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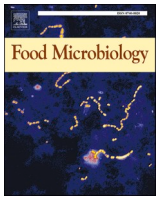

Comparing resistance of bacterial spores and fungal conidia to pulsed light and UVC radiation at a wavelength of 254 nm

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ABSTRACT

Pulsed light (PL) inactivates microorganisms by UV-rich, high-irradiance and short time pulses (250 μs) of white light with wavelengths from 200 nm to 1100 nm. PL is applied for disinfection of food packaging material and food-contact equipment. Spores of seven *Bacillus* ssp. strains and one *Geobacillus stearothermophilus* strain and conidia of filamentous fungi (One strain of *Aspergillus brasiliensis, A. carbonarius and Penicillium rubens*) were submitted to PL (fluence from 0.23 J/cm^2 to 4.0 J/cm^2) and UVC (at λ = 254 nm; fluence from 0.01 J/cm^2 to 3.0 J/cm^2). One PL flash at 3 J/cm^2 allowed at least 3 log-reduction of all tested microorganisms. The emetic *B. cereus* strain F4810/72 was the most resistant of the tested spore-forming bacteria. The PL fluence to 3 log-reduction ($F_{3\ PL}$) of its spores suspended in water was 2.9 J/cm^2 and $F_{3\ UVC}$ was 0.21 J/cm^2, higher than $F_{3\ PL}$ and $F_{3\ UVC}$ of spores of *B. pumilus* SAFR-032 2.0 J/cm^2 and 0.15 J/cm^2, respectively), yet reported as a highly UV-resistant spore-forming bacterium. PL and UVC sensitivity of bacterial spores was correlated. *Aspergillus* spp. conidia suspended in water were poorly sensitive to PL. In contrast, PL inactivated *Aspergillus* spp. conidia spread on a dry surface more efficiently than UVC. The $F_{2\ PL}$ of *A. brasiliensis* DSM1988 was 0.39 J/cm^2 and $F_{2\ UVC}$ was 0.83 J/cm^2. The resistance of spore-forming bacteria to PL could be reasonably predicted from the knowledge of their UVC resistance. In contrast, the sensitivity of fungal conidia to PL must be specifically explored.

1. Introduction

Pulsed light (PL) and ultraviolet (UV)-based technologies are used in food industries to inactivate pathogenic and spoilage microorganisms in clear liquids as well as surfaces of foods and food-contact materials such as packaging and process equipment (Beck et al., 2015; Bintsis et al., 2000; Garvey and Rowan, 2019; Kramer et al., 2017; Mahendran et al., 2019). The inactivation of spores of bacteria and conidia of fungi by such technologies has received a particular attention because of their high resistance to both UV and PL (Koutchma et al., 2009; Kramer et al., 2017). Furthermore, spore-forming bacteria like *Bacillus cereus* and *Clostridium botulinum* are human pathogens associated with occasionally severe foodborne poisonings (Wells-Bennik et al., 2016). Contamination by bacterial spores and fungal conidia is the initial cause of food spoilage (Snyder and Worobo, 2018; Wells-Bennik et al., 2016). Continuous UVC light applied in food industry environments can be emitted by different sources (mercury, amalgam, or excimer lamps, and light emitting diodes for instance) (Koutchma et al., 2009). Among those, low-pressure vapor mercury lamps emit a monochromatic spectrum at a wavelength of λ = 254 nm. In contrast, PL delivers a polychromatic spectrum of white light comprising visible spectrum, UV and near IR with wavelengths ranging from λ = 200 nm–1100 nm (Gómez-López et al., 2012). Technically, xenon lamps of PL devices generate short-duration (i.e. < 1 ms) and high-power (irradiance about 10 MW/m^2) light pulses. At a given fluence, PL efficiency mainly depends (i) on the UVC proportion (short-wave UV at wavelengths <280 nm) determined by the discharge voltage accumulated in the capacitor and released for lamp ignition and (ii) on the number of flashes (Gómez-López et al., 2022). The germicidal effect of UV radiations is generally attributed to photochemical damages of microbial DNA. UVC wavelengths are the most effective in inactivating bacterial spores (Setlow, 2014; Taylor et al., 2020). UVC photons are absorbed by microbial DNA and thereby induce DNA damages like pyrimidine dimers formation impairing microbial growth or DNA strand breaks (Moeller et al., 2007). According to previous reports, UVC wavelengths clearly play a major role in this mechanism (Bohrerova et al., 2008; Levy et al., 2012). Thus, to which extent can we estimate the

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relative or absolute resistance of bacterial spores and conidia to PL from their resistance to a monochromatic UVC source? We explored this question in comparing the sensitivity to PL and UVC of spores of eight bacteria and conidia of three filamentous fungi. The selected strains belonged to different species and application fields. These include reference strains for sterilization studies, foodborne pathogens or representatives of microorganisms contaminating food environments.

2. Materials and methods

2.1. Strains, growth, sporulation and spore conservation conditions

The eight strains of spore-forming bacteria and the three fungal strains used in this study as well as their growth and sporulation conditions are listed in Table 1. *B. atrophaeus* DSM675 is a reference for sterilization by dry heat, ethylene oxide and hydrogen peroxide (Sella et al., 2015). *B. cereus* ATCC10876, *B. cereus* F4810/72 (AH187), *B. thuringiensis* 407 and *B. weihenstephanensis* KBAB4 belong to the wide *B. cereus sensu lato* group that includes foodborne pathogens (Ehling--Schulz et al., 2019). *B. pumilus* DSM492 is a reference for sterilization by γ-radiations (Prince, 1977). *B. pumilus* SAFR-032 was identified as exceptionally UV-resistant (Vaishampayan et al., 2012). *Geobacillus stearothermophilus* DSM5934 is a reference for sterilization by moist-heat (The International Pharmacopoeia, 2020). *Aspergillus brasiliensis* DSM1988 is a reference for sterilization by UV (VDMA, 2016). *A. carbonarius* DSM872 and *Penicillium rubens* DSM848 (formerly *P. chrysogenum* DSM848) are representatives of molds commonly found in food production environments.

