ORIGINAL ARTICLES AAEM

Ann Agric Environ Med 2005, 12, 253–260

MICROMYCETES, PRODUCERS OF TOXINS, DETECTED ON STORED VEGETABLES

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Lugauskas A, Repečkienė J, Novošinskas H: Micromycetes, producers of toxins, detected on stored vegetables. *Ann Agric Environ Med* 2005, **12**, 253–260.

Abstract: In 2003–2004, investigations of mycological contamination of stored and newly harvested vegetables were carried out. The aim of the study was to detect fungal species able to synthesize toxic metabolites, which are spread on vegetables under various conditions. For mycological investigations, samples of carrots, onions and cabbage were taken from storehouses with different storage periods and conditions. *Penicillium expansum*, *P. nalgiovense*, *Mucor silvaticus* and *Penicillium verrucosum* were more frequently detected on carrots, *Penicillium expansum* - on onions and *Aspergillus niger*, *Botrytis cinerea*, *Mucor hiemalis*, *Penicillium funiculosum* and *Penicillium expansum* - on cabbages. Storing conditions of vegetables influenced distribution of different fungal species. Primary screening using CYA and YES test-media showed that 46.7% of tested strains may be evaluated as toxin producers. The ability of fungi to produce mycotoxins depends on their growth substrata. According to Rf and fluorescence in the UV of compounds comparing with standards, such toxins as patulin, cytochalasin and penitrem were identified. The ability of *Penicillium expansum* Sv-168-1 growing on different foodstuff and especially potato to produce patulin was confirmed quantitatively.

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Key words: fungi, mycotoxins, carrots, onions, cabbage.

INTRODUCTION

In most European countries the demand for organic products is continuously increasing. A safe organic food supply requires the development of detection methods, identifying mycotoxin risks in the production chain, determining critical points of harvesting, storing or processing. Some environmental conditions promote the development of various microorganisms that contaminate vegetables in places of their growth, ripening, harvesting and storage. The propagules of micromycetes infect plant origin material from the soil and air. Ability to adapt to changing environmental conditions enables them to contaminate stored vegetables and to cause their spoilage during storage [3, 13, 24].

Carrots, cabbages and onions are among the most popular vegetables and important components of various organic food products. Microorganisms of some species intensively developing on vegetables make them unsuitable for consumption. These include species of *Alternaria* (carrots, cabbages, cauliflowers, peppers, tomatoes), *Botrytis* (green beans, carrots, onions, peppers), *Colletotrichum* (beans, onions, tomatoes), *Fusarium* (carrots, potatoes). Highly toxigenic fungi such as *Fusarium oxysporum* were isolated from carrots, *F. solani* from cabbages, *Penicillium lanosum* from onions [2, 6].

In storehouses of organic products there is always plenty of dust with various soil fungi propagules, therefore workers in such premises are at risk of work-related respiratory and skin diseases [10, 32]. It was

Received: 18 July 2005
Accepted: 30 September 2005

estimated that the concentrations of total *Fusarium* species in grain dust samples significantly correlated with the concentrations of total fusariotoxins. Especially high levels of mycotoxins are estimated in small farms unequipped with ventilation systems [21]. The same trends of mycotoxin distribution may be evident in storehouse dust of vegetables. Therefore, the workers of vegetable processing facilities could be exposed to micromycetes and their produced mycotoxins pose a risk of respiratory diseases. Various toxic compounds produced by certain micromycete species are especially hazardous. Aiming to evaluate the potential hazard to the health of people and animals caused by mycotoxins, scientists investigated the contamination of food and feed with micromycetes to determine the synthesised mycotoxins [12, 14, 23, 32, 33].

The aim of the research was to investigate the diversity of micromycete species - potential mycotoxin producers - spread on vegetables stored under various conditions during winter, and that of new yield, to select strains synthesizing toxic secondary metabolites.

