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PENICILLIUM STRAINS ISOLATED FROM GRAPES GROWN IN THE CENTRAL SLOVAK WINE REGION

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ABSTRACT

A total of 10 wine producing grapes were collected from the Central Slovak region in 2011 and 2012, which involved 7 vineyards. Mycological analysis was carried out for the detection of fungi using standard media with focus on genera *Penicillium*. The exogenous mycobiota was determined by the method of direct placing of berry samples on agar plates and the endogenous mycobiota by the same method but the berry samples were first superficially sterilized. The resulting Penicillia were identified morphologically. The potentially toxigenic isolates were analysed for mycotoxin production by TLC method. In general representatives of *Penicillium* genus were isolated with higher frequency from the surface of the berries than from their interior. In regard to species *Penicillium chrysogenum* seems to be the most wide-spread in wine grape berries from the Central Slovak wine growing area because was detected more frequently than other species. In total, 546 fungal isolates belonging to 12 *Penicillium* species. Overall, two species were dominant: *P. chrysogenum* and *P. expansum*. Four potentially toxigenic species isolated from endogenous mycobiota were tested for their toxigenic ability. Out of 10 strains, all of them produced at least one mycotoxin. Of all 16 potentially toxigenic strains from exogenous mycobiota, 88% produced at least one mycotoxin. Therefore study concluded, that wine producing grapes seems to be risk products.

Keywords: *Penicillium*, wine grapes, TLC method, mycotoxins

INTRODUCTION

Molds are ubiquitous with various genera commonly found on grapes. Common examples include *Aspergillus, Botrytis* and *Penicillium*, and, to a lesser extent, *Phytophthora*, *Moniliella*, *Alternaria* and *Cladosporium* (**Rosa *et al.*, 2002**). Mold growth plays an important role in the physical and chemical stability as well as the sensory properties of the future wine. For example, uncontrolled proliferation of mold on grapes just prior to harvest rapidly leads to growth of secondary contaminants (yeasts and bacteria), which, in turn, leads to a deteriorative state called "rot". Recognizing the importance of mold growth to wine quality, grape contracts generally include specification for the extent of infections. Although not tolerant of ethanol, molds are ubiquitous in wineries and are present on surfaces as well as in the air (**Donnelly, 1977**). Molds are capable of growth on the outer and inner surfaces of wooden storage containers and on cork in bottled wines where seepage has occurred. Aside from esthetic problems of growth on these surfaces, molds produce sensorially powerful metabolites that are perceivable at parts-per-billion or parts-per trillion concentrations. As such, these compounds can play a significant role in wine quality (**Fugelsang and Edwards, 2007**).

Fungi play a substantial role in spoilage of fruits and vegetables, because of their pathogenicity to the harvested products. Fungal spoilage of fruits causes substantial financial losses to fruit growers and processors and may pose a health threat to the consumer if the contaminating fungi produce mycotoxins. During the various stages of pathogenesis, however, some of these fungi may generate different mycotoxins – secondary metabolites that are toxic to humans and animals that consume the products. The major mycotoxigenic fungi that attack harvested fruits and vegetables are *Aspergillus*, *Penicillium* and *Alternaria* species – and the mycotoxins produced by them in the host tissues, e.g., aflatoxins, ochratoxin A, patulin and alternaria toxins. Some of these mycotoxins are known to be carcinogenic, and most of them are highly stable during processing and, therefore, can reach the consumer. Thus, although consumers will likely reject a visibly moldy or rotten fruit, processed fruit products may still form a significant source of these mycotoxins, if decayed or moldy fruit is not removed before processing or packaging and pose a serious threat to human and animal health throughout the world (**Barkai-Golan and Paster, 2008**).

Some fungi are highly specialized pathogens, attacking only certain types of fruits while others have a more general ability to invade fruit tissue (**Drusch and Ragab, 2003**). The presence of toxin-producing fungi on fruit does not necessarily imply that mycotoxins will be present since toxin production is influenced by many factors including environmental conditions, type, cultivar and nutritional status of the fruit, the microbial load on the fruit, and strain of the fungus (**Drusch and Ragab, 2003; Sanchis and Magan, 2004**).

Penicillium species are frequently associated with spoilage of foods and feeds, and are therefore of great economic importance. The majority of *Penicillium* species can easily be isolated from the soil, where they take part in the decomposition of organic substances, but they are also among the most common airborne fungi, capable of inducing allergic reactions in sensitive patients. Being common airborne fungi, *Penicillium* spp. can easily be isolated from surfaces of healthy plants, including fruits and vegetables. Several *Penicillium* species are among the most common agents of postharvest diseases and they attack a wide range of fruits and vegetables (**Barkai-Golan, 2001**). *Penicillium* is a diverse genus, with more than 200 recognized species. *Penicillium* species are common pathogens of stored grapes. These include *P. aurantiogriseum, P. brevicompactum, P. chrysogenum, P. citrinum, P. crustosum, P. cyclopium, P. decumbens, P. frequentans, P. glabrum, P. stoloniferum, P. viridicatum*, among others (**Snowdon, 1990; Benkhemmar *et al.*, 1993**). *Penicillium expansum* is an example of the destructive postharvest pathogens that cause a large part of the economic losses that occur during storage and shipment (**Barkai-Golan, 2001**). However, in addition to losses they cause in many fruits and vegetables, various *Penicillium* species that are involved in postharvest decay of fruits and vegetables may produce a variety of mycotoxins – secondary metabolites, toxic to animals and humans who consume contaminated foods – during their life cycle in the host fruits (**Andersen and Frisvad, 2004**). It is important to know that the fungus can grow at 0 °C and that decay can progress, albeit slowly, at this temperature, and can be spread from diseased to sound fruits, by contact between them, during months of cold storage. When the fruits are transferred to warmer shelf-life conditions, rapid development takes place.

