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Effect of X-irradiation on Citrus Canker Pathogen *Xanthomonas citri* subsp. *citri* of Satsuma Mandarin

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ABSTRACT

Citrus canker caused by *Xanthomonas citri* subsp. *citri* (*Xcc*) is one of the most important bacterial diseases of citrus. *Xcc* has been strictly regulated in order to prevent its spread, because citrus canker has not been founded in many countries including European Union and Australia. Methyl Bromide was restricted the use by Montreal Protocol, which had been used as a disinfectant. Therefore, application of ionizing radiation on the agricultural production is raised as an eco-friendly alternative. In this study, the effects of X-irradiation on *Xcc* growth either in the suspension or on the surface of citrus fruits were investigated. The suspension containing 1×10^7 cfu/ml of *Xcc* was irradiated with different absorbed doses of X-irradiation ranging from 50 to 400 Gy. The results showed that *Xcc* was fully dead at 400 Gy of X-irradiation. To determine the effect of X-irradiation on quarantine, the *Xcc*-inoculated citrus fruits were irradiated with different X-ray doses at which *Xcc* was completely inhibited by an irradiation dose of 250 Gy. The D_{10} value for *Xcc* on citrus fruits was found to be 97 Gy, indicating the possibility of direct application on citrus quarantine without any side sterilizer. Beside, presence of *Xcc* on the surface of asymptomatic citrus fruits obtained from citrus canker-infected orchards was identified. It indicated that the exporting citrus fruits need any treatment so that *Xcc* on the citrus fruits should be completely eliminated. Based on these results, ionizing radiation can be considered as an alternative method of eradicating *Xcc* for export of citrus fruits.

Ⅰ. INTRODUCTION

Citrus canker caused by *Xanthomonas citri* subsp. *citri* (*Xcc*) is one of the most serious bacterial diseases of many commercial citrus varieties, resulting in significant economic losses worldwide (Schaad et al., 2006). *Xcc* has been recently reclassified from *Xanthomonas axonopodis* pv. *ctiri* (Schaad et al., 2006; Vauterin et al., 1995). The pathogen is spread by rain splash and enters its host plant through stomata and wounds. They cause distinctive necrotic raised lesions surrounded by oily, water-soaked margins and yellow chlorotic rings on leaves, stems, and fruits (Graham et al., 2004; Schubert et al., 2001). Severe infection causes defoliation, dieback and fruit drop, which have serious economic consequences (Gottwald et al., 2002).

The disease first appeared in Southeast Asia and India, but it is now present in more than 30 countries such as Japan, South and Central Africa, South America, and USA (Das, 2003; Gottwald et al., 2001; Mohammadi et al., 2001; Stall and Civerolo, 1991). Satsuma mandarin, one of the main cultivar of citrus in Korea, is resistant variety against *Xcc* so that citrus canker usually isn't much of a problem in Korea (Kim et al, 2014). However, some varieties were very susceptible to Xcc, such as Sweet orange, Grapefruit and Mexican lime so that they cause severe economic losses (Graham and Gottwald., 1991). In some locations, the efforts to eradicate *Xcc* have failed and continued active eradication campaigns are being performed (Schubert et al., 2001). In contrast, *Xcc* has not been detected in many countries such

as European Union or Australia (Del Campo et al., 2009), where *Xcc* was considered as a quarantine organism and strict regulatory programs were implemented to prevent the spread of citrus canker disease. Consequently, only the disinfected and asymptomatic citrus fruits are allowed to be exported (Golmohammadi et al., 2007). However, because even asymptomatic citrus fruits were suspected of harboring *Xcc*, a completely eradication system is very essential for export.

The use of antibiotic bactericides such as streptomycin and copper sulfate has controlled the Citrus canker disease. However, the continued use of chemical bactericides is limited. Because chemical bactericides are induce the bacterial resistance and harmful to human health and environment (Willianson et al., 2007). Besides, as an eradication measure, methyl bromide (MeBr) has been used for effective control of nematodes, fungi, and insects on more than 100 crops in the world. However, MeBr is identified as an ozone-depleting substance and it may cause serious problems to human health and environment (Bell et al., 1996; Penner, 1999; Watson et al., 1992). Under the Montreal Protocol, developed countries were required to completely phase out MeBr use by 2015 (Fields and White, 2002; Gareau, 2010; Osteen, 2003). Therefore, it is necessary to develop an eco-friendly quarantine treatment for replacing the use of chemicals.