MATERIALS AND METHODS

Investigated premises. The mycological analyses of vegetables stored during winter in storehouses (2003 year yield) and those of new 2004-year yield were performed. Vegetables were stored in various storehouses belonging to State and private farms (located in Kaunas and Kėdainiai districts, Lithuania). Their temperature, humidity and ventilation conditions varied.

Carrots and cabbages were stored by 2 methods: cold storage and in bulk storage. A crate storage system was used in cold storage. Vegetables were brought from the fields and containerized; the containers were loaded on 5 floors and brought to cold storage where aeration along crates was used. At temperature of 0.1±0.1°C and relative air humidity of 99–99.5% for carrots and 0±0.1°C, 94–95% for cabbages was maintained. This storehouse has been in use for about 5 years.

In another storehouse, carrots were stored in bulk without ventilation or mechanical refrigeration. The pile of carrots was 1.5–1.7 m deep, storage conditions were not regulated; temperature was 6–8°C and relative air humidity - 96–98%.

Cabbages were stored in bulk with a mechanical ventilation system. The pile of cabbages was 1.6–1.8 m high, temperature of blowing air was not regulated; storage temperature was 6–8°C and relative air humidity - 96–98%.

Onions after harvesting were dried in storehouse with outside air to 8–9% of outer peel humidity. They were stored in bulk with a mechanical ventilation system. The size of the pile was 500 t, and was 3.5 m deep. Ventilation was maintained manually, 350 m^3/h per ton. Keeping temperature was 6–8°C and relative air humidity 75–80%.

Some carrots, cabbages and onions were kept in a combined stone storehouse for vegetables. Its period of use was more than 30 years. Vegetables were kept in separate boxes with an autonomic system of microclimate maintenance. Keeping temperature was 0–1°C and relative air humidity was not regulated.

Isolation of fungi. For the mycological investigations, 18 samples of vegetables: 6 of carrots, 5 of onions and 7 of cabbages were taken. A piece of visibly infected vegetable was cut off with a sterile scalpel and placed on malt agar medium with chloramphenicol (50 mg/l). Fungi were cultivated for 7–10 days at a temperature of 28°C. The analysis of each sample was performed in 4 replications [28]. Pure micromycete strains were isolated on standard Czapek, malt and corn extract media and identified according to manuals [8, 15, 18, 25, 29, 30]. Detection frequency (%) of dominant species was calculated.

Evaluation of the micromycete toxicity. Selection of fungi able to synthesize secondary metabolites was tested applying cultivation on standard Czapek agar, Czapek yeast extract agar (CYA) and yeast extract-sucrose agar (YES) media for 7–14 days at 28°C temperature. Strains - potential producers of mycotoxins - showed significant changes in the colour of colonies and abundant excretion of pigment into CYA and YES media [14].

Thin-layer chromatography method was used for the estimation of toxins produced by fungi. Silica gel 60 with fluorescent indicator UV254 (Mackerey-Nagel) was used. Selected fungal strains were grown on malt agar (MA) for 14 days; biomass was collected and extracted with 96° ethanol. CYA and YES media were used additionally for estimation of the influence of substrata on the ability to produce secondary metabolites. 0.05–0.1 ml of extract and standard toxins were analysed chromatographically. The separation of compounds was carried out in the system of solvents toluol-etilacetat-formic acid (5:4:1). Produced mycotoxins were identified according to Rf (distance of compound/distance of solvent) and to fluorescence in the UV, as compared with standards [4, 11].

The amount of patulin produced by fungus *Penicillium expansum* sv-168-1 under different growing conditions was determined using international standard [16].

RESULTS AND DISSCUSION

Contamination of vegetables by fungal propagules depended upon various factors: their growing technologies, chosen sorts and climatic conditions during the vegetation period. Species of micromycetes detected on vegetables of the 2003 and 2004 - year yield and stored by different means are shown in Table 1. It is evident that storing conditions influenced distribution of different micromycte species. For example, a lower number of fungal species was detected on the carrots stored in cold storage, in comparison with vegetables stored in the old stone combined storehouse. It was noticed that the species composition of fungi isolated from vegetables stored in combined storehouse was more diverse and significantly differed from those obtained in cold storage. The

Table 1. Species of micromycetes isolated from vegetables stored under different conditions.

