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Effects of ultraviolet-c treatment on growth and mycotoxin production by Alternaria strains isolated from tomato fruits

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ABSTRACT

Large amounts of tomato fruits and derived products are produced in China and may be contaminated by *Alternaria* mycotoxins, which may have the potential risks for human health. There is thus an increasing interest in reducing the mycotoxins. In the present study, 26 *Alternaria* strains isolated from tomato black rots were identified according to morphological and molecular grounds, and their mycotoxigenic abilities for alternariol (AOH), alternariol monomethyl-ether (AME) and tenuazonic acid (TeA) were also investigated. The results showed that *A. alternate* was the predominant species with incidence values of 65.4% (17/26), followed by *A. brassicae* (7/26) and *A. tenuissima* (2/26). *A. alternate* isolates showed the highest capacity for AOH, AME and TeA production among the studied isolates either *in vitro* or *in vivo*, suggested that *A. alternata* may be the most important mycotoxin-producing species in tomato fruits. Thus, UV-C irradiation was used to reduce the mycotoxin produced by *A. alternata* in our study. The results showed that low dose of UV-C irradiation (0.25 kJ/m^2) could effectively inhibit mycotoxins production and penetration in tomatoes. Upon treatment with UV-C, there was 79.6, 76.4 and 51.4% of reduction in AOH, AME and TeA penetration when compared to untreated fruits. This may be associated with the enhanced phenolics by UV-C irradiation. In fact, the induced phenolics were including *p*-coumaric, ferulic and pyrocatechuic acids, of which *p*-coumaric acid (1.0 mM) displayed the highest reduction of TeA with 60.2%, whereas ferulic acid (1.0 mM) showed strong inhibitory effects on the AOH and AME production by 59.4 and 79.1%, respectively. Therefore, the application of UV-C irradiation seems to be a promising method for reducing the potential risk of *Alternaria* mycotoxins in fruits and also for enhancing phenolics of processing products.

1. Introduction

Tomatoes (*Solanum lycopersicum*) are widely consumed as fresh and pastes, sauces, ketchups and juices in diets and the health benefits of consumption may contribute to the lower incidence of the cancers and cardiovascular diseases due to their high antioxidant activities (Bravo et al., 2012; Liu et al., 2018). However, fresh tomatoes are very easily infected by *Alternaria* species that cause the black rots of fruits in the field and during harvest and storage (Somma et al., 2011). Fruit decays not only reduce yields quantitatively, but also deteriorate the quality and nutritive values of fruits due to the production of *Alternaria* mycotoxin, such as alternariol (AOH), alternariol monomethyl ether (AME) and tenuazonic acid (TeA). Exposure to these toxins may cause genotoxic, mutagenic, carcinogenic, and cytotoxic effects on both human and animals (Wang et al., 2017). Recently, a widespread natural occurrence of *Alternaria* mycotoxins, sometimes even in very high amounts, has been reported in tomatoes as well as their derived products worldwide (Lopez et al., 2016; Terminiello et al., 2006; Van de Perre et al., 2014; Zhao et al., 2015). Therefore, it is important to identify the mycotoxigenic abilities of fungi in order to evaluate the potential risks for human health related to the consumption of fresh tomatoes or their derived products.

Recent investigations have explored the availability of mycotoxin produced by *Alternaria* strains isolated from tomatoes (Andersen et al., 2015; Meena et al., 2017; Somma et al., 2011), suggesting that *Alternaria* species can occur in different habitats and geographical regions, and may thus have different metabolite profiles and toxic potential. However, no information is available for the toxicogenic potential of

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Alternaria strains isolated from tomatoes cultivated in China, although *Alternaria* mycotoxins with the highest concentrations of 1.7 mg/kg have been found in China commercial tomato byproducts (Zhao et al., 2015).

China is the main consumer, producer, and trader of tomatoes in the world, and most of China production is exported as processed byproducts, mainly to the European Union and Turkey. Taking into account the increasing significance and potential risks of *Alternaria* mycotoxin, it is urgent to find an effective method to control postharvest diseases and inhibit the mycotoxin production. UV-C treatment, among the variety of non-chemical approaches, has been demonstrated to be an ideal method in postharvest decay management, especially in reducing the mycotoxins, including aflatoxins, patulin, ochratoxin A and deoxynivalenol (Isman and Biyik, 2009; Jubeen et al., 2012; Popović et al., 2018; Selma et al., 2008; Tikekar et al., 2014). However, the efficacy of UV-C irradiation for removal of *Alternaria* mycotoxin contamination has not been investigated yet.

UV-C irradiation can not only control the disease development, but also induce the biosynthesis of phenolic compounds in tomato fruits (Bravo et al., 2012; Liu et al., 2018). As a product of the secondary metabolism, phenolic compounds are considered to be part of the defense mechanisms in plants (Wang et al., 2017). Moreover, previous studies suggested that these compounds can effectively inhibit the mycotoxin accumulation (Ferruz et al., 2016; Pani et al., 2014; Wang et al., 2017). To our knowledge, limited information is available on the inhibitory effects of phenolics on the *Alternaria* mycotoxin production. Therefore, the aim of the present study was to identify the small-spored *Alternaria* responsible for tomato black rots and their mycotoxigenic ability for AOH, AME and TeA. Furthermore, the effects of UV-C irradiation and induced phenolics to inhibit *Alternaria* mycotoxin production were investigated. The results of this study could be applicable to improve the safety and quality of tomato fruits, and also to extend the shelf life of fruit for commercial purpose.

