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Natural Occurrence of Fungi and Fungal Metabolites in Moldy Tomatoes

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Fresh tomatoes, homegrown and from supermarkets, with developing fungal lesions were collected. Each lesion was sampled, and the resulting fungal cultures were identified morphologically and extracted for analyzes of secondary metabolites. The tomatoes were incubated at 25 °C for a week, extracted, and analyzed for fungal metabolites. Extracts from pure cultures were compared with extracts from the moldy tomatoes and fungal metabolite standards in two HPLC systems with DAD and FLD detection. The results showed that *Penicillium tularense*, *Stemphylium eturmiunum*, and *S.* cf. *lycopersici* were postharvest spoilers of fresh tomatoes. The results also showed that *P. tularense* could produce janthitrems, paspalinine, paxilline, and 3-*O*-acetoxypaxilline, that *S.* cf. *lycopersici* could produce stemphols, and that *S. eturmiunum* could produce infectopyrone and macrosporin when grown in pure culture. This study is the first to report on the detection of tentoxin, paxillines, janthitrems, verrucolone, infectopyrone, macrosporin, and stemphols in naturally contaminated tomatoes.

KEYWORDS: ***Alternaria*; metabolite profiling; mycotoxins; *Penicillium*; qualitative detection methods; *Stemphylium***

INTRODUCTION

Because of their thin skin, tomatoes are very susceptible to fungal decay, and *Alternaria* is the most common fungus on moldy tomatoes (*1*). Sun scorch and chilling injuries also allow other fungi, such as *Cladosporium* and *Botrytis*, to cause postharvest decay (*1*). Less frequently, tomatoes are spoiled by *Aspergillus*, *Fusarium*, and *Penicillium* (*1*−*4*). Only a limited number of fungal species from the aforementioned genera have been associated with serious postharvest spoilage of tomatoes: *Alternaria alternata* (*2*, *5*), *Alternaria tenuissima* (*5*), *Alternaria arborescens* (*6*), *Cladosporium herbarum* (*1*), *Botrytis cinerea* (*1*), *Aspergillus flavus* (*4*), *Aspergillus ochraceus* (*2*, *4*), *Fusarium oxysporum* (*1*), *Penicillium citrinum* (*2*), *Penicillium expansum* (*7*, *8*), and *Penicillium olsonii* (*6*).

Relatively few fungal metabolites have been reported as naturally occurring in moldy tomatoes and tomato products: alternariol, alternariol monomethyl ether, and tenuazonic acid from *Alternaria* (*5*); aflatoxin from *Aspergillus* (*4*); and cyclopiazonic acid from *Penicillium* or *Aspergillus* (*3*). This is probably an underestimation since different species of *Alternaria*, *Aspergillus*, *Fusarium*, and *Penicillium* are known to produce many different mycotoxins and other toxic metabolites in pure culture (*9*, *10*). Knowing which fungal metabolites to look for in moldy tomatoes can be difficult because (i) it depends on which fungi have been spoiling the tomatoes, (ii) the metabolite production of a fungus is very often species specific, (iii) identification of the naturally occurring fungi to species level demands specialist knowledge, (iv) many fungal metabolites have not yet been structurally elucidated, and (v) standards of relevant metabolites are not commercially available for analysis. All these difficulties are remedied by direct comparison of extracts of the moldy tomatoes with metabolite profiles from the isolated fungi in pure cultures. This can be done by qualitative, multimetabolite analyses of both the fungi and the moldy tomatoes in the same detection system to establish if the same metabolites have been produced.

The purpose of this study is to show that the fungi responsible for lesions on the tomatoes also produce metabolites later in the spoilage process. The aim of this paper is to focus the attention of legislators and the food industry on the potential problem of co-occurring fungal metabolites other than aflatoxin in tomato products.

MATERIALS AND METHODS

Tomato Samples. Twenty samples of moldy tomatoes, nine Danish and seven Spanish bought at the local markets and four Danish home grown, were analyzed for fungal growth. Each tomato was examined for visible growth, and each fungal lesion was sampled with a plastic inoculation needle without breaking the skin or otherwise damaging the tomato. After sampling, each tomato was placed in a beaker and incubated for 7 days at 25 °C in darkness to simulate worst case.