Lysogeny broth (LB), Tryptone soy broth (TSB), Malt extract agar (MEA) and Plate count agar (PCA) were purchased from Biokar diagnostics (Beauvais, France). The Nutrient broth used for Fortified nutrient agar (FNA) plates (Fernández et al., 1999) was purchased from Biokar and glucose, and salts and minerals used for FNA preparation were purchased from Sigma-Aldrich® (Steinheim, Germany). Bacteria and fungal conidia stock cultures were stored at −20 °C in a 40 % (v/v) glycerol solution.

2.2. Bacterial spore and conidia preparation

To prepare bacterial spore suspensions, a loopful of a bacterial glycerol stock culture was inoculated into 3 mL of the appropriate nutrient broth, incubated under gentle shaking (200 rpm) at temperatures and times indicated in Table 1. Volumes of 200 μL of this culture were spread on FNA plates (Fernández et al., 1999), and then incubated at the appropriate time and temperature (Table 1). After incubation, all tested strains showed over 90% sporulation by microscope examination (Olympus microscope BX61, Rungis, France). Spores were detached from agar plates with 2 × 3 mL of demineralized sterile water. The suspension was centrifuged for 15 min at 7000×*g* and the pellet was suspended in 25 mL of sterile demineralized water. This operation was performed twice. Then, the spore suspension was twice centrifuged at 5000×*g* and twice at 4000×*g* for 15 min. After the last wash, the purified suspension was collected in 2 mL of demineralized sterile water, heated at 80 °C for 10 min to inactivate vegetative cells, and stored at 4 °C until use within 6 months. Spore counts were determined by spreading 100 μL volumes of decimal serial dilutions in demineralized sterile water on plate count agar (PCA) plates. The spore suspensions contained from 10^7 to 10^{10} spores Colony Forming Unit per mL (CFU/mL).

Conidia suspensions were prepared from cultures on malt extract agar (MEA). A loopful of a fungal glycerol stock culture was spread on MEA and incubated at the appropriate temperature (Table 1). After 3 days, conidia were harvested and suspended in Ringer solution containing 0.01 % (w/v) Tween 80. Volumes of 200 μL of this conidia suspension were spread on MEA plates and incubated at the appropriate temperature for 7 d or 14 d (Table 1). Conidia were gently harvested using a sterile cotton swab and suspended in 10 mL Ringer solution (Merck Millipore, Germany) containing 0.01 % (w/v) Tween 80 and filtered (if needed) through sterile glass wool to remove mycelium and cell debris. Conidia were then washed twice by centrifugation and suspended again in 5 mL of Ringer solution, stored at 4 °C and used within one month. The conidia suspensions containing 10^7–10^8 CFU/mL were free of mycelium and cell debris (spore proportion >95 %) as determined by phase-contrast microscopy under a 1000× magnification.

2.3. Pulsed light, UVC (254 nm) devices and measurements of fluence and UVC efficiency

PL resistance of bacterial spores and conidia was tested using a previously described lab-scale equipment (Claranor, Avignon, France) (Clair et al., 2020; Esbelin et al., 2016; Levy et al., 2012). This consists in a stainless-steel box with an optical system containing three xenon lamps with their optical reflector system and an adjustable lab-elevator for placing the samples below the lamps at the desired distance. The optical system is an assembly of three polished aluminium reflectors (one for each lamp) with calculated profile to insure an almost uniform fluence on a 10 cm × 10 cm square surface. The PL equipment delivers short-time pulses (250 μs) of polychromatic light with broad spectrum (200 nm–1100 nm) emitted by xenon flash lamps. Tests were performed

Table 1
Strain used and culture conditions of the tested microorganisms.

Strain designation	Growth conditions (Temperature/medium/time)	Sporulation conditions (Temperature/medium/time)	Spore or conidia storage (Temperature/medium)	Survivor counts (Temperature/medium/time)
Bacillus atrophaeus DSM675	30 °C/LB/16 h	30 °C/FNA/10 d	4 °C/demineralized sterile water	30 °C/PCA[b]/24 h–48 h
Bacillus cereus ATCC10876[a]	30 °C/LB/16 h	30 °C/FNA/7 d		
Bacillus cereus F4810/72 (AH187)	30 °C/LB/16 h	30 °C/FNA/7 d		
Bacillus pumilus DSM492[a]	30 °C/LB[b]/16 h	30 °C/FNA/7 d		
Bacillus pumilus SAFR-032[a]	30 °C/LB/16 h	30 °C/FNA/7 d		
Bacillus thuringiensis 407[c]	30 °C/LB/16 h	30 °C/FNA/7 d		
Bacillus weihenstephanensis KBAB4[c]	30 °C/LB/16 h	30 °C/FNA/7 d		
Geobacillus stearothermophilus DSM5934	55 °C/TSB[b]/16 h	55 °C/FNA/10 d		55 °C/PCA/48 h
Aspergillus brasiliensis DSM1988	30 °C/MEA[b]/3 d	30 °C/MEA/14 d	4 °C/Ringer solution	30 °C/MEA/48 h–72 h
A. carbonarius DSM872	30 °C/MEA[b]/3 d	30 °C/MEA/14 d		
Penicillium rubens DSM848	23.5 °C/MEA[b]/3 d	23.5/MEA/7 d		23.5 °C/MEA/48 h–72 h

[a] DSM: Deutsche Sammlung von Mikroorganismen; SAFR: Spacecraft Assembly Facility; ATCC: American Type Culture Collection.
[b] LB: Lysogeny broth; FNA: Fortified Nutrient Agar; MEA: Malt Extract Agar; PCA: Plate Count Agar; TSB: Tryptic Soy Broth.
[c] INRAE laboratory collection.

