Chapter 2

Alternaria Species and Their Associated Mycotoxins

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Abstract

The genus *Alternaria* includes more than 250 species. The traditional methods for identification of *Alternaria* species are based on morphological characteristics of the reproductive structures and sporulation patterns under controlled culture conditions. Cladistics analyses of "housekeeping genes" commonly used for other genera, failed to discriminate among the small-spored *Alternaria* species. The development of molecular methods achieving a better agreement with morphological differences is still needed. The production of secondary metabolites has also been used as a means of classification and identification. *Alternaria* spp. can produce a wide variety of toxic metabolites. These metabolites belong principally to three different structural groups: (1) the dibenzopyrone derivatives, alternariol (AOH), alternariol monomethyl ether (AME), and altenuene (ALT); (2) the perylene derivative altertoxins (ATX-I, ATX-II, and ATX II); and (3) the tetramic acid derivative, tenuazonic acid (TeA). TeA, AOH, AME, ALT, and ATX-I are the main. Certain species in the genus *Alternaria* produce host-specific toxins (HSTs) that contribute to their pathogenicity and virulence. *Alternaria* species are plant pathogens that cause spoilage of agricultural commodities with consequent mycotoxin accumulation and economic losses. Vegetable foods infected by *Alternaria* rot could introduce high amounts of these toxins to the human diet. More investigations on the toxic potential of these toxins and their hazard for human consumption are needed to make a reliable risk assessment of dietary exposure.

Key words *Alternaria* species, Taxonomy, Mycotoxins, Grains, Fruits, Vegetables

1 Introduction

The genus *Alternaria* includes more than 250 species of ubiquitous dematiaceous hyphomycetes [1–4]. It is widely distributed in the environment and its spores can be isolated from several different habitats. Some saprotrophic species are commonly found in soil, air, or indoor environments [5]. However, most are plant pathogens that cause pre- and postharvest damage to agricultural products including cereal grains, fruits, and vegetables [6]. The genus can infect more than 4000 host plants. Its spores are among the most common and potent airborne allergens and sensitization to *Alternaria* allergens has been determined as an important onset of childhood asthma in arid regions [7].

Antonio Moretti and Antonia Susca (eds.), *Mycotoxigenic Fungi: Methods and Protocols*, Methods in Molecular Biology, vol. 1542,
DOI 10.1007/978-1-4939-6707-0_2,

2 Taxonomy

2.1 Morpho-Taxonomy

The genus was first described by Nees [8] with *A. tenuis* as the type. It is characterized by the production of large brown or dark conidia with both longitudinal and transverse septa (phaeodictyospores), borne from inconspicuous conidiophores, and with a distinct conical narrowing or "beak" at the apical end. These structures can be solitary or produced in various patterns of chains. Several subsequent descriptions of additional *Alternaria* species have been made by Elliot [9], Wiltshire [10], Neergaard [11], Joly [12], Simmons [13], and Ellis [1, 2]. The traditional methods for identification of *Alternaria* species are primarily based on morphological characteristics of the reproductive structures, including shape, color, size, septation, and ornamentation. However, due to the wide diversity of species and the complexity of these structures, identification solely based on these characteristics can be extremely laborious and time consuming, becoming restricted to experts in this field.

Several attempts to organize the genus in subgeneric groups to simplify its classification have been proposed, either formally or informally [14]. A common segregation consists in the distinction of two groups according to conidia size, the "large-spored" (conidia size 60–100 μ) and "small-spored" (conidia <60 μ) *Alternaria*. The small-spored species are cosmopolitan saprotrophs, plant pathogens, allergens, and mycotoxin producers, being the most commonly reported group in foods. Its taxonomy is still under revision, and there is a need for their accurate identification in a broad range of disciplines.

More recently, Simmons [3] developed a classification based on the species group concept, organizing the genus into a number of species groups distinguished by sporulation patterns and conidia morphology, each of which is typified by a representative species, for instance the *A. alternata*, *A. tenuissima*, *A. infectoria*, *A. porri*, or *A. brassicicola* species group. This subgeneric level classification arranges the morphologically diverse assemblage of *Alternaria* spp. and allows a generalized discussion of morphologically similar species.

A further attempt to simplify the identification of *Alternaria* species was introduced by Simmons and Roberts [15]. Their study involved a large number of small-spored *Alternaria* with the utilization of the three-dimensional sporulation pattern as a tool for categorizing species group. They described six major sporulation groups (1–6), each one associated with a representative species. The definition of stable sporulation patterns under controlled culture conditions and the grouping of similar species have been particularly valuable among the small-spored catenulate *Alternaria*, which represent the most challenging in terms of accurate diagnostics due to their complex three-dimensional sporulation patterns [6].

Simmons has intended to cover the entire genus in his series of taxonomic essays in *Alternaria Themes and Variations* [16–20], describing at least 296 taxa sufficiently distinctive to be maintained in an initial assembly of named species. His identification manual [4] summarizes descriptions and illustrations of the maintained species based on the examination of stable isolates in axenic culture.

