

## OCCURRENCE OF *ASPERGILLUS FLAVUS* ON CEREALS IN HUNGARY

BEÁTA TÓTH<sup>1</sup>, NIKOLETT BARANYI<sup>2</sup>, ADRIENN BERKI<sup>2</sup>, ORSOLYA TÖRÖK<sup>1</sup>, ÉVA KÓTAI<sup>1</sup>, ÁKOS MESTERHÁZY<sup>1</sup>, JÁNOS VARGA<sup>2</sup>

<sup>1</sup> Cereal Research Nonprofit Ltd., 6726 Szeged, Alsó kikötő sor 9.

<sup>2</sup> University of Szeged, Faculty of Science and Informatics, Department of Microbiology, 6726 Szeged, Közép fasor 52.  
jvarga@bio.u-szeged.hu

### ABSTRACT

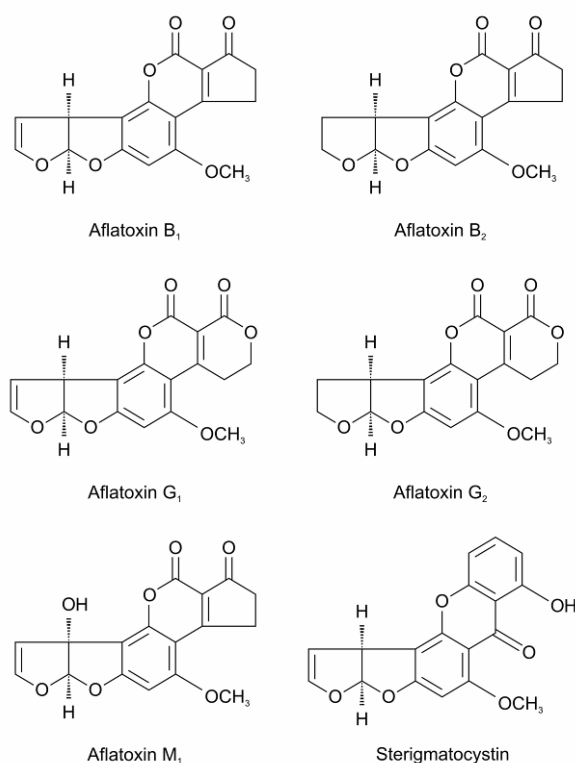
Climate change affects the occurrence of fungi and their mycotoxins in our foods and feeds. A shift has recently been observed in the occurrence of aflatoxin producers in Europe, with consequent aflatoxin contamination in agricultural commodities in several European countries not facing with this problem before including Northern Italy, Serbia, Slovenia, Croatia, Romania and Ukraine. Although aflatoxin contamination of agricultural products is not treated as a serious threat to Hungarian agriculture due to climatic conditions, these observations led us to examine the mycobiota and mycotoxin content of different cereals including maize, wheat and barley collected from different locations in Hungary. The surface-sterilized cereal seeds were placed on selective media, and the isolated fungal strains were identified using morphological and sequence-based methods. Several *Aspergillus flavus* isolates were identified, which are potential aflatoxin producers. This species was identified on cereal seeds in different regions of Hungary. Maize, wheat and barley seeds were contaminated with infection rates of 0.83%, 3.17% and 2%, respectively. Further studies are in progress to examine the aflatoxin producing abilities and genetic variability of the isolates, and mycotoxin content of the cereal samples.

**Keywords:** cereals, *Aspergillus flavus*, aflatoxins, sequence-based identification, climate change

### INTRODUCTION

Mycotoxins are secondary metabolites of filamentous fungi which are harmful to animals and humans, and able to provoke various disease symptoms (VARGA et al., 2009a). Aflatoxins are among the most important mycotoxins, which are produced by species assigned to the *Aspergillus* genus. Among the numerous aflatoxins described, aflatoxin B<sub>1</sub> (Figure 1) is the most toxic aflatoxin, being a potent genotoxic carcinogen in laboratory animals and there is strong evidence for its liver carcinogenicity in humans (WILD and TURNER, 2002). Aflatoxin B<sub>1</sub> exhibits hepatocarcinogenic and hepatotoxic properties, and is referred to as the most potent naturally occurring carcinogen. The International Agency for Research on Cancer has classified aflatoxin B<sub>1</sub> as a group I carcinogen (IARC, 1982). The most important producer, *Aspergillus flavus* is also an important pathogen of various cultivated plants including maize, cotton and peanut, and cause serious yield losses throughout the world. Since aflatoxin production is favoured by moisture and high temperature, *A. flavus* is able to produce aflatoxins in warmer, tropical and subtropical climates (VARGA et al., 2009b). Consequently, aflatoxin contamination of agricultural products in countries with temperate climate, including Central European countries is not treated as a serious health hazard. However, climate change associated with global warming seems to change the scenario. Recently, several papers have dealt with the effects of climate change on the appearance of aflatoxin producing fungi and aflatoxins in foods (PATERSON and LIMA, 2010; TIRADO et al., 2010; MIRAGLIA et al., 2009; COTTY and JAIME-GARCIA, 2007). Based on these studies, aflatoxin producing fungi and consequently aflatoxins are expected to become more prevalent with climate change in countries with temperate climate. Indeed, several recent reports have indicated the occurrence of aflatoxin

