Closing report

NKFI-ID: 116609

Title: **The role of the genera *Bipolaris* and *Curvularia* in mycotoxin contamination of agricultural products**

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The main objective of the project was to assess the prevalence of *Bipolaris* and *Curvularia* species. We also planned to examine the role of B*ipolaris* species in fumonisin contamination of cereals in Hungary. Since most of these species are necrotrophic leaf pathogens, mainly silage samples were planned to be examined from different regions of the country. We tried to clarify whether besides *Fusarium* and *Aspergillus* species these fungi can also contribute to fumonisin burden in animals. In addition to fumonisins we planned to clarify whether these species can contribute to the sterigmatocystin contamination of agricultural products. As part of the research on the mycotoxin producing potential of these economically important species, we investigated the correlation of the presence of genes involved in the biosynthesis of fumonisins and the observed toxin producing abilities. We also investigated the applicability and phylogenetic informativeness of a fragment of the GPDH gene used for species delineation by Manamgoda et al. (2014). Parallel to this work we examined the applicability of other genes for example RNA polymerase II (RPB) and a putative ribosome biogenesis protein (Tsr1) used in species identification of other genera. According to our objectives the research project will constitute the five basic fields listed below:

The following main research tasks were planned to achieve these objectives:

1. Examination of the phylogenetic applicability of the GPDH gene for exact species identification, and testing other genes previously used in our studies for Aspergilli.

2. Primer design for some genes taking part in the biosynthesis of fumonisins and screening the presence of these genes in all isolates. Development of a PCR based technique to distinguish between producing and non-producing isolates.

3. Isolation and identification of environmental *Bipolaris* and *Curvularia* isolates from different field crops in Hungary.

4. Optimisation of the culture conditions to obtain well detectable amounts of fumonisins and/or sterigmatocystin.

5. Measuring the toxin producing abilities by using HPLC and HPLC-MS techniques.

Examination of the phylogenetic applicability of the GPDH gene for exact species identification, and testing other genes previously used in our studies for Aspergilli.

For phylogenetic studies reference sequences were downloaded from the GenBank based on the well-established previous studies. A total of 107 *Curvularia* and 40 *Bipolaris* sequences were used as reference in our experiments. Nucleic acid was isolated from 79 strains previously deposited in the Szeged Microbiology Collection (SZMC) by the use of the Epicentre MasterPure™ Yeast DNA Purification Kit. Amplification and sequencing of the ITS region and TEF sequences was successful from each strain. Despite of several attempt with various amplification conditions and different enzymes, we were not able to amplify the desired part of the GPDH gene. In most cases, four amplicons with size very close together were formed during the reaction, otherwise no successful amplification was observed. Previously, we hypothesized that the members of this two genera may contain more than one copy of this gene, which are not transcriptionally active, but we found no evidence for this by the thorough examination of the available genomes. We have examined more than 400 available sequences covering almost all species of the two genera. Based on sequence alignment of the available sequences we designed new primer-pairs for the amplification of part of the GPDH gene. Our primers GPDHKSfor 5'-TTYATYGARCCYCACTAYGCTGTAAG-3' and GPDHKSrev 5'-TYACAACCTTCTTYGCMCCHCCCTTCA-3' successfully amplified a ~350 bp part of the GPDH gene. To extend the amplifiable region we tested our primers in combination with the previously published gpd1 and gpd2 (Berbee et al., 1999) primers. Only the combination of GPDHKSfor and gpd2 resulted in successful amplification of a single fragment indicating that the primer gpd1 has more than one binding site in the genome. For further experiments we used this combination to amplify a part of the GPDH gene.

To obtain more information about the phylogenetic relations of *Bipolaris* and *Curvularia* species we tested the applicability of several primers used by our group in previous studies. We successfully amplified a part of the RNA polymerase II largest (*RPB1*) and second largest (*RPB2*) subunit from several species. According to our results these loci can be applied successfully for the exact species identification. We also amplified a part of the calmodulin gene of the several isolates using cmd5 and cmd6 or CF1 and CF4 primers but the amplification was not successful in every case. Based on the obtained sequence data it seems that this gene will not be suitable for exact differentiation between the members of these two genera. We also amplified a part of the minichromosome maintenance complex component 7 (*MCM7*) with primers mcmF709 and mcmR1340. The amplification was successful in every examined isolate and according to the phylogenetic examination this region is suitable for the identification at the species level however, further work is needed to prove this. Testing the applicability of sequences of a part of the beta tubulin gene (*BenA*) is also in progress. We also tested three other loci namely acl1 ATP citrate lyase (*acl1*), chaperonin containing TCP1 subunit 8 (*cct8*) and ribosome biogenesis protein (*tsr1*). Despite several attempts involving numerous isolates we had no amplicons for *acl1* and *cct8* by the use of aclR943/aclR230 and cct8F660/cct8R1595 primers, respectively. The amplification of a part of the tsr1 gene by the tsrF1626 and tsrR2434 primer pair was also unsuccessful in several cases. Based on our results RPB1 and RPB2 could be successfully used for the identification of *Bipolaris* and *Curvularia* species, however there are only limited data available for these sequences especially for the genus *Bipolaris*.

Phylogenetic analyses were conducted using the ITS, TEF and GPDH sequences of 147 reference strains and those amplified from 79 strains from the SZMC culture collection. During the initial experiments the intron-freee TEF sequences were aligned by MAFFT v7.149b with the L-INS-i option while the ITS region was aligned using PRANK v.140603 with a guide tree obtained from a previously performed Maximum Likelihood inference, based on an alignment prepared using MAFFT v7.149b. As ITS sequences contain large number of indels, we

converted them to a binary matrix by FastGap 1.2 and added the matrix to the dataset. GPDH sequences also contain one intron with important indel information therefore a binary matrix based on indels was also generated. The ITS dataset was partitioned by ITS1, 5.8S rRNA, ITS2 and the binary matrix, while the GPDH alignment was partitioned based on the exon and intron regions plus the binary matrix. In subsequent experiments we performed all alignment using Canopy v0.1.4. All ML analyses was carried out with the aid of RAxML under the GTR+GAMMA model with 1000 bootstrap replicates.

