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MYCOBIOTA OF SLOVAK WINE GRAPES WITH EMPHASIS ON *ASPERGILLUS* AND *PENICILLIUM* SPECIES IN THE SMALL CARPATHIAN AREA

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ABSTRACT

The Slovak wine-growing region is divided into six viticulture areas. The largest in size and the most important over the centuries has been the Small Carpathian area (around 5800 ha of vineyards) spreads in the western of Slovakia. The objectives of this study were: to gain more knowledge about mycobiota on grapes originating from Slovakia, with a focus on genera *Aspergillus* and *Penicillium* and their ability to produce mycotoxins in *in vitro* conditions by thin layer chromatography method. From the twelve vineyards were collected 14 samples of wine grapes (white 6, blue 8) during harvesting 2011, 2012 and 2013. Fifty wine grapes per bunch (approximately 7-8 berries per plate) that showed no symptoms were randomly selected on Dichloran Rose Bengal Chloramphenicol agar medium. The plates were then incubated aerobically at 25 ±1 °C for 5 to 7 days in the dark. Of these samples were identified 22 genera. Ninety-three percent of samples were colonies by the genus *Penicillium* and 79% by the genus *Aspergillus*. During the survey, 251 isolates belonging to 14 *Penicillium* species (*P. aurantiogriseum, P. citrinum, P. coprophylum, P. crustosum, P. expansum, P. funiculosum, P. glabrum, P. griseofulvum, P. chrysogenum, P. oxalicum, P. polonicum, P. purpurogenum, P. roqueforti* and *P. thomii*) and 37 isolates belonging to 7 *Aspergillus* species (*A. clavatus, A. flavus, A.* section *Nigri, A. ostianus, A. parasiticus, A. versicolor* and *A. westerdijkiae*) were isolated and identified from exogenous contamination. The main occurring penicillium species of the samples were *P. chrysogenum* (36% Fr), followed *P. crustosum* (29% Fr), *P. griseofulvum* (21% Fr) and *P. expansum* (21% Fr). The main occurring aspergillus species of the samples were *A.* section *Nigri* (64%). Thirteen potentially toxigenic species were tested for their toxigenic ability. It was confirmed the production of various mycotoxins such as aflatoxin B_1, G_1, citrinin, griseofulvin, patulin, cyclopiazonic acid, penitrem A, roquefortin C and sterigmatocystin. Out of 124 strains, 84% produced at least one mycotoxin.

Keywords: wine grapes; Slovak Republik; fungi; mycotoxin

INTRODUCTION

Grapevine can be attacked by a number of fungi and fungus-like organisms which affect the berries and cause loss of quality and influence the taste of the wine **(Pitt and Hocking, 2009)**. Several fungi are pathogenic to grapevines, infecting the roots, trunk, canes, leaves and berries **(Pearson and Goheen, 1988)**. Fungi which commonly infect berries include the mildew pathogens *Erysiphe necator* and *Plasmopara viticola*, as well as *Alternaria* spp., *Aspergillus* spp., *Botrytis cinerea*, *Cladosporium* spp., *Penicillium* spp., *Epicoccum* spp. and *Rhizopus* spp. **(Bellí et al., 2004; Sage et al., 2002)**. During maturation, the spoilage agents, *Aspergillus*, *Botrytis*, *Penicillium* and *Rhizopus*, increase their incidence. When the temperature is higher than 37 °C, species in *Aspergillus* section *Nigri*, usually called "black aspergilli", predominate **(Valero et al., 2005)**. At harvest time the conditions are optimal for fungal invasion, especially if physical damage has occurred on berries.