There are still discrepancies among the use of morphological characters as criteria of identification for small-spored *Alternaria* species. Those classifications based on conidial size as the primary taxonomic criterion concluded that all isolates whose spore dimensions fall within the range described for *A. alternata* should be considered to belong to this species. Nishimura et al. [21] proposed naming all pathogen species indistinguishable from *A. alternata* by conidial size, which were host-specific toxin producers, as pathotypes of *A. alternata*. Thus, several species were included in this collective group, such as *A. gaisen* (Japanese pear pathotype), *A. citri* (citrus pathotype), and *A. mali* (apple pathotype), as shown in Table 1. Rotem [22] named these pathotypes as special forms of *A. alternata* (e.g., *A. alternata* f. sp. *lycopersici* for the tomato pathotype). Several adverse consequences of these approaches have been pointed out in many subsequent scientific works. They criticized the inclusion of large amounts of discriminating data in the literature under a single nondiscriminating name [23]. Moreover, it has been demonstrated that some pathotypes can spontaneously lose the capacity of producing the host-specific toxin, with a consequent loss of pathogenicity. It has also been suggested that lateral

Table 1
Host-specific toxins of plant pathogen *Alternaria* species

Disease	Pathotype	Species (synonym)	Toxins
Black spot of Japanese pear	*A. alternata* Japanese pear pathotype	*Alternaria gaisen* Nagano *(A. kikuchiana* Tanaka)	AK
Black spot of strawberries	*A. alternata* strawberry pathotype	*A. alternata* f. sp. *fragariae* Dingley	AF
Brown spot of tangerine	*A. alternata* tangerine pathotype	*A. tangelonis* Simmons *(A. citri* tangerine pathotype)	ACT
Leaf spot of rough lemon	*A. alternata* rough lemon pathotype	*A. limoniasperae* Simmons *(A. citri* rough lemon pathotype)	ACR
Brown spot of tobacco	*A. alternata* tobacco pathotype	*A. longipes* Mason	AT
Alternaria blotch of apple	*A. alternata* apple pathotype	*A. mali* Roberts	AM
Stem canker of tomato	*A. alternata* tomato pathotype	*A. arborescens* Simmons *(A. alternata* f. sp. *lycopersici* Keissl)	AAL

gene transfer of toxin genes might occur, indicating that toxin production is not a stable character. Thus a system for classifying the small-spored *Alternaria* species based on pathotype is not a practical or desirable system for *Alternaria* taxonomy [24]. The extended use of this system has led to the general belief that *A. alternata* is the most abundant small-spore taxon in nature.

2.2 Molecular Taxonomy

With the advancement of molecular techniques, several studies have examined taxonomic relationships among *Alternaria* spp. using a variety of methods in an attempt to establish consensus with contemporary morphological based species. Most of them have been focused on small-spored catenulate *Alternaria*, which show little resolution in their molecular phylogeny. However, cladistics analyses of "housekeeping genes" commonly used for other genera, such as the mitochondrial large subunit (mtLSU) ribosomal DNA, internal transcribed spacer (ITS), β-tubulin, translation elongation factor α, calmodulin, actin, and chitin synthetase, failed to discriminate among the small-spored species, except for the *A. infectoria* species group. Analyses of RAPD and PCR-RFLP data were effective to distinguish small-spored from large-spored species, such as *A. porri* or *A. solani* [25, 26], and provided resolution among some of the most common small-spored species groups. Pryor and Michailides [27] obtained separate clusters for *A. infectoria*, *A. arborescens*, and a combined *A. alternata/A. tenuissima* cluster. These last two species groups have proved to be the most difficult to discriminate by molecular techniques, although they can be distinguished by culture in standardized conditions. Roberts et al. [28] reported that RAPD analyses resolved the small-spored morphological groups or species *A. gaisen*, *A. longipes*, *A. tenuissima* sp.-grp., *A. arborescens* sp.-grp., and *A. infectoria* sp.-grp. Peever et al. [29] found that an endopolygalacturonase (endoPG) gen and two anonymous loci were sufficiently variable to differentiate members of the *A. alternata* sp.-grp., with general agreement, but not strict congruence between morphological classification and the phylogeny. This research was expanded by Andrew et al. [24], using OPA1-3, OPA10-2, and endoPG, founding strict agreement between morphology and phylogenetic lineage for isolates classified in the *A. arborescens* group, but not for the *A. alternata* and *A. tenuissima* groups.