We examined the applicability ITS, TEF and GPDH sequences for exact species identification. We focused primarily on the GPDH region as this gene was chosen as a secondary barcode for the two genera. We found that among *Bipolaris* species the resolving potential of GPDH sequences was good, the majority of the species were distinguishable, however, some species pairs had short branches (Figure 1).

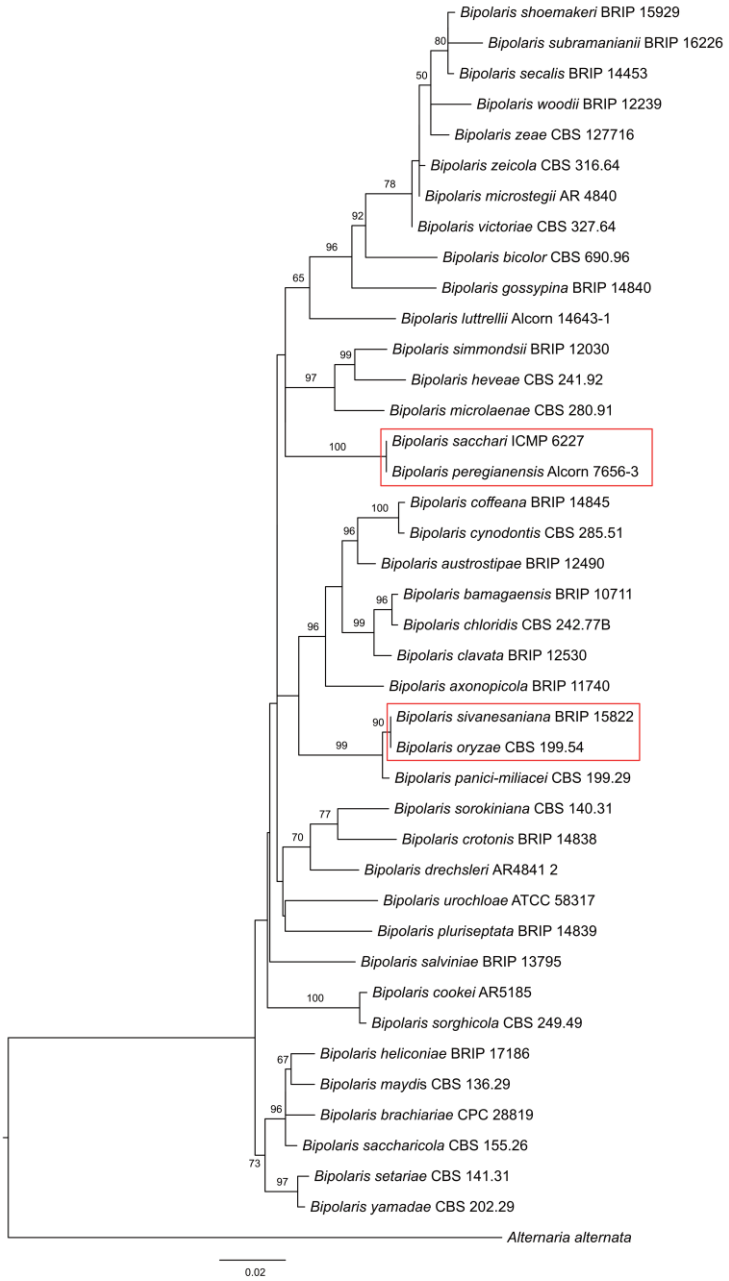

Figure 1. Maximum likelihood phylogram of *Bipolaris* species based on GPDH sequences. Species with identical sequences highlighted with red square.

Only two species pairs had completely identical sequences, namely *B. sacchari*/*B. peregianensis* and *B. sivanesaniana*/*B. oryzae*. These species can be reliably distinguished by the analysis of either TEF or ITS sequences.

In the case of *Curvularia* species three species pairs *C. crustacea*/*C. ryleyi*, *C. gudauskasii*/*C. harveyi* and *C. lunata*/*C. chiangmaiensis* had identical GPDH sequences (Figure 2). The ITS region had moderate resolving potential as several species shared identical sequences. Although some species had identical TEF sequences the above mentioned three species pairs were distinguishable based on this gene. Our results show that TEF and GPDH sequences alone are not completely suitable as secondary barcode, but with a combined use every *Curvularia* and *Bipolaris* species can be identified at the species level.