The major *Penicillium* mycotoxin associated with fruit and vegetable decay, and the most studied one, is patulin (**Andersen and Frisvad, 2004**). Patulin is a mycotoxin characteristic of infected fruits. Although *Penicillium expansum* is the main pathogen responsible for patulin production in apples, fruit and vegetable decay may produce a variety of mycotoxins during their life cycle in the host. These include ochratoxin A, citrinin, penicillic acid, citreoviridin, penitrem,

roquefortine C and others, which may elicit toxicological effects in humans and animals.

Factors known to affect production of these mycotoxins in fruit include the fruit type and cultivar, the physical and chemical properties of the fruit, geographical location where the fruit is grown and harvested, climate, pre-harvest treatments, method of harvest, the ripening state of the fruit, the microbial load, presence of surface defects on the fruit, post-harvest treatments and storage conditions. Temperature and relative humidity are environmental factors that influence fungal growth and mycotoxins production. Mycotoxin accumulation in fruits can occur in the field, during harvest, post-harvest and during storage. The best approach to prevent contamination is to prevent mold growth at all stages of production. Gentle and sanitary handling of the fruit during harvest and in storage and processing facilities is essential for reducing fungal decay and mycotoxin production in fruits (**Jackson and Al-Taher, 2008**).

The objective of our study was to monitor the occurrence of mycobiota in wine grape samples collected from the Central Slovak region, in this manuscript with focus on genera *Penicillium*. Special emphasis was laid on the ability of some potentially toxigenic penicillia to produce some selected mycotoxins.

MATERIAL AND METHODS

Study area

Slovak republic has 6 distinct wine-growing zones (the Small Carpathians, the Southern Slovak, the Nitra, the Central Slovak, the Eastern Slovak and the Tokaj wine regions). They spread from the west to the east of the country along its southern and south-western borders. We had samples from the Central Slovak wine region, which is divided to 7 subregions. The subregion is the area with the same soil and climate conditions. The time between sample collection and laboratory analysis was less than 24 hours. Ten samples – 5 of white grape varieties and 5 of blue grape varieties were mycologically analyzed.

Table 1 Wine grape varieties used in the study from the Central Slovak region

Village	Subregion	Grape variety	Date of harvest	Date of analyses
1. Hontianske Moravce	Hontiansky	Konkordia	26.09.2011	27.09.2011
2. Veľký Krtíš	Modrokamenský	Pinot gris	16.10.2011	17.10.2011
3. Veľký Krtíš	Modrokamenský	Pinot noar	16.10.2011	17.10.2011
4. Veľký Krtíš	Modrokamenský	Sauvignon	16.10.2011	17.10.2011
5. Vinica	Vinický	Blue frankish	30.09.2012	01.10.2012
6. Šahy	Ipeľský	Perle of Zala	30.09.2012	01.10.2012
7. Sebechleby	Hontiansky	Svätovavrinecké	30.09.2012	01.10.2012
8. Čamovce	Fiľakovský	Pálava	17.10.2012	17.10.2012
9. Rimavská Sobota	Gemerský	Blue frankish	17.10.2012	17.10.2012
10. Kráľ	Tornaľský	Müller Thurgau	17.10.2012	17.10.2012

Mycological analysis of grapes

Four wine grape varieties were collected from the end of September to the middle of October in the harvest time 2011 and six wine grape varieties from the end of September to the middle of October 2012 (Table 1). The berries from the vineyards sampled were generally in good condition without visible damage. A total of 50 berries (6 - 7 healthy berries per bunch) from each sample were plated on Dichloran Rose Bengal Chloramphenicol agar medium (DRBC) (MERCK, Germany), and incubated at 25 °C in the dark for one week. In this way was determined an exogenous mycobiota. Fifty another grapes were surface-disinfected in 1% NaClO for 1 min according methods of **Magnoli *et al.* (2003)** and 3 times rinsed by submersion in sterile distilled water (total amount 1L), dried, plated in the same medium and incubated at 25°C in the dark for 7 days. In this way was determined an endogenous mycobiota.