with a charging voltage of 2500 V. Strains suspended in water or deposited onto polystyrene (PS) Petri dishes were exposed to PL fluence between 0.23 and 4 J/cm^2 obtained by varying both flash number (from 1 to 4 at 1 s intervals) and the distance between xenon lamps and the sample using the lab-elevator (from 20.3 cm to 2.4 cm). Fluence measurements were performed using a Gentec joulemeter equipped with an UP-17 sensor adapted for high power measurement (Gentec-eo Electro-Optics Inc., Quebec, Canada) and connected to a display device (MAESTRO, Gentec-eo). Fluence homogeneity was verified and the relative standard deviation (% RSD) was less than 7.2 % on the width of the treated area. To determine UVC efficiency, a neutral filter or a pass band filter cutting off wavelengths <280 nm was placed onto the joulemeter at a distance of 4.4 cm from the lamps and the transmitted fluence was measured. The UVC efficiency (%) was calculated using equation (1):

$$UVC\ (\%) = \left(\frac{F_{200-1100nm} - F_{280-1100nm}}{F_{200-1100nm}}\right) \times 100 \quad (1)$$

where $F_{200\text{-}1100nm}$ is the fluence transmitted through the neutral filter and $F_{280\text{-}1100nm}$ the fluence through the passband filter.

For UVC resistance tests, a UVC chamber was equipped with two 1.6 cm diameter and 30 cm long low pressure mercury lamps (G5- TUV 8 W germicidal UVC, PHILIPS) emitting germicidal UV at a 253.7 nm peak according to the manufacturer's specifications. The lamps were placed in parallel at a distance of 16.5 cm from the inoculated Petri dishes. The irradiance delivered in experimental conditions was measured with the Gentec-eo joulemeter equipped with an UP-12 E Gentec-eo detector connected to the MAESTRO display device. The measured irradiance was 3.37 ± 0.68 mW/cm^2. Fluence is equal to irradiance × exposure time and ranged between 0.01 J/cm^2 and 2.98 J/cm^2 (*i.e.* with a UVC exposure lasting from 3 s to nearly 900 s). Fluence homogeneity was verified and the relative standard deviation (% RSD) was less than 3.2 % on the width of the treated area.

2.4. Inactivation of spores and conidia suspended in water by PL and UVC

One mL of purified spore/conidia suspension containing 10^7–10^8 spores CFU/mL or 10^5–10^6 conidia CFU/mL was transferred into a 55 mm diameter Petri dish. The dish was gently shaken, resulting in a 0.42 mm-deep (i.e. the height of a 1 mL-volume in a cylinder of 55 mm diameter) spore suspension spread over the whole Petri dish surface. After PL or UVC exposure, 100 µL of the spore suspensions were pipetted, then appropriate serial decimal dilutions in demineralized sterile water were plated on agar and incubated at temperature and for time given in Table 1.

2.5. PL and UVC inactivation of spores and conidia deposited by spraying on PS Petri dishes

Selected spores or conidia were sprayed on PS Petri dishes of 9 cm diameter using a previously described airbrush method, with minor modifications (Levy et al., 2011). Briefly, 100 µL of suspension containing 10^8–10^9 spores CFU/mL or 10^6–10^7 conidia CFU/mL were poured into a 2 mL Mecafer AG-1 airbrush tank (Mecafer SA, Valence, France) and sprayed at a distance of approx. 20 cm on sterile Petri dishes placed vertically and perpendicularly to the spray direction. The deposition of sprayed cells on the dish bottom and not on the dish wall was obtained with a home-designed Petri dish holder. After spraying, inoculated Petri dishes were dried overnight in the dark at room temperature, and then exposed to PL or UVC. Cells deposited on unexposed (control) Petri dishes, PL and UVC-exposed Petri dishes were placed with 100 mL of a Ringer and tween 80 (0.01 %) solution into sterile plastic bags (Stomacher, UK) and detached by manual shaking for 1 min. One mL of the appropriate serial decimal dilutions was transferred into a Petri dish and covered with molten agar (pour plate counting). Using this spraying technique, average counts on five replicate plates inoculated with a suspension containing *A. brasiliensis* conidia at a concentration of 6.9 log CFU/mL were (mean ± standard deviation) 5.8 ± 0.1 log CFU/plate, i.e. a percentage standard deviation lower than 2 %. This indicates the high reproducibility of the inoculum level obtained by spraying.

2.6. Fitting of survival curves

A modified Weibull model was chosen to fit microbial reduction curves. The model of Albert and Mafart (2005) describes sigmoidal survival curves with shoulder and tail. Equation (2) of the model is:

$$\log_{10}\frac{N_F}{N_0} = \log_{10}\left[\left(1 - \frac{N_{res}}{N_0}\right) \times 10^{-\left(\frac{F}{F_1}\right)^p} + \left(\frac{N_{res}}{N_0}\right)\right] \quad (2)$$

where F is the applied fluence (J/cm^2), N_F (CFU) is the number of surviving spores or conidia after treatment at fluence F, N_0 (CFU) is the initial number of spores or conidia, N_{res} (CFU) is the residual microbial concentration (at the end of the observation), F_1 is the fluence allowing the first 10-fold reduction and p is a parameter, which determines the curve convexity or concavity (upward concavity, $p < 1$; upward convexity, $p > 1$). The fluence allowing n log reduction (F_n) was calculated using Equation (3):

$$F_n = F_1 \times \left[\left(\log_{10}\left(\frac{1 - \frac{N_{res}}{N_0}}{10^{-n} - \frac{N_{res}}{N_0}}\right)\right)^{\frac{1}{p}}\right] \quad (3)$$

F_1, p and N_0 were calculated using the Microsoft® Excel 2016 solver function.

2.7. Inclusion and exclusion criteria for data comparison with literature

PL and UVC spore and conidia resistance determined in this work was challenged with data from literature selected with the following criteria: (i) only data about *Bacillus* spp. spores and *Aspergillus* spp. conidia resistance to PL or UVC (254 nm) were considered; data related to vegetative cells were excluded; (ii) survival tests performed on suspensions in sterile water, phosphate buffered saline (PBS), NaCl solution or on suspensions spread on PS or agar were considered; survival tests on food surfaces were excluded.

