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Characterization of *Alternaria* strains from Argentinean blueberry, tomato, walnut and wheat

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Alternaria species have the ability to produce a variety of secondary metabolite, which plays important roles in food safety. Argentina is the second largest exporter of fresh and processed food products to Europe, however, few studies on *Alternaria* mycotoxins and other bioactive secondary metabolites have been carried out on Argentinean cereals, fruit and vegetables. Knowing the full chemical potential and the distribution of *Alternaria* spp. on crops, it is necessary to establish a toxicological risk assessment for food products for human consumption. In the present study, 87 *Alternaria* strains from different substrates (tomato, wheat, blueberries and walnuts) were characterized according to morphology and metabolite production. Aggressive dereplication (accurate mass, isotopic patterns and lists of all described compounds from *Alternaria*) was used for high-throughput evaluation of the chemical potential. Four strains belonged to the *Alternaria infectoria* sp.-grp., 6 to the *Alternaria arborescens* sp.-grp., 6 showed a sporulation pattern similar to that of "M" according to Simmons, 1 to that of *Alternaria vaccinii*, and the remaining 70 constituted a diverse group belonging to morphological groups "G" and "H". The cluster analysis yielded 16 almost identical dendrograms and grouped the *Alternaria* strains into four clusters and 11 singletons and outlier groups. The chemical analysis showed that AOH and AME were the most common metabolites produced, followed by TEN, ALXs and TeA. The *A. infectoria* sp.-grp. had no metabolites in common with the rest of the strains. Several secondary metabolites isolated from large-spored *Alternaria* species or other fungal genera were detected, such as dehydrocurvularin, pyrenochaetic acid and alternarienonic acid. The strains isolated from tomato produced lower amounts of metabolites than strains from blueberries, walnut and wheat, although individual strains from tomato produced the highest amount of some metabolites. The *A. infectoria* sp.-grp. was unique to cereals, whereas strains classified as belonging to the *A. arborescens* sp.-grp or having sporulation pattern "M" were only isolated from tomatoes. Otherwise, no clear association between substrate and identity could be found. The analyses in the study show that at least 75% of the Argentinean strains are able to produce potential mycotoxins.

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1. Introduction

The genus *Alternaria* contains both plant pathogenic and saprophytic species that may affect the ornamental and crop plants in the field or cause pre- and postharvest spoilage of the plant fruits or kernels. Most *Alternaria* species have the ability to produce a variety of secondary metabolites, which may play important roles in either plant pathology or food quality and safety of agricultural produce (EFSA, 2011; Logrieco et al., 2003, 2009). Alternariol (AOH), alternariol monomethyl ether (AME), tenuazonic acid (TeA), tentoxin (TEN) and altenuene (ALT) are the main *Alternaria* compounds thought to pose a risk to human health (EFSA, 2011), however, food relevant *Alternaria* species are able to produce many more metabolites (Ostry, 2008). Importantly, toxicological data are limited to the abovementioned metabolites, and even these data are incomplete, with neither good bioavailability studies nor long term clinical studies. AOH and AME are mutagenic and highly active in cell based assays, but data on whole animal studies is absent in the literature (Prelle et al., 2013). Their presence in cereal grain has been suggested to be associated with high levels of human oesophageal cancer in China (Liu et al., 1992). TeA has been reported to exert antiviral, antitumor, antibacterial, cytotoxic and phytotoxic properties, as well as being acutely toxic in living organisms and its LD_{50} value in mice is similar to that of the *Fusarium* mycotoxin deoxynivalenol (Asam and Rychlik, 2013). Altertoxins (ALXs) have been reported to be more potent mutagens and acutely toxic to mice than AOH and AME (Scott, 2004) and recently, high genotoxic potency of ALX II in mammalian and human cells was demonstrated (Fleck et al., 2012; Schwarz et al., 2012). Other *Alternaria* metabolites are reported to be phytotoxins, i.e. being toxic to plants (Montemurro and

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Visconti, 1992), while the role of many other *Alternaria* metabolites, such as infectopyrones, phomapyrones and novae-zelandins, is unknown. However, *Alternaria* spp. produce a variety of other metabolites for which there are no reports on function, toxicity or if they are produced in the plants. A main reason for this is the lack of availability of the pure substances of which only AME, TeA, AOH, TEN and ALT are available.

The morphological diversity within *Alternaria* is considerable and great efforts have been made to organize taxa into subgeneric species-groups and species (Simmons, 2007). Polyphasic approaches, combining traditional morphology, molecular sequence analysis and secondary metabolite profiling, have been successful for the identification of large-spored, plant pathogenic *Alternaria* species (Andersen et al., 2008; Brun et al., 2013). However, other polyphasic studies of e.g. the *Alternaria infectoria* sp.-grp. (small-spored saprophytic and endophytic species) showed an overabundance in morphological, chemical and molecular variations that made species identification, chemotaxonomy and even molecular phylogeny unachievable (Andersen et al., 2009). Other small-spored, saprophytic species-groups, such as the *Alternaria arborescens* and *Alternaria tenuissima* sp.-grps., are equally challenging in terms of accurate identification. Several polyphasic studies based on morphology and chemotaxonomy of small-spored, food associated species have only achieved separation at species-group level (Andersen et al., 2001; 2002; Polizzotto et al., 2012; Serdani et al., 2002). Due to lack of molecular variation (Andrew et al., 2009), a molecular study recently pooled the *A. arborescens* and the *A. tenuissima* species-groups with *A. alternata* into one section (Lawrence et al., 2013) now comprising more than 50 species based on nucleotide sequence data (Woudenberg et al., 2013).

Argentina is the second largest exporter after Brazil of fresh and processed food products to Europe (ec.europa.eu, 2014), but few studies on mycotoxins and other bioactive secondary metabolites have been carried out on *Alternaria* spp. from Argentinean cereals, fruit and vegetables. Most studies on secondary metabolite production have been done on food-borne *Alternaria* spp. from Europe, North America and South Africa (Andersen et al., 2005, 2006, 2009; Andersen and Thrane, 1996a; Polizzotto et al., 2012; Serdani et al., 2002). Previous studies have demonstrated the presence of *Alternaria* spp. in Argentinean mouldy tomatoes (Pose et al., 2004) and *Alternaria* mycotoxins have been found in Argentinean commercial tomato by-products (Terminiello et al., 2006). *Alternaria* spp. were also found as the dominant part of the mycobiota in wheat from Argentina's main wheat producing area, known as V-South (Patriarca et al., 2007). Most of the *Alternaria* strains found in these studies were able to produce compounds suspected of being mycotoxins and some of the samples were contaminated with high levels of AOH, AME and TeA (Azcarate et al., 2008). Argentinean blueberries also contained *Alternaria* spp. (Greco et al., 2012) with a high percentage of strains being able to produce mycotoxins. In walnuts cultivated in Argentina, *Alternaria* was the second predominant genus and natural occurrence of *Alternaria* toxins was demonstrated, especially the "Criolla" variety was highly contaminated (Unpublished results, AP).