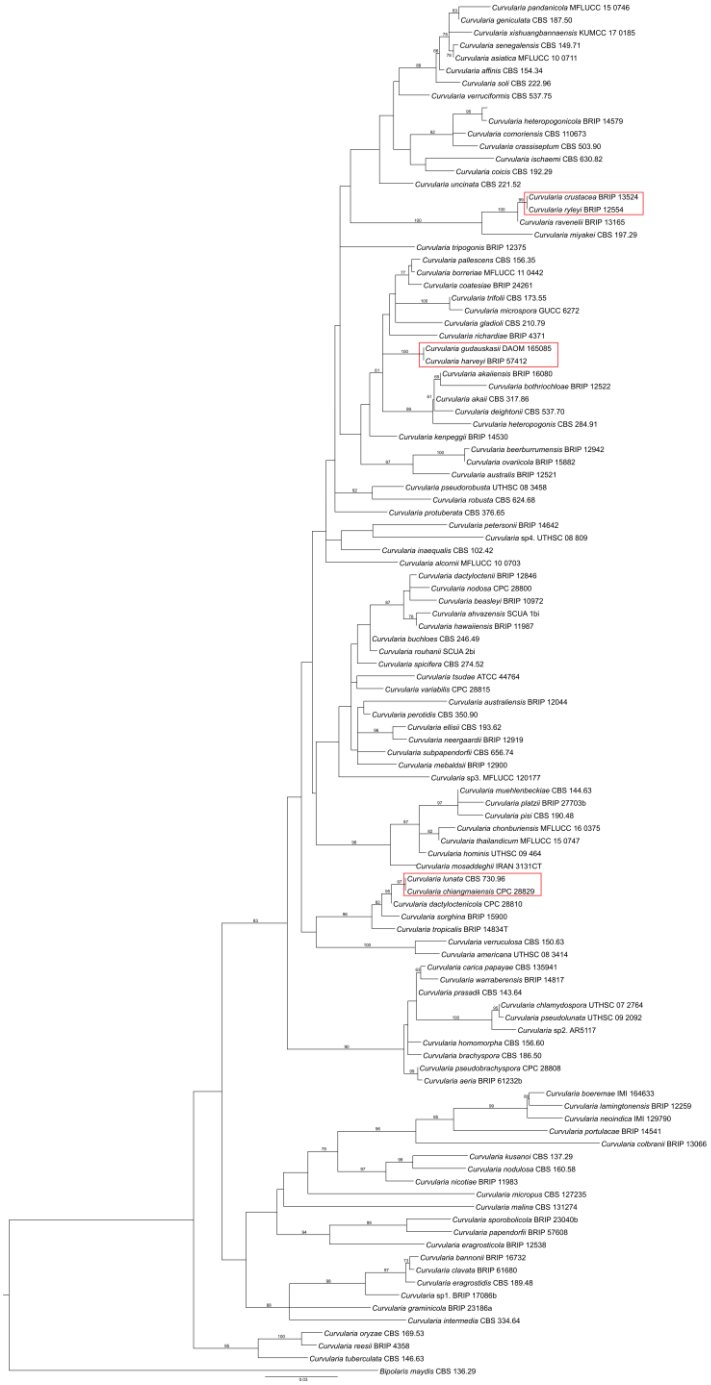

Figure 2. Maximum likelihood phylogram of *Curvularia* species based on GPDH sequences. Species with identical sequences highlighted with red square.

During the implementation period we examined several isolates originated from keratomycosis cases from India. In the case of four strains the exact species identification was not possible. The four isolates formed a separate clade based on the examined TEF, ITS and GPDH sequences and represented two undescribed species (Figure 3).

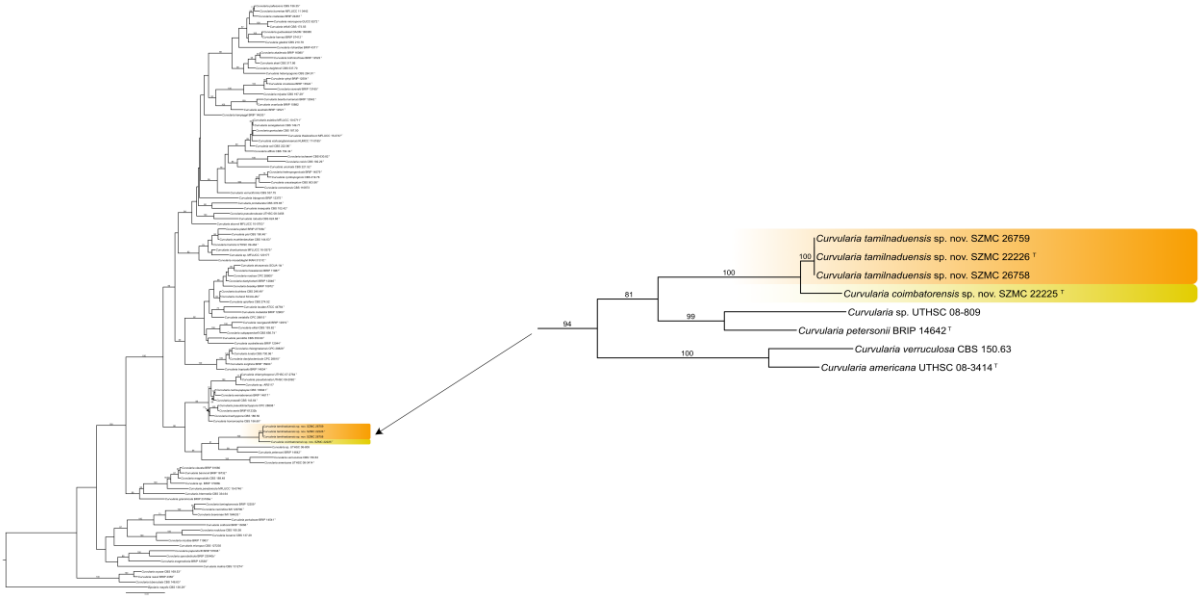

Figure 3. ML phylogenetic tree showing the position of the two new *Curvularia* species.

Our group described the two species under the name of *C. coimbatorensis* and *C. tamilnaduensis*. The results of this project have been published in an open-acces journal (accepted in 2019):

Kiss, N.; Homa, M.; Manikandan, P.; Mythili, A.; Krizsán, K.; Revathi, R.; Varga, M.; Papp, T.; Vágvölgyi, C.; Kredics, L.; Kocsubé, S. New Species of the Genus *Curvularia*: *C. tamilnaduensis* and *C. coimbatorensis* from Fungal Keratitis Cases in South India. *Pathogens* 2020, *9*, 9.

The phylogenetic techniques and secondary metabolite analyses used in these studies were also applied in other experiments during the grant period therefore the OTKA identifier has been indicated in an abstract (Power of Microbes in Industry and Environment 2016) and 4 articles published in Frontiers in Microbiology, Toxins, Frontiers in Cellular and Infection Microbiology and PloS One.

