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AFLP variability, toxin production, and pathogenicity of *Alternaria* species from Argentinean tomato fruits and puree

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ABSTRACT

Large amounts of tomato fruits and derived products are produced in Argentina and may be contaminated by *Alternaria* toxins. Limited information is available on the genetic variability, toxigenicity, and pathogenicity of *Alternaria* strains occurring on tomato. We analyzed 65 *Alternaria* strains isolated in Argentina from tomato fruits affected by black mould and from tomato puree, using amplified fragment length polymorphisms (AFLPs) technique. AFLP analysis resolved the set of strains in 3 main clusters (DICE similarity values of 58 and 60%) corresponding to *A. alternata/tenuissima* (44 strains), *A. arborescens* (15 strains) and to an unknown group (6 strains). Most of the representative strains, belonging to each AFLP cluster, when cultured on rice, produced tenuazonic acid (up to 46,760 mg/kg), alternariol monomethyl ether (AME, up to 1860 mg/kg), and alternariol (up to 70 mg/kg). The toxin profile related to the strains was not related to any AFLP cluster, except for AME which was produced at lower level by *A. arborescens*. Most of strains were pathogenic on two types of commonly cultivated tomato fruits. These findings provide new information on the variability within the *Alternaria* species complex associated with tomato disease.

1. Introduction

Alternaria species occur in different habitats worldwide, causing infections to a wide range of plants and agricultural commodities, due to their pathogenic and saprophytic nature (Thomma, 2003). Diseases caused by *Alternaria* species can affect several important crops such as cereals, oil crops, vegetables, and different fruit plants (Logrieco et al., 2009; Thomma, 2003). Among these, a well-known *Alternaria* plant disease is the black mould of tomato, characterized by black lesions on green and ripe fruits (Pearson and Hall, 1975). Tomato fruits are easily infected because of their thin skin and weak tissues that allow a rapid penetration and growth of the different *Alternaria* infecting species (Pitt and Hocking, 1997). However, some species are also involved in post-harvest infection before fruit processing (Rotem, 1994; Wilson and Wisniewski, 1994). Therefore, the risk of contamination of industrialized products derived from pre and post-harvest infected tomato fruits, such as tomato sauce, juice, pulp, or puree, is very high (Ostry, 2008). Several of these species of *Alternaria* produce toxic metabolites which can be harmful for human health (Logrieco et al., 2009).

Among the *Alternaria* mycotoxins, tenuazonic acid (TA), alternariol (AOH), and alternariol monomethyl ether (AME) are the most occurring on plants. TA is a metabolite characterized by toxicity towards animals (Ostry, 2008), antibacterial activity (Gallardo et al., 2004) and it is considered as a possible causal factor of Onyalai, a human haematological disorder (Steyn and Rabie, 1976). TA has been shown also to inhibit of protein production and cell proliferation in three mammalian cell lines (Zhou and Qiang, 2008). AOH and AME, two toxins frequently found in combination, were reported to be mutagenic (Schrader et al., 2001), and Lehmann et al. (2006) reported also an estrogenic activity and genotoxic effects of AOH in mammalian cells.

Large volumes of tomato fruits and derived products are produced in Argentina (680,000 tonnes of tomato fruits produced on 18,000 hectares in 2008; FAOSTAT source). Most of the crop is used in processed products, where toxin contamination may result from preformed *Alternaria* toxins in tomato fruits. Recently Terminiello et al. (2006) reported contamination by TA, AOH, AME in Argentinean tomato puree that could pose serious risks for human health related to the consumption of these products. *Alternaria alternata* and *A. tenuissima* were considered the dominant species on tomato fruits analyzed, but only a morphological identification was provided (Terminiello et al., 2006).

