International Journal of Food Microbiology 378 (2022) 109824

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

UV tolerance of *Lactococcus lactis* 936-type phages: Impact of wavelength, matrix, and pH

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ARTICLE INFO

Keywords:
UVA, UVB, UVC inactivation
LEDs
Dairy bacteriophages
Non-thermal treatment
Light-shielding
Tailing

ABSTRACT

Ultraviolet C (UVC) radiation is a widely used technology for the disinfection of surfaces, air flows, water and other liquids. Although extensive research has been conducted on the UV tolerance of bacteriophages used as surrogates for waterborne viruses, limited information is available on phages relevant to food processing. Phages of dairy starters may reach high numbers in dairy facilities and cause fermentation failure with great economic losses for the dairy industry. Here, the UV tolerance of virulent phages, belonging to the 936-group (*Skunavirus*) of *Lactococcus lactis* subsp. *diacetylactis* F7/2, was assessed, employing both host infectivity loss and qPCR assays. A highly heat-tolerant phage (P680) and a less heat-tolerant phage (P008) were exposed to UV radiation at 265 nm (UVC), 285 nm (UVB) and 365 nm (UVA), respectively, in an aqueous suspension, using UV Light-Emitting-Diodes (LEDs) in a static set-up. UVC at 265 nm achieved the highest total inactivation, leading to a 4 $\log_{10}$ reduction of the phage titer at a UV dose of 327 and 164 mJ/cm^2 for P680 and P008, respectively. UVB at 285 nm achieved similar inactivation levels, while UVA at 365 nm did not cause major reductions. Phages were also suspended in yoghurt serum of pH 5.5 and pH 7.0 and exposed to UVC radiation at 265 nm. The heat-tolerant phage P680 was more UV tolerant for all wavelengths, matrices and pH values tested. A higher aggregation degree together with less DNA damage was observed for both phages at pH 5.5, especially for phage P680, indicating a UV light-shielding effect. Interestingly, there were indications of some phage survivors exhibiting higher UV tolerance on re-exposure, pointing out a need for further investigation. Our results show that UV LEDs emitting at 265 nm and 285 nm are efficient in reducing the phage population significantly, but also underline that 936-type phages are relatively UV resistant. A further understanding of the main factors influencing UV efficiency could enable future use of the UV technology as an alternative or complement to thermal treatment for phage inactivation.

1. Introduction

Lactococcus lactis (*L. lactis*) is one of the most important species of lactic acid bacteria (LAB), used in starter cultures and a major contributor to milk fermentation in the production of several cheese varieties. The majority of *L. lactis* phages belong to the 936-group (genus *Skunavirus*, family *Siphoviridae*, order *Caudovirales*), a group of virulent phages that are responsible for fermentation failure with significant financial losses for the dairy industry. The phages of the 936-group are double-stranded (ds) DNA phages with a long, non-contractile tail and an isometric capsid (Geagea et al., 2018). The persistence of this group in the dairy environment is attributed to their high heat tolerance, their ability to become airborne and spread fast within the dairy facilities, and the sensitivity of the starter cultures (Madera et al., 2004). Raw milk is the main source of phage contamination, containing up to 10^4 plaque-forming units (PFU)/mL (McIntyre et al., 1991) and active phages can

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https://doi.org/10.1016/j.ijfoodmicro.2022.109824
Received 19 March 2022; Received in revised form 17 June 2022; Accepted 26 June 2022
Available online 1 July 2022

be recovered after low temperature/long time (63 °C/30 min) or high temperature/short time (72 °C/15 s) pasteurization (Atamer et al., 2009, 2013; Atamer and Hinrichs, 2010; Mahony et al., 2012).

Up-concentration of whey cream and whey protein, to be added in cheese production to increase yield and improve organoleptic characteristics, can lead to a phage concentration up to 10^{10} PFU/mL in the whey concentrate (Atamer et al., 2013; Atamer and Hinrichs, 2010). The pore size of ultrafiltration membranes, used for the up-concentration of whey products (0.01–0.1 μm) retains most of the phage particles (length: ca. 0.2 μm, capsid diameter: ca. 0.05 μm) from the whey solution (Atamer, 2022; Atamer et al., 2013; Samtlebe et al., 2015). Such high phage population levels are not easily eliminated, and whey protein, casein and salts may further protect the phages against heat treatment (Atamer et al., 2010; Müller-Merbach et al., 2005). Cheese brines, which can be continuously reused for years for the immersion of cheese blocks, also allow for the long-term accumulation of *L. lactis* phages since the phages can pass through the microfiltration membranes (0.1–10 μm) used for periodical brine reconditioning. The high salt content (approx. 18 % (w/v)) does not let the bacteria, and thereby the phages, proliferate, but they can survive for long periods and proliferate when salinity is reduced (Neve et al., 2005). Finally, whey water permeate may, after varying degrees of filtration, be used for different purposes to save water and could potentially be another source of phage contamination. Although some water treatments such as ultrafiltration retain most of the phages and reverse osmosis has very small pores (pore size <0.001 μm), bacteria and even yeast have occasionally been found to pass to the permeate side (Stoica et al., 2018; Vitzilaiou et al., 2019).

The International Dairy Federation suggests a treatment of 90 °C/15 min to achieve complete phage inactivation (Mahony et al., 2012). However, high heat/long time treatments can deteriorate the functional properties of dairy products and negatively affect the texture, smell and taste (Chawla et al., 2021; Guglielmotti et al., 2012; Michel et al., 2021). Moreover, *L. lactis* phages have been found to (i) survive even skim milk spray drying (190 °C) with $\leq$1.80 $\log_{10}$ phage titer reduction and to (ii) remain stable during skim milk powder storage for months and whey powder storage for years (Chopin, 1980; Wagner et al., 2017).

Ultraviolet C (UVC) radiation (200–280 nm) has a high germicidal efficiency against a wide spectrum of microorganisms (Kebbi et al., 2020; Li et al., 2019). Especially the 265 nm wavelength targets directly the peak of DNA/RNA absorption. The microorganisms experience UV-induced formation of pyrimidine dimers in the DNA (mainly thymine-thymine dimers) which will hinder further replication. UVC has been found to have an equivalent effect to thermal pasteurization for microbial inactivation, with no or little effect on the quality characteristics of dairy products (Buhler et al., 2019; Chawla et al., 2021). Therefore, UVC treatment may be an efficient non-thermal alternative or complement to heat inactivation. UVB (280–315 nm) radiation inactivates cells in the same way as UVC, but also by damaging proteins/enzymes as it is close to the protein peak absorption (approx. 280 nm), while UVA (315–400 nm), applied in high doses, inactivates cells by the generation of reactive oxygen species (ROS) which damage cell membrane lipids and proteins, enzymes and DNA (Kebbi et al., 2020; Li et al., 2019).

Due to the wide industrial application of UV technologies for the microbial disinfection of drinking water, several studies have investigated the UV efficiency towards waterborne viruses such as Adenovirus (HAdV2) and bacteriophages (MS2, Q_β, ΦX174), acting as surrogate models for other waterborne pathogens, using low-pressure mercury lamps (LPML) at 254 nm or UV Light-Emitting-Diodes (LEDs) at different wavelengths (Beck et al., 2017; Bowker et al., 2011; Kim et al., 2017; Oguma, 2018; Rattanakul and Oguma, 2018; Rodriguez et al., 2014; Yoshinobu et al., 2011). However, to our knowledge, there are few studies investigating the UV tolerance of LAB phages, using LPML (Atamer, 2022; Greene and Babel, 1948; Michel et al., 2021) and no studies that employ UV LEDs. Viruses and phages used as surrogates are relatively tolerant towards UV treatment compared with pathogenic bacteria such as *Listeria monocytogenes*, *Legionella pneumophila*, *Pseudomonas aeruginosa*, *E. coli* O157:H7, *Salmonella enterica*, and even *Bacillus subtilis* spores (Beck et al., 2017; Green et al., 2018; Rattanakul and Oguma, 2018). Several reasons for this high UV tolerance have been suggested including a small genome organization (Kim et al., 2017; Rodriguez et al., 2014), light-shielding due to phage aggregation (Nieuwstad and Havelaar, 1994), genome recombination within the host (Luria and Dulbecco, 1949; Mattle and Kohn, 2012) and the presence of more UV resistant subpopulations (Cutler et al., 2011).

