sciendo

DOI: 10.2478/fv-2025-0020

FOLIA VETERINARIA, 69, 2: 81—90, 2025

ORIGINAL ARTICLE

IDENTIFICATION OF OCHRATOXIN A-PRODUCING MICROMYCETES

Soňa Pivka, Pavlina Jevinová, Monika Pipová, Ivana Regecová

Department of Food Hygiene, Technology and Safety, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovakia

OPEN ACCESS

***Correspondence:** sona.pivka@uvlf.sk

Citation: Pivka, S., Jevinová, P., Pipová, M., Regecová, I., 2025: Identification of ochratoxin A-producing micromycetes. Folia Veterinaria, 69, 2, 81–90.

Received: May 6, 2025

Accepted: May 26, 2025

Published: June 16, 2025

Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

This study focused on the identification of microscopic filamentous fungi isolated from food commodities (chicken eggs, fermented green coffee beans, and dried marjoram) and the evaluation of their ability to produce ochratoxin A (OTA). A total of 179 isolates (93 from eggshells, 34 from coffee, 52 from marjoram) underwent phenotypic and genotypic identification. Phenotypic traits were assessed macroscopically and microscopically; genotypic identification was performed using conventional PCR and verified by ITS (internal transcribed spacers) region sequencing. Three complementary methods were used to assess OTA production: detection of the *otanps*PN gene, cultivation on coconut cream agar, and thin-layer chromatography. Identified species included *Aspergillus niger*, *A. ochraceus*, *A. westerdijkiae*, *A. carbonarius*, *A. tubingensis*, and *Penicillium verrucosum*. Molecular analysis corrected the misclassification of two *A. carbonarius* and one *A. tubingensis* isolate, reidentified as *A. niger*. OTA production was confirmed by all methods in most isolates. Notably, only one *A. niger* isolate from coffee carried the biosynthetic gene. Two *A. tubingensis* isolates, despite being considered non-ochratoxigenic, also showed OTA production. The findings underscore the importance of combining morphological and molecular tools in fungal identification and highlight the need for multi-method approaches in evaluating the toxigenic risk of foodborne fungi.

Key words: chicken egg, coffee, marjoram, microscopic filamentous fungus, mycotoxin

INTRODUCTION

Microscopic filamentous fungi (MFF) are widely distributed microorganisms capable of colonizing a variety of food substrates at different stages of processing, storage, and distribution. Their presence in food products is undesirable not only due to the deterioration of sensory properties but, more importantly, because of their ability to produce secondary metabolites with potent toxic effects—mycotoxins. One of the most hazardous and frequently

monitored mycotoxins within the food chain is ochratoxin A (OTA), predominantly produced by species belonging to the genera *Aspergillus* and *Penicillium* [1, 2, 3].

Contamination by OTA-producing microscopic filamentous fungi has been demonstrated across a range of food commodities, including cereals, coffee, dried fruits, herbs, spices, or wine. The contamination can occur either primarily (during cultivation) or secondarily (during harvesting, drying, storage, or transportation) [4, 5, 6, 7, 8]. OTA is remarkably stable under high temperatures and standard food processing conditions, implying that even processed foods may pose a significant risk to consumers [9].

Preventive measures and control of OTA contamination are implemented at all stages of food production and processing. An integral part of this effort is the effective and sensitive detection of microscopic fungi and their toxic metabolites. Although traditional phenotypic methods based on morphological characteristics are valuable, they exhibit limitations in distinguishing closely related species [10]. Consequently, increasing emphasis is placed on molecular analyses, which enable precise species-level identification and the detection of specific toxigenic lineages. Complementary methods, such as chemical analyses of mycotoxins, serve to confirm the production capacity of toxic metabolites under defined conditions [11].

From a public health perspective, OTA is classified as a possible human carcinogen (Group 2B) by the International Agency for Research on Cancer (IARC), exhibiting nephrotoxic, immunotoxic, hepatotoxic, and teratogenic effects [12]. Long-term exposure, even at low doses, may lead to serious chronic diseases, making OTA one of the most intensively monitored substances in food safety [13]. OTA is rapidly absorbed and distributed but slowly eliminated and excreted, leading to potential accumulation in the body, mainly due to binding to plasma proteins and a low rate of metabolism. Plasma half-lives range from several days to several weeks, which poses a serious risk to humans [12].

Although extensive monitoring has been conducted for OTA in cereals and coffee, fewer studies have focused on fungal contamination in animal-origin products such as eggs. Moreover, the role of less common substrates like dried herbs (e.g., marjoram) remains underexplored. Identifying OTA-producing fungi in such commodities is critical to understanding hidden routes of contamination and improving food safety controls.

This study was designed to fill these gaps by applying a polyphasic approach to identify and characterize OTA-producing fungi from chicken eggshells, green coffee beans, and dried marjoram. By integrating phenotypic, genotypic, and chemical analyses, we aimed to evaluate both species diversity and the toxigenic potential of the isolates.