The identification of species in *Alternaria* (Nees:Fr.) genus has been mainly based on morphological traits such as conidial shape and the type of catenulation, even though these traits can have a great intraspecific variability. Molecular tools have been recently used with

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increasing efficiency for *Alternaria* species identification (Logrieco et al., 2009; Peever et al., 2004). Amplified fragment length polymorphisms (AFLPs) represent a powerful, highly reproducible, PCR-based DNA-fingerprinting technique (Vos et al., 1995). Because a large number of polymorphic loci can be investigated in a single experiment, the AFLP technique has become one of the major methods used for investigation of genetic diversity of fungal species (Fahleson et al., 2003, Leissner et al., 1997; Majer et al., 1996).

The objectives of this work were (a) to investigate by AFLP the species composition and genetic variability of *Alternaria* species occurring on tomato fruits affected by black mould and tomato puree in Argentina, (b) to evaluate their mycotoxin production, and (c) to test their pathogenicity on tomato fruits.

2. Materials and methods

2.1. Fungal strains

Among the 65 strains studied here, a set of 57 strains of *Alternaria* species was isolated from 38 tomato fruits of two different types affected by black mould (28 strains from "Redondo" type and 29 strains from "Perita" type, belonging to Platense, Petitt, Rhodas, and Superman varieties). The tomatoes were collected in 2004/2005 years from 8 fields in Buenos Aires province. A further 8 strains were isolated from 30 samples of Argentinean commercial tomato puree. Infected tissue portions of each tomato fruit and 0.2 ml of each tomato puree sample were inoculated on Dichloran Chloramphenicol Malt Extract Agar (DCMA) (Pitt and Hocking, 1997). Petri dishes were incubated for 7 days at room temperature under fluorescent lamps, with a 12-h/12-h day/night photoperiod. The plates were observed under the stereomicroscope and single-conidial cultures, representing each suspected *Alternaria* colony, were obtained on Water–Agar (WA) and plated on Potato–Carrot–Agar (PCA). Plates were stored at 25 °C under cool-white fluorescent lamps with an alternating 8/16 light/dark cycle (Simmons and Roberts, 1993) to induce sporulation. The strains were stored and deposited in the Institute of Sciences of Food Production (ISPA) Collection, Italy (ITEM accession: http://www.ispa.cnr.it/Collection/).

Four strains used in this work as reference strains were *A. alternata*, EGS 34.016; *A. tenuissima*, EGS 34.015; *A. arborescens*, EGS 39.128; and the *A. infectoria* ex type, EGS 27.193 (kindly provided by Dr. E. G. Simmons, Mycological Services, Crawfordsville, IN).

2.2. DNA extraction

Each strain was grown on PDA and inoculated in 100 ml of Wickerman's medium (glucose 4%, malt extract 0.3%, yeast extract 0.3% and peptone 0.5%). The shaken cultures (120 rpm), incubated at 25 °C, were filtered after 2 days and lyophilized. Genomic DNA was isolated from powdered lyophilized mycelia by using the E.Z.N.A. (EaZy Nucleic Acid Isolation) Fungal DNA Miniprep Kit (Omega Bio Teck Inc., USA), according to the manufacture's protocol. Quality and quantity of DNA were checked by comparison with standard DNA markers (1 kb DNA Ladder, ready-to-use, with fragments ranging from 250 bp to 10,000 bp, Fermentas GmbH, St. Leon-Rot, Germany) on 0.8% agarose gel.

