Toxigenic fungi in human environment

A. Krikštaponis,
J. Stakėnienė,
A. Lugauskas

Institute of Botany,
Žaliųjų ežerų 49,
LT-2021 Vilnius, Lithuania

Fungal species compositions on vegetable-born food products and in the air and dust of dwellings were studied in 1996–2000. Seven food selling-storage places and 14 residences were investigated, 179 samples of 94 names of food products as well as 50 air and 118 dust samples were surveyed. Ability of 393 fungal isolates to produce secondary metabolites grown on Czapek – yeast extract and yeast extract – sucrose agar media was tested, 124 strains were regarded as active producers of secondary metabolites.

Key words: microscopic fungi, mycotoxins, food, living environment

INTRODUCTION

Adequate nutrition and clean environment are the basic factors predetermining the quality of human life. Healthy food ought to be biologically valuable, free of chemical pollution and microbiological contamination. Taking into consideration the fact that activities of people are concentrated indoors, microbiological pollution of the premises directly influences the state of health of the occupants. One of the hazards associated with fungi is their ability to produce toxic secondary metabolites – mycotoxins which present a group of heterogeneous organic compounds hazardous for living organisms, including humans. Arising with dust, fungal spores can be inhaled, mycotoxins easily diffuse, penetrate the alveoli and get into the blood. Consequently, the risk of inhalable mycotoxicoses arises [1, 2]. Food polluted by mycotoxins can cause alimentary toxicoses, moreover, mycotoxins may be carcinogenic, nephrotoxic, hepatoxic, haemorrhagic, oestrogenic or cause inflammatory effects. According to the literature, the most common producers of mycotoxins are fungi from the genera *Aspergillus*, *Penicillium*, *Fusarium* [3].

The aim of the study was to determine the species of toxigenic fungi most common on food and in living premises, and to evaluate their abilities to produce secondary metabolites.

MATERIALS AND METHODS

The study was performed in 1996–2000. In order to detect food-deteriorating fungi, specimens of fruit, vegetables, and other vegetable-born food products were collected from 7 food selling-storage places at a different time of the year. 179 samples of 94 above-mentioned products, grown and made in Lithuania or brought from other countries were investigated. Isolation of fungi was performed according to the methods described by R. A. Samson et al., 1992 [4]. The plating method was used when a specimen was evidently damaged by one fungal species, and the diluting was applied when several fungal species were suspected [4, 5].

For the presence of toxigenic fungi in living environment, 14 residences considered as mould-problematic were surveyed; 50 air and 118 dust samples were investigated. A Krotov 818 slit-to-agar single stage impactor (cut-off 0.5 μm) was used for sampling total airborne propagules, simultaneously the settle plate method for direct airborne fungi isolation was applied. Dust samples were taken by sterile swabs and placed directly onto agar. Standard solid malt extract, corn extract, Czapek and Sabouraud dextrose media were used for fungi isolation and cultivation [6].

The ability of the isolated fungi to produce secondary metabolites (some of them could be toxic) was tested according to J. C. Frisvad et al. (1988) – the pure cultures were grown on secondary metabolism-stimulating CYA (Czapek yeast extract agar), YES (yeast extract-sucrose agar), and standard Czapek agar as a control [7]. Obvious changes of typical colours of developing fungal colonies as well as pigmentation of medium were registered.

RESULTS AND DISCUSSION

From food specimens, 288 fungal species ascribed to 93 genera and from the living environments 274 species belonging to 85 genera were isolated and identified. The vast majority of the identified species were attributed to mitosporic fungi; additional-

ly, there were isolates from the divisions of *Zygomycota, Ascomycota*, and *Basidiomycota*.