In this study, we aimed to investigate the UV tolerance of the heat-tolerant phage P680 and the less heat-tolerant phage P008 in a UV LED collimated beam set-up, employing UV LEDs at 265 nm (UVC), 285 nm (UVB) and 365 nm (UVA). To compare the UV tolerance of these two lactococcal phages, UV exposure was conducted in aqueous suspension and in filtered yoghurt serum. Moreover, pH 5.5 and 7.0, were employed, to induce different aggregation levels of the phage particles as assessed by epifluorescence microscopy. Phage inactivation was determined by combining two methods, a double-agar layer plaque assay for host infectivity loss and quantitative PCR (qPCR) for DNA damage, designing for the first time primers that target directly regions with several consecutive thymine bases in the phage DNA.

2. Materials and methods

2.1. Bacteriophages and host strain

Phages P680 and P008 and the host *L. lactis* subsp. *diacetylactis* F7/2 were supplied from the phage and strain collection of Max Rubner-Institute (Federal Research Institute of Nutrition and Food, Department of Microbiology and Biotechnology, Kiel, Germany). They belong to the 936-group (*Skunavirus*) of virulent lactococcal phages, the most frequently isolated phages from failed cheese fermentations (Geagea et al., 2018). Phage P680, originally isolated from a Quarg cheese sample, is a highly heat-tolerant phage, requiring 100 °C/20 min for a 9-$\log_{10}$ reduction (Atamer et al., 2009). In contrast, phage P008, widespread in German dairies, is a less heat-tolerant phage, requiring 70 °C/20 min for a 9 $\log_{10}$ reduction (Atamer et al., 2013; Atamer and Hinrichs, 2010; Müller-Merbach et al., 2005). Phage P008 will henceforth be referred to as heat-sensitive.

2.2. Phage propagation

To prepare the high concentration stock solutions of phages P680 and P008, an overnight culture of *L. lactis* subsp. *diacetylactis* F7/2, grown at 30 °C, was made by inoculating host-colony material in tubes with M17 broth (M1029, HIMEDIA, Germany) containing 0.5 % glucose (CAS 14431437, Millipore, Germany). One hundred μL from the overnight culture was added into each of two tubes having 10 mL M17 broth containing 5 mM $CaCl_2$ (C3881, Sigma-Aldrich, Denmark), 2 mM $MgCl_2$ (CAS 7791186, Millipore, Germany) and 0.5 % glucose. After incubation for 3 h at 30 °C, 10 μL of phage P680 or P008 (from fridge-stock phage collection) were added into one of the tubes with the host-broth culture, and both tubes were incubated for an additional 2 h at 30 °C. Thereafter, half of the host-broth culture from the second tube was added to the first tube with the phage-host culture and both tubes were incubated for 2 h more at 30 °C. Finally, the residual host-broth culture from the second tube was poured into the first tube with the phage-host solution and incubated overnight at 30 °C. The phage-host suspension was then centrifuged at 4000*g* for 10 min (Eppendorf Centrifuge 5920R, Denmark) and the supernatant was filtered through 0.45 μm pore filters (Q-Max^R Syringe Filters, Frisenette, Denmark), collecting approx. 20 mL of phage stock solution, stored in the fridge (4 °C). The phage concentration of the final P680 and P008 phage stock solutions was determined by double-agar layer plaque assay.

2.3. Inocula preparation for UV exposure in SM buffer at 265, 285, 365 nm

At first, the UV exposure of phages P680 and P008 was conducted at 265 nm, 285 nm and 365 nm in SM buffer [0.1 M NaCl, 10 mM $MgSO_4{\cdot}H_2O$ (CAS 14168731, Sigma-Aldrich, Denmark) and 50 mM Trizma·HCl, pH 7.5 (CAS 1185531, Sigma-Aldrich, Denmark)]. The phage inocula were prepared right before each UV exposure trial by diluting the high concentration phage stock solutions in SM buffer to a final concentration of approx. 8 $\log_{10}$ (PFU/mL) and mixing by shaking. The final inocula concentration was determined by double-agar layer plaque assay [P680: 7.6 $\log_{10}$ (PFU/mL), P008: 8.3 $\log_{10}$ (PFU/mL)].

2.4. Inocula preparation for UV exposure in yoghurt serum at 265 nm

The phage suspensions were subsequently prepared in yoghurt serum and exposed to 265 nm. Yoghurt with 0.1 % fat content was purchased (Coop 365 Økologi, Yoghurt Naturel, Thise, Denmark), centrifuged at 4200*g* for 30 min and the supernatant, i.e., the yoghurt serum, was filtered through a 0.45 μm filter (Jet Biofil, China), using a vacuum pump. The filtrate (pH 4.5 ± 0.1) was collected in two different bottles and subsequently diluted by a factor of 10 in each bottle, using autoclaved MilliQ water, to increase the %UV transmittance (%UVT) of the yoghurt serum matrix and facilitate the UV dose calculation. The pH value was set at pH 5.5 ± 0.1 in one bottle and pH 7.0 ± 0.1 in the other, using 1 M NaOH solution (CAS 1310732, Avantor, Denmark) and filtered again through a 0.22 μm filter (Jet Biofil, China), using a vacuum pump. To prepare the phage inocula, the high concentration phage stock solutions were diluted accordingly in the yoghurt serum of each pH at a final concentration of approx. 8 $\log_{10}$ (PFU/mL), right before each UV exposure trial, and mixed by shaking. The final inocula concentration was determined by double-agar layer plaque assay [P680: 8.0 $\log_{10}$ (PFU/mL), P008: 8.5 $\log_{10}$ (PFU/mL)].

2.5. UV exposure

For the UV exposure experiments, the PearlLab Beam™ (AquiSense Technologies, Erlganger, KY, USA) was used (Fig. 1), a compact UV LED collimated beam device with three wavelengths at 265, 285 and 365 nm, respectively. UV LEDs are narrowband polychromatic emission lamp sources. Therefore, the full width at half-maximum bandwidth (FWHM) for the UV LEDs at 265 nm was 13 nm with a peak at 268 nm, 12 nm with a peak at 284 nm for the UV LEDs at 285 nm and 10 nm with a peak at 369 nm for the UV LEDs at 365 nm (Supplementary data/Fig. S1). The experimental protocol was structured with the technical support of the UV LED manufacturer to calculate the Average Germicidal Fluence Rate (E_{avg}, mW/cm^2) for each wavelength by the following equation:

$$E_{avg} = E_0 \times PF \times WF \times SF \times RF \times DF,$$

where E_0 is the Incident Irradiance or Incident Fluence Rate (mW/cm^2), measured with an ILT2400 radiometer connected to a SED005 sensor (International Light Technologies, Peabody, MA, USA). PF is the Petri Factor, calculated with the ILT2400 radiometer/SED005 sensor for each wavelength, as the average of the ratios of the irradiance every 5 mm across the Cartesian grid around the central position and the irradiance at the central position. WF is the Water Factor, calculated by measuring the depth of the microbial suspension in the petri dish (l = 10 mm) and the %UVT of the microbial suspension for a path length of l = 10 mm over the narrowband wavelength emission of each UV LED peak, to account for the polychromatic emission. This procedure was conducted using Quartz Suprasil® cuvettes (dimensions: 48 mm × 12.5 mm × 12.5 mm, 100-QS, Hellma® Analytics, Germany) with a 10 mm path length and the UV–Vis 1800 Spectrophotometer (Shimadzu Corporation, Japan). From %UVT of the microbial suspension, the absorbance (a) is calculated as the negative natural logarithm of %UVT. The WF is finally calculated using the equation: WF = (1–10^{-al}) / (al * ln (10)). RF is the Reflection Factor, accounting for the beam reflection when the light passes between air and water. DF is the Divergence Factor, accounting for the UV beam's divergence from collimation. The sensor, connected to a radiometer, was measuring the Incident Irradiance of each UV LED. The SF is the Sensor Factor, introduced in the Average Fluence Rate equation to correct for the variation of the sensor sensitivity across the narrowband polychromatic wavelength emission of the UV LEDs. To calculate the SF, the radiant power over the sensor spectral range was calculated, relative to the peak wavelength of each UV LED, and weighted by the sensor calibration values, as recorded by the manufacturer company. These values can be found in the Supplementary data (Table S1).

