In vitro effect of innovative light with photodynamic microbicidal activity in bacteria

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Introduction

Light energy emitted in the visible light spectrum (VIS) is a non-harmful form of radiance and can result in photodynamic microbicidal effect in bacterial species, including multidrug resistant strains (1). Photosensitizing chromophores, like porphyrins, are present in bacteria and are sensitive to light in the visible spectrum, particularly in the visible blue-violet spectrum (400-420 nm). These wavelengths result in excitation of porphyrins and subsequent production of singlet oxygen ($^1O_2$) and other reactive species (RS). These products damage cytoplasmic and membrane structures, causing reduction of survival. The Biovitae ® light, used in this study, emits white light from a light-emitting diode (LED) system but no UV spectrum. This technology has a microbicidal power on bacterial strains and viruses and, unlike wavelengths in the UV (ultraviolet) spectrum, does not harm mammalian cells (2).

Materials and Methods


Fig. 1A

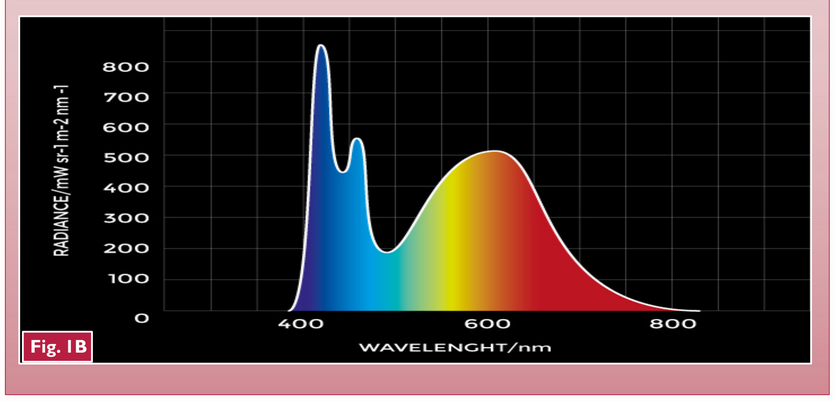

Fig. 1B

Fig. 1A The strip used for the experiments, consisting of 13 Biovitae® LEDs emitting wavelengths in the 400–420 nm range and delivering three different peaks within, and 37 conventional white light LEDs. The LEDs are powered at a constant current of 500 mA.
Fig. 1B Relative radiometric spectral distribution of the Biovitae® strip.

The tested light was provided by the *Nextense* company, and it uses a special combination of frequencies which cover the visible spectrum and create a multispectral interfering wave system for microbial eradication (MIME): 400-420 nm, 400-450 nm, 400-700 nm at an intensity of 3,51 mW/cm^2, 5,85 mW/cm^2, 12,53 mW/cm^2, respectively. The tests (n=28) were performed *in vitro* using 96-well plates and *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) strains, previously cultured in LB medium for 24 h at 37°C.

We obtained an average optical density (OD_{595nm}) for *E. coli* of 0.140 A (1.1 x 10^8 cells/ml) and for *S. aureus* of 0.150 A (1.2 x 10^8 cells/ml) using Victor spectrophotometer (PerkinElmer). The absorbance of the empty culture medium was subtracted from the bacteria containing samples. The bacterial stock solutions were also diluted 1:2 and 1:4. Plates with bacteria were exposed to antimicrobial light for six hours, and bacterial growth monitored over 24 hours.

Results

During light exposure, a slight and steady increase in growth was observed for both *E. coli* and *S. aureus*. However, 18 hours after light exposure, bacterial growth significantly ($P \leq 0{,}001$) decreased compared to probes not exposed to the light. Comparison of the corresponding samples with Student's *t*-Test showed that growth reduction appears to be higher significant in the 1:2 and 1:4 dilution of both bacteria strains.