From single infected berries the whole cluster may be affected causing mummified clusters covered with green mould *Penicillium expansum*. Green mould produce mycotoxins **(Abrunhosa et al., 2001; Serra et al., 2006)** for example patuline which is however degraded during fermentation and by sulphurization. Berries affected by green mould have an off-flavor and even a small amount of infected berries add a mouldy taste to the wine **(Kassemeyer and Berkelmann-Löhnertz, 2009)**. **Samson et al., (2004)** considered 15 species provisionally accepted in *Aspergillus* section *Nigri*, four of those producing ochratoxin A (OTA) and only two occurring on grapes, raisins and in wine – *Aspergillus carbonarius* and to a lesser extent *A. niger*. Ochratoxin A is the main mycotoxin of concern in grape products. OTA is produced primarily when *Aspergillus carbonarius* infects berries before harvest. The relatively few toxigenic strains of the related species, *Aspergillus niger* is by far the most common species of *Aspergillus* present on grapes **(Leong et al., 2007)**. The aflatoxigenic species, *Aspergillus flavus* and *Aspergillus parasiticus*, have occasionally been isolated from grapes **(Sáez et al., 2004)**. Toxigenic isolates of *Aspergillus ochraceus* have also only occasionally been isolated from grapes. Generally, the colonisation of grape bunches by black aspergilli and other fungi occurs when berry skin damage allows the entry into fruit tissues, where the low pH and high sugar content under aerobic

conditions provide a competitive advantage for moulds. However, fungal invasion may occur without visible symptoms **(Bellí et al., 2007)**.

The aim of our study was to detect mycobiota on grapes and species of genera *Aspergillus* and *Penicillium* and potentially toxigenic producing species tested by thin layer chromatography for the ability to produce selected mycotoxins in *in vitro* conditions.

MATERIAL AND METHODOLOGY

Study area

Twelve vineyards were studied (Modra, Zeleneč, Svätý Martin, Doľany, Dolné Orešany, Dvorníky, Pezinok, Moravany n. Váhom, Gajary, Skalica, Bratislava – Rača) during a 3-year period (2011 – 2013) in Small Carpathian wine-growing region. 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. The largest in size and the most important over the centuries has been the Small Carpathian area (around 5800 ha of vineyards) spreads in the western of Slovakia. The Small Carpathian wine region is divided to 12 subregions. The subregion is the area with the same soil and climate conditions. Wine-growing zones are defined as geographic regions with distinct climatic conditions for grape cultivation. The Small Carpathian wine-growing region has medium climates and abundant moisture.

Samples

Samples were collected from early September to late October, in the maturation stage harvest. Fourteen samples: 6 of white grape varieties (Sauvignon, 2 x Pinot Blanc, 2 x Green Veltliner, Riesling) and 8 of blue grape varieties (2 x Cabernet Sauvignon, 2 x André, 4 x Blue Frankish) were mycologically analyzed. Three kilograms of samples were collected at the time of technological ripeness. Picked grapes were stored at 4 ±1 °C and analyzed within 24 h after harvest.

Mycological analysis of grapes

A total of 50 berries (7 – 8 berries per bunch) from each sample were plated in Dichloran Rose Bengal Chloramphenicol agar medium (DRBC) and incubated at 25 ±1 °C in the dark for one week. The spore-producing filamentous fungi detected were identified to genus level based on morphological characters according to the manual of **Pitt and Hocking (2009)**. Different media were used for the taxonomic identification of obtained fungi according to that used for standard strains. Specifically, *Penicillium* and *Aspergillus* strains were identified down to the species level first using Malt extract agar (MEA) **(Pitt and Hocking, 2009)**, Czapek yeast extract agar (CYA) **(Samson et al., 2002a)**, Czapek yeast extract with 20% sucrose agar (CY20S) **(Pitt and Hocking, 2009)**, Yeast extract agar (YES) **(Samson et al., 2010)**, Creatine-Sucrose agar (CREA) **(Samson et al., 2010)** and identified to species level according to the manuals of **Samson et al., (2002a), Samson and Frisvad (2004), Pitt and Hocking (2009)**. The berries from the vineyards sampled were generally in good condition without visible damage.

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. (1999)** as follows:

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

Where: 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, griseofulvin, ochratoxin A, aflatoxin B_1, G_1 were carried out on YES agar and intracellular roquefortin C, penitrem A, cyclopiazonic acid and sterigmatocystin 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, griseofulvin as a blue spot, ochratoxin A as a blue-green spot, aflatoxin B_1 as a blue spot, aflatoxin G_1 as a green-blue spot and sterigmatocystin as a reddish spot.

