Application of 405 nm visible light to selected bacterial species in animal husbandry

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Abstract

The antimicrobial effect of light at specific wavelengths is currently used for sanitation procedures in various types of facilities. The aim of this study was to verify the bactericidal and fungicidal activity of 405 nm light as a safer alternative to ultraviolet C (UVC) radiation that could be used for disinfection in animal housing. Commercially available lamp located in the experimental room was used to emit the 405 nm radiation. For most of the bacterial species tested, there was no decrease in colony forming units after 8-h and 24-h radiation. Significant ($P < 0.01$) reduction in the number of colonies was observed for 8-h and 24-h radiation application in the case of *Bordetella bronchiseptica* when grown on trypticase soy agar. There was also a significant reduction ($P < 0.01$) in the number of colonies for *Staphylococcus aureus* on trypticase soy agar after 24-h radiation exposure. The results indicate a partial bactericidal effect of radiation depending on the type of bacterium irradiated, the type of nutrient medium used and the duration of radiation exposure. However, the effect of the method used in this study cannot be described as disinfectant. In the context of practical application of the technology, the factors mentioned above need to be further investigated.

Disinfection, light spectrum, prevention

Environmental sanitation in facilities with large numbers of animals is the key strategy to prevent the spread of pathogens. Although chemical disinfectants are among the tools traditionally used for microbial load control, with increasing knowledge there has also been an increase in interest in the development of new decontamination technologies (Maclean et al. 2014). One of these technologies is the use of the natural properties of light. The antimicrobial effects of light at specific wavelengths are now the subject of scientific work, with particular attention being paid to wavelengths falling in the ultraviolet spectrum. Ultraviolet C (UVC) radiation with a wavelength of 240–260 nm is being commonly used to decontaminate air and medical devices (Andersen et al. 2006; Reed 2010) for its effect of inactivation of bacterial, viral, and fungal agents (Tseng and Li 2007; Nakpan et al. 2019). Although this technology shows satisfactory disinfection potential, its negative side effects on the organism limit its use to unoccupied spaces only (Leung and Ko 2021). The main ones include the harmful effects on the eyes and skin, or more severely, genotoxic effects (Sterenborg et al. 1988; Sliney 2013).

The germicidal activity of blue light in the 405 to 450 nm range has been described as an alternative to UVC radiation with the advantage of use even in the presence of animals and humans. The underlying mechanism of microbial inactivation is thought to be related to light absorption by the photosensitizers porphyrins and flavins (Dai et al. 2012; Bumah et al. 2017). Exposure to light of this wavelength induces an oxygen-dependent photoexcitation in exposed cells, where the excited porphyrins react with oxygen or cellular components to form reactive oxygen species (ROS), causing oxidative damage and cell death (Maclean et al. 2008). The generation of ROS is associated with direct damage

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to biomolecules (proteins, lipids, and nucleic acids), which are essential components of pathogen cells. Previous studies document that ROS can lead to loss of cell membrane permeability mediated by lipid oxidation (Hadi et al. 2020). It has also been found that partial damage of bacterial cell is enough to make it more susceptible to inactivation by light. This suggests the practical use of 405–450 nm light at least in the context of final decontamination, i.e., its application after a previous disinfectant application that has already resulted in partial disruption of bacterial activity (McKenzie et al. 2014). Recent studies document the use of blue light in disinfection in the food industry (Wu et al. 2022) but also in the treatment of dental infections, acne, and fungal skin infections (Al Hamzi et al. 2019; Bumah et al. 2020; Zhao et al. 2022). Blue light has also been used to successfully treat burns and wounds in mice (Zhang et al. 2014). Amodeo et al. (2023) conducted a study in a hospital setting. Although 405 nm radiation reduced microbial contamination, the authors also recommended the use of other disinfection methods.

Although the use of devices emitting light of these wavelengths in animal husbandry has great potential, information regarding its application in disinfection procedures is lacking. Therefore, the aim of this study was to investigate under experimental conditions the effect of 405 nm radiation generated by commercially manufactured equipment on selected pathogenic bacteria that may be present in these facilities.

Materials and Methods

Bacterial strains and agar media

The following strains were used in this study: reference strains *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923; isolates provided by the diagnostic laboratory of the Department of Infectious Diseases and Microbiology, *Salmonella enterica* serotype Typhimurium dgc*, Pseudomonas aeruginosa* 706/23, *Bordetella bronchiseptica* 632/23, *Pasteurella multocida* 136/23, *Staphylococcus pseudintermedius* 689/23, *Streptococcus canis* 484/22 and *Enterococcus faecium* 555/23.