Sándor Kocsubé, Giancarlo Perrone, Donato Magistà, Jos Houbraken, János Varga, Jens C. Frisvad, Robert A. Samson Evidence for the monophyletic origin of Aspergilli based on multilocus phylogenetic analysis *Power of microbes in industry and environment 2016*

Sonderegger, C., Váradi, G., Galgóczy, L., Kocsubé, S., Posch, W., Borics, A., Dubrac, S., Tóth, GK., Wilflingseder, D., Marx, F. (2018). The Evolutionary Conserved γ-Core Motif Influences the Anti-*Candida* Activity of the *Penicillium chrysogenum* Antifungal Protein PAF. *Frontiers in Microbiology*, *9*, 1655. http://doi.org/10.3389/fmicb.2018.01655

Szabo, B., Toth, B., Toth Toldine, E., Varga, M., Kovacs, N., Varga, J., Kocsube, S., Palagyi, A., Bagi, F., Budakov, D., Stojšin, V., Lazić, S., Bodroža-Solarov, M., Čolović, R., Bekavac, G., Purar, B., Jocković, D., Mesterházy, A. (2018) A New Concept to Secure Food Safety Standards against *Fusarium* Species and *Aspergillus Flavus* and Their Toxins in Maize. *Toxins* 2018, 10(9), 372; doi:10.3390/toxins10090372

Kartali, T., Nyilasi, I., Szabó, B., Kocsubé, S., Patai, R., Polgár, T. F., … Papp, T. (2019). Detection and Molecular Characterization of Novel dsRNA Viruses Related to the *Totiviridae* Family in *Umbelopsis ramanniana*. *Frontiers in cellular and infection microbiology*, *9*, 249. doi:10.3389/fcimb.2019.00249

Vigneshwari, A., Rakk, D., Németh, A., Kocsubé, S., Kiss, N., Csupor, D., … Szekeres, A. (2019). Host metabolite producing endophytic fungi isolated from Hypericum perforatum. *PloS one*, *14*(5), e0217060. doi:10.1371/journal.pone.0217060

Primer design for some genes taking part in the biosynthesis of fumonisins and screening the presence of these genes in all isolates. Development of a PCR based technique to distinguish between producing and non-producing isolates.

Publicly available genome sequences of *Cochliobolus heterostrophus* (*B. maydis*) C4 and C5, *Cochliobolus sativus* (*B. sorokiniana*) ND90Pr, *Cochliobolus miyabeanus* (*B. oryzae*) ATCC 44560, *Cochliobolus victoriae* (*B. victoriae*) FI3, *Cochliobolus carbonum* (*Bipolaris zeicola*) 26-R-13 and *Cochliobolus lunatus* (*Curvularia lunata*) m118 were analysed for secondary metabolite gene clusters by the use of antiSMASH 2.0 software package. We focused primarily on type I polyketide synthases (PKS) during the search. In *C. heterostrophus* C4 we identified 12, while the C5 strain contained 11 type I PKSs. *C. sativus*, *C. miyabeanus*, *C. victoriae*, *C. carbonum* and *C. lunata*, strains contained 11, 12, 12, 11 and 9 type I PKSs, respectively. The gene cluster responsible for sterigmatocystin biosynthesis was not identified in any of the examined genomes. In the genomes of *C. heterostrohus* C4 and C5 as well as *C. sativus* the biosynthetic genes responsible for fumonisin production were found, however in the case of *C. carbonum* and *C. sativus* the genes were not organized in cluster and some of the required genes were missing. Based on the results of antiSMASH we carried out a thorough search for genes other than the PKS responsible for fumonisin biosynthesis. In C4 and C5 strains we identified 11 (Figure 1.) homologues with genes involved in fumonisin biosynthesis in *Fusarium verticillioides*. The genes found were *fum1*, *fum3*, *fum6*, *fum7*, *fum8*, *fum10*, *fum13*, *fum14*, *fum15*, *fum19* and *fum21*. All of the genes were located on a single scaffold (scaffold 8 in *C. heterostrophus* C4 and scaffold 14 in *C. heterostrophus* C5) and organised in a cluster spanning ~41.5 kbp in the genome. Other genes which are part of the fumonisin biosynthetic cluster in Fusarium verticillioides were also found with high similarity scores (*fum11*, *fum2*, *fum16*, *fum17*, *fum18*, *mpu1*, *npt1*, *png1*, *wdr1*, *znf1* and *orf21*) however, these were located on different scaffolds. Two genes had no homologues in the genom of the two *B. maydis* isolates, namely *zbd1* and *orf20*. Genes that were identified in the cluster are also present in fumonisin producing *Aspergillus niger* and *Tolypocladium inflatum* strains (Figure 4).

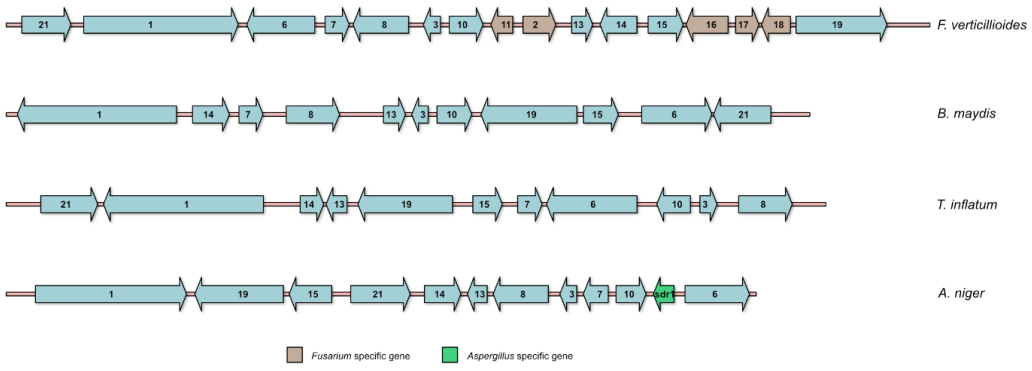

Figure 4. Organisation of fumonisin biosynthetic genes in *F. verticillioides*, *B. maydis*, *T. inlatum* and *A. niger*. Sizes of arrows and the space between them are proportional to the gene lengths and the intergenic regions.