Our findings contribute to a more nuanced understanding of OTA risk across food matrices and underscore the need for precise diagnostic methods in food microbiology. Therefore, systematic monitoring and research focused on toxigenic fungal species are crucial prerequisites for safeguarding public health.

MATERIALS AND METHODS

Isolation of microscopic filamentous fungi

The strains of microscopic filamentous fungi were isolated from the surface of moldy eggshells (n=90), fermented green coffee samples (n=3), and dried marjoram samples (n=7) according to the instructions of STN EN ISO 21527-1/O1 and STN EN ISO 21527-2/O1 [14, 15].

Phenotypic identification

Phenotypic identification of microscopic filamentous fungi isolates was carried out according to the criteria established by several authors [10, 11, 17, 16]. Isolated colonies presumed to belong to different mold genera or species were inoculated onto the surface of yeast extract sucrose agar (YES), malt extract agar (MEA), Czapek yeast extract agar (CYE), Czapek yeast autolysate agar (CYA), and 25 % glycerol nitrate agar (G25N; Hi-Media, Mumbai, India). Incubation was performed for 7 days at 25 °C (YES, MEA, CYE, and CYA) and 5 °C (CYA and G25N). Macroscopic evaluation focused on the colony growth rate, color, texture, corrugation, and the presence of exudates and pigments. Microscopic evaluation was conducted on agar preparations stained with lactophenol cotton blue using an optical microscope. Micromorphological structures were measured and documented using the B-290TB optical microscope and Optika Vision Lite 2.13 software (OPTIKA® Microscopes, Ponteranica, Italy). Isolates preliminarily classified as *Penicillium* and *Aspergillus* underwent the creatine test as described by Frisvad and Samson [16].

DNA isolation

Total genomic DNA of MMF was isolated using a two-step procedure involving zirconium and glass beads, Proteinase K (Macherey-Nagel GmbH & Co., Dueren, Germany), ultrasonic disruption, and the commercially available E.Z.N.A.® Fungal DNA Mini Kit (OMEGA Bio-tek, Inc., Norcross, GA, USA) according to Regecová et al. [18]. DNA purity and concentration were measured using a BioSpec spectrophotometer (SHIMADZU, Kyoto, Japan).

Genotypic identification by PCR

Fungal isolates were identified using conventional PCR methods. Primers used in this study (Table 1) were selected based on available literature and synthesized commercially by Amplia Ltd. (Bratislava, Slovakia).

Amplification was carried out in a 20 µL reaction volume containing 4.0 µL of HOT Firepol® Blend Master Mix (Solis BioDyne, Tartu, Estonia), 0.5 µL of each primer (10 pmol/µL concentration), and 1–10 ng of template DNA using a TC-512 thermal cycler (Techne, Staffordshire, UK). The PCR cycling conditions included an initial denaturation at 95 °C for 12 minutes, followed by 40 cycles of denaturation at 95 °C for 20 seconds, primer annealing at 46–60 °C for 60 seconds (depending on the primers), and elongation at 72 °C for 2 minutes. Amplification was terminated by cooling to 6 °C.

Detection of PCR products

Amplified PCR products were detected by electrophoresis on a 1% agarose gel prepared in TBE buffer and stained with GelRed™ Nucleic Acid Gel Stain (Biotium, Inc., Fremont, CA, USA). Amplicons were visualized by UV transillumination using a Mini Bis Pro® system (DNR Bio-Imaging Systems Ltd., Neve Yamin, Israel). A 100 bp

Table 1. Primers used and their characteristics

Primer name	Primer sequence (5′-3′)	Annealing temperature (°C)	PCR product size	Gen-Bank-EMBL accession number	Study
Penicillium spp.					
ITS 212d	AAATATAAATTATTTAAAACTTTC	46	336 bp	LC 317718.1	[19]
ITS 546	CTGGATAAAAATTTGGGTTG				
Penicillium verrucosum					
VERF	TCGTAACAAGGTTTCCGTAGG	59	607 bp	DQ681351.1	[20]
VERR	TTTCCTTCCGCCTTATTGAT				
Aspergillus spp.					
AspNest1	TCTTGGTTCCGGCATCGAT	55	240 bp	AF138288	[21]
AspNest2	TGACAAAGCCCCATACGCT				
AspNest3	GAAGAACGCAGCGAAATGC		146 bp		
AspNest4	AACACACAAGCCGTGCTTGA				
Aspergillus ochraceus					
OCRAF	CTTTTTCTTTTAGGGGGCACAG	60	430 bp	FM995527	[22]
OCRAR	CAACCTGGAAAAATAGTTGGTTG				
Aspergillus niger					
ASPU	ACTACCGATTGAATGGCTCG	46	295 bp	TIMM0113	[23]
Ni1R	ACGCTTTCAGACAGTGTTCG				
Aspergillus carbonarius					
CAR1	GCATCTCTGCCCCTCGG	53	389 bp	GQ888685.1	[24]
CAR2	GGTTGGAGTTGTCGGCAG				
Aspergillus westerdijkiae					
WESTF	CTTCCTTAGGGGTGGCACAG	60	430 bp	FN185739	[22]
WESTR	CAACCTGATGAAATAGATTGGTTG				
ITS1–5.8S–ITS2 region					
ITS1	TCCGTAGGTGAACCTGCGG	53	variable	-	[25]
ITS4	TCCTCCGCTTATTGATATGC				

DNA ladder (Sigma-Aldrich, Saint Louis, MO, USA) was used as a molecular weight marker.