More recently, Lawrence et al. [7] attempted to assess the phylogenetic relationships among *Alternaria* and closely related genera, using a larger sample of taxa. Based on the analysis of five loci (gpd, *Alt a1*, actin, plasma membrane ATPase, calmodulin) they introduced two new species groups, *A. panax* and *A. gypsophila*, and proposed to elevate eight asexual species groups to the taxonomic status of sections within *Alternaria*, since morphological features of the species groups were not congruent with molecular data. According to their results, the sexual phylogenetic

Alternaria lineage, the *A. infectoria* sp.-grp., did not get the status of section. In another recent work, Woundenberg et al. [30] intended to delineate phylogenetic lineages within *Alternaria* and allied genera based on nucleotide sequence data of parts of the 18S nrDNA (SSU), 28S nrDNA (LSU), the internal transcribed spacer regions 1 and 2 and intervening 5.8S nrDNA (ITS), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), RNA polymerase second largest subunit (RPB2), and translation elongation factor 1-alpha (TEF1) gene regions. Species of *Alternaria* were assigned to 24 *Alternaria* sections, of which 16 were newly described, and 6 monotypic lineages. As a result of this proposed classification many of the most common small-spored species groups described by Simmons [4], such as *A. gaisen*, *A. tenuissima*, *A. arborescens*, *A. longipes*, among others, were enclosed together into the *Alternata* section of which *Alternaria alternata* (Fr. Keissl) was described as the type species. *A. infectoria* remained differentiated from the asexual small-spored species groups as the type species of the *Infectoriae* section, in which other members of the *A. infectoria* sp.-grp. described by Simmons were included.

In a study on the *Alternaria* species causing brown spot of citrus, Stewart et al. [31] found that morphospecies described as citrus pathogens were poorly supported by molecular analyses, sequencing endoPG gen, two anonymous, noncoding SCAR markers OPA1-3 and OPA2-1, and one noncoding microsatellite flanking region Flank-F3. According to their results, citrus brown spot is caused by a maximum of two species of *Alternaria*, and they suggested that taxonomic revision of *Alternaria*-infecting citrus, based on congruent morphological and genetic analyses, is needed. Characterization of *Alternaria* species by morphological and molecular analyses is important in making a correct identification, but might not be sufficient to differentiate between closely related species groups. Lineage sorting, recombination, and horizontal transfer make phylogenetic analyses and species delimitation among small-spored *Alternaria* challenging. Sequencing of "housekeeping genes" or some functional genes has not provided segregation among the small-spored *Alternaria* species. However, this lack of resolution does not necessarily imply that they all belong to the same species; it might indicate that there is little diversity among the isolates on the particular sequences under study. Techniques such as RAPD, which characterizes random priming sites across the entire genome, provided better resolution for the small-spored species. There is still the need for molecular methods that could achieve a better agreement between morphological differences.

2.3 Chemo-Taxonomy

In addition to morphology and molecular analysis, the production of secondary metabolites has been used as a means of classification and identification, taking advantage of the enormous potential of this genus to biosynthesize secondary metabolites.

Chromatographic methods such as thin-layer chromatography, initially, and high-performance liquid chromatography with diode array detection (HPLC-DAD) or combined with mass spectrometry (HPLC-MS), later, have been used in several scientific works to determine the profiles of metabolites produced on standardized laboratory media [32]. Gas chromatography combined with mass spectrometry (GC-MS) has been used for volatile secondary metabolites in *Penicillium* taxonomy [33]. Nowadays, the method of preference for fungal chemotaxonomical studies is HPLC-DAD-MS [34, 35].

Extraction methods are easy to use, less time consuming than morphological characterization, and relatively economic, and they have been successful in differentiating between species in other genera such as *Aspergillus*, *Fusarium*, and *Penicillium* [32]. Secondary metabolite data can be statistically analyzed to determine a characteristic profile for a species or species group, or they can be used to determine species-specific metabolites that could be adopted as chemotaxonomic markers in taxon identification.

Andersen and Thrane [36] reported that the chemical profiles for the *A. infectoria* sp.-grp. contained unique metabolites not identical to any of the known *Alternaria* metabolites, and it could be useful to distinguish between this and the *A. alternata* sp.-grp. Andersen et al. [37] extended their studies demonstrating the chemical and morphological segregation of *Alternaria alternata*, *A. gaisen* (Japanese pear pathotype), and *A. longipes* (tobacco pathotype), when the cultures were grown under standardized conditions. Based on these results they recommended the use of the species names instead of their corresponding *A. alternata* pathotype. In a further work, Andersen et al. [38] found that the secondary metabolite profile from the *A. infectoria* sp.-grp. is chemically very different from both the *A. arborescens* and the *A. tenuissima* sp.-grp. with only a few metabolites in common. *A. arborescens* and *A. tenuissima* sp.-grp. had most of the known *Alternaria* metabolites in common, but they also produced a number of metabolites by which the two species groups can be distinguished. Furthermore, by combining morphological and chemical data obtained by two different methods (HPLC-UV and MS) more host-specific toxin-producing *Alternaria* isolates could be segregated from *A. alternata*. Andersen et al. [23] showed that the analyses of cultural and chemical data allowed to segregate *A. alternata*, *A. longipes* (tobacco pathotype), *A. gaisen* (Japanese pear pathotype, syn. *A. kikuchiana*), *A. tangelonis, A. turkisafria*, and *A. limoniasperae*, which have been segregated as new species from the citrus pathogen complex, but regarded as pathotypes of *A alternata*. The metabolite profile of *A. alternata* was different from those of the five species that are commonly described as *A. alternata* pathotypes. In another work from Andersen et al. [39], chemotaxonomy proved useful to discriminate between *A. dauci*, *A. solani*, and *A. tomatophila* and sets of species-specific metabolites could be selected for each of these species as chemotaxonomic markers.