2. Materials and methods

2.1. Isolation of Alternaria species from tomato fruits

Isolates of *Alternaria* spp. were collected from tomato fruits (*Solanum lycopersicum*) showing black rot symptoms. The rotten fruits were obtained from commercial greenhouse in Beijing, China in 2018. Isolations were carried out from surface-disinfected fruit pieces at the margin of diseased/healthy tissue, which were drenched for 1 min in a 1% sodium hypochlorite solution and washed thoroughly with sterile distilled water for 3 times, and then placed on Petri dishes containing potato dextrose agar (PDA; Becton Dickinson, Franklin Lakes, NJ, USA) amended with streptomycin sulfate (300 mg/L). After 3 to 5 days of incubation at 25 °C, the emerging putative *Alternaria* spp. colonies were transferred to fresh PDA medium and incubated at 25 °C for 7 days. In total 30 single-spore isolates of *Alternaria* spp. were obtained and stored at 4 °C until use.

2.2. Identification of Alternaria spp.

Alternaria spp. isolates were initially identified on the basis of morphological characteristics including shape and structure of conidia, and further confirmed by ITS amplification using universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′). DNA extraction was carried out as described previously (Meena et al., 2017). The PCR amplification mixture (25 μL) contained 1.0 μL DNA template, 1.0 μL each primer (10 μM), 9.5 μL demineralized sterile water, and 12.5 μL GoTaq Master Mix (Promega, Madison, WI, USA) in a T-100 thermal cycler (Bio-Rad, Hercules, CA, USA). The cycling conditions included an initial denaturing step at 94 °C for 10 min, followed by 35 cycles of denaturation at 94 °C for 0.5 min, annealing at 54 °C for 0.5 min, extension at 72 °C for 1 min, and final extension at 72 °C for 5 min. The PCR products were checked by 1% agarose gel electrophoresis. The amplicons were sequenced using the ITS1 and ITS4 primer pairs by Sangon Biotech, Inc. The blast searches were carried out in NCBI nucleotide databases using a nucleotide query. Phylogenetic analyses of all the sequences were performed by maximum likelihood method using DNAMAN 8.

2.3. Preparation of spore suspension

The fungal strain of *A. alternate* FQ 18 was cultured in PDA at 25 °C for 7 days. The plates were then flooded with sterile distilled water containing 0.05% (v/v) Tween 80 and gently rubbed the surface with a sterile glass spreading rod to release spores. Spore suspensions were filtered through four layers of sterile cheesecloth to remove mycelial fragments, and the spore concentration was adjusted to 1×10^5 spores/mL with the aid of a hemocytometer prior to use.

2.4. Inoculation of tomato fruits

Healthy fresh tomatoes (*Solanum lycopersicum* cv. Shengfan 863) were harvested at mature red stage from a commercial greenhouse in Beijing, China. Fruit was selected for uniform size and shape; those with physical injuries or infections were discarded. After surficial disinfection with 0.01% sodium hypochlorite for 2 min and air dried at ambient temperature, tomato fruits were wounded (3 mm deep and 3 mm wide) with a dissecting needle at the equator of each fruit. An aliquot of 20 μL spore suspension of *A. alternata* (1×10^5 spores/mL) was injected into each wound. Afterward, inoculated tomato fruits were stored for 24 h at ambient temperature before UV-C treatment.

2.5. UV-C treatment

Tomato fruits were randomly grouped into five lots. Four lots of fruits were treated by UV-C as previously described (Selma et al., 2008). Specifically, the wound-inoculated tomato fruits were placed on trays and illuminated with G30T8 germicidal UV-C lamp (Philips, Netherlands) (30 W each lamp, peak output at 254 nm) at a distance of 30 cm. The radiation intensity of the lamps was measured by a UV digital radiometer (LS-125, Shenzhen Linshang Technology Co. Ltd., China). Each lot of fruits was exposed to UV-C radiation for 12 s (0.25 kJ/m^2), 24 s (0.50 kJ/m^2), 36 s (0.75 kJ/m^2) or 48 s (1.0 kJ/m^2), respectively. To provide uniform irradiation, the treatment chamber was covered with a protective reflecting inner layer, which enhanced homogeneous distribution of the emitted light and allowed indirect illumination of practically all sides of the chamber. The fifth lot of fruits without UV-C treatment served as the control. Following treatments, both control and UV-C treated fruits were stored in the 20 °C for 11 days. Each treatment consisted of three replicates with 50 fruits per replicate and the experiment was repeated three times. The lesion spot was measured average of two perpendicular radii (mm) of each tomato. The lesion tissue and pericarp of the healthy tissue (0–1.0 cm) around rotten part in tomato fruits was cut into small pieces, frozen in liquid nitrogen immediately, and then stored at −80 °C. The lesion tissue was used to mycotoxins extraction and the healthy tissue (0–1.0 cm) around rotten part was used to the quantification of mycotoxin and phenolic acids.