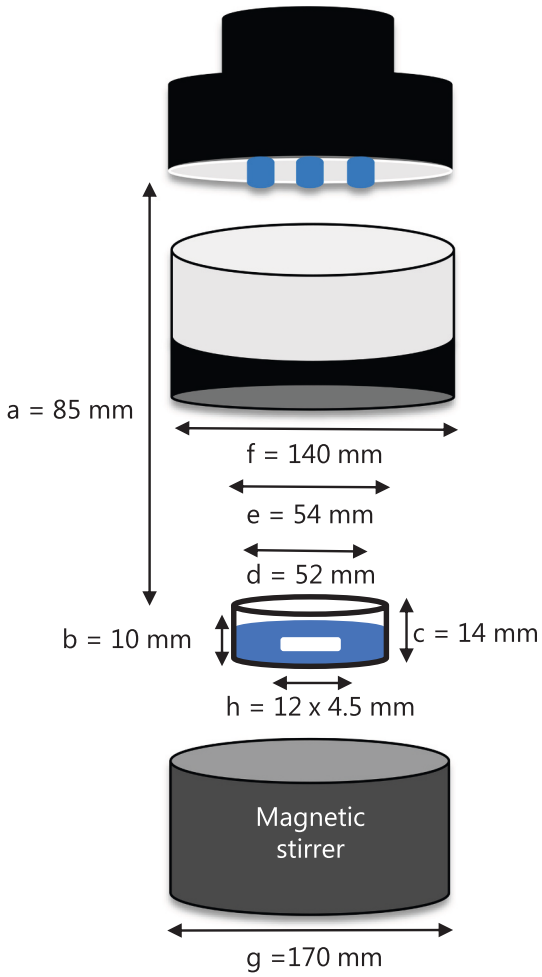

Fig. 1. UV LED PearlLab Beam set-up with dimensions. a) Distance of the UV LEDs from the solution surface. b) Height of the solution in the petri dish. c) Petri dish height. d) Petri dish inner diameter. e) Petri dish outer diameter. f) PearlLab Beam stand diameter. g) Magnetic stirrer diameter. h) Magnet dimensions.

The UV exposure time was selected as the experimental variable for investigating the UV tolerance of the phages at different wavelengths, different matrices, and pH. For all UV experiments at three different wavelengths in SM buffer (pH 7.5) and at 265 nm in yoghurt serum (pH 5.5 and pH 7.0), the phages were exposed to UV radiation for 1, 2, 4, 8, and 16 min, generating different UV doses (Supplementary data/Table S1). To obtain the absorbed UV fluence or UV dose (mJ/cm^2) after each exposure time, the Average Germicidal Fluence rate, E_{avg} (mW/cm^2) was multiplied by the exposure time (s) (Bolton and Linden, 2003):

$$\text{UV dose } \left(\text{mJ/cm}^2\right) = \text{E}_{\text{avg}} \left(\text{mW/cm}^2\right) \times \text{t (s)}$$

2.6. UV exposure experimental set-up

For the UV exposure trials in SM buffer and at three wavelengths, three individual experiments (trials) were conducted in technical duplicates for each phage (n = 6 replicates) (Fig. 2/Part A). For the UV exposure in yoghurt serum (pH 5.5 and pH 7.0) at 265 nm, three individual experiments (trials) were conducted in technical triplicates for each phage (n = 9 replicates) (Fig. 2/Part B). For each trial, a stock inoculum in larger volume was prepared and for each UV exposure time (1, 2, 4, 8 and 16 min), 20 mL of the inoculum were poured into a petri dish (inner diameter: 52 mm, height: 14 mm, Frisenette, Denmark) with a magnetic stir bar (dimensions: 12 × 4.5 mm, Frisenette, Denmark). The height of the solution with the magnetic bar should be equal to the radiometer's sensor height (here 10 mm). The petri dish was placed on a magnetic stirrer (diameter: 170 mm, Hounisen, Denmark) and the UV LED stand was placed on the top. The shutter and UV main switch were turned on simultaneously and each sample was exposed to UV for the desired time under continuous stirring (200 rpm). Two control samples were also prepared for the shortest and longest UV exposure time, 1 and 16 min, respectively, and were handled in the same way as the UV-exposed samples, but with the shutter and UV main switch turned off. Exposure times of control and UV exposed samples were randomized for each trial and phage. After each UV exposure time, the samples (UV-exposed and control) were collected, and infectivity was assessed by double-agar layer plaque assay. For the UV exposure in yoghurt serum (pH 5.5 and pH 7.0) at 265 nm, the phage infectivity and DNA damage were assessed using a double-agar layer plaque assay and qPCR, respectively.

2.7. Infectivity: double-agar layer plaque assay

To enumerate the phages, the UV-exposed or control samples from each UV exposure time were serially diluted in Eppendorf tubes, containing SM buffer. One hundred μL from the chosen serial dilutions were added to 5 mL M17 soft agarose tubes (M17 broth with 0.4 % agarose, 5 mM $CaCl_2$ and 2 mM $MgCl_2$) together with 100 μL of overnight M17 broth host-culture and vortexed shortly. Thereafter, the M17 soft agarose was poured on M17 agar plates (M17 broth with 1.5 % agar, 0.5 % glucose, 5 mM $CaCl_2$ and 2 mM $MgCl_2$). The plates were left to dry and thereafter incubated at 30 °C for 24 h. Plates showing 30–300 plaques were counted in PFU/mL units. The control samples were used to calculate the $\log_{10}$ reduction or $\log_{10}$ (N_0/N) after each UV exposure time, where N_0 is the PFU/mL of the control samples and N is the PFU/mL after *t* min of UV exposure. The $\log_{10}$ (N_0/N) was plotted versus the exposure time (min) and versus the UV dose (mJ/cm^2).

2.8. DNA damage: qPCR

qPCR was also conducted after UV exposure of phages P680 and P008 in yoghurt serum at both pH 5.5 and pH 7.0 to assess the DNA damage and compare it with concomitant infectivity loss. The software CLC Genomics Workbench 20.0.4 was used to align the whole genome sequences of phages P680 (dsDNA, 29,631 bp, Acc. # NC_021852) and P008 (dsDNA, 28,538 bp, Acc. # DQ054536) and to manually detect DNA regions with several consecutive T (thymine bases). The regions with consecutive thymines are those mostly affected by UVC radiation, creating thymine dimers and rendering the DNA unable to be replicated by DNA polymerase. As a result, a clear quantitative decrease in the qPCR fluorescence signal will be detected in the UV-treated samples compared to the controls. Primer sets that target specifically these regions were designed. The designed primer sets were purchased from IDT (Integrated DNA Technologies, Belgium). After extensive optimization assays (data not included), the forward primer (FWD): 5′-GCGTGCTTAACTGGATGA-3′ (nt. 1100-1117 (P680), nt. 1116-1133 (P008), respectively) and the reverse primer (REV): 5′-TGAACTGT-TACCACCTTG-3′ (nt. 1350-1333 (P680), nt. 1366-1349 (P008), respectively) targeting the large terminase gene, *terL*, were chosen, producing a product of 251 bp length for each phage (Supplementary data/Table S2). DNA was extracted from high concentration phage stock solutions (Section 2.2), using the Genomic Mini AX Phage Kit for DNA purification from bacteriophages (A&A Biotechnology, Poland). The DNA concentration was determined by Qubit™ 4 fluorometer (Invitrogen, ThermoFisher Scientific, USA) and the number of DNA copies was calculated according to the following equation:

$$\text{Number of DNA copies} = \frac{\text{Amount (ng)} \times 6.022 \times 10^{23}\ \text{(molecules/mole)}}{\text{Length (bp)} \times 1 \times 10^{9}\ \text{(ng/g)} \times 660\ \text{(g/mole)}},$$