2.3. AFLP analysis

AFLPs were performed by using the Applied Biosystem AFLP Microbial Fingerprinting Kit (Perkin Elmer Corporation, USA). *Eco*RI and *Mse*I restriction enzymes were used for DNA digestions, followed by ligation of *Eco*RI and *Mse*I adapters to the DNA fragments and subsequent preselective amplification. *Eco*RI fluorescent dye-labeled primers were used in three different primer combinations (*Eco*RI-AC FAM/*Mse*I-CC, *Eco*RI-AT NED/*Mse*I-CG, *Eco*RI-G JOE/*Mse*I-T), performed in Gene Amp PCR System 9700 (Applied Biosystems). The samples, combined with deionized formamide and a size standard (GeneScan-500 ROX), were denatured and loaded for electrophoresis on ABI Prims 310 Genetic Analyzer (Applied Biosystems) for 26 min at 15 kV. The peaks on electrophorogram were scored as present (1) or absent (0); the size of the fragments was calculated by the ABI Prism GeneScan Analysis Software (Applied Biosystems) comparing their migration to the standard. For each primer combination a binary matrix of bands with present and absent fragments for each strain was generated by ABI Genotyper 2.5.2 Software (Applied Biosystems). A combined matrix derived by the three primer combinations was analyzed by NTSYS version 2.0.2. software by UPGMA (Unweighted Paired Group Method of Arithmetic Averages) cluster analysis, to generate a similarity tree with the DICE similarity coefficient.

2.4. Mycotoxin production and analysis

Fungal cultures were grown on 12.5 g of autoclaved polished rice with 40% moisture in flasks of 250 ml. Each strain of *Alternaria* was inoculated with three agar plugs (1×1 cm) of 1-week-old cultures. The flasks were incubated in the dark at 25 °C for 21 days (Li et al., 2001).

The method for the detection of *Alternaria* toxins in rice was described by Li et al. (2001). The culture material was homogenized with 30 ml of methanol and filtered through a Whatman filter paper (n. 1). Briefly, the filtrate was clarified with 60 ml of 20% ammonium sulphate and divided into two parts. One part (40 ml) was extracted three times with 10 ml of chloroform. The organic phases were combined, evaporated to dryness, and dissolved in 4 ml of methanol for AOH and AME analysis by high-performance liquid chromatography (HPLC). Another part (20 ml) was adjusted to pH 2 with 6 N HCl and extracted twice for TA with 15 ml of chloroform. TA was then partitioned into 10 ml of 5% sodium bicarbonate, acidified to pH 2 again, and extracted twice with 10 ml of chloroform. The chloroform extracts were combined, washed with 7.5 ml of water, and evaporates to dryness. The residue was made up to 4 ml of methanol and analyzed for TA by HPLC.

The HPLC system consisted of Shimadzu LC-CA liquid chromatography (Shimadzu, Kyoto, Japan) equipped with a Rheodyne sample valve fitted with a 20 µl loop and a Shimadzu SPD-M10Avp UV photodiode array detector. The analytical column was Jupiter 4.6×250mm 5 µ C18 (Phenomenex, USA). Standards of TA, AME and AOH were purchased from SIGMA Chemical Company (St. Louis, MO, USA). The mobile phase was methanol/water (80:20) containing 300 mg ZnSO4.H2O/l, for AOH and AME, and methanol/water (85:15) containing 300 mg of ZnSO4.H2O/l for TA. A flow rate of 0.4 ml/min was used. The wavelength for recording chromatograms was 258 nm for AME and AOH, and 280 nm for TA. A calibration curve was constructed for quantification purposes using the toxins standards and correlating peak-area versus concentration. The spectra were acquired in the range of 200–300 nm. Reference spectra were acquired during the elution of associated standards and used for peak identification by comparison after spectra normalization. The detection limits were 11 µg/kg for TA, 2 µg/kg for AME, and 5 µg/kg for AOH, respectively. The analyses were performed twice, with similar results.