Penicillium strains were isolated and cultivated in MEA (Malt extract agar, **Samson *et al.*, 2010**) and CYA (Czapek yeast agar, **Samson *et al.*, 2010**), Creatine-Sucrose agar (CREA, **Samson *et al.*, 2010**) and Yeast Extract agar (YES, **Samson *et al.*, 2010**). Genus *Penicillium* was identified to species level based on morphological characters according to the manuals of **Pitt and Hocking (1997), Samson and Frisvad (2004)** and **Samson *et al.* (2002a, 2010).**

Results evaluation

The obtained results were evaluated and expressed according to isolation frequency (Fr) and relative density (RD). The isolation frequency (%) is defined as the percentage of samples within which the species or genus occurred at least once. The relative density (%) is defined as the percentage of isolates of the species or genus, occurring in the analyzed sample (**Guatam *et al.*, 2009**). These values were calculated according to **González *et al.* (1996)** as follows:

Fr (%) = (ns / N) x 100 RD (%) = (ni / Ni) x 100

ns – number of samples with a species or genus; N – total number of samples; ni – number of isolates of a species or genus; Ni – total number of isolated fungi.

Toxinogenity analysis

Toxinogenity of selected isolates was screened in *in vitro* conditions by means of thin layer chromatography (TLC) according to **Samson *et al.* (2002b)**, modified by **Labuda and Tančinová (2006).** Extracellular metabolites – citrinin, patulin and griseofulvin were carried out on YES agar and intracellural roquefortin C, penitrem A and cyclopiazonic acid on CYA agar. A few pieces of mycelium with approximate size 5 x 5 mm were cut from colonies and placed in an Eppendorf tube with 500 µL of chloroform: methanol – 2:1 (Reachem, Slovak Republic). The content of the tubes was stirred for 5 min. by Vortex Genie ® 2 (MO BIO Laboratories, Inc. – Carlsbad, CA, USA). The volume 30 µL of liquid phase of extracts along with 10 µL standards (Sigma, Germany) was applied on TLC plate (Alugram ® SIL G, Macherey – Nagel, Germany). The plate was put into TEF solvent (toluene: ethyl acetate: formic acid – 5:4:1, toluene – Mikrochem, Slovak Republic; ethyl acetate and formic acid – Slavus, Slovak Republic). After elution the plate was air-dried. Identification of the metabolites was done by comparison with metabolite standards. Roquefortin C was visible after spraying with $Ce(SO_4)_2$ x 4 H_2O as an orange spot. Cyclopiazonic acid was visible directly in daylight after spraying with the Ehrlich reagent as a violet-tailed spot. Penitrem A after spraying with 20% $AlCl_3$ in 60% ethanol and heating at 130 °C for 8 min as a dark blue spot. Patulin by spraying with 0,5% methylbenzothiazolone hydrochloride (MBTH), (Merck, Germany) in methanol and heating at 130 °C for 8 min and then detectable as a yellow-orange spot. Directly under UV light with a wavelength of 365 nm was visualized citrinin as a yellow-green-tailed spot and griseofulvin as a blue spot.

RESULTS AND DISCUSSION

Fungi of the genus *Penicillium* were found from 10 in 8 wine grape samples, except Pinos gris and Pinos noar from Modrokamenský subregion (2, 3) (Table 2). Nine *Penicillium* species were found, but there occurrence was very sporadically, except 2 subregions – Gemerský (9) and Tornaľský (10). The samples were contaminated by potentially toxicological species *P. expansum* and *P. chrysogenum*. According **Felšöciová *et al.* (2013)** some similar species but in lower number of abundance were isolated from the Nitra wine growing region in 10 analysed samples, namely *P. citrinum, P. corylophilum, P. crustosum, P. decumbens, P. expansum, P. chrysogenum* and *Penicillium* sp.. The occurrence of most was also very sporadically, except *P. crustosum, P. chrysogenum* and *P. expansum*. *Penicillium expansum* spoils grapes, apples, pears, tomatoes, avocados and mangoes. *Penicillium expansum*, the cause of the destructive blue mold rot of pome and stone fruits, is a major concern for human health, because of the production of patulin during pathogenesis. The factors affecting *P. expansum* growth and patulin formation include fungal strain, host fruit cultivar, and storage conditions, especially the storage atmosphere. Mould growth normally occurs only where the surface tissue of fruits has been damaged. Infection commonly follows insect or storm damages during pre-harvest, rough gathering at harvest or strong washing and sorting procedures post-harvest (**Snowdon, 1990**). During storage, infection can occur even at 0 °C, but decay proceeds slowly during cold storage, and usually develops rapidly only when fruits are returned to warm temperatures (**Paster *et al.*, 1995**).

The percentages of a colonized samples by *Penicillium* species (isolation frequency) was low (Table 3), except *Penicillium* sp. (50%). However these isolates could not be taxonomically identified because of contamination. *Penicillium crustosum* was detected in 30% of the samples, *P. expansum, P. chrysogenum* and *P. variabile* in 20%. Other samples were contaminated only once (10%). The most presented species of all tested isolates (226) was *P. chrysogenum* (67%) and *P. expansum* (22%). Another isolates were present only under 5%. From the overall endogenous *Penicillium* contamination from the Nitra wine growing region *Penicillium expansum and P. chrysogenum* grew on 20% from 10 tested samples. Other samples were contaminated only once (10%). The most presented species of all tested isolates (37) was *P. crustosum* (35%), follow by *P. chrysogenum* (24%) and *P. expansum* (19%) (**Felšöciová *et al.*, 2013**).

