoculated control (room light only) plates were incubated (48 h) and colonies enumerated. The antifungal properties of blue light combined with ERY (11·4 and 22·8 μmol l^{-1}) on *Penicillium digitatum* (PD) and *Fusarium graminearum* (FG) conidia were determined. Conidial controls consisted of: no light, room light-treated conidia and ERY plus room light. Light-treated (ERY + blue light) conidial samples were exposed only to AR2 (0–100 J cm^{-2}), aliquots spread on potato dextrose agar plates, incubated (48 h, 30°C) and colonies counted. Blue light alone significantly reduced bacterial and FG viability. Combined with ERY, it significantly reduced PD viability. Blue light is lethal to bacteria and filamentous fungi although effectiveness is dependent on light purity, energy levels and microbial genus.

Introduction

As early as 1887, visible blue light was known to be the most effective part of the light spectrum to stimulate phototrophism in plants (Sachs 1887). It is also important as a 'cue' for fungal metabolism, growth, pigment formation, tropism and spore production (Siegel *et al.* 1968; Casas-Flores *et al.* 2006; Purschwitz *et al.* 2006). Blue light signal transduction pathways have been studied in *Neurospora crassa*, which has two major blue light photoreceptors, (i) white collar (WC)-1 and (WC)-2, which control dark to light transition and (ii) VVD, a protein important in the second light signalling system. Together, they control responses to daily changes in light intensity (Linden and Macino 1997; Schwerdtfeger and Linden 2001, 2003).

In vitro lethality of blue light for the bacteria *Escherichia coli*, aerobic methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* (PA) has also been reported (Guffy and Wilborn 2006; Brovko *et al.* 2009). Blue light causes photoexcitation of endogenous bacterial photosensitive porphyrins and subsequent bactericidal reactive oxygen species production (Lavi *et al.* 2004; Lipovsky *et al.* 2008, 2009, 2010). Bacteria without internal light

reactive compounds can be killed with a combination of blue light and nontoxic photoactivatable dyes, such as cationic phenothiazinium dyes (Alves *et al.* 2009) that, together, generate singlet oxygen and reactive oxygen species (Wainwright 1998; Hamblin and Hasan 2004; Tegos *et al.* 2005).

While blue light is an important 'cue' in the asexual development of fungal spores, the combination of it with certain photosensitive dyes is fungicidal. Such dyes include the phenothiazinium dyes, toluidine blue O and dimethylmethylene blue and, combined with blue light, reduce the viability of *Candida* (Jackson *et al.* 1999; Phoenix and Harris 2003).

In recent years, safe chemicals with greater photosensitizing efficacy due to strong absorbance of light (e.g. blue, red or white), lipophilicity with a delocalized positive charge and stable to photodegradation have been identified. They include benzo[a]phenoxazinium chalcogen analogues (BCA) and cationic fullerenes (Tegos *et al.* 2005; Foley *et al.* 2006).

Other visible light wavelengths combined with photosensitizing agents can also reduce fungal viability. White light (polychromatic) combined with BAM-SiPc, an unsymmetrical bisamino phthalocyanine, reduces *Candida albicans* viability (So *et al.* 2010). White light combined with cationic bis- and tris-cationic fullerenes reduces bacterial and *C. albicans* viability after only 10 min of incubation (Tegos *et al.* 2005). Red light is also active against *C. albicans* in the presence of methylene blue, BCA or BAM-SiPc (Foley *et al.* 2006; de Souza *et al.* 2006; So *et al.* 2010). *C. albicans* growth and germ tube formation is inhibited by red light plus methylene blue (Munin *et al.* 2007) and is due to increased permeability (Giroldo *et al.* 2009). Among the compounds with photosensitizing properties is erythrosine (FD&C Red no. 3), a common food dye (Yang and Min 2009).

This study determined the effect of (i) different LEDs producing blue light (peak: 470 nm) and (ii) incubation temperature after exposure to blue light on bacterial viability. Three bacteria used in this study include *Leuconostoc mesenteroides* (LM), a soil-borne bacterium which is the major factor in US sugarcane and sugarbeet deterioration (De Bruijin 2002; Eggleston and Monge 2005); *Bacillus atrophaeus* (BA), a surrogate in experiments for *Bacillus anthracis* (Weber *et al.* 2003), and *P. aeruginosa* which causes serious burn wound infections, colonization of medical equipment (e.g. catheters) as well as contact dermatitis (Yue *et al.* 2007; Lundov *et al.* 2009; Bak *et al.* 2010).