2.6. Extraction and quantification of Alternaria mycotoxins

Analysis of *Alternaria* mycotoxins *in vitro* was conducted as described previously by Meena et al. (2017). The extractions of these mycotoxins were carried out on Potato Dextrose Broth (PDB) medium by using 7 day old cultures. Three agar plugs (3 mm) were cut from the center of each *Alternaria* colony and inoculated in 50 mL PDB medium for 7 days at 25 °C, in the dark. A 5.0 mL aliquot of PDB was extracted with 5.0 mL ethyl acetate containing 1% formic acid by sonication for 60 min. The extracts were evaporated to dryness at 50 °C under a gentle nitrogen stream, and redissolved with 1 mL of acetonitrile/water (3:7, v/v). The obtained solution was forced through a 0.22 µm PTFE membrane filter (Pall, MI, USA) prior to UPLC-MS/MS analysis. Certified standards of mycotoxins, namely alternariol (AOH), alternariol monomethyl ether (AME) and tenuazonic acid (TeA) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

For assessment of *in vivo* mycotoxin production, the lesion tissue and the healthy surroundings (−1.0 cm) in artificially inoculated tomato fruits cut into small portions with a knife and homogenized with a blender at room temperature. Mycotoxins were extracted and analyzed according to our previous study (Wang et al., 2016) with minor modification. Briefly, an amount of 5.0 g of fine homogenized samples were mixed with 5 mL of 50 mM citric acid, and then 20 mL acetonitrile was added and the mixture was homogenized with a high-speed blender (Ultra-Turrax T25, IKA, Staufen, Germany) for 3 min. After the addition of 2.0 g NaCl, the mixture was centrifuged at 10,000 rpm for 5 min. A 4.0 mL aliquot of upper acetonitrile layer was passed through the homemade SPE cartridge and collected. Finally, the cleanup extract was evaporated to dryness at 50 °C under a gentle nitrogen stream, and reconstituted with 1 mL of acetonitrile/water (3:7, v/v). The obtained solution was forced through a 0.22 µm PTFE membrane filter (Pall, MI, USA) prior to UPLC-MS/MS analysis.

The UPLC-MS/MS analysis method of AOH, AME and TeA was estimated according to our previous study (Wang et al., 2016). Quantification was completed according to the standard curves generated from individual compounds in serial dilutions (1–200 ng/mL).

2.7. Extraction and quantification of individual phenolic compounds

Phenolic acid extraction followed the method reported by Crupi et al. (2013) with some modification. Before analysis, the pericarp of the 1 cm around lesion tissue of tomato fruits were grounded to a fine power under liquid nitrogen, and the powder was divided into three subsamples. An amount of 5.0 g of pericarp samples were extracted with 20 mL of 80% (v/v) methanol containing 1% hydrochloric acid. The resultant mixture was ultrasonicated for 30 min at room temperature and then centrifuged at 10,000 rpm for 10 min. The supernatant was collected and the above extraction was repeated twice more. The combined supernatant was transferred to a 50 mL volumetric flask, diluted with extracting solution to volume, mixed, and then filtered through 0.22 µm PTFE membranes (Pall, MI, USA) prior to UPLC-MS/MS analysis. The determination method of individual phenolic compounds was according to our previous study (Wang et al., 2017). Quantification was completed according to the standard curves generated from individual compounds in serial dilutions (1–500 ng/mL).

2.8. The inhibition effects of natural phenolic acids on mycotoxin production

The inhibition effect of natural phenolic acids on mycotoxin production was conducted according to the method described by Wang et al. (2017) with some modification. Natural phenolic compounds (ferulic, *p*-coumaric, pyrocatechuic and chlorogenic acids) were added aseptically to the 50 mL of sterile PDB medium to obtain 1 mM of the final concentration. The spore suspensions were also inoculated to the above PDB medium and the final spore concentration was adjusted to 1×10^5 spores/mL. After the incubation for 7 days at 25 °C, PDB containing *A. alternate* mycelia was filtered through Whatman filter No. 1 and the filters were used for mycotoxin determination. Three replicates were performed for the experimental and control group.

2.9. Statistical analysis

All the experiments were performed in triplicate for each treatment and each replicate included three determinations. Non-linear regression was conducted for the determination of half-inhibition concentration. Statistical analyses were performed with SPSS version 23.0 (SPSS Inc., Chicago, IL, USA). Analysis of variance (ANOVA) was carried out to determine the effects of the treatments, and those means were compared by Duncan's multiple range tests. Differences at $P < 0.05$ were considered as significant.

3. Results

3.1. Characterization of Alternaria spp.

A total of 30 *Alternaria* isolates have been obtained from different greenhouse in China. Out of them 26 *Alternaria* isolates were selected in the present study on the basis of their high pathogenic nature. PCR amplification with universal primer pairs ITS1/ITS4 generated a 570 bp fragment from all 26 *Alternaria* strains. The PCR products were sequenced and the BLAST data analyses of sequences revealed that 17 isolates were *A. alternata* (GenBank Accession numbers MH862229-MH879767), 7 were *A. brassicae* (GenBank Accession numbers JF439445-JF439449) and 2 were *A. tenuissima* (GenBank Accession numbers MH032750). The phylogenetic tree, obtained by DNAMAN 8 software using the maximum likelihood method, was clearly clustered into three groups (Fig. 1). The first clade was subdivided into two further clades. All the *A. alternate* isolates were grouped together in the one cluster and only 2 *A. tenuissima* isolates were grouped in the second clade (Fig. 1). In the last group, we found all isolates with sporulation pattern close to *A. brassicae* (Fig. 1). However, molecular analyses for small-spored *Alternaria* have some challenges to overcome as evolutionary differences caused by lineages sorting and recombination (Andrew et al., 2009), and thus morphological characterization was conducted based mainly on the conidial structure and sporulation pattern of the tested isolates using reference strains.

