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MYCOBIOTA OF SLOVAK WINE GRAPES WITH EMPHASIS ON *ASPERGILLUS* AND *PENICILLIUM* SPECIES IN THE SOUTH SLOVAK WINE REGION

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ABSTRACT

The Southern Slovak wine growing region is warmest part of Slovakia and is suitable for cultivating the grapes for production of wines at high quality. From the eight vineyards were collected 8 samples of wine grapes (white 7, blue 1) during harvesting 2011, 2012 and 2013. The aim of this work was to gain more knowledge about mycobiota on grapes originating from Slovakia, to identify *Aspergillus* and *Penicillium* species according to their morphopogy and evaluate the presence of secondary metabolites (also including intracellular and extracellular mycotoxins) produced in *in vitro* conditions by thin layer chromatography method from fresh grape berries. 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 17 genera. One hundred percent of samples were colonies by the genus *Penicillium* and 75% by the genus *Aspergillus*. During the survey, 135 isolates belonging to 9 *Penicillium* species (*P. aurantiogriseum, P. canescens, P. citrinum, P. crustosum, P. decumbens, P. expansum, P. funiculosum, P. chrysogenum* and *P. purpurogenum*) and 26 isolates belonging to 3 *Aspergillus* species (*A. clavatus, A. flavus* and *A.* section *Nigri*) were isolated and identified from exogenous contamination. The main occurring penicillium species of the samples were *P. expansum* (37.5% Fr), followed *P. citrinum, P. chrysogenum* and *P. crustosum* (25% Fr). The main occurring aspergillus species of the samples were *A.* section *Nigri* (62.5%). Eight potentially toxigenic species were tested for their toxigenic ability. It was confirmed the production of various mycotoxins such as aflatoxin B_1, citrinin, patulin, cyclopiazonic acid, penitrem A and roquefortin C. Out of 34 strains, 56% produced at least one mycotoxin.

Keywords: wine grapes; mycobiota; *Aspergillus* spp.; *Penicillium* spp.; mycotoxin

INTRODUCTION

Fruits contain high levels of sugars and other nutrients, and they possess an ideal water activity for microbial growth, their low pH makes them particularly susceptible to fungal spoilage. The grape microbial ecosystem is composed of highly diverse microorganisms, including fungi **(Rousseaux et al., 2014)**. Grape health status is the main factor affecting the microbial ecology of grapes, increasing both microbial numbers and species diversity **(Barata et al., 2012)**. Climate change (warmer weather, heat waves, higher levels of precipitation and drought) may affect the distribution of fungal population, including those present on grapes, thereby also affecting the presence of mycotoxins or off-flavours in wine **(Paterson and Lima, 2011)**. Generally fungi have been detected in vineyards and on grapes from setting. Many species of fungi can colonize grapes during the withering process, and the most important of these for enhancing wine quality is *Botrytis cinerea* **(Lorenzini et al., 2012)**. *Botrytis cinerea* is widely recognized as the causal agent of gray mold and also generates off-flavors **(Steel et al., 2013)**. However, grape rotting and spoilage can be caused by a variety of fungal species, including *Penicillium, Aspergillus, Alternaria, Cladosporium* and *Rhizopus*. *Aspergillus* and *Alternaria*, followed by *Penicillium*, are the most frequently reported genera on grapes. The genus *Penicillium* seems to be more frequent in temperate and cold climates, such as those in northern Europe, whereas *Aspergillus* is more frequently associated with warmer and wetter regions **(Serra et al., 2006)**. *Aspergillus* spp. are ubiquitous saprophytes present in soils around the world, particularly in tropical and subtropical regions. *Aspergillus* section *Nigri* are the most important mycotoxigenic (ochratoxigenic) fungi present on grapes (5 – 83% infected berries) **(García-Cela et al., 2015)**. Grape aspergilli increase gradually, reaching their maximum values at the