Sequencing

ITS1–5.8S–ITS2 PCR products were sequenced by the Sanger method (SeqMe, Dobříš, Czech Republic). The obtained sequences were analyzed for homology with sequences available in the GenBank-EMBL database using the BLAST program (NCBI, software package 3.40, Bethesda, MD, USA).

Detection of ochratoxin A

The ability of isolates to produce ochratoxin A was determined by detecting the gene encoding its production and by demonstrating its presence in agar media. Detection of the specific *otanpsPN* gene using real-time PCR was performed with specific primers designed by Rodríguez et al. (Table 2) [26].

Amplification was conducted in a 20 µL reaction volume containing 10.0 µL of Q SYBR® Green Supermix (BIO-RAD, USA), 1.0 µL of each primer (10 pmol/µL concentration), and 1–10 ng of DNA using a CFX Opus 96 thermal cycler (BIO-RAD, USA). The PCR conditions included an initial denaturation at 95 °C for 5 minutes, followed by 40 cycles of denaturation at 95 °C for 10 seconds, annealing at 56 °C for 60 seconds, and a melt curve analysis from 65 °C to 95 °C with 5-second increments. The specific melting temperature (Tm) was considered to be 84 ± 0.5 °C. The cultivation screening assay on coconut cream agar (CCA) and thin-layer chromatography (TLC) were performed according to Jevinová et al. (2023) [11].

Statistical analysis

Chi-square (χ^2) tests of independence were used to compare phenotypic and molecular identification results and OTA production capabilities among species, performed in Jamovi software (version 2.6.44.0).

RESULTS

A total of 179 isolates of microscopic filamentous fungi were subjected to phenotypic and genotypic identification, including 93 isolates from the surface of eggshells, 34 from coffee beans, and 52 from marjoram. Based on characteristic macroscopic and microscopic features, 12 isolates were identified as *Penicillium verrucosum*, 5 as *Aspergillus carbonarius*, 43 as *A. niger*, 11 as *A. ochraceus*, 8 as *A. westerdijkiae*, and 4 as *A. tubingensis*.

The isolates were further analyzed using conventional PCR at both the genus and species levels. A total of 78 isolates were assigned to the genus *Penicillium*, of which 8 were specifically identified as *P. verrucosum*. Additionally, 109 isolates were assigned to the genus *Aspergillus*, including 46 identified as *A. niger*, 10 as *A. ochraceus*, 8 as *A. westerdijkiae*, and 3 as *A. carbonarius*. Two isolates preliminarily identified phenotypically as *A. carbonarius* and one isolate identified as *A. tubingensis* were reclassi-

Table 2. Primers for the detection of OTA and their characteristics

Primer name	Primer sequence (5′-3′)	Annealing temperature (°C)	PCR product size	Gen-Bank-EMBL accession number	Study
*otanps*PN					
F-npstr	GCCGCCCTCTGTCATTCCAAG	56	117 bp	AY557343	[26]
R-npstr	GCCATCTCCAAACTCAAGCGTG				

Table 3. Number of isolates identified by the methods used

Species	Commodity	Phenotype	PCR	Sequencing
P. verrucosum	chicken eggs	12	8	8
A. carbonarius	marjoram	5	3	3
A. niger	coffee, marjoram	43	46	46
A. ochraceus	coffee	11	10	10
A. westerdijkiae	coffee	8	8	8
A. tubingensis	coffee, marjoram	4	-	3

fied as *A. niger* by PCR analysis. Three isolates initially classified as *A. tubingensis* based on morphology were not confirmed by PCR.

The fungal strains identified by conventional PCR were subsequently subjected to sequencing for confirmation. Sequencing was also performed on isolates phenotypically identified as *A. tubingensis*. Amplification of the ITS1–5.8S–ITS2 region of ribosomal DNA (rDNA), characterized by low intraspecific polymorphism and high interspecific variability, was carried out using the ITS1 and ITS4 primers. The amplicons were sequenced using the Sanger method. Sequencing validated all species identifications determined by PCR and genetically confirmed three isolates preliminarily classified as *A. tubingensis* by morphology (Table 3).

