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MICROFUNGI OF GRAPES FROM SMALL CARPATHIAN REGION IN SLOVAKIA

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ABSTRACT

A total of 10 samples of grapes (bunches) without apparent fungal contamination were analyzed. The samples were collected during the 2017 and 2018 harvest from Suchá nad Parnou and Vrbové village in Small Carpathian region of Slovakia. Samples were sent to mycological laboratory, where they were stored at 4°C until their analysis. The objectives of this study were to gain more knowledge about mycobiota on grapes originating from Slovakia, with a focus on genus *Penicillium* and its ability to produce mycotoxins in *in vitro* conditions by thin layer chromatography method. For the isolation of fungi were used the direct plating technique on DRBC plates while surface sterilized grapes were used for endogenous mycobiota analysis. The plates were then incubated aerobically at 25 ±1 °C for one week in the dark. Overall, we isolated 818 strains belonging to 14 genera of filamentous microscopic fungi from surface mycobiota of grapes. The most frequent were genera *Alternaria*, *Penicillium*, *Rhizopus*, *Sordaria*, *Aspergillus*, *Botrytis*, *Cladosporium* and *Epicoccum*. The main occurring *Penicillium* species of the samples were *P. expansum* (60% Isolation frequency, 93% Relative density). A total of 388 isolates belonging to 12 genera were obtained from endogenous mycobiota. The most frequent and the most abundant genera were *Alternaria*, *Cladosporium* and *Penicillium*. From 3 different *Penicillium* species the most common was again *P. expansum* (30% IF, 92.5% RD). The selected isolates – *P. citrinum, P. expansum*, and *P. chrysogenum* were tested for their toxigenic ability. Out of 69 strains, 74% produced at least one mycotoxin as revealed by the method used here.

Keywords: grape, mycobiota, *Penicillium* sp., mycotoxins, TLC method

INTRODUCTION

The incidence of filamentous fungi and toxin levels in grapes and wines varies depending on the variety of grapes, the wine region, agricultural practices, weather conditions, the harvest and the winemaking process (**Freire *et al.*, 2017**). The mycobiota frequently isolated from grapes includes the genera *Aspergillus, Penicillium, Mucor, Rhizopus, Alternaria, Cladosporium, Botrytis* and *Fusarium* **(Trinidad *et al.*, 2015)**.
Penicillium is a diverse fungal genus of ascomycetous fungi and contains more than 350 species **(Visagie *et al.*, 2014)** playing various roles in natural ecosystems, agriculture, and biotechnology. Species of *Penicillium* are ubiquitous soil fungi, preferring cool and moderate climates commonly present in organic materials. Most of the species are saprophytes and live mainly on organic biodegradable substances **(Kirk *et al*., 2008)**. *Penicilli* have a large economic impact on human life. They have two sides – a good and beneficial one and a bad and economically destructive one. Many species of *Penicillium* are of proven importance because of their widespread occurrence and ability to produce a wide range of bioactive metabolites, including antibacterial, antifungal, immune suppressants, cholesterol-lowering agents etc. **(Petit *et al.*, 2009)**. Some species produce toxins and may render food inedible or even dangerous **(Visagie *et al.*, 2013)**. *Penicillum expansum* can cause rot in grapes, but does not usually attack grapes before harvest. Aside from losses in fruit, this species is regarded as the major producer of patulin, although this species produces many other toxic metabolites such as citrinin, roquefortine C or chaetoglobosins among others **(Andersen *et al.*, 2004)**.
Mycotoxins are secondary metabolities produced by filamentous fungi either pre- or postharvest and which can contaminate agricultural food and feed products and have detrimental effects on human and animal health. Much of the research on mycotoxins, including recent research on the effects of climate change on mycotoxins, has focused on *Aspergillus, Fusarium,* and *Penicillium* species, as they are the major mycotoxin-producing fungi in field crops and stored products in the world **(Paris *et al.*, 2015)**.

The most important mycotoxin in wine is ochratoxin A (OTA) **(Amézqueta *et al.*, 2009; Varga and Kozakiewicz, 2006)**. The role of OTA producing penicillia in contaminating wine is of interest, although isolation from grapes is considered infrequent. However, **Mikušová *et al.* (2010)** isolated OTA-producing *P. verrucosum* from Slovakian grapes. **Rousseaux *et al.* (2014)** reported OTA-producing *Penicillium* species from grapes in northern Italy and France, suggesting they could be involved in OTA contamination. The production of OTA from Chinese penicillia **(Zhang *et al.*, 2016)** requires confirmation **(Perrone *et al.*, 2017)**. In general, isolating OTA fungi from grapes should not exclude penicillia. The aim of our study was to monitor the mycobiota of grapes and determine the characteristic mycotoxin production profiles of *Penicillium* strains (patulin, citrinin, and roquefortin C) isolated from grapes for wine production in the Small Carpathian region of Slovakia.

MATERIAL AND METHODS

Study area

Grape samples were collected in moderate region of Slovakia, as it can be common in the Middle Europe areas, from Terra Parna winery, Suchá nad Parnou and from Sabo winery, Vrbové in Small Carpathian wine region during the years 2017 and 2018 (Table 1). 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 (4260 ha of vineyards) spreads in the western of Slovakia **(ÚKSÚP, 2019a)**. The Small Carpathian wine region is divided into 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 **(ÚKSÚP, 2019b)**. The Small Carpathian wine-growing region has medium climates and abundant moisture.

