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«TRAINING ACTIVITIES ON FOOD CONTAMINATION CONTROL AND MONITORING WITH SPECIAL REFERENCE TO MYCOTOXINS»

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TOXIGENIC PENICILLIA OCCURRING IN FEEDS AND FOODS, WITH SPECIAL REFERENCE TO MOULD-FERMENTED SAUSAGE AND CHEESE

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Toxigenic penicillia occurring in feeds and foods, with special reference to mould-fermented sausage and cheese

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Summary

Penicillia frequently occur in feeds and foods. Many isolates of this genus are toxigenic and they impair the health of domestic animals, produce residues in organs and meat due to carry over, form mycotoxins in mouldy meat products and may be hazardous for mould-fermented foods. Observations gathered in the West German Federal Centre for Meat Research are summarised.

Toxigenic penicillia

We studied 1481 Penicillium isolates originating from various foods and feeds (Eckardt et al. 1979). These isolates represented 42 species, according to the nomenclature of Raper and Thom (1949), revised by Samson, Stolk and Hadlok (1976), Samson, Hadlok and Stolk (1977) and Samson, Eckardt and Orth (1977). The predominant species encountered are listed in Table 1. Using chemical methods (TLC) we demonstrated the production in malt extract agar of 20 different mycotoxins (Table 2) by 828 (55,9 %) of the isolates. Some isolates, e.g. of P. simplicissimum, produced up to four different mycotoxins in malt extract agar; some Penicillium species included isolates which produced several mycotoxin combinations (Leistner & Eckardt 1979b). Nevertheless, the mycotoxin pattern of an isolate can be used as an aid in the identification of penicillia (Eckardt et al. 1978, Leistner & Eckardt 1979b). In the brine shrimp-test 998 (67,4 %) of the 1481 isolates proved toxigenic (Eckardt et al. 1979). Considering the chemical as well as the biological assays 1166 (78,7 %) of the 1481 Penicillium isolates investigated must be regarded as toxigenic. Therefore, most of the penicillia occurring in feeds or foods must be regarded as potential mycotoxin producers.

Mycotoxicosis

Moulds of the genera Penicillium, Aspergillus and Fusarium are important for meat producing animals as well as for meat and meat products. However, these genera are of variable significance for mycotoxicosis, as well as carry-over, mould growth on meats and as starter cultures (Table 3). Penicillia in feeds may cause mycotoxicosis in animals. We investigated an outbreak of illness in 20 breeding sows, of which 16 died within a few weeks (Hofmann & Mintzlaff 1982). The symptoms of the diseased animals

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Table 1. *Predominant Penicillium species in a group of 1481 isolates originating from feeds and foods*

Species	No. of isolates	Species	No. of isolates
P. verrucosum var. cyclopium	505	P. critrinum	39
		P. variable	33
P. chrysogenum	197	P. brevicompactum	29
P. verrucosum var. verrucosum	150	P. corylophilum	25
		P. griseofulvum	25
P. roquefortii	80	P. rugulosum	18
P. camembertii	69	P. islandicum	18
P. frequentans	68	P. simplicissimum	15
P. nalgiovense	49	Others (26 species)	94
P. expansum	42	Unidentified	25

Table 2. *Mycotoxins produced in malt extract agar by 1481 Penicillium isolates originating from feeds and foods*

Mycotoxin	No. of isolates*	Mycotoxin	No. of isolates*
Cyclopiazonic acid	226	Ochratoxin A	39
'S-toxin'+	164	Rugulosin	30
Penicillic acid	140	Verruculogen TR_1	19
Patulin	82	Roquefortine	15
Brevianamide A	63	Fumitremorgen B	14
Citrinin	63	Citreoviridin	7
Penitrem A	62	Viridicatumtoxin	3
Xanthomegnin	61	Erythroskyrin	1
PR-toxin	55	Islanditoxin	1
Griseofulvin	43	Luteoskyrin	1

* Some are multiple toxin producers

+ 'S-toxin' is an undefined mycotoxin, frequently produced by P. verrucosum var. cyclopium; it has been detected by Still in our laboratories in 1978 (pers. commun.)