The ability of fungal isolates to produce ochratoxin A (OTA) was assessed using three methods. OTA production was confirmed by at least one method in 70 isolates (8 eggs, 18 coffee, 44 marjoram). TLC and coconut agar assays correlated strongly with molecular detection of the *otanpsPN* gene (detected in 73 isolates). Among *A. niger* isolates from coffee, the ability to produce OTA was rare; gene encoding OTA production was detected only in one isolate (Table 4). Unexpectedly, OTA production was confirmed in three *A. tubingensis* isolates, suggesting possible variation in toxigenic capacity.

Statistical analysis showed no significant differences between phenotypic and PCR identification ($p = 0.138$), nor between phenotypic identification and sequencing ($p = 0.138$). PCR and sequencing identification results were in complete agreement.

Statistical analysis also showed no significant differences among species in OTA production capability across the different detection methods ($p > 0.05$; coconut cream agar: $p = 0.508$; TLC: $p = 0.508$; real-time qPCR: $p = 0.305$).

DISCUSSION

This study focused on the identification and characterization of microscopic filamentous fungi isolated from the surface of chicken eggshells, fermented green coffee beans, and dried marjoram. Combining phenotypic and genotypic methods, several potentially toxigenic species of the genera *Penicillium* and *Aspergillus* were identified.

Of the 179 isolates, the majority originated from eggshell surfaces (93 isolates), suggesting that eggs can serve as significant carriers of environmental contaminants, including filamentous fungi, consistent with previous findings [27, 28, 29, 30]. Notably, the ochratoxigenic species *P. verrucosum*, typically associated with cereal substrates, was also identified among the eggshell isolates, as previously described by Schmidt-Heydt et al. [31].

In green coffee beans (34 isolates), species of sections *Nigri* (*A. niger*, *A. tubingensis*) and *Circumdati* (*A. westerdijkiae*, *A. ochraceus*) were predominantly detected, aligning with reports of thermotolerant and xerotolerant fungi in coffee [32, 33, 34, 35]. Although coffee is considered a high-risk commodity for OTA contamination, we observed notable variability in OTA production among isolates.

Dried marjoram (52 isolates) exhibited the highest contamination rate with OTA-producing fungi, primarily *A. niger*, *A. carbonarius*, and *A. tubingensis*, supporting findings by Dimić et al. [36] and other studies [37, 38, 39].

Phenotypic identification based on macroscopic and microscopic traits was verified by conventional PCR and sequencing of the internal transcribed spacer (ITS) region. While phenotypic methods allowed preliminary classification, molecular techniques revealed misclassifications, particularly distinguishing *A. niger* from *A. tubingensis*

Table 4. Number of isolates producing ochratoxin A

Species	Commodity	Number of isolates	qPCR	CCA	TLC
P. verrucosum	chicken eggs	8	8	8	8
A. carbonarius	marjoram	3	3	3	3
A. niger	coffee	4	1	0	0
	marjoram	42	41	40	40
A. ochraceus	coffee	10	10	9	9
A. westerdijkiae	coffee	8	8	8	8
A. tubingensis	coffee	1	1	1	1
	marjoram	2	1	1	1

and *A. carbonarius*. These findings highlight the morphological similarity within section *Nigri* and the essential role of molecular methods [10, 40]. Results corroborate previous reports emphasizing the necessity of ITS sequencing and PCR for accurate species-level identification in morphologically similar fungi [41].

Sequencing fully confirmed PCR-based identifications, and variability in ITS amplicon sizes proved the genomic region's suitability for distinguishing related species. This underscores the robustness of molecular approaches in mycological diagnostics.

Ochratoxin A (OTA) production was evaluated using three methods: *otanps*PN gene detection, cultivation on coconut cream agar, and TLC. Among the *Aspergillus* species, *A. ochraceus* is widely recognized as the principal OTA producer [42], followed by *A. carbonarius* and *A. niger*, which are also frequently implicated in OTA contamination of food commodities [43]. Additionally, *A. westerdijkiae* has been identified as a significant OTA-producing species, with toxigenic potential comparable to that of *A. ochraceus* [44].

While *P. verrucosum*, *A. ochraceus*, *A. carbonarius*, and *A. westerdijkiae* showed OTA production by all methods, among coffee-derived *A. niger* isolates, the biosynthetic gene was detected in only one, and no OTA was visible on agar, diverging from reports of consistent OTA production in this species [35, 45, 46]. This discrepancy may be attributed to lower incubation temperatures and shorter duration (25 °C and 5 °C for 7 days), which are known to suppress OTA biosynthesis [47]. According to Medina et al., elevated temperatures (e.g., 37 °C) may stimulate secondary metabolite biosynthesis as a stress response [48]. Similarly, Belli and Akar note that OTA is produced during the stationary growth phase, typically after 14 days of incubation [49].

