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A new roller conveyer system of non-thermal gas plasma as a potential control measure of plant pathogenic bacteria in primary food production

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ABSTRACT

We have developed a novel roller conveyer plasma device, which generates gas plasma *via* atmospheric pressure dielectric barrier discharge (APDBP) on rollers. High voltage and earth electrodes are incorporated into the rollers, which are covered with a silicon sheet. Bactericidal activity of the device was measured against *Xanthomonas campestris* pv. *campestris* (*Xcc*), which was spotted onto aluminium plates and subjected to gas plasma treatment generated by APDBD. After treatment, viable cell number of *Xcc* decreased with a decimal reduction time (*D* value) of 0.90 min (initial population: 5.0×10^8 CFU/ml) and below 0.34 min (initial population: 9.8×10^5 CFU/ml). APDBD treatment induced slight morphological changes to *Xcc* along with significant degradation of lipopolysaccharides (LPS) and degradation/oxidation of genomic DNA to form 8-hydroxy-2'-deoxyguanosine (8-OHdG). Reactive chemical products (hydrogen peroxide, nitrite and nitrate), ultraviolet light (UV-A) and slight temperature elevations were observed during operation of the device. Our results suggest hydrogen peroxide and nitrite generated during operation of the device cause oxidation and degradation of the genomic DNA as well as the degradation of LPS, which are potential mechanisms for inactivation. In addition, the successful inactivation of *Xcc* on cabbage leaf by the device suggests the method could have practical applications.

1. Introduction

Soil-borne pathogens infect plants and can sometimes prevent their normal growth. Interactions between soil organisms and plants can kill seedlings and even adult trees. For most soil-borne plant diseases, pathogen spread occurs predominantly between plants that have grown as close neighbours (Anderson & May 1986). In modern times, the development of efficient transportation systems for agricultural food products has inadvertently allowed the rapid spread of pathogens. Thus, these plant pathogens cause both environmental and economic problems because they can have a devastating impact on crop production. In addition, previous studies have shown that the transfer of agricultural food products, such as vegetables, from the farm to the packaging plants presents potential cross-contamination (Davies & Breslin, 2003; Wang & Ryser, 2014). Moreover, there is a significant contamination hazard during food processing e.g., cutting plants (Scott & Bloomfield, 1990) as well as quality hazard.

Hygienic practice should meet microbiological criteria for ensuring public health protection under the general principles of Good Hygienic Practices (GHPs) and Good Manufacturing Practices (GMPs) described at the international level by the Codex Alimentarius Commission (CAC/RCP 1-1969, 2009). For example, in the European Union, GHPs included in the annex to regulation (EC) No 852/2004 of the European Parliament and of the Council of 24 April 2004 on the hygiene of foodstuffs (The European Parliament and the council of the European Union, 2004) and in USA current GHPs (CGMPs) are defined in the Code of Federal Regulations-Part

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110 (US Food and Drug Administration, 2015). In Japan, such issues are regulated by the Food Sanitation Act and relevant laws (Government of Japan, 2006). Wherever possible, it is recommended that a HACCP (Hazard Analysis and Critical Control Point)-system based approach should is implemented to enhance food safety (CAC/RCP 1-1969, 2009).

There are numerous methods to kill microorganisms. These include thermal processing (thermal treatment, microwave heating, infrared heating, radio-frequency heating, and ohmic heating) and non-thermal processing (ozonation, irradiation technology, high-pressure processing, pulsed electric field technology, and electrolyzed oxidized water treatment) (Neetoo & Chen, 2014). Currently, there are few readily available *in situ* methods for disinfection that potentially could facilitate future application and implementation of a HACCP system. Consequently, there is a need to develop novel generic techniques to efficiently and safely decontaminate foods during the food processing procedure.

Xanthomonas campestris pv. *campestris* (*Xcc*) is a bacterial pathogen that causes black rot, which is a kind of quality defect and considered the most important worldwide disease of crucifers, attacking all cultivated brassicas, radishes and numerous cruciferous weeds (Williams, 1980). Black rot is prevented and controlled by adopting various different strategies such as using disease-free seeds, employing good sanitary practices, managing insects and weeds, planting varieties of crops with partial resistance to black rot and chemical control (Seebold, Bachi, & Beale, 2008). However, these control measures are confined to the fields and are not applicable once the crop has been harvested. Therefore, we were interested in the development of an efficient post-harvest inactivation method for *Xcc*.

Gas plasma is the fourth state of matter and is generated by subjecting gases to a high electric field, resulting in a mixture of partially ionized gas with dissociated molecules, photons, electrons, free radicals, and other reactive species as well as positive and negative ions (Shintani & Sakudo, 2016). The use of non-thermal atmospheric gas plasma is a potential alternative to conventional methods for the decontamination of food products (Afshari & Hosseini, 2014; Niemira, 2012; Shintani & Sakudo, 2016; Yagyu et al., 2016). This methodology offers a broad range of inactivation against viruses, fungi and bacteria, including those forming spores and biofilms (Kelly-Wintenberg et al., 1999; Pan et al., 2013). In the food industry, gas plasma is a powerful tool for surface decontamination of not only foods but also food packaging materials such as plastic bottles, lids and films without any associated adverse effects (Pankaj et al., 2014).