Condition of storage	Year of yield harvesting	Isolated micromycete species
Carrots		
Cold storage	2003	*Mortierella alpina* Peyronel, *Mucor hiemalis* Wehmer, *M. luteus* Linnem., *M. mucedo* Fresen., *M. silvaticus* Hagem, *Penicillium corymbiferum* Westling, *P. expansum* Link, *P. granulatum* Bainier, *P. nalgiovense* Laxa, *P. oxalicum* Currie et Thom, *P. rugulosum* Thom, *P. steckii* K.M. Zalessky, *P. viridicatum* Westling, *Sclerotinia sclerotiorum* (Lib.) de Bary, *Septoria carotae* Nagorny, *Serpula lacrymans* (Wulfen) J. Schrőt, *Sporotrichum olivaceum* (Link et Fr.) Fr., *Trichoderma viride* Pers.
–"–	2004	*Alternaria* sp., *Aspergillus* sp., *Aureobasidium pullulans* G. Arbnaud, *Fusarium oxysporum* Schltdl., *F. sambucinum* Fuckel, *Mortierella alpina* Peyronel, *Mucor hiemalis* Wehmer, *Penicillium clavigerum* Demelius, *P. corymbiferum* Westling, *P. expansum* Link, *P. frequentans* Westling, *P. rugulosum* Thom, *Rhizomucor pusillus* (Lindt) Schipper, *Rhizopus oryzae* Went ex Prins. Geerl., *Sclerotium rolfsii* Sacc., *Septoria carotae* Nagorny, *Verticillium tenerum* (Nees ex Pers.) Link
Bulk storage	2003	*Aspergillus niger* Tiegh., *Mucor silvaticus* Hagem, *Mucor* sp., *Penicillium corymbiferum* Westling, *P. expansum* Link, *P. nalgiovense* Laxa, *P. olivinoviride* Biourge, *P. steckii* K.M. Zalessky, *P. verrucosum* Dierckx
Combined storehouse	2004	*Alternaria dauci* (J.G. Kűhn) J.W. Groves et S. Hughes, *A. radicina* Meier, Drechsler et E.D. Eddy, *Fusarium moniliforme* J. Sheld, *F. solani* (Mart.) Appel et Wollenw., *Mucor murorum* Naumov, *Penicillium corymbiferum* Westling, *P. granulatum* Bainier, *P. lanosoviride* Thom, *Pythium sylvaticum* W. A. Campb. et F. F. Hendrix, *Rhizoctonia solani* J.G. Kűhn, *Trichoderma viride* Pers.
Onions		
Bulk storage	2003	*Aspergillus restrictus* G. Sm., *Galactomyces geotrichum* E.E. Butler et L.J. Petersen, *Mucor albo-ater* Naumov, *Paecilomyces niveus* Stolk et Samson, *Penicillium jensenii* K.M. Zalessky, *P. fellutanum* Biourge
–"–	2004	*Aspergillus niger* Tiegh., *Choanephora cucurbitarum* (Berk. et Ravenel) Taxt, *Cladosporium cladosporioides* (Fresen.) G.A. de Vries, *Mucor hiemalis* Wehmer, *Penicillium aurantioviolaceum* Biourge, *P. expansum* Link, *P. spinulosum* Thom, *P. tardum* Thom
Combined storehouse	2004	*Aureobasidium pullulans* G. Arnaud, *Mucor murorum* Naumov, *M. piriformis* A. Fisch., *M. racemosus* Fresen., *Penicillium claviforme* Bainier, *P. corymbiferum* Westling, *P. digitatum* Sacc., *P. expansum* Link, *P. funiculosum* Thom, *P. verruculosum* Peyronel, *Trichoderma viride* Pers.
Cabbages		
Cold storage	2003	*Alternaria brassicae* (Berk.) Sacc.), *Aspergillus niger* Tiegh., *Botryotinia porii* (v. Beyma) Whetzel, *Botrytis cinerea* Pers. et Fr., *Cladosporium herbarum* (Pers.) Link ex Gray, *Fusarium avenaceum* (Fr.) Sacc., *Mucor hiemalis* Wehmer, *Penicillium expansum* Link, *P. funiculosum* Thom, *P. nalgiovense* Laxa, *P. olivinoviride* Biourge, *P. paxilli* Bainier, *P. verrucosum* Dierckx, *Penicillium* sp., *Sporotrichum aurantiacum* (Bull. ex Fr.) Fr.
–"–	2004	*Botrytis cinerea* Pers. et Fr., *Penicillium claviforme* Bainier, *P. corymbiferum* Westling, *P. cyclopium* Westling, *P. decumbens* Thom, *P. expansum* Link, *P. granulatum* Bainier, *P. martensii* Biourge, *P. oxalicum* Currie et Thom, *P. roquefortii* Thom, *P. variabile* Sopp, *Piptocephalis arrhiza* v. Tiegh. et le Monnier, *Scopulariopsis brevicaulis* (Sacc.) Bainier, *Trichoderma viride* Pers.
Bulk storage	2003	*Absidia spinosa* Lendn., *Aspergillus niger* Tiegh., *Botrytis cinerea* Pers. et Fr., *Mucor hiemalis* Wehmer, *M. luteus* Linnem., *M. mucedo* Fresen., *M. racemosus* Fresen., *Oidiodendron rhodogenum* Robak, *Penicillium expansum* Link, *P. olivinoviride* Biourge, *P. sublateritium* Biourge, *P. verrucosum* Dierckx, *Rhizopus oryzae* Went ex Prins. Geerl., *Sporotricum aurantiacum* (Bull. ex Fr.) Fr.
Combined storehouse	2004	*Mucor hiemalis* Wehmer, *Penicillium corymbiferum* Westling, *P. expansum* Link, *P. funiculosum* Thom, *Penicillium* sp., *Trichoderma viride* Pers.

