EKOLOGIJA. 2006. Nr. 3. P. 1–13

Micromycetes as toxin producers detected on raw material of plant origin grown under various conditions in Lithuania

Albinas Lugauskas,

Jūratė Repečkienė,

Loreta Levinskaitė,

Rimutė Mačkinaitė,

Audrius Kačergius,

Vita Raudonienė

Institute of Botany,
Žaliųjų Ežerų 49,
LT-08406 Vilnius, Lithuania.
E-mail: lugauskas@botanika.lt

In 2003–2006, micromycetes – potential producers of mycotoxins – were detected and their taxonomic dependence as well as ability to produce toxins under various growth conditions were assessed. Accumulation regularities of toxin-producing micromycetes in vegetables, fruit, berries, herbs, grain, seeds and their products were found out. Factors determining fungal abilities to produce various toxins and excrete them into food raw material are discussed. Potential producers of toxic secondary metabolites were detected during processing of food raw material of plant origin. Such micromycetes were *Alternaria alternata, A. citri, A. cucumerina, Aspergillus candidus, A. clavatus, A. flavus, A. fumigatus, A. niger, A. ochraceus, A. parasiticus, A. sulphureus, A. ustus, A. versicolor, Cylindrocarpon destructans, Drechslera sorokiniana, Fusarium culmorum, F. equiseti, F. graminearum, F. moniliforme, F. oxysporum, F. poae, F. proliferatum, F. solani, F. sporotrichioides, Penicillium aurantiogriseum, P. chrysogenum, P. claviforme, P. clavigerum, P. commune, P. corylophilum, P. crustosum, P. expansum, P. funiculosum, P. griseofulvum, P. palitans, P. spinulosum, P. variabile, P. viridicatum.* The toxin-producing ability of fungi isolated from food raw material was tested and primary screening of active producers was conducted. The taxonomic position of strains of some toxicogenic fungi using molecular genetic methods was revised. The diversity and peculiarities of isolated micromycetes in relation to substrate specificity, climate conditions and the environment, particularly dustiness, moisture and temperature, are pointed out.

Key words: micromycetes, food raw material, products, mycotoxins, temperature, moisture, sanitary state

INTRODUCTION

Micromycetes get onto food raw material by various ways and from various sources; nevertheless, the main source of fungal contamination of food plants growing in fields is soil. Different microbial communities are established in soil; furthermore, interdependent relations are formed inside them, determining not only the role of each microbial species inside the community, but also external relations with other environmental and soil biota. Micromycetes growing under such complicated conditions strengthen their properties that enable them to establish in the particular environment, to develop and prevent growth of competitors. For such purposes, many micromycetes produce and excrete toxic secondary metabolites; e.g., *Fusarium moniliform*e and *F. proliferatum* when growing on maize, wheat and barley synthesize fumanisin B (Morin et al., 1999). It is indicated that fungi of the genera *Rhizopus* and *Neurospora* are able to accumulate aflatoxin B_1 produced by *Aspergillus flavus* (Naut, 1989). While growing on common and specific substrates, many fungi of the genus *Penicillium* synthesize various toxins and other metabolites (Boutrif, Bessy, 2000; Stakėnienė et al., 2001; Samson, Frisvad, 2004). Rather high numbers of micromycetes get onto raw material of plant origin from the air together with dust and other contaminants (Dutkiewicz et al., 2001; Lugauskas et al., 2002; Krysińka-Trasczyk et al., 2003; Repečkienė et al., 2005; Šveistytė et al., 2005).

Microbial contamination of food and its outcome are studied worldwide, as microorganisms and their metabolites are an important agent of human diseases and death as well as losses of natural resources and human work products. The diversity of microorganisms and their adapting abilities enforce man to concern about this invisible but aggressive micro-world, to learn more about it, to acquire how to regulate microbial activity,

apply it for useful purposes or limit it (Rabie et al., 1997; Samson et al., 2000; Thrane, 2004). Micromycetes have specific biological properties that enable them to adapt easily to changeable environmental conditions and to deteriorate various plant substrates. Due to their activity, every year about 30% of raw material and products are lost (Mills, 1990; Mills et al., 1995; Tauxe, 2002; Doohan et al., 2003; Lugauskas, 2005; Mačkinaitė, Kačergius, 2005). When sanitary requirements are not met, micromycetes can evoke ecological problems and cause various plant, bird, animal and human diseases. The interest in fungal secondary metabolites – mycotoxins and their release into the environment has been growing constantly in the world, and new toxic fungal metabolites and diseases caused by them have been detected. Micromycetes of the *Penicillium expansum* species, widespread in various countries and particularly in Europe, could be an example. According to the literature, *P. expansum* can produce citrinin, ochratoxin A, patulin, penitrem A, rubratoxin B and some other compounds. It has been reported that chaetoglobosin A and communesin B were produced consistently by all isolates. Patulin and roquefortin C were produced by 98% of the isolates. Exponsolides A/B and citrinin were detected in 91 and 85% of the isolates, respectively. Chaetoglobosins and communesins were detected in naturally infected juices and potato pulp, whereas neither patulin nor citrinin were found. Because most *P. expansum* isolates produce patulin, citrinin, chaetoglobosins, communesins, roquefortine C and exponsolides A and B, food contaminated with this fungus should be ideally examined for chaetoglobosin A as well as patulin (Anderson et al., 2004). It is reported that *P. expansum* is of concern, especially in fruit products because of its production of patulin. A specific regulation for patulin at a level of 50 μg/kg has been set by most countries in Europe, and several quantitative methods have been developed (Ritieni, 2003). It is pointed out that citrinin synthesized by the mentioned fungi has nephrotoxic effects in mammals. Individually, metabolites from *P. expansum* are known to have different toxic effects (Pfohl-Leszkowicz, 2002; Cole, Schweikert, 2003).

It has been reported that mouldy feed containing ochratoxin A caused renal cancer in male Fisher rats (Mantle, 2004). Ochratoxin, also dangerous for human health, is detected in dust, as conidia of toxigenic fungi are associated with dust (Shoug et al., 2001). Abundance of micromycetes, the level of their toxicity and pathogenicity are determined by climatic and other environmental factors (Doohan et al., 2003; Stakėnienė, Lugauskas, 2004; Kačergius et al., 2005). Recently, the necrotic effect of ochratoxin A of *Penicillium polonicum* on animal tissues has been indicated (Miljkovic et al., 2003). New methods are being created for the detection and quantification of *Fusarium culmorum* and *F. graminearum, F. poae* and *F. avenaceum* in cereals by PCR assays (Parry, Nicholson, 1996; Schiling et al., 1996), assessment of ultrafloxacin and morfloxacin cultures of *Trichoderma viride* (Parshikov et al., 2002) and PCR-based detection and identification of fungi producing trichotecenes in food grains (Kačergius, Mačkinaitė, 2005), etc.

Data on micromycetes and their occurrence in the environment and food products, fungal potential toxicity and pathogenicity and their significance for human health increase every year (Cole et al., 2003; Lugauskas et al., 2003; Lugauskas et al., 2004; Levinskaitė et al., 2005; Lugauskas, Krasauskas, 2005).

The aim of the present work was to highlight the micromycetes that contaminate food raw material of plant origin grown, processed, stored and sold under local conditions of Lithuania; to evaluate the level of spreading of fungi and fungal ability to synthesize and release toxic secondary metabolites dangerous for human health.

CONDITIONS AND METHODS

Samples of food products originated from grains, seeds, fruit, vegetables and food products of plant origin were analyzed according to the methods of Kudryasheva (1986), Andrews et al. (1997), Rabie et al. (1997) and Samson et al. (2000). Analyses were conducted in triplicates. Direct isolation of micromycetes and imprint methods were used for hard products (grains, seeds and nuts, etc.) and for the products when, after visual inspection, a single contaminant was supposed to develop.

To get pure cultures, isolates were cultivated on standard Czapek, worth and corn agars at 26±2 ˚C for 5–7 days. The isolates were ascribed to taxonomic groups following Ainsworth and Bisby's Dictionary of the Fungi (Hawksworth et al., 1995). Micromycetes were identified according to handbooks (Raper, Thom, 1949; Raper et al., 1965; Gams, 1971; Ellis, 1971, 1976; Ģčëüźī, 1974; Domsch, 1980; Plaats-Niterink, 1981; Ramirez, 1982; Nelson et al., 1983; Brandenburg, 1985; Samson, Reenen-Hoekstra, 1988; Kozakiewicz, 1989; Samson et al., 2000; Kiffer, Morelet, 2000; Klich, 2002; Watanabe, 2002). Distribution frequency (%) was evaluated according to Booth (1971) and Мирчинк (1988).

