Research Note

Mycotoxin Production and Postharvest Storage Rot of Ginger (Zingiber officinale) by Penicillium brevicompactum

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ABSTRACT

Twenty naturally infected ginger (Zingiber officinale) rhizomes displaying visible mold growth were examined to identify the fungi and to evaluate the presence of fungal secondary metabolites. Penicillium brevicompactum was the predominant species isolated from 85% of the samples. Mycophenolic acid was identified from corresponding tissue extracts. Because mycophenolic acid is a potent immunosuppressant and synergistic mycotoxicosis studies involving human consumption have not been carried out on this metabolite, spoilage of commercially marketed produce caused by P. brevicompactum is a concern. This is the first reported occurrence of mycophenolic acid in commercially sold plant food products.

Many described mycotoxicoses in domestic animals result from the consumption of a food product contaminated with a known toxigenic species and often linked with a well-known toxin. In a dog, a temporary tremorgenic toxicity (lasting 36 h) was induced by penitrem A following accidental consumption of moldy walnuts (Juglans sp.) infected with Penicillium crustosum (14). Other toxins include aflatoxins from Aspergillus flavus that cause aflatoxicosis and ochratoxin A from Penicillium verrucosum that causes porcine nephropathy (20). In other mycotoxicoses, the problem appears to be multifactorial. In Balkan endemic nephropathy, the ochratoxin A-producing species P. verrucosum or Aspergillus ochraceus were first thought to be important factors. Later, researchers determined that ochratoxin A and penicillic acid may act synergistically (18) or that Penicillium aurantiogriseum may be involved because of its production of nephrotoxic glycopeptides (12). In other cases, a dominant fungal species involved in a mycotoxicosis may be identified but that fungus does not produce known mycotoxins. For example, Penicillium brevicompactum is a very common fungus worldwide (4) and has been reported from many foods (6, 7, 15), but none of its secondary metabolites (8) have been claimed to be mycotoxins in the strict sense of the word. However, the secondary metabolites from P. brevicompactum can be biologically very active. Mycophenolic acid is now used in its mycophenolic acid form, mofetil, as an immunosuppressive agent for patients with heart and kidney transplants (2). Mycophenolic acid may cause secondary mycotoxicosis by lowering the immune response of humans, thus making them more susceptible to bacterial infections and food-borne diseases. Natural occurrence of mycophenolic acid may therefore be of importance. Mycophenolic acid has until now been found only as a naturally occurring product in blue cheeses and Manchego cheese (10, 11). Because we detected many strains of P. brevicompactum growing actively on ginger (Zingiber officinale) rhizomes, we investigated whether this metabolite naturally occurred on this plant product.

MATERIALS AND METHODS

Twenty moldy ginger rhizome samples were collected from a grocery store in Kgs. Lyngby, and fungal strains were isolated and identified by colony morphology and secondary metabolite profiling. High-pressure liquid chromatography (HPLC) analysis was performed on the moldy samples, and the isolated fungal strains were grown on Czapek yeast autolysate (CYA) and yeast extract sucrose (YES) agars, and the analyses were compared (for recipes, see Samson et al. (15). The Czapek Dox broth and yeast extract used were manufactured by Difco (Becton Dickinson, Sparks, Md.), saccharose was manufactured by Fluka (Sigma, St. Louis, Mo.), and the agar (BBB 10030 SO-BI-Gel) was purchased from Bie and Berntsen (Rødovre, Denmark). All chemicals used in the micronutrients formulation were purchased from Sigma.

Isolation of fungal strains. Conidia were removed from the moldy ginger samples using a flame-sterilized inoculation loop, transferred to vials containing spore suspension medium, and vortexed, and a loopful of suspension medium was streaked across the surface of a petri dish containing CYA agar. Streak plates were incubated in the dark at 25°C for 5 days. Plates were then removed and visually inspected, and plates containing mixed colonies were identified and streak plated once more to obtain axenic cultures. Axenic cultures were three-point inoculated onto CYA and YES agars and incubated in the dark at 25°C for 9 days.

Culture identification. Axenic cultures were provisionally identified on the basis of colony morphology, and strains were sorted into species groups. All cultures were extracted for thin-layer chromatography (TLC) and HPLC analysis according to the
FIGURE 1. (A) HPLC trace at 210 nm of a moldy ginger tissue extract depicting the presence of Penicillium brevicompactum secondary metabolites. (B) Segment of moldy ginger used in isolation and extraction (open arrow indicates region of mold growth).

method of Smedsgaard (16). Extracts were evaporated to dryness in a Christ rotational vacuum concentrator and resuspended in 250 μl of methanol. Using 10-μl disposable glass capillary tubes, each extract was spotted in duplicate onto Silicagel 60, Merck Art 5721 TLC plates by discharging the capillary tube contents in a line 5 mm in length positioned parallel to the base of the TLC plate. The silica plates were developed using the two solvent systems described by Filtenborg et al. (5). Secondary metabolite profiles were visualized under UV light (366 nm and 254 nm) and visible light prior to and following anisaldehyde spray (for toluene-ethyl acetate-90% formic acid; 5:4:1, vol/vol/vol) and cerium sulfate spray (for dichloromethane-acetone-isopropanol; 85:15:20, vol/vol/vol) in accordance with the method of Frisvad and Thrane (8). Isolates were sorted based upon profile similarity into species groupings. Representative CYA and YES extracts from each tentative species groupings (determined by TLC profiling) were analyzed by reverse phase HPLC using diode array detection according to the method of Frisvad and Thrane (9) to confirm species identification. Extracts were filtered by syringe through a Titisan polytetrafluoroethylene membrane filter (pore size, 0.45 μm; filter size, 13 mm) prior to HPLC injection.