producing fungi and consequently aflatoxin contamination in agricultural commodities in several European countries that did not face with this problem before, including Northern Italy (GIORNI et al., 2007), Romania (TABUC et al., 2009), Serbia (JAKSIC et al., 2011), Slovenia (TORKAR and VENGUST, 2007) and Croatia (BILANDZIC et al., 2010). Regarding Hungary, RICHARD et al. (1992) examined the mycotoxin producing abilities of 22 isolates collected from various sources in Hungary, and none of the isolates were found to produce aflatoxins. However, more recently, BORBÉLY et al. (2010) have examined mycotoxin levels in cereal samples and mixed feed samples collected in eastern Hungary, and detected AFB<sub>1</sub> levels above the EU limit in some of the samples. Besides, DOBOLYI et al. (2011) identified aflatoxin producing *A. flavus* from maize kernel collected in various parts of Hungary. These observations led us to examine the occurrence of potential aflatoxigenic species in Hungarian cereals. The obtained isolates were identified using morphological and molecular methods.



**Figure 1. Chemical structures of aflatoxins and sterigmatocystin**

## MATERIALS AND METHODS

### Sample collection

The samples were collected from various cereal growing regions of Hungary in 2010 and 2011. The cereals examined included wheat, maize and barley. The samples were surface sterilized using ethanol, and plated onto dichloran rose bengal (DRBC) media (KING et al., 1979). Plates were incubated at 25 °C in darkness and monitored periodically for characteristic mycelium growing from the kernels. Outgrowing mycelia were purified and transferred to malt extract agar (MEA) and/or Czapek-Yeast Extract agar (CYA) media without antibiotics. Isolates were subcultured as single conidia on MEA, PDA and CYA

plates (SAMSON et al., 2004).

### **Genotypic studies**

The cultures used for the molecular studies were grown on malt peptone (MP) broth for 2 days, and DNA was extracted from the mycelia using the Masterpure™ yeast DNA purification kit (Epicentre Biotechnol.) according to the instructions of the manufacturer. Part of the calmodulin gene was amplified and sequenced as described previously (PILDAIN et al., 2008). Calmodulin sequences were compared using nucleotide-nucleotide BLAST (blastn) with default settings (<http://blast.ncbi.nlm.nih.gov>; ALTSCHUL et al., 1990) to the Genbank database, and to our own sequence database. Species identification was determined from the lowest expect value of the BLAST output.

## **RESULTS**

Several *Aspergillus* species have been identified recently which are able to produce aflatoxins. However, *A. flavus*, *A. parasiticus* and *A. nomius* are the economically most important species regarding aflatoxin contamination of agricultural products (VARGA et al. 2009b). These species can readily be distinguished using sequence analysis of part of their  $\beta$ -tubulin or calmodulin genes (VARGA et al., 2011). In this study, we examined the occurrence of potential aflatoxin producing fungi in cereals in Hungary. The surface-sterilized cereal seeds were placed on selective media, and the isolated fungal strains were identified using morphological and sequence-based methods. The number of primary isolates of each sample was restricted upon the grounds of colony and microscopic features and only the diverging ones were maintained for further investigations. Among the examined samples, several isolates were found to be members of section *Flavi* of the genus *Aspergillus* based on colony morphology and microscopic features (*Figure 2*). Species assignment of the isolates was carried out using partial sequence analysis of the calmodulin gene. In spite of their high morphological variability, concerning the colour of colonies, sporulation, sclerotium formation, and conidiophore structures, all isolates proved to belong to the *Aspergillus flavus* species based on calmodulin sequence data. The proportion of the positive samples varied between 0.83% and 3.17%, depending on the cereal examined (*Table 1*).