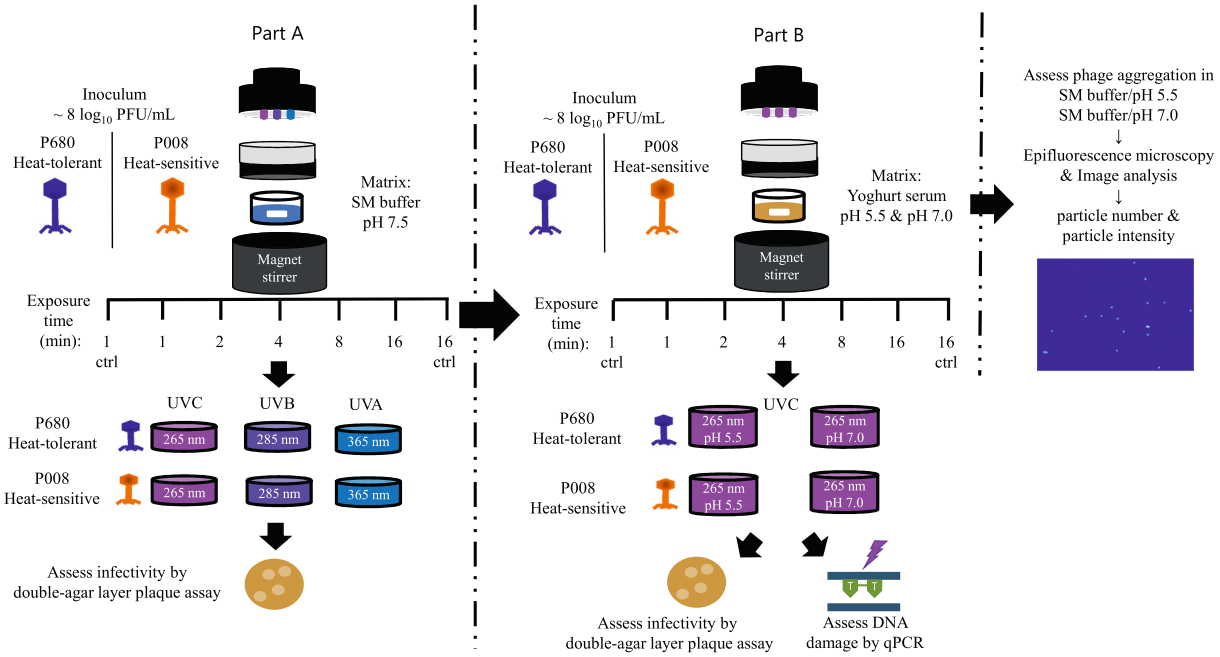

Fig. 2. Experimental design for UV exposures in SM buffer/pH 7.5 at 265, 285 and 365 nm (Part A) and in yoghurt serum/pH 5.5 and pH 7.0 at 265 nm (Part B).

where Amount (ng) = the amount of the DNA in the sample, 6.022×10^{23} molecules/mol = Avogadro's constant, Length (bp) = the length of the DNA, 1×10^9 (ng/g) = converting factor, 660 g/mol = average mass of 1 bp of dsDNA. The samples with the known number of DNA copies were stored in the fridge (4 °C). For each qPCR reaction, the DNA stock samples were serially diluted by a factor of 10 and added to 96-well microtiter plates to create the standard curve, from which the DNA concentration of control and UV-exposed samples could be determined.

To determine the DNA copies after each UV exposure for each phage, pH, trial, and UV exposure time, 200 μL from the UV-exposed and control phage samples were collected and DNA was extracted using the A&A Genomic Mini AX Phage Kit. For each qPCR reaction, a 15 μL MasterMix was prepared, containing 1 μL from each primer (10 μM concentration), 3 μL DNA-free water and 10 μL SYBR green stain (RealQ Plus 2× Master Mix Green Low ROX, AMPLIQON, Denmark). To this mix, 5 μL of DNA template was added, originating either from the DNA phage stock samples for standard curves or the UV-exposed phage DNA samples or the control DNA samples. For each scenario, negative control samples were also included with DNA-free water instead of a DNA template. For the qPCR reactions, the CFX96 Optics Module (Bio-Rad, Denmark) was used. For the data acquisition and analysis, the software CFX Maestro (Bio-Rad, Denmark) was used. Each qPCR assay was performed in biological duplicates and triplicate wells were made for each scenario (n = 6 replicates). The qPCR program included heating at 50 °C/2 min and 95 °C/10 min, followed by 40 cycles of DNA denaturation at 95 °C/15 s, primer annealing at 53 °C/20 s and DNA polymerase extension at 60 °C/40 s. At the end of each cycle, the fluorescence signal was read. To check for primer-dimers and reaction specificity, a melting curve was also conducted by heating the DNA at 95 °C and then decreasing gradually the temperature to 60 °C. For each qPCR assay, the number of DNA copies per sample was calculated using the software-generated standard curve equation and the threshold cycle (Ct) value from the corresponding wells. The $\log_{10}$ reduction in DNA copies after each UV exposure time was calculated as $\log_{10}$ (D_0/D), where D_0 is the number of DNA copies/μL of the control samples and D is the number of DNA copies/μL after *t* minutes of UV exposure. The $\log_{10}$ (D_0/D) was then plotted versus the exposure time (min) and versus the UV dose (mJ/cm^2).

2.9. Epifluorescence microscopy and particle intensity analysis

Epifluorescence microscopy was conducted for both P680 and P008 phages to investigate whether there were any differences in particle intensity, indicating the presence of phage aggregates, at the two pH values of pH 5.5 and pH 7.0, respectively (Fig. 2). For this purpose, the protocol of Noble and Fuhrman (1998) was used. First, high concentration phage stock solutions were prepared as described in Section 2.2 to a final concentration of 10 $\log_{10}$ (PFU/mL). One μL of the high concentration stocks was diluted into 2.5 mL of SM buffer (pH 5.5 and pH 7.0). The resulting solutions were filtered through 0.02 μm Al_2O_3 anodisc filters (Whatman: 6809-6002, Merck, Denmark) to fix the phage particles on the filter surface to facilitate microscopic observation. All incubation and drying steps were done in the dark at room temperature. The anodiscs were dried for 10–15 min and placed on 100 μL-droplets of 400× diluted SYBR Gold 10,000× Nucleic Acid Stain (S11494, ThermoFisher Scientific, Denmark) for staining for 30 min and then they were dried for 10–15 min. Each anodisc was then mounted on a glass microscopic slide with 15 μL of anti-fading/mounting solution [50 % glycerol and 50 % Phosphate Buffer Solution (0.05 M Na_2HPO_4 and 0.85 % (w/v) NaCl, pH 7.5)]. A glass coverslip was mounted above, by adding 15 μL of anti-fading/mounting solution, and the microscope slide was placed on the inverted microscope (Carl Zeiss, Denmark) and observed with immersion oil under a 100× objective lens (1000× final magnification) and fluorescent light at 490 nm. Ten images were captured for each scenario (P680/pH 5.5, P680/pH 7.0, P008/pH 5.5, P008/pH 7.0), using the software RSImage version 1.9.2 (n = 10 replicates). Image J (https://imagej.nih.gov/ij/), an image processing software [National Institutes of Health (NIH) & Laboratory for Optical and Computational Instrumentation (LOCI), University of Wisconsin, USA], was used to analyze the captured images and measure the number and the intensity (mean gray value) of the particles per image, by the following functions: the images were transformed into RGB Colour, the colour threshold was adjusted to RGB colour space, and the green channel threshold was adjusted accordingly to remove noise. Finally, the function analyze particles was used, applying a particle size threshold set at ≥10 pixel^2. The particles were shown as ellipses and the mean gray value for each particle was recorded.

2.10. Statistical analysis

Two-way Analysis of Variance (ANOVA) and Tukey's Honest Significant Difference (HSD) post hoc test with a 95 % confidence interval were conducted to assess the differences in the dependent variables $\log_{10}$ (N_0/N) or $\log_{10}$ (D_0/D) for the independent variables of UV exposure time (0, 1, 2, 4, 8, 16 min), wavelength (265 nm, 285 nm and 365 nm), matrix (SM buffer, yoghurt serum), pH (pH 5.5 and pH 7.0) and phage (P680 and P008). For the epifluorescence microscopy, Welch Two Sample *t*-test with 95 % confidence interval was conducted to compare the differences in the intensity between the different pH for each phage and between the phages for each pH. R studio software (Version 4.1.1 (2021-08-10)) was used for data handling, plotting and analysis.