Recently, the use of ionizing radiation as a promising phytosanitary treatment is increasing all over the world (Hallman, 2011). Thousands of researches have reported that irradiation enhances the quality, safety, and marketability of fruits and vegetables (Abu-Tarboush et al., 1997; Jeong, 2014; Mostafavi et al., 2013). Irradiation does not leave any residues and does not make food harmful for

consumers. Also, comparisons of fruit quality against traditional MeBr fumigation showed that irradiation provided better overall quality than MeBr fumigation (Drake and Neven, 1997).

The sterilization mechanisms for irradiation of living cells are well known (Aquino, 2012). Irradiation causes injury to plant cells, insects or microorganisms on the produce due to gamma irradiation, e-beams, or X-irradiation (Kader, 1986). Ionizing radiation exerts its effect directly by breaking the chromosomal DNA and indirectly by producing oxygen radicals which can disrupt membranes and interfere with the DNA (Elgazzar and Kazem, 2015; Niemira, 2003). Irradiation treatment up to 10 kGy has been considered a safe and effective technology since 1981 by several international food organizations (World Health Organization, 1981). In fact, its application can eliminate the pathogenic organisms without causing any toxicological hazard and loss of quality of fruits (World Health Organization, 1981; Youssef, 1994).

It has been reported that ionizing radiation of citrus fruits prevents decay and extends the shelf-life (Mahmoud et al., 2010). However, the effect of X-irradiation on *Xcc*, which is important for citrus quarantine, has not been reported. The objective of this study was extended to evaluate the *in vitro* antibacterial activity of X-irradiation against *Xcc* so that determine the X-irradiation sensitivity. The quarantine system using X-irradiation could be applied to the citrus fruits being exported. For this purpose, we determined the accurate dose of X-ray which completely eliminates *Xcc* either in the suspension or on the surface of Satsuma mandarin fruits.

Ⅱ. MATERIALS AND METHODS

1. Isolation of the pathogen

The bacterium was isolated from infected leaves of Satsuma mandarin showing necrotic raised lesions at a citrus orchard in Topyung, Jeju. The experiments were carried out in a clean bench to prevent secondary contamination. Edges of a citrus canker lesion on a leaf were cut into small pieces (5×5 mm) with a blade. The surface of leaf disks were sterilized with 1% sodium hypochlorite solution (NaClO) for 30 sec, rinsed in sterile distilled water three times each for 1 min. Then, the samples were sterilized again with 70% ethanol for 30 sec and likewise rinsed. The samples were divided into four parts and placed in the micro tube filled with 5% of peptone solution. They were shaken at room temperature for 2 h at 70 rpm by using a shaker (CR300, Finemould Precision Ind. Co., Korea). An aliquot of 100 μl of the solution was inoculated into tryptic soy agar medium (TSA: Becton, Dickinson and company, France) and incubated at 28℃ for 48 h. The stock cultures were maintained on 20% glycerol at -80℃.

The bacteria were determined by sequence analysis of ribosomal DNA for identification of *Xcc*. The genomic DNA was extracted from the bacteria using a genomic DNA extraction kit (Dneasy Blood & Tissue Kit 56, QIAGEN™, Germany) according to manufacturer's instruction. PCR amplification of the internal transcribed spacer region (ITS) of 16S/23S ribosome was carried out using primer 2

(5’-CACGGGTGCAAAAAATCT-3’) and primer 3 (5’-TGGTGTCGTCGCTTG TAT-3’) (Hartung et al., 1993). Polymerase chain reaction (PCR) was performed in volume of 40 μl containing final concentrations of 10x PCR buffer (iNtRON Biotechnology Inc., Seoul, Korea), 2.5 mM dNTP, 0.5 μM each primer (2 and 3), 10 ng of template DNA, and 5.0 U of *Taq* DNA polymerase (iNtRON Biotechnology Inc., Seoul, Korea). PCR amplifications were run in Thermal Cycler TP600 (TaKaRa, Japan) with an initial denaturation step at 94℃ for 3 min; followed by 36 cycles of denaturation at 94℃ for 45 s, annealing at 60℃ for 45 s and elongation at 72℃ for 45 s; plus a final extension at 72℃ for 5 min. The PCR products were visualized on 1% agarose gels stained with 0.01% ethidium bromide under ultraviolet light (I-MAX-H250, coreBiosystem, Seoul, Korea). Nucleotide sequences of the PCR products were identified at a commercial analysis service (Macrogen Inc., Seoul, Korea). The sequences were compared to those in the GeneBank database using the NCBI BLAST program.