## **CONCLUSIONS**

During a survey of aflatoxin producing molds in Hungarian cereal samples in 2010 and 2011, 0.8-3.17% of the maize, wheat and barley samples were found to be contaminated with potentially aflatoxigenic *Aspergillus flavus* isolates. Examination of the aflatoxin producing abilities of the isolates and mycotoxin content of the cereal samples is in progress. A thorough investigation of the mycobiota of other agricultural products also seems to be necessary to estimate the potential effects of climate change on the occurrence of mycotoxin producing Aspergilli in Hungary.



Figure 2. Occurrence of *Aspergillus flavus* in maize seeds collected in Kőszárhegy (left) and Iregszemcse (right)

Table 1. Occurrence of *Aspergillus flavus* on various cereals in Hungary (2010-2011)

Cereal	No. of locations	No. of hybrids/cultivars analyzed	No. of seed samples analysed	No. of <i>A. flavus</i> isolates identified	% of <i>A. flavus</i> -infected samples
Maize	18	6	2160	18	0.83
Wheat	6	4	1200	38	3.17
Barley	3	1	150	3	2.00

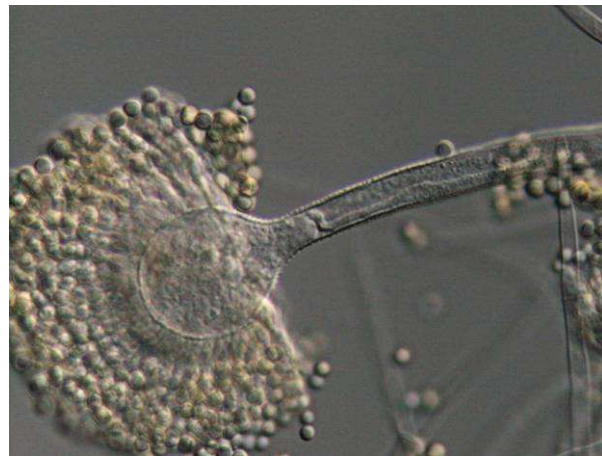
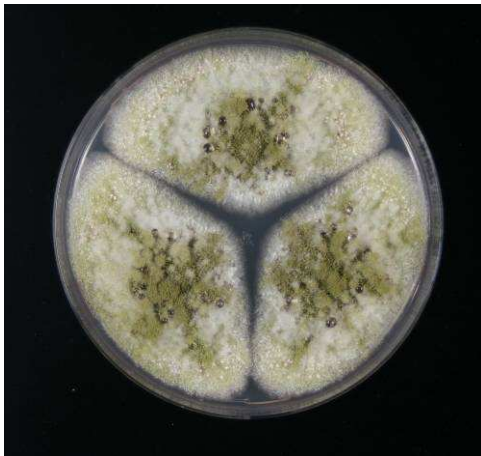


Figure 3. *Aspergillus flavus* colony morphology on CYA medium (left) and microscopic picture of a conidial head (right)

#### ACKNOWLEDGEMENTS

This work was supported by OTKA grant Nos. K84122 and K84077, and by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences (B. Tóth). The project is co-financed by the European Union through the Hungary-Serbia IPA Cross-border Cooperation Programme (ToxFreeFeed, HU-SRB/1002/122/062), and by the European Union and co-funded by the European Social Fund (“Broadening the knowledge base and supporting the long term professional sustainability of the Research University Centre of Excellence at the University of Szeged by ensuring the rising generation of excellent scientists”; TÁMOP-4.2.2/B-10/1-2010-0012).