beginning of veraison and ripening **(Battilani and Pietri, 2002)**. As *Aspergillus* species are not considered primary pathogens, various grape damage, such as attack by other fungi or mechanical injury, dramatically increases the risk of fungal infection by these species **(Serra et al., 2006)**. Some vineyard fungal species are capable of producing toxic secondary metabolites (mycotoxins) in infected tissue, which may contaminate grapes and grape products such as wine, grape juice and dried vine fruit. The mycotoxins of greatest significance include aflatoxins, citrinin, patulin, ochratoxin A (OTA) and fumonisin B_2 **(Susca et al., 2010)**. However, of the above mentioned mycotoxins, maximum levels for grape products, juices and/or wine are established for OTA. Only a few species produce OTA among the *Aspergillus niger* aggregate, *A. carbonarius*, *A. japonicas*, *A. ochraceus* (only occasionally isolated from grape) **(Serra et al., 2005)**. *Penicillium verrucosum*, which is more frequently found on cereals is known to produce OTA and citrinin, is rarely found on grapes **(Rousseaux et al., 2014)**. We focus particularly on descriptions of the fungal microbiota on grapes and species of genera *Aspergillus* and *Penicillium* responsible for the production of mycotoxins and off-flavors.

MATERIAL AND METHODOLOGY

Study area

Slovakia is a country located in Central Europe with climate conditions similar to those of the neighboring winemaking countries: the Czech Republic, Hungary, and Austria. Winemaking is concentrated in the southern part of the country, primarily on the southern, south-eastern, and southwestern slopes of the Carpathians Mountains, which take up approximately two thirds of Slovakia's total area. Slovakia's wine growing territory is divided into six regions. South Slovak wine growing region is situated north from river Danube, belongs to continental climate zone. It is warmest part of Slovakia and the vineyards are planted on loess uplands, silty clay soils with good water holding, which are suitable for growing of red grape varieties. The average air temperature in the period from May to September range from 13 °C to 23 °C, the average temperature in growing season is 18.5 °C. Higher average temperatures are contributing to production of saccharides substances in grapes, which will reflect in superior fullness of wine. Higher temperature allows to cultivate the grapes for production of wines at high quality and wines with attribute with average natural content of sugar around 23 °NM. Altogether 8 Slovakian vineyards were studied (Gbelce, Mužla, Pribeta, Nové Zámky, Abrahám, Nesvady, Veľký Meder and Šamorín) during a 3-year period (2011 – 2013) in South Slovak wine growing region.

Samples

Eight samples of 5 different grape varieties – 7 of white grape varieties (3 x Welschriesling, 2 x Riesling, 1x Green Veltliner, 1x Chardonnay) and 1 of blue grape variety Blue Frankish were involved in the study. Samples were collected in autumn of 2011 – 2013, in the maturation stage harvest. 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. We used conventional identification techniques, such as macroscopic and microscopic observations, with guidelines by **Pitt and Hocking (2009)** facilitating the identification of isolated microorganisms. 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:

$$Fr\ (\%) = (ns / N) \times 100;\ RD\ (\%) = (ni / Ni) \times 100$$

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 B1, G1 were carried out on YES agar and intracellular 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 Geni® 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(SO4)2 x 4 H2O as an orange spot. Penitrem A after spraying with 20% AlCl3 in 60% ethanol and heating at 130 °C for 8 min as a dark blue spot. Cyclopiazonic acid was visible directly in daylight after spraying with the Ehrlich reagent as a violet-tailed 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 and aflatoxin B1 as a blue spot and aflatoxin G1 as a green-blue spot.

RESULTS AND DISCUSSION

A total of 1377 isolates of microscopic fungi were obtained. The most abundant moulds belong to the genera Alternaria, Cladosporium and Penicillium. They were found with 100% frequency. The higher frequency was detected in Fusarium (100%), Epicoccum, Rhizopus (87.5%), Botrytis, Aspergillus (75%) and Mucor (62.5%) but with lesser relative density. Table 1 lists the fungal isolated from grape berries at harvest time from 2011 to 2013.