The anti-*Candida* properties of combined extracellular photosensitizers and light have been published (So *et al.* 2010). However, no data on the effect of monochromatic light and photosensitizers on filamentous fungi have been reported. This study also investigated the effect of blue light with and without erythrosine (ERY) on the viability of nongerminated and germinating filamentous conidia. The fungi studied were *Penicillium digitatum* (PD) that causes rot in stored citrus and *Fusarium graminearum* (FG), which produces potent mycotoxins and renders harvested wheat unsafe when postharvest factors allow naturally occurring fungi to grow (Magan *et al.* 2010).

Results and discussion

Bacteria

Leuconostoc mesenteroides only grew only at 25°C with no growth at the other postlight treatment temperatures. Array 2 light (pure blue) had no effect on LM. However, Array 1 light (impure blue) significantly reduced colony-forming units (CFU) beginning at 150 J cm^{-2}, and a viability loss of about 80% was observed at 180 J cm^{-2}.

In contrast to the results with LM, both LED arrays reduced the CFU of BA although a difference was observed in the effect of the two arrays. Array 1 (Table 1) achieved significant CFU reduction beginning at 40 J cm^{-2}, with approximate 100% losses observed at 80 J cm^{-2} at incubation temperatures of 25 and 30°C, which were much lower energy levels than needed to achieve similar results with Array 2 at any incubation temperature.

Significant CFU reduction of BA was not observed using Array 2 below 100 J cm^{-2}, while approximately 100% viability reduction was achieved at 300 J cm^{-2}. This energy level was much higher than that needed from Array 1 to achieve similar viability loss and indicates that blue light with trace levels of other wavelengths produced by Array 1 was more active against BA than pure blue light (Array 2).

After exposure to Array 1, cells incubated post-treatment at 37°C displayed less viability loss at 60 J cm^{-2} than did cells incubated at 25 and 30°C, but this difference was not significant. In contrast, no temperature effect was observed with cells exposed to Array 2.

Pseudomonas aeruginosa was more susceptible to blue light than the other bacteria and both arrays (Table 1) significantly reduced viability. An 8 J cm^{-2} energy dose from Array 1 saw the greatest CFU reduction (approximately 84%) with cells incubated at 25°C, a suboptimum temperature for this bacterium. CFU reductions were approximately 58–54% at 10 J cm^{-2} with cells grown at 30 and 37°C (the optimum incubation temperature), respectively.

An 8 J cm^{-2} energy dose from Array 2 caused the greatest viability loss, approximately 96%, when PA was incubated at 37°C after light treatment. In comparison,

Table 1 Effect of blue light (470 nm) on *Bacillus atrophaeus* and *Pseudomonas aeruginosa* viability (Per cent of control viability means)