The species-specific PCR used for re-identification of fungal strains was described elsewhere (Kačergius, Mačkinaitė, 2005), and restriction fragment analysis of amplified rDNA of *Alternaria* and *Ulocladium* fungal strains was carried out by standart PCR with ITS4 and ITS5 primers (White et al., 1990) synthesized by Fermentas GmbH (Lithuania). PCR products in ~600 bp size were digested for 4 h, with 2 units *HinfI* restriction endonucleases (Fermentas GmbH, Lithuania). The restriction fragments were separated by electrophoresis in TAE buffer using 1.5 % agarose (Fermentas GmbH, Lithuania) gels containing 0.4 μg/ml ethidium bromide.

The analysis of fungal secondary metabolites was carried out by thin-layer chromatography (TLC) using toxin standards (Sigma). The standards were used in

1 mg/ml concentration, the solvent system of toluene / ethyl acetate / formic acid (90%) (5:4:1) was chosen. Precoated plates of silica gel (20 by 20 cm, 0.20 mm thick (Macherey-Nagel, Germany), containing a fluorescence indicator (F254) were used. After extraction of mycelium in chloroform / methanol mixture (2:1), 20 µl of extract was placed onto TLC plates and after elution and drying examined under UV light (254/366 nm).

Statistical analysis was performed to assess the dominating micromycete genera by the LSD test.

The pure cultures of mycromycete strains with a high mycotoxin production level have been deposited in the collection of the Institute of Botany, Vilnius, Lithuania.

RESULTS AND DISCUSSION

In 2003–2006, an investigation of the ecological state of berries, vegetables, grains and other products of plant origin was performed. The facilities of their growth, sorting, marketing and processing differed depending on grown cultures, stored production and their amounts, storage time and conditions (temperature and humidity regimes, quality and application of preventive and disinfection measures, processing technologies of grown production and other factors). The obtained data on isolated and identified micromycetes allow to state that micromycetes, potential toxin producers, abound in all investigated samples. Analysis of the data and comparison with results of other authors has shown that the total number of micromycetes in premises where food products are kept, correlate with the number of potentially toxic micromycete species (the correlation coefficient R = 0.92) and the number of different products tested (R = 0.81).

The distribution frequency of the most often detected *Penicillium expansum* was 13.2% and *Aspergillus niger* 12.8%. These micromycetes prevailed reliably over micromycetes, whose distribution frequency in all premises was no more than 6%, and *Penicillium granulatum* (8.8%) reliably prevailed over micromycetes whose distribution frequency was no more than 3.2% (Table 1).

In many samples the following micromycetes were found: *Acremonium strictum, Aspergillus niger, Botrytis cinerea, Fusarium moniliforme, Geotrichum candidum, Penicillium biforme, P. chrysogenum, P. claviforme, P. digitatum, P. expansum, P. funiculosum, P. granulatum, P. italicum, P. palitans, P. spinulosum, P. verrucosum, P. viridicatum, Rhizopus oryzae, R. stolonifer, Sclerotinia sclerotiorum*.

Potatoes are very often used for daily nutrition. From potatoes grown in Lithuania and stored in various storehouses, micromycetes common in soil were isolated. The following fungi were most often found on deteriorated potatoes: *Acremonium strictum, A. charticola, Mortierella hyalina, Fusarium solani, F. oxysporum, F. equiseti, Rhizopus stolonifer, Verticillium alboatrum* and *Trichoderma harzianum,* more rarely – *Geotrichum candidum*, *Rhizopus oryzae* and *Sclerotinia sclerotiorum.* Rots caused by *Rhizoctonia solani* and *Sclerotinia fuckeliana* are particularly dangerous to potatoes stored in piles. When potatoes are harvested in damp weather and poured into big piles without preliminary sorting and drying, huge losses due *Rhizoctonia solani* activity are suffered. From potatoes the following micromycetes were isolated: *Acremonium roseum, Fusarium equiseti, Gliocladium deliquescens, Penicillium corymbiferum, P. expansum, P. funiculosum, P. lanosoviride, P. palitans, P. spinulosum, P. variabile, P. verruculosum* and *Verticillium alboatrum.*

Table 1. **Statistical analysis (according to LSD test) of the most often detected micromycete species**

Micromycete species	Distribution frequency, %	Reliability of distribution frequences of micromycete species (p)				
		Penicillium expansum	*Aspergillus niger*	*Penicillium granulatum*	*Penicillium claviforme*	*Penicillium spinulosum*
Penicillium expansum	13.2					
Aspergillus niger	12.8	0.8973				
Penicillium granulatum	8.8	0.1218	0.1560			
Penicillium claviforme	6.0	**0.0101**	**0.0145**	0.3019		
Penicillium spinulosum	5.6	**0.0069**	**0.0101**	0.2456	0.8973	
Penicillium italicum	5.4	**0.0057**	**0.0084**	0.2203	0.8464	0.9485
Sclerotinia sclerotiorum	5.1	**0.0038**	**0.0057**	0.1756	0.7649	0.8464
Penicillium chrysogenum	4.9	**0.0031**	**0.0047**	0.1560	0.6985	0.7962
Penicillium biforme	4.5	**0.0020**	**0.0031**	0.1218	0.6056	0.6985
Rhizopus oryzae	4.2	**0.0013**	**0.0020**	0.0937	0.5186	0.6056
Penicillium digitatum	4.0	**0.0011**	**0.0017**	0.0818	0.4777	0.5613
Rhizopus stolonifer	4.0	**0.0011**	**0.0017**	0.0818	0.4777	0.5613
Botrytis cinerea	3.4	**0.0005**	**0.0008**	0.0533	0.3663	0.4387
Alternaria alternata	3.2	**0.0004**	**0.0007**	**0.0458**	0.3331	0.4015
Acremonium strictum	3.1	**0.0003**	**0.0005**	**0.0393**	0.3019	0.3663

Statistical analysis was performed using LSD test. Statistically reliable differences are shown in bold fonts.

Table 2. **Micromycetes detected on grain, flour, bread and baked goods**

Product	Sampling site	Number of samples	Species of isolated micromycetes
Wheat	Mill	14	*Acremonium cerealis, A. strictum, Fusarium culmorum, Mucor globosus, Penicillium biforme, P. chrysogenum, P. expansum, P. fellutanum, P. paxilli, P. restrictum, Rhizopus oryzae*
Wheat flour	Mill	14	*Aspergillus flavus, A. niger, A. puniceus, A. penicillioides, Botrytis cinerea, Eurotium herbariorum, E. chevalieri, Fusarium culmorum, F. graminearum, F. moniliforme, Mortierella hyalina, Mucor mucedo, M. racemosus, Penicillium biforme, P. chermesinum, P. chrysogenum, P. commune, P. diversum, P. expansum, P. lanosocoeruleum, P. simplicissimum, P. verrucosum, P. viridicatum, Rhizopus oryzae*
	Bakehouse	5	*Aspergillus flavus, A. niger, Cladosporium herbarum, Fusarium graminearum, Rhizopus oryzae*
Rye flour	Mill	14	*Aspergillus flavus, Fusarium culmorum, Penicillium biforme, P. chrysogenum, P. daleae, P. expansum, P. roqueforti, P. velutinum, P. verrucosum, P. viridicatum*
	Bakehouse	5	*Aspergillus flavus, A. penicillioides, Cladosporium herbarum, Fusarium moniliforme, Penicillium biforme, P. chrysogenum, P. expansum, P. roqueforti, P. urticae, P. verrucosum, Ulocladium chartarum*
Rye malt	Bakehouse	2	*Aspergillus flavus, A. niger, Rhizopus oryzae*
Bread	Supermarket	14	*Aspergillus niger, Penicillium expansum, P. oxalicum, P. spinulosum, Rhizopus oryzae, Trichoderma viride*
	Premises for food preparation	10	*Penicillium lanosogriseum*
	Bakehouse	10	*Absidia glauca, Aspergillus awamori, A. cervinus, Aureobasidium pullulans, Cladosporium herbarum, C. resinae, Eurotium herbariorum, Penicillium biforme, P. brevicompactum, P. paxilli, P. verrucosum*
	Manufacturing premises	10	*Aspergillus flavus, A. niger, Cladosporium herbarum, Eurotium herbariorum, Penicillium biforme, P. expansum, P. oxalicum, P. roqueforti, P. spinulosum, P. verrucosum*
Baked goods	Supermarket	14	*Aspergillus niger, A. oryzae, Mortierella hyalina, Penicillium claviforme, P. cyclopium, P. expansum, P. granulatum, P. implicatum, P. olivinoviride, P. roqueforti, P. spinulosum, P. verrucosum*
	Premises for food preparation	10	*Aspergillus duricaulis, Penicillium aurantioviride, P. corylophilum, P. spinulosum, P. verrucosum, Syncephalastrum racemosum*

Under conditions of Lithuania, other vegetables of the *Solanaceae* family (tomatoes, paprica and aubergines) are often damaged by *Alternaria alternata, Botrytis cinerea, Sclerotinia sclerotiorum*, more rarely by fungi of the genus *Penicillium* such as *P. italicum, P. chrysogenum* and *Geotrichum candidus, Paecilomyces inflatus, Torula convuluta.* The diversity of micromycetes on tomatoes grown in Lithuania and sold in wholesale premises was low. Most often the fungi *Aspergillus caesiellus*, *A. niger, Cladosporium cladosporioides, Penicillium corylophilum, Rhizopus stolonifer* were found. *Fulvia fulva* isolated from tomatoes is known as *Cladosporium fulvum*, a causative agent of tomato leaf mold. The fungus *Rhizopus oryzae* was often isolated; *Alternaria solani,* which causes tomato early blight, and *Penicillium granulatum* were more rare.