Table 1 Wine grape varieties used in the study from the Small Carpathian region

No of samVillage	Subregion	Grape variety	Date of harvest	Date of analyses
1. Suchá nad Parnou	Orešanský	Alibernet	27.09.2017	28.09.2017
2. Suchá nad Parnou	Orešanský	Blue Portugal	27.09.2017	28.09.2017
3. Suchá nad Parnou	Orešanský	Rheinriesling	27.09.2017	28.09.2017
4. Vrbové	Vrbovský	Pálava	28.09.2017	29.09.2017
5. Vrbové	Vrbovský	Dornfelder	28.09.2017	29.09.2017
6. Vrbové	Vrbovský	Alibernet	04.09.2018	05.09.2018
7. Vrbové	Vrbovský	Cabernet Sauvignon	04.09.2018	05.09.2018
8. Vrbové	Vrbovský	Dornfelder	04.09.2018	05.09.2018
9. Vrbové	Vrbovský	Pálava	04.09.2018	05.09.2018
10. Vrbové	Vrbovský	Chardonnay	04.09.2018	05.09.2018

Sampling

Two samples of red grapes (Alibernet, Blue Portugal) and one sample of white grape (Rheinriesling) were collected from Suchá nad Parnou and one sample of white grape (Pálava) and one sample of red grape (Dornfelder) were collected from Vrbové in the final stages of maturation of the berries (harvest season), at the end of September, 2017.
Three samples of red grapes (Alibernet, Cabernet Sauvignon and Dornfelder) and two samples of white grapes (Pálava and Chardonnay) were collected from Vrbové at the beginning of September, 2018. The sample comprised 3 bunches of grapes collected across two diagonal transects. Grape samples were put directly each into a sterile plastic bag. Samples were brought into the laboratory and kept at 5 °C till fungal analysis.

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. In this way was determined an exogenous mycobiota. Another 50 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) to remove incidental surface contaminants, 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. The identification of fungal taxa was based on macroscopic and microscopic features, with guidelines by **Pitt and Hocking (2009)**. *Penicillium* strains were isolated and cultivated on MEA (Malt extract agar) **(Samson *et al.*, 2010)**, 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)**. From the pure cultures, genus *Penicillium* was identified to species level based on macroscopic and microscopic characteristics according to the manuals of **Pitt and Hocking (2009), Samson and Frisvad (2004)** and **Samson *et al.* (2002a, 2010)**.

Results evaluation

The obtained results were evaluated and expressed according to isolation frequency (IF) 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:
IF (%) = (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 and patulin were carried out on YES agar and intracellular roquefortin C on CYA agar. A few plugs of mycelium were removed from different points of the colony 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 of 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. Patulin detection was achieved by spraying with 0.5% methylbenzothiazolone hydrochloride (MBTH) (Merck, Germany) in methanol and heating at 130 °C for 8 min and then detected as a yellow-orange spot under visible light. Citrinin was detected directly as an intense yellow-green streak under ultraviolet light (365 nm).

RESULTS AND DISCUSSION

The filamentous fungi identified in 4 white and 6 red grape varieties from surface mycobiota during the years 2017 and 2018 are indicated in table 2. A total of 818 strains belonging to 14 genera were identified. From varieties Alibernet (1), Blue Portugal (2) and Rheinriesling (3) from Suchá nad Parnou in 2017, the highest number of isolates (from 89 to 102) with 8 or 9 genera were found. All samples were colonised by genera *Alternaria, Aspergillus, Cladosporium, Epicoccum* and *Penicillium*, concretely *P. expansum*.

Table 2 Fungi identified in Slovak wine grapes from exogenous mycobiota from 2017 to 2018 by the direct plating method

Fungal taxa	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
Alternaria	36	16	7	35	9	93	90	70	54	73
Arthrinium	3	-	2	2	-	-	-	-	-	-
Aspergillus	3	4	1	1	-	1	1	-	-	-
Aureobasidium	-	-	-	-	-	-	-	-	1	-
Botrytis	2	6	-	1	1	-	-	2	-	12
Cladosporium	19	13	31	2	-	-	-	-	-	2
Epicoccum	8	12	20	-	-	1	2	-	-	-
Eurotium	6	-	-	-	-	-	-	-	-	-
Fusarium	6	-	-	2	2	-	-	-	-	-
Mucor	-	3	-	8	-	-	-	-	-	-
Penicillium	19	29	29	2	4	1	3	-	-	1
P. aurantiogriseum	-	-	-	-	1	-	-	-	-	-
P. brevicompactum	-	-	-	-	1	-	-	-	-	-
P. citrinum	-	-	-	-	-	-	3	-	-	-
P. expansum	19	29	29	2	2	-	-	-	-	1
P. glabrum	-	-	-	-	-	1	-	-	-	-
Rhizopus	-	4	7	5	-	4	4	7	5	2
Sordaria	-	2	4	5	-	9	4		1	2
Trichoderma	-	-	-	1	-					1
∑	102	89	101	64	16	109	104	79	61	93