2.4 Polyphasic Taxonomy

Most recently, a polyphasic approach, which integrates three sets of data, such as morphological characteristics, molecular analyses, and secondary metabolite profiling, has been applied to segregate *Alternaria* species, especially in an attempt of differentiate the complicated small-spored species. The combination of all the information provided by different perspectives represents a powerful tool for classification of this complex genus.

The polyphasic approach was applied by Andersen et al. [40] to characterize strains from the *A. infectoria* sp.-grp. and closely related species. The *A. infectoria* sp.-grp. could be separated from *Embellisia abundans*, *Chalastospora cetera*, and *Alternaria malorum* based on morphology, secondary metabolite profiles, and molecular classification. From the chemical analysis, the main factor segregating *A. infectoria* was the capability of producing altertoxins and novae-zelandins. Sequence analyses of ITS, *gpd*, and translocation elongation factor 1α showed two clades, one with all the *A. infectoria* sp.-grp. strains and one with the rest of the species tested. This polyphasic approach revealed that *A. malorum* var. *polymorpha* and *A. malorum* strains do not belong in *Alternaria*, but in the *Chalastospora* genus, as several distinct species.

Another polyphasic study was carried on to characterize endophytic *Alternaria* strains isolated from grapevine [41]. A pooled cluster analysis was obtained by combining morphological, molecular, and chemical data. The species were morphologically identified as members of the *A. arborescens* and *A. tenuissima* species group, and the RAPD analysis confirmed these results and showed that they were molecularly distinct from strains belonging to the *A. alternata* sp.-grp. The strains were also grouped in the same way by chemotaxonomy, with strains producing metabolites typical of these species groups.

3 *Alternaria* Toxins

Alternaria spp. can produce a wide variety of toxic metabolites which play an important role in plant pathogenesis. About 70 toxic metabolites of *Alternaria spp.* have been characterized to date. These bioactive compounds with different chemical structure also exhibit different biological activities and functions and under certain conditions of temperature and humidity could accumulate in vegetable foods and be harmful to humans and animals [42, 43]. These metabolites belong principally to three different structural groups: (1) the dibenzopyrone derivatives, alternariol (AOH), alternariol monomethyl ether (AME), and altenuene (ALT); (2) the perylene derivative altertoxins (ATX-I, ATX-II, and ATX II); and (3) the tetramic acid derivative, tenuazonic acid (TeA). TeA, AOH, AME, ALT, and ATX-I are the main *Alternaria* mycotoxins that can be found as contaminants of food commodities. Of

particular health concern is the association found between *Alternaria* contamination in cereal grains and the high levels of human esophageal cancer in China [44, 45].

3.1 Alternariol, Alternariol Monomethyl Ether, and Altenuene

The mutagenicity and carcinogenicity of AME and AOH, and their relevance to the etiology of human esophageal cancer, were studied. These mycotoxins were the main toxic compounds found in grains in an area with high incidence of esophageal cancer. AME and AOH might cause cell mutagenicity and transformation, and could combine with the DNA isolated from human fetal esophageal epithelium and promote proliferation of human fetal esophageal epithelium in vitro. Also, squamous cell carcinoma of the fetal oesophagus could be induced by AOH [42, 45]. The mutagenicity of AOH in Chinese hamster V79 cells and in mouse lymphoma L5178Y tk+/− cells (MLC) was investigated. The mutagenic potency of AOH was about 50-fold lower than that of the established mutagen 4-nitroquinoline-*N*-oxide in both cell lines. The mutagenicity of AOH may have an incidence on the carcinogenicity of this mycotoxin [46].

AOH inhibited metabolic activity and cellular proliferation of porcine granulosa cells. In the regulation of female fertility the hormone progesterone (P4) plays an important role. AOH and AME inhibited P4 secretion in cultured porcine granulosa cells, so their reproductive cycles in pig and other mammalian species may be affected [47].

Cell proliferation studies on human endometrial adenocarcinoma cell line (Ishikawa) and Chinese hamster V79 cells indicated that AOH inhibited cell proliferation by interfering with the cell cycle [48]. AOH induces oxidative DNA damage and DNA strand breaks [49]. AOH and AME act as topoisomerase poisons, which contribute to their genotoxic properties and might cause DNA damage in human colon carcinoma cells. DNA topoisomerases are enzymes regulating DNA topology during transcription, replication, chromosome condensation, and maintenance of genome stability. When interference with the activity of topoisomerases occurs the DNA integrity could be affected [50].

There are very few toxicological data on altenuene, indicating that it has a low acute toxicity and a low-to-moderate antimicrobial activity [51, 52].

3.2 Altertoxins

ATXs are mutagenic in the Ames test when Salmonella strains TA98 and TA100 were used.