On the basis of the available genomes we designed primer pars targeting the *fum1*, *fum3* and *fum8* genes (Table 1).

Table 1. Sequences of the designed primers and the length of the amplified region.

	Sequence	Amplicon size
Bfum1f	5'-GTAGACGGATTTCATAGTCCATCA-3'	~500 bp
Brum1r	5'-TCGCACAGCTTCGTGAAGCGCA-3'	
Bfum3f	5'-GCGCCAACACTATCAACGT-3'	~470 bp
Bfum3r	5'-GTAACCGTCCATCCAGAGAC-3'	
Bfum8f	5'-TGTCGTTACAGCTGCTAGCT-3'	~560 bp
Bfum8r	5'-AGGAAACTGCCGAGGTACGT-3'	

By using the three primer sets we screened 37 isolates representing 8 species from the major clades of *Bipolaris* (*B. oryzae*, *B. maydis*, *B. sorokiniana*, *B. microlaenae*, *B. eleusines*, *B. sivnesaniana*, *B. zeae* and *B. secalis*) and one *Johnalcornia aberrans* (previously *B. aberrans*) isolate available in the SZMC collection. Only *B. maydis* (SZMC 13039B, SZMC 13033, SZMC 3091 and SZMC 13014) and *B. sorokiniana* (SZMC 13045, SZMC 13091 and SZMC 13005) isolates contained all three genes. We also tested 23 Curvularia isolates belonging to 10 species (*C. lunata*, *C. australiensis*, *C. nodosa*, *C. hawaiiensis*, *C. spicifera*, *C. caricae-papayae*, *C. akaiiensis*, *C. muehlenbeckiae*, as well as the recently described *C. coimbatorensis* and *C. tamilnaduensis*). Despite several attempts we were not able to amplify the fragments of the three fumonisin biosynthetic genes from any of the species.

Regardless of the unsuccessful amplification of fumonisin biosynthetic genes from all *Curvularia* species we decided to include them in further experiments.

Isolation and identification of environmental *Bipolaris* and *Curvularia* isolates from different field crops in Hungary.

During the implementation period we screened several silage products for the presence of *Bipolaris* and *Curvularia* species. Samples were inoculated onto dichloran rose Bengal chloramphenicol agar (DRBC) and dichloran 18% glycerol agar (DG18) plates. None of the silage samples were found to be contaminated by *Bipolaris* or *Curvularia* species. The vast majority of the isolates were the members of the genera *Aspergillus, Penicillium, Fusarium* and *Alternaria*. Besides this, crop samples from agricultural areas of Szeged, Makó and Békéscsaba were also monitored for the presence of *Bipolaris* and *Curvularia* species during the spring and summer. Despite the large number of samples we found only a few isolates belonging to the genera of interest. Only four *Curvularia spicifera,* and seven *Bipolaris sorokiniana* isolates were found during the implementation period. Our results indicate that the prevalence of *Bipolaris* and *Curvularia* species is low in our country.

Optimisation of the culture conditions to obtain well detectable amounts of fumonisins and/or sterigmatocystin.

To investigate the fumonisin producing ability of *Bipolaris* and *Curvulari*a species we selected 20 isolates representing eight species (3 *B. maydis*, 2 *B. sorokiniana*, 1 *B. zeae* 2 *B. oryzae*, 2 *B. microlaenae*, 3 *C. spicifera*, 3 *C. australiensis*, 2 *C. hawaiiensis* and 2 *C. lunata*). All isolates were inoculated on Czapek Yeast Autolysate (CYA) agar, CYA supplemented with 20% sucrose (CYA20), Yeast Extract Sucrose (YES) agar, Malt Extract agar (MEA), Czapek-Dox Agar supplemented with 8% sorghum, modified V8 agar (20% Bio vegetable cocktail from Biopont Kft supplemented with 0.2% $CaCO_3$ and 1.5% agar) and rice. The samples were incubated in darkness at 25 °C and 30 °C for 10 days. The whole agar content of the Petri dishes from each media was homogenized and extracted with of MeOH/H_2O (3:1 v/v). All samples were vacuum-dried and re-dissolved in 1 ml extraction solvent. Identification of fumonisins and sterigmatocystin was carried out by HPLC-MS technique using a LC-MS 2010A (Shimadzu) instrument. Separation was performed on a Gemini NX (Phenomenex) column (150x2 mm, 3 µm) thermostated at 40 °C. The mobile phase consisted of A: H_2O + 0.1% formic acid, and B: MeOH/MeCN (1/1 v/v) + 0.1% formic acid with flow rate of 0.2 ml/min. The gradient program was the following: 0 min 20% B, 1 min 20% B, 5 min 95% B, 9 min 95% B, 10 min 20% B, 25 min 20% B. The MS instrument equipped with an ESI source operated in positive ion mode using air as nebulizing and drying gas with flow of 1.5 L/min. Positive ions were acquired in selected ion monitoring mode with voltages of the detector and the interface 1.5 kV and 4.5 kV, respectively. Both the curved desolvation line (CDL) and Q-array were run with voltages of 25 V. The CDL and the heatblock temperature were set to 250 °C and 200 °C.

Under these experimental conditions none of the examined isolates produced detectable amount of sterigmatocystin and the majority of the isolates were atoxigenic regarding fumonisins, except three *B. maydis* strains. Fumonisins were not detectable in modified V8 agar, YES and rice. We were able to identify fumonisin-like isomers in CYA, CYA20 and sorghum media in the case of all examined *B. maydis* isolates however, only in trace amounts. The highest amounts of isomers were produced on MEA, but we were not able to identify them exactly as the concentration of suspicious compounds were low. According to our experiments the applied temperatures had no influence on the amount of the produced toxin.