comparatively small number of species isolated from cabbage stored in combined storehouse may be explained by low contamination of these vegetables in the fields.

The carrots of the 2003-year yield stored in the farmers storehouses during winter were contaminated with fungi of 22 species. The detection frequency of *Penicillium expansum* and *P. nalgiovense* was 100%, of *Mucor silvaticus* and *Penicillium verrucosum* - 66.7%. *Septoria carotae* and *Serpula lacrymans* fungi were found exceptionally on carrots. *Serpula lacrymans* was distributed in a cold storehouse with rather good storing conditions; however, wooden boxes were badly damaged by these fungi (Fig. 1). *Serpula lacrymans* has been ascertained as able to produce such mycotoxins as variegatorubin, xerocomic and atromentic acids; furthermore, the action of the spores of this micromycete increased sensibility of organism [26, 34]. The latter example apparently demonstrates that containers produced from non-quality and properly prepared wood cannot be used.

Figure 1. *Serpula lacrymans* on carrots stored in wood boxes in cold storage.

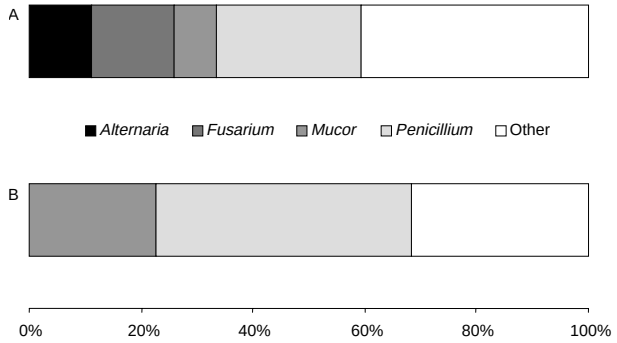

Figure 2. Fungal genera composition on stored (A) and harvested (B) carrots.