From carrots grown in Lithuania and kept in various storehouses or premises of the market system, fungus *Sclerotinia sclerotiorum* which causes watery soft rot was sometimes isolated, while fungus *Verticillium alboatrum* was frequently detected and its distribution frequency was more than 20%. The micromycetes *Fusarium moniliforme, F. proliferatum F. redolens, Myrothecium roridum, M. verruraria* and abundant fungi of the genus *Penicillium* isolated from carrots are potential toxin producers. In some premises, fungi of the species *Myrothecium verrucaria* were particularly abundant. Fungi *Aspergillus niger, Cladosporium cladodporioides, Geotrichum candidum, Synchytrium endobioticum,* and micromycetes of the genus *Penicillium* were isolated from a carrot salad, were they apparently could have occurred from improperly maintained premises, particularly walls damaged by fungi. Other umbellifer plants such as parsnips, root parsleys and celeries were mostly contaminated by typical soil micromycetes which usually not manifest as pathogens, but are often found together with rot agents. These fungi were: *Acremonium strictum, Mortierella polycephala, Penicillium claviforme, P. expansum* and *P. italicum*. Root-crops of celery seedlings were damaged by *Verticillium alboatrum,* and dill sprays were mostly contaminated by *Acremonium strictum, Cladosporium cucmerinum, Penicillium digitatum* and micromycetes of the genus *Penicillium*.

Specific products are vegetables from the mustard family. The following fungi were isolated from radish: *Acremonium strictum, Cladodporium cladosporioides, Fusarium oxysporum, Geotrichum candidum, Sclerotinia sclerotiorum* and *Verticillium album.* On Japanese rads, *Mucor hiemalis* and *Penicillium paxilli* were detected, and on black radish *Fusarium moniliforme, F. solani, Nectria ventricosa, Penicillium paxilli, P. verruculosum* and *Rhizoctonia solani* were found. These fungi of the genera *Fusarium* and *Penicillium* are potential producers of mycotoxins, thus, radish contaminated by these fungi is harmful for human health.

Various fungi contaminate cabbages. The following fungi were detected: *Absidia glauca, Acremonium charticola, Alternaria alternata, A. brassicicola, A. fumigatus, A. niger, Botrytis cinereus, Gliocladium viride, Mucor hiemalis, M. racemosus, Penicillium expansum, P. granulatum, P. stoloniferum, Perenospora brassicae, Piptocephalis lemannieriana* and *Sclerotinia sclerotiorum*. Together with cabbages, micromycetes easily get into in dwellings and spread there during handling of cabbages, then some of them start to develop on food products and other substrates.

Vegetables from the cucumber family are most often damaged by fungi *Alternaria alternata, Aspergillus fumigatus, Botrytis cinerea, Cladosporium cucumerinum, Mucor murorum, Penicillium meleagrinum*. Fungi *Gliocladium album, Mucor racemosus, Phoma cava, Torula herbarum* and other micromycetes distributed on different substrates contaminate these vegetables.

Despite the natural immunity of members of the onion family, various fungi and particularly *Penicillium* can deteriorate them. Fungi *Penicillium claviforme, P. clavigerum, P. corymbiferum, P. granulatum, Sclerotinia sclerotiorum* were isolated from leeks. Garlics were contaminated by *Aspergillus niger, Cladosporium cladosporioides, Fusarium oxysporum, Mucor circinelloides* and *M. silvaticus*. Onions growing in fields, stored and realized in the market are deteriorated by various micromycetes, such as *Chrysosporium farinicola, Dipodascus ingens, Epicoccum purpurescens, Geotrichum candidum, Fusarium oxysporum, Penicillium atramentosum, P. claviforme, P. decumbens, P. digitatum, P. expansum, P. funiculosum, P. impactum, P. italicum, P. lanosum, P. ochrochloron, P. restrictum* and *P. spinulosum*. Fungi of the *P. spinulosum* species isolated from onions (distribution frequency 15%) are active producers of toxins. Onions were also contaminated by fungi *Epicoccus purpurescens, Penicillium adametzii, P. claviforme, P. implicatum, P. piscarium, P. verrucosum, Phoma fimeti, Rhizopus cohnii, Sclerotinia sclerotiorum, Trichoderma viride* and *Verticillium alboatrum.*

Fruit and berries, especially desiccated ones, grown in other countries prevail in the market of Lithuania. Fruit grown in Lithuania comprise only a little part of all fruit in premises were fruit are stored, sorted and sold. From the microbiological point of view, these storehouses are interesting in two aspects: 1) there are a plenty of micromycetes occurred from the local environment and brought together with fruit from other countries, 2) a sanitary aspect – the composition of local fungal species producing toxins is supplemented with micromycetes – toxin producers brought from abroad. Strawberries grown in Lithuania and sold in the market were contaminated by *Sclerotinia sclerotiorum* (distribution frequency up to 60%), which is known to cause strawberry white mold. Fungi *Absidia butleri, Cladosporium cladosporioides* and *Penicillium melinii* also were isolated from these berries. Raspberries were contaminated by fungi *Alternaria alternata, Chrysosporium inops, Cladosporium cladosporioides, Drechslera biseptata, Mucor piriformis, Penicillium citreoviride* and *Sclerotinia sclerotiorum.* Fungi *Aspergillus niger, Cladosporium cladosporioides, Rhizomucor pusillus* and *Sphaerotheca mors-uvae* were isolated from gooseberries, and *Aspergillus niger, Chrysosporium inops, Cladosporium herbarum, Sclerotinia sclerotiorum* were found on black currants. Some of the fungi mentioned above produce metabolites toxic to plants and warm-blooded animals. However, this problem has been analyzed insufficiently. We paid more attention to plant pathogens, such as fungi of *Sphaeroteca mors-uvae* species, which induce leaf shedding, withering, stem deformity and often plant death.

Most stone-fruits such as cherries, sweet cherries, plums, peaches, apricots, nectarines and others sold in Lithuanian markets and other selling places are brought from abroad. Cherries grown in Lithuania and sold in markets were contaminated by *Aspergillus niger, Aureobasidium pullulans, Ctenomyces serratus, Fusarium proliferatum, Geotrichum fermentans, Hormonema prunorum, Penicillium biforme, P. oxalicum, P. restrictum* and *Sclerotinia sclerotiorum.*

Apples grown in Lithuania and stored in various storehouses were mostly deteriorated by fungi of the genus *Penicillium*, especially *P. expansum* (distribution frequency about 50%), *P. granulatum* (32%), *P. digitatum* (18%), *P. claviforme, P. clavigerum* and *P. italicum* (about 14%). Sometimes fungi *Chaetomium globosum, Epicoccus purpurescens, Monilia implicata, Myrothecium roridum* and *Trichosporiella cerebriformis* were also detected on apples. These fungi are potential mycotoxin-producers, thus, fruit damaged by these micromycetes can be dangerous to human health as can cause chronic ailments or other diseases.

Distinctive food of plant origin is nuts, seeds, desiccated fruit and berries and other dry products. They accumulate various nutritional components: fats, proteins, vitamins and microelements. Differently from natural fruit and vegetables, they have little amounts of water, thus, according to other authors, they are heavily contaminated by micromycetes from the *Aspergillus, Rhizopus* and *Mucor* genera, which are able to develop in the presence of low humidity (Simsekli et al., 1999; Klich, 2002). Nuts, various desiccated fruit and seeds are brought from abroad, therefore fungi detected on

them are rare in Lithuania. For example, peanuts imported from Iran were contaminated by the aflatoxin producer *Aspergillus flavus* together with a very rare fungus in Lithuania, *Chaetomium aureum,* which was found in all tested samples. There is a lack of information about biological and physiological properties of this fungus. From flax-seed packed in small bags, fungi *Penicillium chrysogenum* and *Rhizopus oryzae* were isolated, on caraway seeds *Aspergillus candidus, A. flavus, A. niger, Fusarium* spp., *Penicillium daleae* and *P. expansum* were detected, on dried calamus leaves, used for bread baking and kept in a bakehouse fungi *Alternaria alternata, Aspergillus awamori, Aureobasidium pullulans, Mortierella isabellina, Mucor mucedo* and *Penicillium funiculosum* were found.