	B. atrophaeus						*P. aeruginosa*					
	Array 1†			Array 2‡			Array 1			Array 2		
J cm^{-2}	25°C	30°C	37°C	25°C	30°C	37°C	25°C	30°C	37°C	25°C	30°C	37°C
2	§	§	§	§	§	§	65·9 ± 16·4	85·1 ± 8·9	65·8 ± 14·9	§	§	§
4	§	§	§	§	§	§	40·0* ± 11·9	67·1* ± 7·5	60·0* ± 17·5	§	§	§
5	§	§	§	§	§	§	§	§	§	66·2* ± 21·7	55·5* ± 10·0	14·6* ± 3·5
6	§	§	§	§	§	§	47·3* ± 12·5	62·3* ± 8·5	65·8* ± 15·3	§	§	§
8	§	§	§	§	§	§	16·0* ± 7·6	42·1* ± 4·1	46·2* ± 13·3	38·4* ± 13·0	43·1* ± 12·1	3·5* ± 1·0
10	89·5 ± 8·0	92·7 ± 4·4	91·1 ± 5·1	§	§	§	12·1* ± 5·1	40·5* ± 6·9	44·2* ± 14·9	28·0* ± 10·6	31·1* ± 8·4	0·8* ± 0·5
20	86·6 ± 4·7	89·5 ± 2·9	92·5 ± 4·7	§	§	§	§	§	§	19·1* ± 8·4	22·8* ± 6·3	1·9* ± 1·0
30	§	§	§	§	§	§	§	§	§	16·4* ± 7·4	20·9* ± 6·8	1·5* ± 0·6
40	71·0* ± 7·3	54·0* ± 10·6	79·5* ± 4·1	§	§	§	§	§	§	16·1* ± 6·8	22·2* ± 8·5	0·6* ± 0·4
50	§	§	§	§	§	§	§	§	§	15·5* ± 6·4	20·4* ± 5·9	3·5* ± 1·6
60	16·0* ± 7·2	12·9* ± 6·6	53·0* ± 11·7	§	§	§	§	§	§	§	§	§
80	0·0* ± 0·0	0·7* ± 0·1	12·9* ± 0·3	§	§	§	§	§	§	§	§	§
100	0·0* ± 0·0	0·0* ± 0·0	0·0* ± 0·0	74·3 ± 5·4	63·5 ± 7·7	75·0 ± 3·5	§	§	§	§	§	§
150	§	§	§	69·9* ± 7·5	44·0* ± 7·1	50·1* ± 9·2	§	§	§	§	§	§
200	§	§	§	27·6* ± 8·1	49·0* ± 5·6	57·1* ± 5·1	§	§	§	§	§	§
250	§	§	§	2·1* ± 0·8	6·4* ± 2·5	4·4* ± 1·4	§	§	§	§	§	§
300	§	§	§	0·5* ± 0·2	1·1* ± 0·4	0·6* ± 0·3	§	§	§	§	§	§

*Significantly ($P < 0·001$) lower than the control viability means.
†Array 1: LEDs, 2·2 mW cm^{-2} (LEDtronics, Torrance, CA, USA).
‡Array 2: LEDs, 80 mW cm^{-2} (CREE, Durham, NC, USA).
§Not done.

cells incubated at 25 and 30°C showed approximately 62 and 57% CFU loss, respectively.

With both Arrays, lethality increased with the amount of energy to which PA was exposed and suggests that for PA, Array 2 (pure blue light) was slightly better than Array 1 (blue light with traces of other wavelengths) as a bactericide.

Fungi

Penicillium digitatum. Nongerminated conidia: Blue light (Array 2) or ERY alone did not reduce the viability of PD nongerminated conidia (Fig. 1) when compared with the viability control means (no light or ERY treatments). In contrast, nongerminated conidia treated with blue light and 11·4 μmol l^{-1} ERY significantly reduced CFU counts by about 40 and 70% with blue light of 80 and 100 J cm^{-2}, respectively, when compared with the conidial control.

Blue light plus 22·8 μmol l^{-1} ERY showed significant CFU reduction of approximately 25% at 40 J cm^{-2} when compared to the conidial (no light or ERY) control. CFU losses increased to approximately 80 and 95% when the blue light energy exposure was increased to 80 and 100 J cm^{-2}, respectively. CFU counts at these two energy levels in the presence of ERY (22·8 μmol l^{-1}) were significantly lower than with blue light or ERY alone controls.

Germinating conidia: The blue light and ERY control CFU for the germinating conidia (Fig. 2) were not significantly lower than those for the conidial (no light or ERY) control means. Germinating conidia were much more susceptible than nongerminated conidia to a treatment of blue light plus ERY. The viability losses (approximately 80–98%) with blue light energy levels of 40–100 J cm^{-2} in combination with ERY at 11·4 μmol l^{-1} were significantly lower than the CFU of the conidial (no light or ERY), blue light and ERY controls. Viability losses of approximately 95–98% were observed when blue light (40–100 J cm^{-2}) was combined with 22·8 μmol l^{-1} ERY. The combination of light and ERY also significantly reduced CFUs in comparison to the blue light and ERY alone controls.

Fusarium graminearum. Nongerminated conidia: Figure 3 shows that no significant viability loss was observed for the FG nongerminated conidia treated with only ERY at 11·4 and 22·8 μmol l^{-1} as well as conidia treated with blue light (20–100 J cm^{-2}) alone. However, significant viability losses of 80, 95 and 100%, were observed for conidia exposed to blue light energy levels of 40, 80 and 100 J cm^{-2}, respectively, in the presence of 11·4 μmol l^{-1} ERY when compared with the conidial control. When the ERY concentration was increased to 22·8 μmol l^{-1}, the viability loss was 100% when combined with a blue light energy value of 40 J cm^{-2}.