TABLE 1. Isolation frequency of Penicillium species from the 20 moldy ginger samples

<table>
<thead>
<tr>
<th>Penicillium species isolated</th>
<th>No. of ginger samples</th>
<th>Isolation rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>brevicompactum</td>
<td>17</td>
<td>85</td>
</tr>
<tr>
<td>crustosum</td>
<td>11</td>
<td>55</td>
</tr>
<tr>
<td>polonicum</td>
<td>7</td>
<td>35</td>
</tr>
<tr>
<td>cyclopium</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>aurantiogriseum</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>steckii</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>bialowiezense</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>freii</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>allii</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>commune</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>viridicatum</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>expansum</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>discolor</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

HPLC analysis of moldy ginger. Tissue explants consisting of approximately 3 to 5 mm of ginger tissue underneath and including exposed conidiophores and hyphae of the moldy ginger samples were placed in 14-ml screw-cap vials to which 1.5 ml of extraction solvent was added (dichloromethane-ethyl acetate-methanol; 3:2:1, vol/vol/vol). A control sample was similarly prepared using visually uninfected ginger. The vials were placed in a sonication bath for 60 min, and the extraction solvent was then removed to 2-ml HPLC vials and evaporated to dryness using a vacuum centrifuge. The dried extracts were resuspended in 500 μl of methanol, filtered, and analyzed by reverse phase HPLC as previously described to identify the presence of fungal metabolites. Metabolite identifications were confirmed by comparison of UV absorbance spectra and retention indices with those of the secondary metabolite profiles generated from the fungal cultures and with authentic standards.

RESULTS AND DISCUSSION

Fungal infections occurred in wounded regions, where a section of the ginger rhizome had been physically removed. These regions were densely sporulating, gray-green in appearance (due to conidia), and slightly floccose and ranged in size from 5 to 20 mm in diameter (average size 16 mm in diameter; see Fig. 1B). P. brevicompactum was the predominant species isolated from the 20 moldy ginger samples surveyed, with an isolation rate of 85%. P. crustosum Thom, Penicillium polonicum Zaleski, and Penicillium cyclopium Westling were isolated from 55, 35, and 25% of the samples, respectively. Several other Penicillium species were isolated at a rate of ≤10% (see Table 1).

HPLC examination of the moldy ginger tissue revealed the presence of several P. brevicompactum secondary metabolites (see Fig. 1A). Asperphenamate, mycophenolic acid, and Raistrick phenols were identified based on the UV spectra and corresponded with metabolite standards and metabolite profiles obtained from culture extracts of the isolated P. brevicompactum strains. Identification of these metabolites confirmed the presence of P. brevicompactum in the moldy ginger tissue. Expected secondary metabolites associated with the remaining fungi were not identified in...
the analyzed tissue extracts. Failure to detect these metabolites suggests insignificant fungal growth or transient conidia removed from the \textit{P. brevicompactum}–infected regions. The three samples from which \textit{P. brevicompactum} was not isolated and secondary metabolites were not found in the tissue extracts were from the smallest of all the infected regions (average size approximately 5 mm in diameter). The amount of fungal material extracted may have been too small relative to the ginger tissue, masking the fungal metabolites during analysis.

Ginger is known to produce several antifungal metabolites \((1)\), making it resistant to fungal invasion by many species; however, \textit{Pythium aphanidermatum} (Edson) Fitzpatrick, \textit{Pythium myriotylum} Drechsler, and \textit{Fusarium oxysporum} f. sp. \textit{zingiberi} Trujillo are known field pathogens of ginger, and crop losses due to these fungi have prompted the development of rapid diagnostic tools for field analysis \((13, 19)\). The results from this study indicate that \textit{Penicillium brevicompactum} has the ability to rot ginger, entering the rhizome through wounded regions. Because \textit{Penicillium} rot has never been reported as a field pathogen of ginger, crop losses during cultivation are not likely to threaten growers; however, postharvest crop losses during storage are a possibility. Losses due to postharvest infection more likely will be noticed by the marketer. Aside from handling during transport and storage, ginger rhizomes are routinely subjected to wounding by consumers removing smaller segments when purchasing ginger. Because \textit{P. brevicompactum} is a fairly ubiquitous fungus, occurring most often in buildings and especially in association with foodstuffs, a potential exists for product losses due to infection with this fungus during storage.

Of the several \textit{P. brevicompactum} metabolites identified in the infected tissue, only mycophenolic acid has been previously tested for bioactivity. Mycophenolic acid has antibiotic, antifungal, antiviral, and antitumor activity \((2)\). Although it has a wide range of bioactivities, mycophenolic acid appears to have relatively low acute toxicity, as determined by oral 50% lethal dose \((LD_{50})\) trials using mice and rats \(LD_{50} 700\) and 2,500 mg/kg, respectively \((3)\). However, as for ochratoxin A in swine \((17)\), consumption of mycophenolic acid may also lower the immune response of humans, resulting in a higher incidence of bacterial infections (secondary mycotoxicosis). To the best of our knowledge this is the first report of mycophenolic acid found in naturally infected food stuffs sold for human consumption.

ACKNOWLEDGMENTS

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REFERENCES