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L. LEISTNER

TOXINOGENIC PENICILLIA OCCURRING IN FEEDS AND FOODS

Centre of International Projects, GKNT

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L. LEISTNER

Federal Centre for Meat Research, Federal Republic of Germany

SUMMARY

Penicillia frequently occur in feeds and foods. Many isolates of this genus are toxinogenic, and thus 1. impair the health of domestic animals, 2. cause residues in organs and meat due to carry over, 3. synthesize mycotoxins in moldy meat products, and 4. may be hazardous for mold-fermented foods. The results of our laboratory gathered in these respects are summarized in this contribution.

TOXINOGENIC PENICILLIA

We studied 1481 Penicillium isolates originating from various foods and feeds[1]. These isolates represented 42 species, according to the nomenclature of Raper und Thom [2], revised by Samson et al.[3,4,5]. 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 per cent) of the isolates. Some isolates (e.g. of P. simplicissimum) produced
3515 up to four different mycotoxins in malt extract agar, and some Penicillium species included isolates which produced se-

veral mycotoxin combinations[6]. Nevertheless, the mycotoxin pattern of an isolate can be used as an aid in the identification of penicillia[6,7]. In the brine shrimptest 998 (67.4 per cent) of the 1481 isolates proved toxinogenic[1]. Considering the chemical as well as the biological assays 1166 (78.7 per cent) of the 1481 Penicillium-isolates investigated must be regarded as toxinogenic. Therefore, most of the penicillia occurring in feeds or foods must be regarded as potential mycotoxin producers.

Table 1

Predominant Penicillium species in a group of 1481 isolates originating from feeds and foods

Species	N of isolates	Species	N of isolates
P. verrucosum var. cyclopium	505	P. variabile	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. sim plicissium	15
P. nalgiovense	49	Others (26 species)	94
P. expansum	42	Unidentified	25
P. citrinum	39		

Table 2

Mycotoxins produced in malt extract agar by 1481 Penicillium isolates originating from feeds and foods

Mycotoxin	# of isolates+	Mycotoxin	# of isolates+
Cyclopiazonic acid	226	Ochratoxin A	39
"S-toxin"[a]	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

MYCOTOXICOSIS

Molds of the genera Penicillium, Aspergillus and Fusarium are apparently important for meat producing animals as well as for meat and meat products. However, these genera are of various significance for mycotoxicosis, the carry over, mold growth on meats and as starter cultures (Table 3).

Penicillia in feeds amy cause mycotoxicosis in animals.

3815

a) "S-toxin" is an undefined mycotoxin, frequently produced by P. verrucosum var. cyclopium; it has been detected by Dr. Paul Still (USA) in our laboratories in 1978.

We investigated an outbreak of illness in 20 breeding sows, of which 16 died within a few weeks[8]. The symptoms of the diseased animals were: cachexia, abscesses, aparalysis, rhinitis, pneumonia, and slight hepatitis. Their feed (oats and barley) contained molds of the P. viridicatum series in large numbers (10^6-10^7 per gram), 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 a factoral disease.

Table 3

Important molds 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

CARRY OVER

Mycotoxins present in the feed may cause 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, and is of particular importance for milk and milk products.

Of the toxins synthesised by penicillia of most concern for the carry over is at present ochratoxin A (OT-A). This toxin occurs in barley, maize, oats, wheat, rye etc., and causes residues in hogs and poultry[9,10]. OT-A is a nephrotoxin and residues are most likely to be found in the blood and kidneys, however, also in liver and muscle[9,11]. From tissues OT-A depletes rather slow, since the PL_{50} for hogs is about 4 days[12]. Denmark is the only country which has established ligal tolerances for OT-A residues in hogs: Discolored kidneys of hogs are collected during meat inspection and are analysed for OT-A; if the kidney contains more than 25 ppb OT-A (earlier the limit was 10 ppb), then the carcass is condemned. In Denmark 2336 discolored hog kidneys were analysed in 1982, and 229 (9.8 per cent) were found to contain more than 25 ppb OT-A, i.e. 229 carcasses were discarded.

In our laboratory we analysed in 1982/83 for OT-A blood and kidneys from healthy hogs slaughtered in West Germany, with a detection limit of about 0.2 ppb. Of 261 blood samples 40 (15.3 per cent) proved positive[13]. Of 177 normal hog kidneys (without adverse color, size and shape), which passed meat inspection and were bought in 1983 from butcher shops all over West Germany, we found 32 (18.1 per cent) to be positive for OT-A[14]. Fortunately, the detected amounts of OT-A in blood and kidneys generally were below 3 ppb, and only a few samples contained up to 10 ppb. However, since OT-A has been demonstrated to cause after oral administration of large doses carcinomas in male mice[15,16], and to be strongly immunosuppressive in low concentrations[17], even the occurrence of low residues of OT-A in hogs is of some concern.