## REFERENCES

- ALTSCHUL, S.F., GISH, W., MILLER, W., MYERS, E.W., LIPMAN, D.J. (1990): Basic local alignment search tool. *Journal of Molecular Biology*, Vol. 215, pp. 403–410.
- BILANDZIC, N., VARENINA, I., SOLOMUN, B. (2010): Aflatoxin M<sub>1</sub> in raw milk in Croatia. *Food Control*, Vol. 21, pp. 1279-1281.
- BORBÉLY, M., SIPOS, P., PELLE, F., GYŐRI, Z. (2010): Mycotoxin contamination in cereals. *Journal of Agroalimentary Processes and Technologies*, Vol. 16, pp. 96-98.
- COTTY, P.J., JAIME-GARCIA, R. (2007): Influences of climate on aflatoxin producing fungi and aflatoxin contamination. *International Journal of Food Microbiology*, Vol. 119, pp. 109-115.
- DOBOLYI, C., SEBŐK, F., VARGA, J., KOCSUBÉ, S., SZIGETI, G., BARANYI, N., SZÉCSI, Á., LUSTYIK, G., MICSINAI, A., TÓTH, B., VARGA, M., KRISZT, B., KUKOLYA, J. (2011): Aflatoxin-termelő *Aspergillus flavus* törzsek előfordulása hazai kukorica szemtermésben. *Növényvédelem*, Vol. 47, pp. 125-133 (in Hungarian).
- GIORNI, P., MAGAN, N., PIETRI, A., BERTUZZI, T., BATTILANI, P. (2007): Studies on *Aspergillus* section *Flavi* isolated from maize in northern Italy. *International Journal of Food Microbiology*, Vol. 113, pp. 330-338.
- IARC (International Agency for Research on Cancer) (1982): The evaluation of the carcinogenic risk of chemicals to humans. IARC Monograph Supplement 4. International Agency for Research on Cancer, Lyon, France, p. 292.
- JAKSIC, S.M., PRUNIC, B.Z., MILANOV, D.S., JAJIC, I.M., ABRAMOVIC, B.F. (2011): Fumonisin and co-occurring mycotoxins in North Serbian corn. *Proceedings of National Sciences Matica Srpska Novi Sad*, Vol. 120, pp. 49-59.
- KING, A.D.JR., HOCKING, A.D., PITT, J.I. (1979): Dichloran-rose bengal medium for enumeration and isolation of molds from foods. *Applied and Environmental Microbiology*, Vol. 37, pp. 959–964.
- MIRAGLIA, M., MARVIN, H.J., KLETER, G.A., BATTILANI, P., BRERA, C., CONI, E., CUBADDA, F., CROCI, L., DE SANTIS, B., DEKKERS, S., FILIPPI, L., HUTJES, R.W., NOORDAM, M.Y., PISANTE, M., PIVA, G., PRANDINI, A., TOTI, L., VAN DEN BORN, G.J., VESPERMANN, A. (2009): Climate change and food safety: an emerging issue with special focus on Europe. *Food Chemistry and Toxicology*, Vol. 47, pp. 1009-1021.
- PATERSON, R.R.M., LIMA, N. (2010): How will climate change affect mycotoxins in food? *Food Research International*, Vol. 43, pp. 1902-1914.
- PILDAIN, M.B., FRISVAD, J.C., VAAMONDE, G., CABRAL, D., VARGA, J., SAMSON, R.A. (2008): Two novel aflatoxin-producing *Aspergillus* species from Argentinean peanuts. *International Journal of Systematic and Evolutionary Microbiology*, Vol. 58, pp. 725-735.
- RICHARD, J.L., BHATNAGAR, D., PETERSON, S., SANDOR, G. (1992): Assessment of aflatoxin and cyclopiazonic acid production by *Aspergillus flavus* isolates from Hungary. *Mycopathologia*, Vol. 120, pp. 183–188.
- SAMSON, R. A., HOEKSTRA, E. S., FRISVAD, J. C. (2004): *Introduction to Food- and Airborne Fungi*. 7<sup>th</sup> edition. CBS Fungal Biodiversity, Center, Utrecht, Netherlands.
- TABUC, C., MARIN, D., GUERRE, P., SESAN, T., BAILLY, J.D. (2009): Molds and mycotoxin content of cereals in southeastern Romania. *Journal of Food Protection*, Vol. 72, pp. 662-665.
- TIRADO, M.C., CLARKE, R., JAYKUS, L.A., MCQUATTERS-GOLLOP, A., FRANK, J.M. (2010): Climate change and food safety: A review. *Food Research International*, Vol. 43, pp. 1745-1765.
- TORKAR, K.G., VENGUST, A. (2007): The presence of yeasts, moulds and aflatoxin M<sub>1</sub> in

raw milk and cheese in Slovenia. *Food Control*, Vol. 19, pp. 570-577.

VARGA, J., TÉREN, J., RIGÓ, K., TÓTH, B., KOCSUBÉ, S. (2009a): Gombák másodlagos anyagcseretermékei: mikotoxinok, gombamérgek. JATE Press, Szeged (in Hungarian).

VARGA, J., FRISVAD, J.C., SAMSON, R.A. (2009b): A reappraisal of fungi producing aflatoxins. *World Mycotoxin Journal* Vol. 2, pp. 263-277.

VARGA, J., FRISVAD, J.C., SAMSON, R.A. (2011): Two new aflatoxin producing species, and an overview of *Aspergillus* section *Flavi*. *Studies in Mycology*, Vol. 69, pp. 57-80.

WILD, C. P., TURNER, P. C. (2002): The toxicology of aflatoxins as a basis for public health decisions. *Mutagenesis*, Vol. 17, pp. 471-481.