Based on morphological traits, a higher variability in colony morphology was observed on potato dextrose agar (PDA) medium. The 17 isolates identified as *A. alternate* based on molecular analyses produced colonies of dark olivaceous colors. Conidia were ovoid, ellipsoid or obpyriform with 3–5 transverse septa and 0–3 longitudinal septa. Mean

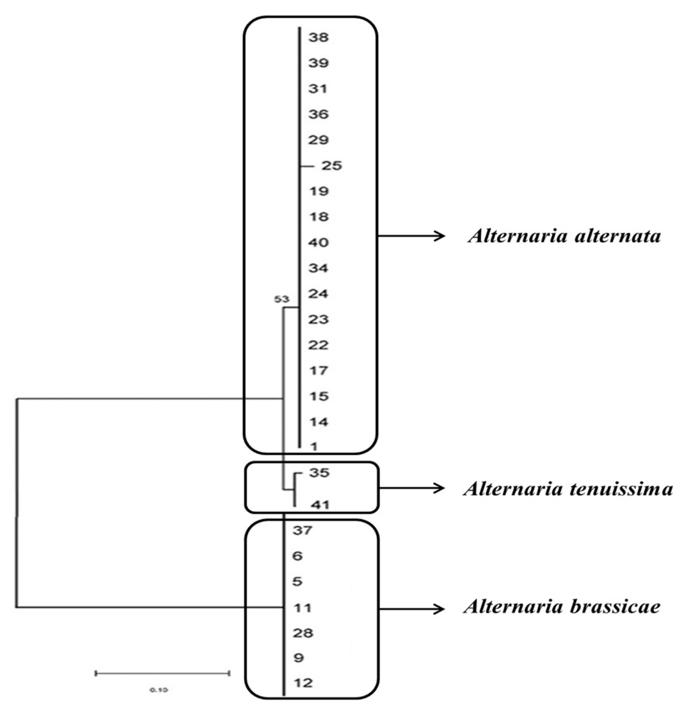

Fig. 1. Phylogenetic tree constructed based on the ITS rDNA sequences using maximum likelihood method. Numbers at the nodes indicate the bootstrap values (> 50%) of 1000 replicates.

conidial length ranged from 24.5 to 37.8 μm. These features matched those of *A. alternata* reference strain ATCC 66981. The 2 isolates identified as *A. tenuissima* produced colonies of olive gray color and the appearance of colonies was cottony. Conidia were ovoid to obclavate with 1–4 transverse and 0–2 longitudinal septa. Mean conidial length ranged from 22.8 to 48.5 μm. Those characters correspond to *A. tenuissima* morphotype. The 7 isolates identified as *A. brassicae* produced colonies of brown, white or olivaceous green color. Conidia were ovoid to obclavate with 6–12 transverse and 0–6 longitudinal septa. Mean conidial length ranged from 56.5 to 104.2 μm. Those characters correspond to *A. brassicae* morphotype.

The combined molecular and morphological identification of *Alternaria* spp. showed that *A. alternate* was the predominant species causing black rot on tomato with incidence values of 65.4% (17/26). *A. brassicae* was the second most common species with frequency of 26.9% (7/26), whereas *A. tenuissima* was identified in frequency of 7.7% (2/26).

3.2. Mycotoxin production by Alternaria spp.

A total of 26 *Alternaria* isolates were analyzed for mycotoxin production after incubation for 7 days either *in vitro* or *in vivo* (Table 1). For each strain, production of alternariol (AOH), alternariol monomethyl-ether (AME) and tenuazonic acid (TeA) were evaluated. Overall, 88.5% of the tested strains were able to produce at least one mycotoxin, of which 17.6% and 11.8% of the *A. alternata* isolates was found to individually produce AOH and TeA, respectively (Supplementary Data, Table S1). Only three strains (2 *A. alternata* and 1 *A. brassicae*) were found not to produce all three tested toxins (Supplementary Data, Table S1). Most of the *Alternaria* isolates were able to simultaneously produce AOH and AME. AOH was produced *in vitro* by *A. alternata*, *A. brassicae* and *A. tenuissima* isolates at a range of 17.3 to 1589.0, 49.8 to 344.1 and 115.3 to 173.7 μg/kg, respectively (Table 1). Similarly, *A. alternata* isolates produced AME at a range of 38.2 to 1132.4 μg/kg, *A. brassicae* isolates produced AME at a range of 17.0 to 103.8 μg/kg and *A. tenuissima* isolates produced AME at a range of 29.5 to 61.1 μg/kg (Table 1). *A. alternata* isolates produced significantly higher mean concentration of AOH and AME than the other two species (Table 1). Compared with AOH and AME, TeA was the most abundant toxin, produced at a mean concentration of 23.6, 29.9 and 13.7 mg/kg by *A. alternata*, *A. brassicae* and *A. tenuissima* isolates, respectively (Table 1). However, only fewer tested isolates can produce TeA (Table 1). Concomitant production of all three mycotoxins was evident in 17.6%, 28.6%, and 50.0% of the *A. alternata*, *A. brassicae* and *A. tenuissima* isolates, respectively (Supplementary Data, Table S1).

Analysis of mycotoxin production in artificially inoculated fruit showed that the mean concentrations of *in vivo* mycotoxin production were significantly higher than those *in vitro* (Table 1). More specifically, the mean AME produced *in vivo* by the *A. alternata*, *A. brassicae* and *A. tenuissima* isolates was 1.9, 4.2 and 6.2 fold higher than those *in vitro* production after incubation for 7 days, respectively (Table 1). However, no difference in the frequency of mycotoxin production was observed between PDB and artificially inoculated fruits (Supplementary Data, Table S1). It is noteworthy that overall, *A. alternata* showed the highest capacity for toxin production compared with the other two species either *in vitro* or *in vivo* (Table 1). The highest concentration of AOH and AME was 1.4 mg/kg *in vivo* produced by *A. alternata* FQ 18 strain, whereas the highest concentration of TeA was 309.1 mg/kg produced by FQ 34 strain, followed by FQ 18 (Supplementary Data, Table S1). Since FQ 18 strain was able to simultaneously produce triple mycotoxins with relative high concentration, it was selected for further analyze the effects of ultraviolet-C (UV-C) on controlling the mycotoxin accumulations in tomato fruits.