Different *Alternaria* spp. can be found in different habitats and geographical regions and may therefore have different metabolite profiles and hence a different toxic potential. To understand the full chemical potential of saprophytic, food associated *Alternaria* spp. and their distribution on crops and food products, it is necessary to establish a toxicological risk assessment for agricultural products for human consumption. The purpose of this work was therefore to characterize Argentinean *Alternaria* strains isolated from four different substrates with respect to morphology and metabolite production and compare them with well-characterized representative *Alternaria* strains from other parts of the world. One aim was to test ultra-high performance liquid chromatography-high resolution mass spectrometry (UHPLC–HRMS) with a new high-throughput approach, aggressive dereplication (Klitgaard et al., 2014), which is based on matching all described compounds from *Alternaria* and related genera with data on accurate mass and isotopic patterns, and further strengthen via high resolution tandem mass spectrometry (MS/HRMS) matching structures and fragmentation patterns. Another aim was to evaluate the chemical potential these *Alternaria* species pose to different Argentinean food products for both local consumption and export.

2. Materials and methods

2.1. Fungal strains

Eighty-seven fungal isolates belonging to the genus *Alternaria* Nees were used in this study (AP strains from the last author's collection); 50 were isolated from black-mould affected tomatoes (*Lycopersicon esculentum*) collected directly in the field from La Plata, Buenos Aires province, Argentina; 21 from symptomless wheat grain (*Triticum aestivum*) cultivated in the Argentinean wheat production area known as V-South (La Pampa and South West Buenos Aires provinces); 11 from symptomless blueberries (*Vaccinium angustifolium*) of the O'Neal variety cultivated in Buenos Aires province, Argentina; and 5 from symptomless walnuts (*Juglans regia*) of "Criolla" variety cultivated in the province of Catamarca, Argentina. Twelve representative strains from the IBT collection at the Department of Systems Biology, DTU, Denmark, belonging to four "small-spored" *Alternaria* species-groups were used for comparison (BA strains from the first author's collection). AP strains are maintained in the fungal culture collection, at Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina and BA strains in the IBT culture collection at the Department of Systems Biology, Danish Technical University, Denmark.

2.2. Micro- and macro-morphological examination

All 99 strains were inoculated on Potato Carrot Agar (PCA; Simmons, 2007) and Dichloran Rose Bengal Yeast Extract Sucrose agar (DRYES; Samson et al., 2010) and grown under standardized conditions (Andersen et al., 2005; Simmons, 2007). The unsealed PCA plates (9 cm diameter, plastic) were incubated in one layer for 7 days at 23 °C under an alternating cycle consisting of 8 h of cool white fluorescent daylight and 16 h darkness. The lamps (TLD, 36 W/95o, Philips) were placed 40 cm from the plates. The DRYES plates (9 cm diameter, plastic) were packed in perforated plastic bags and incubated for 14 days in the dark at 25 °C. A temporary working collection of the strains was made from agar blocks with conidia from PCA placed in cryo-tubes and kept at 5 °C.

The morphological characteristics of the strains were recorded from PCA after 7 days of growth. The three-dimensional sporulation patterns of the cultures were examined directly on the plates using a stereo-microscope. Further examination (length of primary conidiophores, branching types and origin of branching, conidial shapes, sizes, colours and ornamentation, etc.) was done at ×400 magnification using slide preparations made with adhesive tape mounted in lactophenol. Colony characteristics (e.g. colour, texture, diameter) were recorded from DRYES after 7 days of growth. The morphological characteristics of each strain were registered and compared to reference strains.

2.3. Metabolite extraction

The metabolite profiling was done on the 14-day-old DRYES cultures using a micro-scale extraction method modified for *Alternaria* metabolites (Andersen et al., 2005). Three agar plugs (6 mm ID) were cut from the centre of the three colonies and the nine plugs were placed in a 2 ml screw top vial. Then 1.0 ml ethyl acetate containing 1% formic acid (vol/vol) was added to each vial and the plugs were extracted by sonication for 60 min. The extract was transferred to a clean 2 ml vial, evaporated to dryness in a gentle stream of N_2 and re-dissolved in 400 μl methanol. The methanol extract was filtered through a 0.45 μm

filter into a clean 2 ml vial and kept at −18 °C prior to HPLC analysis. Selected cultures were grown and extracted in triplicate from different culture plates.

2.4. UHPLC–HRMS analyses

Analyses were performed using ultra-high-performance liquid chromatography (UPHLC) with diode array detector (DAD) and high resolution (HR) maXis 3G QTOF mass spectrometer (MS) (Bruker Daltonics, Bremen, Germany) equipped with an ESI source and connected to an Ultimate 3000 UHPLC system (Dionex, Sunnyvale, USA) equipped with a Kinetex 2.6-μm C_{18}, 100 mm × 2.1 mm column (Phenomenex, Torrance, CA) (Klitgaard et al., 2014). A linear water–acetonitrile gradient was used (buffered with 20 mM formic acid) starting from 10% (vol/vol) acetonitrile and increased to 100% in 10 min, maintained for 3 min before returning to the starting conditions. MS was performed in ESI^+ and ESI^- in the scan range m/z 100–1000, with a mass accuracy < 1.5 ppm (Klitgaard et al., 2014). UV/VIS spectra were collected at wavelengths from 200 to 700 nm. Data processing was performed using DataAnalysis 4.0 and Target Analysis 1.2 (Bruker Daltonics) by the *aggressive dereplication approach* (Klitgaard et al., 2014). This method is based on accurate mass and isotopic patterns from a list of putative compounds and can handle many thousands of data inputs in a very short time. For this study, a database of 235 known and putative *Alternaria* compounds was used, tentatively identifying them based on accurate mass (deviation < 1.5 ppm) and isotopic pattern (isotope fit < 50) (Klitgaard et al., 2014). A further database of 1500 reference standards and tentatively identified compounds was also used along with a small 50 compound database of blank peaks (BPs) observed in sample blanks. All major peaks (observed in the BP chromatograms) not tentatively identified by the approach were added to the search list as unknown compounds for mapping. All major peaks (known and unknown) for the 99 extracts were subsequently ordered in a data matrix.

To further verify the tentative identification from the above UHPLC–HRMS analysis selected extracts were reanalysed on an Agilent 1290 UHPLC with a photo diode array detector equipped with a 40 mm flow cell scanning 200–640 nm (more sensitive than the DAD on the Bruker instrument), and coupled to an Agilent 6550 qTOF (Santa Clara, CA, USA) equipped with a dual electro spray (ESI). Separation was performed at 60 °C and at a flow of 0.35 ml/min on a 2.1 mm ID, 250 mm, 2.7 μm Agilent Poroshell phenyl hexyl column using a water–acetonitrile gradient solvent system, with both water and acetonitrile containing 20 mM formic acid. The gradient started at 10% acetonitrile and was increased to 100% acetonitrile in 15 min, kept for 4 min, returned to 10% acetonitrile in 1 min, and equilibrating for the next sample for 4 min. Samples were analysed in both ESI^+ and ESI^- scanning *m/z* 50 to 1700, also making automated data-dependent MS/HRMS on all major detected peaks, using collision energies of 10, 20 and 40 eV for each MS/MS experiment (Kildgaard et al., 2014). Peaks identified on the maXis, where reference standards were not available, had their MS/HRMS spectra searched by *reversed* and *similarity search* in a 900 in-house MS/MS library. Furthermore, MS/HRMS fragmentations were compared to the tentative structure(s) using the MassHunter Molecular Structure Correlator programme which uses a systematic bond disconnection approach (Hill and Mortishire-Smith, 2005) correlate MS/HRMS spectrum to the structure. Further UV/Vis spectra were compared to original data of the published structure of the dereplicated candidate(s) (Nielsen et al., 2011).