Grains and processed products such as flour and grits, etc. almost always are more or less contaminated by propagules of various micromycetes. Due to the high content of hydrocarbons, proteins, vitamins, microelements and low humidity in these products, the micromycetes that are rarely detected on other products of plant origin can contaminate grains and processed products.

Grains kept in various bins, mills, bakehouses and special capacitances were mostly contaminated by *Acremonium cerealis, A. strictum, Aspergillus flavus, A. niger, A. penicillioides, A. puniceus, Cladosporium herbarum, Fusarium culmorum, F. graminearum, F. moniliforme, Mortierella hyalina, Penicillium biforme, P. chrysogenum, P. expansum, P. paxilli, P. roqueforti, P. palitans, P. viridicatum, Rhizopus oryzae* and other fungi (Table 2).

In the flour samples taken in a bread bakehouse, fungi *Cladosporium herbarum* and *Ulocladium chartarum* were found. They could get into products from moldy walls and cellings in premises were raw material was stored. Under other conditions, food raw material of this group was mostly contaminated by micromycetes of the genus *Penicillium*; e.g., the distribution frequency of *P. simplicissimum* reached 22%. Bread and other baked goods (doughnuts, hardtacks, rolls and bisquits with different fillings and additives such as apples, curd, coconut shaves, poppyseeds and raisins) kept in various places were frequently damaged by micromycetes of the genera *Aspergillus* and *Penicillium*, but also other saprophyte micromycetes were detected in some samples. It should be stressed that both microbial contamination of breadhouse production and flour quality were determined by the same factor – the mycological state of manufacturing premises. For example, biscuits and cakes baked in a supermarket and bread baked in a bakehouse were contaminated by the same micromycetes, which were also detected on additives of baked goods, e. g., *Aspergillus niger* was found in cake flavourings and *A. awamori* and *Aureobasidium pullulans* on calamus. Hardtacks with raisins were contaminated by *Syncephalastrum racemosum* (distribution frequency about 39%), which is spread in soil and grains in countries of tropical and subtropical climate. Recently these micromycetes have been found on stored dry products, as they can grow at low water activity (a_w); they could have been brought here with raisins and other products from tropics.

Fungi of the genus *Fusarium* damage mostly wet grain. Data on the distribution of these fungi on grains are presented in Table 2, where the distribution frequency of different *Fusarium* species in internal grain tissues is shown.

Wheat grains were mostly contaminated by micromycetes *Fusarium poae, F. avenaceum* and *F. sporotrichioides,* barley by *F. poae, F. sporotrichioides* and *F. avenaceum*, oats by *F. poae,* rye by *F. sporotrichioides,* triticale by *F poae* and buckwheat by *F. equiseti.*

Identification of *Fusarium* fungi presented difficulties, as the morphological and cultural peculiarities are not always clearly manifested by fungi. Thus, to specify *Fusarium* species, polymerase chain reaction was applied using species-specific primers. Part of the data are presented in Figs. 1–3. Using this method, 42 genomic DNA samples of various *Fusarium* fungi were obtained. The species identity of 21 strains was confirmed and of 10 strains revised, and the latter fungi were ascribed to other species. The species of 10 fungal strains remain unclear and they are being examined further.

To compare fungi of two close fungi, *Alternaria alternata* and *Ulocladium consortiale*, the comparatable method of restriction fragment length polymorphism analysis was used. Results are presented in Fig. 4.

During polymerase chain reaction, there were amplified four ITS fragments of *Alternaria* and three of *Ulocladium* strains digested with Hinfl endonuclease. Restrictase analysis showed that all these strains falled into three groups: the first group consisted of all three *Ulocladium consortiale* strains, the second of three *Alternaria alternata* strains, and the third of only one *A.*

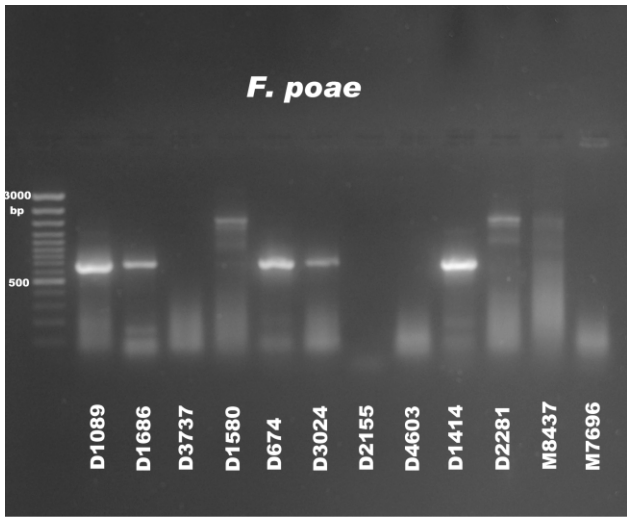

Fig. 1. Species-specific PCR amplification using primer pairs Fp8F and Fp8R specific for *Fusarium poae*

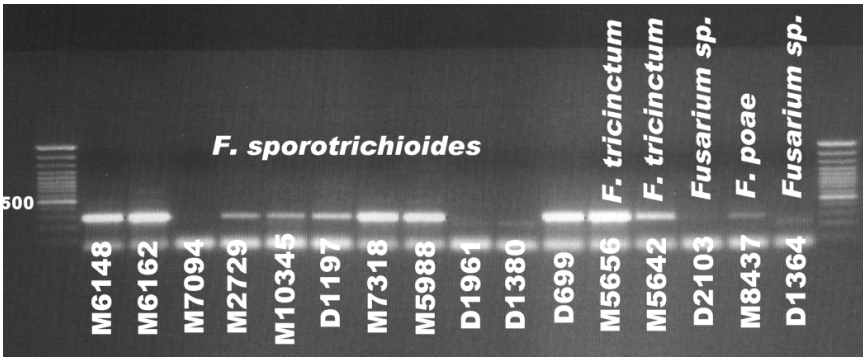

Fig. 2. Species-specific PCR with FspITS2K (F) and P28SL (R) primers specific for *F. sporotrichioides* for re-identification of fungi from the genus *Fusarium*

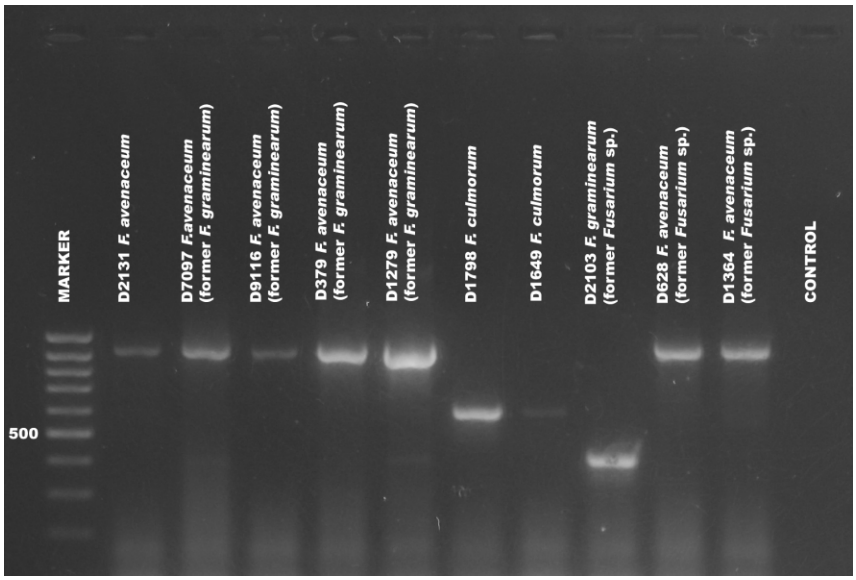

Fig. 3. Amplification of species-specific DNA fragments employing multiplex PCR with four pairs of primers to specify the morphological identification of fungal cultures (MARKER – molecular weight standard 100 bp ladder (1031, 900, 800, 700, 600, **500**, 400, 300, 200, 100, 80 bp), CONTROL – control without DNA)