The pathogen were identified as *Xcc*, then they were grown on TSA medium at 28℃ for 24 h. *Xcc* on TSA medium were suspended with 10 ml of distilled water. Then, the concentration of suspension was adjusted to 1×10^7 cfu/ml for inoculation.

2. Semi-selective medium

A semi-selective medium (SSM) was used to detect *Xcc* selectively (Dezordi et al., 2009). SSM was prepared by adding the following compounds: peptone 5.0 g, beef extract 3.0 g, sucrose 5.0 g, soluble starch 10.0 g, agar 15.0 g, CaCl2 0.25 g, Tween 80 10.0 ml, 1% crystal violet solution 150 μl in 1 L total volume with distilled water. And cephalexin 50.0 mg, methyl thiophanate 10.0 mg, and chlorothalonil 10.0 mg were added after autoclaving the culture medium.

3. X-irradiation of the suspension of *Xcc*

The stored culture of *Xcc* was inoculated into tryptic soy broth medium (TSB: Becton, Dickinson and company, France) and incubated at 28℃ for 48 h at 120 rpm using a shaking incubator (HB-201SL, Hanbaek Scientific Co., Korea). An aliquot of 10 ml of the suspension was transferred to a conical test tube and the concentration was measured by a UV-visible spectrophotometer (Optizen POP, Mecasys co., Korea) at a wavelength of 600 nm. The final concentration was adjusted to 1 x 10^7 cfu/ml with sterile distilled water for the experiment. In 3 independent trials, the suspensions of *Xcc* were irradiated using a X-irradiator (10MeV Linear Electron accelerator, MB10/8-635, Mevex, Canada) in GeV Korea. All 3 tubes were exposed to different absorbed doses of 0, 50, 100, 150, 200, 250, 300, 350, and 400 Gy at a dose rate of 0.7 kGy/h. Alanine dosimeters (ES 200-

2106/E2044562, Bruker Biospin, Germany) were used for calibration of the absorbed doses. Following treatment, both irradiated and non-irradiated suspensions of *Xcc* were serially diluted with sterile water. Then 100 μl of each dilute was spread on SSM in triplicate. After incubation at 28**℃** for 3 days, the number of colonies was counted.

4. X-irradiation of citrus fruits

To assess the strength of the doses needed to eradicate *Xcc* on the surface of citrus fruits, the following experiments were carried out. Satsuma mandarin fruits were purchased from a local market in Jeju. The fruits measuring about 60 mm of diameter were selected for the experiments. Fruits were washed under running water, and then dried and cleaned to remove any dirt and microorganisms. The fruit samples were sprayed with *Xcc* suspension at a concentration of 1 x 10^7 cfu/ml and 0.01% Tween 20 evenly until dew moist. After the fruits were completely dried at room temperature, the citrus fruits were exposed to X-irradiation at absorbed doses of 0, 30, 50, 100, 150, 200, 250, and 300 Gy in the same way as the *Xcc* suspension mentioned above. After irradiation, the fruits were placed in a beaker which was filled with sterile water until the fruits were submerged and shaken at 100 rpm at 28℃ for 2 h. Then 100 μl of the washing solution was prepared by plating on the SSM and incubated at 28℃ for 3 days.

5. Determination of the D_{10} value

The radiation sensitivity of *Xcc* was measured by the D_{10} value which is defined as the radiation dose required reducing the microbial population by 90%. After incubating the suspension or the washing solution of *Xcc* at 28℃ for 3 days, the colony forming units (CFU) of *Xcc* were counted. The D_{10} value was determined by the survival curve method. The number of CFU per sample was plotted as $\log_{10}$ against radiation doses. Each experiment was carried out three times and the D_{10} value was generated. The slopes of the individual survivor curves were calculated with linear regression using a graphic program of Microsoft Excel.

6. Detection of *Xcc* on the surface of asymptomatic fruits by colony PCR

Asymptomatic Satsuma mandarin fruits were obtained from citrus canker infected orchards. The microorganisms on the surface of the fruits were isolated on the SSM using the same methods by which *Xcc* was isolated from the X-irradiated fruits. Among various growing epiphytic bacteria, 6 colonies per plate were randomly selected, spread on TSA media, and incubated at 28℃ for 3 days.