3. Results

3.1. Infectivity loss after UV exposure in SM buffer at 265, 285, and 365 nm

The initial P680 population of 7.6 $\log_{10}$ (PFU/mL) was significantly decreased after 1 min of UV exposure in SM buffer by 2.0 ± 0.3 $\log_{10}$ at 265 nm (UV dose: 20 mJ/cm^2) and by 2.3 ± 0.2 $\log_{10}$ at 285 nm (UV dose: 36 mJ/cm^2) ($P < 0.05$) (Fig. 3A, B). However, a reduction in the inactivation rate (tailing effect) was observed for the longest exposure times. For 265 nm, a 4-times (UV dose: 82 mJ/cm^2) and 16-times (UV dose: 327 mJ/cm^2) higher UV exposure time led to a significant phage titer decrease of 3.1 ± 0.8 $\log_{10}$ and 4.1 ± 0.7 $\log_{10}$, respectively ($P < 0.05$). For 285 nm, the tailing effect was more pronounced since an additional significant reduction to 3.6 ± 0.4 $\log_{10}$ was achieved only after exposure at 16 min (UV dose: 578 mJ/cm^2) ($P < 0.05$). For the initial P008 population of 8.3 $\log_{10}$ (PFU/mL), high inactivation was observed at both 265 nm and 285 nm. UV exposure at 265 nm for 2 min (UV dose: 41 mJ/cm^2) led to a 1.9 ± 0.2 $\log_{10}$ reduction and to sequential 1 $\log_{10}$ reductions after 4, 8 and 16 min (UV doses: 82, 164 and 327 mJ/cm^2, respectively) ($P < 0.05$). At 285 nm, the inactivation rate was similar to 265 nm apart from the last 16 min-exposure (UV dose: 578 mJ/cm^2), where it was dramatically decreased ($P > 0.05$). UV exposure at 365 nm had little effect on both P680 and P008 phages leading to a total 0.4 ± 0.2 $\log_{10}$ reduction for P680 and 0.7 ± 0.2 $\log_{10}$ reduction for P008, although the generated UV doses were considerably higher [Fig. 3A, C (as insert in 3B)] ($P > 0.05$) (Table 1).

When the UV tolerance at 265 nm between the two phages is compared, P008 was more UV tolerant up to 2 min of UV exposure compared to P680, but P680 became more resistant at the longer exposure times, resulting in a 4.1 ± 0.7 $\log_{10}$ titer reduction after 16 min of UV exposure versus 5.0 ± 0.4 $\log_{10}$ reduction for P008. The same trend was observed for 285 nm. The average UV doses that correspond to each exposure time for the experiments conducted are included in Table 1. Additional information can be found in Supplementary data (Table S1).

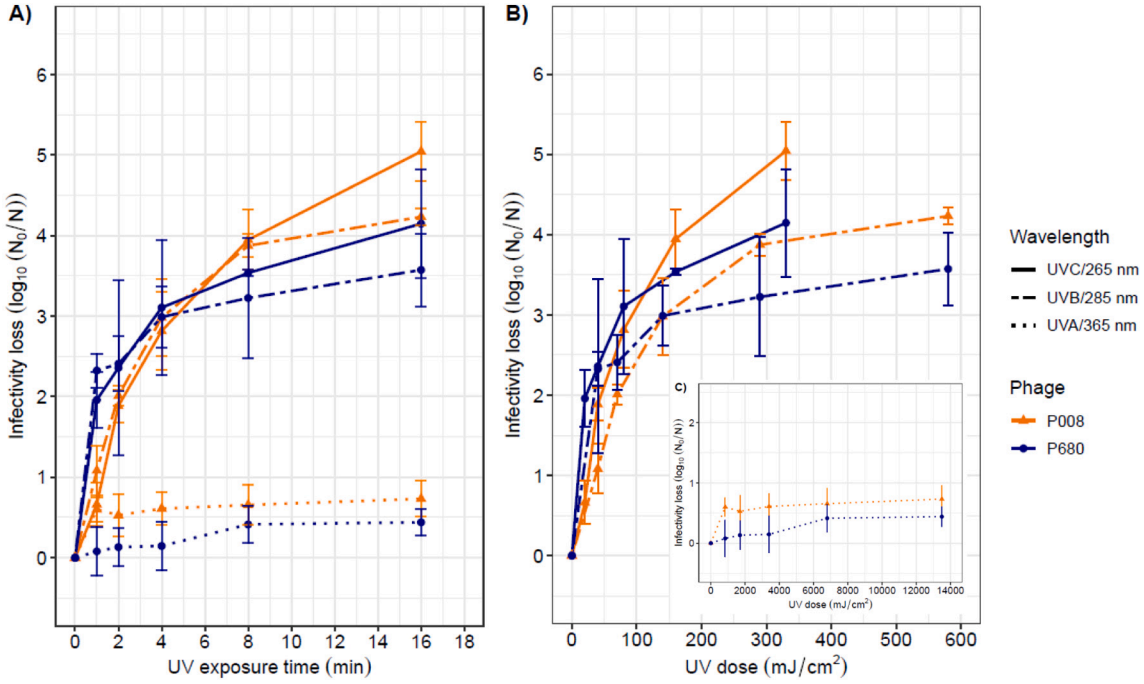

Fig. 3. UV inactivation in SM buffer/pH 7.5 at UVC/265 nm (solid line), UVB/285 nm (dashed line) and UVA/365 nm (dotted line) for P680 (heat-tolerant phage: blue colour) and P008 (heat-sensitive phage: orange colour). UV inactivation is represented as infectivity loss in $\log_{10}$ (N_0/N), where N_0 is the PFU/mL of the control samples and N is the PFU/mL of the UV exposed samples. UV inactivation is plotted versus UV exposure time (min) for UVC, UVB and UVA (A) and versus UV dose (mJ/cm^2) for UVC, UVB (B) and UVA (C). The standard deviation was calculated from n = 6 replicates per scenario.

Table 1
Average values of UV doses, Incident Irradiance (E_0) and %UVT for P680 and P008 phage suspensions, exposed to UV radiation under different wavelengths, matrices, and pH values. Additional data is included in Supplementary data (Table S1).

Wavelength	265 nm			285 nm	365 nm
Matrix	SM buffer/ pH 7.5	Yoghurt serum/pH 5.5	Yoghurt serum/pH 7.0	SM buffer/ pH 7.5	SM buffer/ pH 7.5
UV exposure time (min)	UV doses (mJ/cm^2)				
1	20	17	18	36	845
2	41	34	36	72	1,690
4	82	68	71	145	3,380
8	164	136	142	289	6,761
16	327	273	285	578	13,521
E_0 (mW/cm^2)	0.228	0.220	0.220	0.527	16,229
%UVT	45.7	32.5	36.1	55.2	87.8

3.2. Infectivity loss after UV exposure in yoghurt serum at 265 nm

The initial P680 population of 8.0 $\log_{10}$ (PFU/mL), suspended in yoghurt serum/pH 5.5, was reduced by 2.0 ± 0.4 $\log_{10}$ after 1 min of UV exposure (UV dose: 17 mJ/cm^2) ($P < 0.05$). A tailing effect was observed for the longer exposure times with an 8-times longer exposure needed (UV dose: 136 mJ/cm^2) for a further significant 1 $\log_{10}$ reduction ($P < 0.05$) (Fig. 4A, C). The same trend was observed at pH 7.0, but the tailing effect here was less pronounced. For phage P008 in yoghurt serum/pH 5.5, the initial population of 8.5 $\log_{10}$ (PFU/mL) was decreasing with a high inactivation rate, having a 1.1 ± 0.2 $\log_{10}$ reduction after 1 min, 2.3 ± 0.6 $\log_{10}$ reduction after 2 min, 3.6 ± 1.0 $\log_{10}$ reduction after 4 min and finally 5.0 ± 0.6 $\log_{10}$ reduction after 8 min of UV exposure (UV doses: 17, 34, 68 and 136 mJ/cm^2, respectively). However, at 16 min (UV dose: 273 mJ/cm^2) the inactivation rate was dramatically decreased ($P > 0.05$). The inactivation rate was similar at pH 7.0, but a tailing effect was observed for the longer exposure times of 8 and 16 min with no further significant decrease ($P > 0.05$).