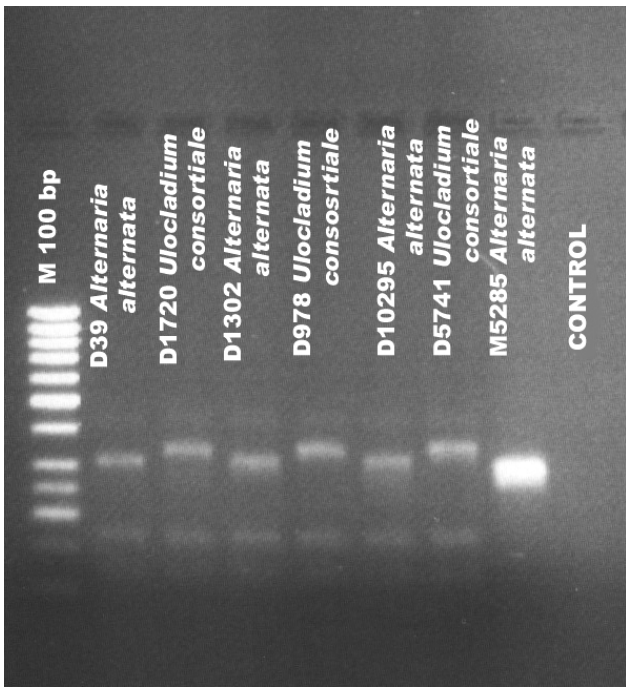

Fig. 4. Restriction patterns of polymerase chain reaction-amplified fungal rDNA digested with *HinfI* and analyzed in 1.5% agarose gel (MARKER – molecular weight standard 100 bp ladder (1031, 900, 800, 700, 600, **500**, 400,300, 200, 100, 80), CONTROL – control without DNA)

alternata strain 145285. Thus, it became clear that the genus *Alternaria* according its morphological properties is rather heterogenic, and further genetic investigation is necessary to ascribe fungi to species and separate them from the close genera *Ulocladium* and *Stemphylium*.

To screen fungal strains with an intensive synthesis of toxic secondary metabolites, 508 strains belonging to 53 genera and 156 species were grown on media enhancing the secondary metabolisms: YES (yeast extract-sucrose agar) and CYA (Czapek yeast extract agar). The growth of micromycetes on these media was compared with their growth on Czapek agar. According to Samson et al. (2000), fungi growing on these media change colony color and release abundant pigments into media, and this is related to fungal ability to produce toxins. For examination, the fungi were distributed into three groups: frequently isolated fungi, fungi of the genus *Penicillium*, and more rare fungi on these substrata. There were 166 strains of fungi (exception the genus *Penicillium* fungi) frequently detected in food samples tested for toxin production. They belonged to 21 genera and 86 species. Many of these fungi are active producers of toxic secondary metabolites. It was noticed that after seven days colonies of 69 fungal strains became pigmented and released pigments into media, whereas the other colonies of 36 fungal strains only changed color without pigment release into media (Table 4).

Activity of various *Penicillium* strains in the synthesis of pigments and their release into media should be stressed. Analysis of 216 strains of 74 species of this genus showed that a strong colony pigmentation and pigment diffusion into media were characteristic of 121 strains, and only colony pigmentation change was manifested by 51 strains (Table 5).

Applying the same method, the ability of fungi more rarely detected on raw material of plant origin to produce and excrete secondary metabolites by changing colony and medium color was tested (Table 6). It should

Table 3. **Distribution of micromycetes of the genus *Fusarium* in internal grain tissues of various cereals**

Micromycete	Number of isolates								
	wheat	barley	oats	rye	triticale	rape	pease	buckwheat	Total
Fusarium									
acuminatum	3	-	-	-	-	-	-	-	3
F. avenaceum	28	26	-	8	-	-	16	10	88
F. chlamidosporum	1	-	-	-	-	-	-	-	1
F. culmorum	5	11	-	1	-	-	-	-	17
F. equiseti	-	-	-	-	-	-	-	47	47
F. graminearum	12	14	1	8	1	-	-	1	37
F. graminum	-	1	-	2	1	-	-	10	14
F. heterosporum	-	1	-	-	-	-	-	-	1
F. moniliforme	-	-	-	1	-	-	-	-	1
F. poae	34	119	86	-	13	-	4	8	264
F. proliferatum	-	-	1	-	-	-	-	-	1
F. sambucinum									
var. *minus*	-	3	-	-	3	-	3	-	9
F. semitectum	7	-	-	1	-	-	-	10	18
F. solani	-	-	-	-	-	1	-	-	1
F. sporotrichioides	16	56	10	12	1	-	7	12	114
F. tricinctum	-	1	-	1	-	-	-	2	4
Fusarium sp.	6	-	-	-	-	2	-	1	9

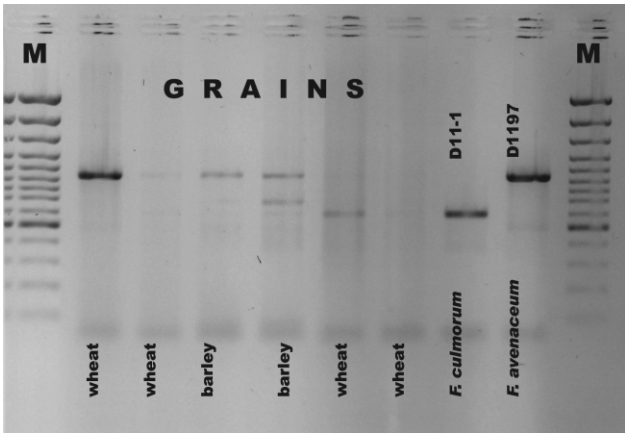

Fig. 5. Analysis of total DNA grains using multiplex PCR with species-specific primers. Two last lines represent specific DNA fragments characterized *F. culmorum* and *F. avenaceum* respectively (M – molecular weight standard 100 bp ladder (3000, 2000, 1500, 1200, 1031, 900, 800, 700, 600, **500**, 400, 300, 200, 100 bp)

be noted that fungi presented in Table 6 were found in raw material at different frequency. Some of them were detected more frequently; the others were isolated only once. Among 34 strains belonging to 31 species, 16 strains changed colony color and released pigments into media, and 14 of the strains tested only changed colony color. For further analysis the following fungi were selected: *Chaetomium aureum*, *Cylindrocarpon destructans, Embellisia allii, Myceliophthora vellerae, Sporotrichum pruinosum, Trichthecium roseum* and *Volutella ciliata.*

If to summarize the results of primary screening of toxin-producing micromycetes, it is clear that a half of tested strains were able to synthesize intensively secondary metabolites. Nevertheless, not all tested strains synthesized pigments in seven days; about one fourth of the fungi did not produce pigments during this period. Fungi *Aspergillus* and *Penicillium* produced pigments most intensively.

Using thin layer chromatography, 64 strains of *Alternaria alternata* were tested for their ability to produce toxic metabolites *in vitro*. There were four toxic compounds produced by *Alternaria* fungi, determined as alternariol, alternariol methyl ether, alternuene and tenuazonic acid. Six strains of the genus *Alternaria* synthesized all the four mentioned toxins, 49 strains produced 2–3 compounds each and seven strains one compound each. Only two strains in this investigation did not produce toxic metabolites.

While analysing the ability of fungal strains to produce toxic compounds, the main producers of trichotecene type A in Lithuanian grains were ascertained (Nicholson et al., 1988). They were *Fusarium culmorum, F. graminearum* and *F. sporotrichioides*. T-2 toxin, HT-2 toxin, diacetoxyscirpenol and neosolanol were detected in the mycelium filtrates of these fungi. The main source of trichotecene type B, nivalenol, was identified to be *F. culmorum*. It should be noted that we found few *F. graminearum* and *F. culmorum* strains producing deoxynivalenol.

During the long-term investigation, the method of polymerase chain reaction (PCR) was applied for analysis of total DNA. Thus, using specific primers it is possible to determine the species of fungi detected in the foodstuff (Fig. 5). Evaluation of the ratio of PCR product to the initial DNA concentration allows

Table 4. **Primary screening of micromycetes able to synthesize toxic secondary metabolites**

Fungal genera	Fungal species	Number of tested strained	Results after 7 days	
			TKP*	KP**
Acremonium	*A. charticola, A. kiliensis, A. pinkertoniae, A. roseum, A. strictum, A. murorum, A. terricola*	16	4	6
Alternaria	*A. alternata, A. citri, A. cucumerina*	5	3	1
Aspergillus	*A. athecius, A. caesellius, A. candidus, A. duricaulis, A. fisheri, A. flavus, A. fumigatus, A. niger, A. ochraceus, A. penicillioides, A. clavatus, A. terreus, A. restrictus, A. versicolor, A. A. ustus*	34	14	10
Botrytis	*B. allii, B. bifurcata, B. cinerea*	5	2	1
Chrysosporium	*C. merdarium, C. farinicola, C. inops*	4	2	0
Cladosporium	*C. cladosporioides, C. cucumerinum, C. sphaerospermum, C. herbarum, C. tenuissimum*	11	8	1
Eupenicillium	*E. brefeldianum*	5	3	1
Eurotium	*E. herbariorum, E. niveoglaucus*	2	1	1
Fusarium	*F. anthophilum, F. equiseti, F. merismoides, F. monilogorme, F. oxysporum, F. proliferatum, F. redolens, F. solani, F. poae, F. sporotrichioides, F. heterosporum*	17	6	4
Gliocladium	*G. catenulatum, G. deliquescens, G. virens, G. viride*	7	4	1
Myrothecium	*M. roridum, M. verrucaria*	2	1	1
Mucor	*M. circinelloides, M. hiemalis, M. lusitanicus, M. mucedo, M. murorum, M. oryzae, M. racemosus, M. ramannianus, M. silvaticus*	12	5	1
Nectria	*N. haematococca, N. ventricosa*	2	1	1
Paecilomyces	*P. javanicus, P. inflatus*	2	0	0
Perenospora	*P. farinosa, P. parasitica*	2	2	0
Pythium	*P. aphanidermatum, P. ultimum*	3	2	1
Rhizopus	*R. oryzae, R. stolonifer*	10	2	3
Sclerotinia	*S. fuckeliana, S. rolfsii, S. sclerotiorum*	12	4	1
Trichoderma	*T. aureoviride, T. harzianum, T. viride, T. hamatum*	4	1	0
Ulocladium	*U. consortiale, U. botrytis, U. chartarum*	5	1	1
Verticillium	*V. album, V. alboatrum*	5	3	1
Total		166	69	36

Note. * TKP – changed colony pigmentation and pigment released into media. ** KP – changed colony pigmentation.