Identification of the isolated epiphytic bacteria was performed by colony PCR. Colony PCR is a very easy to perform, effective technique that allows rapid amplification of DNA fragments and screening of a large number of bacterial colonies (Cao et al., 2009). For direct PCR amplification, the PCR reaction mixtures contained 1x PCR buffer, 1.5 mM of $MgCl_2$, 1.0 unit of *Taq* DNA polymerase

(Invitrogen Corp, San Diego, CA, USA), 250 µM of each dNTP, 3.6 mM of each primer Xac01 (5'-CGC CAT CCC CAC CAC CAC CAC GAC-3') and Xac02 (5'-AAC CGC TCA ATG CCA TCC ACT TCA-3') (Coletta-Filho et al., 2006), and distilled water up to a total volume of 20 µl. All isolated bacteria stored in the Eppendorf tube were centrifuged at 12,000 rpm for 5 min. The bacterial pellet was diluted with 1 ml of sterile water, of which 1 µl was directly added to the PCR tubes as a DNA template. PCR amplification and analysis of the nucleotide sequences were carried out by the same methods as those used for identification of *Xcc* mentioned above.

Ⅲ. RESULTS AND DISCUSSION

1. Effect of X-irradiation on *Xcc*

In order to determine the effect of X-irradiation on *Xcc,* the *Xcc* suspension was irradiated with absorbed doses ranging from 0 to 400 Gy of X-ray. The number of colonies of *Xcc* was significantly decreased with increasing radiation doses of X-ray and *Xcc* was completely inactivated at 400 Gy, which was the total lethal dose (Fig. 1, Table 1). In addition, to determine the dose of X-irradiation for application in quarantine, Satsuma mandarin fruits inoculated with *Xcc* were irradiated with various doses ranging from 0 to 300 Gy. Similar to that in the *Xcc* suspension, in the washing solution from the inoculated fruits, the number of surviving *Xcc* was decreased with the increasing level of X-irradiation (Table 2). However, the total lethal dose was > 250 Gy for the surface of citrus fruits, which was lower than that for the *Xcc* suspension (Tables 1 and 2). Probably, it need stronger X-irradiation to sterilize *Xcc* in the suspension which contained higher concentration in of *Xcc* than that on the surface of the citrus fruit.

It is well known that ionizing radiation results in inactivation of microorganisms including insects, fungi, bacteria and viruses. (Aquino, 2012). Some bacterial populations such as those of *Escherichia coli*, *Salmonellae.* spp, and *Campylobacter jejuni* were reduced with increasing doses of γ-irradiation. They were

completely inactivated at 2.5 kGy, the total lethal dose (Clavero et al., 1994; Lee et al., 2006). In our previous study, *Xcc* was very sensitive to γ-irradiation and its total lethal dose was between 300 and 400 Gy (Kim et al., 2014). This dose was lower than that for *E. coli* which is known to be a microorganism sensitive to radiation (Mayer-Miebach et al., 2005). Also, in this study it was demonstrated that *Xcc* was very sensitive to X-irradiation, as it is to γ-irradiation (Table 1). Additionally in case of mold strains, the lethal doses for *Rhizopus stolonifer*, *Botrytis cinerea*, *Botrytis elliptica,* and *Aspergillus flavus* were 2.8 kGy, 4 kGy, 2 kGy, and 10 kGy, respectively (Aquino et al., 2005; Jeong et al., 2014; Kim and Yun, 2014; Yoon et al., 2014), which were higher compared with that for *Xcc*. This high sensitivity of *Xcc* to ionizing radiation may be useful for its application in agricultural production in which elimination of *Xcc* is required for export to the *Xcc*- free countries.