For each reference, the fluence allowing 1 to 5-log reduction was extracted from tables and survival curves. For comparison purpose, the relative resistance (RR) for each strain was calculated using the following formula (Equation (4)):

$$RR = \frac{\sum_{i=1}^{n}\left(\frac{F_x}{F_{xref}}\right)}{n} \quad (4)$$

F_x is the fluence for x log-reduction for the tested strain, F_{xref} the fluence for x log-reduction for the reference strain and n the number of fluence data reporting x log-reduction. *B. pumilus* DSM492 and *A. brasiliensis* DSM1988 were taken as a reference strain for *Bacillus* spp. and *Aspergillus* spp., respectively, and RR = 1 for both strains. Data are summarized in Supplementary Table 2.

2.8. Statistical analysis

Mean values and standard deviation were calculated on data from at least three independent experiments performed at different dates and using different spore preparations. The root-mean-square error (RMSE) was calculated to evaluate the variability between the model fitting and experimental data. Means were compared using Tukey's multiple

comparisons test. Linear regression and Pearson correlation coefficient calculation were performed with GraphPad Prism 8.0. For each survival curve, two replicate counts in untreated samples were performed for tests in water and three replicate counts for tests on PS Petri dishes.

3. Results

3.1. Bacterial spore resistance to UVC and PL

Fig. 1 shows survival curves of spores of *B. pumilus* strains DSM492 and SAFR-032 suspended in sterile distilled water, as examples of the survival curves obtained with the test strains. The fitting of experimental data to the model was satisfactory. RMSE is expressed in the same units as the predicted variable. RMSE were <0.12 log reductions for PL (Fig. 1A) and <0.06 log reductions for UVC (Fig. 1B) and therefore much lower than the reported log reductions (most of which were between 1 and 6). One flash of PL at a fluence of 1.51 J/cm^2 allows for a log-reduction of 5.8 ± 0.61 (mean ± standard deviation, n = 3) and 2.0 ± 0.16 for *B. pumilus* DSM492 and *B. pumilus* SAFR-032, respectively (Fig. 1A). A UVC fluence of 0.11 J/cm^2, corresponding to an exposure time of 50 s, allows for log-reductions of 5.3 ± 0.24 and 1.9 ± 0.08 for *B. pumilus* DSM492 and *B. pumilus* SAFR-032, respectively (Fig. 1B). Similar survival curves were determined with spores of six other strains *B. atrophaeus* DSM675, *G. stearothermophilus* DSM5934, *B. cereus* F4810/72, *B. cereus* ATCC10876, *B. weihenstephanensis* KBAB4 and *B. thuringiensis* 407, exposed to PL fluence ranging from 0.23 J/cm^2 to 4 J/cm^2 and UVC fluence ranging from 0.01 J/cm^2 to 0.33 J/cm^2. Fluence values to achieve 1, 2, 3, 4 and 5 log-reduction were derived from survival curves fitted with the non-linear model of Albert and Mafart (2005) (Supplementary data Table 1).

The fluences allowing for two and three log-reduction (Supplementary data Table 1) by PL ($F_{2\ PL}$ or $F_{3\ PL}$) and UVC ($F_{2\ UVC}$ or $F_{3\ UVC}$) were plotted for the eight strains on a correlation diagram (Fig. 2A and B). Among the strains tested, *B. cereus* F4810/72 spores were the most resistant to both UVC and PL, followed by spores of *B. pumilus* SAFR-032 sand *B. cereus* ATCC10876. Spores of strains from the *B. cereus sensu lato* group showed significant differences in resistance to PL and to UVC treatments: *B. cereus* F4810/72 spores were 4–5 times more resistant than *B. weihenstephanensis* spores (Fig. 2). The scatterplots showed that the resistance of spores to UVC and PL (Fig. 2) is significantly correlated (Pearson's test, $p < 0.0001$), with correlation coefficients (R^2) equal to 0.95 and 0.96 for F_2 and F_3, respectively. The ratio $F_{2\ PL}/F_{2\ UVC}$ was comprised between 5 % and 10 %, which is less than the estimated UVC proportion in PL, which was estimated at 12 % (Table 2).

3.2. Fungal conidia resistance

Preliminary tests showed that PL has nearly no effect on *A. brasiliensis* conidia suspended in water (Supplementary Fig. 1) contrarily to *Bacillus* sp. spores (see section 3.1). Several causes could explain this phenomenon. Damages to DNA and/or oxidative damages may be lower, and DNA repair systems, commonly reported as major factors contributing to fungal resistance to UV, may be more effective in cells suspended in water (Braga et al., 2015). The photothermal effect inherent to PL could be attenuated because of heat dissipation in water (Kramer et al., 2017). UV absorption by water is not a plausible cause: this is very low in our device because of the very short optical pathway (0.42 mm) through the water suspension of conidia (Quickenden and Irvin, 1980). The comparison of the sensitivity of fungal strains to PL and UVC was consequently performed with conidia spread on PS Petri dishes. Fig. 3A shows survival curves for PL exposure of conidia of *A. brasiliensis* DSM1988, *A. carbonarius* DSM872 and *Penicillium rubens* DSM848. For comparison, survival curves of *B. pumilus* and *G. stearothermophilus* spores obtained in the same condition (i.e. with spores sprayed on PS Petri dishes) were added to Fig. 3A. One flash of PL at fluence 0.75 J/cm^2 caused a conidia log-reduction greater than 5 for the three tested strains (Fig. 3A). The PL fluences allowing for 3 ($F_{3\ PL}$) and 5 ($F_{5\ PL}$) log-reductions of conidia were not significantly different ($p > 0.05$) between *A. brasiliensis* and *P. rubens*. In contrast, $F_{3\ PL}$ and $F_{5\ PL}$ values of *A. carbonarius* were significantly lower ($p < 0.05$) than the ones *P. rubens* (Fig. 3C), and $F_{5\ PL}$ of *A. carbonarius* was significantly lower ($p < 0.001$) than the one of *A. brasiliensis*.