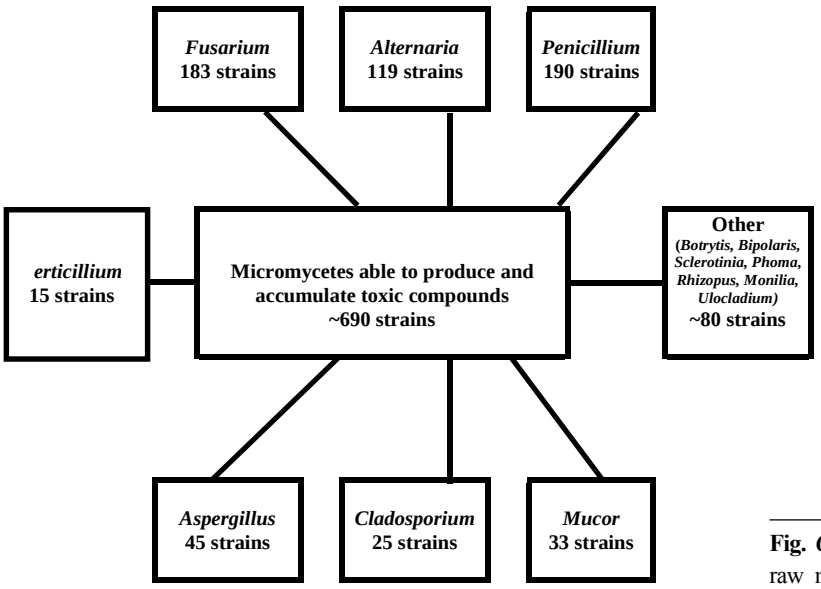

Fig. 6. Mycotoxin-producing fungi isolated from food raw material of plant origin

assessing the fungal contamination level of grains or other raw materials of plant origin.

Basing on the analysis of the obtained data, the diversity of micromycetes – potential toxin producers and abundance of toxic compounds produced by these fungi and released into the environment where they can pose threat to health should be pointed out. During this work, a collection of toxin-producing micromycetes isolated from raw material and products of plant origin was accumulated (Fig. 6).

Table 5. **Primary screening of the genus *Penicillium* micromycetes able to synthesize toxic secondary metabolites**

Species of the genus *Penicillium*	Number of tested species	Results after 7 days		Species of the genus *Penicillium*	Number of tested species TKP*	Results after 7 days	
		TKP*	KP**			KP**	KP**
P. atramentosum	1	1	-	*P. lanosogriseum*	2	1	1
P. aurantioviolaceum	2	2	-	*P. lanosoviride*	5	2	2
P. aurantiacus	2	1	1	*P. lanosum*	1	-	1
P. biforme	6	2	1	*P. lividum*	3	1	1
P. brevicompactum	1	1	-	*P. martensii*	1	1	-
P. canescens	2	1	1	*P. meleagrinum*	1	-	1
P. capsulatum	2	0	1	*P. melinii*	1	1	-
P. chermesinum	2	2	-	*P. miczynskii*	1	-	-
P. chrysogenum	5	3	2	*P. nalgiovense*	1	-	-
P. cyaneofulvum	1	1	-	*P. nigricans*	1	-	1
P. cyclopium	3	3	-	*P. notatum*	3	2	1
P. citreoviride	3	2	1	*P. ochrochloron*	1	-	-
P. citrinum	2	1	1	*P. olivinoviride*	2	1	1
P. claviforme	8	7	0	*P. oxalicum*	7	2	2
P. clavigerum	5	3	2	*P. palitans*	6	2	1
P. commune	1	1	-	*P. paxilli*	6	3	1
P. corylophilum	4	3	1	*P. piceum*	2	2	-
P. corymbiferum	1	-	1	*P. piscarium*	1	1	-
P. daleae	1	1	-	*P. purpurescens*	2	2	-
P. damascenum	1	-	1	*P. purpurogenum*	2	2	-
P. decumbens	2	0	1	*P. putterilli*	1	1	-
P. digitatum	7	2	2	*P. raciborskii*	1	1	-
P. diversum	2	1	0	*P. restrictum*	2	1	0
P. duclauxii	1	1	-	*P. reticulosum*	1	1	-
P. expansum	13	9	2	*P. roqueforti*	1	-	1
P. fellutanum	2	0	0	*P. silvaticum*	1	1	-
P. frequentans	1	1	-	*P. spinulosum*	7	3	2
P. funiculosum	5	2	1	*P. steckii*	3	1	1
P. fuscum	1	-	1	*P. stoloniferum*	4	3	1
P. godlewskii	3	1	2	*P. tardum*	2	0	1
P. granulatum	11	9	2	*P. urticae*	1	1	-
P. herqui	1	1	-	*P. variabile*	4	3	0
P. implicatum	4	1	1	*P. varians*	1	1	-
P. islandicum	3	2	1	*P. velutinum*	1	0	0
p. italicum	12	7	3	*P. verrucosum*	4	2	0
P. janthinellum	1	1	-	*P. verruculosum*	4	3	1
P. lanosocoeruleum	3	2	1	*P. viridicatum*	2	1	1
Total				74	216	121	51

Note. *TKP – changed colony pigmentation and pigment released into media. **KP – changed colony pigmentation.

CONCLUSIONS

1. Data of the present investigation allow to state that raw material of plant origin is heavily contaminated by propagules of various microorganisms, origination and sources of which as well as species diversity depend on the ecological conditions under which plants are grown and harvest is handled, stored, processed, sold and used.

2. Micromyces comprise a large part of microorganisms contaminating raw material of plant origin. Most of these micromycetes are able to produce toxic substances and release them mostly as secondary metabolites into their nutritive substrate. When a fungus producing toxic metabolites grows on food raw material, the latter becomes dangerous to human and animal health.

3. Mycotoxins are produced by micromycete strains belonging to various genera and species. Toxin abundance and composition are determined by genetic peculiarities and the environment where a toxin producer grows. A collection of toxin-producing micromycetes isolated from row material and products of plant origin was accumulated.

Table 6. **Primary screening of fungi, more rarely detected on food raw material, able to synthesize toxic secondary metabolites**

Fungal species	Number of tested species	Results after 7 days		Fungal species	Number of tested species	Results after 7 days	
		TKP*	KP**			KP**	KP**
Annellophorella magdalensis	1	0	0	*Myceliophthora vellerae*	1	-	1
				Oidium oospora	1	1	-
Arthroderma tuberculatum	1	1	-	*Piptocephalis lemmonnieriana*	1	1	-
Ascochyta pruni	1	-	1				
Aureobasidium prunicola	1	0	0	*Pleospora infectoria*	1	1	-
Bdellospora helicoides	1	-	1	*Rhinocladiella spinifera*	1	1	-
Chaetomium aureum	1	1	-	*Rhizoctonia solani*	2	1	1
Cylindrocarpon destructans	1	-	1	*Rhizomucor pusillus*	1	1	-
Cylindrocephalum stellatum	1	1	-	*Sphaerotheca mors-uvae*	1	-	1
Cylindrocladium soparium	1	1	-	*Sporotrichum pruinosum*	1	-	1
Cunninghamella elegans	1	-	1	*Thielaviopsis basicola*	1	1	-
Dipodascus aggregatus	1	-	1	*Tilachlidium pinnatum*	1	1	-
Embellisia allii	1	1	-	*Trichthecium roseum*	1	1	-
Geotrichum fermentans	1	-	1	*Verticillium trifidum*	1	-	1
Hyalodendron lignicola	3	0	2	*Volutella ciliata*	1	0	0
Hormonema prunorum	1	1	-	*Wardomyces dimerus*	1	-	1
Leptodontium boreale	1	1	-				
Total				31	34	16	14

Note. *TKP – changed colony pigmentation and pigment released into media. **KP – changed colony pigmentation.