The filamentous fungi identified from samples by the direct plating method are indicated in Table 1. Without surface disinfection, a total of 2774 strains belonging to 20 genera were identified. The three most abundant genera found by descending order were *Alternaria* (42%), *Cladosporium* (33%) and *Penicillium* (9%). *Epicoccum, Botrytis, Fusarium, Rhizopus, Trichoderma* were detected in more than 1% of the berries analyzed. The remaining 12 genera were detected in less than or equal to 1% of the berries. The genus *Alternaria, Cladosporium, Fusarium,*

Rhizopus, Trichoderma colonised 100% of samples, followed *Epicoccum, Penicillium* (93%, each) and *Aspergillus* (79%). In our study was also unidentified *Mycelia sterilia* without creation fruiting bodies.

RESULTS

The *Aspergillus* and *Penicillium* strains were isolated and identified to species level. The isolation rates for *Aspergillus* from the berries were 79%. The relative densities were low (Table 1). Table 2 shows the number of isolates and isolation frequency (%) of *Aspergillus* spp. The species of *Aspergillus* section *Nigri* were the predominant in mycobiota. The species of *A. clavatus* and *A. flavus* were the other most important species recorded with high isolation frequency.

The incidence of *Penicillium* species on agar DRBC revealed the occurrence of 13 different *Penicillium* species (Table 3) with high frequency – 93% (Table 1). The relative density was 9%. From the 251 *Penicillium* strains identified, the most frequent were *Penicillium chrysogenum* (64%), *P. crustosum* (12%) and *P. griseofulvum* 8% of the isolates. Isolation frequency among species was maximum for *P. chrysogenum* (36%), *P. crustosum* (29%), *P. expansum* and *P. griseofulvum* (21%, each).

In total 124 isolates representing 13 potentially toxigenic species were tested for their toxigenic ability (Table 4). Out of 124 strains, 84 % produced at least one mycotoxin as revealed by the method used here. Positive toxigenity was detected in *A. clavatus, A. parasiticus, P. crustosum* and *P. chrysogenum*. *Aspergillus flavus* produced aflatoxin B_1 and cyclopiazonic acid (CPA, 2 out of 5 strains screened, each) but did not produce aflatoxin G_1. *Aspergillus ostianus* produced sterigmatocystin and did not produce OTA and citrinin. Ochratoxin A production was tested also in 7 strains belonging to *Aspergillus* section *Nigri*. Among them, the production of ochratoxin A was not confirmed. *Penicillium citrinum* produced citrinin (1 out of 2), *Penicillium expansum* produced roquefortin C (RC), patulin (3 out of 5) and citrinin (two out of 5), *P. griseofulvum* produced CPA, RC, griseofulvin (12 out of 13) and patulin (10 out of 13). Negative toxigenity was detected in *A. versicolor, P. coprophilum* and *P. roqueforti.*

DISCUSSION

The native mycobiota of seven grape varieties (14 samples) grown in Small Carpathian wine-growing region (Slovak Republic) has been studied. We used plating methods without surface disinfection to detect the sporulating fungi colonizing the grape surface. Most of the fungi found are ubiquitously distributed, such as the field fungi *Alternaria* and *Cladosporium*, which occur commonly in the air, plant surfaces, debris and soil. *Alternaria, Cladosporium* and *Penicillium* occured with high isolation frequency and relative density. **Magnoli et al., (2003)** showed that *Alternaria* (80% of the samples), *Aspergillus* (70%), *Cladosporium* (40%) and *Penicillium*

Table 1 Fungi identified in Slovak wine grapes from 2011 to 2013 by the direct plating method.

Fungal taxa	No.	Fr (%)	RD (%)
Absidia	1	7	<1
Acremonium	4	7	<1
Alternaria	1180	100	42
Arthrinium	3	21	<1
Aspergillus	37	79	1
Botrytis	59	71	2
Cladosporium	901	100	33
Epicoccum	90	93	3
Fusarium	59	100	2
Gibberella	3	7	<1
Geotrichum	1	7	<1
Chaetomium	1	7	<1
Mucor	25	43	1
Nigrospora	1	7	<1
Penicillium	251	93	9
Phoma	2	14	<1
Rhizopus	43	100	2
Trichoderma	54	100	2
Ulocladium	1	7	<1
Mycelia sterilia	58	79	2
Total isolates	**2774**		

Note: No. – number of isolates, Fr – isolation frequency, RD – relative density.