The comparative sensitivity of strains exposed to UV differs significantly from that exposed to PL. The survival curves of *A. brasiliensis* and *A. carbonarius* conidia exposed to UVC showed an upward concavity with a pronounced tail at about 2-log reduction, while the one of *P. rubens* conidia showed a sharp decrease (Fig. 3B). A UVC dose of 1.8 J/cm^2 (exposure time = 600 s) allows for a log-reduction of 2.1 ± 0.2 and 2.3 ± 0.1 for *A. carbonarius* and *A. brasiliensis*, respectively. In comparison, a UVC dose of 0.23 J/cm^2 (exposure time = 100 s) was sufficient to cause more than 5 log-reduction for *P. rubens* conidia. Overall, the resistance of the three strains to PL was relatively similar, while *Aspergillus* conidia were markedly more resistant to UVC than *P. rubens* conidia (Fig. 3D). In this sense, *P. rubens* behaves similarly to *B. pumilus* and *G. stearothermophilus*, which exhibit a very similar sensitivity to PL in the three first log reduction cycles, and a comparable sensitivity to UVC (Fig. 3). Taken together, these data suggest that conidia of *A. brasiliensis* and *A. carbonarius* are more resistant to UVC than other conidia-forming molds and spore-forming bacteria. This greater

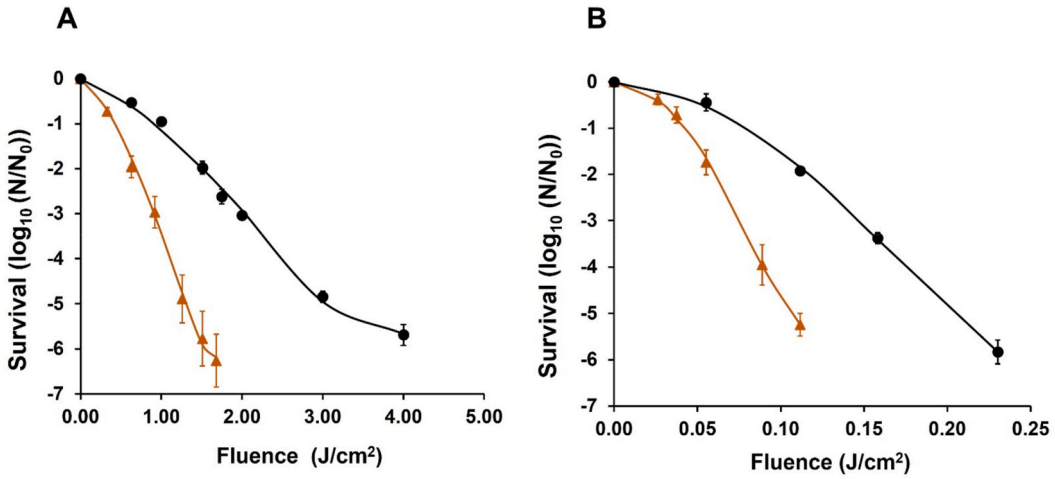

Fig. 1. Inactivation of *B. pumilus* DSM492 (▲) and *B. pumilus* SAFR-032 (black●) spores suspended in water by pulsed light (A) and UVC at λ = 254 nm (B). Symbols represent the mean of three biological replicates (different spore preparations) and bars show standard deviation. Lines represent curves that have the best fit to data points with the model of Albert and Mafart (2005).

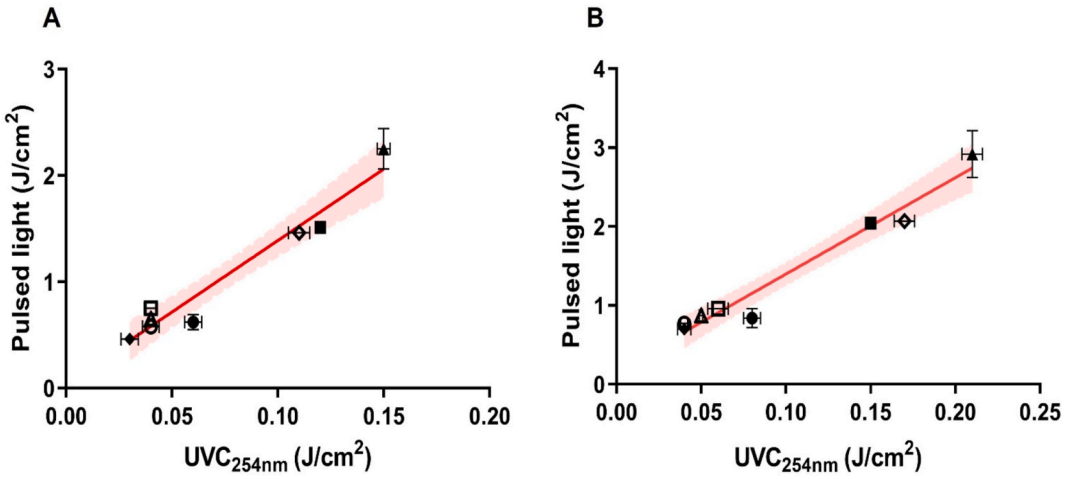

Fig. 2. Relationship between pulsed light and UVC (λ = 254 nm) fluence allowing for (A) a 2 log-reduction (F_2) and (B) a 3 log-reduction (F_3) of spores of eight strains suspended in water. Symbols represent the mean of three biological replicates and bars represent standard deviation. F_2 and F_3 were calculated with the Albert and Mafart model (2005). Strains are, from bottom left to top right: *B. weihenstephanensis* KBAB4 (♦), *B. thuringiensis* 407 (○), *G. stearothermophilus* DSM5934 (Δ), *B. atrophaeus* DSM675 (□), *B. pumilus* DSM492 (●), *B. pumilus* SAFR-032 (■), *B. cereus* ATCC10876 (◊) and *B. cereus* F4810/72 (▲). The red line corresponds to the linear regression with a Pearson's correlation coefficient (R^2) of 0.95 ($P < 0.0001$) and 0.96 ($P < 0.0001$) for F_2 and F_3, respectively. Filled areas represent the 95 % confidence interval. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Fluence (J/cm^2) allowing a two log-reduction (F_2) of bacterial spores and conidia of different strains (in bold) with pulsed light ($F_{2\ PL}$) and UVC (λ = 254 nm) ($F_{2\ UVC}$) and ratio $F_{2\ UVC}/F_{2\ PL}$.