Fungi from the genus *Penicillium* prevailed among the isolated fungi. More common species, known as toxin producers, and their detection frequencies are presented in Table 1. Fungi from the genus *Aspergillus* were common too, especially in living environments, but the detection frequency was lower compared with that of the genus *Penicillium.* It must be emphasized that heavily sporulating fungi from the genus *Aspergillus* are known as the most potent mycotoxin (aflatoxins, ochratoxins, cyclopiazonic acid, gliotoxin) producers. A substantional fraction of these metabolites may be accumulated in air-borne spores and when inhaled can provoke acute or chronic mycotoxicoses. The *Fusarium* fungi were rare in the air and dust samples, but abundant on food, especially on vegetables. These fungi are known as producers of DON, T-2, fumonisin, zearalenone toxins. Species *Alternaria alternata*, *Mucor pusillus*, and *Rhizopus stolonifer* were widespread in human environment. Usually they are regarded as allergenic, and their capabilities to produce mycotoxins are not more profoundly investigated in contrast to those of the fungi from the genera *Penicillium*, *Aspergillus*, or *Fusarium*.

It should always be kept in mind that the chemical composition of many fungal metabolites has not been studied, and mycotoxins having not been reported for a species does not mean that the species does not produce them. Therefore, the ability of 393 fungal isolates ascribed to 54 genera and 176 species to synthesize secondary metabolites, some of them possible mycotoxins, was tested (Table 2). On cultivating the strains on CYA and YES media it was revealed that 124 strains produced secondary metabolites very actively; the following strains could be mentioned: *Aspergillus candidus* PSK-5, *A. niger* LR-7, *A. penicilloides* GR-8, *Penicillium chermesinum* MS-2, *P. chrysogenum* O-18, *P. claviforme* M-26, *P. clavigerum* PR-8, *P. expansum* BL-9, *P. funiculosum* S-2, *P. granulatum* S-42, *P. italicum* MA-3, *P. palitans* BR-22, *P. spinulosum* S-22, *P. stoloniferum* KO-15, *P. verrucosum* JR-1, *Alternaria alternata* PA-3, *Cladosporium cladosporioides* PO-10, *Gliocladium catenulatum* BL-51, *Mucor hiemalis* M-44, *Rhizopus oryzae* PO-4, *Sclerotinia sclerotiorum* KO-36, *Trichothecium roseum* LR-15, and others.

Table 1. **Toxigenic fungi isolated from the indoor environments and food**

Fungus	Species detection frequency, %			Produced toxins
	Residences			
	Air*	Dust*	Food**	
Alternaria alternata (Fr.) Keissl.	30.8	20.3	0.6	Alternariols, tenuazonic acid
Aspergillus candidus Link	20.0	8.5	0.3	Candidulin, terphenyllin, kojic acid, xanthoascin
A. flavus Link	–	2.5	–	Aflatoxins, aflatrem, aspergillic acids, paspalinin
A. fumigatus Fresen.	22.0	8.5	0.1	Fumigaclavines, fumigillin, fumigatin, fumitoxins, fumitremorgins, verruculogen
A. niger Tiegh.	34.6	17.1	5.3	Malformins, naphthoquinones, nigragillin
Eurotium herbariorum	34.6	4.9	–	Sterigmatocystin
Fusarium moniliforme J. Sheld.	6.0	–	0.5	Moniliformin, zearalenone
F. oxysporum Schltdl.	2.0	–	0.5	Moniliformin, fusaric acid, enniatins, naphthoquinones
F. solani (Mart.) Appel et Wollenw.	6.0	–	0.1	Naphthoquinones, fusaric acid
F. sporotrichioides	2.0	–	0.1	Trichothecenes A, butenolide
Mucor pusillus Lindt	20.0	16.9	0.3	Tetracyclic triterpenes, obtusifoliol, sillucin,
Penicillium chrysogenum Thom	24.0	12.7	1.7	Roquefortine C, PR-toxin, xanthocillin X, penicillin
P. decumbens Thom	26.0	4.2	0.5	Decumbin
P. expansum Link	76.9	45.5	3.2	Patulin, citrinin, roquefortine C
P. oxalicum Currie et Thom	30.8	21.1	–	Oxaline, secalonic acid D, roquefortine C
P. spinulosum Thom	20.0	7.6	2.0	Penitrem A
P. stoloniferum Thom	12.0	4.2	0.4	Mycophenolic acid
P. verrucosum Dierckx	36.5	17.1	2.3	Ochratoxin A, citrinin
P. granulatum	–	1.7	3.0	Patulin
P. italicum Wehmer	8.0	2.5	2.3	Deoxybrevianamide E
P. funiculosum Thom	20.0	5.9	1.2	11-deacetoxywortmannin
Rhizopus stolonifer (Ehrenb. ex Fr.) Vuill.	15.4	14.6	1.5	Ergoline alcaloids

