**SPECIES OF GENUS ASPERGILLUS ON GRAPE SLOVAK ORIGIN**

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**ABSTRACT**

The aim of this study was to detect species of genus Aspergillus from wine grapes (berries, surface sterilized berries - endogenous mycobiota, from damaged berries and grape juice) of Slovak origin. We analyzed 20 samples of grapes, harvested in 2011 from various wine-growing regions. For the isolation of species we used the method of direct plating berries, surface-sterilized berries (using 0.4% freshly pre-paried chlorine), and damaged berries on DRBC (Dichloran Rose Bengal Chloramphenicol agar). For the determination of fungal contamination of grape juice, we used plate-dilution method and DRBC as medium. The cultivation in all modes of inoculation was carried at 25 ± 1°C, for 5 to 7 days. After incubation Aspergillus isolates were inoculated on the identification media. Representatives of the genus Aspergillus were isolated from 13 samples berries, 7 samples of surface-sterilized berries, 4 samples of damaged berries and 9 samples of grape juice. Overall, representatives of aspergilli were detected in 90% of samples (75 isolates). In this way we focused on the detection of potential producers of ochratoxin A belonging to the genus Aspergillus. Isolates, potential producers of ochratoxin A (Aspergillus niger aggregate and Aspergillus westerdijkiae), were after their identification inoculated on YES medium (Yeast Extract Sucrose Agar) and after 14 days of incubation at 25 ± 1°C, in the dark, we tested them for their ability to produce ochratoxin A using thin layer chromatography. Out of the 16 isolates from isolated potential producers of ochratoxin A none of the isolates of Aspergillus niger aggregate (13 tested) produced ochratoxin A. The isolate of Aspergillus westerdijkiae (1), isolated from the surface-sterilized berries, produced ochratoxin A.

**Keywords:** Aspergillus, ochratoxin A, grape

**INTRODUCTION**

The fungal genus Aspergillus was established in 1729, and includes species that are adapted to a wide range of environmental conditions. Many aspergilla produce mycotoxins in food that may be toxic, mutagenic or carcinogenic in animals. Contamination of grapes by different moulds occurs during preharvesting, harvesting and grape processing. The fungal growth begins in grapes if temperature and humidity are suitable. Rotting and spoilage of grape bunches that are heavily infected with moulds alter in chemical composition and secondary metabolities such as mycotoxins. These mycotoxin hazards (Serra et al., 2006). Mould growth in wine is strongly inhibited by ethanol and anaerobic conditions, during the fermentation process (Otteneder et Majerus, 2000), but possible occurrence of mycotoxins in final products from grapes is high. The occurrence of ochratoxin in wine and fruit juices is a result of poor agricultural practices (Otteneder et Majerus, 2000; Zimmerli et Dick, 1996). In wine the most important mycotoxin is the ochratoxin A (OTA) which is not appreciably degraded during wine making, fermentation process, and storage (Delage et al., 2003). OTA was first detected in wines by Zimmerli and Dick (1995, 1996). Since then, the presence of OTA in imported and locally produced wines has been reported from a number of European and other countries (Varga et Kozakiewicz, 2006). According to studies, the source of OTA in this products are OTA-producing strains from the group Aspergillus section Niger (A. carbonarius and A. niger aggregate) (Valero et al., 2005; Hocking et al., 2007). OTA is produced primarily when A. carbonarius infects berries before harvest. The relatively few toxigenic strains of the relates species, A. niger, may also contribute to OTA contamination, as A. niger is by far the most common species of Aspergillus present on grapes (Chulze et al., 2006).

The aim of our study was to detect species of genus Aspergillus from wine grapes of Slovak origin. The isolates of potential producers of ochratoxin A were tested for their ability to produce this mycotoxin in vitro.

**MATERIAL AND METHODS**

**Samples**

We analyzed 20 samples of grapes, harvested in year 2011 from various wine-growing regions of Slovakia. We analyzed grape variety Chardonnay (number of samples 1, 3, 5), mix (2), Velšek Riesling (4, 7, 11, 12), Riesling (6 and 13), Grünér Veltliner (8), Pinot blanc (9 and 17), Konkordia (10), Pinot gris (14), Pinot noir (15), Sauvignon (16), Cabernet Sauvignon (18), Tramin (19) and Limberger (20).

**Mycological analysis**

For the isolation of species we used the method of direct plating berries, surface-sterilized berries (using 0.4% freshly pre-paried chlorine), and damaged berries on DRBC (Dichloran Rose Bengal Chloramphenicol agar). For the determination of fungal contamination of grape juice, we used plate-dilution method and DRBC as medium. The cultivation in all modes of inoculation was carried at 25 ± 1°C, for 5 to 7 days. After incubation Aspergillus isolates were inoculated on the identification media. We use CYA (Czap Yeast Extract agar), MEA (Malt Extract agar), CY20S (Czap Yeast Extract agar with 20 % Sucrose) as the identification media. In all cases, cultivation proceeded for 7 days in the dark at 25 ± 1°C. To determine particular species, diagnostic literature was used as follows: Klich (2002), Samson et al. (2002, 2010), Samson et Varga (2007). The results were expressed according to isolation frequency (Fr): Fr(%) = (ns/N) x 100
The cultivation for screening of ochratoxin A was carried out on YES (Yeast Extract agar). Isolates were cultivated for 14 days in the dark at 25 ± 1°C. In each tested isolate, 3 pieces of mycelium together with the cultivation medium on an area of approximately 5 x 5 mm were cut from colonies and extracted in 1000 ml of chloroform-methanol (2:1, v/v) on vortex for 2 minutes. 20 μl of liquid phase from extracts along with standard ochratoxin A was visualized directly under UV light (365 nm) as a bluish-green spot.