Isolation and Identification of Fungi from Lesions. Isolation of fungal material from the tomato lesions was done by gently rubbing the lesion with a disposable plastic inoculation needle and transferring the fungal material to V8 juice agar (V8) (*11*) as a streak inoculation. The V8 plates were incubated for 7 days at 20−23 °C under an

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alternating light/dark cycle consisting of 8 h of cool-white daylight fluorescent lamps followed by 16 h of darkness. The different fungal colonies were identified to genus level using a stereomicroscope and inoculated as three-point inoculations onto specific media for identification. *Alternaria, Stemphylium*, and *Ulocladium* were inoculated onto Dichloran Rose Bengal Yeast Extract Sucrose Agar (DRYES) (*12*) and Potato Carrot Agar (PCA) (*11*); *Penicillium* onto Czapek yeast extract agar (CYA) (*13*), malt extract agar (MEA according to Blakeslee) (*13*), and yeast extract sucrose agar (YES) (*13*); *Botrytis* onto YES and PCA; *Cladosporium* onto DRYES and V8; and *Verticillium* onto YES and MEA. All media contained 1 mL of trace metal solution (1.0 g $ZnSO_4 \cdot 7H_2O$ and 0.5 g of $CuSO_4 \cdot 5H_2O$ per 100 mL) according to Filtenborg et al. (*14*). DRYES, YES, CYA, and MEA were packed seven high in perforated plastic bags and incubated at 25 °C in darkness for 7 days. PCA and V8 were packed in one layer and incubated for 7 days at 20−23 °C under alternating light as mentioned previously. Morphological identification was done on MEA, PCA, or V8 after 7 days of growth, whereas metabolite profiling was done on YES and MEA or DRYES after 7−14 days of growth. Identification of *Alternaria* was done using (*15*, *16*) *Botrytis* and *Cladosporium* (*17*), *Penicillium* (*17*, *18*), *Stemphylium* (*19*, *20*), and *Ulocladium* (*21*). All fungal cultures are held at the IBT Culture Collection at BioCentrum-DTU, Denmark.

Extraction of Pure Cultures. Nine 6 mm agar plugs were cut from DRYES (*Alternaria, Cladosporium, Stemphylium*, and *Ulocladium*), YES and CYA (*Penicillium*), and YES (*Botrytis* and *Verticillium*) and placed separately in 2 mL vials. One milliliter of ethyl acetate/formic acid (200:1, v/v) was added to each vial, and the plugs were extracted for 60 min by sonication. Each extract was transferred to a clean 2 mL vial and evaporated to dryness in a rotary vacuum concentrator. The dried residue was redissolved ultrasonically in 500 μL of methanol for 10 min and filtered through a 0.45 μm filter into a clean 2 mL vial prior to HPLC analyses.

Extraction of Naturally Infected Samples. Eight samples of naturally infected tomatoes were used as worst case examples and extracted after 7 days of incubation at 25 °C. Each sample was weighed, and 1:1 (w/v) ethyl acetate/formic acid (100:1, v/v) was added. The sample was mashed with a fork to a pulp and left to stand for an hour. The extract was filtered through a Whatman filter paper (no. 1), evaporated to dryness, and redissolved in 10 mL of methanol by sonication for 30 min. The extract was then diluted to approximately the same concentration as the extracts of the pure cultures, filtered through a 0.45 μm filter into a clean 2 mL vial, and analyzed in the same HPLC system.