Table 2 The overall endogenous *Penicillium* contamination of tested various types of grape

***Penicillium* species**	**1.**	**2.**	**3.**	**4.**	**5.**	**6.**	**7.**	**8.**	**9.**	**10.**
P. canescens							1			
P. citrinum				1						
P. crustosum						1	1	3		
P. expansum									17	33
P. funiculosum					1					
P. glabrum						1				
P. griseofulvum							1			
P. chrysogenum									133	18
P. variabile							1	5		
Penicillium sp.	1			1	2	4		1		
∑	1			2	3	6	4	9	150	51

Legend: 1-10 subregions; 1- Hontiansky; 2-4 – Modrokamenský; 5 – Vinický; 6 – Ipeľský; 7 – Hontiansky; 8 – Fiľakovský; 9 – Gemerský; 10 - Tornaľský

Table 3 Isolation frequency and relative density of endogenous strains from genus *Penicillium*, isolated of grapes (n=10) harvested in Central Slovak wine region

***Penicillium* species**	**No of isolates**	**Isolation frequency (%)**	**Relative density (%)**
P. canescens	1	10	0.4
P. citrinum	1	10	0.4
P. crustosum	5	30	2
P. expansum	50	20	22
P. funiculosum	1	10	0.4
P. glabrum	1	10	0.4
P. griseofulvum	1	10	0.4
P. chrysogenum	151	20	67
P. variabile	6	20	2.6
Penicillium sp.	9	50	4

Legend: n = number of samples

Eight *Penicillium* species were found more frequently in exogenous contamination compared to endogenous because all wine growing region was colonised by the genus *Penicillium* (Table 4). *Penicillium* strains were present on grapes in higher numbers. The highest proportion of *Penicillium* species was determined in Fiľakovský subregion (8), namely *P. crustosum, P. expansum, P. chrysogenum* and *Penicillium* sp.. From a quantitative perspective the highest proportion was found in Tornaľský subregion (10) – 141 isolates. *Penicillium* species in exogenous contamination in the Nitra wine growing region were present on grapes also in higher numbers, where *Penicillium* sp. colonised most samples (5 out of 10). The quantitative representation of the colonized samples was not as high as in the samples of the Central Slovak region. The highest abundance was recorded for the species *P. chrysogenum* (36) (**Felšöciová *et al.*, 2013**).

Table 4 The overall exogenous *Penicillium* contamination of tested various types of grape

***Penicillium* species**	**1.**	**2.**	**3.**	**4.**	**5.**	**6.**	**7.**	**8.**	**9.**	**10.**
P. aurantiogriseum			8							
P. crustosum						5	2	8		
P. expansum	1							4	19	53
P. funiculosum					1					
P. griseofulvum							1			
P. chrysogenum			13	4				6	60	87
P. polonicum		1								
P. purpurogenum										1
Penicillium sp.	5	7			10	3	9	12		
∑	6	8	21	4	11	8	12	30	79	141

Legend: 1-10 subregions; 1- Hontiansky; 2-4 – Modrokamenský; 5 – Vinický; 6 – Ipeľský; 7 – Hontiansky; 8 – Fiľakovský; 9 – Gemerský; 10 - Tornaľský

With regard to species representation, the most frequently encountered *Penicillium* were *Penicillium* sp. (60%), follow by *P. chrysogenum* (50%), *P. expansum* (40%) and *P. crustosum* (30%) (Table 5). Colonization of another species was established once. The largest number of isolates belongs to *P. chrysogenum* (170) and *P. expansum* (77). From this point of view, the relative density of both species was the highest (53%) and (24%) respectively. The obtained results of the study in the Nitra wine growing region showed a high isolation frequency of *Penicillium* sp. (50%) exactly as in our wine grape samples from the Central Slovak region, follow by *P. citrinum, P. crustosum* and *P. chrysogenum* (20%). The largest number of isolates belongt to *P. chrysogenum* (37) and *P. crustosum* (34). From this point of view, the relative density was 28% and 26% respectively (**Felšöciová *et al.*, 2013**).

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Table 5 Isolation frequency and relative density of endogenous strains from genus *Penicillium*, isolated of grapes (n=10) harvested in Central Slovak wine region

***Penicillium* species**	**No of isolates**	**Isolation frequency (%)**	**Relative density (%)**
P. aurantiogriseum	8	10	2.5
P. crustosum	15	30	4.7
P. expansum	77	40	24
P. funiculosum	1	10	0.3
P. griseofulvum	1	10	0.3
P. chrysogenum	170	50	53
P. polonicum	1	10	0.3
P. purpurogenum	1	10	0.3
Penicillium sp.	46	60	14