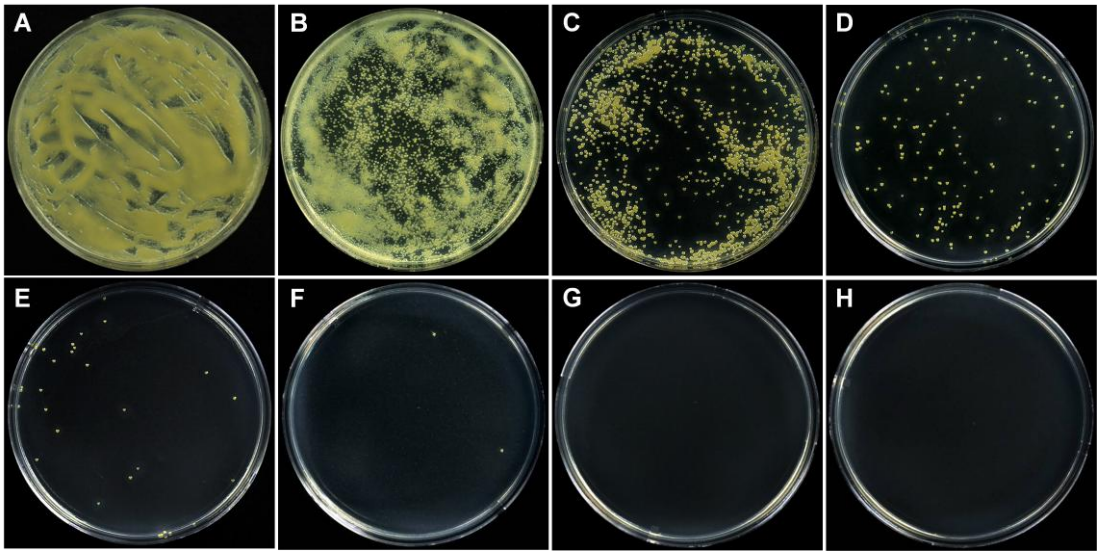

Fig. 1. Suppression of colony formation in the suspension of *Xcc* on semi-selective medium. The suspension of *Xcc* was exposed by 0 (A), 100 (B), 150 (C), 200 (D), 250 (E), 300 (F), 350 (G) and 400 (H) Gy of X-irradiation. The plates were spread with 100 μl of the bacterial suspension and incubated at 28℃ for 3 days. The concentration of the *Xcc* suspension was 1 x 10^7 cfu/ml.

Table 1. Suppression of *Xcc* population in the suspension by irradiation with different X-ray doses

Dose of X-ray (Gy)	Number of *Xcc*[a] (Log cfu/ml)	Inhibition rate[c] (%)	Duncan's test[d] (*p=0.001*)
control	6.9 ± 0.2[b]	-	a
50	5.5 ± 0.5	20.3	b
100	4.4 ± 0.5	36.2	bc
150	3.8 ± 0.3	44.9	cd
200	2.8 ± 0.6	59.1	de
250	2.0 ± 0.7	71.0	e
300	0.7 ± 0.5	89.9	f
350	0.4 ± 0.4	94.2	f
400	0.0 ± 0.0	100.0	f

[a]The concentration of the suspension of *Xcc* was 1 x 10^7 cfu/ml.

[b]Means ± standard deviation from 3 separate experiments containing 3 replication of *Xcc* suspensions in each experiment.

[c]Inhibition rate (%) = [1 – Log (cfu/ml) after X-irradiation/Log (cfu/ml) before X-irradiation] x 100

[d]Means followed by different letters in the same column differ significantly according to Duncan's multiple range test (DMRT).

Table 2. Suppression of *Xcc* population in the washing solution from Satsuma mandarin fruits with different doses of X-irradiation

Dose of X-ray (Gy)	Number of *Xcc*[a] (Log cfu/ml)	Inhibition rate[c] (%)	Duncan's test[d] (*p=0.001*)
control	3.1 ± 0.1[b]	-	a
30	2.4 ± 0.3	22.6	ab
50	2.2 ± 0.2	29.0	b
100	1.6 ± 0.1	48.4	c
150	0.8 ± 0.3	74.2	d
200	0.2 ± 0.3	93.6	d
250	0.0 ± 0.0	100.0	d
300	0.0 ± 0.0	100.0	d

[a]The concentration of the suspension of *Xcc* was 1 x 10^7 cfu/ml.

[b]Means ± standard deviation from 3 separate experiments containing 3 replication of washing solutions in each experiment.

[c]Inhibition rate (%) = [1 – Log (cfu/ml) after X-irradiation/Log (cfu/ml) before X-irradiation] x 100

[d]Means followed by different letters in the same column differ significantly according to Duncan's multiple range test (DMRT).

2. D_{10} value for *Xcc*

To describe the effect of X-irradiation on *Xcc* more clearly, the D_{10} values for both *Xcc* suspension and washing solution were calculated by the survival curves. The number of surviving *Xcc* after X-irradiation was expressed as $\log_{10}$ cfu/ml and plotted against the irradiation dose (Fig. 2). The D_{10} value for *Xcc* in the suspension was 69 Gy (Fig. 2A), whereas the D_{10} value for *Xcc* in the washing solution was 97 Gy (Fig. 2B). The higher D_{10} value for *Xcc* on the surface of Satsuma mandarin may be caused by the lower water content rather than those in the suspension. Normally, microorganisms are resistance to irradiation in dry condition in which free radicals forming from H_2O molecules is lower than in moisture condition (Aquino, 2012). The slope of the graph indicated that inactivation of *Xcc* was directly correlated to the increasing X-irradiation dose.