Varga et al. (2007) examined the mycobiota of grape berries from 25 Hungarian and Czech vineyards. Most of the fungi isolated from Hungarian grapes belonged to genera *Aspergillus*, *Penicillium*, *Botrytis*, *Alternaria*, *Trichoderma* and *Cladosporium*. From the Czech grapes were isolated *Alternaria*, *Cladosporium*, *Penicillium*, *Rhizopus*, *Epicoccum* and *Aspergillus*. Our mycobiota were very similar. No ochratoxigenic microfungi (*Aspergillus carbonarius*, other black aspergilli, *Aspergillus ochraceus*, *Penicillium verrucosum* or *Penicillium nordicum*) were identified in grapes sampled in Hungarian or Czech vineyards. From two different agro-climatic regions in Spain were sampled in 2011 and 2012 in order to determinate the grape mycobiota **(García-Cela et al., 2015)**. The most common mycobiota isolated in both years were *Alternaria*, *Cladosporium* and *Penicillium*. Colonies belonging to *Aureobasidium*, *Botrytis*, *Eurotium*, *Eppicoccum*, *Fusarium*, *Mucor* and *Trichoderma* were occasionally observed in the samples.

Mikušová et al. (2010) identified the fungi in the grape samples in three out of the six most important Slovakia wine making areas – Small Carpathian, Nitrian and South Slovakian in the harvest year 2008. The following genera of fungi were identified in the range of 1 – 4%: *Cladosporium*, *Epicoccum*, *Rhizopus*, *Ulocladium*, *Trichoderma* and *Trichothecium*. The genera *Aspergillus* (11.4%), *Fusarium* (11.4%), *Penicillium* (29.7%), and *Alternaria alternata* (14.8%) were considered to be predominant among the toxigenic fungi. Also, according to our results belonged the mention genera to the most frequent, but the percentage incidence was much higher and ranges from 75% to 100%. According to the results of **Mikušová et al. (2010)** relative density was lower and did not exceed 2%, and in our case reached the limit to 34.3%.

Rousseaux et al. (2014) summarized the various genera

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

Fungal taxa	No.	Fr (%)	RD (%)
Alternaria	472	100	34.3
Aspergillus	26	75	1.9
Aureobasidium	1	12.5	<1
Botrytis	106	75	7,7
Cladosporium	363	100	26.4
Epicoccum	40	87.5	2.9
Fusarium	55	100	4.0
Gibberella	2	12.5	<1
Harzia	3	12.5	<1
Mucor	11	62.5	<1
Paecilomyces	1	12.5	<1
Penicillium	135	100	9.8
Phoma	7	37.5	<1
Rhizopus	41	87.5	2.9
Sordaria	5	37.5	<1
Trichoderma	6	25	<1
Ulocladium	6	25	<1
Mycelia sterilia	97	87.5	7.0
Total isolates	**1377**		

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

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

Aspergillus species	No.	Fr (%)	RD (%)
A. clavatus	1	12.5	3.8
A. flavus	3	25	11.5
A. section *Nigri*	21	62.5	80.8
A. sp.	1	12.5	3.8
Total isolates	**26**		

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

of filamentous microscopic fungi on grapes from different countries of the world. From these studies which were not carried out in the same vineyard and year and on the same variety seven of the genera of filamentous fungi identified by conventional or molecular techniques (excluding *B. cinerea*) were predominated among isolates: *Alternaria*, *Acremonium*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium* and *Rhizopus*. The percentages of the total microflora accounted for by these seven predominant genera varied considerably between vineyards (geographic location): *Alternaria* (2.8% – 80%), *Acremonium* (0.3% – 0.8%), *Aspergillus* (1% – 79.7%), *Cladosporium* (4.4% – 92.2%), *Fusarium* (0.5% – 18%), *Penicillium* (2.3% – 31%) and *Rhizopus* (0.8% – 2.4%). *Acremonium* was not detected in our samples, however *Alternaria* was the most frequently isolated genus (100% Fr, 34.3% RD).