The germinating conidia were resistant to ERY alone at 11·4 and 22·8 μmol l^{-1} when compared with the conidial control (Fig. 4). In contrast, blue light alone at energy levels of 40, 80 and 100 J cm^{-2} significantly reduced conidial viability by approximately 36, 42 and 47%, respectively. Combining blue light with ERY (11·4 μmol l^{-1}) produced a significant viability loss of about 90 and 100% at 40 and 80 J cm^{-2}, respectively. Blue light combined with ERY (22·8 μmol l^{-1}) displayed

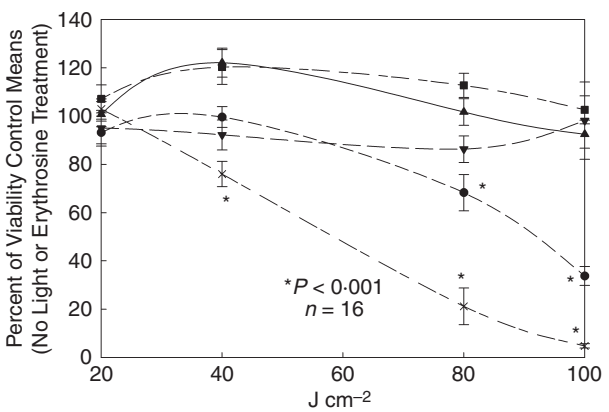

Figure 1 Effect of blue light from Array 2 with and without erythrosine (11·4 and 22·8 μmol l^{-1}) on the viability of nongerminated *Penicillium digitatum* conidia. ▲ Nongerminated *P. digitatum* + erythrosine (11·4 μmol l^{-1}) only; ▼ Nongerminated *P. digitatum* + blue light only; ● Nongerminated *P. digitatum* + blue light + erythrosine (11·4 μmol l^{-1}); ■ Nongerminated *P. digitatum* + erythrosine (22·8 μmol l^{-1}) only; X Nongerminated *P. digitatum* + blue light + erythrosine (22·8 μmol l^{-1}).

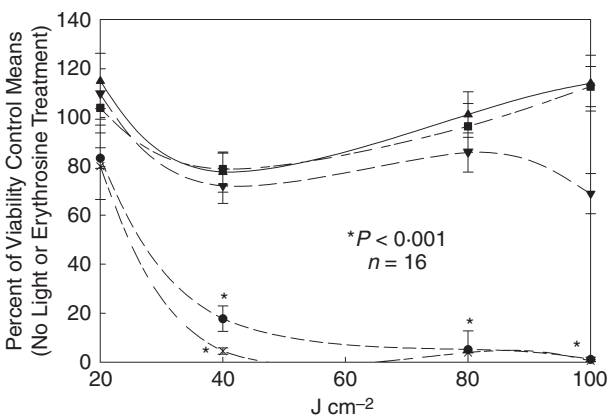

Figure 2 Effect of blue light from Array 2 with and without erythrosine (11·4 and 22·8 μmol l^{-1}) on the viability of germinating *Penicillium digitatum* conidia. ▲ Germinating *P. digitatum* + erythrosine (11·4 μmol l^{-1}) only; ▼ Germinating *P. digitatum* + blue light only; ● Germinating *P. digitatum* + blue light + erythrosine (11·4 μmol l^{-1}); ■ Germinating *P. digitatum* + erythrosine (22·8 μmol l^{-1}) only; X Germinating *P. digitatum* + blue light + erythrosine (22·8 μmol l^{-1}).

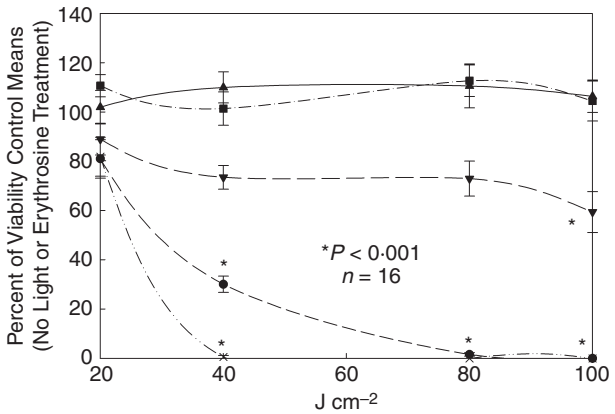

Figure 3 Effect of blue light from Array 2 with and without erythrosine (11·4 and 22·8 μmol l^{-1}) on the viability of nongerminated *Fusarium graminearum* conidia. ▲Nongerminated *F. graminearum* + erythrosine (11·4 μmol l^{-1}) only; ▼ Nongerminated *F. graminearum* + blue light only; ● Nongerminated *F. graminearum* + blue light + erythrosine (11·4 μmol l^{-1}); ■ Nongerminated *F. graminearum* + erythrosine (22·8 μmol l^{-1}) only; X Nongerminated *F. graminearum* + blue light + erythrosine (22·8 μmol l^{-1}).