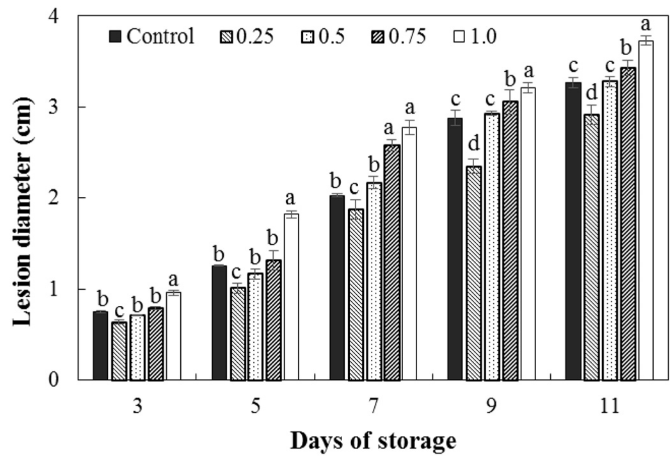

Fig. 2. Effect of UV-C treatment (kJ/m^2) on lesion diameter of tomato fruits inoculated with *A. alternata* FQ 18 strain during storage at 20 °C. Different small letters on the columns of the same day mean significant differences at $P < 0.05$. Statistical significance of the difference was confirmed according to Duncan's multiple range test. Vertical bars indicate ± standard errors. Each value is the mean of three replicates.

3.3. Effect of UV-C treatment on disease development in artificially inoculated fruits

In the present study, 0.25 kJ/m^2 UV-C treatment significantly inhibited the disease development of black rot on ripen tomato fruits wound-inoculated with *A. alternate* FQ 18 strain (Fig. 2). The lesion diameters of black rot on tomato fruits treated with 0.25 kJ/m^2 UV-C were 19.2% and 15.6% smaller ($P < 0.05$) than those on the untreated fruits after storage for 9 d and 11 d, respectively. However, no significant difference was observed on the lesion diameter between

Table 1
Production of alternariol (AOH), alternariol monomethyl-ether (AME) and tenuazonic acid (TeA) on PDB medium (*in vitro*) and on artificially inoculated tomato fruits (*in vivo*) of mycotoxigenic *A. alternata*, *A. tenuissima* and *A. brassicae* isolates originating from tomato fruits showing black rots symptoms after the 7-day incubation period.

Species	Isolates[a]	Medium	AOH			AME			TeA		
			Isolates[b] (%)	Mean (μg/kg)	Range (μg/kg)	Isolates[b] (%)	Mean (μg/kg)	Range (μg/kg)	Isolates[b] (%)	Mean (mg/kg)	Range (mg/kg)
Alternaria alternata	17/26	*In vitro*	76.5	210.2	17.3–1189.0	58.8	167.5	38.2–1132.4	29.4	23.6	10.9–37.0
		In vivo	76.5	300.2	24.5–1403.4	58.8	488.7	35.7–1393.4	29.4	90.9	21.3–309.1
Alternaria brassicae	7/26	*In vitro*	85.7	164.6	49.8–256.9	85.7	64.7	17.0–103.8	28.6	29.9	29.2–30.6
		In vivo	85.7	233.1	155.6–298.8	85.7	335.8	62.6–646.3	28.6	226.4	174.8–277.9
Alternaria tenuissima	2/26	*In vitro*	100.0	144.5	115.3–173.7	100.0	45.3	29.5–61.1	50.0	13.7	13.7
		In vivo	100.0	335.2	101.6–568.8	100.0	326.9	289.9–363.8	50.0	30.8	30.8

[a] Numbers of small-spored *Alternaria* spp. isolates from all 26 *Alternaria* strains.
[b] Percentage of *Alternaria* spp. isolates exhibiting mycotoxin capacity.

Table 2
Effect of 0.25 kJ/m^2 UV-C treatment on mycotoxins production of rotten tissue in tomato fruits inoculated *A. alternata* FQ 18 strain during storage at 20 °C.

Alternaria mycotoxins (mg/kg)	Treatment	Days of storage		
		3	7	11
AOH	Control	0.82 ± 0.03 a	1.40 ± 0.05 a	2.56 ± 0.12 a
	UV-C	0.51 ± 0.02 b	0.79 ± 0.04 b	1.42 ± 0.06 b
AME	Control	0.86 ± 0.04 a	1.39 ± 0.06 a	2.29 ± 0.24 a
	UV-C	0.56 ± 0.01 b	0.88 ± 0.03 b	1.44 ± 0.05 b
TeA	Control	40.39 ± 2.03 a	68.61 ± 2.26 a	106.03 ± 4.67 a
	UV-C	28.07 ± 1.70 b	43.43 ± 3.08 b	67.50 ± 1.72 b

Each value represents means ± standard deviations of three replicates; different small letters in the same storage period within a column mean significant different at $P < 0.05$. Statistical significance of the difference was confirmed according to Duncan's multiple range test. AOH, alternariol; AME, alternariol monomethyl-ether; TeA, tenuazonic acid.

0.50 kJ/m^2 of UV-C and control, whereas there was an increased susceptibility to disease with higher dose (> 0.75 kJ/m^2) of UV-C treatment (Fig. 2). These results suggested that the application of low dose (0.25 kJ/m^2) of UV-C irradiation is effective to control the *Alternaria* rots on the ripen tomato fruits.