Strains	Fluence to 2-log-reduction by exposure to:		$F_{2\ UVC}/F_{2\ PL}$ (%)
	Pulsed light	UVC (λ = 254 nm)	
Geobacillus stearothermophilus DSM5934	0.46 ± 0.08[c]	0.015 ± 0.003	3.2
Bacillus pumilus DSM492[a]	0.35 ± 0.00	0.017 ± 0.003	4.9
Geobacillus stearothermophilus DSM5934	0.65 ± 0.06	0.035 ± 0.001	5.4
***Penicillium rubens* DSM 848**[a,b]	0.43 ± 0.03	0.025 ± 0.004	5.9
Bacillus atrophaeus DSM675	0.75 ± 0.03	0.044 ± 0.003	5.8
Bacillus thuringiensis 407	0.58 ± 0.01	0.035 ± 0.004	6.1
Bacillus cereus F4810/72	2.25 ± 0.19	0.145 ± 0.003	6.5
Bacillus weihenstephanensis KBAB4	0.46 ± 0.03	0.033 ± 0.004	7.1
Bacillus pumilus SAFR-032	1.51 ± 0.04	0.116 ± 0.001	7.7
Bacillus cereus ATCC10876	1.46 ± 0.02	0.113 ± 0.005	7.7
Bacillus pumilus DSM492	0.62 ± 0.07	0.061 ± 0.004	9.7
***Aspergillus brasiliensis* DSM1988**[a,b]	0.39 ± 0.01	0.830 ± 0.077	210
***Aspergillus carbonarius* DSM 872**[a,]	0.34 ± 0.02	1.29 ± 0.28	380

[a] Conidia or spores were spread on a polystyrene carrier.
[b] Fungal conidia in bold characters.
[c] Mean ± standard deviation ($n = 3$).

resistance does not correspond to greater resistance to PL, although UVC wavelengths are dominant in PL efficiency.

4. Discussion

The spores of the bacterial strains tested in this work showed a rather wide range of sensitivity to both PL and UVC enabling the establishment of correlations between the technologies. *B. cereus* F4810/72 produced the most resistant spores to both PL and UVC, surpassing even the ones of *B. pumilus* SAFR-032, yet identified as particularly resistant to UV, solar and cosmic radiations (Link et al., 2004; Vaishampayan et al., 2012). *B. cereus* F4810/72 belongs to the *B. cereus sensu lato* group III according to the Guinebretière et al. (2008) taxonomic scheme. This strain was isolated during an emetic episode of foodborne poisoning and became a reference for emetic-toxin producing *B. cereus*, but is not known for any particular PL- or UV-resistance (Ehling-Schulz et al., 2005; Guinebretière et al., 2008). Nevertheless, the resistance data to PL and UVC (at λ = 254 nm) obtained by Abbas et al. (2014) with the same strain *B. cereus* F4810/72 are similar to the ones obtained in the present work. This confirms the relatively high resistance of *B. cereus* F4810/72 strain to UVC and PL. We suggest that *B. cereus* 4810/72 could serve as an interesting model strain of spore-forming bacteria for both applied and fundamental studies on UV inactivation. The other strains belonging to the *B. cereus sensu lato* group (*B. cereus* ATCC10876, *B. weihenstephanensis* KBAB4 and *B. thuringiensis* 407) showed lower and diverse resistance levels, despite sharing genetic proximity. The *G. stearothermophilus* strain was among the most sensitive strains, confirming the absence of correlation between resistance to heat and resistance to UVC and PL. Notably, the resistance of bacterial spores to PL, expressed as fluence allowing for 2 or 3 log-reduction, was significantly correlated with the resistance to UVC. This correlation aligns with the major role played by UVC of the PL spectrum in inactivation of spores and vegetative cells of bacteria (Levy et al., 2012; Wang et al., 2005; Woodling and Moraru, 2007). However, inactivation by PL cannot be simply predicted from inactivation by UVC. The UVC efficiency of the device used in this work was 12 %. In other words, 12 % of the nominal PL fluence emitted by a continuous UVC source would theoretically achieve the same reduction in spore numbers. Table 2 shows that, in our tests, this percentage was more likely comprised between 3 % and 10 % (5 % and 10 % for spores treated as suspensions in water), depending on the bacterial strain. One reason may be that physical measurements for UVC efficiency equally consider all wavelengths of the PL spectrum (200–280 nm), while wavelengths in this range have different efficacy (Chen et al., 2009).