Legend: n = number of samples

From endogenous and exogenous mycobiota were isolated 546 strains of 12 *Penicillium* species, namely *P. aurantiogriseum, P. canescens, P. citrinum, P. crustosum, P. expansum, P. funiculosum, P. glabrum, P. griseofulvum, P. chrysogenum, P. polonicum, P. purpurogenum* and *P. variabile*. *Penicillium* species were presented in all 9 types of wine berries. The most colonized subregions by *Penicillium* were Gemerský (9) with Blue frankish grape variety and Tornaľský (10) with Müller Thurgau grape variety. According **Felšöciová *et al.* (2013)** some similar species but in lower number of abundance were isolated from grapes in the Nitra wine growing region: *P. citrinum, P. corylophilum, P. crustosum, P. decumbens, P. expansum* and *P. chrysogenum*. In a later study in French vineyards with a variety of climatic conditions the following species were reported by **Bejaoui *et al.* (2006)**: *P. brevicompactum, P. expansum, P. spinulosum, P. glabrum, P. crustosum, P. citrinum,* and others. *Penicillium verrucosum*, which has frequently been isolated from cereals, in which it causes ochratoxin contamination in temperature climates, was no isolated from grapes in Italy (**Battilani *et al.*, 2003**), France (**Sage *et al.*, 2002; Bejaoui *et al.*, 2006**) or southern Spain (**Bellí *et al.*, 2004**). From our Slovak wine grape samples has not been isolated yet. In a study on fungal population in wine grapes in France, **Sage *et al.* (2002)** reported on the occurrence of several different *Penicillium* species, of which *P. brevicompactum* was the most common. In another heavily molded sample, *P. thomii* was the dominant species, and other species, such as *P. chrysogenum, P. citrinum, P. griseofulvum* and *P. purpurogenum*, were recorded infrequently. The dominance of *P. thomii* in harvested grapes was also reported in two grape-growing areas in Italy (**Battilani *et al.*, 2003**). In our study we didn't isolated any of their dominant species.

Table 6 The overall endogenous and exogenous *Penicillium* contamination of tested various types of grape wine samples

***Penicillium* species**	**1.**	**2.**	**3.**	**4.**	**5.**	**6.**	**7.**	**8.**	**9.**	**10.**
P. aurantiogriseum			8							
P. canescens							1			
P. citrinum				1						
P. crustosum						6	3	11		
P. expansum	1							4	36	86
P. funiculosum					2					
P. glabrum						1				
P. griseofulvum							2			
P. chrysogenum			13	4				6	193	105
P. polonicum		1								
P. purpurogenum										1
P. variabile							1	5		
Penicillium sp.	6	7		1	12	7	9	13		
∑	7	8	21	6	14	14	16	39	229	192

Legend: 1-10 subregions; 1- Hontiansky; 2-4 – Modrokamenský; 5 – Vinický; 6 – Ipeľský; 7 – Hontiansky; 8 – Fiľakovský; 9 – Gemerský; 10 – Tornaľský

From 546 *Penicillium* strains isolated from the both type of isolation the largest number of isolates belongs to *P. chrysogenum* (321), *P. expansum* (127) and *Penicillium* sp. (55) (Table 7), so these species represented the highest relative density 59%, 23% and 10%, respectively. On the other hand, their occurrence was not so high, *Penicillium* sp. was detected the most. Other remaining penicillia has been reported in only a small number. Similar results were obtained even in 2011 (**Felšöciová *et al.*, 2013**), when also the largest number of isolates belongs to *P. chrysogenum* (46) and *P. crustosum* (47), so they occurred with the highest relative density (27%, 28%, respectively). A high isolation frequency was detected in *Penicillium* sp. (60%) and *P. chrysogenum* (40%). *Penicillium* sp. were part of an endogenous and exogenous mycobiota in all analyzed samples. **Serra *et al.* (2005)** reported that from 885 *Penicillium* strains identified, the most frequent were *Penicillium brevicompactum* and *P. thomii* which represented 29% of the isolates and *P. glabrum/spinulosum* 14%. In our study we didn't isolated any of them.

Table 7 Isolation frequency and relative density of endogenous and exogenous strains from genus *Penicillium*, isolated of grapes (n=10) harvested in Central Slovak wine region

***Penicillium* species**	**No of isolates**	**Isolation frequency (%)**	**Relative density (%)**
P. aurantiogriseum	8	10	1.5
P. canescens	1	10	0.2
P. citrinum	1	10	0.2
P. crustosum	20	30	3.7
P. expansum	127	40	23.3
P. funiculosum	2	1	0.4
P. glabrum	1	1	0.2
P. griseofulvum	2	1	0.4
P. chrysogenum	321	50	58.8
P. polonicum	1	10	0.2
P. purpurogenum	1	10	0.2
P. variabile	6	20	1.1
Penicillium sp.	55	70	10.1

Legend: n = number of samples

Mycotoxins are a chemically diverse group of toxic secondary metabolites produced by filamentous fungi also belonging to the genus *Penicillium*. Despite efforts to control fungal contamination of foods, mycotoxin-producing fungi are ubiquitous contaminants of nature and make their way into fruit in the field or orchard and at any time during harvesting, processing, storage and marketing. Although most foods have chemical and/or physical properties that permit fungal or microbial spoilage, fruits are susceptible to fungal rather than microbial spoilage due to their high water activity (a_w) and sugar content, and presence of organic acids which impart the fresh of fruit a low pH (**Tournas and Katsoudas, 2005**). Mycotoxins can be present in intact (fresh) fruits, juices, wines, canned fruits and dried fruit products.