Measuring the toxin producing abilities by using HPLC and HPLC-MS techniques.

The experiments were repeated with the use of MEA as culturing media involving the three *B. maydis* isolates. The agar content of four Petri dishes was subjected to extraction with MeOH/H_2O (3:1 v/v). Despite the higher concentration of the extracts the identification of the structure of the compound by fragmentation failed, presumably because of the high melanin content of the samples. The extracts were cleaned-up by using SAX column and the fumonisin content was measured. After the clean-up the compound with protonated molecular weight of *m/z* 706,4 was still present in the extracts. We were able to detect the presence of fumonisin isomer-like compounds in MEA medium on 25 °C and 30 °C, as well. Presence of FB1 was not detectable. Based on the positive ion chromatogram we surmised that the samples contained FB2 or FB3 isomers (Figure 5).

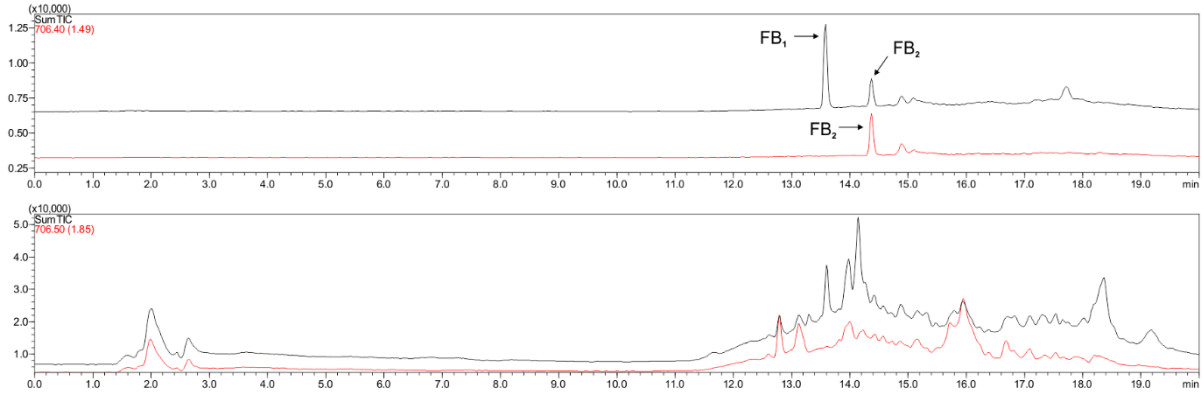

Figure 5. HPLC chromatogram of FB2. Red curve shows the selected ion chromatogram of FB2-like isomer detected in *B. maydis* SZMC 13014 (CBS 130.26) isolate.

As the three *B. maydis* isolates had identical chromatograms regarding the fumonisin like isomers, we decided to continue the experimetns only with isolate SZMC 13014 (CBS 130.26) During further analyses of extracts from *B. maydis* SZMC 13014 we determined the exact molecular masses and the composition of the molecules by Orbitrap mass analyser. The extracts were analysed by a Dionex Ultimate 3000 UHPLC system equipped with a membrane degasser, a binary pump, a standard autosampler and a thermostable column compartment. The compounds were separated using a Gemini-NX C18 (3 µm, 50 x 2 mm) column equipped with a Gemini-NX C18 guard column (5 µm, 4 x 2 mm), thermostated at 25 °C. Mobile phase A consisted of water containing 0.1 % formic acid, while methanol containing 0.1% formic acid served as mobile phase B. The gradient elution was performed as follows: 0 min, 20% B; 1 min, 20% B; 5 min, 95% B; 9 min, 95% B; 10 min, 20% B; 25 min, 20% B. The flow rate was set to 0.2 ml/min. The injection volume was 5 µl.

Mass analyses were performed using a Q Exactive Plus hybrid quadrupole-Orbitrap mass spectrometer coupled to the abovementioned UHPLC system. Ionization of analytes was performed using heated electrospray interface (HESI) in positive electrospray ionization. The temperature of ion transfer capillary, spray voltage, sheath gas flow rate and the auxiliary gas flow rate were set to 350 °C, 3.5 kV, 30 and 15, respectively. The compounds were detected in PRM mode monitoring the characteristic molecular ions of the known fumonisins and fumonisin-like compounds. The extracts were analysed in full scan – ddms2 mode either.

Beside the protonated molecular ion of the fumonisin B2 (*m/z* 706.3551) other molecules (m/z 687.3412, 705.3517, 715.3835 and 723.3623) with the same polarity were detected (Figure 6). MS2 experiments revealed similar fragmentation pattern of the abovementioned molecules to that of fumonisins however, the composition of the fragments predicted by the Xcalibur software was different.