The resistance of fungal conidia to PL was quite similar to the resistance of bacterial spores. In contrast, *A. brasiliensis* and *A. carbonarius* conidia showed a much lower sensitivity to UVC

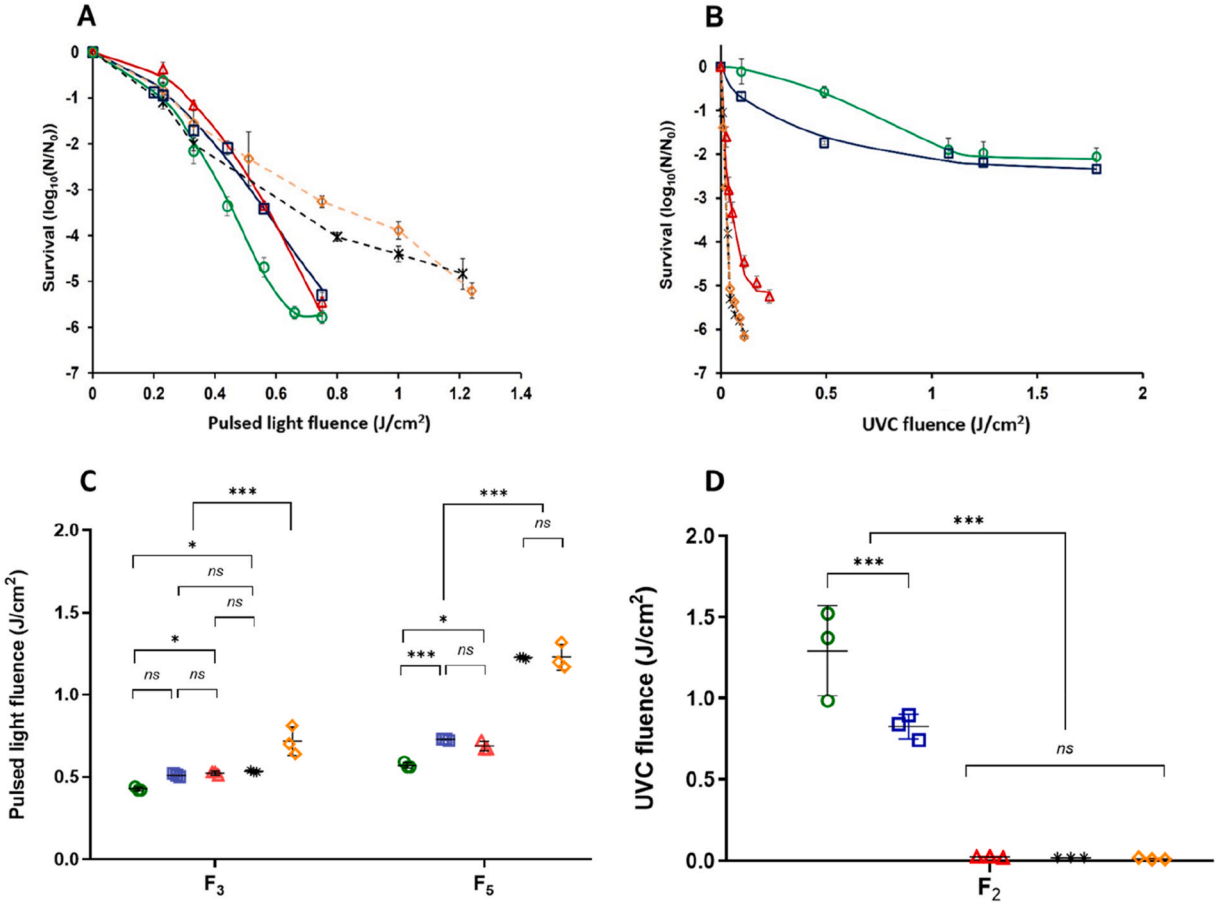

Fig. 3. Inactivation of *A. brasiliensis* DSM1988 (blue ■), *A. carbonarius* DSM872 (green ●) and *P. rubens* DSM848 (red ▲) conidia, *B. pumilus* DSM492 (black *) and *G. stearothermophilus* DSM5934 (orange ◊) spores sprayed on a polystyrene carrier and exposed to pulsed light (A, C) and UVC (B, D) (λ = 254 nm). Lines represent the best fit to the experimental data points with the model of Albert and Mafart (2005). Symbols represent the mean of three biological replicates and bars show standard deviation. Panel (C) shows the pulsed light fluence allowing 3 (F_3) and 5 (F_5) log-reduction and panel (D) the UVC fluence allowing 2 (F_2) log-reduction. Asterisks represent the significance of the difference of means (Tukey's multiple comparison test; *** p-value <0.001, * p-value <0.05, ns, non-significant at p-value >0.05). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

compared to the bacterial spores. Their ratio between PL and UVC resistance was 210 % and 380 %, respectively, and was much higher (between 3 % and 10 %) than for all other tested strains (Table 2). The high efficiency of PL compared to UVC against *Aspergillus* conidia could be attributed to a combination of factors. (i) The wide spectrum of PL includes UVB (λ = 280–320 nm) and UVA (λ = 320–400 nm) to which conidia are sensitive (Braga et al., 2015). Interestingly, García-Cela et al. (2015) demonstrated the deleterious effects of UVB and UVA on conidia from different *Aspergillus* spp. (ii) The irradiance of PL lamps is very high. Levy et al. (2012) reported a lower PL efficiency against *A. niger* conidia when fractioning the PL dose and thus lowering the irradiance of each flash, while high irradiance/short-time and low-irradiance/long-time treatments should give the same photochemical effect (Gómez-López and Bolton, 2016), as observed on *B. subtilis* spores (Rice and Ewell, 2001).

The inter-strains differences reported in our study likely rest on the presence and/or abundance of factors contributing to the resistance of microorganisms to UV. Thus, the resistance of microorganisms to PL and UVC damages is attributed to (i) genetic factors such as DNA repair systems, and (ii) structural factors such as the composition of the cell membrane and cell wall, and specialized pigments absorbing light radiations at wavelengths lethal to microorganisms (Kramer et al., 2017). The high resistance of conidia, and in particular of *Aspergillus* conidia, to UV is generally assigned to a high content in melanin that absorbs damaging UV photons (Braga et al., 2015). Esbelin et al. (2013) reported the major protecting role of melanin in *A. niger* conidia against UVC (λ = 254 nm) and PL. Anderson et al. (2000) showed that pigments of *A. niger* black conidia strongly absorbed in the region 240 nm–280 nm corresponding to UVC, unlike the pink pigments of *Fusarium culmorum* conidia that were also more sensitive. A thick cell wall, an accumulation of compatible solutes like polyols preventing oxidative damage and large amounts of secondary metabolites, including pigments, are other conidia cell attributes that could explain their resistance to UVC stress (Blachowicz et al., 2020; Braga et al., 2015; Dijksterhuis, 2019).