Phage P008 showed higher UV tolerance than phage P680 for the first 2 min of exposure at both pH, but phage P680 became more resistant at the longer exposure times, especially at pH 5.5, resulting in a total reduction of 3.4 ± 0.3 $\log_{10}$ after 16 min of UV exposure versus 5.3 ± 0.7 $\log_{10}$ for P008. No significant differences were found for the phage inactivation rate between the two different matrices of SM buffer and yoghurt serum ($P > 0.05$).

3.3. DNA damage after UV exposure in yoghurt serum at 265 nm

For phage P680, no significant decrease in the initial 5 $\log_{10}$ DNA copies/μL was observed, reaching a total 1.0 ± 1.3 $\log_{10}$ reduction for pH 5.5 and 1.0 ± 0.1 $\log_{10}$ reduction for pH 7.0 after 16 min of UV exposure ($P > 0.05$) (Fig. 4B, D). The initial P008 phage population of 7 $\log_{10}$ DNA copies/μL was significantly reduced after 8 min of UV exposure at 265 nm by 1.8 ± 0.5 $\log_{10}$ at pH 5.5 and by 2.6 ± 0.8 $\log_{10}$ at pH 7.0 ($P < 0.05$). No further significant decrease was observed ($P > 0.05$). The DNA damage rate was higher at pH 7.0 than at pH 5.5 for both phages. Both the infectivity and DNA damage of phage P680 were less affected by UV radiation, especially at pH 5.5.

3.4. Epifluorescence microscopy and particle intensity analysis

Phage P680 had a significantly higher average particle intensity and a wider distribution than phage P008 at both pH levels ($P < 0.05$), while both phages had a significantly higher particle intensity and a wider distribution at pH 5.5 compared with pH 7.0 ($P < 0.05$) (Fig. 5). Selected images from the epifluorescence microscopy for each phage and pH are included in the Supplementary data (Fig. S2).

4. Discussion

Our results show that among 265 nm (UVC), 285 nm (UVB) and 365

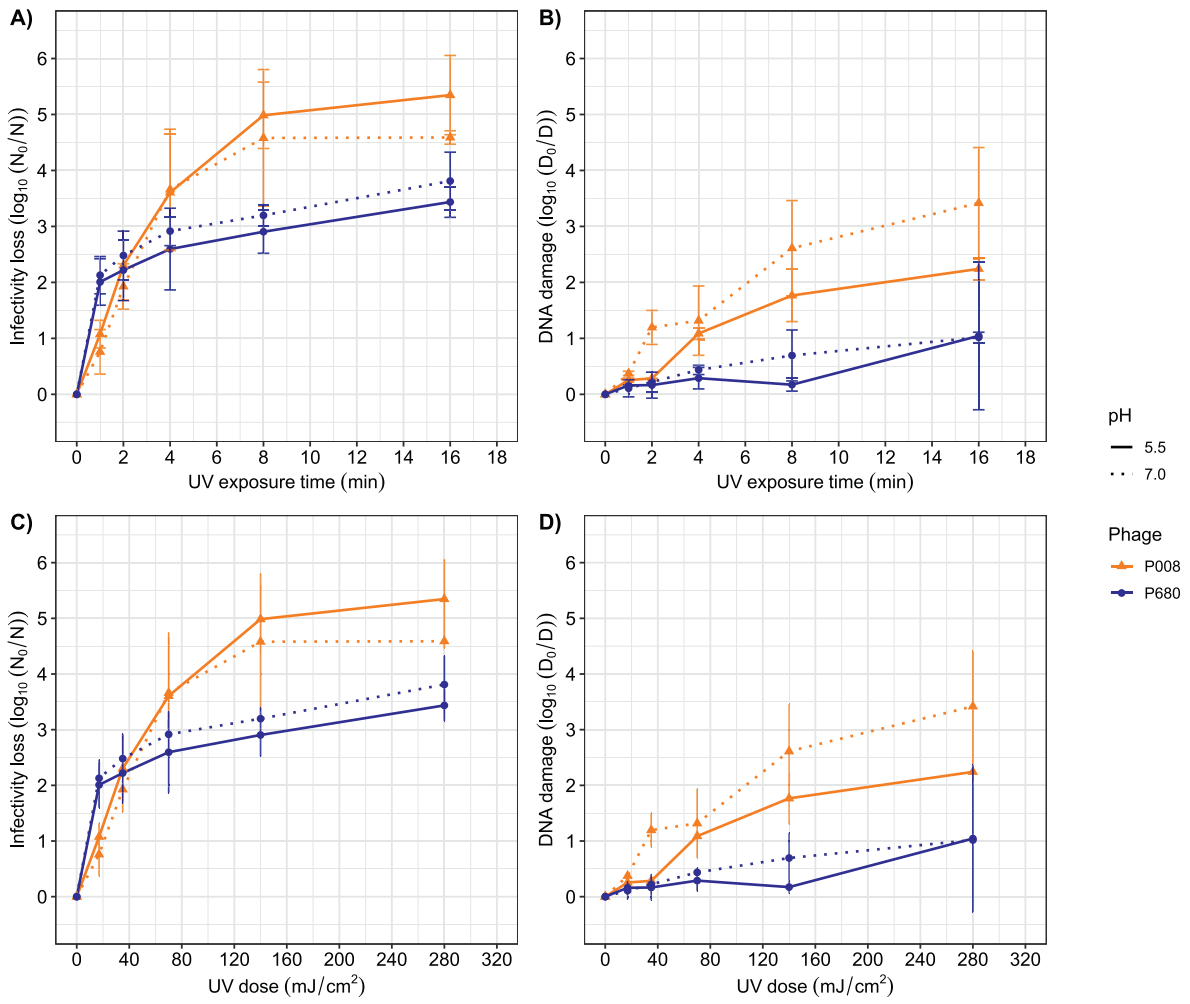

Fig. 4. UV inactivation in yoghurt serum/pH 5.5 (solid line) and pH 7.0 (dotted line) at UVC/265 nm for phage P680 (heat-tolerant phage: blue colour) and phage P008 (heat-sensitive phage: orange colour). UV inactivation is represented as infectivity loss in log_{10} (N_0/N), where N_0 is the PFU/mL of the control samples and N is the PFU/mL of the UV exposed samples (A and C) and as DNA damage in log_{10} (D_0/D), where D_0 is the number of DNA copies/μL of the control samples and D is the number of DNA copies/μL of the UV exposed samples (B and D). UV inactivation is plotted versus UV exposure time (min) (A and B) and versus UV dose (mJ/cm^2) (C and D). The standard deviation was calculated from n = 9 replicates per scenario.

nm (UVA), UVC LEDs emitting at 265 nm achieved the highest phage inactivation at similar fluences (UV doses) (Fig. 3A, B). Time wise the inactivation rates at 285 nm were in the same range as those at 265 nm, but lower and with a more pronounced tailing effect when fluences were compared. Exposure to 365 nm did not practically decrease the phage population.

The infectivity loss was plotted against the UV exposure time to facilitate the comparison of the three different wavelengths, used in this study, within the same graph. However, the Incident Irradiance (E_0) of 265 nm (0.228 mW/cm^2) was only half of that of 285 nm (0.527 mW/cm^2) and both were much lower than that of 365 nm (16,229 mW/cm^2). According to the equation for the UV dose calculation (UV dose = Irradiance × time), more energy is delivered per time unit for UV LEDs at 285 nm and even more for the UV LEDs at 365 nm, when compared with UV LEDs at 265 nm. Therefore, the UV LEDs at 285 nm will generate a higher UV dose for the same exposure time, being more efficient, time wise. In contrast, when the efficiency is compared fluence wise, i.e., for the same energy delivered, the UV LEDs at 265 nm will be more efficient, since they are the closest to the DNA/RNA absorption peak. Indeed, by plotting the infectivity loss against the UV doses (mJ/cm^2), we observed that the higher efficiency of the UV LEDs at 265 nm became even clearer, compared to 285 nm for both phages (Fig. 3B). This is in line with several other studies (Beck et al., 2017; Chevremont et al., 2012; Nyangaresi et al., 2018; Oguma et al., 2013; Rattanakul and Oguma, 2018; Song et al., 2019). The efficiency of the UV LEDs at 365 nm remains low, since they are far from the DNA absorption peak, although they have a very high fluence rate (Fig. 3C).