Table 3. Moulds important in Germany for meat producing animals as well as for meat and meat products

Genus	Myco-toxicosis	Carry-over	Mouldy meats	Starter-cultures
Penicillium	+	++	+++	+++
Aspergillus	++	++	++	0
Fusarium	+++	(+)	0	0

+++ Very important, ++ important, + occasionally important, (+) slightly important, 0 not important

were: cachexia, abscesses, paralysis, rhinitis, pneumonia and slight hepatitis. Their feed (oats and barley) contained moulds of the P. viridicatum series in large numbers (10^6 - 10^7/g) and the mycotoxin viomellein as well as ochratoxin B methylester. Toxins produced by aspergilli or fusaria were not detected. Apparently, in this outbreak Penicillium toxins in the feed have lowered the resistance of the hogs for viral and bacterial infections and contributed to their death which probably was caused by microorganisms rather than the mycotoxins themselves.

Carry-over

Mycotoxins present in feed may lead to the presence of mycotoxin residues in organs, meat and fat if they are taken up by the animals with the feed and are resorbed but not quickly eliminated from the tissues. The carry-over of aflatoxins into milk, eggs, organs and meat has been thoroughly investigated; it is of particular importance in the case of milk and milk products.

Of the toxins formed by penicillia of most concern for the carry-over is at present ochratoxin A (OTA). This toxin occurs in barley, maize, oats, wheat, rye etc. and causes residues in hogs and poultry (Krogh et al. 1974, Elling et al. 1975). OTA is a nephrotoxin and residues are most likely to be found in the blood and kidneys, but also in liver and muscle (Krogh et al. 1974, Rutqvist et al. 1978). From tissues OTA disappears rather slowly, since the RL_{50} for hogs is about 4 days (Galtier, Alvinerie & Charpenteau 1981). Denmark is the only country imposing legal tolerances for OTA residues in hogs: discoloured and/or enlarged kidneys of hogs are collected during meat inspection and analysed for OTA; the carcass is condemned if the kidney contains more than 25 µg/kg OTA (previously the limit was 10 µg/kg). In Denmark 2336 discoloured and/or enlarged hog kidneys were analysed in 1982, and 229 (9,8 %) were found to contain more than 25 µg/kg OTA, i.e. 229 carcasses were discarded.

In 1982 - 83 we analysed in our laboratory blood and kidneys from healthy hogs slaughtered in West Germany for OTA with a detection limit of about 0,2 µg/kg. Of 261 blood samples 40 (15,3 %) proved positive (Hofmann 1983). Of 300 normal hog kidneys without adverse colour, size or shape, which passed meat inspection and were bought in 1983 from butcher shops all over West Germany, we found 41 (13,7 %) to be positive for OTA (Scheuer, Bernard & Leistner 1983). Fortunately, the detected amounts of OTA in blood and kidneys generally were below 3 µg/kg, and only a few samples contained up to 10 µg/kg. However, since OTA has been demonstrated on oral administration of large doses to cause carcinomas in male mice (Kanisawa & Suzuki 1978, Bendele et al. 1983), and to be strongly immunosuppressive in low concentrations (Röschenthaler et al. 1981), even the occurrence of low residues of OTA in hogs is of some concern.

Mouldy meats

Undesirable penicillia grow quite frequently on meat products, especially on fermented sausages (salami) and raw hams. Experimental inoculation with toxigenic isolates revealed that 10 out of 15 Penicillium toxins investigated are formed not only in culture media but also in salami and/or raw ham (Table 4). Most of the data listed in Table 4 have been obtained at our laboratory (Hofmann, Leistner & Trapper 1981); a detailed discussion of the mycotoxin production in meats was given by Leistner and Eckardt (1981). Mouldy ham is more hazardous than salami since it is not protected by a casing. In meats with mould growth on the surface most mycotoxins are present in the first 5 mm below the surface. Therefore, hazards can be minimised by cutting off an adequate slice. Of course, it would be preferable to prevent all undesirable mould growth on meat products. This can be attempted by smoking, treatment with sorbate or pimaricin, an adjustment or vacuum packaging. In West Germany salami and raw ham are usually smoked. Since 1977 a treatment of these products with potassium sorbate suggested by our laboratory (Leistner, Maing & Bergmann 1975) has been legal. Meat products are dipped into a potassium sorbate solution (200 g/L); however, in the first 15 mm zone of the treated salami or raw ham the residue is legally not permitted to exceed 1500 mg/kg sorbic acid.