HPLC-UV Analyses. Extracts of moldy tomatoes, fungi in pure cultures, and relevant metabolite standards were analyzed on a HP-1100 high performance liquid chromatograph equipped with a diode array detector (Agilent) and a fluorescence detector (Agilent). Approximately three UV spectra were collected per second from 200 to 600 nm along with chromatographic traces at 210 and 280 nm, all with a 4 nm resolution. Fluorescence signals were collected using an excitation at 230 nm and measuring emission at 333 and 450 nm. Furthermore, emission spectra were collected from 300 to 700 nm with two spectra per second and a resolution of 10 nm. Two different column/gradient systems were used. In both systems, the column temperature was 40 °C, and a linear acetonitrile/water gradient was used. Both eluents contained 50 μL/L (v/v) trifluoroacetic acid. System 1: separations were done on a 125 × 2 mm i.d., 3 μm Hypersil BDS-C_{18} cartridge column (Agilent) with a 10 × 2 mm i.d. Superspher 100 RP-18 guard column (Agilent) with a the flow rate of 0.3 mL/min. A linear gradient, starting at 10% acetonitrile, changed to 50% acetonitrile in 30 min, then changed again to 100% acetonitrile in 10 min and maintained for 5 min, was used. The solvent composition was returned to starting conditions in 8 min followed by 6 min equilibration. System 2: separation was done on a 100 × 2 mm i.d. Luna C_{18} column (Phenomenex) with a 10 × 2 mm i.d. Superspher 100 RP-18 guard column (Agilent) at a flow rate of 0.4 mL/min. A linear gradient going from 15% acetonitrile to 100% acetonitrile in 20 min was used. 100% acetonitrile was held for 5 min before the gradient was returned to starting conditions in 3 min followed by a 5 min equilibration. All chemicals used were of analytical grade, acetonitrile (HPLC grade) was from ScanLab, trifluoroacetic acid was from Merck, and the water was Milli-Q grade (Waters). A homologous series of alkylphenones was analyzed as external retention time references and used to calculate a bracketed retention index (RI) for each detected compound in both HPLC systems (*22*).

Standards from the IBT Metabolite Collection of 3-*O*-acetoxypaxilline, aflatoxin B_1, AK-toxin, α-paxitriol, altenuene, alternariol, alternariol monomethyl ether, altertoxin I, AM-toxin, aspergillic acid, β-paxitriol, circumdatin A, circumdatin C, circumdatin B, citrinin, cyclopiazonic acid, dehydroxypaxilline, infectopyrone, janthitrem B, ochratoxin A, paspaline, paspalinine, patulin, paxilline, penicillic acid, radicinin, roquefortine C, stemphone, tentoxin, tenuazonic acid, and verrucolone were used to confirm identity of individual peaks by comparing RI values and UV spectra in both HPLC systems. All standards are held at the IBT Metabolite Collection at BioCentrum-DTU, Denmark (*23*).

Table 1. Fungal Species Isolated from Tomato Lesions and Their Production of Toxic Metabolites in Pure Culture

fungal species	metabolite in pure culture
A. tenuissima	
sp.-grp. 1	altertoxin I, altersetin, tentoxin, tenuazonic acid
sp.-grp. 2	alternariols, altertoxin I, tentoxin, tenuazonic acid
sp.-grp. 3	alternariols, altertoxin I, tenuazonic acid
sp.-grp. 4	alternariols, altersetin, tenuazonic acid
B. cinerea	alternariol
C. sphaerospermum	
P. chrysogenum	meleagrin, penicillin, roquefortine C, secalonic acid
P. expansum	chaetoglobosins, citrinin, communesins, patulin, roquefortine C
P. olsonii	verrucolone
P. cf. *restrictum*	
P. tularense	janthitrems, paspalinine, paxilline, 3-*O*-acetoxypaxilline
S. cf. *lycopersici*	stemphols
S. eturmiunum	infectopyrone, macrosporin
U. atrum	
Verticillium sp.	

RESULTS

Isolation and Identification of Fungi from Lesions. Fifty-five fungal colonies were isolated from 20 tomato samples before incubation. Most of the tomatoes examined in this study had more than one lesion. Identification showed that there was only one fungal species in most lesions but that different lesions on the same tomato sometimes yielded different fungal genera or species. Most of the lesions were located near the stem-end of the tomato. At genus level, the most dominant genus was *Alternaria* (ca. 40%), followed by *Penicillium* (ca. 25%), *Stemphylium* (ca. 15%), and *Cladosporium* (ca. 10%). **Table 1** shows the identity of the fungal species collected from the tomatoes. The most dominant fungi on the moldy tomatoes belonged to the *A. tenuissima* species-group. Four different types were found with different morphology and different metabolite profiles. The second most dominant fungal species was *P. olsonii* followed by *S. eturmiunum* and *S.* cf. *lycopersici*. *Cladosporium sphaerospermum, P. chrysogenum, P. expansum, P.* cf. *restrictum*, and *P. tularense* were isolated less frequently. *Botrytis cinerea, Ulocladium atrum*, and *Verticillium* sp. were only isolated twice each. Neither *Fusarium* nor *Aspergillus* was ever recovered from any lesions in this study.