In this study, it was observed that the D_{10} value for *Xcc* was relatively lower than that of other microorganisms (Fig. 2). For example, the D_{10} value for *E. coli*, *Salmonella* spp., and *Yersinia enterocolitica* was 360, 610, and 150 Gy, respectively (Sommers and Boyd, 2006). Also, in the case of mold strains such as *A. flavus, B.cinerea,* and *Curvularia geniculata*, their D_{10} values, i.e. between 1.0 to 2.5 kGy, were higher than that of *Xcc* (Maity et al., 2011; Yoon et al. 2014). Normally, the D_{10} values for viruses are higher than those of bacterial strains or fungi, which were calculated to range from 3 to 5 kGy (Grieb et al., 2005). These differences in irradiation sensitivity of pathogens may be due to their chemical and physical structure or their ability to recover from the radiation injury (Aquino, 2012; Farkas,

2006).

On the other hand, it should be assessed whether the quality of citrus fruits can be negatively affected by irradiation. Application of ionizing radiation is increasingly being accepted for reducing the risk of pathogenic bacteria in foods. Irradiation dose up to 10 kGy is considered safe and does not affect the nutritional quality of foods (World Health Organization, 1981; Youssef, 1994). Irradiation at a dose of 1 to 3 kGy can be used to enhance the microbial safety without a significant loss in the quality attributes of Chinese cabbage (Ahn et al., 2005). Moreover, there was no significant increase in the mutagenicity between irradiated and non-irradiated fruits (Van Kooij et al., 1978).

These differences of irradiation sensitivity on quality of fruits and vegetables may be correlated with DNA sequences and cell sizes (Aquino, 2012). Loss of vitamin was almost occurred by the increasing levels of radiation. Even at the low doses of 0.3 to 0.75 kGy, vitamin C in fruits was destroyed up to 11 % and, vitamin B1 and vitamin E are also reduced after exposure to commercial levels of irradiation range from 1 kGy to 4.5 kGy (Mitchell et al., 1992). Moreover, higher levels of irradiation were occurred destruction of vitamin A and K in food (Stevenson, 1994).

So most pathogens should be necessary to use of combination of irradiation with other treatments such as hot water, chlorination, Ag nano-particle and nano-silica silver. However, *Xcc* of low dose of irradiation was considered unnecessary the combined treatments. Also effects of low dose irradiation for quality maintenance in vegetables and fruits have gained increasingly importance in agricultural industry (Farkas, 1998; Fan, 2012). In our study, the low D_{10} value at 300 Gy of *Xcc* would be

a prerequisite for application of irradiation as a phytosanitary treatment to eradicate the bacteria.

In the case of citrus fruits, no differences in quality of fruits were detected after an X-irradiation dose of even 300 Gy (Jeun et al., 2015), which is also the recommended dose of ionizing radiation for eradication of *Xcc*. Therefore, a low D_{10} value of 300 Gy for *Xcc* may be a prerequisite for the application of irradiation as a phytosanitary treatment to eradicate *Xcc*.