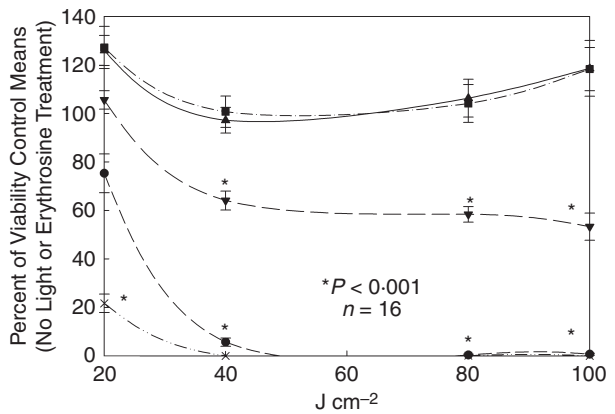

Figure 4 Effect of blue light from Array 2 and erythrosine (11·4 and 22·8 μmol l^{-1}) on the viability of germinating *Fusarium graminearum* conidia. ▲ Germinating *F. graminearum* + erythrosine (11·4 μmol l^{-1}) only; ▼ Germinating *F. graminearum* + blue light only; ● Germinating *F. graminearum* + blue light + erythrosine (11·4 μmol l^{-1}); ■ Germinating *F. graminearum* + erythrosine (22·8 μmol l^{-1}) only; X Germinating *F. graminearum* + blue light + erythrosine (22·8 μmol l^{-1}).

even greater viability reduction, with significant lower CFU counts of about 80 and 100% at 20 and 40 J cm^{-2}, respectively.

Light in the 405–470 nm (blue) spectral range is bactericidal for PA and methicillin-resistant *S. aureus* (Guffy and Wilborn 2006; Enwemeka *et al.* 2009). Results presented here show that blue light also reduces LM and BA viability. In general, the data show blue light emitted by Array 2 did not reduce viability as effectively as that from Array 1. Manufacturer data sheets indicate that Array 2 LEDs emit a more pure blue light (peak 470 nm) than Array 1 LEDs that emit trace amounts of indigo (420–450 nm) cyan (500–510 nm) and green (520–535 nm).

No significant difference in PA viability was observed at the three incubation temperatures (25, 30 and 37°C) in combination with Array 1 light. In contrast, when Array 2 was the light source, a significantly lower number of CFU survived at 37°C, its optimal growth temperature, when compared with the two lower temperatures. Visible light induces cell death in *E. coli* due to ROS induction (Lipovsky *et al.* 2010), and that higher incubation temperature enhances ROS formation in *E. coli* (Pal *et al.* 2009). These observations may explain the significant viability loss with PA at 37°C (optimum growth temperature) when compared with the two lower incubation temperatures.

We believe this is the first report showing blue light, with or without a photosensitizer, significantly reduces nongerminated and germinating filamentous fungal conidial viability. Against the test fungi, *in vitro*, ERY at 11·4 and 22·8 μmol l^{-1}, in the presence of blue light significantly reduced nongerminated conidial viability with activity slightly greater against germinating conidia. Antifungal activity paralleled increases in ERY concentration and light energy.

Data indicate blue light alone can significantly reduce FG viability. This was not expected because previous studies indicated that blue light is used by some fungi as a 'cue' for metabolism, growth, pigment formation, tropism and spore production (Siegel *et al.* 1968; Casas-Flores *et al.* 2006; Purschwitz *et al.* 2006).

Results suggest visible blue light alone can (i) significantly reduce BA, PA and LM viability, (ii) significantly reduce the viability of FG or (iii), when combined with the food grade photosensitizer, ERY, reduces the viabilities of nongerminated and germinating conidia of PD.