Production Fungal Metabolites in Pure Culture. The production of known metabolites from each of the identified

Table 2. Occurrence of Toxic Metabolites in Individual Moldy Tomatoes Analyzed by HPLC-DAD

sample	metabolite in moldy tomatoes
400	alternariols, tentoxin, tenuazonic acid
401	3-*O*-acetoxypaxilline, paxilline
402	3-*O*-acetoxypaxilline, janthitrem B, paxilline, verrucolone, janthitrem analogues
403	infectopyrone, tenuazonic acid
404	macrosporin, stemphol
405	macrosporin, stemphol
406	infectopyrone, stemphol, stemphol analogues
407	tentoxin, tenuazonic acid

species isolated from tomato lesions is also given in **Table 1**. All four *Alternaria* cf. *tenuissima* isolates produced tenuazonic acid, three produced alternariols, and two produced tentoxin. The metabolic diversity in the *Penicillium* species was greater. The metabolite profiles of *P. chrysogenum* and *P. expansum* included roquefortine C, *P. olsonii* produced verrucolone, and *P. tularense* was found to produce paspalines, paxillines, and janthitrems. *B. cinerea* produced alternariol, while *S. eturmiunum* produced infectopyrone and macrosporin, and *S.* cf. *lycopersici* produced stemphol. No known metabolites were detected in any of the *Cladosporium*, *P.* cf. *restrictum*, *Ulocladium*, or *Verticillium* isolates. In addition to the known metabolites, all the fungal extracts also contained compounds that were unique to the respective species but of unknown structure.

Occurrence of Fungal Metabolites in Moldy Tomatoes. Eight individual tomatoes, representing different fungi lesions, were used as worst-case samples. After one week of incubation at 25 °C, the initial black and gray lesions had spread, and most of the tomatoes were covered completely by greenish or grayish fungal mycelium. Two of the tomatoes had collapsed and were leaking, while the others had maintained their shape. After the extraction, the metabolites detected in the eight moldy tomatoes were identified by comparing the tomato extracts with extracts of fungi in pure culture and standards from the IBT Fungal Metabolite Collection in HPLC system 1. The presence and identity of the metabolites were confirmed by analyzing the samples, extracts, and standards in HPLC system 2.

The results of all eight HPLC analyzes are given in **Table 2**. The results showed that samples 400 and 407 contained only *Alternaria* metabolites, and in both samples tentoxin and tenuazonic acid were detected. In addition, alternariol, alternariol monomethyl ether, and a tenuazonic acid analogue (same UV spectrum as tenuazonic acid but different retention time) were also detected in sample 400. Samples 401 and 402 both contained *Penicillium* metabolites. Sample 401 contained 3-*O*-acetoxypaxilline and paxilline from *P. tularense*. *Stemphylium* metabolites were detected in samples 404−406. Samples 404 and 405 contained stemphol from *Stemphylium* cf. *lycopersici* and macrosporin from *S. eturmiunum*. In sample 405, a stemphol analogue was also detected. Sample 403 contained metabolites from both *Alternaria* and *Stemphylium*. In this sample, infectopyrone and the two unidentified metabolites from *S. eturmiunum* were detected together with tenuazonic acid from *A.* cf. *tenuissima*. In none of the moldy tomato samples were aflatoxin B_1, altenuene, citrinin, cyclopiazonic acid, ochratoxin A, patulin, or any other known fungal metabolite detected.