The *Aspergillus* and *Penicillium* strains were isolated and identified to species level. The isolation rates for Aspergillus from the berries were 75% but the relative densities were low (19%, Table 1). Table 2 shows the number of isolates, isolation frequency (%) and relative density (%) of *Aspergillus* spp.

From the 26 *Aspergillus* strains identified, the species of *Aspergillus* section *Nigri* were the predominant in mycobiota, because they were the most frequent (62.5%) of the isolates and relative density among species was maximum again for *A.* section *Nigri* (80.8%). The molds belonging to the species *Aspergillus niger* and *Alternaria alternata* are common grape pathogens **(Diguta et al., 2011)**.

Rousseaux et al. (2014) introduced, that thirty-six species of *Aspergillus* have been isolated from grapes in vineyards around the world. In our case it was 3 species. *Aspergillus carbonarius* and *A. niger* aggregates belong to section *Nigri* (black aspergilli) are the species most frequently isolated, accounting for 50 – 98.5% of all *Aspergillus* strains isolated **(Rousseaux et al., 2014)**. They have also been identified as the principal producers of OTA and A. carbonarius are responsible for OTA contamination worldwide **(Somma et al., 2012)**. **Felšöciová et al. (2015)** from Small Carpathian winemaking region during the years 2011 and 2013 identified 6 different *Aspergillus* species from the 37 *Aspergillu*s strains. The species of *Aspergillus* section *Nigri* were also the predominant in mycobiota (64% Fr). The species of *A. clavatus* and *A. flavus* were the other most important species recorded with high isolation frequency (21% Fr).

Table 3 lists selected *Penicillium* strains isolated from grape berries. A total of 135 isolates belonging to genus *Penicillium*, were obtained.

The spectrum of identified species includes 9 species. The most frequent species were P. expansum (37.5%), *P. citrinum*, *P. crustosum* and *P. chrysogenum* (25%, each). Relative density among species was maximum for *P. expansum* (28.1%), *P. funiculosum* (11.1%) and *P. citrinum* (8.1%). *Penicillium expansum* is a pathogen on fruits and it has been isolated from a wide range of fruits including apples, pears, tomatoes, strawberries, grapes and others **(Pitt and Hocking, 2009)**. *Penicillium expansum* has been shown to be able to produce geosmin. Geosmin is the principal molecule responsible for earthy, moldy, damp earth and red beet root odors in grape must and wine **(Darriet et al., 2000)**. The molds belonging to the species *P. expansum* and *P. crustosum* are common grape pathogens **(Diguta et al., 2011)**. The type of infecting moulds influenced the concentration of total polyphenol and anthocyanin as well as colour intensity. *Penicillium expansum* and *P. crustosum* greatly affected these parameters **(Lorenzini et al., 2012)**. The most common sources of *Penicillium citrinum* are cereals, fermented and cured meats, wine grapes, dried vine fruits, coffee beans, dired beans, peppercorns, because of its mesophilic nature, distribution is world wide **(Pitt and Hocking, 2009)**. The species *P. brevicompactum*, *P. crustosum*, *P. chrysogenum*, *P. expansum*, *P. palitans* and *P. polonicum* were identified by **Santini et al. (2014)** from three winemaking regions of Slovakia – Small Carpathian, Nitrian and South Slovakian during the years 2008 and 2009. We found wide spectrum of *Penicillium* spp. but without *P. brevicompactum*, *P. palitans* and *P. polonicum*. **Felšöciová et al. (2015)** from Small Carpathian winemaking region during the years 2011 and 2013 identified 13 different Penicillium species from the 251 Penicillium strains. 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). **García-Cela et al. (2015)** isolated from Spanish grape samples in 2011 these species *P. angulare*, *P. aurantiogriseum*, *P. crustosum*, *P. erythromellis*, *P. expansum*, *P. glabrum*, *P. northofagi*, *P. oxalicum*, *P. purpurogenum*, *P. ramulosum*, *P. simile*, *P. vasconiae*, *P. westlingii* and Talaromyces trachyspermus, while in 2012 *P. brevicompactum*, *P. citrinum*, *P. glabrum*,