ATX-I, ATX-II, and ATX-III are more potent mutagens and acute toxins to mice than AOH and AME [42, 53]. ATX-I was studied by Schrader et al. [54] with and without nitrosylation, using Ames Salmonella strains TA97, TA102, and TA104. ATX-I was mutagenic in strain TA102 and weakly mutagenic in strain TA104. Nitrosylation of ATX-I enhanced mutagenicity. ATX-I was

also assessed for mammalian mutagenicity in Chinese hamster V79 lung fibroblasts and rat hepatoma H4IIE cells. ATX-I was not mutagenic in either V79 cells or H4IIE cells, but nitrosylated ATX-I was also directly mutagenic in mammalian test systems.

ATX-II is highly mutagenic in the Ames test and is a potent mutagen in cultured Chinese hamster V79 cells. ATX-II is at least 50 times more potent as a mutagen than AOH and AME. ATX-II does not affect the cell cycle but causes DNA strand breaks of V79 cells [55].

ATX-I and -II have been studied in the Caco-2 cell system, which is a widely accepted in vitro model for human intestinal absorption and metabolism. Caco-2 cells are derived from a human colonic tumor and form a monolayer with tight junctions similar to the human intestinal epithelium. ATX-I was well absorbed from the intestinal lumen and ATX-II intestinal absorption was very low. It must be expected that ATX-II will act primarily in the digestive tract and that ATX-I will reach blood circulation and act systemically [56].

3.3 Tenuazonic Acid

TeA is toxic to several animal species, e.g., mice, chicken, and dogs. In dogs, it caused hemorrhages in several organs. Increasing TeA doses in chicken feed suppressed weight gain and increased internal hemorrhages. TeA is more toxic than AOH, AME, and ALT. TeA is not mutagenic in bacterial systems [42, 53, 57]. Precancerous changes were observed in esophageal mucosa of mice [58]. Sorghum grain colonized by *Phoma sorghina* that contained TeA was associated with the human hematological disorder known as Onyalai in Southern Africa [42].

Using *Chlamydomonas reinhardtii, Vicia faba* root tip, and three mammalian normal cell lines, toxicity of TeA was examined. The growth and chlorophyll concentration of *C. reinhardtii* were inhibited. TeA also inhibited the proliferation of 3 T3 mouse fibroblasts (3 T3 cells), Chinese hamster lung cells (CHL cells), and human hepatocytes (L-O2 cells). These results suggested that TA inhibited protein biosynthesis in the cells [59].

3.4 Host-Specific Toxins

Certain species in the genus *Alternaria* produce low-molecular-weight compounds known as host-specific toxins (HSTs) that contribute to their pathogenicity and virulence. Plants that are susceptible to the pathogen are sensitive to the toxin and all isolates that fail to produce HSTs lose pathogenicity to the plants. These host-specific forms have been earlier designed as pathotypes of *A. alternata*, as it is mentioned above, but this classification has not been accepted widely because of difficulties in the discrimination of small-spored *Alternaria* species with few morphological characteristics [60, 61]. In more recent works they were assigned to other species, as it is shown in Table 1.

Simmons and Roberts [15], based in three-dimensional conidiation patterns for differentiating similar species in the *Alternaria*

small-spored groups, sorted the isolates from black spot lesions of Japanese pear into six conidiation groups or species groups. Molecular phylogenetic studies have failed in resolving species groups and host association within the small-spored *Alternaria* species [24].

Chemical structures of HSTs have been determined. Toxins of the Japanese pear, strawberry, and tangerine pathotypes were found to be similar metabolites that are esters of the epoxydecatrienoic acid (EDA). The Japanese pear pathotype produces AK toxins I and II. Both toxins exhibit toxicity only on susceptible pear cultivars. The strawberry pathotype affects strawberry-susceptible cultivars. This pathotype was also pathogenic to susceptible Japanese pear in laboratory and produces AF toxins I, II, and III. AF toxin I is toxic to both strawberry and pear, AF toxin II is toxic only to pear, and toxin III is highly toxic to strawberry and slightly to pear. The tangerine pathotype affects tangerines and mandarins and was also found pathogenic to Japanese pear cultivars. The tangerine pathotype produces ACT toxins I and II. ACT toxin I is toxic to both citrus and pear.

The chemical structure of AM toxin I from the apple pathotype was elucidated as a cyclic tetrapeptide and the rough lemon pathotype produces ACR toxins. The major toxin, ACR toxin I, is a C19 polyalcohol with a dihydropyrone ring.

The tomato pathotype produces AAL toxins which are similar to fumonisins. It is known that fumonisins, very toxic mycotoxins produced by *Fusarium* species, can cause leukoencephalomalacia and pulmonary edema syndrome in animals and are associated to human esophageal cancer and neural tube defects. Fumonisins and AAL toxins together are called sphinganine analog mycotoxins (SAMT) due to their structural similarity to sphinganine, which is the backbone precursor of sphingolipids. AAL toxins and fumonisins show similar toxicity to plants and mammalian cells and also exhibited inhibitory activity to ceramide synthase, which is involved in sphingolipid biosynthesis. AAL toxins are produced by the tomato pathogen.