In total 10 endogenous *Penicillium* strains representing 4 potentially toxigenic species were tested for their toxigenic ability, namely *P. citrinum, P. crustosum, P. expansum* and *P. chrysogenum* (Table 8). Of all potentially toxigenic strains all of them were positive except one - *P. expansum*. Citrinin produced 1 out of 3 strains screened. Three of them (*P. citrinum, P. expansum* and *P. chrysogenum*) were also tested in total 8 endogenous strains from the Nitra wine-growing region (**Felšöciová *et al*., 2013**). Out of 8 strains, 50% produced at least one mycotoxin as revealed by the method used here.

Table 8 Toxinogenity of selected *Penicillium* strains, isolated from endogenous mycobiota of wine grapes

***Penicillium* species**	**C**	**P**	**PA**	**RC**
P. citrinum	1*/1**			
P. crustosum			1/1	1/1
P. expansum	1/3	3/3		3/3
P. chrysogenum				5/5

Legend: C – citrinin; P – patulin; PA – penitrem A; RC – roquefortin C; * positive isolates; ** number of tested isolates

In total 16 exogenous strains representing 4 potentially toxigenic species were tested for their toxigenic ability, namely *P. expansum, P. griseofulvum, P. hordei* and *P. chrysogenum* (Table 9). *Penicillium expansum* produced citrinin (4 out of 8 strains screened) and all of them produced patulin and roquefortin C. Patulin is produced by fungi belonging to several genera including *Penicillium*, *Aspergillus* and *Byssochlamys*. *P. expansum* which, in addition to patulin, citrinin, roquefortine C may produce penicillic acid, cyclopiazonic acid, and other secondary metabolities such as chaetoglobosins, communesin B, and expansolides (**Andersen *et al.*, 2004**). *Penicillium expansum* is believed to be the major fungal species contributing to patulin in apple products or in fruit. Its production in grapes has been associated with moldy berries (**Abrunhosa *et al.*, 2001**). Although patulin can occur in many moldy fruits including pears, berries, mangoes, plums, apricots and tomatoes, the major source of patulin contamination is apples with blue rot, and in apple cider or juice pressed from moldy fruit. Patulin has been demonstrated to be acutely toxic (**Dailey *et al.*, 1977**), genotoxic (**Alves *et al.*, 2000**), teratogenic (**Dailey *et al.*, 1977; Roll *et al.*, 1990; Sugiyanto *et al.*, 1993**) and possibly immunotoxic (**Sorenson *et al.*, 1985; Escuola *et al.*, 1988**) to animals. At present, there are no published toxicological or epidemiological data to indicate whether consumption of patulin is harmful to humans. However, there is a desire to limit patulin levels in apple products since infants and young children are major consumers of these foods and the effects of long-term exposure to patulin are not yet known. Many countries have set regulatory limits for patulin in apple products of 50 µg/L or less (**Drusch and Ragab, 2003**). Surveys of the presence of patulin in cherries, strawberries, raspberries, mulberries, peaches, grapes, pears and other fruits indicated low incidence and levels of the toxin (**Drusch and Ragab, 2003, Piemontese *et al.*, 2005).** Patulin production was not confirmed by *P. griseofulvum*, but this species produced griseofulvin, roquefortin C and cyclopiazonic acid. From six *P. chrysogenum* tested isolates five produced roquefortin C. The production roquefortin C by *P. hordei* was not confirmed.

Table 9 Toxinogenity of selected *Penicillium* strains, isolated from exogenous mycobiota of wine grapes

Penicillium species	**C**	**G**	**P**	**RC**	**CPA**
P. expansum	4*/8**		8/8	8/8	
P. griseofulvum		1/1	0/1	1/1	1/1
P. hordei				0/1	
P. chrysogenum				5/6	

Legend: C – citrinin; G – griseofulvin; P – patulin; RC – roquefortin C; CPA – cyclopiazonic acid; * positive isolates; ** number of tested isolates

CONCLUSION

Ten grape samples were collected from Konkordia, Pinot gris, Pinot noar, Sauvognon, Perle of Zala, Svätovavrinecké, Pálava, Müller Thurgau and 2x from Blue frankish grape varieties in the Central Slovak wine growing zone. Penicillia were isolated in endogenous and exogenous mycobiota. *Penicillium* contamination in endogenous mycobiota at harvest time was lower than in exogenous, where 226 isolates of 9 *Penicillium* species were found. One species less was isolated from exogenous mycobiota but with higher representation of *Penicillium* strains (320). Mycological endogenous and exogenous survey indicated the presence of 12 species of the *Penicillium* genus: *P. aurantiogriseum, P. canescens, P. citrinum, P. crustosum, P. expansum, P. funiculosum, P. glabrum, P. griseofulvum, P. chrysogenum, P. polonicum, P. purpurogenum, P. variabile* and *Penicillium* sp.. *Penicillium* sp. was the most frequent isolated in 70% of the samples according to our study. The species isolated in smaller frequency were *P. chrysogenum* (50%), *P. expansum* (40%) and *P. crustosum* (30%). The largest number of isolates belongs to *P. chrysogenum* (321) and *P. expansum* (127). From this point of view, the relative density of both species were the highest (59%) and (23%). Four potentially toxigenic species isolated from endogenous mycobiota were tested for their toxigenic ability by thin layer chromatography, namely *P. citrinum, P. crustosum, P. expansum* and *P. chrysogenum*. Out of 10 strains, all of them were positive on screening mycotoxin. Also 4 potentially toxigenic species isolated from exogenous mycobiota were tested for their toxigenic ability, namely *P. expansum, P. griseofulvum, P. hordei* and *P. chrysogenum*. Out of 16 strains, 75% produced at least one mycotoxin.