Legend: 1, 6 - Alibernet, 2 - Blue Portugal, 3 – Rheinriesling, 4, 9 – Pálava, 5, 8 – Dornfelder, 7 - Cabernet Sauvignon, 10 - Chardonnay

The lower number of isolates (64) were isolated from the white variety Pálava (4) from Vrbové in 2017 but on the other hand with the highest number of genera (11). The least isolates and genera of filamentous microscopic fungi were isolated from the red grape variety Dornfelder (5) from Vrbové in 2017. In 2018, the red grape varieties Alibernet (6), Cabernet Sauvignon (7) and the white variety Chardonnay (10) reached approximately the same quantitative and qualitative representation of micromycetes from Vrbové with the dominance of the genus *Alternaria*. The lowest quantitative and qualitative representation of micromycetes was isolated from the Pálava (9) and Dornfelder (8) varieties again with the dominance of the genus *Alternaria*.

Alternaria spp. are ubiquitous plant pathogens that may invade fruit (**Asam *et al.*, 2010**). Fungal contamination of grapes could occur before or during harvest and processing **(Prendes *et al*., 2016)**. Globally, *Alternaria* has been isolated from grapes, musts, wines, and raisins. *Alternaria* cause rot bunch of damaged berries, scrape and pedicels **(Kakalíková *et al*., 2009; Steel *et al*., 2013)**. The exposure to *Alternaria* toxins has been linked to a great variety of adverse effects to both human and animal health **(Dall'Asta *et al*., 2014)**. There are not specific international regulations of *Alternaria* toxins in food **(Trinidad *et al*., 2015)**.

Without surface disinfection, a total of 388 strains belonging to 12 genera were identified during the years 2017 and 2018, while *Alternaria* colonised all the samples examined (Table 3). In 2018, we isolated a lower number of genera from our tested samples (from 2 to 4) than in 2017 (from 3 to 8). Species *Penicillium expansum* were dominated from the samples of Terra Parna (1-3) in 2017. In 2018, *P. expansum* was not detected. The occurrence of *Penicillium* spp. in other samples was generally low. The highest number of isolates was detected in the genus *Alternaria* (53) in the grape variety Pálava (4), however in most samples it was dominated.

Table 3 Fungi identified in Slovak wine grapes from endogenous mycobiota from 2017 to 2018 by the direct plating method

Fungal taxa	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
Alternaria	7	15	8	53	10	37	41	45	11	25
Arthrinium	6	2	3	1	-	-	-	-	-	-
Aspergillus	-	-	-	-	1	-	-	-	-	-
Botrytis	-	1	2	9	-	-	-	-	-	1
Cladosporium	9	11	18	-	1	2	2	5	2	-
Epicoccum	3	1	-	-	-	-	1	-	-	-
Fusarium	-	1	-	-	-	-	-	-	-	-
Rhizopus	-	-	-	1	-			3	1	-
Penicillium	10	2	25	-	-	1	4	-	-	-
P. citrinum	-	-	-	-	-	-	3	-	-	-
P. expansum	10	2	25	-	-	-	-	-	-	-
P. chrysogenum	-	-	-	-	-	1	1	-	-	-
Sordaria	1	1	-	1	-	-	-	-	-	-
Talaromyces	-	-	-	2	-	-	-	-	-	-
Trichoderma	-	-	-	2	-	-	-	-	-	-
∑	36	34	56	69	12	40	48	53	14	26

Legend: 1, 6 - Alibernet, 2 - Blue Portugal, 3 – Rheinriesling, 4, 9 – Pálava, 5, 8 – Dornfelder, 7 - Cabernet Sauvignon, 10 - Chardonnay

Data in table 4 show that, fourteen fungal genera namely *Alternaria, Arthrinium, Aspergillus, Aureobasidium, Botrytis, Cladosporium, Epicoccum, Eurotium, Fusarium, Mucor, Penicillium, Rhizopus, Sordaria* and *Trichoderma* were identified from fresh grape samples. *Alternaria* was the most frequently occurring genus (100%), followed by *Penicillium, Rhizopus* (80%, each), *Sordaria* (70%), *Aspergillus, Botrytis* (60%, each), *Cladosporium* and *Epicoccum* (50%, each). The most abundant genus from exogenous mycobiota was *Alternaria* (59%), followed by *Penicillium* (11%), *Cladosporium* (8%), *Epicoccum* and *Rhizopus* (5%, each) of all the fungi found. The remaining genera were detected in less than 5% of all the isolates. From 5 different *Penicillium* species the most frequent and most abundant was *P. expansum*. *Alternaria* is the main component of wine grape mycobiota from different winemaking regions worldwide **(Magnoli *et al*., 2003; Rousseaux *et al*., 2014; Prendes *et al*., 2015; Tančinová *et al*., 2015)**. *Alternaria* growth on grapes has been reported in several countries such as Argentina, Brazil, Spain, Italy, Portugal, USA, Slovakia, Hungary and Czech Republic. The incidence of *Alternaria* in grapes has been reported in different percentages with respect to total mycobiota: 5–23% in USA, 25% in Italy, 80% in Argentina, 3–18%, 24%, 17% in different studies in Portugal, 75%, 3–58%, 13% in Spain, and *A. alternata* and *A. tenuissima* were reported in 16–19% in Slovakia **(Prendes *et al*., 2016)**.