2.5. Data treatment

A matrix was constructed based on 99 strains and their production of 57 metabolites with both known and unknown chemical structures. The presence or absence of a particular metabolite was scored as 1 or 0, respectively, for each strain. The binary matrix was subjected to cluster analysis in NTSYS (pc version 2.20q (Exeter Software, Setauket, USA)) without standardization using Jaccard (J) and simple matching (SM) similarity coefficients and Unweighted Pair Group Method with Arithmetic mean (UPGMA) clustering method (Andersen et al., 2008). The matrix was also analysed by Yule (Y) as correlation coefficient and UPGMA in NTSYS.

3. Results

3.1. Morphological and cultural characterization

After 7 days of growth on PCA under standardized light conditions, all 99 *Alternaria* strains were examined morphologically according to the extended keys and sporulation definitions in the identification manual by Simmons (2007). Four strains (AP025, AP039, AP060 and AP067) out of the 87 Argentinean *Alternaria* strains showed sporulation patterns that matched the sporulation pattern of the *A. infectoria* sp.-grp. (corresponding to morphological group "K" in Simmons (2007)) compared with the representative strains (BA1208 and BA1240). They produced smooth, light coloured conidia arranged in branched chains usually with long, geniculate secondary conidiophores with several conidiogenous loci. Their colonies on DRYES were flat, woolly and ranged from pinkish white to greyish white, which also matched the colony characteristics of the representative strains. Strains belonging to the *A. infectoria* sp.-grp. all originated from wheat samples.

Six strains (AP015, AP016, AP018, AP022, AP073 and AP075) showed the characteristic sporulation pattern of the *A. arborescens* sp.-grp. (corresponding to the morphological group "L" in Simmons (2007)) with long distinct primary conidiophores, occasionally presenting some sub-terminal branches, and with a terminal cluster of branching conidial chains. The branching pattern was mostly defined by secondary conidiophores originating from the conidial apex. Colonies on DRYES were sulcate, dark green with a slightly lighter centre. These six strains, originating from tomato, matched the four representative strains for the *A. arborescens* sp.-grp. (BA0961, BA1343, BA1382 and BA1422) in both micro- and macro morphology.

Six other strains (AP004, AP012, AP014, AP021, AP076 and AP095) exhibited a sporulation pattern similar to that of "M" in Simmons (2007). Primary conidiophores were aerial and cobweb-like, but not truly arborescent, with lumps of conidia in short branching chains scattered along the hyphae. Two strains (AP004 and AP012) had a macro-morphological resemblance to the *A. arborescens* sp.-grp. whereas the other four had khaki coloured colonies on DRYES with a flat, hairy texture and did not resemble any of the representative strains. All six strains originated from tomatoes.

One strain, AP023, isolated from tomato, exhibited a sporulation pattern resembling that of *A. vaccinii* E.G. Simmons (2007) with exceptional long secondary conidiophores (up to 190 μm) located in the morphological group "G" in Simmons (2007), but with smooth conidia (16–46 × 10 μm) (Fig. 1a). Colonies on DRYES were pale green, flat, granulated and with undulating edges.

The remaining 70 Argentinean AP strains constituted a diverse group, where no strain was identical to any other or to the four representative *A. tenuissima* sp.-grp. strains (BA0853, BA0879, BA0888 and BA0925), however, all 70 strains belonged to the morphological groups "G" and "H" in Simmons (2007). Many resembled the sporulation pattern of *A. tomaticola* E.G. Simmons (2007) in group "H" with dark conidia of varying shapes becoming gradually smaller towards the apex of the chains (Fig. 1b). Micro-morphologically, they all were small-spored (largest conidium body smaller than 40 μm) and characterized by three-dimensional sporulation patterns with conidia formed in short chains of 3–10(–15) conidia, borne on primary conidiophores of varying length. Formation of branching conidial chains was infrequent and, when present, it occurred via secondary conidiophores mainly originating from the conidial body. The length of the secondary conidiophores also varied: some strains had medium long (4–70 μm) or long

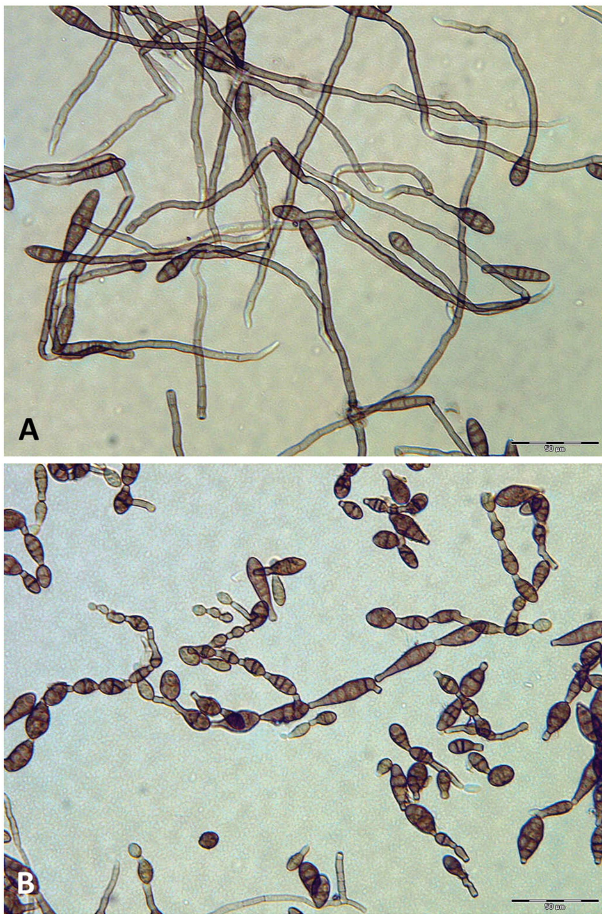

Fig. 1. Conidia, secondary conidiophores and sporulation pattern of two *Alternaria* strains after 7 days of growth on PCA in alternating cool white daylight. A: strain AP023, resembling *A. vaccinii*, located in cluster 2e. B: strain, resembling *A. tomaticola*, AP026 located in cluster 2d. Scale bars: 50 μm.

(4–110 μm) straight, unbranched secondary conidiophores, whereas secondary conidiophores of most strains were short (4–30 μm) geniculate. On DRYES these 70 strains exhibited flat colonies ranging in colour from light green to greyish with granular texture or sulcate colonies varying from brownish to yellow green with a velvety texture. Twenty AP strains were sent to E.G. Simmons for identification: "The 20 isolates you sent are very much what I would expect to see as miscellaneous small-spored isolates from a single host. Except for a few of yours, I have seen scores or hundreds of practically identical strains — most of them undescribed and probably none of them in the main part of the Manual" (Simmons, personal communication Oct. 06, 2011).