The extract of sample 402 in **Figure 1D**, as the 210 nm signal, shows the peaks of verrucolone (**1**), paxilline (**2**), and janthitrem B (**3**). The fluorescence signal of the same extract in **Figure 1C** shows that the extract also contains two janthitrem analogues

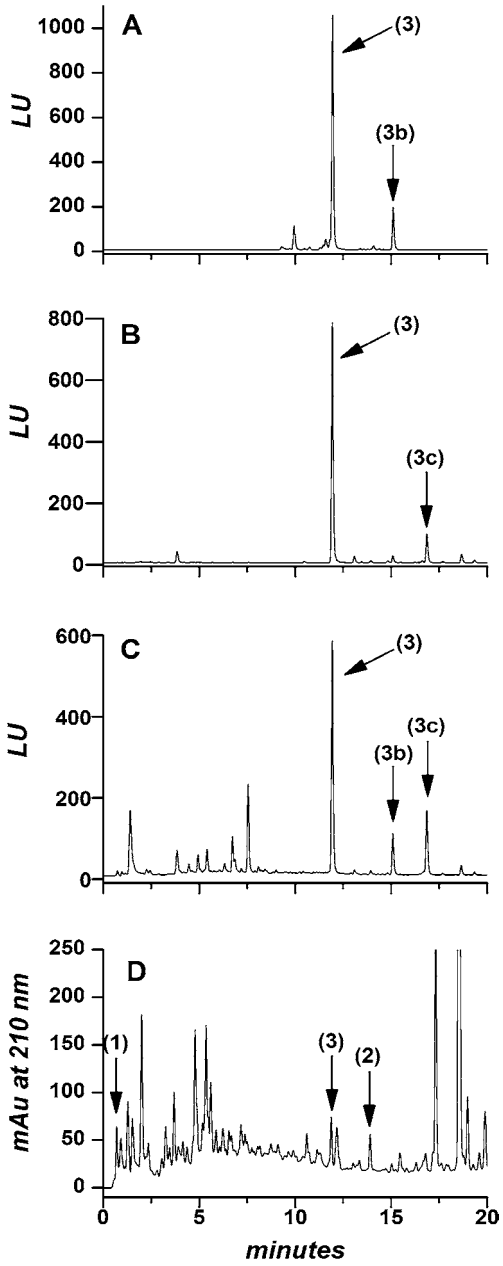

Figure 1. HPLC chromatograms of extracts of moldy tomato, fungal culture, and standard: (**A**) fluorescence signal ($E_x = 230/E_m = 450$) of the standard of janthitrem B (**3**) and one janthitrem analogue (**3b**); (**B**) fluorescence signal ($E_x = 230/E_m = 450$) of *P. tularense* in pure culture; (**C**) fluorescence signal ($E_x = 230/E_m = 450$) of the moldy tomato sample (no. 402) showing janthitrem B (**3**) and two janthitrem analogues (**3b** and **3c**); (**D**) UV signal (210 nm) of the moldy tomato sample (no. 402) showing verrucolone (**1**), paxilline (**2**), and janthitrem B (**3**).

(**3b** and **3c**) in addition to janthitrem B (**3**). For comparison, **Figure 1B** shows the fluorescence signal of *P. tularense* extract from pure culture, and **Figure 1A** is the fluorescence signal of the janthitrem B standard. As can be seen from **Figure 1**, the detection of the janthitrems is much more sensitive using fluorescence detection than UV detection. The extract of sample 406 in **Figure 2C**, as the 210 nm signal, is showing the peaks of infectopyrone (**4**) and stemphol (**5**) together with two stemphol analogues (**5b** and **5c**). For comparison, **Figure 2D** and **2B** show the 210 nm signals of *S.* cf. *lycopersici* and *S. eturmiunum* extracts, respectively, from pure culture, and **Figure 2A** is the 210 nm signal of the infectopyrone (**4**) standard. As can be seen from **Figure 2**, stemphol produced by *S. eturmiunum*