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REFERENCES

ABRUNHOSA, L., PATERSON, R.R.M., KOZAKIEWICZ, Z., LIMA, N., VENÂNCIO, A. 2001. Mycotoxin production from fungi isolated from grapes. *Lett. Appl. Microbiol.*, 32(4), 240-2.

ALVES, I., OLIVEIRA, N.G., LAIRES, A., RODRIQUES, A.S., RUEFF, J. 2000. Induction of micronuclei and chromosomal aberrations by the mycotoxin patulin in mammalian cells: role of ascorbic acid as a modulator of patulin clastogenecity. *Mutagenesis*, 15(3), 229-34.

ANDERSEN, B., FRISVAD, J.C. 2004. Natural occurrence of fungi and fungal metabolites in moldy tomatoes. *J. Agric. Food Chem*., 52(25), 7507-13.

ANDERSEN, B., SMEDSGAARD, J., FRISVAD, J.C. 2004. Ochratoxin A in grapes and wine. *Eur. J. Plant Pathol.,* 108, 639-43.

ANDERSEN, B., SMEDSGAARD, J., FRISVAD, J.C. 2004. *Penicillium expansum*: consistent production of patulin, chaetoglobosins and other secondary metabolites in culture and their natural occurrence in fruit products. *J. Agric. Food Chem*., 52(8), 2421-8.

BARKAI-GOLAN, R. 2001. Postharvest Diseases of Fruits and Vegetables. Amsterdam : Elsevier, 442 p. ISBN 9780444505842.

BARKAI-GOLAN, R., PASTER, N. 2008. Mycotoxins in Fruits and Vegetables. USA : Elsevier, 395 p. ISBN 978-0-12-374126-4.

BATTILANI, P., PIETRI, A., BERTUZZI, T., LANQUASCO, L., GIORNI, P., KOZAKIEWICZ, Z. 2003. Occurrence of ochratoxin A producing fungi in grape grown in Italy. *J. Food Prot*., 66(4), 633-6.

BEJAOUI, H., MATHIEU, F., TAILLANDIER, P., LEBRIHI, A. 2006. Black aspergilly and ochratoxin A production in French vineyards. *Int. J. Food Microbiol*., 111(1), 46-52.

BELLÍ, N., MARÍN, S., DUAIGÜES, A., RAMOS, A.J., SANCHIS, V. 2004. Ochratoxin A in wines, musts and grape juice from Spain. *J. Sci. Food Agric*., 84, 591-4.

BENKHEMMAR, O., LAHLOU, H., DUPONT, J., BOMPEIX, G., BOUBEKRI, C., EL MNIAI H.. 1993. Identification of different species of *Penicillium* causing deterioration of Moroccan table grapes during storage. *Mycopathologia*, 124, 27-30.