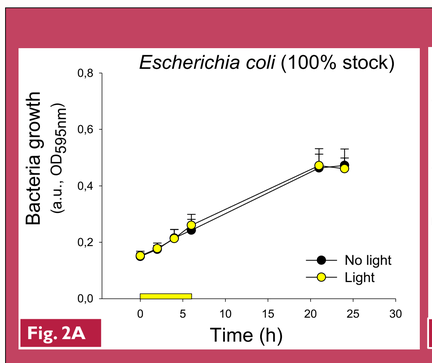

Fig. 2A

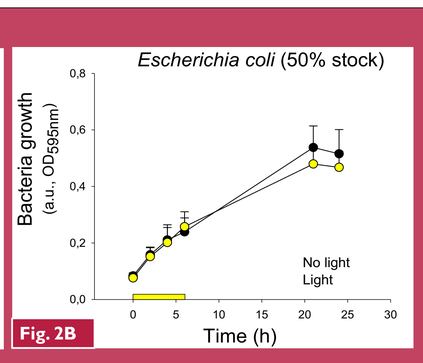

Fig. 2B

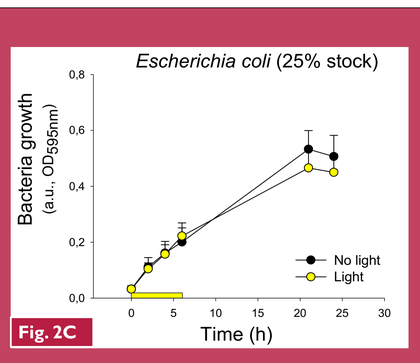

Fig. 2C

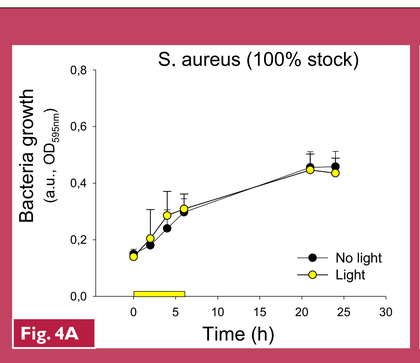

Fig. 4A

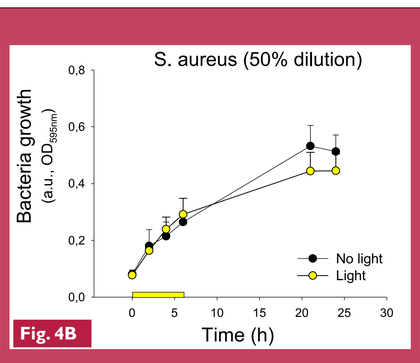

Fig. 4B

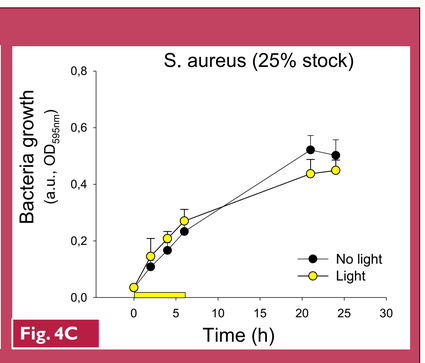

Fig. 4C

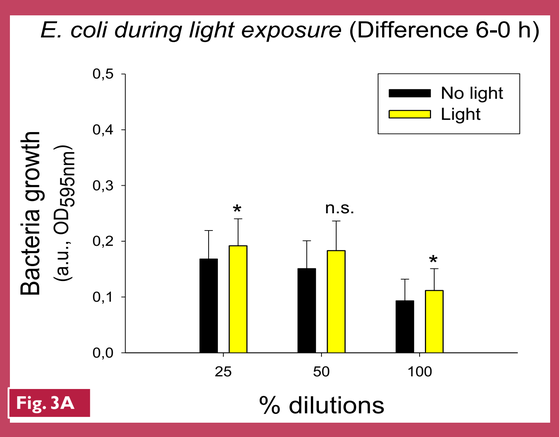

Fig. 3A

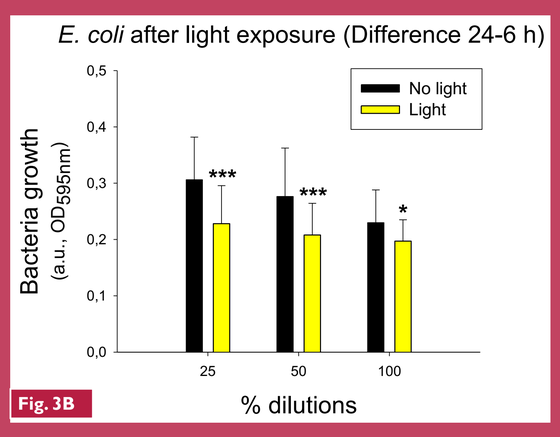

Fig. 3B

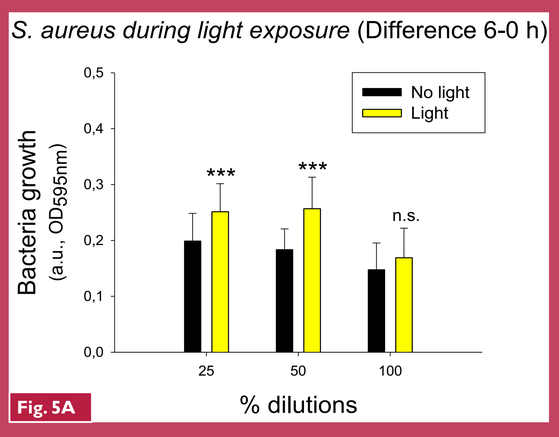

Fig. 5A

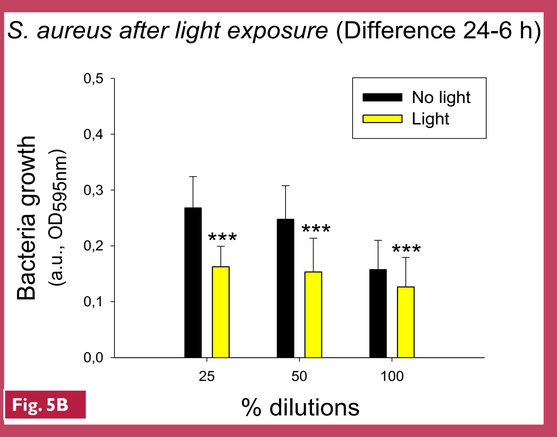

Fig. 5B

Fig. 2 (A, B,C) Time curve during light and no light exposure in *Escherichia coli;*
Fig. 3A Effect on *E.coli* growth during light exposure (Difference between 6 and 0 hours).
Fig. 3B Effect on *E.coli* growth after light exposure (Difference between 24 and 6 hours).
Student's t-Test: * = P<0,05; ** = P<0,005; *** = P<0,001, n.s. = Not significant

Fig. 4 (A, B,C) Time curve during light and no light exposure in *Staphylococcus aureus.*
Fig. 5A Effect on *S. aureus* growth during light exposure (Difference between 6 and 0 hours).
Fig. 5B Effect on *S. aureus* growth after light exposure (Difference between 24 and 6 hours).
Student's t-Test: * = P<0,05; ** = P<0,005; *** = P<0,001, n.s. = Not significant

Discussion and conclusion

The antimicrobial action of Biovitae ® light is resulted to be evident both for Gram-negative and Gram-positive bacteria. Although during light exposure bacterial growth seems to be stimulated, the post light growth significantly ($P \leq 0{,}001$) decreased the bacterial survival, especially for *S. aureus*, which showed identical values for all dilutions used in the study. It would be interesting to investigate whether these LEDs can also be used in the veterinary medicine in order to reduce the need for antibiotics in various fields.

References:
1. *St Denis TG, Dai T, Izikson L, Astrakas C, Anderson RR, Hamblin MR, Tegos GP. All you need is light: antimicrobial photoinactivation as an evolving and emerging discovery strategy against infectious disease. Virulence. 2011 Nov-Dec;2(6):509-20.*
2. *European Commission, Directorate-General for Health and Food Safety, Opinion on biological effects of UV-C radiation relevant to health with particular reference to UV-C lamps, European Commission, 2018.*