2.5. Pathogenicity

A set of 23 representative strains, belonging to all the three AFLP clusters previously characterized (Fig. 1), was tested for pathogenicity on tomato fruits. In particular, 18 strains of the *A. tenuissima/alternata* AFLP cluster, 4 strains of the *A. arborescens* AFLP cluster and one of the 6 unknown strains, were tested. The pathogenicity and host specificity for each strain was determined on two types of fruit (Redondo var. Bonanza and Perita var. Roma) just after picking, by means of the toothpick-inoculation technique (Serdani et al., 2002). Halved toothpicks

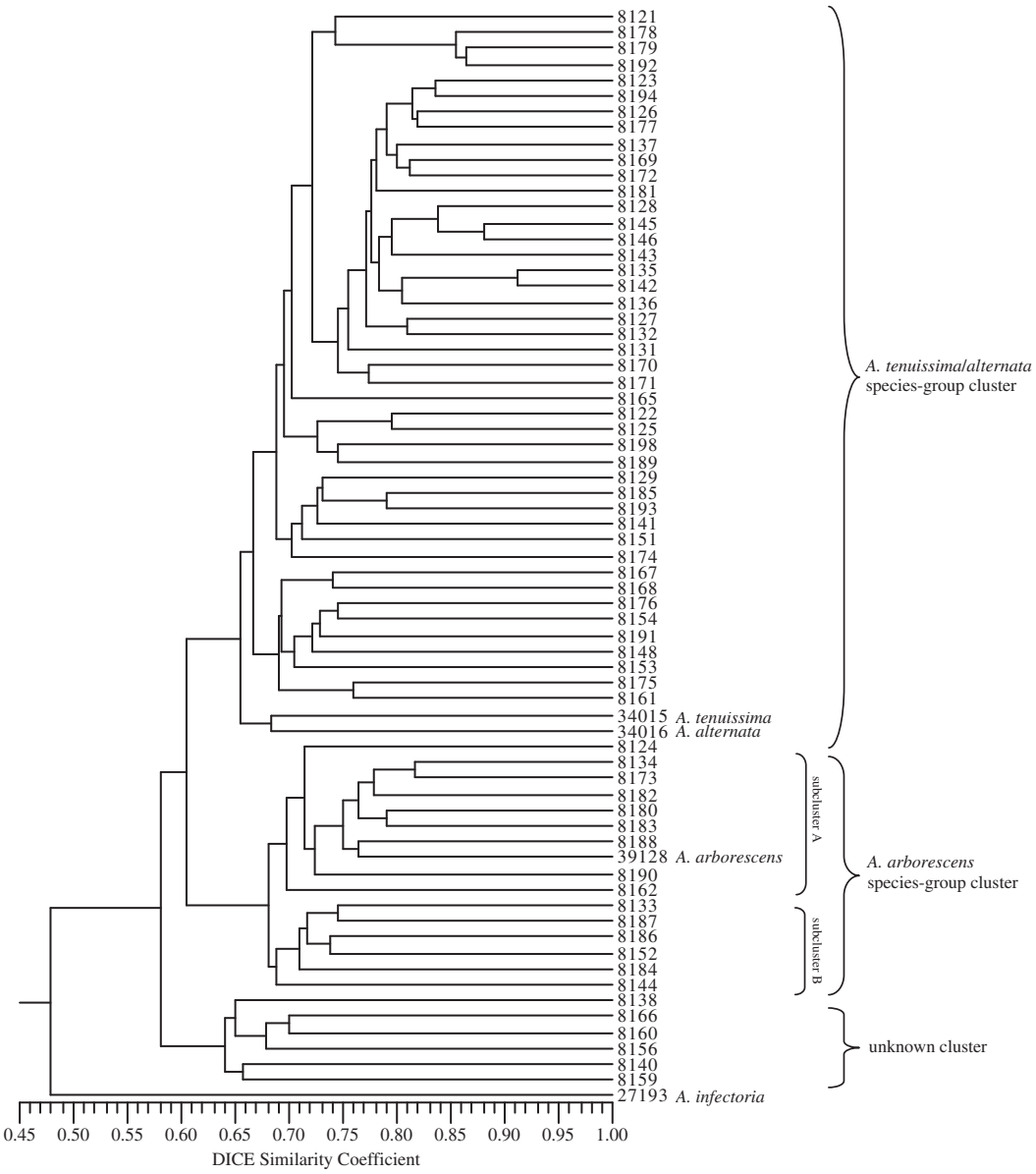

Fig. 1. Similarity tree derived by AFLP fingerprinting analysis on a population of *Alternaria* spp. strains isolated from tomato fruits affected by black mould and four species-group reference strains. Clustering analysis was performed with UPGMA method, by using DICE similarity coefficient.