3.2. Cluster analysis of metabolite profiles

The cluster analysis generated in NTSYS by UPGMA using the Jaccard coefficient of the binary, 99 × 57 data matrix (99 strains × 57 metabolites) yielded 16 almost identical dendrograms and grouped the 99 *Alternaria* strains into four clusters and 11 singletons and outlier groups. One of the dendrograms is shown in Fig 2. The variation within each of the clusters is quite large, suggesting that few metabolite profiles were identical or even similar.

The four AP strains in Cluster 4 all belonged to the *A. infectoria* sp.-grp. or group "K" together with the two representative strains (BA1208 and BA1240) and had no metabolites in common with the other 93 strains. Cluster 1a contains 23 AP strains and only one of the representative strains of *A. tenuissima* sp.-grp. (BA0853) and two strains (AP 053 and AP041) in Cluster 1b. All 26 strains belonging to morphology group "G" or "H". Cluster 2 contains the majority of the strains (50 AP strains), and six of the representative strains: two strains belonging to the *A. arborescens* sp.-grp., two strains belonging to the *A. tenuissima* sp.-grp. and the two *A. alternata* strains. Cluster 2 could be divided into six smaller clusters. Cluster 2a contained strains with an "M" sporulation pattern and Cluster 2b strains with an arborescens or "L" sporulation pattern, including the two *A. arborescens* sp.-grp. representatives (BA1342 and BA1422). Cluster 2c held two strains belonging to the *A. tenuissima* sp.-grp. (BA0879 and BA0888) and five AP classified morphologically as "G" or "H" sporulation pattern. Cluster 2d consisted purely of AP strains and also belonged to sporulation pattern "G" or "H". In Cluster 2e, twelve AP strains again identified as "G" or "H" grouped with one strain with sporulation pattern "M" (AP076), the one resembling *A. vaccinii* (AP023) and the two *A. alternata* representatives (BA0922 and BA0923) from group "J", while Cluster 2f contained four AP strains (AP006, AP009, AP038 and AP089), morphological classification "G" or "H". Cluster 3 held only two representative strains (BA0961 and BA1382), which belong to the *A. arborescens* sp.-grp. or group "L". Strain BA0961 is the type culture and is the only strain able to produce the AAL toxins.

3.3. Production of known mycotoxins, metabolites and unknown compounds

Table 2 gives the production of known potential *Alternaria* mycotoxins, such as alternariols, altenuene, tentoxin and tenuazonic acid, while Table 3 gives the production of known metabolites that are novel to small-spored *Alternaria* species and species-groups. Eighteen major undescribed metabolites were detected, some clearly derivatives of alternariol, tentoxin, and tenuazonic, while others presumable belong to new compound classes, based on their elemental composition UV/Vis and MS/MS spectra. Altogether these unknowns only contributed to an average of 11% of the total peak area, although with some strains producing up to 74% of the total peak area as unknowns (Supplementary Table 1). It should be noted that tenuazonic acid ionizes much more strongly than the many polyketide metabolites and thus the relative peak area of unknowns is underestimated in extracts containing tenuazonic acid.

From Tables 2 and 3 it can be seen that the six strains belonging to the *A. infectoria* sp.-grp. had no metabolites in common with the other 93 *Alternaria* strains, which is also illustrated in Fig. 2 (between cluster 4 and the remaining strains). These six *A. infectoria* sp.-grp. strains all produced infectopyrone and phomapyrone A, as shown in Table 2, but none of the well-known *Alternaria* metabolites, such as the alternariols, altertoxins or tenuazonic acid. Two of the strains (AP060 and AP067) also produced the related novae-zenlandin A and B. Several phomapyrones not previously reported from the *A. infectoria* sp.-grp., were produced by AP025, P039, BA1208, AP060, and BA1240.

The most common metabolites in small-spored *Alternaria* species, alternariol (AOH) and alternariol monomethyl ether (AME), were produced by all strains in clusters 2a to 2f, whereas only half of the remaining 37 small-spored *Alternaria* strains produced these potential mycotoxins. Tenuazonic acid (TeA) was produced by 62 of the 93 small-spored *Alternaria* strains located in clusters 1a, 2a–d and 3. Tentoxin (TEN) was produced by 67 of the 93 small-spored *Alternaria* strains, however, producing strains were scattered amongst all clusters except cluster 4. The *Alternaria* strains that produced altertoxins I–III (ALX I–III) were found in all clusters except clusters 4 and 2a and in the singletons lying outside the clustering.

The only strain able to produce AAL toxins (automated search included 24 AAL isomers as well as 30 fumonisin isomers) was the type culture of *A. arborescens* (BA0961). None of the other 98 strains in the *A. arborescens* sp.-grp. or other groups produced these toxins.

The two representatives of *A. alternata* (BA0922 and BA0923) presented almost identical metabolite profiles consisting of AOH, AME, altenuene (ALT) and altenusin (ALN). Strain AP023, which resembled *A. vaccinii*, had a very similar profile to the two *A. alternata* strains and produced AOH, AME, ALT, TEN, ALN, altenuisol, amongst other

