Growth and Aflatoxin Production by Aspergillus parasiticus in the Presence of Lactobacillus casei

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ABSTRACT

When a mineral salts-glucose broth was inoculated simultaneously with Aspergillus parasiticus and Lactobacillus casei and incubated at 28 C for 10 days, (a) larger numbers of L. casei survived than when the bacterium was grown alone, (b) growth of A. parasiticus initially was more rapid but total growth during the incubation was comparable to that of the mold growing alone, (c) production of aflatoxin was less than when the mold grew alone and (d) degradation of aflatoxin by the mold was somewhat greater than when the mold grew alone. Growth of L. casei for 3 days before adding A. parasiticus resulted in (a) better survival of L. casei than when it grew alone but not better than when the two organisms were added to broth simultaneously, (b) slower growth of A. parasiticus than when it grew alone, (c) production of less aflatoxin than when the mold grew alone or when both organisms were added to broth simultaneously and (d) no apparent degradation of aflatoxin during the 10-day incubation period.

Aflatoxins are a group of a growing list of fungal secondary metabolites of economic and public health importance (2,11). Such metabolites include the mycotoxins that are produced by certain strains of Aspergillus flavus and Aspergillus parasiticus, and that occasionally appear as contaminants of agricultural commodities, including foods. These secondary metabolites, the aflatoxins, are potent hepatocarcinogens of several species of animals and have been implicated by epidemiological studies as both acute intoxicants and hepatocarcinogens in man (11). Aflatoxin has been found in some foods and feeds; production of the mycotoxin in such commodities can be influenced by several factors, including temperature, aw, pH, available nutrients and competitive growth of other microorganisms (11). Interaction between microorganisms grown together in an ecological niche may change availability of nutrients or result in production of volatile and/or non-volatile end products which may stimulate, inhibit, or have no influence on growth of fungi or on production and accumulation of a mycotoxin (11,13,14). Aflatoxin production and growth of A. parasiticus can be altered when the mold grows with other fungi or bacteria (1,11,13,14). Only limited research has been done on how competitive growth influences aflatoxin production, even though mold growth and aflatoxin production often are likely to occur in a competitive environment. Examples of studies on competitive growth and aflatoxin production by a toxigenic aspergillus are those by Weckbach and Marth (13) and Wiseman and Marth (14).

Accordingly, the objective of this investigation was to determine the effect of Lactobacillus casei, a bacterium sometimes of importance in cheese ripening, on growth and aflatoxin production by A. parasiticus. Results of the study are in this report.

MATERIALS AND METHODS

Cultures

The culture of A. parasiticus NRRL 2999 was obtained from the Northern Regional Research Center. The mold was grown on slants of mycological agar (Difco) at 28 C for 7 days before use. Conidia were harvested (13) and served as inoculum for experiments. L. casei ATCC 393 came from the American Type Culture Collection and was used as inoculum after subculturing for several times in APT broth (Difco).

Preparation of test materials

Sufficient of the L. casei culture to provide 10^8 cells/ml was added to a 500-ml Erlemeyer flask containing 100 ml of APT (3%glucose) broth. Then, sufficient of the suspension of conidia to provide 650,000 spores/flask was added to the medium (treatment A), or the conidia were added after L. casei had grown for 3 days (treatment B). Inoculated flasks of medium were incubated quiescently for 10 days at 28 C. Each trial included five flasks of each of the mixed cultures and the control culture. Aflatoxin in a mixture of broth and blended mycelial mat, amount of growth, number of L. casei/ml (APT agar) and pH of the broth were determined. Mold growth, dry weight of mycellum, was determined as previously described by El-Gendy and Marth (10).

Extraction and quantitation of aflatoxin

Aflatoxin in 100 ml of a mixture of broth and blended mycelial mat was extracted, using the method described by Shih and Marth (12). Extracts containing aflatoxin were stored in glass-stoppered volumetric flasks at -20 C until the amount of aflatoxin was to be determined. Aflatoxins B_1 and G_1 were separated by spotting 5 or 10 μl of the chloroform extract on thin-layer chromatographic plates pre-coated with 0.50 mm of Adsorbosil-I; coated plates had been activated at 100 C for 40 min. Plates were developed in an equilibrated tank containing chloroform:methanol:water (98:1:1, v/v/v). The concentration of aflatoxin B_1 and G_1 was measured by fluorometric procedures described by Shih and Marth (12).