were autoclaved five times in distilled water, and once in potato dextrose broth and then placed on 90-mm Petri dishes containing PDA. Toothpicks were inoculated with the *Alternaria* strains and colonized within 2 weeks. Each tomato was inoculated with toothpicks (8 mm depth), one of which was free of fungal growth as control. For each strain three replicates were done on both tomato cultivars. Inoculated tomatoes were covered with plastic bags, and incubated for 7 days at 25 °C. After the incubation period, external and internal lesion diameter and appearance were evaluated. The degree of pathogenicity was assessed by calculating the diameter of the lesion and was confirmed according to Koch's postulates (Koch, 1884, 1891). The host specificity was determined in agreement to the capacity of the strain to produce or not the lesion. With respect to lesion diameter on tomato fruits, three different levels of pathogenicity were established: strains slightly pathogenic (lesion diameter between 0.1 and 1.3 cm), moderately pathogenic (lesion diameter between 1.4 and 2.6 cm) and very pathogenic (lesion diameter between 2.7 and 3.8 cm).

Statistical analysis on data was performed by using the Mann–Whitney *U* test, a non-parametric significance test.

3. Results

3.1. AFLP fingerprint analysis

The analysis of the 65 *Alternaria* strains from tomato and the four species-group reference strains by using AFLP generated a binary matrix with 507 fragments derived by three primer combination. The primer combination *Eco*RI-AC/*Mse*I-CC and *Eco*RI-AT/*Mse*I-CG gave 145 and 161 fragments, respectively; a higher number of fragments (201) were retained by the primer pair *Eco*RI-G/*Mse*I-T, due to the single selective nucleotide of the *Eco*RI primer. The resulting polymorphic peaks were 96.3%, among which 12.6% were specific for the reference strain EGS 27.193 (species-group *A. infectoria*), 8.7% for EGS 39.128 (*A. arborescens*), 4.7% for EGS 34.016 (*A. alternata*) and 3.5% for EGS 34.015 (*A. tenuissima*). The similarity tree obtained by the cluster analysis was shown in Fig. 1. Three main clusters were formed with DICE similarity values of 58 and 60% (Fig. 1). A main cluster of 44 strains with a similarity value of about 60% with respect to the other 2

clusters was formed and included the reference strains EGS 34.016 and EGS 34.015, so that discrimination between the *A. alternata* and *A. tenuissima* groups was not achieved by AFLP analysis. A second cluster comprising 15 strains (lower DICE similarity index among the strains belonging to the clusters 70%) included the reference strain of *A. arborescens*, EGS 39.128. This cluster was composed of two sub-clusters (A and B) comprising 9 strains and the reference strain of *A. arborescens* (cluster A), and 6 strains (cluster B), respectively. A third main cluster, referred to as unknown cluster (Fig. 1), comprised 6 strains (ITEM 8138, 8140, 8156, 8159, 8160, 8166), with similarity values ranging from 64% to 70%. The identification of these strains at a morphological level indicated that strains ITEM 8138, 8160, and 8166 were *A. alternata*, while ITEM 8140, 8156, and 8159 were *A. tenuissima*. The reference strain EGS 27.193, representing the *A. infectoria* species-group, had only 48% as the highest similarity value with the other strains, indicating that none of the strains shared a similar AFLP profile to *A. infectoria*.

