A Research Note

Production of Mycotoxins by Sorbate-Resistant Molds

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ABSTRACT

Nine strains of sorbate-resistant molds were grown in YES broth for 10 d at 21°C in the presence and absence of 3000 ppm sorbate. Following the incubation period, the cultures were extracted with chloroform and the extracts were tested for the presence of mycotoxins. Two of the extracts contained ochratoxin A and another extract contained an unidentified substance similar to cyclopiazic acid. None of the strains produced mycotoxins in the presence of 3000 ppm sorbate.

Some molds are sensitive to the inhibitory action of sorbic acid but others are not. The second group includes molds which can metabolize sorbic acid to 1,3-pentadiene, a volatile compound with a hydrocarbon-like odor (3). By metabolizing sorbic acid, the molds are able to reduce the amount of or eliminate the chemical from the growth medium, and thus create a more favorable environment for growth (1,3).

In 1966, Marth et al. (3) demonstrated that some molds in the genus Penicilium could grow in the presence of up to 7100 ppm potassium sorbate. The molds were isolated from natural and processed Cheddar cheese previously treated with sorbic acid and were able to degrade sorbic acid and produce 1,3-pentadiene. Finof et al. (1) reported in 1982 that some penicillia, also isolated from cheese, could grow in the presence of up to 12,000 ppm potassium sorbate. These molds were also able to metabolically reduce the amount of or eliminate sorbic acid from the growth medium. The ability to metabolize sorbic acid to 1,3-pentadiene appears to be restricted to molds in the genus Penicilium; strains of several of the species able to metabolize sorbic acid also can produce mycotoxins (2).

The purpose of this study was to determine if sorbate-resistant molds produce mycotoxins in the presence or absence of sorbic acid.

MATERIALS AND METHODS

Cultures of molds used in this study were obtained from the culture collection in the Food Microbiology Laboratory, Department of Food Science, University of Wisconsin-Madison, Madison, Wisconsin. Stock cultures were maintained at 2°C on slants of YM agar (Difco). All cultures were sorbate-resistant except for Aspergillus parasiticus NRRL 2999, a known aflatoxin producer, which was included as a positive control. Cultures were inoculated separately into 50 ml of sterile YES broth (2% yeast extract, 20% sucrose), with and without 3000 ppm potassium sorbate (Pfizer, New York), adjusted to pH 5.5 and incubated at 21°C for 10 d without agitation.

Following incubation, the mycelium and liquid contained in each flask were homogenized by blending at high speed for 2 min in a 300-ml blender cup. The mixture was then removed using Whatman No. 1 filter disks fitted into a modified Büchner funnel, described by Yousef and Marth (5), to which vacuum was applied from an aspirator. The filtered solution was extracted twice in a separatory funnel, each time with 50 ml of chloroform and the chloroform extract was cleared of water and denatured protein with a saturated NaCl solution. Chloroform extracts were combined in a round-bottom flask, then chloroform was evaporated by use of a rotary evaporator. The resulting dry films were dissolved in 2 ml of chloroform and were screened for the presence of mycotoxins by thin layer chromatography (TLC) according to the method of Scott et al. (4). Mycotoxin standards were obtained from Sigma Chemical Co. (St. Louis, MO).

RESULTS AND DISCUSSION

Several of the sorbate-resistant molds produced mycotoxins that were detected by TLC (Table 1). Two strains produced a substance identified as ochratoxin A, and another strain produced an unidentified substance with properties similar to those of cyclopiazic acid. A. parasiticus NRRL 2999 produced aflatoxins B1, B2, and G1. No mycotoxins were detected in uninoculated media or in media containing 3000 ppm sorbate. Mycotoxin production by the sorbate-resistant molds was suppressed when the medium contained 3000 ppm sorbate.
### TABLE 1. Mycotoxins produced by sorbate-resistant molds when grown in the presence or absence of sorbate in YES broth at pH 5.5.

<table>
<thead>
<tr>
<th>Culture</th>
<th>Sorbate concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Penicillium cyclopium-8</td>
<td>-</td>
</tr>
<tr>
<td>Penicillium roqueforti-22</td>
<td>-</td>
</tr>
<tr>
<td>Penicillium puberulum-33</td>
<td>-</td>
</tr>
<tr>
<td>P. cyclopium (atypical)-40</td>
<td>-</td>
</tr>
<tr>
<td>Penicillium crustosum-42</td>
<td>-</td>
</tr>
<tr>
<td>Penicillium lanoso-viride-44</td>
<td>-</td>
</tr>
<tr>
<td>Penicillium sp. K1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Ochratoxin A</td>
</tr>
<tr>
<td>Penicillium sp. K2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>Penicillium sp. S2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Ochratoxin A</td>
</tr>
<tr>
<td>Aspergillus parasiticus NRRL2999</td>
<td>Aflatoxins NG&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>B&lt;sub&gt;1&lt;/sub&gt;, B&lt;sub&gt;2&lt;/sub&gt;, G&lt;sub&gt;1&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Denotes no mycotoxin detected.
<sup>b</sup> Fluorescent spot on TLC plate unidentified, properties similar to cyclopiazic acid; no standard was included.
<sup>c</sup> Mold of genus Penicillium, species not identified.
<sup>d</sup> NG = no growth.

These preliminary results indicate that some sorbate-resistant molds in the genus *Penicillium* have the potential to produce toxins. Since sorbate-resistant molds are more likely to grow in sorbate-treated foods than are sorbate-sensitive strains, mycotoxin production is a major concern. However, our preliminary results indicate that 3000 ppm sorbate suppresses mycotoxin production by the sorbate-resistant strains of molds available to us. The molds tested in this study were able to metabolize sorbate to the volatile 1,3-pentadiene and thus eventually eliminate the preservative from the growth medium. It is possible that once sorbate has been eliminated, mycotoxin production by the molds could begin. Since mycotoxin production by sorbate-resistant molds is a matter of concern, more work should be done to determine conditions that allow or prevent mycotoxin production by sorbate-resistant molds.

**ACKNOWLEDGMENT**

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**REFERENCES**


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**Nielsen and Zeuthen, con't. from p. 155**