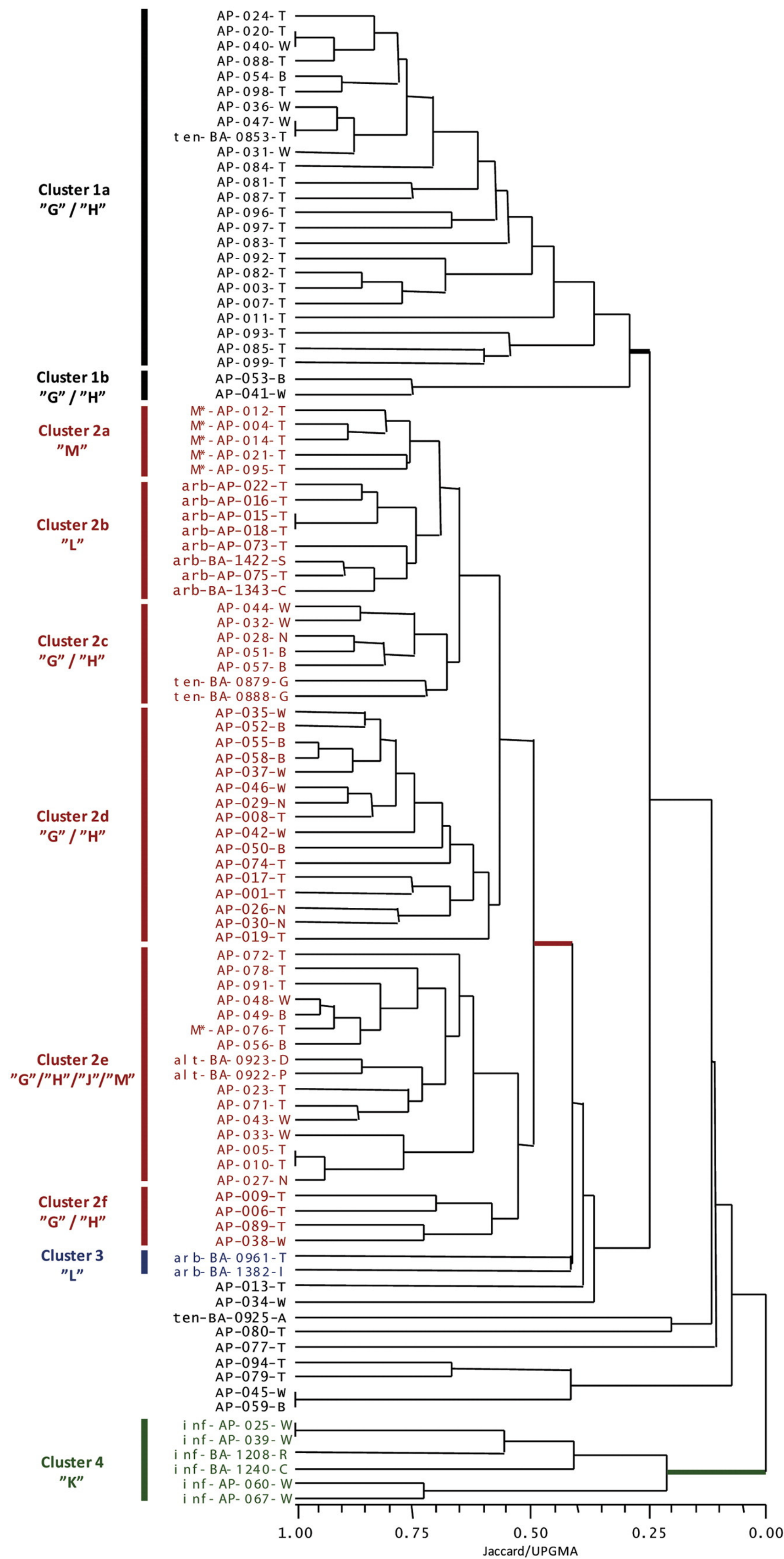

Fig. 2. Dendrogram (Jaccard/UPGMA) of the 87 Argentinean *Alternaria* strains and the twelve representative *Alternaria* strains based on binary metabolite profiles. Sample codes: Species-group ID/Strain ID/substratum code according to Table 1. Substratum code: A: air; B: blueberry; C: barley; D: Devil's trumpet; G: Grape; I: insect gall; N: walnut; P: peanut; R: river mud; S: salt marsh; T: tomato; W: wheat.

Table 1
Strains used in this study with morphological ID and origin.

Strain number	Species/species-group	Substratum	Country	Other number
AP 001	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 003	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 004	*Alternaria* sp. (M[a])	Tomato fruit, mouldy	Argentina	
AP 005	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 006	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 007	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 008	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 009	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 010	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 011	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 012	*Alternaria* sp. (M[a])	Tomato fruit, mouldy	Argentina	
AP 013	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 014	*Alternaria* sp. (M[a])	Tomato fruit, mouldy	Argentina	
AP 015	*A. arborescens* sp.-grp.	Tomato fruit, mouldy	Argentina	
AP 016	*A. arborescens* sp.-grp.	Tomato fruit, mouldy	Argentina	
AP 017	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 018	*A. arborescens* sp.-grp.	Tomato fruit, mouldy	Argentina	
AP 019	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 020	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 021	*Alternaria* sp. (M[a])	Tomato fruit, mouldy	Argentina	
AP 022	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 023	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 024	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 025	*A. infectoria* sp.-grp.	Wheat grain	Argentina	
AP 026	*Alternaria* sp.	Walnut kernel	Argentina	
AP 027	*Alternaria* sp.	Walnut kernel	Argentina	
AP 028	*Alternaria* sp.	Walnut kernel	Argentina	
AP 029	*Alternaria* sp.	Walnut kernel	Argentina	
AP 030	*Alternaria* sp.	Walnut kernel	Argentina	
AP 031	*Alternaria* sp.	Wheat grain	Argentina	
AP 032	*Alternaria* sp.	Wheat grain	Argentina	
AP 033	*Alternaria* sp.	Wheat grain	Argentina	
AP 034	*Alternaria* sp.	Wheat grain	Argentina	
AP 035	*Alternaria* sp.	Wheat grain	Argentina	
AP 036	*Alternaria* sp.	Wheat grain	Argentina	
AP 037	*A. infectoria* sp.-grp.	Wheat grain	Argentina	
AP 038	*Alternaria* sp.	Wheat grain	Argentina	
AP 039	*Alternaria* sp.	Wheat grain	Argentina	
AP 040	*Alternaria* sp.	Wheat grain	Argentina	
AP 041	*Alternaria* sp.	Wheat grain	Argentina	
AP 042	*Alternaria* sp.	Wheat grain	Argentina	
AP 043	*Alternaria* sp.	Wheat grain	Argentina	
AP 044	*Alternaria* sp.	Wheat grain	Argentina	
AP 045	*Alternaria* sp.	Wheat grain	Argentina	
AP 046	*Alternaria* sp.	Wheat grain	Argentina	
AP 047	*Alternaria* sp.	Wheat grain	Argentina	
AP 048	*Alternaria* sp.	Wheat grain	Argentina	
AP 049	*Alternaria* sp.	Blueberry	Argentina	
AP 050	*Alternaria* sp.	Blueberry	Argentina	
AP 051	*Alternaria* sp.	Blueberry	Argentina	
AP 052	*Alternaria* sp.	Blueberry	Argentina	
AP 053	*Alternaria* sp.	Blueberry	Argentina	
AP 054	*Alternaria* sp.	Blueberry	Argentina	
AP 055	*Alternaria* sp.	Blueberry	Argentina	
AP 056	*Alternaria* sp.	Blueberry	Argentina	
AP 057	*Alternaria* sp.	Blueberry	Argentina	
AP 058	*Alternaria* sp.	Blueberry	Argentina	
AP 059	*Alternaria* sp.	Blueberry	Argentina	
AP 060	*A. infectoria* sp.-grp.	Wheat grain	Argentina	
AP 067	*A. infectoria* sp.-grp.	Wheat grain	Argentina	
AP 071	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 072	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 073	*A. arborescens* sp.-grp.	Tomato fruit, mouldy	Argentina	
AP 074	*A. arborescens* sp.-grp.	Tomato fruit, mouldy	Argentina	
AP 075	*A. arborescens* sp.-grp.	Tomato fruit, mouldy	Argentina	
AP 076	*Alternaria* sp. (M[a])	Tomato fruit, mouldy	Argentina	
AP 077	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 078	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 079	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 080	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 081	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 082	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 083	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 084	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 085	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 087	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 088	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 089	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 091	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 092	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 093	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 094	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 095	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 096	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 097	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 098	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
AP 099	*Alternaria* sp.	Tomato fruit, mouldy	Argentina	
BA 853[b]	*A. tenuissima* sp.-grp.	Tomato fruit, mouldy	Denmark	
BA 879[b]	*A. tenuissima* sp.-grp.	Grape berry, mouldy	Denmark	
BA 888	*A. tenuissima* sp.-grp.	Grape berry, mouldy	South Africa	
BA 922[b]	*A. alternata*	Peanut	India	EGS 34-016; CBS 916.96
BA 923[b]	*A. alternata*	Devil's trumpet	India	EGS 34-039
BA 925	*A. tenuissima* sp.-grp.	Air	USA	EGS 30–134

Table 1 *(continued)*

Strain number	Species/species-group	Substratum	Country	Other number
BA 961[b]	*A. arborescens* (Type)	Tomato stem	USA	EGS 39–128; CBS 102605
BA 1208	*A. incomplexa* (Type)	River mud	USA	EGS 17–103; CBS 121330
BA 1240	*A. infectoria* sp.-grp.	Barley grain	New Zealand	EGS 43–162
BA 1343[b]	*A. arborescens* sp.-grp.	Barley grain	Denmark	
BA 1382[b]	*A. arborescens* sp.-grp.	Insect gall on oleander	Spain	
BA 1422[b]	*A. arborescens* sp.-grp.	Salt marsh	Slovenia	

[a] Sporulation pattern according to Simmons (2007).
[b] Used in the study of Polizzotto et al. (2012).

metabolites. However, in common with the two *A. alternata* strains, it did not produce TeA.