From the twelve vineyards in the Small Carpathian area were collected 14 samples of wine grapes (white 6, red 8) during harvesting 2011, 2012 and 2013 **(Felšöciová *et al.*, 2015c)**. In these samples were identified 22 genera and 79% of samples were colonized by the genus *Aspergillus*. During the survey, 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 mycobiota. The main occurring aspergillus species of the samples were *A.* section *Nigri* (64%). Black aspergilli have been reported as the predominant fungi from Spanish wine and liqueur grapes at harvesting time, constituting from 88.7% to 98.5% of the total *Aspergillus* isolates **(Bau *et al.*, 2005, 2006; Gómez *et al.*, 2006)**. In our research *Aspergillus* isolates were found in grape samples but with low relative density (1%).

By the endogenous (surface-disinfected) plating method were identified 12 different genera from the 388 fungal strains (Table 4): *Alternaria, Arthrinium, Aspergillus, Botrytis, Cladosporium, Epicoccum, Fusarium, Penicillium, Rhizopus, Sordaria, Talaromyces* and *Trichoderma*. Data in the same table showed, that *Alternaria* was the most frequently occurring genus (100%), followed by *Cladosporium* (80%) and *Penicillium* (50%). The 3 most abundant genera found by descending order and with the highest relative density were *Alternaria* (65%), *Cladosporium* (13%) and *Penicillium* (11%). From 3 different *Penicillium* species the most common in grape samples was again *P. expansum*. **Felšöciová *et al.* (2015a**) investigated an endogenous mycobiota of grapes from Eastern wine region of Slovakia. A total of 582 isolates were obtained that belonged to 10 genera: *Alternaria, Aspergillus, Botrytis, Cladosporium, Epicoccum, Fusarium, Mucor, Penicillium, Rhizopus,* and *Trichoderma*. The most frequent were 4 genera *Alternaria, Aspergillus, Botrytis* and *Penicillium* with 100% frequention. The relative density of genera *Alternaria, Cladosporium* and *Penicillium,* were the highest (42%, 19.6% and 15.8%, respectively) as in our results. Frequency of isolation of *Penicillium* spp. in Spanish vineyards was low **(Bau *et al*., 2005, 2006; Bellí *et al*., 2006; Gómez *et al*., 2006)**. In other surveys made in European vineyards with different climatic conditions, *Penicillium* was the predominant genus **(Abrunhosa *et al.*, 2001; Serra *et al*., 2005, 2006)**. *Penicillium expansum*, the responsible agent of blue mould on fruits, has been reported in grapes in different studies **(Abrunhosa *et al.*, 2001; Serra *et al.*, 2005, 2006; Felšöciová and Kačániová, 2019).**

Table 4 The occurrence, isolation frequency and relative density of filamentous microscopic fungi in exogenous and endogenous mycobiota of grapes (n=10) harvested in Small Carpathian wine region

Fungal taxa	No of isolates exo	Isolation frequency (%)	Relative density (%)	No of isolates endo	Isolation frequency (%)	Relative density (%)
Alternaria	483	100	59	252	100	65
Arthrinium	7	30	0.9	12	40	3
Aspergillus	11	60	1	1	10	0.3
Aureobasidium	1	10	0.1	-	-	-
Botrytis	24	60	3	13	40	3
Cladosporium	67	50	8	50	80	13
Epicoccum	43	50	5	5	30	1
Eurotium	6	10	0.7	-	-	-
Fusarium	10	30	1	1	10	0.3
Mucor	11	20	1	-	-	-
Penicillium	88	80	11	42	50	11
P. aurantiogriseum	1	10	-	-	-	-
P. brevicompactum	1	10	-	-	-	-
P. citrinum	3	10	-	3	10	-
P. expansum	82	60	-	37	30	-
P. glabrum	1	10	-	-	-	-
P. chrysogenum	-	-	-	2	20	0.5
Rhizopus	38	80	5	5	30	1
Sordaria	27	70	3	3	30	0.8
Talaromyces	-	-	-	2	10	0.5
Trichoderma	2	20	0.2	2	10	0.5
Total	818			388		

Legend: No - number of isolated micromycetes from exogenous mycobiota/ from endogenous mycobiota

The toxigenic profile of the 69 *Penicillium* isolates representing *P. citrinum, P. expansum* and *P. chrysogenum* from the Slovak grapes is shown in Table 5. Patulin, citrinin and roquefortin C production was tested by thin layer chromatography method in *in vitro* condition. Almost all the isolates were able to produce mycotoxins. From the exogenous mycobiota 44 strains were tested, namely *P. citrinum* and *P. expansum*. Positive toxigenity was detected for citrinin by *P. citrinum* and *P. expansum*, for roquefortin C by *P. expansum* and 35 strains out of 41 screened produced patulin. From the endogenous mycobiota 25 strains were tested, namely *P. citrinum, P. expansum* and *P. chrysogenum*. Positive toxigenity was detected for citrinin and roquefortin C by *P. expansum* (100%, each) and 10 strains out of 21 produced patulin. All tested strains of *P. citrinum* were positive for citrinin and one strain of *P. chrysogenum* did not produce roquefortin C. Out of 69 strains, 74% produced at least one mycotoxin as revealed by the method used here.