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

***Penicillium* species**	**No.**	**Fr (%)**	**RD (%)**
P. aurantiogriseum	10	12.5	7.4
P. canescens	2	12.5	1.5
P. citrinum	11	25	8.1
P. crustosum	16	25	1.2
P. decumbens	2	12.5	1.5
P. expansum	38	37.5	28.1
P. funiculosum	15	12.5	11.1
P. chrysogenum	10	25	7.0
P. purpurogenum	4	12.5	3.0
P. sp.	27	62.5	20.0
Total isolates	**135**		

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

P. griseofulvum, *P. mariae-crucis*, *P. minioluteum*, *P. olsonii*, *P. oxalicum*, *P. pinophilum*, *P. purpurogenum*, *P. sizovae* and *Talaromyces* sp. were identified. Ochratoxigenic *Penicillium* species were not found. We isolated from these species only *P. aurantiogriseum*, *P. citrinum*, *P. crustosum*, *P. expansum* and *P. purpurogenum*. Fifty-nine different species of Penicillium have been isolated from grapes in vineyards around the world **(Rousseaux et al., 2014)**. A predominant species of *Penicillium* isolated from grapes differs between vineyards and vintages. For example, *Penicillium chrysogenum* is the species most frequently isolated in Argentina **(Magnoli et al., 2003)**, which is one of the most frequently isolated in our grapes. *Penicillium chrysogenum* occasionally caused spoilage in stored grapes and is not known as a pathogen. It is a ubiquitous fungus and occupies a very range of habitats. **(Pitt and Hocking, 2009)**. *Penicillium brevicompactum* has been identified as the *Penicillium* species most frequently isolated from French and Portuguese vineyards **(Sage et al., 2002; Serra et al., 2005, 2006)**. However, other studies have identified *Penicillium expansum* as the species most frequently isolated from Portuguese **(Abrunhosa et al., 2001)** and French **(La Guerche et al., 2005)** vineyards. **Diguta et al. (2011)** identified P. spinulosum as the most frequently isolated species of *Penicillium*, followed by *P. expansum* and *P. minioluteum*, for the 2008 vintage, in Burgundy. In our samples *P. spinulosum* and *P. minioluteum* were not detected. La **Guerche et al. (2005)** identified *P. expansum* as the predominant species isolated from Bordeaux vineyards. Thus, the distribution of *Penicillium* species, which may generate organoleptic defects, depends on both vineyard and vintage.

Mycotoxins are toxic secondary metabolites detected in various foods (cereals, vegetables, fruits) and drinks (beer, wine) following the development of certain fungi, especially *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp. and *Trichothecium roseum* **(Scott, 2012)**.

In total 34 isolates representing 8 potentially toxigenic species were tested for their toxigenic ability (Table 4). Out of 34 strains, 56% produced at least one mycotoxin as revealed by the method used here.

Aspergillus flavus produced aflatoxin B1 and cyclopiazonic acid (CPA, 1 out of 3 strains screened, each) but did not produce aflatoxin G1. Aflatoxins, including aflatoxin B1 are the most frequently studied of the other mycotoxins, due largely to their highly hepatotoxic nature. In Portugal, 27 aflatoxin B1-producing strains have been isolated from grapes **(Serra et al., 2005)**, 43% of *Aspergillus flavus* isolates from grapes in Lebanon and 23% of *A. flavus* isolates from grapes in Tunisia have been shown to produce this aflatoxin **(El Khoury et al., 2008; Melki Ben Fredj et al., 2009)**.