MOLDY MEATS

Undesirable penicillia grow quite frequently on meat products, especially on fermented sausages (salami) and raw hams. Experimental inoculation with toxinogenic isolates revealed that 10 out of 15 Penicillium toxins investigated are synthesized 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 worked out at our laboratory[18]; a detailed discussion of the mycotoxin production in meats was given by Leistner et al.[19]. Moldy raw ham is more hazardous than salami, since it is not protected by a casing. Most mycotoxins in meats with mold growth on the surface are present in the first 5 mm beneath the surface. Therefore, hazards can be minimised by cutting off an adequate slice. Of course, preferably would be to prevent all undesirable mold growth on leat products. This can be attempted by smoke application, sorbate or pimaricin treatment, a_w-adjustment or vacuum packaging. In West Germany smoke is generally applied to salami and raw ham. Since 1977 a treatment of such products with potassium sorbate, which has been suggested by our laboratory[20], is legal. Meat products are dipped into a 20 per cent potassium sorbate solution, however, in the first 15 mm zone of the treated salami or raw ham not more than 1500 ppm sorbic acid are tolerated as residue.

Table 4

Production of mycotoxins in culture media and meat products

Produced in malt extract agar	Produced in salami and/or raw ham	Produced in malt extract agar	Produced in salami and/or raw ham
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	+	Xanthomegin	n.i.
Patulin	(+)		

+: produced; (+): slightly produced; -: not produced; n.i.: not investigated

MOLD-FERMENTED FOODS

For fermented foods made in the Orient molds of the genera *Rhizopus*, *Mucor*, *Amylomyces*, *Actinomucor*, *Monascus*, *Aspergillus*, and *Neurospora* are essential for fermentation
3515 processes[21,22]. On the other hand, for mold-fermented Western foods, such as cheeses and sausages, only molds of the genus

Penicillium are desirable. In both parts of the world for many years traditional processes were used, in which the desired molds, often associated with bacteria and/or yeasts, became predominant in a particular food, because the environmental conditions are ideally for their growth. More recently, selected molds are added as starter cultures to these foods.

In this contricution starter cultures for mold-fermented meats (salami and raw hams) as well as cheeses (Roquefort and Camembert type) will be discussed, with reference to work carried out in our laboratories.

Mold-fermented raw sausages (salami) are in Europe equally important as the smoked products, however, they are produced frequently only in countries in the southern or southeastern parts of Europe (Table 5). A whitish mold-cover on the surface gives these sausages a typical appearance, contributes to the characteristic flavor of the products, and delays rancidity[19].

Mold-fermented sausages of the salami type are traditionally produced in ripening rooms with an inherent "houseflora" of molds. At a temperature of 20-10°C, a relative humidity of 95-75 per cent, and a ripening time of several weeks or months, the sausages in these rooms develop a heavy mold cover on the surface, which should be uniform and whitish to gray, without greenish, brown or black mold spots. The whitish or gray growning molds are primarily representatives of Penicillium and sometimes of Scopulariopsis, the greenish molds are again Penicillium or Aspergillus, and the brown or black spots are caused by Cladosporium, Alternaria or Aspergillus[23]. In most

Table 5

Estimated percentage of the fermented sausages (salami), which are produced in different countries with a desirable mold-cover on the surface

Country	Per cent	Country	Per cent
Rumania	100	Soviet Union	0
Italy	95	Czecho-Slovakia	0
Bulgaria	90	Netherlands	0
Hungary	80	Finland	0
Switzerland	70	Norway	0
Spain	60	Sweden	0
France	60	Denmark	0
Austria	30	Great Britain	0
Belgium	5	Ireland	0
West Germany	5	Canada	0
DDR	1	Australia	0
United States	1	Japan	0
Yugoslavia	1	South Africa	0
Poland	1		

countries where mold-fermented salami is produced, the sausages are never smoked, however, in Hungary the salami in the initial ripening phase is lightly smoked, and then transfered to ripening rooms where the desired mold flora develops.

