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BACTERIAL INACTIVATION USING PULSED LIGHT

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Pulsed light is a new method intended for the decontamination of food surfaces using short, high frequency pulses of an intense broad spectrum. The effects of broad spectrum pulsed light on the survival of *Listeria monocytogenes* Scott A, *Listeria monocytogenes* CNL, *Pseudomonas fluorescens* MF37 and *Photobacterium phosphoreum* SF680 populations on agar and in a liquid medium were investigated during this study. The sterilisation system generated 1.5 J cm^{-2} per pulse with eight lamps for 300 μs. In the case of surface-seeded cells, a 7.8, 8.14 and >7.14 log reduction was obtained for *L. monocytogenes*, *Ps. fluorescens* MF37 and *Ph. phosphoreum*, respectively, after a single pulse of treatment. Inactivation levels were lower for depth-plated cells: indeed, 10 pulses of treatment achieved 1.6, 2.03 and 4.78 log reductions for *L. monocytogenes* ScottA, *L. monocytogenes* CNL and *Ps. fluorescens* MF37, respectively. After 5 pulses, *Ph. phosphoreum* exhibited a 4.6 log reduction. Similarly, bacterial cells in suspension treated with 3 pulses were reduced by 0.52, 0.8, 2.07 and 2.05 for *L. monocytogenes* ScottA, *L. monocytogenes* CNL, *Ps. fluorescens* MF37 and *Ph. phosphoreum*, respectively. No resistance to pulsed light was observed during our experiments.

Keywords: pulsed light, treatment, bacteria, inactivation, efficiency

Illness caused by the consumption of contaminated food and food-borne diseases remain major health problems throughout the world. For this reason, foods contaminated with pathogenic micro-organisms are a considerable public health concern (MEAD et al., 1999). Several technologies aimed at reducing or eliminating the microbiological risks associated with contaminated foods have been evaluated to date. Pulsed light is a novel, non-thermal decontamination technology for food products which uses short time, high frequency pulses of an intense broad spectrum. During this

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treatment, energy is stored in a high power capacitor and then released to a specially designed xenon lamp unit. The high-voltage, high-current pulse applied to the lamp causes it to emit an intense pulse of light focused on the treatment area, which typically lasts for a few hundred microseconds. This produces several high-energy pulses per second; in this way, micro-organisms are inactivated effectively during the pulse more rapidly than using conventional methods. This technology has potential applications for the treatment of foods, packaging and processing equipment for the food, medical and pharmaceutical industries, water and air. Pulsed light is effective in inactivating bacteria, fungi, viruses and protozoa, and its efficiency is much greater and in a much shorter time than with continuous UV treatment (DUNN et al., 1995; HUFFMAN et al., 2000; ROBERTS & HOPE, 2003; TAKESHITA et al., 2003; FEUILLOLEY et al., 2006). MCDONALD and co-workers (2000) demonstrated that the inactivation of *Bacillus subtilis* spores by pulsed UV-light was significantly more successful than using continuous UV light. Identical levels of inactivation of *Bacillus subtilis* were achieved with 4 mJ cm^{-2} pulsed UV light and 8 mJ cm^{-2} continous UV light. The efficiency of the pulsed light system depends to some extent upon the ease with which the organisms to be killed can be directly illuminated (DUNN et al., 1995).

The aim of this study was to investigate the efficiency of broad spectrum pulsed light in the bacterial inactivation of *L. monocytogenes* Scott A, *L. monocytogenes* CNL, *Pseudomonas fluorescens* MF37 and *Ph. phosphoreum* on solid (agar surface-seeded and agar depth-plated cells) and liquid media.

1. Materials and methods

1.1. Bacterial strains, media and culture conditions

Listeria monocytogenes Scott A and *Listeria monocytogenes* CNL, *Pseudomonas fluorescens* MF37 and *Photobacterium phosphoreum* SF680 were used to study the inactivating effects of pulsed light. The species of micro-organisms used during these experiments, the culture media and the incubation temperatures are summarised in Table 1. *L. monocytogenes* Scott A and *L. monocytogenes* CNL were provided by the Institut Pasteur (Paris, France). The MF37 strain of *Ps. fluorescens* is a natural rifampicin-resistant mutant of the psychrotrophic strain MF0 isolated from raw milk (BURINI et al., 1994). *Ph. phosphoreum* SF680 was isolated from smoked salmon at IFREMER (Nantes, France).

