Research Note

Aflatoxin-Producing Strains of Aspergillus flavus Isolated from Cheese

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ABSTRACT

Contamination by fungi of the genus Aspergillus with special reference to the possible detection of aflatoxin-producing strains of Aspergillus flavus was studied in 52 samples of commercial cheeses made with different types of milk (8 of cow’s milk, 12 of ewe’s milk, 13 of goat’s milk, and 19 of milk mixtures of various species: cow, ewe, and goat) produced in southern Spain. The frequency of appearance of various species of Aspergillus, A. glaucus, A. niger, A. nidulans, A. sulphureus, A. Terreus, and A. flavus, in the different types of cheese was determined. In 4 (2 of goat’s milk cheese and 2 of cheeses made with milk from various species) out of 52 samples (7.69%), aflatoxin-producing strains of A. flavus were detected.

Key words: Cheese, aflatoxins, Aspergillus flavus

Cheese is an adequate substrate for mold growth, given suitable conditions of temperature and moisture (19). However, little comparative work has been done on the relative ability of different types of cheese to support actual fungal growth. Mycotoxin-producing molds require oxygen (3) and thus the packaging of cheese is an important factor. Scott (19) considers that prolonged storage can permit mold growth, particularly if the package is opened. It is thus important to know what kind of fungi are present in all cheeses, both visibly and not visibly moldy. Also it must be emphasized that the presence of fungal growth does not necessarily imply concomitant formation of mycotoxins (19).

In Chapman and Sharpe’s opinion (5), molds are a common form of spoilage of dairy products, particularly of unripened cheeses. Molds also occur very frequently on the surface of hard cheeses during ripening and curing. Some molds, such as Aspergillus and Penicillium species are able to grow at very low temperatures, such as 4 to 10°C. Their growth may result in a musty off flavor and their appearance is commercially undesirable, often resulting in downgrading of the cheese, and there is the potential that mycotoxins might be produced.

Detection of the formation of known toxic metabolites is mainly achieved by using chromatographic and spectrophotometric methods. Besides physicochemical methods that allow the detection of the type and concentration of known metabolites, various biological methods are suitable as screening tests for the detection of unknown toxic substances (6).

The objective of this work was the detection of aflatoxin-producing strains of Aspergillus flavus, using chick embryo toxicity as a presumptive test. Thin-layer chromatography (TLC) has been used for confirmation of the presence of A. flavus in commercial cheeses produced in the South of Spain.

MATERIALS AND METHODS

Preparation and sampling

A total of 52 samples of commercial cheeses made with different types of milk produced in the South of Spain were analyzed: 8 of cow’s milk, 12 of ewe’s milk, 13 of goat’s milk and 19 milk mixtures of various species, cow, ewe, and goat. Samples were diluted by mixing 25 ± 0.1 g with 75 ml of 0.1% Bacto-peptone (Difco Laboratories, Detroit, MI) in buffered sterile water (pH 7.2). Further dilutions were made as required.

Isolation and identification of molds

Potato dextrose agar (PDA, Difco) plates were incubated at 25°C for 7 days according to Standard Methods for the Examination of Dairy Products (14). The macroscopic and microscopic characteristics of the molds grown on PDA were observed and compared to descriptions and illustration given by Raper and Fennell (16) and Fassatiňová (8).

Detection of aflatoxin-producing strains of Aspergillus flavus

Use of chick embryo in screening. The method employed was proposed by Jayaraman et al. (10) using yeast extract sucrose (YES) broth incubated at 25°C for 10 days (7) as culture. The aflatoxin determination was made from broth by a double extrac-


TABLE 1. Frequency of occurrence of Aspergillus spp. and aflatoxin-producing strains of A. flavus in 52 samples of Spanish cheeses

<table>
<thead>
<tr>
<th>Cheese from milk of (no. samples)</th>
<th>Aspergillus spp.</th>
<th>A. glaucus</th>
<th>A. niger</th>
<th>A. nidulans</th>
<th>A. sulphureus</th>
<th>A. terreus</th>
<th>A. flavus*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow (n = 8)</td>
<td>12.50</td>
<td>12.50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ewe (n = 12)</td>
<td>8.33</td>
<td>8.33</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goat (n = 13)</td>
<td>38.46</td>
<td>7.69</td>
<td>5.26</td>
<td>7.69</td>
<td>7.69</td>
<td>15.38</td>
<td></td>
</tr>
<tr>
<td>Mixedb (n = 19)</td>
<td>21.05</td>
<td>1.92</td>
<td>5.76</td>
<td>1.92</td>
<td>1.92</td>
<td>1.92</td>
<td>7.69</td>
</tr>
<tr>
<td>Total</td>
<td>21.15</td>
<td>1.92</td>
<td>5.76</td>
<td>1.92</td>
<td>1.92</td>
<td>1.92</td>
<td>7.69</td>
</tr>
</tbody>
</table>

* In all cases, and by means of the presumptive biological test in chicken embryos and the confirmative TLC test, the A. flavus strains detected were aflatoxigenic.

b Cheese made with a mixture of milks from various species (cow, ewe, and goat).

tion with 50-ml volumes of chloroform. The chloroform phase was filtered through anhydrous sodium sulfate on pale Whatman No. 1 filter paper. The filtrate was dried by rotary vacuum evaporator and the residue stored at −20°C until analysis.