The carrots of the 2004-year yield were examined mycologicaly after harvesting and transporting to the storehouses. The fungi of 27 species were detected, but detection frequency only of *Penicillium corymbiferum* reached 66.7%. It was noticed that the fungi of the genera *Alternaria* Nees and *Fusarium* Link were not found on the carrots of the 2003-year yield, while on the samples of the new yield the species of the mentioned genera reached 18.5 and 14.8% of all isolated species. The reason for this phenomenon may be the different climatic conditions during the carrot vegetation in the mentioned years. The *Alternaria* genus fungi are known as producers of such harmful mycotoxins as radicinin, altertoxin, alternariol and tenuazonic acid [26, 34].

It was noticed that the species composition of fungi detected on new harvest vegetables is more diverse in comparison with stored vegetables, because a great number of fungal propagules passed on them with soil (Fig. 2). Vegetables are contaminated with fungi in fields and a part of them continues to grow during storage. A wide distribution of the *Penicillium* Link genus fungi in storehouses may pose a hazard to human health [20].

In total, 38 fungal species were isolated from the examined carrot samples. The majority belonged to the *Penicillium* (13), *Mucor* Fresen. (5) and *Fusarium* (4) genera. Some species (*Mucor hiemalis*, *Penicillium expansum*, *P. granulatum*, *P. rugulosum*, *Septoria carotae*, *Trichoderma viride*), were isolated from both 2003 and 2004 years yield (Tab. 2). The majority are known as toxin producers.

The number of detected species on the onions stored during winter was not great - 6 fungal species were isolated. *Galactomyces geotrichum* and *Mucor* alboater were found more frequently.

Eighteen fungal species were isolated from the newly harvested onions, 4 (22.2%) of them belonged to the genus *Mucor*, 9 (50%) - to the genus *Penicillium*. *Penicillium expansum*, which is known as potential producer of mycotoxins (citrinin, patulin, etc.) [27], was found on all of the examined onion samples.

Cabbages, differently from carrots and onions, are leaf vegetables, but the fungal species composition revealed on them was similar to root vegetables carrots. 23 species of fungi were isolated from the samples of cabbage stored during winter. The differences between the amount of isolated species and the storage conditions were not significant: 6–9 species were found on cabbages from various storehouses. The majority were ascribed to the genera *Mucor* and *Penicillium* (17.4% and 39.1%, correspondingly).

On the new harvested cabbages, 18 species of fungi were detected. The part of species from the genus *Mucor*

Table 2. Fungal species detected on stored and harvested carrots and their ability to produce toxins.

Fungi	Detection frequency, %	Produced main toxins (according to [5, 34])
Mortierella alpina Peyronel	33.3	–
Mucor hiemalis Wehmer	33.3	ergoline alcaloids
Mucor silvaticus Hagem	33.3	–
Penicillium corymbiferum Westling	66.7	roquefortin, patulin
P. expansum Link	66.7	citrinin, patulin, roquefortin
P. granulatum Bainier	33.3	patulin
P. nalgiovense Laxa	50.0	cyclopiazonic acid
P. rugulosum Thom	33.3	rugulosin
P. steckii K. M. Zalessky	33.3	citromycotin
Serpula lacrymans (Wulfen) J. Schrőt	33.3	variegatorubin, xerocomic, atromentic acids
Trichoderma viride Pers.	33.3	trichodermin, T-2 toxin, viridin, viridiol

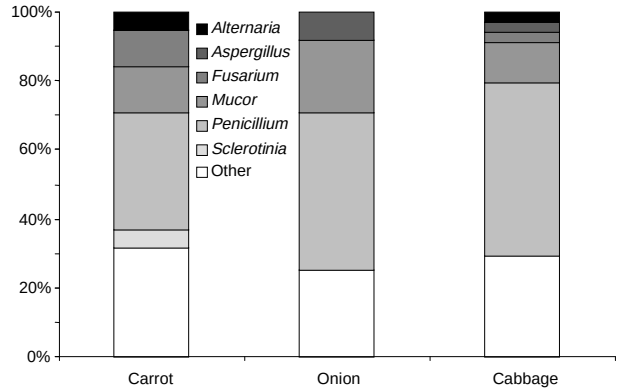

Figure 3. Distribution of fungal species belonging to different genera on various vegetables.

