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Pulsed light inactivation of murine norovirus, Tulane virus, *Escherichia coli* O157:H7 and *Salmonella* in suspension and on berry surfaces

Yaoxin Huang, Mu Ye, Xinang Cao, Haiqiang Chen*

Department of Animal and Food Sciences, University of Delaware, Newark, DE 19716-2150, USA

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ABSTRACT

Pulsed light (PL) inactivation of two human norovirus (HuNoV) surrogates, murine norovirus (MNV-1) and Tulane virus (TV), and two bacterial pathogens, *Escherichia coli* O157:H7 and *Salmonella*, were evaluated. The viruses and bacteria were suspended in phosphate buffered saline (PBS) to final populations of ~6 log PFU/mL and ~6 log CFU/mL, respectively. Both viral and bacterial suspensions were then irradiated by PL for different durations and the reductions of each microorganisms were determined. MNV-1 and TV were significantly ($P < 0.05$) more resistant to PL treatment than *Salmonella* and *E. coli* O157:H7 in PBS suspension. MNV-1, *Salmonella* and *E. coli* O157:H7 were also inoculated on strawberries and blueberries and the PL inactivation of each microorganism was determined. Lower inactivation of each microorganism was achieved on berry surfaces than in PBS suspension. This study shows that PL can induce rapid inactivation of MNV-1, TV, *Salmonella* and *E. coli* O157:H7 in clear suspension with viruses more resistant to PL treatment than bacteria. The efficacy of PL treatment is substantially influenced by food surface structure.

1. Introduction

Human Noroviruses (HuNoV) are recognized as the leading cause of gastroenteritis. Research on HuNoV has to rely on proper surrogates since HuNoV is non-cultivatable. Some common HuNoV surrogates include murine norovirus (MNV), feline calicivirus, and more recently, Tulane virus (TV) (Farkas et al., 2008). MNV shares many biological and molecular properties with HuNoV (Wobus et al., 2006). However, the limitations of MNV are that it differs in several aspects from HuNoV such as clinical manifestations, host receptors, pathogenesis, and infected cell types (Tan and Jiang, 2010). In addition, MNV uses sialic acid as a functional receptor (Taube et al., 2009), whereas HuNoVs use histo-blood group antigens (HBGAs) as cellular receptors and infect digestive epithelial cells *in vivo* (Tan and Jiang, 2005; Tan et al., 2008). Genomic sequence analysis showed that TV was closely related to HuNoV (Farkas et al., 2010). More importantly, similar to HuNoV, TV recognizes type A and B HBGAs as receptors for infection (Farkas et al., 2010). Many groups have studied the resistance of TV to a range of chemical and physical treatments such as chlorine, UV irradiation, ethanol, pH, heat and high pressure (Hirneisen and Kniel, 2013; Li et al., 2013; Tian et al., 2013). However, to our knowledge, study of pulsed light (PL) inactivation of TV has not been reported. PL is effective against a broad spectrum of microorganisms including bacteria (Rowan et al., 1999; Woodling and Moraru, 2007), viruses (Belliot et al., 2013; Lamont et al., 2007; Roberts and Hope, 2003), and spores (Jun et al., 2003). In the US, the use of PL technology was approved by the U.S. Food and Drug Administration in 1996 for decontamination of food or food contact surfaces (21CFR179.41).

Berries have been frequently involved with outbreaks of foodborne pathogens in recent years. Frozen berries have caused multiple large-scale HuNoV outbreaks in 2005 and 2009 (Friedman et al., 2005; Sarvikivi et al., 2012). In 2012, the largest foodborne outbreak in Germany was associated with HuNoV-contaminated frozen strawberries, which affected more than 11,000 people (Mäde et al., 2013). In 2011, fresh strawberries from a farm in Oregon were linked with an *Escherichia coli* O157:H7 outbreak, which caused at least 15 cases of sicknesses and two deaths (Laidler et al., 2013). In 2009, a multistate outbreak of *Salmonella* Muenchen was reported due to consumption of blueberries, which caused 14 cases of illnesses (CDC, 2009).

The objective of this study was to assess the PL inactivation of TV, MNV-1 and two bacterial pathogens (*Salmonella* and *E. coli* O157:H7) in phosphate buffered saline (PBS) and on strawberries

* Corresponding author.
E-mail address: haiqiang@udel.edu (H. Chen).

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and blueberries. This is the first study describing the use of PL to inactivate TV. The potential application of this nonthermal technology was evaluated.

2. Materials and methods

2.1. Viruses and cell lines

The detailed information regarding the MNV strain (MNV-1), murine macrophage cell line RAW 264.7, and the growing of MNV-1 was provided in our previous publication (Huang and Chen, 2015). The concentration of virus was performed using the method described by Hwang et al. (2014) with minor modifications. Briefly, the virus was concentrated by centrifugation at 95,000*g* for 3 h at 4 °C in a Surespin 630 (36 mL) rotor (Thermo Scientific). The pellet was mixed with 0.5 mL PBS (pH 7.2) and incubated overnight at 4 °C. The pellets were then resuspended in PBS and pooled together. Each tube was rinsed with 0.5 mL PBS and the rinsate was combined with previously pooled concentrated MNV stock to get a final titer of ~8.7 log PFU/mL.