1.2. Pulsed light equipment

The pulsed light generator employed in the present study was a model RDT 350 from La Calhène (Rush City, USA). In this apparatus, the treatment of the samples was carried out in a circular chamber (diameter 270 cm) containing eight xenon lamps with

emitted light ranging from 200 to 1200 nm (Fig. 1). The eight xenon lamps placed 13.5 cm from the sample to be treated were generating a light flux of 1.5 J cm^{-2} during the 300 μs of one flash.

Table 1. Culture conditions for the micro-organisms studied

Name	Media		Incubation temperature
	Liquid[a]	Solid[b]	
Listeria monocytogenes Scott A	BHI	TSA-YE	37 °C
Listeria monocytogenes CNL 895807	BHI	TSA-YE	37 °C
Pseudomonas fluorescens MF37	NB	TSA	28 °C
Photobacterium phosphoreum SF680	BHI + 2%NaCl	BHA + 2%NaCl	17 °C

[a] Liquid media codes and sources: BHI, Heart Infusion Broth (Merck, Darmstadt, Germany); NB, Nutritive Broth (Merck).
[b] Solid media codes and sources: TSA, Trypticase Soy Agar (Merck); TSA-YE, Trypticase Soy Agar supplemented with 0.6% yeast extract (Biorad, Marnes-La-Coquette, France); BHA, Heart Infusion Agar (Merck)

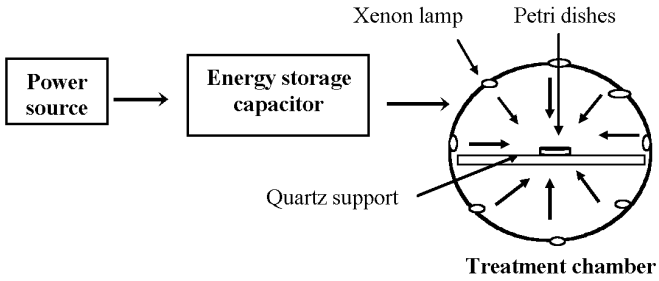

Fig. 1. Schematic diagram of pulsed light treatment system

1.3. Sample preparation and pulsed light treatment

The cells to be treated by pulsed light were prepared as agar surface-seeded cells, agar depth-plated cells and suspended cells, representing solid and liquid food systems.

For agar surface-seeded cells, after 24 h of incubation in broth medium, a 0.1 ml sample of the cell suspension from each inoculum was surface-seeded onto solid media. Surface-inoculated solid media plates were treated with 1 pulse, after which, the control and treated plates were incubated for 24 to 72 h and the colonies enumerated.

For agar depth-plated cells, the 24 h bacterial culture was serially diluted. A 0.5 ml sample of the cell suspension from each dilution was then mixed with 15 ml agar medium. After cooling, the open dishes were subjected from 1 to 10 pulses with the flash lamps. After incubation for 48 h, the colonies for the appropriate dilutions were enumerated.

For suspended cells, 20 ml (2 mm depth) of a culture of each micro-organism was transferred into standard, 15 cm diameter Petri dishes. The open dishes were then positioned in the treatment chamber. Suspended cell samples were treated with 1 or 3 pulses. Both control and treated samples were serially diluted followed by the surface plating of 0.1 ml samples on agar medium. All plates were incubated for 24 to 48 h and then the colony counts were recorded.

For agar surface-seeded, agar depth-plated and suspended cells in Petri dishes, only the xenon lamps from the top would contribute to photon illumination.

1.4. Pulsed light-induced resistance

In order to investigate the possibility that the four strains studied might acquire resistance to pulsed light treatment, a 0.1 ml sample of each bacterial culture was agar surface-seeded. The inoculated plates were then subjected to 1 pulse. After an incubation time of 48 h and the counting of survivors, one colony from each bacterial culture was removed, and cultured for 24 h in a liquid medium. A 0.1 ml sample of each bacterial culture was agar surface plated and flashed again with 1 pulse. The experiment was repeated 3 times.