Here, we describe the development of a novel plasma apparatus for the disinfection of agricultural food products. The apparatus, termed a roller conveyer plasma device, generates gas plasma in air *via* an atmospheric pressure dielectric barrier discharge (APDBD) and can be utilized as an automated disinfection system during the sorting of vegetables. In addition, we investigated the influence of gas plasma treatment on the components of *Xcc* as well as determining the principal mechanism of inactivation.

2. Materials and methods

2.1. Study plan

To enhance food safety, key hygiene control at each process is recommended wherever possible using a potential HACCP-based approach, in addition to embracing the general principle of food hygiene (CAC/RCP 1-1969, 2009). However, there are only a limited number of methods for minimizing microbiological risk that can utilize an *in situ* system during the sorting of vegetables. These considerations prompted us to design and fabricate a novel roller conveyer plasma device, which is well suited to the disinfection of vegetables during sorting on rollers. Here, we have extended this work by addressing a number of additional issues as follows. Firstly, we aimed to assess the effectiveness of our roller conveyer plasma device by analyzing the inactivation of *Xcc*, which is a key plant pathogenic bacteria. We also identified the likely inactivating factors generated during the operation of the roller conveyer plasma device. Finally, we elucidated the mechanism of inactivation by analyzing the biochemical changes induced in *Xcc* after treatment.

2.2. Roller conveyer plasma device

In 2014, we designed and fabricated a unique gas plasma apparatus composed of rolling electrodes and a high voltage power supply (Fig. 1). The apparatus is our original design and is not manufactured commercially. The electrode comprised a plastic rod (diameter = 30 mm) covered with a thin aluminium and silicon sheet, which was placed at an interval of 50 mm from an earth electrode. The high voltage electrode was then connected to an alternating power supply (10 $kV_{peak\ to\ peak}$ (kV_{p-p}), 10 kHz; LHV-10AC, Logy electric Co. Ltd., Tokyo, Japan). Plasmas were generated on the silicon sheet when electrically conductive samples, such as metals or vegetables, contacted both the high-voltage electrode and earth electrode.

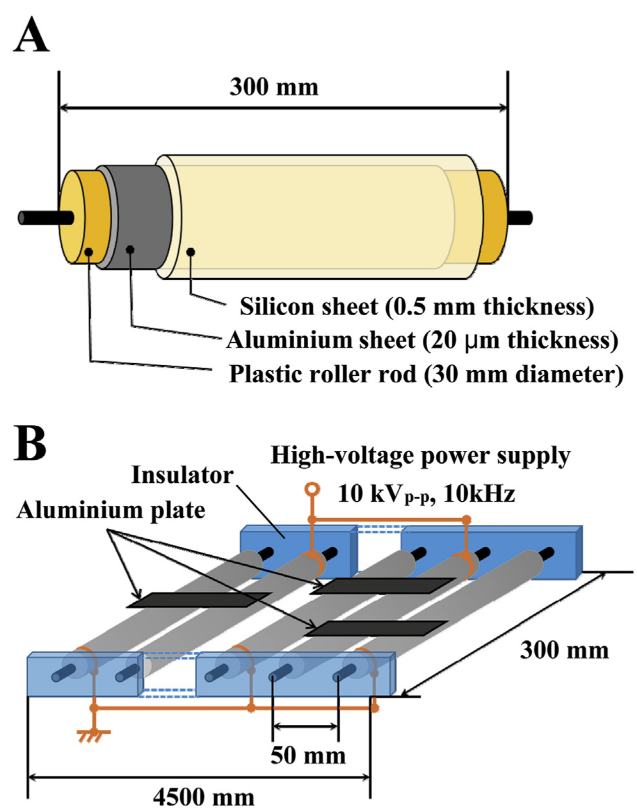

Fig. 1. Schematic representation of a roller conveyer plasma device producing gas plasma by atmospheric pressure dielectric barrier discharge (APDBD). (A) The electrode is a plastic roller rod covered with an aluminium sheet (20 μm thickness) and silicon sheet (0.5 mm thickness). The aluminium sheet is connected to a high-voltage power supply [10 $kV_{peak\ to\ peak}$ (10 kV_{p-p}), 10 kHz]. (B) The position of the aluminium plate and electrodes during operation of the roller conveyer apparatus is shown. *Xanthomonas campestris* pv. *campestris* (*Xcc*) suspended in distilled water (20 μl) was spotted and dried onto an aluminium plate (0.3 mm thickness). Plasma was generated at the grounding position between the aluminium plate and silicon sheet by the mechanism of APDBD. Spots of *Xcc* on the aluminium plate were set onto the grounding position.