Interestingly, OTA production was detected in three *A. tubingensis* isolates, a species generally considered non-ochratoxigenic [39, 50, 51, 52]. Similar findings by Gherbawy et al. suggest that under certain conditions, *A. tubingensis* may produce OTA, emphasizing the need for individual strain assessment [53]. This highlights the need for individualized evaluation of toxigenic potential, even in species not classically considered OTA producers.

Statistical analysis showed strong concordance between phenotypic and molecular identification, confirming the reliability of PCR and ITS sequencing while acknowledging the limitations of morphology-based approaches [10].

This study provides novel insights into the presence and toxigenic profiles of microscopic filamentous fungi in commonly consumed food matrices of both animal and plant origin. It demonstrates that even species traditionally considered non-toxigenic may pose potential risks under specific environmental conditions. By integrating morphological and molecular methods with chemical detection of mycotoxins, the study highlights the necessity of a multi-tiered approach to ensure accurate fungal identification and mycotoxin risk assessment. These findings contribute valuable knowledge to the fields of food safety, mycotoxicology, and fungal diagnostics and may support future improvements in regulatory monitoring practices.

CONCLUSIONS

This study addressed the identification of microscopic filamentous fungi isolated from chicken egg surfaces, fermented green coffee beans, and dried marjoram and evaluated their ability to produce ochratoxin A (OTA). By combining phenotypic methods with molecular (PCR, ITS sequencing) and chemical approaches, several species of *Aspergillus* (*A. niger*, *A. tubingensis*, *A. ochraceus*, *A. carbonarius*, *A. westerdijkiae*) and *Penicillium verrucosum* were successfully identified.

Molecular analyses confirmed the reliability of phenotypic identification but also revealed discrepancies among closely related species, emphasizing the need for integrated methods. OTA production testing through coconut cream agar, thin-layer chromatography, and PCR detection of the *otanpsPN* gene showed a high level of concordance, with the highest proportion of positive isolates found in marjoram. Integrated approach revealed species previously underrecognized as OTA sources, including *P. verrucosum* in eggs and A. *tubingensis* in herbs. The occurrence of OTA production in isolates traditionally deemed non-toxigenic highlights the necessity for ongoing verification and strain-specific evaluation.

The study highlights the importance of rigorous monitoring for OTA-producing fungi in both plant- and animal-derived foods. Combining morphological, molecular, and chemical analyses provides a comprehensive strategy for fungal identification and mycotoxin risk assessment, contributing valuable insights for food safety and public health protection.

Future research should focus on studies refining food safety standards and supporting regulatory efforts in mitigating mycotoxin exposure risks globally, such as study of environmental and genetic factors influencing OTA biosynthesis in atypical producers like *A. tubingensis*; longitudinal studies assessing OTA accumulation across production and storage phases; and development of rapid, field-deployable molecular assays for early screening of OTA producers in diverse food environments.

Data Availability Statement

The data and materials generated during the current study are available from the corresponding author upon reasonable request.

Ethical Statement

No ethical approval was needed for this study.

Conflict of Interest

The authors declare no conflict of interest.

Funding

This work was funded by the Scientific Grant Agency of the Ministry of Education, Research, Development and Youth of the Slovak Republic and the Slovak Academy of Sciences, grant number (VEGA 1/0038/25).

Generative AI Statement

The authors declare no generative AI and AI-assisted technologies were used in writing the manuscript.

Authors' Contributions

Soňa Pivka: writing—original draft, writing—review and editing, conceptualization, visualization, investigation, methodology, formal analysis, data curation. Pavlina Jevinová: writing—review and editing, visualization, investigation, methodology, data curation. Monika Pipová: methodology, data curation. Ivana Regecová: methodology, data curation.