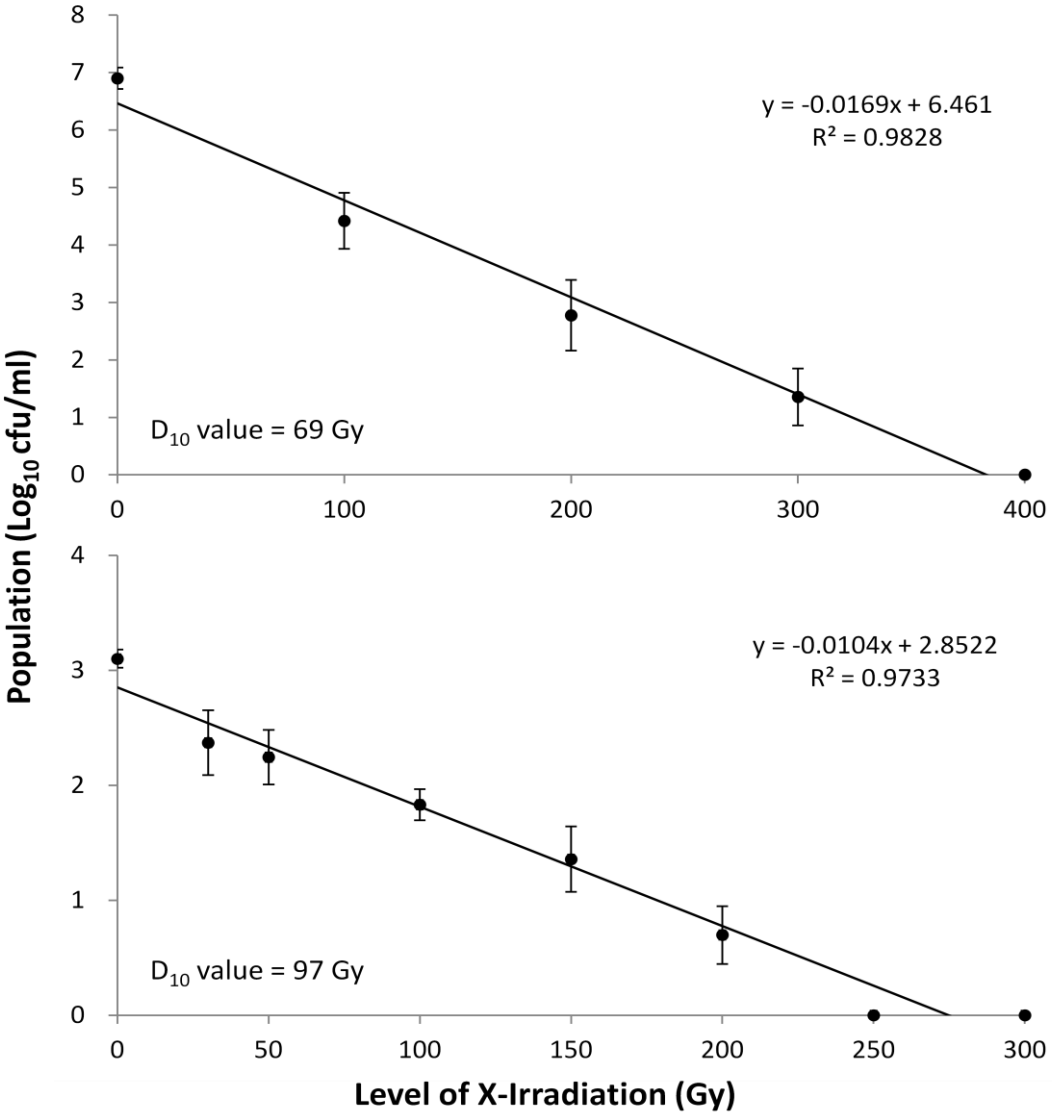

Fig. 2. Survival curves of *Xcc* in the suspension (A) and in the washing solution from Satsuma mandarin fruits inoculated with *Xcc* (B) after X-irradiation with various doses. The vertical bars indicated the standard deviation of three separating replications of each experiment.

3. Detection of *Xcc* on the surface of asymptomatic fruits by colony PCR

To verify the presence of *Xcc* on the surface of asymptomatic Satsuma mandarin fruits obtained from citrus canker-infected orchards, microorganisms from the surface of the fruits were identified by colony PCR. Colony PCR is a method for amplifying DNA fragments by PCR using the single colony of organisms without isolating pure DNA. It is a rapid, reliable, and highly accurate detection method, and it is suitable for screening a large number of environmental isolates (Sheu et al., 2000).

Yellow colonies were randomly selected to evaluate the presence of *Xcc* on asymptomatic Satsuma mandarin fruits (data not shown). Each isolated colony was transferred onto TSA media and used as a DNA template. Total 4 among the 54 selected colonies were identified as *Xcc* by colony PCR using primers Xac01 and Xac02 (Fig. 3). The results proved the presence of *Xcc* on asymptomatic citrus fruits, which has the potential ability to spread to susceptible citrus trees causing citrus canker disease. Similar results were reported, which suggested that amplification was observed in asymptomatic leaves of ‘Natal’, ‘Pera’, and ‘Valencia’ sweet orange varieties in *Xcc*-infected areas (Coletta-Filho et al., 2006). Also, *Xcc* was detected in 67 out of the 90 samples from leaves with and without symptoms, which were collected from canker-infected pomelo orchards (Kositcharoenkul et al., 2011). Therefore, there is a need for treatment of the citrus fruits being exported so that *Xcc* bacteria present on the citrus fruits are completely eradicated. Based on these results, X-irradiation as a sterilization strategy is the best way to eliminate all *Xcc* bacteria

present even on asymptomatic citrus fruits.