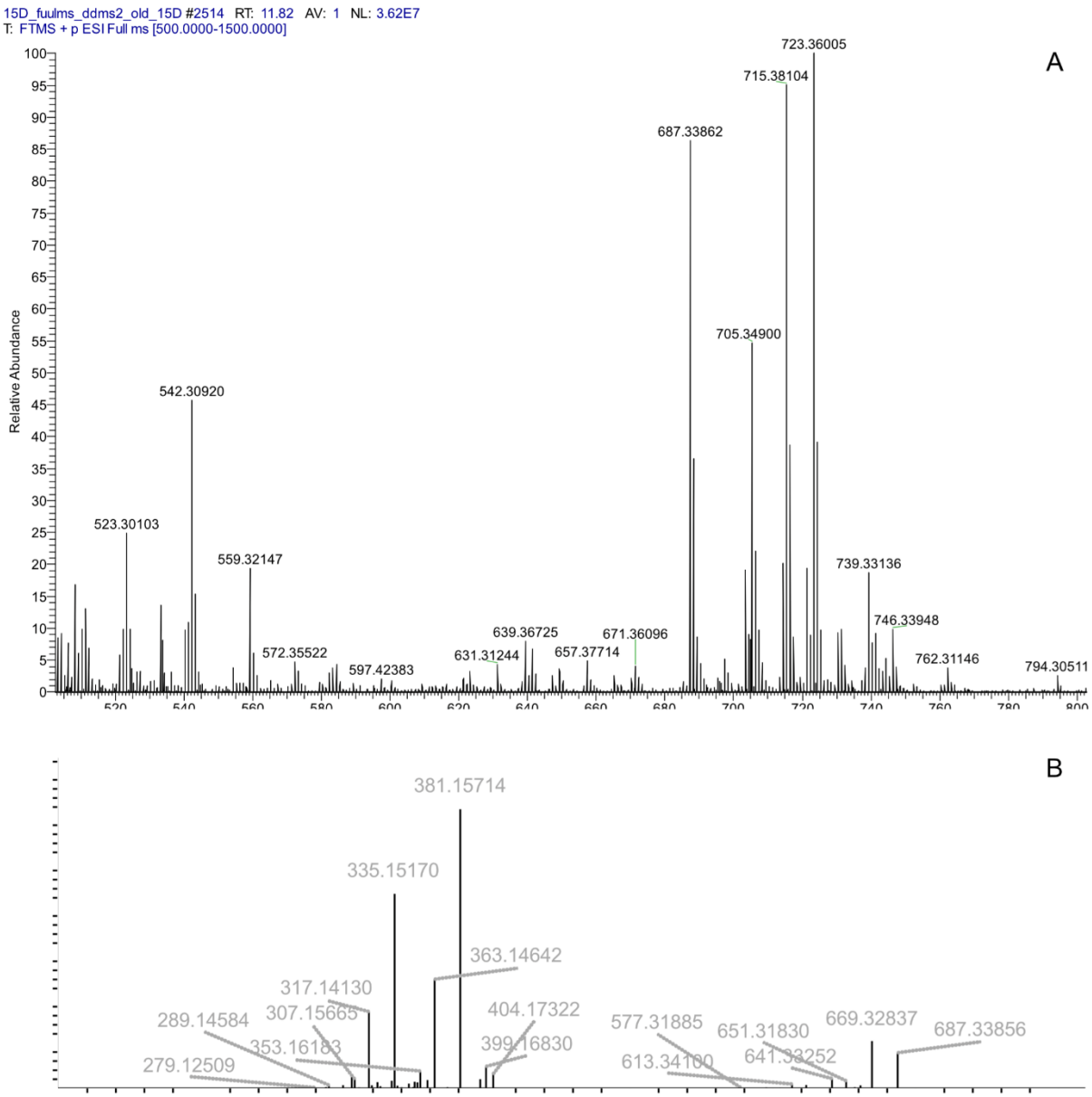

Figure 6. A, Full-scan spectra of fumonisin-like compounds at similar retention time as FB2. **B**, MSMS spectra of the protonated molecular ion of m/z 704

The MS^2 spectra of fumonisins usually contain three groups of product ions due to the esterified hydroxyl groups. In our case, fragmentation of the protonated molecular ions resulted only two fragment groups. As we were not able to detect the fumonisin backbone, and we could not explain the origin of the *m/z* 381 fragment ion, MS^3 experiments were performed by the use of an ion trap instrument. Despite the high similarity with fumonisin B2 the results of MS^3 did not prove that the compounds are fumonisins.

Within the frames of the project we analysed the metabolite profile of several *Bipolaris* and *Curvularia* isolates. By the mass analysis of extracts performed by a Q Exactive Plus hybrid quadrupole-Orbitrap mass spectrometer we determined the exact molecular mass and composition of the molecules. Based on previous results we hypothesized that *B. maydis* and *B. sorokiniana* isolates may be capable of producing fumonisins however, the extended analyses have revealed that none of the examined isolates are able to produce this mycotoxin.

We also examined the full metabolite profile of several isolates (Table 2). After the chromatographic separation, mass analyses were performed by a Q Exactive Plus hybrid quadrupole-Orbitrap mass spectrometer coupled to the above mentioned UHPLC system. The mass spectra were acquired by a high-resolution full-scan, data-dependent MS method. Raw data files were processed using Compound Discoverer™ software (v.2.0; Thermo Scientific, Fremont, CA, USA). An in-house exact mass database, including 138 known *Bipolaris* and *Curvularia* and 543 Aspergillus metabolites, was constructed and used for the selection of the potential metabolites. As experimental MS/MS data were not available for most of the compounds, they were subjected to *in silico* fragmentation to obtain the predicted MS/MS data. Most of the compounds are not available commercially as standard therefore, potential metabolites were assessed on the basis of exact mass and predicted MS/MS spectra. Only compounds found in high amounts in the samples were taken into account (Table 2).

Table 2. List of compounds found to be produced in high amounts by *Bipolaris* and *Curvularia* species.