(30%) species were the predominant microfungi in harvested grapes from Argentina. In Spanish wine grapes the most prevalent reported genera were *Alternaria*, yeasts, *Aspergillus*, *Cladosporium*, *Rhizopus* and *Penicillium* **(Bellí et al., 2006)**.

Also, in some other research from Tunisia, *Aspergillus* (33%), *Botrytis* (23%), *Alternaria* (13%), *Cladosporium* (11%) and *Penicillium* (8%) have been isolated as the most frequent fungi genus **(Fredj et al., 2007)**. In three-year a study, **Serra et al., (2006)** investigated the fungal species present on the surface of grape berries from Portuguese vineyards in four winemaking regions. According to their results, in more humid climates, *Botrytis* was the main pathogen and spoiling agent, and the incidence of black *Aspergillus* was minimal. *Alternaria*, *Botrytis*, *Cladosporium* and *Penicillium* were four of the most frequent genera in all the regions. *Botrytis*, *Cladosporium* and *Penicillium* were also reported as the predominant mycobiota by **Abrunhosa et al., (2001)** in Portugal. In 41 samples of grape fruits grown in Eastern Spain the most infected samples were *Cladosporium*, *Alternaria* and *Aspergillus* section *Nigri* **(Sáez et al., 2004)**.

Certainly the *Aspergillus* species are present worldwide, in all the grape products and under all environmental conditions, most frequent in warmer regions and heat-generating substrates **(Somma et al., 2012)**. Our results agree with this because this genus represented 1% of all the fungi found in the region so the occurrence of *Aspergillus* spp. in our samples was generally low. *Aspergillus* section *Nigri* were the most prevalent, followed by *A. clavatus* and *A. flavus*. The isolation frequency of *Aspergillus* section *Nigri* in our contaminated samples was 64% and relative density 40% in non-disinfected grapes. From the thirteen samples of wine grapes in Czech Republic, a Slovak neighbouring country, **Ostrý et al., (2007)** were not found ochratoxigenic microfungi, e. g. *Aspergillus carbonarius*, and other species of section *Nigri*, *A. ochraceus*, *Penicillium*

Table 2 *Aspergillus* species identified in Slovak wine grapes from 2011 to 2013 by the direct plating method.

***Aspergillus* species**	**No.**	**Fr. (%)**
A. clavatus	7	21
A. flavus	6	21
A. section *Nigri*	15	64
A. ostianus	1	7
A. parasiticus	1	7
A. versicolor	1	7
A. sp.	6	43
Total isolates	**37**	

Note: No. – number of isolates, Fr – isolation frequency.

Table 3 *Penicillium* species identified in Slovak wine grapes from 2011 to 2013 by the direct plating method.

***Penicillium* species**	**No.**	**Fr. (%)**
P. aurantiogriseum	2	7
P. citrinum	3	14
P. coprophylum	1	7
P. crustosum	30	29
P. expansum	17	21
P. funiculosum	1	7
P. glabrum	1	7
P. griseofulvum	21	21
P. chrysogenum	160	36
P. oxalicum	3	7
P. purpurogenum	2	14
P. roqueforti	1	7
P. thomii	3	14
P. sp.	6	29
Total isolates	**251**	

Note: No. – number of isolates, Fr – isolation frequency.

Table 4 Toxinogenity of selected strains, isolated from exogenous mycobiota of wine grapes.