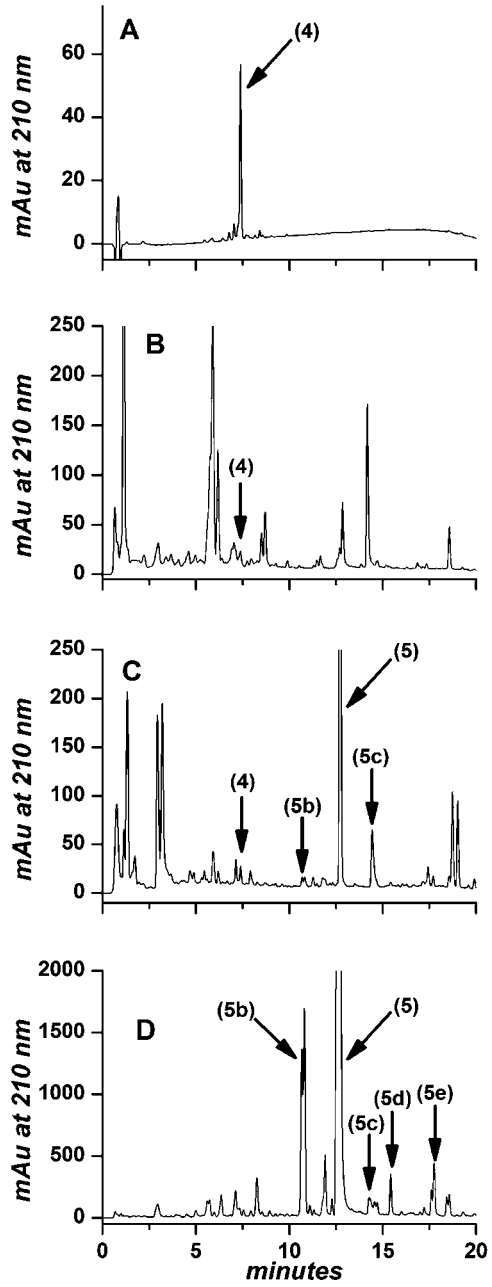

Figure 2. HPLC chromatograms of extracts of moldy tomato, fungal culture, and standard: (**A**) UV signal (210 nm) of the standard of infectopyrone (**4**); (**B**) UV signal (210 nm) of *Stemphylium eturmiunum* in pure culture showing infectopyrone (**4**); (**C**) UV signal (210 nm) of the moldy tomato sample (# 406) showing infectopyrone (**4**), stemphol (**5**), and two stemphol analogues (**5b** and **5c**); (**D**) UV signal (210 nm) of *S.* cf. *lycopersici* in pure culture showing stemphol (**5**) and four stemphol analogues (**5b**, **5c**, **5d**, and **5e**).

is the dominating peak in the chromatogram. **Figures 3** and **4** show the normalized UV spectra and structures, respectively, of the different fungal metabolites in samples 402 and 406.

DISCUSSION

Spoilage Fungi and Their Metabolites in Pure Culture. *Alternaria* is considered to be the major postharvest spoiler of fresh tomatoes (*1*, *5*), which is in accordance with our findings. The results in this study showed that approximately 40% of moldy tomatoes grown and/or sold in Denmark were spoiled by *Alternaria*. Results published by Harwig et al. (*7*) showed *Alternaria* on 37% of moldy tomatoes from Ontario, Canada. Mislivec et al. (*2*) found that 47 and 60% of moldy tomatoes from the East and Midwest of the U.S, respectively, were spoiled by *Alternaria*, while the amount of *Alternaria* spoilage in California was only 23%. The dominant spoiler of Californian tomatoes was *Aspergillus* with >57% (*2*). A study by Muhammad et al. (*4*) on decaying tomatoes from Nigeria showed no *Alternaria* at all but found that the dominating fungus also was *Aspergillus*. These reports and our results could suggest, although the studies are few, that there is a climatic/geographic difference in the composition of the tomato associated mycobiota, which favors *Aspergillus* in dryer and hotter climates and *Alternaria* in more humid and temperate climates. *Aspergillus*, which is considered a storage fungus thriving at elevated temperatures on spices and foods with low water activity (*1*), may be more common on tomatoes that previously thought.