Table 5 Toxinogenity of selected *Penicillium* strains, isolated from exogenous and endogenous mycobiota of wine grapes

***Penicillium* species**	**patulin**	**citrinin**	**roquefortin C**
Toxinogenity of exogenous mycobiota			
P. citrinum	nt	3*/3**	nt
P. expansum	35/41	41/41	41/41
Toxinogenity of endogenous mycobiota			
P. citrinum	nt	3/3	nt
P. expansum	10/21	21/21	21/21
P. chrysogenum	nt	nt	0/1

Legend: * - number of isolates with ability to produce mycotoxin, ** - number of tested isolates, nt – not tested

Patulin is a tetraketide lactone produced by a variety of moulds, in particular, *Aspergillus, Penicillium,* and *Byssochlamys* species **(Puel *et al.*, 2010)**. The main producer of patulin is *Penicillium expansum*, which contaminates mainly apple and apple products, and also other fruits like cherries, blueberries, plums, bananas, strawberries, and grapes, processed grape juice and fermenting wine **(Abrunhosa *et al.*, 2001)**. *Penicillium expansum* is found frequently in botrytized grapes **(Russell *et al.*, 2018)**. The occurrence of patulin in wine is low because it is well-known to be degraded partially by the fermentation process **(Moss and Long, 2002)**. Patulin is thermal resistant, causes gastrointestinal problems, including ulceration, distension and bleeding, skin rashes, and is known to be mutagenic, immunotoxic, and neurotoxic mycotoxin **(Medina *et al.*, 2005)**. Patulin is known to be antibacterial, cytotoxic and perhaps even anticarcinogenic **(Kumar *et al.*, 2018)**. It is classified in group 3 as not classifiable as to its carcinogenicity to human by IARC **(Varga *et al.*, 2015)**. Out of 62 screened strains in our research, 73% produced patulin. The predominant *Penicillium* species was not only *Penicillium expansum* as in our study, but also *Penicillium chrysogenum* (100 %) from Tokaj wine region **(Felšöciová *et al.*, 2015b)**. *Penicillium expansum* produced patulin (15 out of 18 strains screened), citrinin (13 out of 18 strains screened) and all of them produced roquefortin C.

Citrinin is a pentaketide derivate produced principally by species of the genera *Penicillium* and *Aspergillus* (*A. terreus, A. niveus*). *Penicillium citrinum*, the main producer of citrinin, has been isolated from grapes, *P. expansum* could produce this mycotoxin, too. Citrinin, a hepato-nephrotoxic compound, is not degraded during alcoholic fermentation and may be present in very small amounts in wine **(Samson *et al.*, 2011)**. All 68 tested strains were positive on citrinin in our study. The metabolite citrinin, a characteristic yellow-lemon pigment, was also produced by all strains of *P. expansum* from grapes and must under laboratory conditions in Small Carpathian wine growing region of Slovakia in the year 2017 **(Felšöciová *et al.*, 2018)**.

Roquefortin C is the most widespread mycotoxin produced by various fungi, particularly species from the *Penicillium* genus, and is known as an important neurotoxic (paralytic) secondary metabolite **(Cole and Cox, 1981)**. Out of 63 tested strains, 98% produced roquefortin C. A total of 10 wine producing grapes were collected from the Central Slovak region in 2011 and 2012, which involved 7 vineyards (**Felšöciová *et al.*, 2014)**. Twenty five potentially toxigenic species were tested for their toxigenic ability on roquefortin C, namely *P. crustosum, P. expansum, P. griseofulvum, P. chrysogenum* and *P. hordei*. Positive toxinogenity was also high, 92 % strains produced mentioned mycotoxin. **Felšöciová *et al.* (2015c)** also tested 68 *Penicillium* strains on roquefortine C from Small Carpathian winemaking region from exogenous mycobiota of grapes which all were positive.

CONCLUSION

From 10 samples of wine grapes from exogenous and endogenous mycobiota were isolated 1206 strains belonging to 15 genera. *Alternaria, Cladosporium* and *Penicillium* were the most common genera within the surface and endogenous colonisation. The most abundant genus was *Alternaria* almost in all samples. During the survey six *Penicillium* species were isolated: *P. aurantiogriseum, P. brevicompactum, P. citrinum, P. expansum, P. glabrum* and *P. chrysogenum*. Of the 130 fungi of the *Penicillium* genus isolated from grapes, 119 (91%) were *P. expansum*, which shows a higher risk of the toxin in grapes and their derivatives from the region. The present work showed the toxigenic potential of the *Penicillium* species isolated from wine grapes, which indicates the potential risk of mycotoxin accumulation in the fruits. Extracts of fungal strains were analysed for patulin, citrinin and roquefortin C by thin-layer chromatography. Patulin was produced by 73% (45/62), citrinin was produced by 100% (68/68), roquefortin C production was observed in 98% (62/63). Our results showed that the *Penicillium* species commonly isolated from grapes are a source of the mycotoxins patulin, citrinin and roquefortin C. Altogether, these results indicate that the higher presence of potentially toxigenic genera *Alternaria* and *Penicillium* in wine grapes could represent a risk in the health of wine consumers.