RESULTS AND DISCUSSION

Simultaneous inoculation of the culture medium with

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Incubation (days)</th>
<th>No. L. casei/ml (× 10⁴)</th>
<th>Mycelial dry wt (g/100ml)</th>
<th>pH</th>
<th>Aflatoxin (µg/100ml)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>B₁</td>
</tr>
<tr>
<td>L. casei</td>
<td>0</td>
<td>0.111</td>
<td>—</td>
<td>7.10</td>
<td>—</td>
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<tr>
<td></td>
<td>3</td>
<td>1520</td>
<td>—</td>
<td>3.65</td>
<td>—</td>
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<tr>
<td></td>
<td>7</td>
<td>60</td>
<td>—</td>
<td>3.45</td>
<td>—</td>
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<tr>
<td></td>
<td>10</td>
<td>1.4</td>
<td>—</td>
<td>3.50</td>
<td>—</td>
</tr>
<tr>
<td>A. parasiticus</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>7.10</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>—</td>
<td>0.50</td>
<td>3.70</td>
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<td>6.80</td>
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<td>—</td>
<td>1.95</td>
<td>7.45</td>
<td>1017.1, 1272.9</td>
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<tr>
<td>L. casei plus</td>
<td>A. parasiticus (A)ᵇ</td>
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<td>0.111</td>
<td>7.10</td>
<td>—</td>
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<td>3</td>
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<td>3.65</td>
<td>—</td>
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<td>1.75</td>
<td>7.60</td>
<td>522.0, 233.8</td>
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</table>

ᵃTreatment A = Inoculated simultaneously with both organisms.
ᵇTreatment B = Inoculated with L. casei, incubated 3 days and then inoculated with A. parasiticus. The 0-day value represents the culture when mold spores were added.

L. casei and A. parasiticus resulted in the following differences, when compared to the behavior of either culture growing alone. First, the population of L. casei in the mixed culture did not decrease during the incubation as happened when L. casei grew alone (Table 1). This is probably attributable to the increase in pH of the mixed culture during incubation and persistence of a low pH in the culture of L. casei, causing inactivation of many cells.

Second, growth of A. parasiticus, as measured by mycelial dry weight, initially was more rapid in the mixed culture than when the mold grew alone, but mold growth at the end of the incubation was comparable in both cultures. It is possible that a bacterial metabolite may stimulate mold growth early during the incubation. Weckbach and Marth (13) obtained somewhat similar results by A. parasiticus when it grew together with Acetobacter aceti.

Third, production of aflatoxin (B₁, G₁ and total) by A. parasiticus was markedly less, particularly after 3 days of incubation, when L. casei was present than when it was absent. Weckbach and Marth (13) obtained a similar result when A. parasiticus was grown together with Brevibacterium linens.

Fourth, degradation of aflatoxin between 7 and 10 days was somewhat greater when L. casei was present than when A. parasiticus grew alone. This was true for B₁ (30 vs. 36% remained), G₁ (17 vs. 23% remained) and total aflatoxin (23 vs. 27% remained). Degradation of aflatoxin by toxigenic aspergilli is described in a series of papers by Doyle and Marth (3-9).

Allowing L. casei to grow 3 days before adding spores of A. parasiticus (treatment B, Table 1) gave results somewhat different from those just described for the situation in which both microorganisms were simultaneously added to the medium. First, L. casei did not survive as well in this instance as in the former, but survival was better than when the bacterium grew by itself. Second, growth of A. parasiticus appeared to be slower during the first 7 days of incubation than under the other cultural conditions used in this experiment. This reduction in growth was accompanied by the third difference, namely, a reduction in the amount of aflatoxin that was produced. An inadequate supply of one or more nutrients in the medium, as a consequence of substantial growth of L. casei, could have caused the substantial reduction in synthesis of aflatoxin. Finally, there was no evidence that aflatoxin was being degraded by the mold under these conditions. In fact, the amount of aflatoxin (B₁, G₁ and total aflatoxin) was still increasing at the end of the incubation period.

Wiseman and Marth (14) did experiments similar to those just described but used Streptococcus lactis rather than L. casei. Their data suggest that S. lactis had considerably less impact on A. parasiticus than did L. casei, as noted in this report.

These data and those of Weckbach and Marth (13), Wiseman and Marth (14) and Alderman et al. (1) indicate that presence of another microorganism in the culture can affect the behavior of A. parasiticus. Growth and aflatoxin production by the mold can be enhanced, retarded or remain unchanged as a result of another microorganism in the environment. The effect of a particular microorganism seems to be unpredictable. Since A. parasiticus or A. flavus seldom exist as pure
30-min hold time required for pasteurization at this temperature would result in a 500-D reduction of the enzyme activity. Since the D-value decreases as temperature increases, HTST pasteurization would result in an even more rapid reduction in the enzyme activity. The concern that enzyme from cells killed before heating (pasteurization, etc.) would interfere with the test. Second, it is assumed that heating in a complex system, such as a food, will have a protective effect on the enzyme and bacterium. The data for the enzyme in buffer provide a convenient standard against which to compare the enzyme when heated in food systems.

**REFERENCES**


**El-Gendy and Marth, con't. from p. 212**

cultures in food or feed, presence of the competing microflora must be taken into account when considering aflatoxin production in such substrates.

**ACKNOWLEDGMENTS**

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**REFERENCES**