3.4. Effect of UV-C treatment on mycotoxin production in tomato fruits

As was expected, after artificial inoculation with the *A. alternate* FQ 18 strain, the *Alternaria* mycotoxins of rotten tissues were increased during storage reaching the maximum level at 11 days of storage, while 0.25 kJ/m^2 UV-C exposures significantly inhibited the mycotoxin accumulation (Table 2). The AOH, AME and TeA levels of rotten part on tomato fruits treated with 0.25 kJ/m^2 UV-C were 44.5%, 37.1% and 34.5% lower ($P < 0.05$) than those on the untreated fruits after 11 days of storage, respectively.

The results in our study also indicated that all three *Alternaria* mycotoxins could transfer from a rotten part of the inoculated fruit into nearly 1 cm of the surrounding sound tissue after 11 day of storage (Fig. 3). Especially for TeA, the level of surrounding tissues was gradually increased along with the expanded areas of decomposing tissue (Supplementary Data, Fig. S1). However, only relative trace amounts of AOH and AME were found in the surrounding tissues until artificially inoculated fruit stored for 11 days. It is noteworthy that low dose of UV-C treatment can significantly inhibit the mycotoxin penetration from the rotten part to surrounding healthy tissues (Fig. 3). Upon treatment with UV-C, there were 79.6%, 76.4% and 51.4% of reduction in AOH, AME and TeA penetration when compared to untreated fruits after 11 days of storage, respectively.

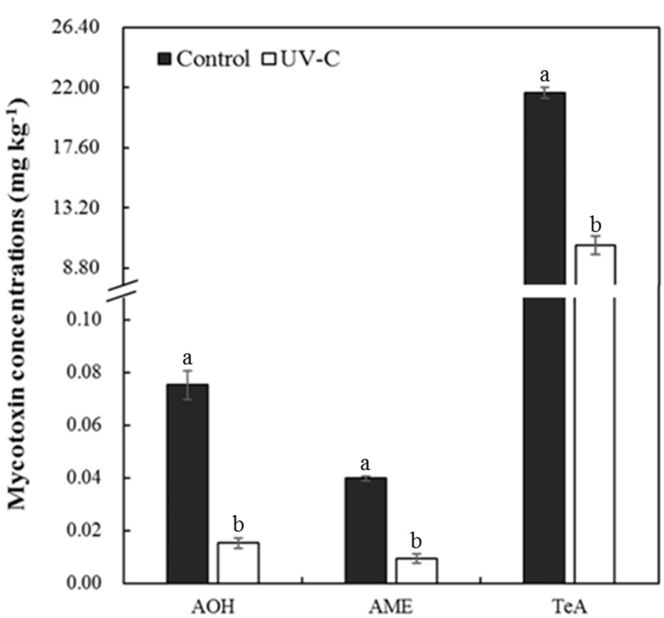

Fig. 3. Effect of UV-C treatment (0.25 kJ/m^2) on mycotoxin levels of surrounding healthy tissues (−1.0 cm) inoculated *A. alternata* FQ 18 strain after 11-day storage at 20 °C. Different small letters on the columns of the same day mean significant differences at $P < 0.05$. Statistical significance of the difference was confirmed according to Duncan's multiple range test. Vertical bars indicate ± standard errors. Each value is the mean of three replicates. AOH, alternariol; AME, alternariol monomethyl-ether; TeA, tenuazonic acid.

3.5. Effect of UV-C irradiation on phenolic acid contents in tomato fruits

Phenolic acids are the most important antioxidant compounds found in tomato fruits and the effect of UV-C irradiation on individual phenolic acids are summarized in Table 3. Regarding the individual phenolic acids, *p*-coumaric acid was the most abundant phenolic compounds in tomato fruits, and the highest content was found at 11 days of storage. Similar increased trend was observed in ferulic acid, whereas it appears that the other phenolic acids increased first and then decreased during storage. In addition, UV-C treatment had an enhancing effect on individual phenolic acids after 11 days of storage except for chlorogenic acid ($P < 0.05$). The *p*-coumaric, ferulic and protocatechuic acid contents of tomato fruits treated with 0.25 kJ/m^2 of UV-C were 8.5%, 12.8% and 15.4% higher ($P < 0.05$) than those of the control fruits after 7 days of storage, respectively. However, UV-C irradiation did not affect the content of *p*-coumaric acid at the early storage period ($P > 0.05$). Moreover, the enhancing effect of UV-C on total phenolic contents was also observed during storage (Table 3).

3.6. Effect of phenolic acid on mycotoxin productions

Previous study had shown that phenolic acids can be considered as mycotoxin inhibitors (Pani et al., 2014; Wang et al., 2017), and thus the anti-mycotoxigenic effects of phenolic acids found in tomato fruits were evaluated. As shown in Table 4, the ferulic acid showed strong inhibition effects on the AOH and AME production by 59.4% and 79.1%, respectively, followed by pyrocatechuic acid. Although *p*-coumaric acid displayed the highest reduction of TeA with 60.2%, it showed a slight inhibition of AOH and AME by 14.3% and 12.5%, respectively. In addition, the low inhibition effects on mycotoxin production were obtained by chlorogenic acid, which only caused 4.0%, 14.1% and 27.2% reduction of AOH, AME and TeA produced by *A. alternata*, respectively (Table 4).