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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), 240–242. https://doi.org/10.1046/j.1472-765x.2001.00897.x

AMÉZQUETA, S., GONZÁLEZ-PEÑAS, E., MURILLO-ARBIZU, M., LÓPEZ DE CERAIN, A. 2009. Ochratoxin A decontamination: A review. *Food Control*, 20(4), 326-333. https://doi.org/10.1016/j.foodcont.2008.05.017

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. https://doi.org/10.1021/jf035406k

ASAM, S., KONITZER, K., RYCHLIK, M. 2010. Precise determination of the *Alternaria* mycotoxins alternariol and alternariol monomethyl ether in cereal, fruit and vegetable products using stable isotope dilution assays. *Mycotoxin Research*, 27(1), 23-28. https://doi.org/10.1007/s12550-010-0071-6

BAU, M., BRAGULAT, M., ABARCA, M., MINGUEZ, S., CABAÑES, F. 2005. Ochratoxigenic species from Spanish wine grapes. *Int J Food Microbiol*., 98(2), 125–130. https://doi.org/10.1016/j.ijfoodmicro.2004.05.015

BAU, M., BRAGULAT, M. R., ABARCA, M. L., MINGUEZ, S., CABAÑES, F. J. 2006. Ochratoxin A producing fungi from Spanish vineyards. In HOCKING, A. D., PITT, J. I., SAMSON, R. A., THRANE, U. *Advances in Food Mycology*. Advances in Experimental Medicine and Biology. New York : Springer, 173–179. https://doi.org/10.1007/0-387-28391-9_10

BELLÍ, N., BAU, M., MARÍN, S., ABARCA, M., RAMOS, A., BRAGULAT, M. 2006. Mycobiota and ochratoxin A producing fungi from Spanish wine grapes. *Int J Food Microbiol*., 111, 40–45. https://doi.org/10.1016/j.ijfoodmicro.2006.03.011

COLE, R. J., COX, R. H. 1981. Handbook of Toxic Fungal Metabolites. New York : Academic Press, 937 p. ISBN : 0121797600. https://doi.org/10.1016/c2009-0-03073-6

DALL'ASTA, C., CIRLINI, M., FALAVIGNA, C. 2014. Mycotoxins from *Alternaria*: toxicological implications. In FISHBEIN, C., HEILMAN, J. M. ed. *Advances in Molecular Toxicology*. Amsterdam : Elsevier B.V., 107– 121. https://doi.org/10.1016/b978-0-444-63406-1.00003-9

FELŠÖCIOVÁ S., KAČÁNIOVÁ, M. 2019. Mycobiota in traditional grapes and grape for ice wine production cultivated in Slovakia. *Scientific Papers: Animal Science and Biotechnologies*, 52(1), 70-77.

FELŠÖCIOVÁ, S., MAŠKOVÁ, Z., KAČÁNIOVÁ, M. 2018. Fungal diversity in the grapes-to-wines chain with emphasis on *Penicillium* species. *Potravinarstvo*, 12(**1**), 379-386. https://doi.org/10.5219/882

FELŠÖCIOVÁ, S., RYBÁRIK, Ľ., TANČINOVÁ, D., MAŠKOVÁ, Z., KAČÁNIOVÁ, M. 2014. *Penicillium* strains isolated from grapes grown in the Central Slovak wine region. *Journal of Microbiology, Biotechnology and Food Sciences*, 3(1), 210-214.

FELŠÖCIOVÁ, S., RYBÁRIK, Ľ., TANČINOVÁ, D., MAŠKOVÁ, Z., KAČÁNIOVÁ, M. 2015a. Microfungi and mycotoxins of grapes from Eastern Slovak wine region. *Journal of Microbiology, Biotechnology and Food Sciences*, 4(1), 12-15. https://doi.org/10.15414/jmbfs.2015.4.special1.12-15

FELŠÖCIOVÁ, S., RYBÁRIK, Ľ., TANČINOVÁ, D., MAŠKOVÁ, Z., KAČÁNIOVÁ, M. 2015b. Microfungi and mycotoxins of grapes from Tokaj wine region. *Journal of Microbiology, Biotechnology and Food Sciences*, 4(1), 16-18. https://doi.org/10.15414/jmbfs.2015.4.special1.16-18

FELŠÖCIOVÁ, S., TANČINOVÁ, D., RYBÁRIK, Ľ., MAŠKOVÁ, Z., KAČÁNIOVÁ, M. 2015c. Mycobiota of Slovak wine grapes with emphasis on *Aspergillus* and *Penicillium* species in the Small carpathian area. *Potravinarstvo*, 9(1), 501-508. https://doi.org/10.5219/529

FREIRE, L., PASSAMANI, F. R. F., THOMAS, A. B., NASSUR, R. D. M. R., SILVA, L. M., PASCHOAL, F. N., PEREIRA, G. E., PRADO, G., BATISTA, L. R. 2017. Influence of physical and chemical characteristics of wine grapes on the incidence of *Penicillium* and *Aspergillus* fungi in grapes and ochratoxin A in wines. *International journal of food microbiology*, 241, 181-190. https://doi.org/10.1016/j.ijfoodmicro.2016.10.027