How representative of the overall diversity of resistance of microorganisms to PL is the present work? Studies reporting PL inactivation data in non-food environments, with properly measured fluence, are few in number. In addition, as previously underlined (Gómez-López and Bolton, 2016; Rowan, 2019), microbial inactivation by PL needs more harmonization to provide comparable research data. Gómez-López et al. (2022) recently published guidelines on appropriate reporting of treatment conditions for emerging technologies including PL. However, a selection of 16 studies carried out in conditions similar to the ones implemented in our work allowed a global comparison. Fig. 4 gives an overview of the relative resistance to UVC and PL of *Bacillus* spp. and *Aspergillus* spp. (different species/strains tested as suspensions or on surfaces). The reference strains used here were *B. pumilus* DSM492 and *A. brasiliensis* DSM1988 for *Bacillus* spp. and *Aspergillus* spp., respectively. The relative resistance of *Bacillus* spp. shows a wide variability ranging from 0.3 to 5 for UVC and from 0.4 to 3.6 for PL. This variability

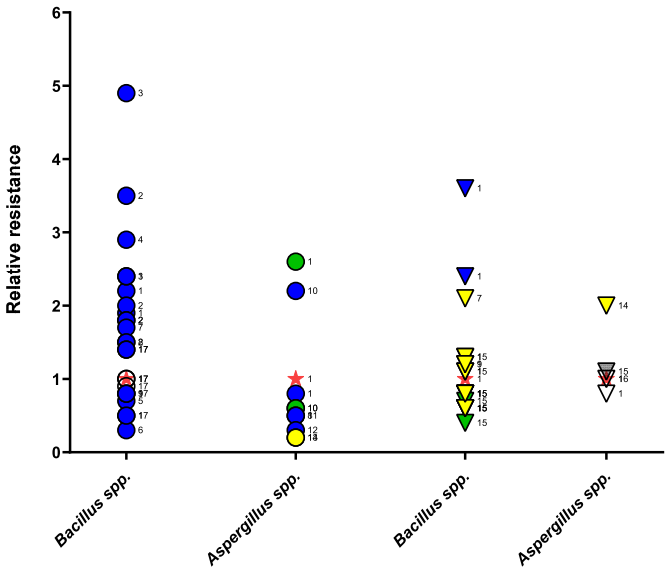

Fig. 4. Relative resistance to UVC (λ = 254 nm) (○) and PL (▽) of *Bacillus* sp. spores compared to spores of *B. pumilus* DSM492 (this work, labeled with ★) and of conidia of *Aspergillus* sp. compared to *A. brasiliensis* DSM1988 (this work, labeled with ★). Spores treated as suspensions in water are labeled in blue (●, ▼). Spores spread on agar are labeled in yellow (●, ▼). Spores spread on a dry surface are labeled in green (●, ▼). Spores treated in conditions not communicated by the authors are labeled in white (○, ▽). Numbers near symbols indicate the reference: (1) this work; (2) (Link et al., 2004); (3) (Boczek et al., 2016); (4) (Beck et al., 2015); (5) (Zhang et al., 2014); (6) (Sholtes et al., 2016); (7) (Abbas et al., 2014); (8) (Clauß, 2006); (9) (Planchon et al., 2011); (10) (Begum et al., 2009); (11) (Taylor-Edmonds et al., 2015); (12) (Narita et al., 2020); (13) (Cortesão et al., 2020); (14) (Esbelin et al., 2013); (15) (Levy et al., 2012); (16) Claranor (unpublished); (17) (Zhao et al., 2023). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

is higher, but consistent with what was observed in the present work (See Supplementary Table 2) and could be attributed to tests conducted with different strains, variations in microbiological test conditions and/or diverse PL sources. With fewer data also, the relative resistance for *Aspergillus* spp. conidia was less variable compared to *Bacillus* spp. spores, mostly ranging from 0.2 to 2.5 for UVC and from 0.8 to 2 for PL.

In conclusion, PL offers per UVC fluence basis a similar capacity as UVC in inactivating spore-forming bacteria. Nonetheless, exposure times to radiations required to achieve a given reduction will be tremendously shorter with PL than with continuous UVC. This allows for faster inactivation in industry, depending on equipment and treatment conditions (such as fluence and number of pulses). PL may be much more efficient than UVC for inactivation of fungal conidia such as *Aspergillus* sp. conidia. In general terms, filamentous fungi forming conidia and spore-forming bacteria are among the most difficult microorganisms to inactivate with UVC. The relative resistance of spore-forming bacteria to PL could be reasonably predicted from the knowledge of their UVC resistance. In contrast, the sensitivity of fungal conidia to PL must be specifically explored, because extrapolation from UVC inactivation data may result in overtreatment by PL. In addition, evaluation of the sensitivity of fungal conidia to PL is very dependent on the peak power of the sources (Levy et al., 2012) and the conditions of treatment (conidia suspended in water were nearly not affected by UVC or PL). This strengthens the need for standardization of methods for evaluating the resistance to PL of microorganisms prone to contaminate food-processing chains.

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CRediT authorship contribution statement

Imed Dorbani: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Alain Berberian:** Resources, Project administration, Methodology, Conceptualization. **Christophe Riedel:** Writing – review & editing, Resources, Project administration, Funding acquisition, Conceptualization. **Catherine Duport:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Frédéric Carlin:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

None.

Data availability

Supplementary data Table 1 and 2 have been versed in an institutional repository and can be viewed at https://entrepot.recherche.data.gouv.fr/dataset.xhtml?persistentId=doi:10.57745/0REQVX

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2024.104518.

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