Mould-fermented foods

For fermented foods made in the Orient moulds of the genera Rhizopus, Mucor, Amylomyces, Actinomucor, Monascus, Aspergillus and Neurospora are essential for fermentation processes (Hesseltine 1983, Gandjar 1983). On the other hand, for mould-fermented Western foods, such as cheese and sausages, only moulds of the genus Penicillium are desirable. In both parts of the world for many years traditional processes have been used, in which the desired moulds, often associated with bacteria

Table 4. Production of mycotoxins in culture media and meat products

Produced in malt extract agar	Produced in salami and/or raw hams	Produced in malt extract agar	Produced in salami and/or raw hams
Brevianamide A	+	Penicillic acid	-
Citreoviridin	+	Penitrem A	-
Citrinin	+	PR-toxin	-
Cyclopiazonic acid	+	Roquefortine	-
Fumitremorgen B	+	Rugulosin	+
Griseofulvin	+	'S-toxin'	n.i.
Mycophenolic acid	-	Verruculogen TR_1	+
Ochratoxin A	+	Xanthomegnin	n.i.
Patulin	(+)		

- Not produced, n.i. not investigated, + produced, (+) slightly produced

and/or yeasts, become predominant in a particular food because the environmental conditions are ideally suited for their growth. More recently, selected moulds have been added as starter cultures to these foods.

In this presentation, only starter cultures for mould-fermented meats (salami and raw hams) as well as cheese (Roquefort and Camembert types) will be discussed with reference to work carried out in our laboratories. In Europe mould-fermented raw sausages (salami) are as important as the smoked products; they are essentially produced in the southern or south-eastern countries of Europe (Table 5). A whitish mould cover on the surface gives these sausages a typical appearance, contributes to the characteristic flavour and delays rancidity (Leistner & Eckardt 1981).

Mould-fermented sausages of the salami type are traditionally produced in ripening rooms with indigenous moulds. At a temperature of 20° - 10°C, relative humidity of 95 - 75 % and a ripening time of several weeks or months, the sausages in these rooms develop a heavy mould cover on the surface. This cover should be uniform and whitish or gray without greenish, brown or black mould spots. The whitish or gray moulds are primarily representatives of Penicillium and sometimes of Scopulariopsis, the greenish moulds are again Penicillium or Aspergillus; the brown or black spots are caused by Cladosporium, Alternaria or Aspergillus (Leistner & Ayres 1967, 1968). In most countries producing mould-fermented salami, the sausages are not smoked; however, in Hungary the salami is lightly smoked in the initial ripening phase and then transferred to ripening rooms to develop the desired mould flora.

Table 5. Estimated proportion of fermented sausages (salami) with desirable mould cover produced in various countries

Country	Proportion (%)	Country	Proportion (%)
Rumania	100	Soviet Union	0
Italy	95	Czechoslovakia	0
Bulgaria	90	Netherlands	0
France	80	Finland	0
Hungary	80	Norway	0
Switzerland	70	Sweden	0
Spain	60	Denmark	0
Austria	30	UK	0
Belgium	5	Ireland	0
West Germany	5	Canada	0
DDR	1	Australia	0
USA	1	Japan	0
Yugoslavia	1	South Africa	0
Poland	1		

The flora indigenous in ripening rooms for salami is mainly composed of penicillia and has only recently been scrutinised for mycotoxin producers. Since about 70 - 80 % of the penicillia are potential toxin producers (Leistner & Pitt 1977, Leistner & Eckardt 1979b, Eckardt et al. 1979), it should be expected that frequently toxigenic penicillia occur on mould-fermented salami. We investigated 28 samples of genuine Hungarian salami, 67 samples of genuine Italian salami and 27 samples of mould-fermented sausages from different manufacturers in West Germany (Leistner & Eckardt 1979a). From these products 175 isolates of penicillia were recovered, identified to the species level and examined with chemical (TLC) and biological (brine shrimp-test) methods for mycotoxin formation in malt extract agar.

Table 6 indicates that from the Hungarian, Italian and German salami, 77,1, 66,2 and 21,1 %, respectively, of the Penicillium isolates formed mycotoxins in malt extract agar (Leistner & Eckardt 1979a). The predominant species recovered and mycotoxins produced of the isolates from Hungarian, Italian and German salami are listed in Table 6. Even the predominant penicillia isolated from Hungarian and Italian salami exhibit a greenish colour on culture media; yet they show whitish growth on the

Table 6. Occurrence of toxigenic Penicillium isolates on mould-fermented salami

Country of origin	Isolates No. investi-gated	No. toxi-genic	Proportion (%)	Predominant species	Predominant mycotoxins
Hungary	48	37	77.1	P. verrucosum var. verrucosum, P. verrucosum var. cyclopium	Ochratoxin A, cyclopiazonic acid
Italy	89	59	66.2	P. verrucosum var. cyclopium, P. chrysogenum	Cyclopiazonic acid, 'S-toxin', ochratoxin A
West Germany	38	8	21.1	P. nalgiovense*, P. verrucosum var. cyclopium	Cyclopiazonic acid (rarely produced)