Ochratoxin A production was tested in 5 strains belonging to *Aspergillus* section *Nigri*. Among them, the production of ochratoxin A was not confirmed. *Penicillium verrucosum* and *P. nordicum*, he only confirmed *Penicillium* species that are able to produce OTA, were not isolated. Ochratoxin A is the main mycotoxin found in the human food chain, it has been shown to be nephrotoxic, hepatotoxic, teratogenic and carcinogenic to animals and, probably, also to humans **(Creppy, 1999)**. Wine is now considered to be the second major source of human exposure to OTA, after cereals. OTA is produced principally by several species of the genera *Aspergillus* (*A. ochraceus*, *A. carbonarius*, *A. niger*) and *Penicillium*. An ability to produce OTA has been reported for 25 – 100% of the A. carbonarius strains isolated from grapes and for 0 – 77% of isolates from the *A. niger* aggregate. Thus, the high abundance of *A. niger* aggregate on grapes is not always correlated with a high level of OTA production **(Rousseaux et al., 2014)**.

Positive toxigenity was detected in *A. clavatus* on patulin. Patulin inhibits the fermenting yeast *Saccharomyces cerevis*iae, it is partially degraded by the addition of sulfur dioxide and completely degraded during alcoholic fermentation **(Díaz et al., 2011)**. It is therefore unlikely to be present in wine.

The genus *Penicillium*, in particular, has been associated with the production of secondary metabolites (including mycotoxins) in food and fruits **(Pitt and Hocking, 2009)**. Considering the 18 total strains examined, the 94.4% of them produced roquefortine C. **Felšöciová et al. (2015)** tested 68 strains on roquefortine C from Small Carpathian winemaking region from exogenous mycobiota which all were positive, too. *Penicillium citrinum* is the main producer of citrinin, a mycotoxin of moderate toxicity **(Pitt and Hocking, 2009)**. The metabolite citrinin, a characteristic yellow-lemon pigment, was produced by all strains of *P. citrinum* under laboratory conditions. Only two strains of *P. expansum* produced this mycotoxin on YES. Citrinin is not degraded during alcoholic fermentation and may be present in very small amounts in wine. However, wine contamination is unlikely, due to the low abundance of citrinin producing species on grapes **(Pitt and Hocking, 2009)**.

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
A. flavus	1*/3**	0/3					1/3		
A. clavatus						1/1			
A. section *Nigri*			0/5						
P. canescens					0/2			0/2	
P. citrinum				5/5					
P. crustosum								4/4	4/4
P. expansum				2/8		3/8			7/8
P. chrysogenum									6/6

Note: * – number of isolates with ability to produce mycotoxin, ** – number of tested isolates, AFB_1 – aflatoxin B_1, AFG_1 – aflatoxin G_1, OTA – ochratoxin A, C – citrinin, G – griseofulvin, P – patulin, CPA – cyclopiazonic acid, PA – penitrem A, RC – roquefortin C.

Penicillium canescens did not produce griseofulvin. *Penicillium crustosum* is the major producer of penitrem A, a powerful neurotoxin and it has been implicated in a tremor syndrome in humans **(Pitt and Hocking, 2009)**. Penitrem A, an intracellular mycotoxin, were produced by all strains of *P. crustosum*. According to **Frisvad et al. (2006)** all isolates of *P. crustosum* produce penitrem A at high levels. Interestingly, this metabolite was not produced by any of the strains of *P. canescens*. Penitrem A is produced only at high moisture levels, above about 0.92 aw, with an optimum around 0.995 aw. This probably explains the relatively low number of reports of poisoning from a very toxic compound produced by a very common fungus **(Pitt and Hocking, 2009)**.

Penicillium expansum, an important producer of citrinin and patulin can cause patulin contamination in must obtaind from grapes **(Samson and Frisvad, 2004)**. Its presence in grapes has been associated with moldy berries, even if patulin is degraded to some extent during the fermentation process **(Abrunhosa et al., 2001)**.

CONCLUSION

Grapes were analyzed by plating methods from South Slovak wine-growing region at the harvest time between 2011 and 2013. From the 1377 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 34 exogenous strains representing 8 potentially toxigenic species, 56% produced at least one mycotoxin. Potential producers of ochratoxin A *Aspergillus* section *Nigri* and roquefortin C *Penicillium* expansum 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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