GÓMEZ, C., BRAGULAT, M., ABARCA, M., MÍNGUEZ, S., CABAÑES, F. 2006. Ochratoxin A-producing fungi from grapes intended for liqueur wine production. *Food Microbiol*., 23(6), 541–545. https://doi.org/10.1016/j.fm.2005.09.007
GONZÁLEZ, H. H. L., MARTÍNEZ, E. J., PACIN, A., RESNIK, S. L. 1999. Relationship between *Fusarium graminearum* and *Alternaria alternata* contamination and deoxinivalenol occurrence on Argentinian durum wheat. *Mycopathologia*, 144(2), 97-102. http://dx.doi.org/10.1023/a:1007020822134
GUATAM, A., SHARMA, S., BHADAURIA, R. 2009. Detection of toxigenic fungi and mycotoxins in medicinally important powdered herbal drugs. *The Internet Journal of Microbiology*, 7(2), 1-8. http://dx.doi.org/10.5580/104b
KAKALÍKOVÁ, L., JANKURA, E., ŠROBÁROVÁ, A. 2009. First report of *Alternaria* bunch rot of grapevines in Slovakia. *Australas Plant Dis Notes*, 4, 68–69.
KIRK, P. M., CANNON, P. F., DAVID, J. C., STALPERS, J. A. 2008. Ainsworth and Bisby's Dictionary of the Fungi (10th edition). Wallingford, UK, CT : CABI, 771 p. ISBN: 978 0 85199 826 8. https://doi.org/10.1108/09504120910969104
KUMAR, A., ASTHANA, M., GUPTA, A., NIGAM, D., MAHAJAN, S. 2018. Secondary metabolism and antimicrobial metabolites of *Penicillium*. In GUPTA, V., RODRIGUEZ-COUTO, S. ed. *New and future developments in microbial biotechnology and bioengineering, Penicillium system properties and applications.* Netherlands, UK, US : Elsevier, 47-68. ISBN: 978-0-444-63501-3. https://doi.org/10.1016/b978-0-444-63501-3.00003-x
LABUDA, R., TANČINOVÁ, D. 2006. Fungi recovered from Slovakian poultry feed mixtures and their toxinogenity. *Annals of Agricultural and Environmental Medicine*, 13(2), 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. http://dx.doi.org/10.1046/j.1472765x.2003.01376.x
MEDINA, A., MATEO, R., LÓPEZ-OCAÑA, L., VALLE-ALGARRA, F., JIMÉNEZ, M. 2005. Study of Spanish Grape Mycobiota and ochratoxin A production by isolates of *Aspergillus tubingensis* and other members of *Aspergillus* section *Nigri*. *Applied and Environmental Microbiology*. 71(8), 4696–4702. https://doi.org/10.1128/aem.71.8.4696-4702.2005
MIKUŠOVÁ, P., RITIENI, A., SANTINI, A., JUHASOVÁ, G., ŠROBÁROVÁ, A. 2010. Contamination by moulds of grape berries in Slovakia. *Food Additives & Contaminants: Part A*, 27(5), 738-747. https://doi.org/10.1080/19440040903571754
MOSS, M. O., LONG, M. T. 2002. Fate of patulin in the presence of the yeast *Saccharomyces cerevisiae*. *Food Additives and Contaminants*, 19(4), 387-399. https://doi.org/10.1080/02652030110091163
PARIS, K., PAULA, M., YIN-JUNG, L., NAHRER, K., BINDER, E. M. 2015. 7 Climate change impacts on mycotoxin production. In BOTANA, L. M., SAINZ, M. J. ed. *Climate Change and Mycotoxins*, 133-152. ISBN 978-3-11-033361-9. https://doi.org/10.1515/9783110333619-009
PERRONE, G., LOGRIECO, A. F., FRISVAD, J. C. 2017. Comments on "Screening and identification of novel ochratoxin A-producing fungi from grapes. Reporting Ochratoxin A production from strains of *Aspergillus, Penicillium* and *Talaromyces*. *Toxins*, 9(2), 65. https://doi.org/10.3390/toxins9020065
PETIT, P., LUCAS, E. M. F., ABREU, L. M., PFENNING, L. H., TAKAHASHI, J. A. 2009. Novel antimicrobial secondary metabolites from a *Penicillium* sp. isolated from Brazilian cerrado soil. *Electronic Journal of Biotechnology*, 12(4), 1-9. https://doi.org/10.2225/vol12-issue4-fulltext-9
PITT, J. I., HOCKING, A. D. 2009. *Fungi and food spoilage*. 3rd ed. London, New York : Springer Science + Business Media, LLC, 519 p. ISBN 978 0-387-92206-5. http://dx.doi.org/10.1016/j.ijfoodmicro.2010.08.005
PRENDES, L. P., MERÍN, M. G., ANDREONI, M. A., RAMIREZ, M. L., MORATA DE AMBROSINI, V. I. 2015. Mycobiota and toxicogenic *Alternaria* spp. strains in Malbec wine grapes from DOC San Rafael, Mendoza, Argentina. *Food Control*, 57, 122– 128. https://doi.org/10.1016/j.foodcont.2015.03.041
PRENDES, L. P., ZACHETTI, V. G. L., PEREYRA, A., MORATA DE AMBROSINI, V. I., RAMIREZ, M. L. 2016. Water activity and temperature effects on growth and mycotoxin production by *Alternaria alternata* strains isolated from Malbec wine grapes. *Journal of Applied Microbiology*, 122(2), 481-492. https://doi.org/10.1111/jam.13351