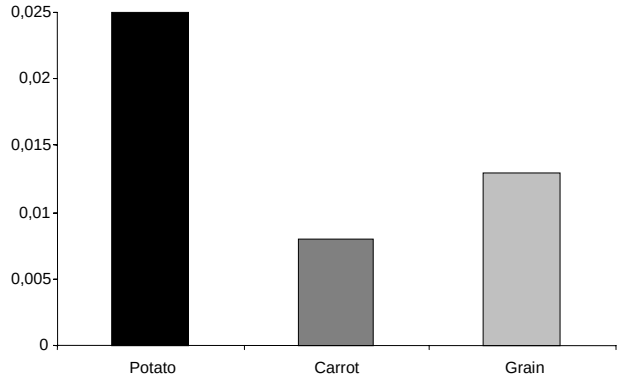

Figure 4. Amount of patulin produced by *Penicillium expansum* Sv-168-1 growing on different foodstuff.

was 16.7%, *Penicillium* - 50%. It was noticed that the complex of dominating species on all examined vegetables was more abundant on stored vegetables, compared to the new harvest (Tab. 3). *Aspergillus niger*, *Botrytis cinerea*, *Penicillium corylophilum*, *P. expansum*, *P. funiculosum*, *P. olivinoviride*, *P. verrucosum*, *Trichoderma viride* are able to synthesize various toxic secondary metabolites [34].

Totally, 34 species of fungi were isolated from cabbages and identified. The genera composition did not vary significantly: 50% of detected species were ascribed to the genus *Penicillium*, 11,8% - to the genus *Mucor*, and 13 species (38.2%) belonged to other genera. Detection frequency of *Aspergillus niger*, *Botrytis cinerea*, *Mucor hiemalis*, *Penicillium funiculosum* was 42.9% and of *Penicillium expansum* - 71.4%. Results of this research correspond to the results of previous investigations, carried out in various preservation and selling premises [24].

Seventy-two species of fungi were isolated from the tested vegetable samples. The majority of isolated species were found on carrots - 52.8%, on cabbages - 47.2%, on onions - 33.3%. Fungal species belonging to the genus *Penicillium* prevailed - 34.2–50% on different vegetables (Fig. 3). Two *Sclerotinia* Fuckel species were found only on carrots. Fungi from the genera *Alternaria* and *Fusarium* were not incident to onions. Thus, the majority of isolated species are known as potential producers of toxic secondary metabolites [5, 34].

Primary screening of toxin producing micromycete strains was carried out. For the investigation, 30 strains were selected according to their detection frequency or knowledge about their ability to produce toxins. The majority of tested strains did not show colour changes or these changes were not significant (33.3 and 20%, correspondingly). Some pigmentation changes were observed in 14 (46.7%) tested strains and 7 (23.3%) of them may be evaluated as toxin producers: *Fusarium* sp. 1-pk, *Alternaria brassicae* (Berk.) Sacc. 2-pk, *Chrysosporium merdarium* (Link ex Grev.) J. W. Carmich. 1-Šal, *Fusarium solani* (Mart.) Appel et Wollenw. F-08, *Alternaria alternata* (Fr.) Keissl. B-230-1, *Penicillium* sp. Sv-161-1, *Penicillium expansum* Sv-168-1. It is assumed that a fungus with a significantly changed colony colour and excreting pigments on CYA and YES media in comparison with Czapek could be a potential producer of mycotoxin [1, 14].

The most strains able to produce various mycotoxins were isolated from onions (80%) (Tab. 4). Cabbage was not significantly contaminated by harmful fungi (33% of treated strains changed pigmentation). The greatest part of mycotoxin producers was detected among strains belonging to the genera *Alternaria* (66.7%) and *Fusarium* (75%).

Table 3. Fungal species frequently detected on storaged and new yield cabbage.