4. Discussion

The economically most important pathogens belonging to the *Alternaria* genus cause considerable postharvest losses of fruits and vegetables (Sanzani et al., 2016). It is the first report on characterizing *Alternaria* spp. isolates associated with black rot of tomato fruit in China. They were conducted using morphological characters and

Table 3
Effect of UV-C treatment (0.25 kJ/m^2) on phenolic acid contents of healthy tissue (−1.0 cm) around rotten fruits inoculated *A. alternata* FQ 18 strain during storage at 20 °C.

Phenolic acid	Treatment	Days of storage		
(μg/kg)		3	7	11
p-Coumaric acid	Control	874.1 ± 8.5 a	961.2 ± 6.8 b	1055.4 ± 16.6 b
	UV-C	906.0 ± 13.8 a	1043.3 ± 9.0 a	1162.9 ± 14.9 a
Ferulic acid	Control	473.6 ± 8.0 b	521.5 ± 12.6 b	569.8 ± 6.6 b
	UV-C	504.9 ± 4.5 a	588.2 ± 16.2 a	625.7 ± 11.4 a
Protocatechuic acid	Control	278.6 ± 15.5 b	223.2 ± 12.3 b	188.4 ± 5.0 b
	UV-C	317.3 ± 6.8 a	257.6 ± 12.2 a	204.0 ± 3.7 a
Chlorogenic acid	Control	542.9 ± 17.5 a	593.7 ± 3.6 a	551.6 ± 14.3 a
	UV-C	556.3 ± 11.4 a	622.4 ± 0.9 a	583.0 ± 8.5 a
Total phenolics	Control	3306.1 ± 50.2 b	3565.6 ± 17.4 b	3357.8 ± 4.2 b
	UV-C	3497.6 ± 23.1 a	3856.7 ± 67.3 a	3705.4 ± 33.2 a

Each value represents means ± standard deviations of three replicates; different small letters in the same storage period within a column mean significant different at $P < 0.05$. Statistical significance of the difference was confirmed according to Duncan's multiple range test.

Table 4
Inhibitory effects of 1.0 mM phenolic standards on mycotoxins production by *A. alternata* FQ 18 strain incubated on PDB for 7 d.

Phenolic acid	Inhibition of mycotoxin production (%)		
	AOH	AME	TeA
p-Coumaric acid	14.3 ± 0.7 c	12.5 ± 0.7 d	60.2 ± 2.1 a
Ferulic acid	59.4 ± 1.3 a	79.1 ± 0.9 a	50.1 ± 0.8 b
Pyrocatechuic acid	51.4 ± 0.4 b	24.3 ± 0.7 b	34.1 ± 1.5 c
Chlorogenic acid	4.0 ± 0.3 d	14.1 ± 0.9 c	27.2 ± 1.1 d

Each value represents means ± standard deviations of three replicates; different small letters within a column mean significant different at $P < 0.05$. Statistical significance of the difference was confirmed according to Duncan's multiple range test. AOH, alternariol; AME, alternariol monomethyl-ether; TeA, tenuazonic acid.

sequence analysis of rDNA-ITS, since the aforementioned gene has been proved adequate for identifying the small-spored *Alternaria* by recent reports (Meena et al., 2017; Naik et al., 2017), and also only a polyphasic approach could be useful in the characterization of the genus *Alternaria*. The present study indicated that the black rot disease on tomato fruits is caused by three distinct species, with *A. alternata* being the prominent, followed by *A. brassicae* and *A. tenuissima*. These results are in agreement with the previous studies (Andersen et al., 2015; Pose et al., 2004; Somma et al., 2011). In addition, this is the first report on the occurrence on tomato of *A. brassicae*, which is common pathogens of Brassica plants (Aneja et al., 2014).

In addition to morphology and molecular analysis, the mycotoxin production has been used as a means of classification. However, chemotaxonomic identification of small-spored *Alternaria* isolates is not a reliable method, but mycotoxin profiling can be useful for verifying the toxicological risk of *Alternaria* strains (Andersen et al., 2015). Our results showed that 88.5% (23/26) of the *Alternaria* isolated from tomatoes was able to produce at least one mycotoxin (AOH, AME and TeA), and most of the strains can co-produce AOH and AME. This is in consistent with several reports on *Alternaria* mycotoxin production on different fruits (Andersen et al., 2015; Kanetis et al., 2015; Meena et al., 2017; Ramires et al., 2018). With respect to the amount of mycotoxin produced, *A. alternate* strains showed the highest production of AOH and AME among the studied isolates, but a relative lower production of mean TeA than the *A. brassicae* strains. The reason for the different capacity of toxin production among species may be influenced by their genetic makeup and also well affected by cultural and environmental conditions (Meena et al., 2017; Nguyen et al., 2018). Indeed, TeA and AME concentrations remarkably increased in the artificially inoculated fruits compared with PDB medium. Notably, TeA was observed to be present in the highest amount produced by *A. alternata* strains, suggested that *A. alternata* may be the most important mycotoxin-producing species in tomato fruits. Previous study on basil plants also showed that *A. alternate* strains seem to have a higher potential for AOH, AME and TeA production than isolates of other species such as *A. arborescens* and *A. tenuissima* (Siciliano et al., 2018). Moreover, TeA was the most abundant one (up to 309.1 mg/kg) compared with AOH and AME, as already reported for tomatoes (Andersen et al., 2015; Van de Perre et al., 2014). A similar trend was observed on wheat (Patriarca et al., 2007). They reported that the highest amount of TeA (up to 9478 mg/kg) was obtained in the *A. alternata* isolates from wheat; while AOH and AME were present at the lower levels (up to 622 and 2352 mg/kg, respectively).