None of the 22 *Alternaria* cultures isolated from tomato lesions in this study could be identified as *A. alternata*, *A. arborescens* (= *A. alternata* f. sp. *lycopersici*), or *A. tomato* reported elsewhere (*2*, *5*, *7*). All 22 *Alternaria* cultures belonged to different, currently undescribed taxa in the *A. tenuissima* species-group (*15*). The taxonomic literature concerning *Alternaria* is under revision, showing that *A. alternata* is not as common as presumed (*17*) and that several species are in fact species-groups covering several distinct species (*15*, *16*). None of the 15 *Penicillium* cultures in this study could be identified as *P. citrinum*, which was found in high numbers by both Mislivec et al. (*2*) and Muhammad et al. (*4*). Like *Aspergillus*, *P. citrinum* was found as the dominant *Penicillium* species on decaying tomatoes in California and Nigeria. It could either be because *P. citrinum* is more prevalent in warmer climates than other *Penicillium* species or because the fungi have been misidentified. In this study, *P. olsonii* was found to be the dominant *Penicillium*, while *P. expansum* was the only identified *Penicillium* in the study of Harwig et al. (*7*). In most studies dealing with moldy tomatoes, *Stemphylium*, *Botrytis*, and *Cladosporium* were not identified to species level, so comparisons were not possible. Other studies report the findings of *Fusarium* and *Geotrichum* (*2*, *7*) and *Rhizopus* (*2*, *24*), but none of these genera were isolates the tomato lesions in this study.

This is the first report on *P. tularense*, *S. eturmiunum*, and *S.* cf. *lycopersici* as postharvest spoilers of fresh tomatoes. Likewise, it is the first report on the production of janthitrems, paspalinine, paxilline, and 3-*O*-acetoxypaxilline by *P. tularense* of stemphol from *S.* cf. *lycopersici* and of infectopyrone and macrosporin from *S. eturmiunum* in pure culture (**Table 1**).

Natural Occurrence of Fungal Metabolites and Their Toxicity. Most studies on fungal metabolites naturally formed in tomatoes and tomato products report on the detection of *Alternaria* metabolites such as alternariol, alternariol monomethyl ether, altertoxin, and tenuazonic acid (*3*, *5*, *25*, *26*), whereas only a few report on *Aspergillus* metabolites, such as aflatoxin B_1 (*4*) and ochratoxin A (*27*). This study is the first to report on multi-metabolite detection of *Alternaria*, *Penicillium*, and *Stemphylium* metabolites in moldy tomatoes and the co-occurrence of (i) alternariols, tentoxin, and tenuazonic acid; (ii) paxillines, janthitrems, and verrucolone; (iii) macrosporin and stemphol; (iv) infectopyrone and stemphol; and (v) infectopyrone and tenuazonic acid in naturally contaminated tomatoes (**Table 2**).

Most metabolites originating from *Alternaria* and *Stemphylium* species have so far have been associated with various plant diseases (*28*), and very little is therefore known about the toxicity of these metabolites in animals and humans. Alternariol

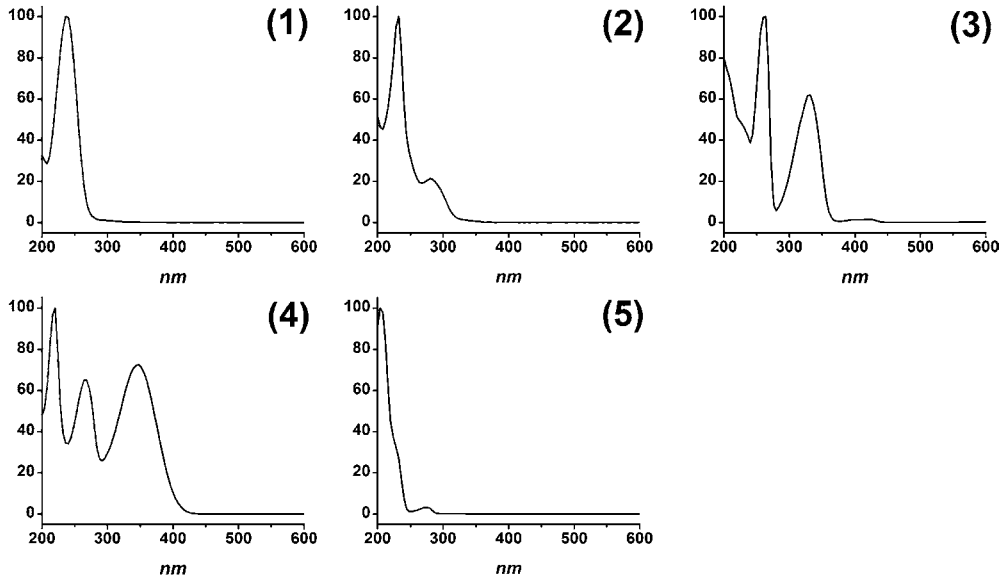

Figure 3. The normalized UV spectrum from HPLC analyzes for each of the *Penicillium* and *Stemphylium* metabolites detected in moldy tomatoes. Verrucolone (= arabenoic acid) (**1**), paxilline (**2**), janthitrem B (**3**), infectopyrone (**4**), and stemphol (**5**).