Several known secondary metabolites previously isolated from large-spored *Alternaria* species or other fungal genera were detected in this study (see Table 3). Dehydrocurvularin, which has not been reported from small-spored *Alternaria* species before, was detected in all six strains in clusters 1b and 2f together with AP013 (singleton), AP030 (cluster 2d) and AP093 (cluster 1a). Pyrenochaetic acid (PyA), known from *Pyrenochaeta terrestris* (Ichihara et al., 1987), was detected in all strains in clusters 2a–f, except in AP019 and AP050 (cluster 2d) and in BA961, AP013 and AP034. Alternarienonic acid (AlA), isolated from an unidentified *Alternaria* sp. (Aly et al., 2008) was detected in all strains in cluster 2, except AP050, the two *A. arborescens* sp.-grp. strains in cluster 3 and AP024 and AP092 in cluster 1.

Table 2
Secondary metabolites (and their abbreviations) known to be produced by small-spored *Alternaria* species and species-groups.

Abb.	Metabolite	Small-spored *Alternaria* spp and sp.-grps.					
		inf (6)	alt (2)	arb (10)	M* (6)	ten (4)	spp (71)
	4Z-Infectopyrone	6	–	–	–	–	–
	Phomapyrone A (=phomenin A)	6	–	–	–	–	–
	Phomapyrone B	5	–	–	–	–	–
	Novae-zelandin A	2	–	–	–	–	–
	Novae-zelandin B	2	–	–	–	–	–
	Phomapyrone E or G	4	–	–	–	–	–
	Phomapyrone F	3	–	–	–	–	–
	Phomapyrone D	3	–	–	–	–	–
AOH	Alternariol	–	2	10	6	2	51
ALT	Altenuene	–	2	10	6	2	37
AME	Alternariol monomethyl ether	–	2	9	6	2	50
	Altenuic acid II	–	2	8	6	2	27
ALN	Altenusin	–	2	5	6	2	21
	Dehydroaltenusin	–	2	2	1	–	10
ALX II	Altertoxin II (=stemphyltoxin II)	–	1	5	1	3	53
	Altertenuol	–	1	–	2	–	7
TEN	Tentoxin	–	–	10	4	3	50
TeA	Tenuazonic acid	–	–	10	5	3	44
	Tenuazonic acid derivative[a]	–	–	9	6	1	42
ALS	Altersetin	–	–	10	4	2	32
	Dihydrotentoxin	–	–	9	–	2	34
ALX I	Altertoxin I (=dihydroalterperylenol)	–	–	4	1	2	51
ALXIII	Altertoxin III	–	–	–	–	1	30
	AAL-toxin	–	–	1	–	–	–

inf: *A. infectoria* sp.-grp.; alt: *A. alternata*; arb: *A. arborescens* sp.-grp.; M*: strains identified as belonging to sporulation group M in Simmons (2007); ten: *A. tenuissima* sp.-grp.; spp: strains with small spores that could not be assigned to any described species, species-group or sporulation pattern.
[a] Isoleucine substituted by valine or norvaline.

Table 3
Secondary metabolites (with their abbreviations) new to small-spored *Alternaria*, but known to be produced by large-spored *Alternaria* species and/or species from other genera.

Abb.	Metabolite	Small-spored *Alternaria* spp and sp.-grps.					
		inf (6)	alt (2)	arb (10)	M* (6)	ten (4)	spp (71)
	3-Methoxy-3-epiradicinol	2	–	–	–	–	–
AlA	Alternarienonic acid	–	2	10	6	3	41
PyA	Pyrenochaetic acid A	–	2	9	6	2	38
	Altechromone A	–	2	7	4	2	18
	cis-Dehydrocurvularin[a]	–	–	–	–	–	9
	11beta-Hydroxy-curvularin[a]	–	–	–	–	–	7

inf: *A. infectoria* sp.-grp.; alt: *A. alternata*; arb: *A. arborescens* sp.-grp.; M*: strains identified as belonging to sporulation group M in Simmons (2007); ten: *A. tenuissima* species-group; spp: strains with small spores that could not be assigned to any described species, species-group or sporulation pattern.
[a] Standards available.

3.4. Correlation between substrate and chemical profile

Raw data from the semi-quantitative UPHLC-HRMS runs of 83 Argentinean *Alternaria* strains (excluding the four strains belonging to the *A. infectoria* sp.-grp. and the twelve representative strains) were sorted into substrate groups and analyzed. Table 4 gives the number of strains (in %) that produced nine selected metabolites based on substrate. As can be seen, the production of individual metabolites is not consistent amongst the 83 strains, not even the production of the most common metabolites AOH and AME. Fig. 3 shows the average amount (blue columns) and the maximum/minimum range (black "error" bars) of the nine metabolites produced by the positive strains from each substrate after growth on DRYES. The amounts are given as peak areas of the individual metabolites and can therefore not be compared between different metabolites, only between substrates within the same metabolite. The initial analysis showed that the strains isolated from tomato on average produced lower amounts (given as total peak area) of metabolites compared to strains from blueberries, walnut and wheat, but also that individual strains from tomato produced the highest amount of some metabolites. As can be seen, the range of metabolite production was wide for nearly all metabolites in all substrate groups, except for the range of TeA in strains from wheat, TEN in strains for walnuts and ALN in strains from blueberries. Furthermore, the average amounts produced by these strains were also the highest compared with the other substrate groups. On the other hand, a low percentage of the strains (59, 40 and 36 %, respectively) was able to produce these metabolites. Fig. 3 also shows that AOH was the only metabolite that the strains isolated from mouldy tomatoes produced in relative high average amounts. The production of AlA was most pronounced in strains isolated from wheat, while PyA was most pronounced in strains isolated from blueberries.

Comparisons of substrate with identification into unique taxa or species-groups, as given in Table 1, show that the *A. infectoria* sp.-grp.

Table 4
Number of Argentinean *Alternaria* strains (83) from the four substrata and number (%) of positive strains for the different mycotoxins (not including the four *A. infectoria* sp.-grp. strains).

Substratum	Samples	% of positive samples								
	Total	AOH	AME	ALX II	ALT	TeA	TEN	ALN	AlA	PyA
Blueberries (B)	11	100	82	91	64	64	91	36	64	64
Walnuts (N)	5	100	100	100	100	80	40	60	100	100
Tomatoes (T)	50	72	72	60	56	68	66	36	62	54
Wheat (W)	17	71	71	82	53	59	88	36	59	65

AOH: alternariol; AME: alternariol monomethyl ether; ALX II: altertoxin II; ALT: altenuene; TeA: tenuazonic acid; TEN: tentoxin; ALN: altenusin; AlA: alternarienonic acid; PyA: pyrenochaetic acid.