Bipolaris maydis SZMC 13014	6-epi-3-anhydroophiobolin B-like compound
	Ophiobolin-like compound
	Ophiobolin A
	Terpestacin
	Sorokinianin
Bipolaris microlaenae SZMC 13015	6-epi-3-anhydroophiobolin B-like compound
	Ophiobolin-like compound
	Ophiobolin A
	Terpestacin
	Ophiobolin-like compound
	Deacetylmycoepoxydiene
	6-epi-3-anhydroophiobolin B-like compound
	Ophiobolin A
	Paecilin B
B. maydis SZMC 13039B	6-epi-3-anhydroophiobolin B-like compound
	Ophiobolin A
	Terpestacin
	Ophiobolin-like compound
	Deacetylmycoepoxydiene
	Paecilin B
B. secalis SZMC 13038	cis-Sativenediol
	Ophiobolin A
	Terpestacin
	Curvulamine
	6-epi-3-anhydroophiobolin B-like compound
	Sorokinianin
	Brefeldin A
	Dihydrobipolaroxin
B. sorokiniana SZMC 13091	cis-Sativenediol
	6-epi-3-anhydroophiobolin B-like compound
	Terpestacin
	Curvulamine
	6-epi-3-anhydroophiobolin B-like compound
	Sorokinianin
B. zeae SZMC 13051	cis-Sativenediol
	Terpestacin
	6-epi-3-anhydroophiobolin B-like compound
	Sorokinianin
	Brefeldin A
	Dihydrobipolaroxin
C. coimbatoriensis SZMC 22225	Radicinol/ 4-epiradicinol
	Isocochlioquinone C
C. tamilnaduensis SZMC 22226	Deacetylmycoepoxydiene
	11-alfa-methoxycurvularin
C. hawaiiensis SZMC 13056	Terpestacin
	Curvulamine
	6-epi-3-anhydroophiobolin B-like compound
	Sorokinianin
	Brefeldin A

C. lunata SZMC 13042	-
C. muehlenbeckiae SZMC 22220	Sorokinianin
	Zeaenol
C. australiensis SZMC 13045B	-
C. spicifera SZMC 13080	Terpestacin
C. nodosa SZMC 13083	Radicinol/4-epiradiciol
	Radicinin
C. akakiiensis SZMC 13037B	Radicinol/ 4-epiradicinol
	Radicinin
	Curvulapyrone
	Paecilin B
C. caricae-papayae SZMC 26793	Radicinol/4-epiradicinol
	Radicinin
	Curvulapyrone

None of the *Aspergillus* metabolites were found in the examined samples. Regarding *Curvularia* and *Bipolaris* metabolites, Ophiobolins were detected in every *Bipolaris* species, but were absent in *Curvularia* species except *C. hawaiiensis* SZMC 13056, which was found to produce a 6-epi-3-anhydroophiobolin B-like compound. Cis-Sativenediol, Sorokinianin and Dihydrobipolaroxin were detected exclusively in *Bipolaris* species. Radicinol/4-epiradicinol, Radicinin, Zeaenol, Curvularopyrone, Isocochlioquinone C and 11-alfa-methoxycurvularin were found exclusively in *Curvularia* species. Isocochlioquinone was produced only by *C. coimbatoriensis* SZMC 22225 while 11-alfa-methoxycurvularin was detected only in *C. tamilnaduensis* SZMC 22226. Zeaenol was found solely in *C. muehlenbeckiae* SZMC 22220 (Table 7). By comparing all 44469 metabolites detected by Compound Discoverer™ software, all *Curvularia* species grouped together suggesting that their metabolite profiles share high similarity. *Bipolaris* species did not form a group, but all of them were separated from *Curvularia* species. Our results suggest that *Bipolaris* species can produce a significantly more diverse set of metabolites than *Curvularia* species (Figure 7).

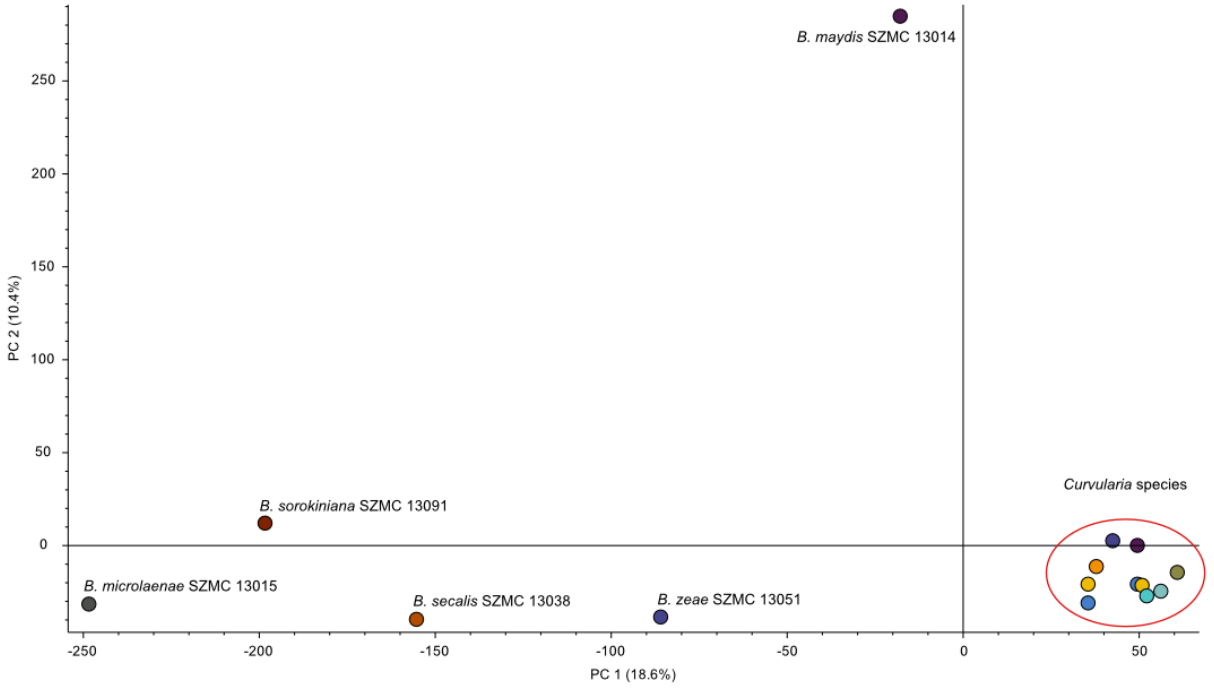

Figure 7. PCA analysis based on the 44469 compounds found by Compound Discoverer.