The mechanism for SAMT to execute their toxicity is through the competitive inhibition of sphinganine N-acetyltransferase (ceramide synthase). This leads to the obstruction of complex sphingolipid biosynthesis, such as the important second messenger ceramide in animal systems, and the accumulation of sphinganine. The inhibition of this enzyme leads to various diseases in animals and humans as ceramides and sphingolipids are ubiquitous constituents of eukaryotic cells and involved in crucial signal transduction of numerous cellular processes. SAMT are also found to induce apoptosis. In addition to their animal toxicity, AAL toxins are known as the causal agent of stem canker in tomato.

The gene clusters involved in HST production have been identified from the Japanese pear pathotype (*AKT* genes), strawberry pathotype (*AFT* genes), tangerine pathotype *(ACT* genes), apple

pathotype (*AMT* genes), rough lemon pathotype (*ACRT* genes), and tomato pathotype (*ALT* genes). There is evidence that these biosynthetic genes were clustered in small chromosomes of <2.0 Mb. These chromosomes appear to be conditionally dispensable (CD) chromosomes, which are not required for growth but that are essential to produce toxin and to cause disease. CD chromosomes, which nonpathogenic strains do not have, suggest that the ability to produce HSTs in the pathotypes could be acquired by intraspecies transfer of CD chromosomes. Protoplast fusion experiments provided evidence for intraspecies transfer of CD chromosomes in *A. alternata*. Hybrid strains between the tomato and apple pathotypes and between the tomato and strawberry pathotypes were made by protoplast fusion [62, 63]. The fusants synthesized two toxins produced by the parental strains and showed pathogenicity to both plants affected by the toxins. The fusants carried two CD chromosomes, one derived from each of the parental strains. It seems that *A. alternata* is able to accept and maintain a small, exogenous chromosome in its genome. This fact could indicate that pathogenicity could be acquired by strains by horizontal transfer of an entire pathogenicity chromosome and this could provide a possible mechanism by which new pathogens arise in nature [60–63].

3.5 Tentoxin

Tentoxin is a cyclic tetrapeptide from plant pathogen *Alternaria spp.* that inhibits chloroplast with the development of chlorotic symptoms on infected tissues. There is no direct effect of tentoxin on chlorophyll synthesis. Two fundamental processes are linked with this fact. The first one is inhibition of energy transfer of the chloroplast-localized CF1 ATPase. This process alone could not be responsible for the chlorosis because tentoxin also completely inhibits the transport of nuclear enzyme polyphenol oxidase (PPO) into the plastid even in etioplasts which should have no CF1 ATPase activity. Without this action PPO has no enzyme activity. Inhibition of these two steps seems to be linked, and both are inhibited in vivo in tentoxin-sensitive plant species and not affected in insensitive species. Tentoxin was also responsible for chlorophyll accumulation through overenergization of thylakoids, but this fact does not explain its effects on PPO processing in etioplasts without thylakoid membranes. The linkage of the β-subunit of proton ATPase to PPO processing remains unexplained [43, 64].

4 Natural Occurrence of *Alternaria* Toxins in Food and Feed

Alternaria species are plant pathogens that cause spoilage of agricultural commodities with consequent mycotoxin accumulation and economic losses. Mycotoxin accumulation in fruits and vegetables may occur in the field and during harvest, postharvest, and storage (Table 2).

Table 2
Natural occurrence of *Alternaria* toxins in food and feed

Food/feed	Mycotoxin range (μg/kg) (No. positive samples/no. total samples)					Country	References
	TeA	AOH	AME	ALT	ATX-I		
Wheat	Max 4224[a] (322/1064)	Max 832[a] (86/1064)	Max 905[a] (33/1064)	Max 197[a] (7/1064)	–	Germany	Muller et al. [65]
Wheat	1001-8814 (12/64)	645-1348 (4/64)	546-7451 (15/64)	–	–	Argentina	Azcarate et al. [66]
Feeding wheat	–	0.3-29 (21)[b]	0.3-133 (21)[b]	ND	–	Czech. Rep.	Zachariasova et al. [67]
Feeding maize	–	0.3-37 (8)[b]	0.3-34 (8)[b]	ND	–	Czech. Rep.	Zachariasova et al. [67]
Feeding oat	–	295-523 (3)[b]	223-444 (3)[b]	ND	–	Czech. Rep.	Zachariasova et al. [67]
Soya beans	–	25-211 (23/50)	62-1153 (22/50)	–	–	Argentina	Oviedo et al. [68]
Tomato sauces	ND	4-33 (11/17)	1-9 (12/17)	ND	ND	Switzerland	Noser et al. [69]
Tomato sauces	ND	4.0-6.8 (5/10)	ND	3.8-4.8 (8/10)	–	Italy	Prelle et al. [70]
Ketchup	10.2-1787 (31/31)	2.5-300 (14/31)	0.32-38 (28/31)	–	–	China	Zhao et al. [71]
Ketchup	ND	4-5 (3/19)	1 (3/19)	ND	ND	Switzerland	Noser et al. [69]
Tomato pure	29-4012 (29/80)	187-8.8 (6/80)	84-1.7 (26/80)	–	–	Argentina	Terminiello et al. [72]
Tomato pure	ND	4-10 (8/24)	1-4 (7/24)	ND	ND	Switzerland	Noser et al. [69]
Red wine	–	0.36-7.5 (5/5)	0.04-0.15 (5/5)	–	-	Germany	Asam et al. [75]
Red wine	–	0.03-7.41 (20/25)	0.01-0.23 (20/25)	–	–	Canada	Scott et al. [75]
White wine	–	0.10-7.59 (6/6)	ND	–	–	Germany	Asam et al. [73]
White wine	–	0.67-1.48 (2/23)	0.02-0.06 (2/23)	–	–	Canada	Scott et al. [75]
Grape juice	–	0.10-1.05 (5/5)	ND	–	–	Germany	Asam et al. [73]