Strains were revived from the glycerol cryostock and grown on Columbia blood agar (CBA) (OXOID, Basingstoke, UK) at 37 ± 0.5 °C for 24 h. All cultures were sub-cultivated before analysis. For analyses, CBA and trypticase soy agar (TSA) (BD, New Jersey, USA) were used. The volume of agar was adjusted to a standard depth of 4 mm. Prior to the experiments, the possible negative effect of radiation on the agar media was tested. Sterile open plates were irradiated from a height of 1 m. After exposure, a bacterial suspension was cultured on the plates. Suspension inoculated on non-irradiated agar served as a positive control. Plates were cultured for 24 h at 37 °C, and the number of colony-forming-units (CFU) was evaluated.

Sample preparation

Well-isolated colonies from overnight culture were selected, suspended in phosphate buffered saline (PBS) and the density was adjusted to 1.0 McFarland (comparable to the density of a bacterial suspension with a 3×10^8 CFU/ml). The suspension was subsequently diluted in PBS up to 10^4 (*S. aureus, S. canis*), 10^3 (*E. coli, S. enterica serotype* Typhimurium*, P. aeruginosa, S. pseudintermedius, E. faecium*) and 10^2 (*B. bronchiseptica, P. multocida*) CFU/ml. One hundred µl of the diluted suspension was spread over surface of 4 CBA plates and 4 TSA plates.

Irradiation of bacterial strains

Exposure of bacterial pathogens to 405 nm radiation was carried out in the experimental room (fluorescence microscopy laboratory at the Department of Infectious Diseases and Microbiology, room dimensions 4.0 × 1.6 × 2.6 m) using a commercially available device of a size 110 × 80 × 1000 mm mounted on the ceiling. The SPECTRA 1 spectrometer (Kvant, Bratislava, Slovakia) was used to verify the emission of light of the required wavelength.

The average ambient temperature during analyses was 31.7 °C, relative humidity 41% and the average CO_2 concentration was 450 ppm. Each analysis was repeated in two independent experiments. Open plates with inoculated bacterial suspensions were placed under the lamp at a distance of 1 m, so that the light fell directly on the surface of agar. One inoculated CBA and TSA plate was exposed to the radiation for 8 h, another CBA and TSA plate was exposed for 24 h. Positive control consisted of two inoculated CBA and TSA plates that were placed in the experimental room in a clean, sealed carboard box protected from the radiation for 8 and 24 h, respectively.

Survivability testing

After irradiation, plates with bacterial suspensions were sealed and wrapped in a microtene bag to prevent further drying of the agar. Afterwards, they were cultured in a thermostat at 37 °C for 24 h. After 24 h, the number of colonies was counted.

Statistical analysis

Data obtained in this study were analysed using Unistat 6.5 for Excel statistical software (Unistat Ltd, London, UK). The evaluation of differences between the number of colonies detected on the plates after experimental intervention and the number of colonies of the positive control (no intervention) for each bacterial species was performed by χ^2 test. A P value ≤ 0.05 was considered significant.

Results

When testing possible negative effects of the radiation on agar media, no significant differences in bacterial growth on pre-irradiated plates compared to non-irradiated plates were observed (data not shown).

Table 1 summarizes the results of the effect of radiation on each bacterial species tested. For most species, there was no decrease in colony counts caused by the 8- and 24-hour radiation. A significant difference ($P < 0.01$) between the number of colonies detected after 8-h radiation (2 vs. 63 CFU/ml [0.3 $\log_{10}$ CFU/ml vs. 1.8 $\log_{10}$ CFU/ml] in the first trial, 1 vs 46 CFU/ml [0 $\log_{10}$ CFU/ml vs. 1.66 $\log_{10}$ CFU/ml] in the second trial) and 24-h radiation (0 vs. 66 CFU/ml [0 $\log_{10}$ CFU/ml vs. 1.82 $\log_{10}$ CFU/ml] in the first trial, 0 vs. 64 CFU/ml [0 $\log_{10}$ CFU/ml vs. 1.81 $\log_{10}$ CFU/ml] in the second trial) and the positive control was found for *B. bronchiseptica* when cultured on TSA. No significant difference in colony counts was found for this pathogen when CBA was used. On TSA, a significant difference ($P < 0.01$) in the number of colonies compared to the control (0 vs. 101 CFU/ml [0 $\log_{10}$ CFU/ml vs. 2 $\log_{10}$ CFU/ml] in the first trial and 0 vs. 97 CFU/ml [0 $\log_{10}$ CFU/ml vs. 1.99 $\log_{10}$ CFU/ml] in the second trial) was also found for *S. aureus*, but only in the case of 24-h exposure. Noteworthy, for most of the isolates tested, colony growth on agar could have been observed during 24-h light exposure already.

Table 1. Colony counts (log CFU/100 µl) for individual bacterial species irradiated by 405 nm wavelength light for 8 and 24 h.