The detailed information regarding the TV strain (MNV-1), monkey kidney cell line LLC-MK2, and the growing of TV was provided in our previous publication (Li et al., 2013). TV was concentrated following the methods described by Farkas et al. (2008) with minor modifications. Briefly, TV was precipitated by mixing equal volume of 20% polyethylene glycol 8000 (PEG) (Fisher Scientific) and 0.6 M NaCl stock solution with viral suspension and incubated at 4 °C overnight. Polyethylene glycol precipitate was collected by centrifugation at 10,000*g* for 45 min and resuspended in 1/30 of the original culture volume of PBS to obtain a viral titer of ~6.7 log PFU/mL.

2.2. Bacterial strains and inoculum preparation

E. coli O157:H7 strain 251 (lettuce outbreak isolate) and *Salmonella enterica* serotype Newport H1275 (sprout outbreak isolate) were used. The preparation and growing of nalidixic acid-resistant mutants of the strains were described in our previous publication (Huang et al., 2013). Bacterial cells were harvested by centrifugation at 3000*g* for 15 min (Sorvall ST16 R, Thermo Scientific). The supernatant was discarded and the pellet was resuspended in 1 mL of sterile PBS to yield a final concentration of ~10^8 CFU/mL.

2.3. PL treatment

The PL treatments were performed with a laboratory scale PL system (Steripulse-XL RS-3000, Xenon Corp.,Wilmington, MA). Information about the PL system and measurement of PL energy received by samples was provide in our previous publication (Huang and Chen, 2014). For PL treatment in suspension, virus stocks and bacterial suspensions were diluted in PBS to final populations of ~6.0 log PFU/mL and ~6.0 log CFU/mL for viruses and bacteria, respectively. Viral or bacterial suspensions (2 mL) were placed in a 35-mm sterile petri-dish, which was centered at the bottom of the PL chamber. The samples were irradiated with PL for 1–16 pulses at distances of 16 cm from the quartz window. After PL treatments, populations of the viral and bacterial samples were determined as described in our previous publications (Huang and Chen, 2015; Li et al., 2013). For PL treatments on berry surfaces, fresh strawberries and blueberries were illuminated with UV-C (254 nm) in a biosafety hood for at least 10 min to lower the load of natural background microbiota. Blueberries (~5 g/sample) and strawberry halves (~10 g/sample) were then spot-inoculated in multiple small droplets on the skin (avoiding the calyx) with 150 μL suspension of *Salmonella*, *E. coli* O157:H7 or MNV-1. Samples were then air-dried in a biosafety hood for 2 h at 22 ± 2 °C to facilitate bacterial and viral attachment. Inoculated berries were irradiated by PL for 6, 12 or 24 s.

2.4. Extraction of MNV-1 from berries

MNV-1 was extracted from the berries as described by Dubois et al. (2002) and Kingsley et al. (2005) with some modifications. Individual berry samples were transferred into a 50 mL conical tube containing 10 mL of vegetable extraction buffer (Huang and Chen, 2015) and 50 μL pectinase (Sigma-Aldrich, St Louis, MO). To release viruses from berries, each tube was vortexed for 5 times and each pulse lasted for 5 s on a Vortex Genie 2 vortexer (Scientific industries, Bohemia, NY) with speed set at 8–9. The liquid portion was transferred to a 15 mL tube and centrifuged at 3000*g* for 10 min at 4 °C. The supernatant was serially diluted in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Life Technologies Corporation) and used for plaque assays as described by Huang and Chen (2015).

2.5. Bacterial recovery and enumeration

After PL treatment, berry samples were transferred into sterile filter bags (Whirl-Pak, Nasco, USA) containing 40 mL (for blueberry samples) or 45 mL (for strawberry sample) of Dey-Engley (D/E) neutralizing broth (Difco). The samples were pummeled in a laboratory stomacher (Seward 400, Seward, London, U.K.) for 2 min at 260 rpm. The homogenate was serially diluted in sterile 0.1% peptone water and surface-plated on tryptic soy agar (Difco Laboratories, Sparks, MD) supplemented with 0.6% yeast extract (Difco) and 50 μg/mL of nalidixic acid (Fisher Scientific) (TSAYE-N). Presumptive colonies of *E. coli* O157:H7 or *Salmonella* formed on the plates were counted after 72-h incubation at 35 °C.

2.6. Statistical analysis

At least three independent trials were conducted for all the experiments. Colony counts and plaque counts were converted to log reductions and standard deviations were calculated. Statistical analyses were conducted using JMP (SAS Cary, NC, USA). One-way analysis of variance and Tukey's one-way multiple comparisons were used to determine significant differences among treatments ($P < 0.05$).

3. Results

3.1. Inactivation of MNV-1, TV, E.coli O157:H7 and Salmonella in PBS by PL

An increasing reduction of MNV-1, TV, *E. coli* O157:H7 and *Salmonella* in PBS was observed as the treatment duration or fluence of PL increased (Table 1). The *E. coli* O157:H7 strain was significantly more sensitive to PL treatment than the *Salmonella* strain ($P < 0.05$). No significant difference ($P > 0.05$) in the PL resistance of MNV-1 and TV was observed after 1-pulse of PL treatment; however, TV showed significantly higher ($P < 0.05$) resistance to PL treatment during longer PL treatments (2–8 pulses). Overall, comparing to the two pathogenic bacteria, the two viruses showed significantly higher resistance to PL treatments ($P < 0.05$).

3.2. Inactivation of MNV-1, E. coli O157:H7 and Salmonella on berry surfaces by PL

The titer of MNV-1 on strawberry was reduced by 0.7–0.9 log units after 6–24 s of PL treatments (Table 2). Increasing PL fluence