1.5. Statistical analysis

All experiments were performed in triplicate using the same culture for the same treatment at the same time for all three trials. Bacterial populations were expressed in log CFU ml^{-1}. Statistically significant differences between treated and untreated cells were tested using an analysis of variance by ANOVA using the STAT-VIEW software system.

2. Results and discussion

2.1. Agar-seeded cells

In order to demonstrate the efficacy of pulsed light on solid surfaces, agar-seeded bacterial cells were treated with pulsed light at 1.5 J cm^{-2}. One pulse achieved the significant inactivation of tested bacteria, ($P<0.0001$) to yield reductions of about >7 log (Fig. 2A). A significant number of publications have documented the ability of pulsed light to destroy microorganisms. The differences between the materials used and experimental conditions under which different studies were performed have given rise to variable results which render any comparison difficult. Data in the literature confirm the effects of light pulse treatment on agar surfaces (MACGREGOR et al., 1998; ROWAN et al., 1999; KRISHNAMURTHY et al., 2004). KRISHNAMURTHY and co-workers (2004) observed a 7.5 log reduction of *Staphylococcus aureus* in agar-seeded cells treated for 5 s or longer by pulsed UV light at an energy of 5.6 J cm^{-2}. Different mechanisms have been proposed to explain the lethal effects of pulsed light, all of them related to the UV part of the spectrum and its photochemical and/or photothermal effects. The pulsed light

process can rapidly and irreversibly disrupt the genetic mechanisms of target bacteria through the photochemical transformation of pyrimidine bases to form dimmers (WEKHOF, 2000; MCDONALD et al., 2000; TAKESHITA et al., 2003). According to WEKHOF (2000), the lethal action of pulsed light can be due also to photothermal effect.

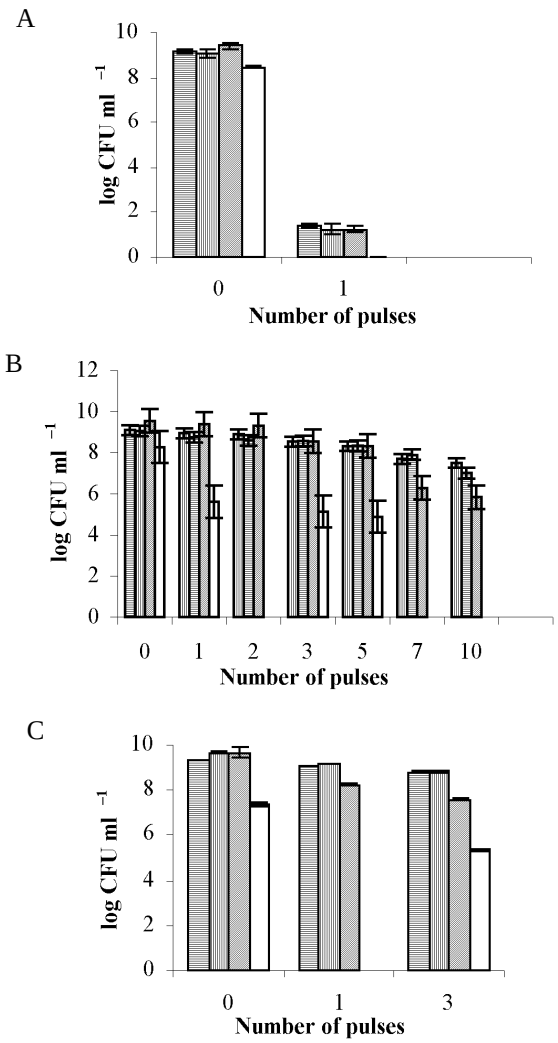

Fig. 2. Bacterial inactivation after pulsed light treatment of surface-seeded plates (A); of agar depth-plated cells (B) and of suspended cells (C). ▤: *Listeria monocytogenes* Scott A; ▥: *Listeria monocytogenes* CNL; ▩: *Pseudomonas fluorescens* MF37; □: *Photobacterium phosphoreum*

It is proposed here that with an energy exceeding 0.5 J cm^{-2}, disinfection is achieved through the rupture of bacteria as a result of overheating caused by the absorption of all UV light from a flash lamp. This overheating can be attributed to a difference between the absorption of UV light by bacteria and that of the surrounding medium. The water contained in bacteria will be vaporised, generating a small steam flow that induces membrane destruction (TAKESHITA et al., 2003).