Species	Agar medium	1st trial				2nd trial			
		8 h 405 nm¥ ($\log_{10}$)	8 h C+ ($\log_{10}$)	24 h 405 nm¥ ($\log_{10}$)	24 h C+ ($\log_{10}$)	8 h 405 nm¥ ($\log_{10}$)	8 h C+ ($\log_{10}$)	24 h 405 nm¥ ($\log_{10}$)	24 h C+ ($\log_{10}$)
Escherichia coli	CBA	2.36	2.40	2.34	2.40	2.31	2.40	2.40	2.36
	TSA	2.32	2.38	2.37	2.37	2.37	2.37	2.36	2.35
Salmonella Typhimurium	CBA	2.44	2.45	2.42	2.45	2.49	2.48	2.45	2.48
	TSA	2.45	2.48	2.31	2.39	2.44	2.44	2.43	2.43
Pseudomonas aeruginosa	CBA	2.35	2.38	2.36	2.30	2.39	2.37	2.37	2.41
	TSA	2.32	2.36	2.25	2.30	2.30	2.37	2.23	2.26
Bordetella bronchiseptica	CBA	1.83	1.79	1.79	1.79	1.81	1.79	1.81	1.82
	TSA	0.30	1.80	0	1.82	0	1.66	0	1.81
Pasteurella multocida	CBA	2.09	2.11	2.12	2.12	1.96	2.02	2.07	2.04
	TSA	0	0	0	0	0	0	0	0
Staphylococcus aureus	CBA	2.02	2.01	2.02	2.03	2.06	2.05	2.01	2.04
	TSA	1.99	1.98	0	2.00	2.03	2.07	0	1.99
Staphylococcus pseudintermedius	CBA	2.02	2.07	2.03	2.05	2.29	2.22	2.24	2.31
	TSA	1.96	2.06	1.97	2.02	2.28	2.28	2.18	2.26
Streptococcus canis	CBA	2.05	2.00	1.99	2.01	2.01	2.04	2.00	2.06
	TSA	2.04	2.05	2.03	2.00	2.06	2.03	1.72	2.01
Enterococcus faecium	CBA	2.24	2.27	2.26	2.19	2.26	2.27	2.26	2.21
	TSA	2.26	2.34	2.26	2.26	2.23	2.25	2.24	2.23

¥ Plates irradiated by 405 nm light; C+: non-irradiated plates, positive control; CBA: Columbia blood agar; TSA: trypticase soy agar

Discussion

Visible light perceived by the naked eye is part of the electromagnetic spectrum, with wavelengths from 380 to 700 nm. Studies of the germicidal properties of the UV spectrum, focusing on its upper and lower limits, have attracted the interest of the scientific community. Visible violet-blue light with a wavelength of 405–470 nm has shown promising results in the disinfection of surfaces and air (Maclean et al. 2014). Previous studies suggest antimicrobial properties through which inactivation of microorganisms including bacteria, viruses and fungi occurs (Amodeo et al. 2022; Frilli et al. 2023). The mechanism of action of violet-blue light is based on production of ROS, which can damage the cellular components of microorganisms and lead to cell death (Maclean et al. 2008). Given the high microbial load that is typical for environments with higher animal numbers, the aim of our study was to validate the inactivation activity of 405 nm wavelength light emitted by a commercial device and its practical application.

Although there was a decrease in the number of microorganisms found in our experiment, the resulting decrease was not sufficient to be evaluated as disinfectant. A reduction of five logarithmic orders of magnitude in the number of microorganisms is required to achieve a disinfectant effect according to the Czech technical standard 1656 (665208) for the minimum requirements for bactericidal activity of chemical disinfectant and antiseptic products used in the veterinary field.

The results of our experiment indicate only partial efficacy in the inactivation process of the selected microorganisms. For most of the isolates tested, there was no reduction in colony counts due to radiation after 8 or 24 h of exposure. Reduction in numbers was only observed for *B. bronchiseptica* and *S. aureus* when applied on TSA, however, the same effect was not observed for CBA. The growth of bacteria can be affected by various influences. According to Kim et al. (2016), the factors influencing the outcome of irradiation include the choice of culture medium and environmental conditions. Recent studies document the amplified effect of radiation when pathogens are exposed on inert surfaces such as polyvinyl chloride (PVC) and acryl, in contrast to nutrient media (Murdoch et al. 2012). An amplified effect has also been found in the presence of biological material such as saliva, blood plasma and faeces as opposed to basal media (Tomb et al. 2017). Although nutrient media may contain photosensitive components likely enhancing the inactivation process, they on the other hand provide a more nutritious environment and greater protection against oxidative stress (Sinclair et al. 2023a). This could explain the difference in results when using the two types of agar media in our study - CBA enriched with 5% defibrinated sheep blood may have provided a more nutritious environment for the bacteria allowing protection against oxidative stress compared to TSA.