REFERENCES

1. **Bryła, M., Ksieniewicz-Woźniak, E., Stępniewska, S., Modrzewska, M., Waskiewicz, A., Szymczyk, K., Szafrańska, A., 2021:** Transformation of ochratoxin A during bread-making processes. *Food Control*, 125, 1, 107950. DOI: 10.1016/j.foodcont.2021.107950
2. **Chen, W., Li, C., Zhang, B., Zhou, Z., Shen, Y., Liao, X., Yang, J., Wang, Y., Li, X., Li, Y., Shen, X. L., 2018:** Advances in biodetoxification of ochratoxin A-A review of the past five decades. *Front. Microbiol.*, 9, 1, 1386. DOI: 10.3389/fmicb.2018.01386
3. **Gu, K., Ryu, D., Lee, H. J., 2021:** Ochratoxin A and its reaction products affected by sugars during heat processing. *Food Chem.*, 348, 129038. DOI: 10.1016/j.foodchem.2021.129038
4. **Alshannaq, A., Yu, J.-H., 2017:** Occurrence, toxicity, and analysis of major mycotoxins in food. *Int. J. Environ. Res. Public Health.*, 14, 6, 632. DOI: 10.3390/ijerph14060632
5. **Arce-López, B., Lizarraga, E., Vettorazzi, A., González-Peñas, E., 2020:** Human biomonitoring of mycotoxins in blood, plasma and serum in recent years: A review. *Toxins*, 12, 3, 147. DOI: 10.3390/toxins12030147
6. **Banahene, J. C. M., Ofosu, I. W., Odai, B. T., Lutterodt, H. E., Agyemang, P. A., Ellis, W. O., 2024:** Ochratoxin A in food commodities: A review of occurrence, toxicity, and management strategies. *Heliyon*, 10, 20, e39313. DOI: 10.1016/j.heliyon.2024.e39313
7. **Banahene, J. C. M., Ofosu, I. W., Odai, B. T., 2023:** Surveillance of ochratoxin A in cocoa beans from cocoa-growing regions of Ghana. *Heliyon*, 9, 7, e18206. DOI: 10.1016/j.heliyon.2023.e18206
8. **Yazdanfar, N., Mahmudiono, T., Fakhri, Y., Mahvi, A., Sadighara, P., Mohammadi, A., Yousefi, M., 2022:** Concentration of ochratoxin A in coffee products and probabilistic health risk assessment. *Arab. J. Chem.*, 15, 104376. DOI: 10.1016/j.arabjc.2022.104376
9. **Kőszegi, T., Poór, M., 2016:** Ochratoxin A: molecular interactions, mechanisms of toxicity and prevention at the molecular level. *Toxins*, 8, 4, 111. DOI: 10.3390/toxins8040111
10. **Houbraken, J., Visagie, C. M., Meijer, M., Frisvad, J. C., Busby, P. E., Pitt, J. I., Seifert, K. A., Louis-Seize, G., Demirel, R., Yilmaz, N., Jacobs, K., Christensen, M., Samson, R. A., 2014:** A taxonomic and phylogenetic revision of *Penicillium* section *Aspergilloides*. *Stud. Mycol.*, 78, 373–451. DOI: 10.1016/j.simyco.2014.09.002