Species	AFB_1	AFG_1	OTA	C	G	P	CPA	PA	RC	STER
A. clavatus						5/5				
A. flavus	2*/5**	0/5					2/5			
A. ostianus			0/1	0/1						1/1
A. parasiticus	1/1	1/1								
A. section *Nigri*			0/7							
A. versicolor										0/1
P. citrinum				1/2						
P. coprophilum					0/1				0/1	
P. crustosum								14/14	14/14	
P. expansum				2/5		3/5			5/5	
P. griseofulvum					12/13	10/13	13/13		13/13	
P. chrysogenum									68/68	
P. roqueforti						0/1			0/1	

Note: * - number of isolates with ability to produce mycotoxin, ** - number of tested isolates, AFB_1,G_1 – aflatoxin B_1,G_1, OTA – ochratoxin A, C – citrinin, G – griseofulvin, P – patulin, CPA – cyclopiazonic acid, PA – penitrem A, RC – roquefortin C, STER – sterigmatocystin.

verrucosum and *P. nordicum*. Occurrence of *Aspergillus* spp. in grapes from Slovakia was surveyed during 2 years 2008 and 2009 by **Mikušová et al., (2012)**. A large number of *Aspergillus* spp., including *A. flavus*, *A. japonicus*, *A, niger*, *A. carbonarius* and *A. ibericus* were identified.

Penicillium is a common component of the grapes mycobiota. This genus is ubiquitous saprophyt whose conidia are easily distributed in the atmosphere **(Serra et al., 2006)**. *Penicillium* is described as being frequent in soils and temperate regions. *Penicillium* was more frequent than *Aspergillus* in all our samples. Isolation frequency among *Penicillium* species was maximum for *P. chrysogenum* (36%), followed by *P. crustosum* (29%), *P. expansum* and *P. griseofulvum* (21%, each). **Cabañes et al., (2002)** found *Penicillium purpurogenum* in all samples of the white Garnacha grape variety that they studied. Their samples were from Tarragona, Spain. *Penicillium purpurogenum* was isolated in our samples, however in low relative density (1%). **Magnoli et al., (2003)** found among *Penicillium* spp. *P. chrysogenum*, as the most frequent species isolated in 22% of the samples what correspond with our results. The most frequent *Penicillium* species in grape berries from Portuguese vineyards in four winemaking regions were *P. brevicompactum, P. thomii* and *P. glabrum/spinulosum* which together accounted for approximately 71% of the strains identified in the genus **(Serra et al., 2006)**. In our study we isolated them by only in a low density. *Penicillium thomii* represented 1% of the isolates and *P. glabrum* 0,4%. The genera *Penicillium* (present in the range 27 – 54%) was predominant in harvest time in grapes from Slovakia, and it was represented by *P. brevicompactum*, *P. chrysogenum, P. crustosum, P. expansum, P. palitans, P. polonicum, P. verrucosum, P. citrinum* and *P. glabrum* **(Mikušová et al., 2012)**. Most of them also were isolated from our samples. It should be noted that despite the differences in geographic location, the varieties studied by the different authors were different as well, which could explain the disagreement of the results found among the samples.

Grapes that are heavily infected with moulds alter in chemical composition and secondary metabolities such as mycotoxins. These mycotoxins of greatest significance in grapes and grape products produced by *Aspergillus* and *Penicillium* spp., include ochratoxin A, aflatoxins, patulin and citrinin **(Magnoli et al., 2003)**. Mycotoxins such as aflatoxin, patulin and citrinin are less common than ochratoxin A in grape and grape products. Ochratoxin A is a mycotoxin with nephrotoxic, nephrocarcinogenic, teratogenic and immunosuppressive properties, which has received growing interest from the scientific community and food committees in the last few years **(Chulze et al., 2006)**. Ochratoxin A is a kidney toxin and probable carcinogen **(Varga and Kozakiewicz, 2006)**. According to studies ochratoxin A producing strains from the group *Aspergillus* section *Nigri* (*A. carbonarius* and *A. niger* aggregate) are the source of ochratoxin A in wines, grapes and dried vine fruits **(Hocking et al., 2007)**. Ochratoxin A is produced primarily when *A. carbonarius* infects berries before harvest. *Aspergillus niger* may also contribute to ochratoxin A contamination. It is by far the most common species of *Aspergillus* present on grapes **(Chulze et al., 2006)**. Toxigenic isolates of *A. ochraceus* have only occasionally been isolated from grapes **(Abrunhosa et al., 2001)**. *Penicillium verrucosum* and *P. nordicum*, the only confirmed *Penicillium* species that are able to produce OTA, were not isolated. Ochratoxin A producers in grapes were isolated in 24.74% in the year 2008 and only one species *Aspergillus niger* (7.01%) in the year 2009 in South Slovak region by **Mikušová et al., (2012)**. The higher presence of *Aspergillus carbonarius* (9.68%) was observed only in year 2008. However, their results confirmed a low production of the OTA, what indicates that there is low risk of OTA contamination of Slovak wine. This conclusion agrees with reported survey data, where OTA has been detected in less than 50% of analysed