* In the samples; ** among all isolated fungal species.

Table 2. **Abilities of certain fungal genera grown on CYA and YES media to produce secondary metabolites**

Genus	Number of tested		Number of secondary metabolite producers:	
	species	strains	pigment exuded into media	changed colour of colony
Acremonium Link ex Fr.	6	14	3	2
Alternaria Nees	3	5	3	0
Aspergillus Mich. ex Fr.	11	30	4	11
Botrytis P. Mich ex Pers.	3	5	2	0
Chrysosporium Corda	2	3	0	1
Cladosporium Link ex Fr.	4	10	9	1
Eupenicillium Ludwig	1	5	0	0
Fusarium Link ex Fr.	9	13	0	5
Gliocladium Corda	4	7	4	0
Myrothecium Tode ex Fr.	2	2	0	1
Mucor Fresen.	9	12	3	2
Nectria (Fr.) Fr.	2	2	1	0
Paecilomyces Bainier	2	2	0	0
Penicillium Link	74	206	73	32
Pythium Pringsh.	2	3	0	1
Rhizopus Ehrenb.	2	10	0	2
Sclerotinia Fuckel	3	13	4	2
Trichoderma Pers. ex Fr.	2	2	0	0
Verticillium Nees	2	5	1	1
Other genera	35	44	18	6

The obtained data allow to state that the human environment can be polluted by fungi producing secondary metabolites. More comprehensive studies applying modern techniques should be conducted in order to monitor the contamination of human environment by toxigenic fungi and to diminish the risks of alimentary or inhalable mycotoxicoses.

References

1. Sorenson WG. 1990. Devel Industrial Microbiol 1990; 31: 205–11.
2. Gravesen S, Frisvad JC, Samson RA. Microfungi. Copenhagen, 1994.
3. Flannigan B, Miller D. In: Health Implications of Fungi in Indoor Environments. Samson RA (Ed.), Amsterdam, 1994.
4. Samson RA, Hochnig AD, Pitt JJ, Knig AD. Modern Methods in Food Mycology. Amsterdam, 1992.
5. Rabie CJ, Lübben A, Marais GJ, Jansen van Vauren H. Int J Food Microbiology 1997; 35: 117–27.
6. Wanner HU, Verhoeff A, Colombi A, Flannigan B, Gravesen S, Mouilleseaux A, Nevalainen A, Papadakis J, Seidel K. Report No. 12. Commission of the European Communities. Brussels, Luxembourg, 1993.
7. Samson RA, Van Reenen-Hoekstra ES. Introduction to Food-borne Fungi. Baarn, 1988.

A. Krikštaponis, J. Stakėnienė, A. Lugauskas

TOKSIGENINIAI MIKROMICETAI ŽMOGAUS APLINKOJE

S a n t r a u k a

1996–2000 m. buvo tirta mikromicetų rūšių, paplitusių ant augalinės kilmės maisto produktų, taip pat gyvenamųjų patalpų ore bei dulkėse, įvairovė. Ištirti 7 maisto prekybos bei sandėliavimo objektai ir 14 gyvenamųjų būstų. Patikrintas 393 išskirtų mikromicetų kamienų, auginant juos ant Čapeko-mielių ekstrakto bei mielių ekstrakto-sacharozės agarizuotų terpių, gebėjimas produkuoti antrinius metabolitus. Nustatyta, kad 124 kamienai buvo aktyvūs antrinių metabolitų producentai.