Grape juice	–	0.03-0.46 (5/10)	0.01-39.5 (5/10)	–	–	Canada	Scott et al. [75]
Apple juice	–	0.16-0.22 (3/4)	ND	–	–	Germany	Asam et al. [73]
Apple juice	24.3-45.3 (2/10)	ND	ND	45.6 (1/10)		Italy	Prelle et al. [70]
Orange juice	–	0.16-0.24 (2/2)	ND	–	–	Germany	Asam et al. [73]
Citrus juice	1.21-4.3 (9/36)	ND	0.11-0.20 (4/36)	–	–	China	Zhao et al. [71]
Dried wine berries	4-18973 (10/13)	52-1308 (11/13)	776-26 (10/13)	4120-48 (7/13)	7.7-159 (11/13)	Slovakia	Mikusova et al. [76]

ND not detected, below the detection limit
– not determined
[a]range not reported
[b]total number of samples
number of positive samples not available in the reference

Vegetable foods infected by *Alternaria* rot could introduce high amounts of these toxins to the human diet if moldy fruit is not removed before processing.

4.1 Tomatoes

Tomatoes are susceptible to fungal decay because of their soft skin. *Alternaria* is responsible of the disease known as "black mold of tomato." Typical lesions are dark brown to black areas, with firm texture that can become several centimeters in diameter. Fruits become more susceptible to fungal invasion during ripening. The disease is favored by warm and rainy weather. Temperature is one of the major factors that affect the shelf life of tomato fruits, and, to control mold growth and toxin accumulation in tomatoes, the temperature should be maintained below 6 °C to avoid infection.

Alternaria mycotoxin occurrence has been reported in tomatoes. TeA was the major toxin produced in naturally infected fruits. Lower levels of AOH and AME were also recorded.

Moldy tomatoes could be used for processed tomato products with the consequent accumulation of toxins in these products. TeA, AOH, AME, ALT, and ALTX were detected in tomato paste, tomato pulp, and tomato puree samples, occasionally in very high amounts [57, 72, 77].

4.2 Apples

Moldy core rot is a factor that reduces apple fruit quality and it is a worldwide problem occurring in most countries where apples are grown. The disease is produced by *Alternaria* spp. Infection occurs via the open calyces, into the core or carpel regions, during fruit ripening and storage or by fungal spores on the fruit surface that enter through wounds formed during harvesting and handling. *Alternaria* strains isolated from rotten apples produced AOH and AME in the whole fruits after inoculation. High levels of mycotoxins were found in processed apple products made with apples affected by moldy core. The natural occurrence of AOH, AME, TeA, ALT, and ALTX in samples of apple juice and apple juice concentrate was reported in several countries [70, 78, 79].

4.3 Citrus Fruits

"Black heart rot" of oranges and lemons caused by *Alternaria* species is described as internal blackening of the fruit. Fruit with these defects should not be used to produce juice because the accumulation of toxins could occur.

Alternaria brown spot is a disease of mandarins, tangerines, and various tangerine hybrids. The pathogen causes necrotic lesions in mature fruit that are unacceptable to consumers. TeA, AME, and AOH were found in rotten samples [71, 79].

4.4 Cereal Grains

Alternaria is the most common genus found in cereal grains in several regions of the world. References from many countries about prevalence of this fungus in cereals indicate a very high incidence with more than 90% of the grains affected. Infected grains develop

a disease called "black point" consisting of a discoloration of the germ and the seed due to mycelial and conidial masses. Small grain cereals such as wheat, triticale, barley, and oats are frequently infected, whereas rice and maize are less susceptible. Black point is known to affect grain quality, giving a grayish color to the flour and by-products with great economic losses. Several *Alternaria* species have been involved. *A. triticina* is the major cause of wheat leaf blight. The *A. infectoria* species group is the casual agent of black point in certain wheat cultivars in Argentina, Australia, North America, and several European countries. Small grain cereals are frequently contaminated with *Alternaria* mycotoxins. Natural occurrence of AOH, AME, and TeA has been reported worldwide in wheat, barley, and oats [57, 65, 66, 80].

4.5 Other Foods

Olives are often affected by *Alternaria*, particularly if the fruits remain in the soil for a long time after ripening. Several *Alternaria* toxins were also found in olive oil as well as in other edible oils (rapeseed, sesame, and sunflower).