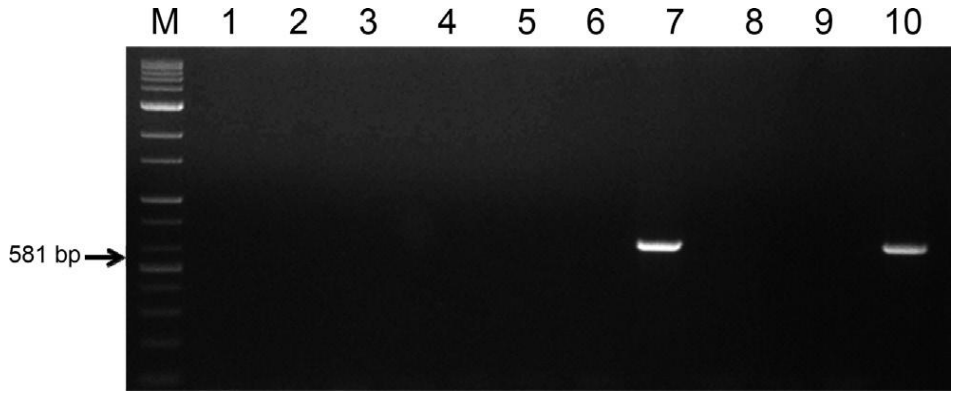

Fig 3. Detection of *Xcc* in washing solution from non-symptomatic fruits using colony PCR. Lanes: 1-kb DNA ladder (BIOFACT, Co. Ltd.) (M), epiphytic bacteria from asymptomatic Satsuma mandarin fruits (1-9), *Xcc* (10). Arrow indicates the *Xcc* specific fragment.

Ⅳ. 적　　요

감귤 궤양병은 *Xanthomonas citri* subsp. *citri* (*Xcc*)에 의해 발생하는 중요한 세균병이다. 감귤 궤양병은 유럽과 오스트레일리아를 포함한 여러 나라에서 발생되지 않았기 때문에 병의 확산을 막기 위하여 철저하게 규제되고 있다. 감귤궤양병을 방제하기 위해서 스트렙토마이신, 보르도액과 같은 화학농약이 사용되고 있으며, 소독훈증제로 메틸브로마이드가 사용되고 있다. 그러나, 최근 몬트리올협약이 성립됨으로써 2015년에는 메틸브로마이드의 사용이 완전히 규제되고 있다. 이에 따라 친환경적인 대체방안으로써 이온화에너지의 적용에 대한 관심이 부상하고 있다. 이 연구에서는 이온화에너지를 적용하여 궤양병균 현탁액과 과실표면에 접종된 궤양병균에 대하여 엑스선의 효과를 관찰하였다. 궤양병균 현탁액을 10^7의 농도로 조절한 후 50Gy 부터 400Gy까지 여러 선량으로 엑스선 조사한 결과, 400Gy의 선량에서 완전히 억제되었다. 또한, 검역에 있어서 엑스선의 효과를 규명하기 위해 감귤궤양병균을 접종한 과실을 사용하여 50Gy 부터 400Gy까지 여러 선량으로 엑스선 조사하였다. 그 결과, 감귤궤양병균은 과실 표면에서 250Gy의 선량으로 완전히 억제되었다. 감귤궤양병균의 엑스선에 대한 D_{10} 값은 97Gy로 측정되었다. 이러한 결과들은 감귤 수출 시 다른 추가적인 소독처리과정 없이 오직 엑스선만을 사용하여 검역에 적용될 수 있다는 가능성을 제시하고 있다. 추가적으로 감귤궤양병이 감염되어 있는 과수원에서 감귤궤양병균 병징을 나타내지 않는 과실을 사용하여 실험을 하였다. 그 결과, 병징을 나타내고 있지 않는 과실에도 감귤궤양병균이

존재한다는 사실을 발견하였다. 따라서 수출 과실에서는 감귤궤양병균의 완전한 박멸을 위한 처리가 필수적으로 적용되어야 한다고 생각된다. 이러한 결과들을 바탕으로, 이온화에너지는 감귤 수출을 위한 감귤궤양병균을 멸균하는 방법으로 적합하다고 고려할 수 있겠다.

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