2.2. Depth-plated cells

By comparison with the surface-seeded cells, only a very weak level of inactivation was observed after one pulse. Indeed, we only obtained 1.6, 2.03 and 4.78 log reductions for *L. monocytogenes* Scott A, *L. monocytogenes* CNL and *Ps. fluorescens* MF37, respectively, after 10 pulses and a 4.6 log reduction for *Ph. phosphoreum* after 5 pulses ($P<0.0001$). Similar conclusions were reached by OZER and DEMIRCI (2005), JUN and co-workers (2003), SHARMA and DEMIRCI (2003) and LAGUNAR-SOLAR and co-workers (2006) when modelling the inactivation of different microorganisms on salmon fillets, corn meal, alfalfa seeds and fruits. Their studies showed that the log reductions achieved were between 1 and 3 after a larger number of pulses. Reducing the depth of the food product also demonstrated an increase in micro-organisms inactivation. MARQUENIE and co-workers (2003) found no suppression of fungal development when treating *Botrytis cinerea* inoculated on strawberries for up to 250 s.

2.3. Suspended cells

This experiment was designed to evaluate the effect of light pulses on bacteria in a liquid culture simulating a turbid liquid food. Like depth-plated cells, the efficacy of inactivation was significantly less ($P<0.0001$), comparing to that achieved with the surface-seeded cells. Indeed, after treatment with 3 pulses, we showed that the log reduction was no more than 1 for *L. monocytogenes* Scott A and *L. monocytogenes* CNL and 2 for *Ps. fluorescens* and *Ph. phosphoreum* (Fig. 2C). The relatively limited rates of cell reduction was probably due to the very high initial cell population (10^7–10^9 CFU ml^{-1}), causing a fall-off in UV intensity through the 2 mm depth of the sample. GHASEMI and co-workers (2003) only achieved an approximate 0.5 log reduction after treating *Escherichia coli* samples with 5 pulses of 9 J.

The degree of bacterial inactivation decreases in the depth of a solid or a liquid medium because of the poor penetration of pulsed light. So, this difference in log reduction may be related to the location of micro-organisms (shadow effects of different structures) and/or protective substances in the culture medium. Moreover, ROBERTS and HOPE (2003) found that the presence of protein substantially inhibited viral inactivation. Because food products are generally opaque, irregular and thick, lower inactivation levels will be attained. The effectiveness of the bacterial inactivation by pulsed light in a suspension or at depth could therefore be enhanced by minimising sample depth or increasing the number of pulses.

2.4. Pulsed light induced resistance

In order to determine whether any resistance developed against pulsed light, bacterial cells surviving from the surface after light pulse treatment were subcultured and subjected again to the light treatment. These micro-organisms did not exhibit any difference in inactivation by light pulses when compared with the original treatment, indicating that no resistance had developed as a result of pulsed light treatment. An induced resistance of micro-organisms was not observed after the pulsed light treatment of *L. monocytogenes* and *Botrytis cinerea* (MARQUENIE et al., 2003; GÓMEZ-LÓPEZ et al., 2005). If a decontamination technology is to be implemented in the food industry, it is important that micro-organisms should not develop any resistance against it, as the development of resistant flora could hamper the long-term efficiency of the technique.

3. Conclusion

The present study clearly demonstrates the potential of pulsed light for the bacterial inactivation. Complete inactivation of these bacteria can be achieved within a few microseconds. The microbicidal effect depends on the intensity of the light, as well as the position of the microbes, so any shadowing of target cells must be avoided. Microbes on the surface are more readily destroyed than those covered with water, or being inside the food matrix. It is clear that it can be used for surface decontamination. If pulsed light is to be used as an alternative to conventional sterilisation process, the experimental parameters must be optimised in order to develop target inactivation levels for specific applications.

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