3.2. Toxin production

A set of 52 strains representative of all the AFLP clusters was investigated for ability to produce AME, AOH, and TA. The results are shown in Tables 1 and 3. Thirty-eight strains of the *A. tenuissima/alternata* AFLP cluster, 10 strains of *A. arborescens* and 4 strains of the unknown cluster were evaluated. All 52 strains except ITEM 8132, ITEM 8144, and ITEM 8198 showed co-production of the three mycotoxins. ITEM 8132 produced only TA and ITEM 8144 and ITEM 8198 produced only AME and AOH. Among the three mycotoxins, TA production was the highest (ranging from 1 to 46,760 mg/kg; average 3190 mg/kg), the AME average was 190 (range 1–1860 mg/kg) and the AOH average production was 20 mg/kg (range 1–70 mg/kg).

3.3. Pathogenicity assay

Among the 23 tested strains, 9% and 17% showed slightly pathogenicity on Redondo and Perita tomato fruit types, respectively; 56% and 61% resulted moderately pathogenic; 35% and 22% of the strains were very pathogenic on Redondo and Perita types, respectively (Table 2). According to AFLP clusters, the 18 strains belonging to *A. tenuissima/alternata* cluster showed an average lesion diameter of 2.3 and 2.1 cm on Redondo and Perita tomatoes, respectively. Values of 2.3 and 1.6 cm were obtained for the *A. arborescens* cluster; the unique strain tested of the unknown cluster, ITEM 8166, produced 3.4 and 2.4 cm lesions, respectively. The strains were moderately pathogenic on Redondo and Perita tomato types, showing average lesions of 2.4 and 2.0 cm, respectively.

By statistical analyses, there were no significant differences in pathogenicity among the three different AFLP clusters, nor did the two varieties show different susceptibility to the disease.

4. Discussion

AFLP analyses revealed a main cluster of *A. alternata/A. tenuissima* group clearly differentiated from a second cluster containing *A. arborescens* reference strain, EGS 39.128 (Fig. 1). Similar results were previously reported by using AFLP and Inter Simple Sequence Repeat investigations (Hong et al., 2006), Random Amplified Polymorphic DNA (RAPD) and Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP; Pryor and Michailides, 2002). Moreover, the reference strain of *A. infectoria* was also clearly different from the whole set of *Alternaria* strains here analyzed, as reported by previous studies (Hong et al., 2006; Pryor and Michailides, 2002). Altogether variability reported for the *Alternaria* population by AFLPs was rather high, with a maximum DICE similarity value of about 90% of AFLP fragments shared among the strains (Fig. 1); no correlation with toxin production or pathogenicity was observed. Moreover, although the genetic variability assessed in this study among the species of *Alternaria* by AFLPs proved to be very high, *A. alternata* and *A. tenuissima* strains were not resolved in two different clusters, showing that these two species could be considered by this criterion as a single taxon. An unknown cluster of six strains

Table 1
Production of tenuazoic acid (TA), alternariol monomethyl ether (AME) and alternariol (AOH) toxins, expressed in ppm, evaluated on 52 strains of *Alternaria* species from Argentinean tomato fruits affected by black mould.