However, UV LEDs emitting close to 265 nm are more complex and expensive to manufacture (Chen et al., 2017; Koutchma et al., 2019; Muramoto et al., 2014). It has furthermore been stated that UV LEDs emitting close to 280 nm can also damage the enzymes responsible for photoreactivation and dark repair, leading to more permanent inactivation results (Li et al., 2017; Nyangaresi et al., 2018). This, together with their lower energy consumption, indicates that the UV LEDs close to 280 nm may have the potential for designing industrial UV disinfection set-ups, where energy consumption and time are crucial factors. However, the impact of these wavelengths on the quality characteristics of the dairy matrices should also be assessed.

The heat-tolerant phage P680 was the most UV tolerant for all the wavelengths, matrices, and pH values tested, while no systematic differences were found between the two matrices. It should be noted, however, that the yoghurt serum matrix used in this study cannot be directly compared to yoghurt or milk, since it was double filtered, and 10-fold diluted to achieve a higher %UVT. To achieve a 4 log_{10} titer reduction of phage P680 and P008 in the aqueous suspension at 265 nm, 327 mJ/cm^2 and 164 mJ/cm^2 are needed, respectively, according to our

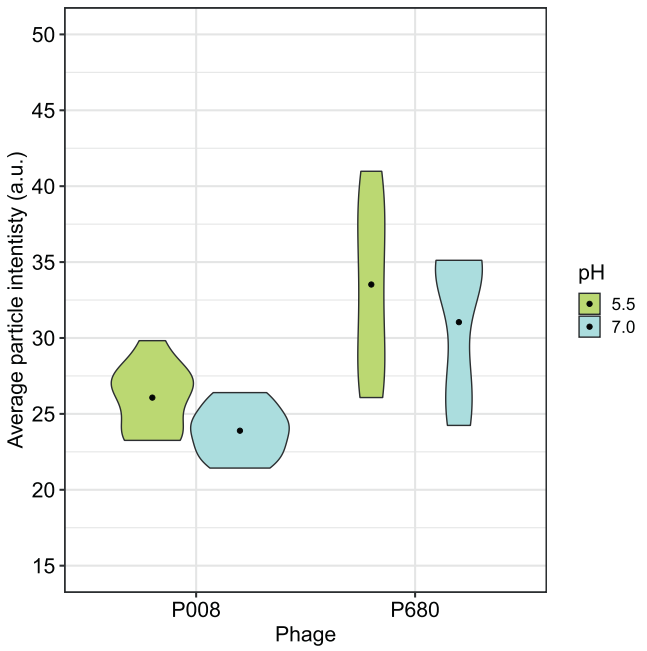

Fig. 5. Average particle intensity or mean gray value (arbitrary units, a. u.) for phages P680 and P008 in SM buffer/pH 5.5 (green) and pH 7.0 (blue). The black dots in each violin area indicate the average particle intensity per pH and phage, calculated from all the particles detected for each scenario. The violin areas represent the distribution of the average particle intensity values of each of the 10 photos for each scenario.

study. When comparing these values with those recently reported from Michel et al. (2021) for phages P680 and P008, using a static LPML set-up at 254 nm, approx. 10 times lower UV doses were needed for phage P680 (30 mJ/cm^2) and P008 (25 mJ/cm^2) to achieve a 4 and 3 $\log_{10}$ titer reduction, respectively, in an aqueous suspension (Table 2). Moreover, Michel et al. (2021) reported a similar UV tolerance for phage P680 and P008 both in aqueous suspension and filtered whey and a limited tailing effect. These differences in phage UV tolerance between the two studies may originate from the use of different lamp sources, set-ups, initial population levels and suspension matrices.

When comparing the observed UV tolerance of phages P680 and P008 of this study, with previously reported inactivation values of common phages used as surrogate models, vegetative bacteria and even some bacterial spores exposed to UVC radiation in aqueous suspensions, phages P680 and P008 seem to be quite UV tolerant (Table 2). For instance, a UV dose of approx. 85 mJ/cm^2 reduced MS2 by approx. 3.5 $\log_{10}$ and a UV dose of approx. 25 mJ/cm^2 reduced the spores of *Bacillus subtilis* by approx. 4 $\log_{10}$, while UV doses of 327 and 164 mJ/cm^2 were needed for a 4 $\log_{10}$ reduction of phages P680 and P008, respectively. Moreover, a UV dose of 7 mJ/cm^2 was sufficient to reduce the pathogenic bacteria *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella enterica* by almost 5 $\log_{10}$. However, for such comparisons the initial microbial population levels should also be taken into account since high starting cell numbers can lead to cell aggregation and light-shielding, connected to higher UV tolerance (Abshire and Dunton, 1981; Chatzisymeon, 2016; Vitzilaiou et al., 2021).

UV light disinfection is a well-established technology in water treatment, recognized by regulatory authorities all over the world such as the U.S. Environmental Protection Agency (U.S. EPA) and the European Food Safety Authority (EFSA) for its efficiency in inactivating waterborne bacteria, protozoa, and viruses. The European authorities recommend a minimum UV dose of 40 mJ/cm^2 at 253.7 nm for a safe drinking water disinfection, based on the fact that this UV dose can lead up to a 4 $\log_{10}$ reduction of drinking water transmittable pathogens (DVGW, 2006; ÖNORM, 2001, 2003). The U.S. EPA is focusing more on the target pathogens and recommends UV doses that result in a 3 $\log_{10}$ reduction of the chlorine resistant protozoa *Cryptosporidium* and *Giardia*, and a 4 $\log_{10}$ reduction of viruses, for a safe water disinfection (USEPA, 2006). The use of UV light for food treatment remains however an emerging technology, more recently approved for foods such as juice and milk as an alternative to thermal pasteurization by the European and U.S. authorities. The food matrix itself is very diverse and the UV dose necessary for sufficient microbial reduction would be affected by various factors, such as the type of food, the microbial load, the UV tolerance of the target pathogenic microorganisms, and the irradiation system. Therefore, the regulatory authorities do not specify minimum or maximum UV doses or log reductions for an efficient UV treatment, but they expect the treatment to follow the good manufacturing practices of each food industry (Koutchma, 2019).

If a UV dose of 40 mJ/cm^2, as suggested in many drinking water guidelines based on LPML/254 nm, is applied for the inactivation of P680 and P008 phages, but using the UV LED set-up at 265 nm, this would only result in a 2–2.5 $\log_{10}$ reduction for the two phages while for e.g., a 4 $\log_{10}$ reduction an 8-times higher UV dose would be needed for phage P680 and a 4-times higher for phage P008.

A 4–5 $\log_{10}$ reduction of the initial 10^{7-8} PFU/mL P680 and P008 population was achievable in our study, both in aqueous suspension and in yoghurt serum, and together with the observation that initial

Table 2
UVC tolerance of common surrogate-phages, bacteria, and phages P680, P008. Experiments were conducted in aqueous microbial suspensions using a UVC LED or LPML static collimated beam set-up.

Wavelength (nm)	Microorganism	UVC dose (mJ/cm^2)	Suspension matrix	Initial population levels (CFU/mL[a] or PFU/mL)	$\log_{10}$ reduction	References
265	MS2[b]	85	Phosphate buffer	10^6	3.5	(Oguma, 2018)
265	Qβ[b]	40	Phosphate buffer	10^{6-7}	4	(Rattanakul and Oguma, 2018)
265	*Legionella pneumophila*	4			4	
265	*Pseudomonas aeruginosa*	5			3	
265	*Bacillus subtilis* spores	25			4	
266	ΦX174[b]	1	Distilled water	10^{8-9}	7	(Kim et al., 2017)
268	*Listeria monocytogenes*	7	0.9 % saline	10^9	4.68	(Green et al., 2018)
268	*E. coli* O157:H7	7	solution		4.88	
268	*Salmonella enterica*	7			4.60	
254 (LPML)	P680	30	Deionised water	10^{8-9}	4	(Michel et al., 2021)
254 (LPML)	P008	25			3	
265	P680	327	SM buffer	10^{7-8}	4	This study
265	P008	164				

[a] CFU/mL: Colony Forming Units/mL.
[b] MS2, Qβ, ΦX174: surrogate phages for human adenovirus and enteric viruses, host: *Escherichia coli*.

membrane filtration may remove 4 $\log_{10}$ units of the phages in the whey (Samtlebe et al., 2015), these data strongly indicate that it could be possible to decrease the phage population in the whey solution by 8–9 $\log_{10}$ units in total by combining efficient filtration with UV LED treatment at 265 or 285 nm. A 5 $\log_{10}$ reduction has been previously observed in filtered whey for phages P680 and P008, with a 10^{8-9} PFU/mL initial population, employing LPML at 254 nm by Michel et al. (2021).