Considering the high capacity of the *Alternaria* isolates to produce mycotoxins, and the heavy contamination of mycotoxin in processed tomato products (Lopez et al., 2016; Terminiello et al., 2006; Van de Perre et al., 2014; Zhao et al., 2015), it is urgent to provide a control system to minimize the fungus growth and inhibit toxin production. UV-C treatment was an ideal method to control postharvest diseases and reduce or degrade mycotoxins (Isman and Biyik, 2009; Jubeen et al., 2012; Popović et al., 2018; Selma et al., 2008) since the standard technology is currently implemented in food processing industry. In the present study, it is found that the low dose (0.25 kJ/m^2) of UV-C treatment effectively controlled postharvest diseases of tomato fruits caused by *A. alternate*, and significantly inhibited the production of AOH, AME and TeA. However, higher doses of UV-C appear to stimulate the disease development. A possible explanation might be associated to the fruit surface tissue damage caused by UV-C irradiation. Charles et al. (2008) indicated that UV-C changed the amount and ultrastructure of the epicuticular wax in tomato and this may make the fruit more vulnerable to the attack by fungi causing postharvest diseases. In addition, our results indicated that *Alternaria* mycotoxins, especially TeA, can transfer from a rotten part of the tomatoes into the surrounding healthy tissue, which may increase the risk of mycotoxins contamination in tomato products because of improper technological processing steps. Indeed, the high frequency and concentration of TeA compared other mycotoxins were reported in tomato derived products (Lopez et al., 2016; Terminiello et al., 2006; Van de Perre et al., 2014; Zhao et al., 2015). Previous studies also showed that patulin is capable of penetrating up to several millimeters into the surrounding healthy tissue (Ostry et al., 2004). However, the very low ability of AOH and AME to spread into healthy surrounding was observed, only trace amounts of AOH and AME were found in the surroundings until the end of storage. The reason for the different ability of penetrating among *Alternaria* mycotoxins may be due to the fact that TeA is a key virulence factor for *A. alternata* infection of its host (Kang et al., 2017). It is noteworthy that UV-C treatment can significantly inhibit the three mycotoxins penetration from the rotten part to surroundings. To our knowledge, this is the first reported study to have demonstrated a reduction of *Alternaria* mycotoxins in fruits subjected to UV irradiation.

The beneficial effect of low doses of UV-C on reducing mycotoxin may be associated with inducing the formation of antifungal metabolites and resistance mechanisms. It is well known that phenolic compounds are part of the plant defense system, while UV-C irradiation seems to induce the accumulation of phenolic compounds due to the activation of their biosynthesis pathway (Bravo et al., 2012; Liu et al., 2018). Our study also observed a significant increase in the individual phenolic compounds, such as *p*-coumaric, ferulic and protocatechuic acids, for tomatoes exposed to low dose of UV-C. Further study in the present indicated that the individual phenolics enhanced by UV-C irradiation had strong inhibition effects on the production of *Alternaria* mycotoxin. Especially ferulic acid (1.0 mM) had the strongest inhibition effects on the AOH and AME production, while *p*-coumaric acid (1.0 mM) displayed the highest reduction of TeA, followed by ferulic and pyrocatechuic acids. The high inhibitory effect of phenolic acids in our study is consistent with the literatures. In fact, ferulic acid (5 μM) reduced 90% of fumonisin B_1 production by *Fusarium verticillioides*, without affecting the growth of the fungus (Beekrum et al., 2003). Recently, biosynthesis of T-2 and HT-2 toxin was significantly reduced by 1.0 mM of ferulic acid (Ferruz et al., 2016). Similarly, *p*-coumaric acid showed the highest inhibition effects on TeA accumulation among nine phenolics (Wang et al., 2017). These results suggested that the phenolics induced by UV-C irradiation may be as an inhibited response to the microbial infection and even the mycotoxin accumulation. Overall, the results of the present study clearly indicated that UV-C treatment may be explored industrially as a non-thermal mode of preservation.

5. Conclusions

In the current study, the combined molecular and morphological identification of *Alternaria* spp. revealed that the main fungal responsible for black rots of tomato fruits were *A. alternata*, *A. brassicae* and *A. tenuissima*, with *A. alternata* being the most frequent. And also, the highest amount of *Alternaria* mycotoxins (AOH, AME and TeA) produced by *A. alternata* isolates suggested that *A. alternata* may be the most important mycotoxin-producing species in tomato fruits. Meanwhile, most of isolates in the present study had a very high capability for production of TeA, AOH and AME either *in vitro* or *in vivo*, especially higher ability of mycotoxin production was observed *in vivo*, suggested a toxicological risk for human consumption of tomato and its byproducts. Thus, UV-C irradiation was used to reduce the *Alternaria* decay in tomatoes and subsequent mycotoxin accumulation in our study. The results showed that UV-C irradiation at 0.25 kJ/m^2 could effectively control the disease development caused by *A. alternata* and inhibit mycotoxins production and penetration in tomatoes. This may be associated with the enhanced effects of UV-C on the accumulation of individual phenolic compounds. In fact, the induced phenolics were including *p*-coumaric, ferulic and pyrocatechuic acids, of which *p*-coumaric acid (1.0 mM) displayed the highest reduction of TeA, whereas ferulic acid (1.0 mM) had the strongest inhibition effects on the AOH and AME production. Therefore, the application of UV-C irradiation seems to be a promising method for reducing the potential risk of *Alternaria* mycotoxins in fruits and also for enhancing phenolics of the processing products. More studies are needed to explore the practical parameters to implement UV technology on a pilot scale, or to further improve the efficacy of mycotoxin reduction by combination with other methods of low environmental impact.

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Declaration of competing interest

The authors declare no competing financial interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2019.108333.

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