11. **Jevinová, P., Koréneková, B., Pipová, M., Regecová, I., 2023:** *Instructions for practical exercises in mycology and mycotoxicology of feed and food*. Košice: University of Veterinary Medicine and Pharmacy in Košice, 2023. ISBN 978-80-8077-794-4.178 pp.
12. **Malir, F., Ostry, V., Pfohl-Leszkowicz, A., Malir, J., Toman, J., 2016:** Ochratoxin A: 50 years of research. *Toxins*, 8, 7, 191. DOI: 10.3390/toxins8070191
13. **Więckowska, M., Szelenberger, R., Niemcewicz, M., Harmata, P., Poplawski, T., Bijak, M., 2023:** Ochratoxin A-The Current knowledge concerning hepatotoxicity, mode of action and possible prevention. *Molecules* (*Basel, Switzerland*), 28, 18, 6617. DOI: 10.3390/molecules28186617
14. **STN EN ISO 21527-1/O1, 2008:** Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of yeasts and moulds. Part 1: Colony count technique in products with water activity greater than 0,95.
15. **STN EN ISO 21527-2/O1, 2008:** Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of yeasts and moulds. Part 2: Colony count technique in products with water activity less than or equal to 0,95.
16. **Frisvad, J., Samson, R., 2004:** Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*. A guide to identif cation of food and airborne terverticillate *Penicillia* and their mycotoxins. *Studies in Mycology,* 1, 1, 1-173.
17. **Tančinová, D., Mašková, Z., Feišociová, S., Dovičičová, M., Barboráková, Z., 2012:** *Introduction to food mycology: A key to the identification of food-important filamentous microscopic fungi.* Nitra: Slovak University of Agriculture in Nitra, 2012. ISBN 978-80-200-1703-1. 286 pp.
18. **Regecová, I., Pipová, M., Jevinová, P., Demjanová, S., Semjon, B., 2020:** Quality and mycobiota composition of stored eggs. *Ital. J. Food Sci.*, 32, 3, 540–561. DOI: 10.14674/IJFS-1702
19. **Pedersen, L. H., Skouboe, P., Boysen, M., Soule, J., Rossen, L., 1997:** Detection of *Penicillium* species in complex food samples using the polymerase chain reaction. *Int. J. Food Microbiol.*, 35, 2, 169–177. DOI: 10.1016/s0168-1605(96)01216-0
20. **Demjanová, S., Jevinová, P., Pipová, M., Regecová, I., 2021:** Identification of *Penicillium verrucosum*, *Penicillium commune*, and *Penicillium crustosum* isolated from chicken eggs. *Processes*, 9, 1, 53. DOI: 10.3390/pr9010053
21. **Buess, M., Cathomas, G., Halter, J., Junker, L., Grendelmeier, P., Tamm, M., Stolz, D., 2012:** *Aspergillus*-PCR in bronchoalveolar lavage for detection of invasive pulmonary aspergillosis in immunocompromised patients. *BMC Infect. Dis.*, 12, 237. DOI: 10.1186/1471-2334-12-237
22. **Gil-Serna, J., Vázquez, C., Sardiñas, N., González-Jaén, M. T., Patiño, B., 2009:** Discrimination of the main Ochratoxin A-producing species in *Aspergillus* section *Circumdati* by specific PCR assays. *Int. J. Food Microbiol.*, 136, 1, 83–87. DOI: 10.1016/j.ijfoodmicro.2009.09.018
23. **Sugita, C., Makimura, K., Uchida, K., Yamaguchi, H., Nagai, A., 2004:** PCR identification system for the genus *Aspergillus* and three major pathogenic species: *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus niger*. *Med. Mycol.*, 42, 5, 433–437. DOI: 10.1080/13693780310001656786
24. **González-Salgado, A., Patiño, B., Vázquez, C., González-Jaén, M. T., 2005:** Discrimination of *Aspergillus niger* and other *Aspergillus* species belonging to section Nigri by PCR assays. *FEMS Microbiol. Lett.*, 245, 2, 353–361. DOI: 10.1016/j.femsle.2005.03.023
25. **White, T. J., 1991:** Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications*. New York: Academic Press, 1990. ISBN -978-0-12372180-8. 315-322 pp.
26. **Rodríguez, A., Rodríguez, M., Luque, M. I., Justesen, A. F., Córdoba, J. J., 2011:** Quantification of ochratoxin A-producing molds in food products by SYBR Green and TaqMan real-time PCR methods. *Int. J. Food Microbiol.*, 149, 3, 226–235. DOI: 10.1016/j.ijfoodmicro.2011.06.019
27. **Gaweł, A., Siedlecka, M., Karwańska, M., Skrok, M., Przychodniak, I., Knap, P., Chmielina, A., Truszko, V., Kuczkowski, M., Bierowiec, K., Woźniak-Biel, A., Szczepankiewicz, B., Bobrek, K., 2025:** Occurrence of fungi on duck egg shells and drug resistance analysis of Aspergillus spp. isolates. *Appl. Sci.*, 15, 2, 860. DOI: 10.3390/app15020860
28. **Melo, M. V. C., , dos Santos, F. C. P., da Silva, I. N. G., Rodrigues, J. L. C., Freire, J. E. C., 2025:** Fungal profile of eggshells from commercial and free-range hens of a supermarket chain in Fortaleza, CE, Brazil. *Biosci. J.*, 40, e40026. DOI: 10.14393/BJ-v40n0a2024-69507
29. **Perez-Nadales, E., Nogueira, M. F., Baldin, C., Castanheira, S., El Ghalid, M., Grund, E., Lengeler, K., Marchegiani, E., Mehrotra, P. V., Moretti, M., Naik, V., Oses-Ruiz, M., Oskarsson, T., Schäfer, K., Wasserstrom, L., Brakhage, A. A., Gow, N. A., Kahmann, R., Lebrun, M. H., Perez-Martin, J., Wendland, J., 2014:** Fungal model systems and the elucidation of pathogenicity determinants. *Fungal Genet Biol.*, 70, 100, 42–67. DOI: 10.1016/j.fgb.2014.06.011