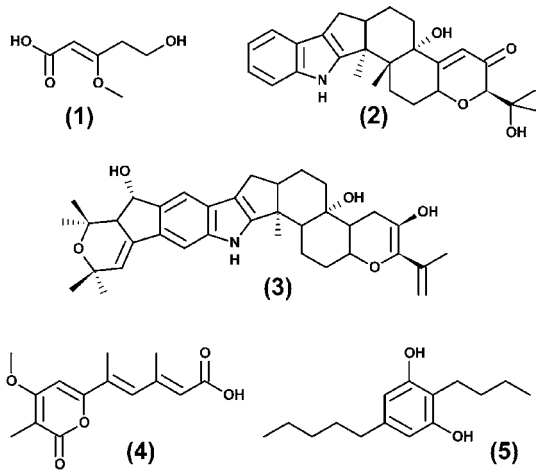

Figure 4. The chemical structure for each of the *Penicillium* and *Stemphylium* metabolites detected in moldy tomatoes. Verrucolone (= arabenoic acid) (**1**), paxilline (**2**), janthitrem B (**3**), infectopyrone (**4**) and stemphol (**5**).

has been reported to have teratogenic effect in pregnant mice, and the effect seems to be synergistic in the presence of alternariol monomethyl ether (*29*). Tenuazonic acid has many diverse activities and has indirectly been associated with the human hematological disorder known as Onyalay (*5*), but no toxicity data exist on tentoxin (*28*), infectopyrone (*30*), macrosporin (*28*), or stemphol (*31*). Among metabolites from *Penicillium*, on the other hand, a large number is known to have adverse effects on animals and humans and have been listed as mycotoxins (*32*). Paxilline has been shown to be genotoxic (*33*) as well as being a tremorgen with a strong effect on the electromyographic activity of smooth muscles (*34*−*37*). Paspalinine also is a tremorgenic mycotoxin with effects similar to those of paxilline and is tremorgenic toward mice, cockerels, and domestic animals after oral administration (*38*). The janthitrems have shown to be tremorgenic also (*32*, *39*−*43*). No toxicity data exists on verrucolone (*44*), 3-*O*-acetoxypaxilline (*32*).

Direct consumption of moldy tomatoes by the consumer is unlikely in the Western world, but the possibility of moldy tomatoes being included in processed tomato products is much more likely. Our worst-case examples have shown that decay is very rapid at 25 °C and that contamination of healthy tomatoes also happens very fast. Fungal growth will of course progress more slowly at lower temperatures in storage prior to processing, but the amount of damaged fruit will be higher, and thus, contamination will affect more fruit.

Evaluation of the concentration of the different metabolites found in this study is difficult, basically because the toxicity of most of the metabolites is unknown. For example, the component that was detected in the largest amount in sample 406 was stemphol, meaning that this metabolite was produced in very high concentrations. If stemphol is not toxic to humans, the problem is minor and is a question of bad quality, which will take a lot of dilution by healthy tomatoes not to show in the finished product. However, with the exception of the paxillines and janthitrems, the toxic and synergistic effects of these metabolites are unknown, and none of the metabolites is covered by any legislation. Whether these metabolites constitute a problem in commercial tomato products remains to be seen. Therefore, systematic testing of tomato products, such as tomato juices, ketchups, and purees, where verrucolone, tentoxin, infectopyrone, macrosporin, stemphols, paxillines, and janthitrems are taken into account, is needed to increase the quality and safety of tomato products.

SAFETY

All work with fungal cultures was done in fume hoods to avoid exposure to fungal spores and volatiles. Preparation of metabolite standards and extractions of fungal cultures and moldy samples were done in fume cupboards while wearing gloves.

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