According to Rodriguez et al. (2014), the phages with the smallest genome sizes are the most UV tolerant. For example, phage MS2 with a linear single-stranded (ss) RNA of 3.6 kb was the most UV tolerant phage, while phage ΦX174 with a circular ssDNA of 5.3 kb and phage T1 with a linear dsDNA of 48.8 kb were the least UV tolerant phage in that study. The same trend has been observed by Kim et al. (2017) and Beck et al. (2015) for phages MS2 and Q_β (ssRNA, 4.2 kb) compared with phages ΦX174 and T7 (dsDNA, 40 kb). Phages P680 and P008 have similar DNA sequences and a similar dsDNA size of 29.6 kb and 28.5 kb, respectively, which is much larger than the genome of phages MS2, ΦX174 and Q_β, but phage P008 and even more so, phage P680 may have a more compact genome organization. This has been previously suggested by Atamer et al. (2010) as the reason behind the high heat tolerance of phage P680. Moreover, phage P008 has two extra possibilities for generating thymine-dimers within the region targeted by the primers (Table S2). This may be responsible for the lower UV tolerance of P008 compared to P680, although it is probably too low to account for the differences observed. The mechanism behind the UV tolerance of phages P680 and P008 needs further investigation.

Geagea et al. (2018) suggested that a 120 bp deletion in the gene of phage P680 coding for a structural protein named tape measure protein (TMP) is partially responsible for the high heat tolerance of P680 and other phages. Interestingly, phages belonging to the 936-group have been found in earlier studies to exhibit resistance also towards various biocides (Hayes et al., 2017). Therefore, several 936-group phages may possess specific genomic characteristics, rendering them tolerant to various stress factors such as heat, biocides and UV radiation. Another potential reason for the higher UV tolerance of phage P680 could be a more UV tolerant capsid, suggested before by Atamer et al. (2010). The aromatic amino acids tryptophan, tyrosine and phenylalanine absorb UVC light at 180–230 nm and 240–300 nm and cysteine residues at 260 nm (Schmid, 2001; Wigginton et al., 2010). Therefore, UVC light may denature the phage structural proteins and the capsid may be more or less UV tolerant based on the amino acid composition. Beck et al. (2018) have previously found that UVC LED radiation at 261 nm and 278 nm led to 11–34 % and 7–20 % protein damage, respectively, in Adenovirus at a UV dose of 400 mJ/cm^2. More studies are needed to focus in the future on the impact of UVC light on phage structural proteins to acquire more knowledge on this phenomenon.

The phages are bio-colloids, and their surface charge is pH-dependent in polar media such as water solutions. The charge of the phage suspension solution in relation to their isoelectric point (IEP) affects their electrostatic interactions and therefore their mobility, their host-adsorption ability and the attachment or repulsion to other phage particles. The IEP is the pH where the net charge of the phage surface is zero, and therefore neither attachment nor repulsion is taking place, while below or above the IEP the net surface charge is becoming positive or negative (Michen and Graule, 2010). Regarding the closely related phages P680 and P008, used in this study, it has previously been shown that the maximum adsorption of P008 phage particles to host cells is at pH 6.4, while below pH 4.8 it is significantly reduced as the phage binding-receptors are affected by the low pH values (Müller-Merbach et al., 2007). In our study, we therefore employed the phage pH-dependent surface charge property and set two pH values, pH 5.5 and pH 7.0, to induce different phage aggregation levels in the suspension matrix, without reducing the phage adsorption, with the aim to observe the impact of aggregation level on UV efficiency. The phages will either have a higher aggregation level at pH 5.5 compared to pH 7.0 or vice versa. To verify this hypothesis, we employed epifluorescence microscopy, calculating the phage particle intensity for the two pH values.

The impact of the different aggregation levels on the UV efficiency was assessed after UVC exposure, by measuring both infectivity loss, by double-agar layer plaque assay (Fig. 4A, C), and DNA damage, by qPCR (Fig. 4B, D). These results were complemented with the particle intensity distribution analysis (Fig. 5). The host infectivity loss at both pH values showed a high initial reduction rate followed by a tailing effect at the longer exposure times. The tailing effect was especially pronounced for P680, which also exhibited less DNA damage and a higher average particle intensity at both pH values, compared to P008. Both phages suffered less genome damage at pH 5.5, where the phage particles were larger. These observations indicate that phage aggregation may lead to UV light-shielding of the phage particles and hence less UV induced DNA damage. Although the DNA damage rate was low, an increasing trend was observed, especially for P008, which revealed smaller particle sizes. However, the host infectivity loss rate was more drastic at the beginning of UV exposure and tailing effect was observed at the longer exposure times. This may be due to the ability of the phages to repair their damaged DNA in the host cell, which is present during the infectivity assay.

Interestingly, more UV resistant survivors were occasionally observed when phage survivors were re-exposed to UVC radiation (data not shown) indicating a potential selection for more UV resistant sub-populations. More research to clarify this is needed since increased UV tolerance after sequential UV exposures has also been noticed for the bacteria *S. aureus* and *E. coli* (Alcántara-Díaz et al., 2004; Shoults and Ashbolt, 2019).

5. Conclusion

UVC LEDs at 265 nm were the most efficient among the three wavelengths tested (265, 285 and 365 nm), reaching a 4 $\log_{10}$ reduction at a UV dose of 327 mJ/cm^2 for phage P680 and 164 mJ/cm^2 for phage P008. UV LEDs at 285 nm achieved a similar inactivation, while 365 nm had little effect on the population. The more heat-tolerant phage P680 was also the most UV tolerant for all the wavelengths, matrices, and pH values tested, but both phages P680 and P008 were more UV tolerant than many previously reported surrogate phages and pathogens. Indications of UV light-shielding, observed as a tailing effect in the host infectivity loss rate and less DNA damage, were found for both phages at pH 5.5, where a higher aggregation degree was induced, in particular for phage P680. Indications of the existence of more UV tolerant phage sub-populations were found on re-exposure to UVC radiation. Phages of the 936-group (*Skunavirus*) of *L. lactis* are found in significant numbers in the dairy environment and their presence can lead to fermentation failure with high yield losses for the dairy industry. Thermal treatment has been proven insufficient for phage elimination. Our results show that UV light could complement thermal treatment for phage elimination in the future or even be an alternative in combination with efficient filtration. However, more research is needed to optimize the technology focusing on UV reactor design, matrix composition and %UVT, phage tolerance, protection mechanisms and the potential emergence of more UV resistant sub-populations.

Funding

This work was supported by a PhD scholarship for Eirini Vitzilaiou co-funded by the Danish Partnership for Resource and Water Efficient Industrial Food Production (DRIP: j.nr. 152-2014-10) and the University of Copenhagen.

CRediT authorship contribution statement

Eirini Vitzilaiou: Conceptualization, Methodology, Validation, Formal analysis, Visualization, Writing-Original Draft, Writing-Review

& Editing. Yuxin Liang: Investigation, Validation, Visualization, Writing-Original Draft, Review. Josué L. Castro-Mejía: Conceptualization, Methodology, Review. Charles M.A.P. Franz: Resources, Review. Horst Neve: Resources, Review. Finn Kvist Vogensen: Conceptualization, Methodology, Validation, Writing-Review & Editing, Supervision. Susanne Knøchel: Conceptualization, Methodology, Validation, Writing-Review & Editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2022.109824.

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