PUEL, O., GALTIER, P., OSWALD, I. 2010. Biosynthesis and toxicological effects of patulin. *Toxins*, 2(4), 613-631. https://doi.org/10.3390/toxins2040613
ROUSSEAUX, S., DIGUTA, C. F., RADOÏ-MATEI, F., ALEXANDRE, H., GUILLOUX-BENATIER, M. 2014. Non-*Botrytis* grape-rotting fungi responsible for earthy and moldy off flavors and mycotoxins. *Food Microbiol*, 38, 104–121. https://doi.org/10.1016/j.fm.2013.08.013
RUSSELL, R., PATERSON, M., VENÂNCIO, A., LIMA, N., GUILLOUX-BÉNATIER, M., ROUSSEAUX, S. 2018. Predominant mycotoxins, mycotoxigenic fungi and climate change related to wine. *Food Research International*, 103, 478-491. https://doi.org/10.1016/j.foodres.2017.09.080
SAMSON, R. A., FRISVAD, J. C. 2004. Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*: new taxonomic schemes, mycotoxins and other extrolites. In *Studies in Mycology 46*, Utrecht, The Netherlands : 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. http://dx.doi.org/10.1007/s11046-005-4201-1
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, p. 283-297. ISBN 90-70351-42-0. http://dx.doi.org/10.1007/s11046-005-4201-1
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.
SAMSON, R. A., PETERSON, S. W., FRISVAD, J. C., VARGA, J. 2011. New species in *Aspergillus* section *Terrei*. *Studies in Mycology*, 69, 39-55. https://doi.org/10.3114/sim.2011.69.04
SERRA, R., BRAGA, A., VENÂNCIO, A. 2005. Mycotoxin-producing and other fungi isolated from grapes for wine production, with particular emphasis on ochratoxin A. *Res Microbiol*., 156(4), 515–521. https://doi.org/10.1016/j.resmic.2004.12.005
SERRA, R., LOURENÇO, A., ALIPIO, P., VENÂNCIO, A. 2006. Influence of the region of origin on the mycobiota of grapes with emphasis on *Aspergillus* and *Penicillium* species. *Mycological Research*, 110(8), 971–978. https://doi.org/10.1016/j.mycres.2006.05.010
STEEL, C. C., BLACKMAN, J. W., SCHMIDTKE, M. 2013. Grapevine bunch rots: Impacts on wine composition, quality and potential procedures for the removal of wine faults. *J Agric Food Chem*, 61(22), 5189– 5206. https://doi.org/10.1021/jf400641r
TANČINOVÁ, D., RYBÁRIK, L., MAŠKOVÁ, Z., FELŠÖCIOVÁ, S., CÍSAROVÁ, M. 2015. Endogenal colonization of grapes berries. *J Microbiol Biotech Food Sci*, 4, 69–73. https://doi.org/10.15414/jmbfs.2015.4.special1.69-73
TRINIDAD, A. V., GANOZA, F. Q., PINTO, V. F., PATRIARCA, A. 2015. Determination of mycotoxin profiles characteristic of *Alternaria* strains isolated from Malbec grapes. *BIO Web of Conferences* 5, p. 02004. EDP Sciences. http://dx.doi.org/10.1051/bioconf/20150502004
ÚKSÚP, 2019a. Home page address: http://www.uksup.sk/ovv-informacie
ÚKSÚP, 2019b. Home page address: http://www.uksup.sk/vinohradnicke-oblasti
VARGA, J., KOZAKIEWICZ, Z. 2006. Ochratoxin A in grapes and grape-derived products. *Trends in Food Science and Technology*, 17(2), 72–81. https://doi.org/10.1016/j.tifs.2005.10.007
VARGA, V., KOCSUBÉ, S., SZIGETI, G., BARANYI, N., TÓTH, B. 2015. *Aspergillus* mycotoxins. In PATERSON, R. R. M., LIMA, N. (Eds.), *Molecular biology of food and water borne mycotoxigenic and mycotic fungi*. Baton Rouge : CRC Press, 165-186. https://doi.org/10.1201/b18645-17
VISAGIE, C. M., HOUBRAKEN, J., FRISVAD, J. C., HONG, S. B., KLAASSEN, C. H. W., PERRONE, G., SEIFERT, K. A., VARGA, J., YAGUCHI, T., SAMSON, R. A. 2014. Identification and nomenclature of the genus *Penicillium*. *Stud. Mycol*., 78, 343-371. https://doi.org/10.1016/j.simyco.2014.09.001
VISAGIE, C. M., HOUBRAKEN, J., RODRIQUES, C., SILVA PEREIRA, C., DIJKSTERHUIS, J., SEIFERT, K. A., JACOBS, K., SAMSON, R. A. 2013. Five new *Penicillium* species in section *Sclerotiora*: a tribute to the Dutch Royal family. *Persoonia - Molecular Phylogeny and Evolution of Fungi*, 31(1), 42-62. https://doi.org/10.3767/003158513x667410
ZHANG, X., LI, Y., WANG, H., GU, X., ZHENG, X., WANG, Y. 2016. Screening and identification of novel ochratoxin a-producing fungi from grapes. *Toxins*, 8(11), 1-14. https://doi.org/10.3390/toxins8110333