Materials and methods

Bacteria

Leuconostoc mesenteroides was grown on MRS agar (de Man *et al.* 1976) medium, while BA and PA were grown on nutrient agar (Difco, Dickson & Co., Sparks, MD, USA) overnight at 25 (LM), 30 (BA) and 37°C (PA). Cell suspensions (3×10^4 ml^{-1} PBS) were prepared and aliquots (25 μl) spread on the respective agar plates (60 × 15 mm).

Photosensitizer compound

Erythrosine, also known as FD&C food colour red no. 3, was obtained from IFC Products (Linden, NJ, USA).

Bacterial exposure to blue light

Inoculated plates were divided into two equal sets. Set 1 served as a control (no blue light group) and placed on the bench top. Set 2 was placed under the respective light array and exposed (0–300 J cm^{-2}) to blue light (peak 470 nm).

Array 1 was comprised of LEDs (2·2 mW cm^{-2}; LEDtronics, Torrance, CA, USA) that emitted blue light with traces of other wavelengths, while Array 2 was comprised of LEDs (80 mW cm^{-2}, CREE, Durham, NC, USA) that produced pure blue light (data obtained from company specification sheets). The energy (mW cm^{-2}) output of the arrays was measured with a Solarmeter Digital Radiometer Model 9.4, 422–499 nm (Solartech, Inc., Harrison Township, MI, USA) to determine the light energy emission at the distance between the LEDs and the inoculated agar plate surfaces. The total energy exposure (J cm^{-2}) was calculated according to the equation:

$$J\,cm^{-2} = mW \times seconds$$

when the desired energy dose (J cm^{-2}) was reached both the control and light-treated plates were placed in the incubator at either 25, 30 or 37°C. Separate runs (four runs per bacterium, $n = 8$) were performed and CFU counted. Array 1 was housed in a standard laboratory room at 22°C, while Array 2 was housed in a chemical hood with the exhaust fan operating to ensure the temperature below the LEDs did not exceed the optimum temperature for the micro-organisms. Temperatures generated the LED arrays were measured and not found to exceed room temperature.

Fungal exposure to blue light alone or with erythrosine. Potato dextrose agar (PDA, Difco) slants were inoculated with either PD or FG, incubated for 7 days (PD and FG at 25 and 30°C, respectively) and then stored (4°C). Prior to *in vitro* testing, fresh suspensions (3×10^4 ml^{-1}) of nongerminated conidia were prepared in 1% potato dextrose broth (1% PDB, Difco) and immediately used in the tests requiring nongerminated conidia. To obtain germinating conidia, suspensions (3×10^4 ml^{-1}) of PD and FG were prepared in 1% PDB and incubated at 25 and 30°C, respectfully, for 7 h.

Three controls sets were used in tests with either nongerminated or germinating conidia and consisted of (i) conidia (25 μl conidia + 225 μl PDB) placed on a bench top for the time period equal to the corresponding J cm^{-2} exposure time; (ii) blue light (no ERY) only (25 μl conidia + 225 μl PDB) exposed to Array 2; and (iii) ERY (11·4 or 22·8 μmol l^{-1}) alone which contained 25 μl conidia + 225 μl of ERY diluted in PDB and not exposed to blue light. Assays were perfomed in sterile 96-well plates (Nunc, Roskilde, Denmark).

Test samples to determine the effect of blue light combined with ERY consisted of either nongerminated or germinated conidia (25 μl) + ERY (11·4 or 22·8 μmol l^{-1} in 225 μl PDB) exposed to Array 2 only as earlier experiments (not shown) showed Array 1 ineffective against the fungi. The amount of light energy to which the conidia were exposed ranged from 0 to 100 J cm^{-2}. Based on the equation (above), the exposure times to the light were 4·3, 8·4, 16·8 or 21·5 min.

After the appropriate time period, aliquots (50 μl) of the controls (conidial, ERY, blue light) as well as the light plus ERY sample were spread on four PDA plates/sample, incubated (48 h, 30°C) and colonies counted. Separately, both nongerminated and germinating conidia types of FG and PD were tested at four different times ($n = 16$).

Statistical analyses. Statistical studies [mean, SEM and significance ($P < 0·001$)] for both the bacterial and fungal samples were performed with SigmaStat (Systat, Richmond, CA, USA).

Acknowledgements

The authors declare that no conflict of interests exist in the reported work.

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