**RESULTS AND DISCUSSION**

Table 1 shows the results from investigation of the colonization of grapes and grape juice by species of genus Aspergillus. We isolated 74 isolates of genus Aspergillus – *A. clavatus, A. fumigatus, A. flavus, A. niger aggregate, A. versicolor* and *A. Westerdijkiae*. In Table 2 is shown mycological colonization and isolation frequency of analyzed samples. We detected species of genus *Aspergillus* from 85 % samples. Thirty-six species of Aspergillus have been isolated from grapes in vineyards around the world (Roussaux et al., 2014). The most significant potential mycotoxin producers occurring in wine - ochratoxin A were detected representatives of *Aspergillus niger aggregate* (12 isolates), which were detected in all methods of isolation and *A. Westerdijkiae* (1 isolate) from the surface sterilized berries. *A. carbonarius*, most important producer of ochratoxin A in wine, has not been identified. Romero et al. (2005) identified Aspergillus as a predominant genus from berries. *Aspergillus niger* was the most common species but only 3 of 293 isolates screened were ochratoxin A producers. *Aspergillus carbonarius* was less common but 96 % of 48 strains screened were ochratoxigenic (Romero et al., 2005). The highest number of isolates, also the highest isolation frequency was observed in *A. clavatus*. Isolation from grape berries from Portugal described Serra et al. (2005). Tančínová et Labuda (2009) in all tested isolates indicate ability to produce mycotoxin patulin. *A. flavus* (1 isolat) is potential producer of aflatoxins. Ability of isolates from wine grapes identified Chummel et al. (2003). *A. fumigatus* (5 isolates) was isolated from 5 samples. According Doaré-Lebrun (source: Rousseaux et al., 2014) this species is able to produce off-flavours – geosmin and earthy odour on grapes. Species *A. versicolor* (4 isolates) was isolated in 3 samples. Occurrence of this species in the berries is reported by Serra et al. (2005, 2006). *A. Westerdijkiae* was detected only in one sample (1 isolate). Díaz et al. (2009) reported the presence of this species on berries, also. Species was separated from species *A. ochraceus* in 2004 and it is an important producer of ochratoxin A (Frissvad et al., 2004).

<table>
<thead>
<tr>
<th>Species</th>
<th>Berries sterilized</th>
<th>Grapes juice</th>
<th>Damages berries</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. clavatus</em></td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>19</td>
</tr>
<tr>
<td><em>A. flavus</em></td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td><em>A. fumigatus</em></td>
<td>3</td>
<td>1</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td><em>A. niger aggregate</em></td>
<td>8</td>
<td>-</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td><em>Aspergillus sp.</em></td>
<td>8</td>
<td>3</td>
<td>18</td>
<td>32</td>
</tr>
<tr>
<td><em>A. versicolor</em></td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td><em>A. Westerdijkiae</em></td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
<td>10</td>
<td>24</td>
<td>74</td>
</tr>
</tbody>
</table>

Legend: A. – *Aspergillus*, Fr – isolation frequency, sp. – species

### Production of ochratoxin A

Ochratoxin A was first detected as a wine contaminant in 1996 and the role of *Aspergillus section Nigri* and *A. carbonarius* in ochratoxin A production discovered in Europe in 1999 (Battilani et al., 2006). We isolated *A. niger* aggregate (12 isolates) and *A. Westerdijkiae* (1 isolate) as a potential producers of ochratoxin A. Isolat *A. Westerdijkiae* was detected as real producer of ochratoxin A detected by TLC method in *in vitro* conditions. Labuda et Tančínová (2006), Dovičičová et al. (2009), Tančínová et al. (2012) similarly referred to the inability of isolates obtained from samples of Slovak origin to produce ochratoxin A. *A. Westerdijkiae* was separated from *A. ochraceus* (Frissvad et al., 2004), and neither of them is referred to as producer of ochratoxin A in grapes and in wine.

### CONCLUSION

Representatives of the genus *Aspergillus* were isolated from 85% of the analysed samples. We isolated by species *A. clavatus, A. fumigatus, A. flavus, A. niger aggregate, A. versicolor* and *A. Westerdijkiae*. Representatives of *A. niger aggregate* did not produce the most significant mycotoxin studied in vine - ochratoxin A. This mycotoxin was produced only by isolates of *A. Westerdijkiae*. The occurrence of potential producers of mycotoxins as well as their mycotoxins in grapes and the vine should be paid more attention to.

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