wine samples, however wines produced and sold in Slovakia had lower level of OTA than imported wines and OTA concentrations found were far below the proposed European limit of 2 $\mu g.L^{-1}$ **(Belajová and Rauová, 2007)**.

Aflatoxins are potent carcinogens, produced by *Aspergillus flavus* and *A. parasiticus* **(Pitt, 2000)**. Aflatoxins and aflatoxin producing strains **(Fredj et al., 2007)** have been detected in wine and must occasionally, as reported in Lebanon and Turkey **(El Khoury et al., 2008)**. So far, aflatoxin contamination in the grape and wine product chains does not seem to be a real risk for human and animal health. Aflatoxins may occur as common contaminants of dried vine fruits in some countries, i.e. Iran **(Feizy et al., 2012)**, Egypt **(Youssef et al., 2000)** and Greece **(Kollia et al., 2013)** at very high levels. The strain of *A. parasiticus* isolate from our study was able to produce *in vitro* aflatoxins B_1 and G_1. Other toxigenic species *A. flavus* produced AFG_1 (2 out of 5) but not produced AFB_1.

Citrinin, a hepato-nephrotoxic compound, also has been detected in grapes before storage **(Bragulat et al., 2008)**. It is produced by different species of *Penicillium*, *Aspergillus* and *Monascus*. Citrinin producing strains *A. ostianus*, *P. citrinum* and *P. expansum* were isolated from our Slovak samples.

Patulin can occur in many moldy fruits including grapes. Patulin causes gastrointestinal problems, skin rashes, and is known to be mutagenic **(Abrunhosa et al., 2001)**. Patulin has been demonstrated to be acutely toxic (**Dailey et al., 1977)**, genotoxic **(Alves et al., 2000)**, teratogenic **(Dailey et al., 1977)**, and possibly immunotoxic **(Escuola et al., 1988)** to animals. Patulin production was confirmed by *P. expansum* and *P. griseofulvum*. The production of patulin by *P. roqueforti* was not confirm. **Tančinová et al., (2015)** analyzed 47 samples of grapes, harvested in 2011, 2012 and 2013 from various wine-growing regions. The potential producers of patulin were isolated from 23 samples berries, 19 samples of surface-sterilized berries and 6 samples of grape juice. Overall, the representatives of producers of patulin were detected in 32 (68.1%) samples (75 isolates). The ability to produce patulin in *in vitro* condition was detected in 82% of isolates of *Penicillium expansum*, 65% of *Penicillium griseofuvum* and 100% of *Aspergillus clavatus*.

Penicillium chrysogenum may produce a very wide range of toxic compounds – roquefortine C, meleagrin and penicillin. These metabolites could be considered as a potential hazard to human health **(Samson et al., 2002a)**. We tested 68 strains on roquefortine C from exogenous mycobiota which all were positive.

CONCLUSION

Grapes were analyzed by plating methods from Small Carpathian wine-growing region at the harvest time between 2011 and 2013. From the 4463 strains detected and identified from exogenous mycobiota, the most frequent genera were *Alternaria*, *Cladosporium* and *Penicillium*. Potentially toxigenic *Aspergillus* and *Penicillium* species were tested for their toxigenic ability by thin layer chromatography. Out of 124 exogenous strains representing 7 potentially toxigenic species, 84% produced at least one mycotoxin. Potential producers of ochratoxin A *Aspergillus* section *Nigri* and roquefortin C *Penicillium chrysogenum* were the most frequent mycotoxigenic species isolated from grapes. In line with the results on OTA content of Slovak grapes, it appears that the mycotoxin does not present a significant hazard to consumers.

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