The "houseflora" in ripening rooms for salami is mainly composed of penicillia, and has only recently been scrutinized for mycotoxin producers. Since about 70-80 per cent of the penicillia are potential toxin producers[24,6,1], it should be expected that frequently toxinogenic penicillia occur on mold-fermented salami. We investigated 28 samples of genuine Hungarian salami, 67 samples of genuine Italian salami, and 27 samples of mold-fermented sausages from different manufactures in West Germany[25]. From these products in total 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 per cent, respectively, of the Penicillium isolates synthesized mycotoxins in malt extracted agar[25]. 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 color on culture media, they show whitish growth on the sausages, because due to the ripening conditions (temerature and relative humidity not above 15°C and 80-85 per cent, respectively) only mycelium growth of these species occurs on salami, and conidia are not formed. In West Germany mold-fermented salami is ripened at higher temeratures (20°C) and relative humidities (95-85 per cent); this is possible since a mold (P. nalgiovense) is generally used as starter culture, which forms white mycelium and conidia

Raw, cured and dryed hams, which are not treated with smoke, exhibit often a similar mold layer on the surface as mold-fermented salami. Mold growth on the surface is e.g.

Table 6

Occurrence of toxinogenic *Penicillium* isolates on mold-fermented salami

Origin of salami	Isolated investig./toxinog.	Species predominant	Mycotoxins predominant
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
W.Germany	38/8 (21,1%)	*P. nalgiovense*+, *P. verrucosum* var. *cyclopium*	Cyclopiazonic acid (rarely produced)

+starter culture

common on Speck (cuts of pork) from Italy, Bündnerfleisch (cuts of beef) from Switzerland, Country Cured Hams (pork hams) from the United States and Kraski Prsut (pork ham) from Yugoslavia. If the relative humidity in the ripening rooms is low, mold growth on the surface of such hams can be avoided; this is often true for Prosciutto di Parma (pork hams) from Italy. The molds growing on the surface of Speck and Bündnerfleisch are predominantly penicillia, and many potential toxinogenic *Penicillium* isolates can be recovered from these products[24,19]. On Country Cured Hams and Kraski Prsut in the earlier stages of the ripening process also penicillia prevail, however, on long ripened products with a low water activity (a_w) molds of the Aspergillus glaucus group are predominant. Experimental inoculations revealed that these aspergilli (especially A. ruber and A. repens) are an indicator of a low a_w, i.e. a long ripening time. The delicious flavor of these products develops during a prolonged ripening, however, the aspergilli are apparently not contributing to the flavour development[23].

Even some mycotoxins produced by penicillia are synthesized in culture media only, many others are also formed in meats if toxinogenic molds grow on them (Table 4). Therefore, for mold-fermented meat products starter cultures should be employed which are neither pathogenic nor toxinogenic, and produce no antibiotics[19]. Our laboratory introduced as starter culture an isolate of P. nalgiovense[26], which was named "Edelschimmel Kulmbach", and now is commercially widely used for salami. This isolate should also be suitable for raw hams, such as Speck[27]. More recently we selected a P. chrysogenum-isolate ("Sp. 1947") for Italian type salami[28]; this is a "green" mold, however, it grows whitish on Italian salami due to the ripening conditions mentioned before. Alsonin France an isolate of P. nalgiovense ("blanche") has been introduced as starter culture for salami[29]. Earlier P. camembertii was recommended in France for this purpose, however, it is not suitable as starter culture for meats, since it produces cyclopiazonic acid.

Cheeses of the Roquefort and Camembert type are traditionally fermented with molds, i.e. P. roquefortii and P. camembertii, which give each type of cheese a characteristic appearance and flavor. 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[6]. Of these isolates 73 (91.3 per cent) proved toxinogenic, and synthesized in malt extract agar the following mycotoxins: PR-toxin (45 isolates), patulin (12), PR -toxin and roquefortin (10), roquefortin (5), and penicillic acid (1). In addition, 10 isolates produced mycophenolic acid. Nevertheless, there is the possibility to select P. roquefortii-isolates as starter culture for cheese, which dont't produce any known mycotoxins in culture media as well as in cheese.

The situation is more complicated with P. camembertii, since this species produces cyclopiazonic acid, as first was demonstrated by our laboratories[30]. This mycotoxin is synthesized in cheese, especially in unrefrigerated products. We investigated 69 isolates of P. camembertii and all produced cyclopiazonic acid[6]. Apparently, until now also other investigations did not succeed in finding a P. camembertii-isolate which is not toxinogenic. Obviously, further efforts should be made to introduce a sound starter culture for Camembert cheese.

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