AFLP cluster	ITEM strain[a]	Toxin production (ppm)		
		TA	AME	AOH
A. tenuissima/alternata	8121[b]	4	120	9
	8122[b]	TR	70	20
	8123[b]	1	150	20
	8125[b]	TR	230	30
	8126[b]	TR	80	10
	8127[b]	10	10	9
	8129[c]	460	400	40
	8131[c]	480	430	30
	8132[c]	24	ND	ND
	8135[c]	TR	220	20
	8136[c]	TR	320	20
	8141[c]	240	290	10
	8142[c]	1	300	7
	8143[c]	1	570	70
	8145[c]	810	490	20
	8146[c]	580	400	20
	8148[d]	1160	90	20
	8151[d]	1920	420	4
	8153[d]	1210	360	30
	8154[d]	1750	400	20
	8198[c]	ND	50	10
	8165[c]	720	70	4
	8167[c]	1120	4	5
	8168[c]	540	40	7
	8169[c]	8	80	20
	8170[c]	1170	5	4
	8171[c]	TR	160	10
	8172[c]	TR	330	30
	8175[d]	1380	400	30
	8176[d]	4440	250	30
	8178[c]	1220	150	20
	8179[c]	330	10	1
	8181[d]	TR	70	3
	8185[d]	1580	30	10
	8189[d]	1	1860	20
	8191[c]	46760	10	10
	8192[c]	21280	10	5
	8193[c]	TR	20	10
	EGS 34.015[e]	ND	TR	ND
	EGS 34.016[e]	ND	TR	ND
A. arborescens subcluster A	8124[b]	20	8	20
	8134[c]	550	30	10
	8162[d]	910	4	5
	8180[d]	1140	140	30
	8182[d]	28720	7	70
	8188[d]	130	10	10
	8190[d]	1460	20	10
A. arborescens subcluster B	8133[c]	5	40	20
	8144[c]	ND	5	2
	8152[d]	TR	1	TR
	EGS 39.128[e]	TR	TR	ND
Unknown	8138[c]	170	300	30
	8156[d]	850	220	20
	8159[d]	990	70	20
	8166[c]	460	30	20
	EGS 27.193[e]	ND	ND	ND

ND = not detected; TR = traces; TA = tenuazonic acid; AME = monomethyl ether; AOH = alternariol.
[a] Isolation source.
[b] Tomato puree.
[c] Tomato Perita.
[d] Tomato Redondo.
[e] Reference strains kindly provided by Dr. E. G. Simmons (Mycological Services, Crawfordsville, IN).

Table 2
Pathogenicity test of 23 *Alternaria* representative strains. Pathogenicity, reported as lesions diameter on tomato fruits and expressed in centimeter, was evaluated on two types of Argentinean tomato fruits (Redondo and Perita).

AFLP cluster	ITEM strain	Pathogenicity (cm)[a,b]	
		"Redondo" var. Bonanza	"Perita" var. Roma
A. tenuissima/alternata (18 Strains)	8198	1.7	2.5
	8165	1.6	1.4
	8167	3.2	3.1
	8168	1.9	2.6
	8169	1.6	1.5
	8170	2.9	1.4
	8171	3.1	3.1
	8172	1.3	1.1
	8175	2.7	2.1
	8176	2.5	1.8
	8178	3.7	2.6
	8179	1.9	2.8
	8181	2.3	2.3
	8185	2.8	3.3
	8189	2.1	1.5
	8191	2.0	0.6
	8192	2.0	1.8
	8193	2.2	2.0
Average *(range)*		2.3 *(1.3–3.7)*	2.1 *(0.6–3.3)*
A. arborescens (4 Strains)	8180	2.4	0.2
	8182	2.1	1.2
	8188	3.6	2.3
	8190	1.2	2.8
Average *(range)*		2.3 *(1.2–3.6)*	1.6 *(0.2–2.8)*
Unknown (1 Strain)	8166	3.4	2.4
Tot (23 strains)		2.4 (1.2–3.7)	2.0 (0.2–3.3)

[a] Diameter of the lesions on tomato fruits.
[b] Statistical analysis was performed by using the Mann–Whitney *U* test.

(ITEM 8138, 8140, 8156, 8159, 8160, and 8166) was formed at a DICE similarity value of 58%. This group could represent a single taxon but further molecular investigations on these strains are necessary to confirm this hypothesis.