* Starter culture

sausages, because due to the ripening conditions (temperature and relative humidity < 15°C and < 80 - 85 % respectively) only mycelium growth of these species occurs on salami, and conidia are not formed. In West Germany, mould-fermented salami is ripened at higher temperatures (20°C) and relative humidities (95 - 85 %); this is possible since a mould (P. nalgiovense) is generally used as starter culture, which forms white mycelium and conidia.

Raw, cured and dried hams which have not been smoked, often exhibit a mould layer on the surface similar to that of mould-fermented salami. Mould growth on the surface is for instance common on Italian speck (cuts of pork), Swiss bündnerfleisch (dehydrated cuts of beef), US country cured hams (pork hams) and Yugoslav kraški pršut (pork ham). With low relative humidity in the ripening rooms, surface mould growth on these hams can be avoided; this is often found for Italian prosciutto di parma (pork ham). The moulds growing on the surface of speck and bündnerfleisch are predominantly penicillia, and many potentially toxigenic Penicillium isolates can be recovered from these products (Leistner & Pitt 1977, Leistner & Eckardt 1981). On country cured hams and kraški pršut penicillia also are prevalent in the early stages of the ripening process; however, on products ripened for a long time, which have a low a_w, moulds of the Aspergillus glaucus group are predominant. Experimental inoculations demonstrated that these aspergilli, especially A. ruber and A. repens, are an indicator of low a_w, i.e. long ripening times. The characteristic flavour of these products develops during prolonged ripening but the aspergilli apparently do not contribute to flavour development (Leistner & Ayres 1967, 1968).

Even though some mycotoxins produced by penicillia are formed in culture media only, many others are also formed in meats if toxigenic moulds grow on them (Table 4). Therefore, for mould-fermented meat products starter cultures should be employed which are neither pathogenic nor toxinogenic and produce no antibiotics (Leistner & Eckardt 1981). Our laboratory introduced as a starter culture an isolate of P. nalgiovense (Mintzlaff & Leistner 1972) which was named 'Edelschimmel Kulmbach' and now is widely used commercially for salami. This isolate should also be suitable for raw hams, such as speck (Mintzlaff & Christ 1973). More recently we selected a P. chrysogenum isolate (Sp. 1947) for Italian type salami (Leistner, Hechelmann & Trapper 1980); this is a 'green' mould; however, it grows whitish on Italian salami due to the ripening conditions mentioned previously. Also in France an isolate of P. nalgiovense ('blanche') has been introduced as starter culture for salami (Vayssier & Guerineau 1979). Earlier P. camembertii was recommended in France for this purpose; however, it is not suitable as a starter culture for meats since it produces cyclopiazonic acid.

Cheese of the Roquefort and Camembert type are traditionally fermented with moulds, i.e. P. roquefortii and P. camembertii which give each type of cheese a characteristic appearance and flavour. P. roquefortii is inoculated into the cheese and grows with dark green conidia, while P. camembertii grows only on the surface of the cheese with white conidia.P. roquefortii produces several mycotoxins, some also in cheese. We investigated 80 P. roquefortii isolates for mycotoxin production in malt extract agar (Leistner & Eckardt 1979b). Of these isolates 73 (91,3 %) proved toxigenic, and produced in malt extract agar the following mycotoxins: PR-toxin (45 isolates), patulin (12), PR-toxin and roquefortine (10), roquefortine (5) and penicillic acid (1). In addition, 10 isolates produced mycophenolic acid. Nevertheless, it should be possible to select P. roquefortii isolates as starter cultures for cheese which do not produce any known mycotoxins in culture media as well as in cheese.

The situation is more complicated with P. camembertii (P. caseicola is synonym) since this species produces cyclopiazonic acid, as first was demonstrated by our laboratories (Still, Eckardt & Leistner 1978). This mycotoxin is formed in cheese, especially in unrefrigerated products. We investigated 69 isolates of P. camembertii and all produced cyclopiazonic acid (Leistner & Eckardt 1979b). Apparently, until now other investigators also have not succeeded in finding a P. camembertii isolate which is not toxigenic. Obviously, further efforts should be made to introduce a safe, beneficial starter culture for Camembert cheese.

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