The solutions were injected into fertile White Leghorn eggs before incubation. Three groups of at least 8 eggs were used for each strain. The injections into the eggs were made into the air space: a hole of about 5-mm diameter was drilled in the shell over the air space. The solution was then deposited on the egg membrane with a syringe, and the hole was sealed with adhesive cellophane tape. The volume injected was 25 μl with a mixture of toluene:ethylacetate:formic acid (6:3:1) before incubation. Three groups of at least 8 eggs were used for the presence of toxins.

The method used was proposed by Romer (17). Activated chromatographic plates (MN Silica Gel G-HR; Merck, Darmstadt, Germany) 0.25 mm thick were developed in the solvent system toluene:ethylacetate:formic acid (6:3:1) (20). The detection of aflatoxins was performed under UV light. Increasing volumes were applied to the plates of the chloroform extracts which corresponded to cultures of A. flavus (12), in addition to an external and an internal standard of the aflatoxins B1 and G1. Standard aflatoxins were purchased from Makor Chem. Ltd., Jerusalem, Israel.

RESULTS AND DISCUSSION

The percent frequency of occurrence of the Aspergillus spp. A. nidulans, A. sulphureus, A. terreus, A. flavus, and aflatoxin-producing strains of A. flavus are summarized in Table 1.

In 9 samples of cheeses out of a total of 52 (17.30%) (4 of a milk mixture, 3 of goat’s milk, 1 of ewe’s milk, and 1 of cow’s milk) some species of the genus Aspergillus were detected. In two samples of goat’s cheese, the simultaneous presence of A. flavus and A. sulphureus and of A. terreus and A. niger, respectively, was observed. A. flavus was identified in 4 samples of cheese, 2 of goat’s milk (15.38%) and 2 of a mixture of milks (10.52%), which represents a percentage of 7.69% of the total of 52 cheeses analyzed. In all cases, and by means of the presumptive tests (biological test in chicken embryos) and confirmative tests (TLC), the A. flavus strains detected turned out to be potentially aflatoxigenic.

In Spain, Calvo et al. (4) found A. flavus in a higher percentage, 52%, of cheeses analyzed. In other dairy products such as yoghurt, Jordano et al. (11), among the species of the Aspergillus genus, detected the presence of A. glaucus, A. flavus, A. fumigatus, A. niger, and A. wentii and also found a greater proportion of samples contaminated by aflatoxin-producing strains of A. flavus, in 7 out of 20 (35%). Conversely, in 94 Teleme-type cheeses Zerfiridis (21) did not isolate any aflatoxigenic strains from among 22 species of Aspergillus in spite of the fact that A. flavus represented 16% of them. Bullerman (1), from 183 strains, only isolated one A. flavus with a toxigenic capacity. Neither did Kivanc (13) detect toxigenic A. flavus in 25 fresh cheeses from a commercial outlet, and white cheese in brine, with no aflatoxins being found in any of the samples.

Jacquet and Tantaoui-Elaraki (9) observed that the center parts of Camembert cheese were not propitious for the growth of A. flavus during the first days of ripening but that they permitted an optimal growth when the cheese had ripened and then became resistant in the rind. In our case, the presence of species of Aspergillus was detected both inside and on the outside of the cheeses; however, A. flavus was only found in the outer surfaces of the product.

Furthermore, Prodromos (15) inoculated the surface of Kefalotyri cheese with spores of A. flavus (CMI 120920) and stored it at 5, 10, 13, and 26°C with 80% relative humidity; growth was only observed at 26°C, with aflatoxins B1 and G1 being detected being detected at depths of up to 8 mm. A similar experiment was carried out by us by inoculating spores of A. parasiticus (NRRL 2999) into 2 batches of cheese, both fresh and ripe, stored at 7, 18 and 25°C for 7, 14, and 28 days, respectively. A high production of aflatoxins was observed only in the fresh cheeses kept at 25°C, and it was verified that the aflatoxins disseminated towards the inside of the cheese; an apparent occurrence in the fresh cheeses but not in the ripe ones (18).

Finally, good manufacturing plant sanitation practices during cheese manufacture and handling is the best way of
minimizing mold growth on cheese (2) and, as a result, the possible hazard of mycotoxin production.

REFERENCES