2003 year yield		2004 year yield	
Species of fungi	Detection frequency, %	Species of fungi	Detection frequency, %
Aspergillus niger[a] Tiegh	50	***Penicillium corymbiferum*** Westling	66.7
***Botrytis cinerea* Pers. et Fr.**	50	***P. expansum*** Link	100
Mucor hiemalis Wehmer	50	***Trichoderma viride*** Pers.	66.7
Mucor mucedo Fresen.	50		
Penicillium expansum Link	50		
P. funiculosum Thom	50		
P. olivinoviride Biourge	50		
P. verrucosum Dierckx	50		
Sporotrichum aurantiacum (Bull. ex Fr.) Fr.	50		

[a] potential mycotoxin producers

Table 4. Distribution of fungi synthesizing secondary metabolites in different genera and on various vegetables.

Genera of fungi	Number of treated strains	Potential mycotoxin producers, %	Vegetables	Number of treated strains	Potential mycotoxin producers, %
Alternaria	3	66.7	Potato	9	66.7
Cladosporium	2	0	Carrot	6	66.7
Fusarium	4	75	Onion	5	80
Mucor	2	50	Cabbage	3	33.3
Penicillium	8	50	Herbs	3	66.7
Other	11	36.4	Other	4	50

Table 5. Secondary metabolites excreted by fungi growing on media of different chemical composition.

Fungi	Number of produced metabolites on different media			Identified toxins
	AM	CYA	YES	
Penicillium sp. Sv-161-1	1	2	3	Not identified
Penicillium expansum Link Sv-168-1	6	3	7	Patulin, cytochalasin, penitrem, tenuazonic acid ?[a]
Fusarium solani (Mart.) Appel et Wollenw. F-08	7	3	4	Patulin ?[a], cytochalasin ?[a]

[a] –? – Small amount of compounds

Table 6. Secondary metabolites produced by fungi grown on malt agar.

Fungal strains	Isolation source	Number of produced metabolites	Identified toxins
Alternaria alternata (Fr.) Keissl. B-230-1	Potato	9	Patulin, cytochalasin, penitrem
Cladosporium chlorocephalum (Fresen.) E.W. Mason et M.B. Ellis 3-pk	Cabbage	1	Sterigmatocystin ?[a]
Ulocladium oudemansii E.G. Simmons B-230-2	Potato	4	Tenuazonic acid, sterigmatocystin
Alternaria pluriseptata (P. Karst et Har.) Jørst. B-230-3	Potato	5	Tenuazonic acid, cytochalasin
Scytalidium lignicola Pesante G-453	Lovage	1	Sterigmatocystin ?[a]
Chrysosporium merdarium (Link ex Grev.) J.W. Carmich. 1-Šal	Sage	2	Tenuazonic acid, patulin ?[a]

[a] –? – Small amount of compounds

Thin-layer chromatography allows qualitative estimation of toxin production and according to Rf comparing with standards and to scintillation in UV the identification of some of them. For example, tenuazonic acid coloured in ferruginous under action of 2% $FeCl_3$ in ethanole. The ability of fungi to produce mycotoxins depends on their growth substrata. The obtained data show that fungi growing on different media produced various metabolites (Tab. 5). Tested *Penicillium* sp. Sv-161-1 and *Penicillium expansum* Sv-168-1 produced the greatest amount of metabolites growing on YES media, but *Fusarium solani* F-08 - on Czapek. Differences of strains toxicity depending on cultures preservation techniques, culture media or sugars used have also been emphasized by other researches [7, 17, 31]. According to Rf and fluorescence in the UV, such toxins as patulin, cytochalasin and penitrem were identified. Very small amounts of some compounds did not allow their exact identification. The remainding compounds were not identified due to the absence of standard toxins.