Alternaria mycotoxins have been reported in many other vegetable foods that are frequently infected by the fungus, such as peppers, melons, mangoes, sunflower, soya beans, raspberries, pecans, and Japanese pears. AOH and AME were detected in several fruit beverages such as grape juices, cranberry nectar, raspberry juice, red wine, and prune nectar [42, 57, 68, 79].

5 *Alternaria* Secondary Metabolite Profiles

The *Alternaria* genus is characterized by its enormous capacity of biosynthesizing secondary metabolites; many of them are known mycotoxins, others are phytotoxins, but the toxicity of most of them is still to be investigated.

It is known that the *A. infectoria* species group has a secondary metabolite profile completely different from the other small-spored species groups. Several works have showed that none of the isolates belonging to the *A. infectoria* sp.-grp. was able to produce any of the known *Alternaria* metabolites, such as alternariols, altenuene, tentoxin, tenuazonic acid, altersolanols, and AAL toxins [38, 40]. These isolates were instead producers of infectopyrone, 4Z-infectopyrone, novae-zelandin A, and novae-zelandin B, metabolites that could be used as chemotaxonomic markers for the *A. infectoria* sp.-grp. [81].

The metabolites confirmed to be synthesized by *A. alternata* include altenuene, alternariol, alternariol monomethyl ether, and altertoxins, but not tenuazonic acid [37, 38]. Although several works in the literature reported the production of tenuazonic acid by *A. alternata* the discrepancies in this genus taxonomy could have led to most of the small-spored *Alternaria* species identified

Table 3
Secondary metabolites most frequently produced by small-spored *Alternaria* species

Metabolite	*A. alternata*	*A. tenuissima*	*A. arborescens*	*A. longipes*	*A. gaisen*	*A. tangelonis*	*A. turkisafria*	*A. limonia sperae*	*A. mali*
Altenuene	+	+[b]	+[b]	–	–	–	–	–	+
Alternariol	+	+	+	-	+	+	+	+	+
Alternariol monomethyl ether	+	+	+	-	+	+	+	+	+
Altersetin	–	+	+	–	+	+	+	+[c]	+[a]
Altertoxin I	+[a]	+	+[b]	+	+	+	+	+	+
Tentoxin	+[b]	+[b]	+[c]	–	+	+	+	–	+
Tenuazonic acid	–	+[a]	+[a]	+	+	+	+	+	+

(+) >90 % isolates
[a]70–90 % isolates
[b]<30 % isolates
[c]<10 % isolates

Table 4
Secondary metabolites most frequently produced by large-spored *Alternaria* species

Metabolite	*A. dauci*	*A. porri*	*A. solani*	*A. tomatophila*
Altenuene	–	–	–	–
Alternariol	+	–	+	+[c]
Alterporriol	–	+	+	–
Altersolanol A	–	+	+	+[b]
Altertoxin	–	–	+[a]	+
Macrosporin	–	+	+	+[b]
Tentoxin	–	+	+/–*	–
Tenuazonic acid	–	–	–	–
Zinniol	+	+	+	–

*discrepant data in literature
[a]70–90 % isolates
[b]50–70 % isolates
[c]<10 % isolates

as *A. alternata*; thus, other small-spored species, whose morphology is closely related to this species, could have been responsible for tenuazonic acid production. Table 3 shows the secondary metabolites most frequently produced by small-spored plant pathogenic and food-contaminant *Alternaria* species.

Large-spored *Alternaria* species can be easily distinguished from the small-spored ones by chemotaxonomy since they have few metabolites in common with them. Alterporriol, altersolanol, and macrosporin are the most frequent compounds biosynthesized by these species. Table 4 shows the most common compounds produced by some plant pathogenic large-spored *Alternaria* species.

6 Conclusions

Species delimitation is important within the *Alternaria* genus, which includes a large number of human and plant pathogenic species, most of them producing a wide range of active metabolites. The correct segregation of species plays a critical role due to the economic importance of *Alternaria* species, especially the small-spored ones, which can contaminate crops of agricultural relevance. Furthermore, for the unambiguous identification of species it is necessary to track the movement of plant pathogens in global trade of foods. The threat of introducing a new pathogen to a different habitat around the world has resulted in rejection of exported crops [31]. The presence of a certain pathogen in food crops is associated with the possible occurrence of secondary metabolites representing a health risk to humans and animals. Thus, incorrect naming of new species or the misidentification of a species could mean significant economic losses.

At present, there are no specific regulations for any of the *Alternaria* toxins in foods. However, these mycotoxins should not be underestimated since they are produced by several *Alternaria* species frequently associated with a wide range of agricultural products and processed plant foods of relevant value in the human diet. More investigations on the toxic potential of these toxins and their hazard for human consumption are needed to make a reliable risk assessment of dietary exposure and better define eventual guidelines on *Alternaria* mycotoxin limits in foods [74].

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http://www.springer.com/978-1-4939-6705-6

Mycotoxigenic Fungi
Methods and Protocols
Moretti, A.; Susca, A. (Eds.)
2017, XI, 383 p. 45 illus., 17 illus. in color., Hardcover
ISBN: 978-1-4939-6705-6
A product of Humana Press