4. Under conditions of Lithuania, raw material and products of plant origin are mostly contaminated by fungi of the *Penicillium, Aspergillus, Alternaria, Fusarium, Eupenicillium, Eurotium, Paecilomyces, Cladosporium, Myrothecium, Acremonium, Rhizomucor, Mucor, Mortierella, Trichotecium, Verticillium* and *Volutella*, etc. genera, nevertheless, their abundance not always coincides with their capacity of toxin production. Ability to produce toxic compounds is often characteristic of one or some strains of a species. The intensity of synthesis of secondary metabolites is limited by the composition of the nutritional substrate and the most important environmental factors: humidity, temperature, light, substrate pH, and the composition and functional peculiarities of the surrounding biota.

5. Using specific primers, the species of fungi detected in the foodstuff tested were determined. Evaluation of the ratio of PCR product to the initial DNA concentration allows assessing the fungal contamination level of grains or other raw material of plant origin.

6. Contamination of food products by fungal toxins is a significant ecological problem closely related to human health. It is often neglected that every micromycete growing on food raw material synthesizes and releases mostly low amounts of various toxic substances, which get into food and on reaching human or animal organisms affect their functions. The toxic effect and outcomes depend on the length of exposure and accumulation peculiarities. At present the effect of only several mycotoxins is more or less investigated, and attempts are made to control them, while the influence of many other metabolites is considered to be insignificant and no attention is paid to their control. The data of the present investigation contribute to the understanding of this important ecological problem.

Received 22 May 2006
Accepted 5 July 2006

References

1. Anderson B., Smedsgard J., Frisvad J. F. 2004. *Penicillium expansum* consistent production of a patulin, chaetoglobosins and other secondary metabolites in culture and their natural occurrence in fruit products. *J. Agric. Food Chem*. Vol. 52. P. 2421–2428.
2. Andrews S., Pardoel D., Harun A., Treolar T. 1997. Chlorine inactivation of fungal spores on cereal grains. *Int. J. Food Microbiol*. Vol. 35. P. 153–162.
3. Booth C. 1971. *Methods in Microbiology*. London, New York. 795 p.
4. Boutrif E., Bessy C. 2000. Global Significance of Mycotoxins and Phycotoxins. In: *Mycotoxins and Phycotoxins in Perspective at the Turn of the Millennium*. Proceedings of the Xth International JUPAC symposium on Mycotoxins and Phycotoxins, 21-25th May, 2000. Guaruja, P. 3–16.
5. Brandenburg W. 1985. *Parasitische Pilze an Gefeazplazen in Europe*. Stuttgart, New York: Gustav Fisher Verlag. 1248 p.
6. Cole R. J., Jarvis B. B., Schweikert M. A. 2003. *Handbook of Secondary Fungal Metabolites*. Amsterdam, Boston, London, New York, Oxford, Paris, San Francisco, San Diego, Singapore, Sydney, Tokyo: Academic Press. Vol. 3. 672 p.

7. Cole R. J., Schweikert M. A. 2003. *Handbook of Secondary Fungal Metabolites*. Amsterdam, Boston, London, New York, Oxford, Paris, San Diego, San Francisco, Singapore, Sydney, Tokyo: Academic Press. Vol. 1–2. P. 1066–819.
8. Domsch K. H., Gams W., Anderson T. H. 1980. *Compendium of soil fungi*. London: Academic Press. Vol. 1. 857 p.
9. Doohan F. M., Brennan J., Cooke B. M. 2003. Influence of climatic factors on *Fusarium* species pathogenic to cereals. *Eur. J. Plant Pathol.* Vol. 103. P. 755–768.
10. Dutkiewicz J., Krysinka-Traczyk E., Skoska C., Sitkowska J., Srazno Z., Golec M. 2001. Exposure to airborne microorganisms and endotoxin in herb processing plants. *Ann. Agric. Environ. Med.* Vol. 8. P. 201–211.
11. Ellis M. B. 1971. *Dematiaceous Hyphomycetes*. Kew: Commonw. Mycol. Inst. 608 p.
12. Ellis M. B. 1976. *More Dematiaceous Hyphomycetes*. Kew: Commonw. Mycol. Inst. 507 p.
13. Gams W. 1971. *Cephalosporium Artige Shimmelpilze (Hyphomycetes)*. Jena: VEB G. Fisher Verlag. 262 p.
14. Hawksworth D. L., Kirk P. M., Sutton B. C., Pegler D. N. 1995. *Ainsworth and Bisby's Dictionary of the Fungi*. 8th edition. CAB International. Cambridge: The University Press. 616 p.
15. Kačergius A., Lugauskas A., Levinskaitė L., Varnaitė R., Mankevičienė A., Bakutis B., Baliukonienė V., Brūkštienė D. 2005. Screening of micromycetes producing toxic substances under various conditions. *Botanica Lithuanica.* Suppl. 7. P. 65–75.
16. Kačergius A., Mačkinaitė R. 2005. PCR-based detection and identification of fungi producing trichotecenes in food grains. *Botanica Lithuanica.* Vol. 11(2). P. 87–99.
17. Kiffer E., Morelet M. 2000. *The Deuteromycetes. Mitosporic fungi. Classification and generic keys*. New Hampsnire: Science Publishers Inc. 273 p.
18. Klich M. A. 2002. *Identification of common Aspergillus species*. Utrecht: Centraalbureau voor Schimmelcultures.
19. Kozakiewicz Z. 1989. *Aspergillus* species on stored products. *Mycol. Papers*. P. 1–188.
20. Krysinska-Trasczyk E., Petrowski J., Kosteczki M., Dutkiewicz J., Kicamz I. 2003. Filamentous fungi and mycotoxins as potential occupation risk factors among farmers harvesting various crops. *Med. Pr.* Vol. 54(2). P. 133–138.
21. Levinskaitė L., Lugauskas A., Valiuškaitė A. 2005. Potential toxin-producing micromycetes on fruit and berries of horticultural plants treated with fungicides. *Botanica Lithuanica.* Suppl. 7. P. 47–54.
22. Lugauskas A. 2005. Potential toxin producing micromycetes of food raw material and products of plant origin. *Botanica Lithuanica*. Suppl. 7. P. 3–16.
23. Lugauskas A., Krasauskas A. 2005. Micromycetes recorded on grain and products of cereal. *Микология и фитопатология*. N 39(6). P. 68–77.
24. Lugauskas A., Krikštaponis A., Šeškauskas V. 2003. Species of Conditionally Pathogenic Micromycetes in the Air of Dwellings and Occupational Premises. *Indoor Built Environ.* Vol. 12. P. 167–177.
25. Lugauskas A., Krikštaponis A., Šveistytė L. 2004. Airborne fungi in industrial environments – potential agents of respiratory diseases. *Ann. Agric. Environ. Med*. Vol. 11. P. 19–25.
26. Lugauskas A., Paškevičius A., Repečkienė J. 2002. *Patogeniški ir toksiški mikroorganizmai žmogaus aplinkoje*. Vilnius: Aldorija.
27. Mačkinaitė R., Kačergius A. 2005. Micromycetes – potential mycotoxin producers in stored wheat grain. *Botanica Lithuanica.* Vol. 11(2). P. 111–118.
28. Mantle P. G. 2005. Occurrence of renal cancer in male Fischer rats given ochratoxin A via mouldy feed up to years: implications for human health and commercial trade. In: *New Tools for Improving Microbial Food Safety and Quality. Biotechnology and Molecular Biology Abstracts.* The 19th International ICFMH Symposium 12–16th September 2004. Portoroz. P. 375.
29. Miljkovic A., Pfohl-Leszkowicz A., Dobrota M., Mantle P. G. 2003. Comparative responses to made of oral administration and dose of ochratoxin A or necrotic extract of *Penicillium polonicum* in rats. *Exp. Toxicol. Pathol.* Vol. 54. P. 305–312.
30. Mills J. T. 1990. Mycotoxins and toxigenic fungi on cereal grains in Western Canada. *Can. J. Phys. Pharmacol.* Vol. 68. P. 982–986.
31. Mills J. T., Seifert K. A., Frisvad J. C., Abramson D. 1995. Nephrotoxigenic *Penicillium* species occuring on farm – stored cereal grains in Western Canada. *Mycophathologia*, Vol. 130. P. 23–28.
32. Morin S., Magon N., Serra J., Ramos A. J., Canela R., Sonchis V. 1999. Fumanisin B_1 production and growth of *Fusarium moniliforme* and *Fusarium proliferatum* on maize, wheat and barley grain. *J. Food Sci*. Vol. 64(5). P. 921–924.
33. Naut M. J. R. 1989. Effect of *Rhizopus* and *Neurospora spp.* on growth of *Aspergillus flavus* and accumulation of aflatoxin B_1 in groundnut. *Mycol. Res.* Vol. 93(4). P. 518–523.
34. Nelson P. E., Dignani M. C., Anaissie E. J. 1983. Taxonomy biology and clinical aspects of *Fusarium* species. *Clin. Microbiol. Rev*. Vol. 3. P. 479–504.
35. Nicholson P., Simpson D. R., Weston G., Rezahoor H. N., Lees A. K., Parry D. W., Joyce D. 1988. Detection and quantification of *Fusarium culmorum* and *Fusarium graminearum* in cereals using PCR assays. *Physiol. Mol. Plant Pathol.* Vol. 53. P. 17–37.
36. Parry D. W., Nicholson P. 1996. Development of a PCR assay to detect *F. poae* in wheat. *Plant Pathol.* Vol. 45. P. 383–391.
37. Parshikov I. A., Moody J. D., Freeman J. P., Williams A. J., Heinze T. M., Sutherland J. B. 2002. Formation of conjugates from utrofloxacin and morfloxacinin cultures of *Trichoderma viride*. *Mycologia.* Vol. 94. P. 1–5.
38. Pfohl-Leszkowicz A., Petkova-Bocharova T., Chernozemsky I. N., Castegnaro M. 2003. Balkan endemic nephropathy and associated urinary tract tumors: a review on aetiological causes and potential role of mycotoxins. *Food Adit. Contam*. Vol. 19. P. 282–302.
39. Plaats-Niterink van der A .J. 1981. Monograph of the genus *Pythium. Studies in Mycology.* Vol. 21. P. 1–242.