In the *Alternaria* population analyzed here, co-production of TA, AME, or AOH was observed for all the strains studied, with the exception of three strains (ITEM 8132, 8144, 8198). This is in agreement with several reports on *Alternaria* toxin production on different hosts (Grabarkiewicz-Szczesna and Chelkowski, 1992; Li et al., 2001; Logrieco et al., 2003). All the strains except one (ITEM 8132) produced AME and AOH, up to 1860 and 70 mg/kg, respectively. The high production of TA compared with AOH and AME found in this study (up to 46,760 mg/kg) is in agreement with results previously reported (Bottalico and Logrieco, 1998; Grabarkiewicz-Szczesna and Chelkowski, 1992; Li et al. 2001; Logrieco et al., 2003; Visconti et al., 1992), although the values obtained here are higher than previously reported (Visconti et al., 1992). The potential for coproduction of the three mycotoxins by most of the 52 strains analyzed here underlines the risk of potential additive and/or synergistic effects on target organisms by these toxic metabolites. The relevant occurrence of *Alternaria* mycotoxins in Argentinean tomato puree (Terminiello et al., 2006), together with the wide capability to produce TA, AME, and AOH at high levels *in vitro* by many strains, is of concern for possible effects on human health. Mycotoxin contamination of tomato fruits can result in a high carry over to the processed products.

The toxin profile was common to most of the strains belonging to the three different AFLP clusters. These data confirm a previous report on the toxin production of *A. alternata*, *A. tenuissima*, and *A. arborescens* species (Bottalico and Logrieco, 1998). With respect to the amount of toxin produced, *A. arborescens* cluster strains showed a higher production of TA and a lower production of AME than the *A. alternata/tenuissima* cluster; AOH production was similar for all the

Table 3
Average values of toxin production of 38 strains of *Alternaria tenuissima/alternata*, 10 strains of *A. arborescens* and 4 unknown strains belonging to different AFLP clusters. Range of values was reported in brackets.

AFLP cluster	Toxin production (ppm)		
	TA	AME	AOH
A. tenuissima/alternata[a]	3,300 *(1–46,760)*	240 *(4–1860)*	20 *(1–70)*
A. arborescens[b]	4,120 *(5–28,720)*	30 *(1–140)*	20 *(2–70)*
Unknown	620 *(170–990)*	150 *(30–300)*	20 *(20–30)*
Tot (52 strains)	3190	190	20

TA = tenuazoic acid; AME = alternariol monomethyl ether; AOH = alternariol.
[a] Only one strain (ITEM 8198) was not able to produce TA.
[b] Only one strain (ITEM 8144) was not able to produce TA.

AFLP clusters. However, a wider set of strains from other sources should be examined before drawing any conclusions.

Pathogenicity tests showed that the level of aggressiveness for most strains of the different species towards tomato fruits ranged from moderate to very pathogenic (Table 2), with no differences at species level and variety. Comparing the diameter of the lesions of the *Alternaria* reference strains showed that the pathogenicity of *A. infectoria* was significantly lower than the other reference strains. These data agree with a previous report on the pathogenicity recorded for *A. infectoria* on pistachio (Pryor and Michailides, 2002).

In conclusion, the *Alternaria* strains that were isolated from infected tomato fruits in Argentina belong to three different main groups, as assessed by the AFLP analysis. Up to now, the evaluation of *Alternaria* species occurring on a specific plant host has been based on morphological identification, which is highly subjective for individual evaluation of the main trait to be analyzed. Only a few studies have based the identification of *Alternaria* species on molecular analyses, such as RAPD, PCR-RFLP, and Internal Transcribed Spacer (ITS) sequences (Dini-Andreote et al., 2009; Kang et al., 2002; Mercado Vergnes et al., 2006; Pryor and Michailides, 2002). In this report the delineation of *Alternaria* species occurring on tomato fruits has been performed for the first time by using AFLP analysis. No links were found between the AFLP clusters and toxin production or pathogenicity.

Natural occurrence of *Alternaria* species on tomato has been often detected. The very high capability for production of TA, AME, and AOH reported in this paper for many strains implies a serious risk for human consumption of tomato, a food that is consumed daily. The lack of regulation for *Alternaria* mycotoxins worldwide increases the risks related to the consumption of contaminated raw commodities. More thorough investigations on the related end-products and their potential toxicity from multi-toxin contamination should be carefully carried out.

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