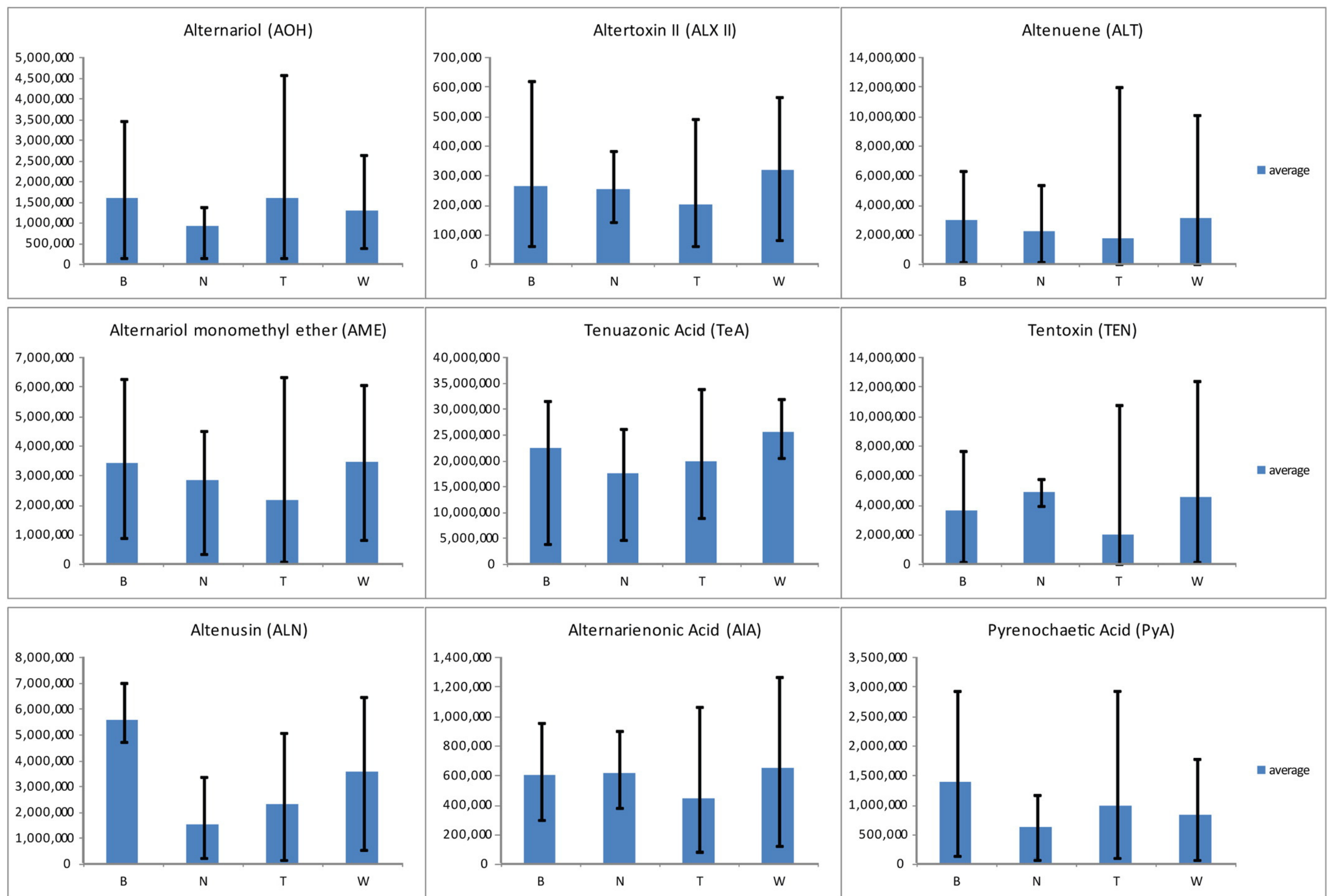

Fig. 3. Column charts of the semi-quantitative production of nine selected metabolites (peak areas) by the 83 strains of Argentinean *Alternaria* according to substratum. Comparisons can only be done between substrata and not between metabolites. Blue columns are average production and black lines are production range. Substratum code: B: blueberry; N: walnut; T: tomato; W: wheat. Y-axes are arbitrary (relative peak areas). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

was unique to cereals, whereas strains classified as belonging to the *A. arborescens* sp.-grp or having sporulation pattern "M" were only isolated from tomatoes. Otherwise, no clear association between substrate and identity could be found.

4. Discussion

4.1. Identification of Argentinean Alternaria strains

The twelve representative *Alternaria* strains, used for comparison in this study, are well-known strains that originate from the collection of E.G. Simmons and/or have been used in several studies on *Alternaria* (Table 1). They have all been characterized morphologically and chemically (Andersen et al, 2001; Andersen et al., 2002), but using molecular methods have been characterized only by RAPD-PCR (Polizzotto et al., 2012), since sequencing of the ITS and other genomic regions only yield resolution at section/species-group level (Woudenberg et al., 2013) and not at species level. *A. alternata* especially has been shown to be distinct from other species (Andersen et al., 2001; Andersen et al., 2005; Polizzotto et al., 2012) and this species contains only a handful of strains, where BA0922 and BA0923 are the most robust ones (Simmons, 2007). None of the Argentinean strains examined in this study showed any resemblance to the two representative strains of *A. alternata*. Erroneously, scores of papers have reported *A. alternata* as the most frequent *Alternaria* species on crop plants and food products (e.g. Barkai-Golan and Paster, 2008; Broggi et al., 2007; EFSA, 2011; Logrieco et al., 2003). *Alternaria alternata sensu stricto* is a rare species and most strains labelled as *A. alternata* are in fact members of the *A. tenuissima* sp.-grp., the *A. arborescens* sp.-grp. or even other *Alternaria* species-groups (Roberts, 2001; Simmons, 1999).

The results from this study show that none of the Argentinean strains studied could be assigned to any particular species based on morphology or metabolite profiles (chemotaxonomy); only to species-groups. The present study parallels the polyphasic study done on the *A. infectoria* sp.-grp. (Andersen et al., 2009) where the morphological diversity did not correspond to the chemical variations in the metabolite profiles. Fifteen Argentinean strains could, however, morphologically be assigned to particular species-groups: the *A. infectoria* sp.-grp. (cluster 4 = group K), the *A. arborescens* sp.-grp. (cluster 2b = group L) and the *A. armoraciae* sp.-grp. (cluster 2a = group M), which corresponded with the metabolite profile analyses. More than 70 Argentinean strains could not confidently be assigned to a well-defined species-group, where both morphology and metabolite profile concurred, only as members of either groups G or H according to Simmons (2007). These two morphological groups, G and H, alone comprise more than 50 described *Alternaria* species where several species are associated with tomato and blueberry (Simmons, 2007). Furthermore, there are more than 250 described *Alternaria* spp. (Simmons, 2007) contrary to the 50 species claimed in EFSA report (2011).