40. Rabie C. J., Lübben A., Marais G. J. 1997. Enumeration of fungi in barley. *Int. J. Food Microbiol*. Vol. 35. P. 117–127.
41. Ramirez C. 1982. *Manual and atlas of the Penicillia*. Amsterdam: Elsevier Biomedical Press.
42. Raper K. B., Fennel D. J., Austwick P. K. C. 1965. *The genus Aspergillus*. Baltimore: The Williams et Wilking Company. 686 p.
43. Raper K. B., Thom C., Fennel D. J. 1949. *A manual of the Penicillia*. Baltimore: The Williams et Wilking Company. 817 p.
44. Repečkienė J., Lugauskas A., Survilienė E., Dambrauskienė E. 2005. Contamination of desiccated herbs by toxin-producing micromycetes. *Botanica Lithuanica*. Vol. 11(2). P. 125–131.
45. Ritieni A. 2003. Patulin in Italian commercial apple products. *J. Agric. Food Chem*. Vol. 51. P. 6086–6090.
46. Samson R. A., Frisvad J. C. 2004. *Penicillium* subgenus; *Penicillium*: new taxonomic schemes, mycotoxins and other metabolites. *Studies in Mycology.* Vol. 49. P. 1–257.
47. Samson R. A., Hoekstra E. S., Frisvad J. C., Filtenborg O. 2000. *Introduction to Food- and Airborne Fungi* (6th edition). Utrecht. 389 p.
48. Samson R. A., Reenen-Hoekstra E. S. van. 1988. *Introduction to food-borne fungi.* 3rd ed. Baarn-Delft: Centraalbureau voor Schimmelcultures. 299 p.
49. Schiling A. G., Möller E. M., Geiger H. H. 1996. Polymerase Chain Reaction-based assays for species-specific detection of *Fusarium culmorum*, *F. graminearum* and *F. avenaceum*. *Phytopathol.* Vol. 36. P. 515–552.
50. Shoug M. A., Eduard W., Stormer F. C. 2001. Ochratoxin A in airborne dust and fungal conidia. *Mycopathologia*. Vol. 151. P. 93–98.
51. Simsekli Y., Gücin F., Asan A. 1999. Isolation and identification of indoor airborne fungal contaminants of food production facilities and warehouses in Burso, Turkey. *Aerobiologia.* Vol. 15. P. 225–231.
52. Stakėnienė J., Lugauskas A. 2004. Presence of toxin producing micromycetes on food of plant origin. *Botanica Lithuanica.* Vol. 10(2). P. 161–167.
53. Stakėnienė J., Lugauskas A., Levinskaitė L. 2001. Presence of potential toxin producing micromycetes on fruit and berries. *Bul. Pol. Acad. Sci. Biol. Sci*. Vol. 49(4). P. 413–420.
54. Šveistytė L., Lugauskas A., Vidgirytė A. 2005. Potential toxin producing micromycetes in the dust of mills and agricultural production storehouses. *Botanica Lithuanica.* Vol. 11(3). P. 179–189.
55. Tauxe R. V. 2002. Emerginging foodborne pathogens. *Int. J. Food Microbiol*. Vol. 78. P. 31–41.
56. Thrane U. 2004. Food safety. Mycotoxins and their producers. In: *New Tools for Improoving Microbiol Food Safety and Quality. Biotechnology and Molecular Biology*. Abstracts. The 19th International ICFMH Symposium, 12–16th September 2004. Portoroz. P. 89.
57. Watanabe T. 2002. *Pictorial Atlas of Soil and Seed Fungi Morphologies of Cultured Fungi and Key to Species.* 2nd edition. London, New York, Washington: CRC Press LLl Borg Raton. 486 p.
58. White T. J., Bruns T., Lee S., Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M. A., Gelfand D. H., Sninsky J. J., White T. J. (eds.). *PCR protocols/A guide to methods and amplifications*. San Diego–New York–Boston–London–Sydney–Tokyo–Toronto. P. 315–322.
59. Кудряшова А. А. 1986. *Микробиологические основы сохранения плодов и овощей*. Москва.
60. Милько А. Л. 1974. *Определитель мукоральных грибов*. Киев: Наукова думка. 203 с.
61. Мирчинк Т. Г. 1988. *Почвенная микология*. Москва: Издательство Московского университета. 220 с.

Albinas Lugauskas, Jūratė Repečkienė, Loreta Levinskaitė, Rimutė Mačkinaitė, Audrius Kačergius, Vita Raudonienė

MIKROMICETAI – TOKSINŲ PRODUCENTAI, APTINKAMI ANT ĮVAIRIOMIS LIETUVOS SĄLYGOMIS IŠAUGINTOS AUGALINĖS MAISTO ŽALIAVOS

Santrauka

2003–2006 m. atliktais tyrimais išaiškinti mikromicetai – potencialūs mikotoksinų producentai, nustatyta jų sistematinė priklausomybė ir geba sintetinti toksinus įvairiomis augimo sąlygomis. Nustatyti mikromicetų, sintetinančių toksinus, kaupimosi daržovėse, vaisiuose, uogose, prieskoniniuose augaluose, grūduose, sėklose bei įvairiuose iš jų pagamintuose produktuose dėsningumai. Aptariami veiksniai, lemiantys mikromicetų galimybes gaminti įvairius toksinus ir juos išskirti į maistui skirtas augalinės kilmės žaliavas. Nustatyti potencialūs toksiškų antrinių metabolitų producentai ruošiant augalinę medžiagą žmonių maistui. Jiems priskirti šitokių rūšių mikromicetai: *Alternaria alternata, A. citri, A. cucumerina, Aspergillus candidus A. clavatus, A. flavus, A. fumigatus, A. niger, A. ochraceus, A. parasiticus, A. sulphureus, A. ustus, A. versicolor, Cylindrocarpon destructans, Drechslera sorokiniana, Fusarium culmorum, F. equiseti, F. graminearum, F. moniliforme, F. oxysporum, F. poae, F. proliferatum, F. solani F. sporotrichioides, Penicillium aurantiogriseum, P. chrysogenum, P. claviforme, P. clavigerum, P. commune, P. corylophilum, P. crustosum, P. expansum, P. funiculosum, P. griseofulvum, P. palitans, P. spinulosum, P. variabile, P. viridicatum.* Patikrinta nuo augalinės maisto žaliavos išskirtų mikromicetų geba sintetinti toksiškus antrinius metabolitus, atlikta pirminė aktyvių producentų atranka. Patikslinta kai kurių mikromicetų producentų padermių sistematinė priklausomybė taikant molekulinius genetinius metodus. Nustatyti toksinai, kuriuos gamina atrinktos mikromicetų padermės. Nurodyta išskirtų mikromicetų įvairovė ir savastis atsižvelgus į substrato specifiškumą, klimato sąlygas, aplinką, ypač dulkėtumą, drėgnį, temperatūrą.

Raktažodžiai: mikromicetai, maisto žaliava, produktai, mikotoksinai, temperatūra, drėgnis, sanitarinė būklė