Dematiaceous fungi are widely spread on vegetables, potatoes and herbs. Analysis by thin-layer chromatography of 5 strains from the genera *Alternaria*, *Cladosporium* Link, *Scytalidium* Pesante and *Ulocladium* Preuss showed that the greatest number of secondary metabolites was synthesized by strain *Alternaria alternata* B-230-1 (Tab. 6). Tenuazonic acid and cytochalasin were identified as secondary metabolites produced by examined *Alternaria* strains. The strains from the genera *Cladosporium* and *Scytalidium* were not determined as toxin producers. Sterigmatocystin was the only toxin preliminary identified from *Scytalidium lignicola* G-453 strain. Tenuazonic acid was detected among *Chrysosporium merdarium* 1-Šal metabolites. Fungi of this species, characterized by yellow pigmentation, were frequently detected on desiccated herbs, they therefore pose a risk to human health.

Toxigenic strains of *Penicillium expansum* and patulin are frequently found on various fruits and vegetables [22, 27]. The ability of *Penicillium expansum* Sv-168-1 to produce patulin was confirmed, quantitatively growing

the fungi on different foodstuffs (Fig. 4). The greatest amount of patulin was estimated when the treated strain was grown on potato - 0.025 mg kg^{-1}. The results show that fungi functioning on various food products are able to contaminate them with different amounts of one or another toxic compounds. Researches by other authors confirmed that strains of *Penicillium* species synthesized sets of secondary metabolites different from those known for these species from the literature [19].

Various chemical compounds were used for limitation of spreading of micromycetes in storehouses. The walls of the storage, wood boards of containers were treated with such desinfection agents as Anolit, Menno Florates, TH4+, CIPS, and steam. It was estimated that on different substrata the used chemicals affect micromycetes unequally. Thus, TH4+ significantly decreased the amount of micromycete on concrete walls, Menno Florates - on wood-wool and wood boards. The used chemical compounds also affect different fungal species. It is important to emphasize that elimination of some species of micromycetes capacitated the development of others, which is why investigations in this field must continued. Nowadays, there are suggestions to integrate biocontrol agents with physical and chemical treatments [9].

The obtained data concerning factors inducing the distribution of mycotoxin producing micromycetes on popular vegetables are important for reducing the possibilities of toxin producers to develop on food raw materials, from the growing stage to the end of their processing.

CONCLUSIONS

The species composition of fungi detected on newly harvest vegetables is more diverse in comparison with stored vegetables because a great number of fungal propagules passed on them with soil. During long-term storing a complex of dominated species formed and toxigenic species composed a significant part among them.

Different micromycete species composition was estimated on the same sort of vegetables depending on their storing conditions. Modern storage with automatic maintenance of suitable temperature and air humidity allows decreasing contamination of vegetables with micromycetes.

In total, 72 fungal species from tested vegetables were isolated and identified. *Penicillium expansum*, *P. nalgiovense*, *Mucor silvaticus* and *Penicillium verrucosum* were more frequently detected on carrots, *Penicillium expansum* - on onions, *Aspergillus niger*, *Botrytis cinerea*, *Mucor hiemalis*, *Penicillium funiculosum* and *Penicillium expansum* - on cabbages.

The data of primary screening showed that 7 tested strains might be evaluated as toxin producers. The largest amount of harmful fungal strains were isolated from onions (80%). The obtained data of thin-layer chromatography showed that fungi growing on different media produced various metabolites. According to Rf and fluorescence in the UV, such toxins as patulin, cytochalasin, penitrem, tenuazonic acid and sterigmatocystin were identified. The greatest number of secondary metabolites was synthesized by strains *Alternaria alternata* B-230-1, *Penicillium expansum* Sv-168-1 and *Fusarium solani* F-08.

The ability of fungi to produce toxic secondary metabolites depends on growing substrata. The highest amount of patulin synthesized by *Penicillium expansum* Sv-168-1 was estimated when the treated strain was grown on potatoes.

The data of investigations are of great importance for proposing preventive measures to producers, enabling the limitation of the spread and functional abilities of harmful fungi and the observation of the quality of products, from the fields where they grow to the processed products.

Acknowledgements

This research was financially supported by the Lithuanian State Science and Studies Foundation.

Grateful thanks to dr. habil. Regina Varnaitė for assistance with thin-layer chromatography and mycotoxin identification.

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