Identification of *Alternaria* strains to species level based on morphology is difficult to none-experts and until usable genes for barcoding/identification are discovered, collective species-group identifications have to suffice. Since most of these small-spored strains produce AOH, AME and other polyketides, genes in this pathway can be targeted for molecular identification. In other genera metabolite pathway genes, e.g. the trichodiene synthase 5 gene in *Stachybotrys* spp., have

successfully been used to resolve closely related taxa into robust species (Andersen et al., 2003). Further work may reveal if there is an association between strains in group G and cluster 1 and between group H and cluster 2.

4.2. Analysis of fungal metabolites

Fast and accurate identification of known *Alternaria* metabolites is essential for assessment of food safety. The analyses in the study show that at least 75 % of the Argentinean strains are able to produce compounds (potential mycotoxins) commonly associated with *Alternaria* (Table 2), such as the AOHs, the ALXs, TeAs and TENs. The results also revealed other metabolites that have not previously been associated with small-spored *Alternaria* (Table 3). Alternarienonic acid (AlA) was produced by 75 % of the strains, including the representative strains of *A. alternata*, but not by any strains in the *A. infectoria* sp.-grp. Less commonly produced mycotoxins/metabolites were ALT (69 %), altersetin (ALS) (57 %), and pyrenochaetic acid (PyA) (69 %). A smaller number of strains also produced altenusin (ALN) (43 %) and altechromone A (40 %). However, many other metabolites are produced by *Alternaria* strains and some with the same or similar elemental compositions. Other metabolites seem to be cyclic polyketides (based on the ratio of oxygen atoms to carbon and number of unsaturations) yielding MC/HRMS spectra, which are difficult to interpret as the main losses are H_2O, CH_3, $O–CH_3$, acetic acid and CO_2. To establish if these metabolites can be found in commodities containing berries, nuts, tomatoes and cereals, further investigations are needed and UHPLC with high resolution mass spectrometry is a promising tool for this.

For profiling of secondary metabolites liquid chromatography (LC) with UV–Vis detection has now been surpassed by LC with High Resolution Mass Spectrometric detection (HRMS) as the data files can be mined for hundreds of known secondary metabolites in seconds (Klitgaard et al., 2014). However, since compounds with identical elemental compositions cannot be differentiated by HRMS alone, reference standards, informative MS/MS spectra or specific UV–Vis spectra are needed (El-Elimat et al., 2013; Nielsen et al., 2011). Mapping such data is still a big challenge as only a handful of the *Alternaria* metabolites are commercially available. One solution could be to use MS–MS libraries to identify compounds automatically. This is the preferred strategy in forensic science and toxicology, for which commercial compound libraries are available (Kildgaard et al., 2014). However, no MS–MS libraries on fungal and plant derived metabolites are currently available, except for the 1300 compound library at DTU, which only contains 50 *Alternaria* compounds (Kildgaard et al., 2014). This is probably due to the lack of requirement to publish an atmospheric pressure MS–MS spectrum (e.g. in MassBank) when publishing new structures from fungi and plants.

4.3. The toxicological potential of Alternaria spp. in Argentinean food products

The results from this study showed that strains with G/H sporulation pattern could be isolated from all four substrates, blueberries, walnuts, tomatoes and wheat. However, comparing metabolite production as a function of substrate showed some interesting variations (Fig. 3). Even though the number of strains from blueberry (11), walnut (5), tomato (50) and wheat (17) was somewhat disproportionate, some trends could be detected. For example, the production of ALN and PyA was higher from strains isolated from blueberries than strains from the other products, suggesting that mouldy blueberries are more likely to contain altenusin and pyrenochaetic acid than e.g. mouldy tomatoes or wheat. Similarly, wheat might be more contaminated with tenuazonic acid and alternarienonic acid than tomatoes, which is in agreement with Muller and Korn (2013) who found TeA in every year in a 10-year survey. On the other hand, mouldy tomatoes seem to be associated with strains with a high potential for AOH and ALX production compared to blueberries, walnuts and wheat. Furthermore, strains isolated from tomato exhibited the biggest range in most metabolite analyses, probably due to the higher number of strains examined.

As the results in this study show, chemotaxonomic identification of small-spored *Alternaria* strains is not a usable method, due to lack of consistency in metabolite production within a species-group combined with the lack of described species matching the metabolite profiles. However, metabolite profiling has a dual purpose as it can be used for partly predicting the toxicological potential of the strain as well as for chemotaxonomic purposes (Frisvad et al., 2008). However, the full chemical potential needs to be taken into account, since true phytotoxins are only produced in the plant during infection. Thus infection trials are necessary to evaluate this as plants can glucosylate or sulfanate fungal metabolites to detoxify them (Berthiller et al., 2011). In such studies where more chemical background comes from the plant itself than from the fungus, LC–HRMS can still be used combined with a targeted approach for known fungal compounds seen from pure culture extracts, while novel compounds need to be detected by a metabolomics multivariant approach (Dunn et al., 2013). However for screening of naturally infected food samples where e.g. only few kernels/fruits are infected, LC–MS/MS on high sensitive triple quadrupole mass spectrometer is needed as more background and less fungal compound will be present in the sample (Abia et al., 2013).

For example, strains from the *A. infectoria* sp.-grp., which the EFSA report (2011) did not take into account, are associated with cereal grain (Andersen et al., 2002; 2009) and produce infectopyrones and phomenins. These metabolites were tested in MRC-5 cells and found to be non-cytotoxic (Ivanova et al., 2010), whereas altertoxins tested in HT29 cells were found to be genotoxic (Schwarz et al., 2012). This suggests that *A. infectoria* sp.-grp. found in food and feeds are of lesser concern than members of the *A. tenuissima* sp.-grp. However, other studies have shown that some members of the *A. infectoria* sp.-grp. are able to produce altertoxin-like metabolites (Andersen et al., 2009; Andersen and Thrane, 1996b), which need to be tested.

Considering that a variety of small-spored species of *Alternaria* is so widely distributed in Argentinean tomatoes and cereals for both domestic use and export, this suggest the need for the development of strategies on monitoring the occurrence of the major *Alternaria* metabolites (AOH, AME, ALX II, ALT, TeA, TEN) in the crops as well as in finished food products, which qualitatively can be achieved with LC-HRMS, while quantification and validated methods require commercially available standards.

5. Conclusions and recommendations

Argentinean small-spored *Alternaria* strains differ morphologically from the described *Alternaria* species (Simmons, 2007), however, their metabolite profiles are similar to food associated *Alternaria* from other parts of the world (Andersen et al., 2009, Polizzotto et al. 2009). Viewed in food safety perspective, both the Argentinean and the EU food safety agencies should prioritize the following 12 *Alternaria* metabolites in their monitor/observation/review programme in order to establish if *Alternaria* contamination of food and feed products constitutes a risk and if statutory guidelines should be made: alternariol, alternariol monomethyl ether, tenuazonic acid and its derivate, tentoxin and dihydrotentoxin, altenuene, altertoxins I–III, alternarienonic acid and pyrenochaetic acid. AAL toxins should not be included, since they are only produced by one strain, which may be a strict plant pathogen. Additionally, cereal and cereal products should be monitored for 4Z-infectopyrone and phomapyrone A, since these commodities also can be contaminated with strains belonging to the *A. infectoria* species-group. Fruit and cereals that are used in manufacturing, such as tomatoes and wheat, should be giving priority over products that are normally consumed fresh, such as blueberries and walnuts.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ijfoodmicro.2014.11.029.

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