The intensity of radiation is also a factor influencing the result. It appears that lower intensities (0.5 mW/cm) may be more effective as opposed to higher intensities (e.g., 50 mW/cm), according to Sinclair et al. (2023b). It is possible that this phenomenon is due to the specific energy levels required to induce photoexcitation of porphyrin molecules in exposed bacteria; the use of higher intensities may be ineffective due to saturation of the porphyrin photoexcitation pathway in the presence of excess photons, which therefore may not necessarily contribute to the inactivation process. In the case of lower intensities, photons can be used more efficiently with less wastage (Maclean et al. 2016).

Kim and Yuk (2017) mention that the sensitivity of cells to 405 nm radiation also differs between bacterial strains and serotypes. For example, *E. coli* strain ST131 was found to be more sensitive to the 410 nm blue light than strain ST648 (dos Anjos et al. 2019). In the case of *Salmonella* Typhimurium, the low amount of endogenous porphyrins in cells does not appear to generate sufficient ROS to disrupt cell membrane integrity. Different

bacterial species also produce different porphyrins; the absorption wavelengths may differ for different photosensitizers. In a practical context, it is a reason why is necessary to emit different wavelengths for optimal stimulation (Nitzan et al. 2004).

Our results are not consistent with the findings of Amodeo et al. (2022), who observed a reduction in bacterial growth in *Salmonella* Typhimurium (2.30 $\log_{10}$), *E. coli* (3.83 $\log_{10}$), and *P. aeruginosa* (3.86 $\log_{10}$) when plates were placed 2–3 m from a radiation source of 967 and 497 μW/cm. In a study by Sinclair et al. (2023a), complete or near-complete inactivation of *S. aureus*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa* occurred under radiation intensities ranging from 0.001 to 2.016 mW/cm; it was found that higher levels of bacterial inactivation were observed with increasing exposure time. Of the Gram-positive bacteria tested in the study, *S. aureus* was found to be the most sensitive species; it correlates with our findings where *S. aureus* was one of the three inactivated species. Increased sensitivity of *S. aureus* was also described in the study by Maclean et al. (2009). In a study by Hoenes et al. (2021), *S. aureus* and *P. aeruginosa* required the lowest radiation dose to achieve a 1 $\log_{10}$ reduction. In the study by Sinclair et al. (2023a), bacteria were approximately 1.5 m from the radiation source and were exposed for 4, 8, and 24 h. The authors further report that irradiance levels were highest in areas directly below the light source and lowest at the furthest measured points, which is confirmed by Boyce et al. (2016). A difference in inactivation of Gram-positive and Gram-negative bacteria was not observed in the study by Sinclair et al. (2023a), however, increased sensitivity of Gram-positive bacteria was observed for bacterial populations dispersed in liquid suspension irradiated with high doses of radiation in a non-nutrient environment over a short period of time (Murdoch et al. 2012; McDonald et al. 2013).

A major limitation of light-based technologies is their activity only on surfaces that are directly exposed to radiation; the effectiveness on covered or shaded surfaces is low, although it is reported that 405 nm radiation can pass through transparent materials (Amodeo et al. 2023). It is also important to note that, for practical use, radiation emitting devices should only complement, not replace, traditional forms of disinfection. Another issue is the safety of use when radiation generating devices run in the presence of animals and humans. Although light at 405 nm has germicidal activity, it is in a relatively harmless wavelength range and is considered safe when used at appropriately low intensities (Maclean et al. 2013). Another advantage of using radiation is that it does not damage disinfected surfaces (Horton et al. 2020).

In conclusion, light of 405 nm wavelength has been previously found to have bactericidal activity against selected pathogen species, but the efficacy of its practical use may be significantly limited compared to the conditions tested in this study (application of shorter exposure time, different location of the pathogen to the light source, different climatic conditions, different composition of the natural carrier/medium in which the pathogen is dispersed). Another limitation of the application of the results obtained in this study in practice is the use of pathogens incubated in laboratory culture conditions, therefore their properties may differ significantly from those found naturally in the environment. Further investigation of the factors mentioned above is needed before applying the technology in practice.

The results of our experiment show that light of 405 nm wavelength does not show sufficient disinfecting effect on selected bacteria. Although the radiation time lasted up to 24 h, a sufficient disinfection effect was not achieved, where the microbial contamination should be reduced by five logarithmic orders of magnitude. An increase in the disinfection effect could have been achieved by influencing other environmental factors (temperature, humidity). In this case, any further decrease in bacterial counts would have been caused by deterioration of the environmental conditions rather than by the co-exposure to 405 nm light.

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