DAILEY, R.E., BROUWER, E., BLASCHKA, A.M., REYNALDO, E.F., GREEN, S., MONLUX, W.S., RUGGLES, D.I. 1977. Intermediate-duration toxicity study of patulin in rats. *J. Toxicol. Environ. Health*, 2(3), 713-25.
DONNELLY, D.M. 1977. Airborne microbial contamination in a winery bottling room. *Am. J. Enol. Vitic.,* 28(3), 176-181.
DRUSCH, S., RAGAB, W. 2003. Mycotoxins in fruits, fruit juices, and dried fruits. *J. Food Prot.,* 66(8), 1514-27.
ESCUOLA, L., THOMSEN, M., BOURDIOL, D., PIPY, B., PEURIERE, S., ROUBINET, F. 1988. Patulin immunotoxicology: effect on phagocyte activation and the cellular and humoral immune system of mice and rabbits. *Int. J. Immunopharmacol.,* 10(8), 983-9.
FELŠÖCIOVÁ, S., RYBÁRIK, Ľ., TANČINOVÁ, D., MAŠKOVÁ, Z. 2013. Occurrence of *Penicillium* species in grapes from Nitra wine region. *J. Microb. Biotech. and Food Sciences* (Special issue on BQRMF), 2, 2136-2148.
FUGELSANG, K.C., EDWARDS, CH.G. 2007. Wine microbiology. Practical Applications and Procedures. Springer Science + Business Media, LLC, NY, 2-nd edition, 393 p., e-ISBN-10: 0-387-33349-5.
GONZÁLES, H.H.L., PACIN, A., RESNIK, S.L., MARTINEZ, E. J. 1996. Deoxynivalenol and contaminant mycoflora in freshly harvested Argentinean wheat in 1993. *Mycopathologia*, 135(2), 129-134.
GUATAM, A., SHARMA, S., BHADAURIA, R. 2009. Detection of toxigenic fungi and mycotoxins in medicinally important powdered herbal drugs. *Internet Journal of Microbiology*, 7(2).
JACKSON, L. S., AL-TAHER, F. 2008. Factors affecting mycotoxin production in fruits. *Mycotoxins in fruits and vegetables*, Barkai-Golan, Paster (editors), Elsevier, 395 p. ISBN 978-0-12-374126-4.
LABUDA, R. - TANČINOVÁ, D. 2006. Fungi recovered from Slovakian poultry feed mixtures and their toxinogenity. *Annals of Agricultural and Environmental Medicine*, 2006, 13, 193-200.
MAGNOLI, C., VIOLANTE, M., COMBINA, M., PALACIO, G., DALCERO, A. 2003. Mycoflora and ochratoxin-producing strains of *Aspergillus* section Nigri in wine grapes in Argentina. *Letters in Applied Microbiology*, 37(2), 179-184.
PASTER, N., HUPPERT, D., BARKAI-GOLAN, R., 1995. Production of patulin by different strains of *Penicillium expansum* in pear and apple cultivars stored at different temperatures and modified atmospheres. *Food Addit. Contam.,* 12 (1), 51-8.
PIEMONTESE, L., SOLFRIZZO, M., VISCONTI, A. 2005. Occurrence of patulin in conventional and organic fruit products in Italy and subsequent exposure assessment, *Food Addit. Contamin.,* 22(5), 437-42.
PITT, J. I., HOCKING, A. D. 1997. *Fungi and food spoilage*. 2nd ed. London : Blackie Academic & Professional, 593 p. ISBN 0-8342-1306-0.
ROLL, R., MATTHIASCHK, G., KORTE, A. 1990. Embryotoxicity and mutagenicity of mycotoxins. *J. Environ. Pathol. Toxicol. Oncol.,* 10(1-2), 1-7.
ROSA, C.A., ROCHA, D.A., PALACIOS, V., COMBINA, M., FRAGA, M.E., DE OLIVEIRA REKSON, A., MAGNOLI, C.E., DALCERO, A.M. 2002. Potential ochratoxin A producers from wine grapes in Argentina and Brazil. *Food Add. Contam.,* 19(4), 408-414.
SAGE, L., KRIBOVOK, S., DELBOS, E., SEIGLE-MIRANDI, F., CREPPY, E.E. 2002. Fungal flora and ochratoxin A production in grapes and musts from France. *J. Agr. Food Chem.*, 50(2), 1306-11.
SAMSON, R. A., FRISVAD, J. C. 2004. *Penicillium subg. Penicillium*: *new taxonomic schemes and mycotoxins and other extrolites*. Utrecht : Centraalbureau voor Schimmelcultures, 260 p. ISBN 90-70351-53-6.
SAMSON, R. A., HOEKSTRA, E. S., FRISVAD, J. C., FILTENBORG, O. 2002a. Introduction to food- and airborne fungi. Utrecht : Centraalbureau voor Schimmelcultures, 389 p. ISBN 90-70351-42-0.
SAMSON, R. A., HOEKSTRA, E. S., LUND, F., FILTENBORG, O., FRISVAD, J. C. 2002b. Method for the detection, isolation and characterisation of food-borne fungi. In SAMSON, R. A., HOEKSTRA, E. S., FRISVAD, J. C., FILTENBORG, O. Introduction to food- and airborne fungi. Utrecht : Centraalbureau voor Schimmecultures, 283-297, ISBN 90-70351-42-0.
SAMSON, R. A., HOUBRAKEN, J., THRANE, U., FRISVAD, J. C., ANDERSEN, B. 2010. Food and Indoor Fungi. Utrecht : CBS – KNAW Fungal Biodiversity Centre, 390 p. ISBN 978-90-70351-82-3.
SANCHIS, V., MAGAN, N. 2004. Environmental conditions affecting mycotoxins. Mycotoxins in Food: Detection and Control (N. Magan, M. Olsen, eds), 174-89. Cambridge, England : Woodhead Publishing.
SERRA, R., BRAGA, A., VENANCIO, A. 2005. Mycotoxin-producing and other fungi isolated from grapes for wine production, with particular emphasis on ochratoxin A. *Research in Microbiology*, 156(4), 515-521.
SNOWDON, A.L. 1990. A colour atlas of post-harvest diseases and disorders of fruits and vegetables. Wolfe publishing Ltd, 1, 302.
SORENSON, W.G., SIMPSON, J., CASTRANOVA, V. 1985. Toxicity of the mycotoxin patulin for rat alveolar macrophage in vitro. *Environ. Res.,* 38(2), 407-16.
SUGIYANTO, J., INOUYE, M., ODA, S. I., TAKAGISHI, Y., YAMAMURA, H. 1993. Teratogenicity of patulin, a mycotoxin in mice. Environ. Med., 37(1), 43-46.
TOURNAS, V.H., KATSOUDAS, E. 2005. Mould and yeast flora in fresh berries, grapes and citrus fruits. *Int. J. Food Microbiol.,* 105(1), 11-7.