30. **Tomczyk, Ł., Stępień, Ł., Urbaniak, M., Szablewski, T., Cegielska-Radziejewska, R., Stuper-Szablewska, K., 2018:** Characterisation of the mycobiota on the shell surface of table eggs acquired from different egg-laying hen breeding systems. *Toxins*, 10, 7, 293. DOI: 10.3390/toxins10070293
31. **Schmidt-Heydt, M., Graf, E., Stoll, D., Geisen, R., 2012:** The biosynthesis of ochratoxin A by *Penicillium* as one mechanism for adaptation to NaCl rich foods. *Food Microbiol.*, 29, 2, 233–241. DOI: 10.1016/j.fm.2011.08.003
32. **Hagos, L., Guta, M., Bacha, K., 2024:** Prevalence of mycotoxigenic fungi and ochratoxin A in coffee (*Coffea arabica* L.). *Cogent Food Agric.*, 10. DOI: 10.1080/23311932.2024.2407524
33. **Hlebová, M., Uzsáková, V., Podhorská, L., Vešelényiová, D., Mrkvová, M., Čuboň, J., Hleba, L., 2022:** Mycobiota and co-occrurence of mycotoxins in green and roasted coffee beans. *J. Microbiol. Biotechnol. Food Sci.*, 11, e5779. DOI: 10.55251/jmbfs.5779
34. **Lu, L., Tibpromma, S., Karunarathna, S. C., Jayawardena, R. S., Lumyong, S., Xu, J., Hyde, K. D., 2022:** Comprehensive review of fungi on coffee. *Pathogens (Basel, Switzerland)*, 11, 4, 411. DOI: 10.3390/pathogens11040411
35. **Maman, M., Sangchote, S., Piasai, O., Leesutthiphonchai, W., Sukorini, H., Khewkhom, N., 2021:** Storage fungi and ochratoxin A associated with arabica coffee bean in post-harvest processes in Northern Thailand. *Food Control*, 130, 108351. DOI: 10.1016/j.foodcont.2021.108351
36. **Dimić, G. R., Kocić-Tanackov, S. D., Tepić, A. N., Vujičić, B. L., Šumić, Z. M., 2008:** Mycopopulation of spices. *Acta Period. Technol.*, 39, 1-9.
37. **Garcia, M. V., Parussolo, G., Moro, C. B., Bernardi, A. O., Copetti, M. V., 2018:** Fungi in spices and mycotoxigenic potential of some *Aspergilli* isolated. *Food microbiol.*, 73, 93–98. DOI: 10.1016/j.fm.2018.01.013
38. **Krstić, M., Stupar, M., Đukić-Ćosić, D., Baralić, K., Svetlana, Đ., 2021:** Health risk assessment of toxic metals and toxigenic fungi in commercial herbal tea samples from Belgrade, Serbia. *J. Food Compos. Anal.*, 104, 104159. DOI: 10.1016/j.jfca.2021.104159
39. **Nascimento, A., Lopez, B., Silva, B., Munaretto, M., Souza, R., Nazário, C., Oliveira, V., Paula, C., Maia, M., Júnior, D., 2024:** Microbiological quality of dehydrated aromatic condiments and spices. *Int. J. Curr. Microbiol. Appl. Sci.,* 13, 3, 1–26. DOI: 10.20546/ijcmas.2024.1303.001
40. **Silva, D. M., Batista, L. R., Rezende, E. F., Fungaro, M. H., Sartori, D., Alves, E., 2011:** Identification of fungi of the genus *Aspergillus* section *Nigri* using polyphasic taxonomy. *Braz. J. Microbiol.,* 42, 2, 761–773. DOI: 10.1590/S1517-83822011000200044
41. **Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. L., Levesque, C. A., Chen, W., et al, 2012:** Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proc. Natl. Acad. Sci. U.S.A.*, 109, 16, 6241–6246. DOI: 10.1073/pnas.1117018109
42. **Mantle, P., 2002:** Risk assessment and the importance of ochratoxins. *Int. Biodeterior. Biodegrad.,* 50, 1, 143-146. DOI: 10.1016/S0964-8305(02)00079-3
43. **Hayat, A., Rhouati, A., Marty, J., Barthelmebs, L., 2012:** Recent advances in ochratoxin A-producing fungi detection based on PCR methods and ochratoxin A analysis in food matrices. *Food Control,* 26, 1, 401–415. DOI: 10.1016/j.foodcont.2012.01.060
44. **Frisvad, J. C., Frank, J. M., Houbraken, J. A. M. P., Kuijpers, A. F. A., Samson, R. A., 2004:** New ochratoxin A producing species of *Aspergillus* section *Circumdati*. *Stud. Mycol.*, 50, 23-43.
45. **Varga, J., Kevei, E., Rinyu, E., Téren, J., Kozakiewicz, Z., 1996:** Ochratoxin production by *Aspergillus* species. *Appl. Environ. Microbiol.*, 62, 12, 4461–4464. DOI: 10.1128/aem.62.12.4461-4464.1996
46. **Frisvad, J. C., Larsen, T. O., Thrane, U., Meijer, M., Varga, J., Samson, R. A., Nielsen, K. F., 2011:** Fumonisin and ochratoxin production in industrial *Aspergillus niger* strains. *PLOS One*, 6, 8, e23496. DOI: 10.1371/journal.pone.0023496
47. **da Silva, S., Pereira, R., Azevedo, N., da Gloria, E., Chalfoun, S., Batista, L., 2020:** Fungi associated to beans infested with coffee berry borer and the risk of ochratoxin A. *Food Control*, 113, 107204. DOI: 10.1016/j.foodcont.2020.107204
48. **Medina, A., Fraga, M. E., Sanchis, V., 2005:** Fungal contamination and mycotoxin production in maize grown in Spain under different environmental conditions. *Mycol. Res.*, 109, 11, 1185-1191.
49. **Belli, N., Akar, T., 2004:** Production of ochratoxin A by *Aspergillus ochraceus* in relation to various environmental conditions. *Food Control*, 15, 1-3, 123-128.
50. **Wang, Y., Wang, L., Liu, F., 2016:** Ochratoxin A producing fungi, biosynthetic pathway and regulatory mechanisms. *Toxins (Basel)*, 8, 3, 83. DOI: 10.3390/toxins8030083
51. **Choque, E., Klopp, C., Valiere, S., Raynal, J., Mathieu, F., 2018:** Whole-genome sequencing of *Aspergillus tubin-*

gensis G131 and overview of its secondary metabolism potential. *BMC Genom.*, 19, 1, 200. DOI: 10.1186/s12864-018-4574-4

52. **Storari, M., Bigler, L., Gessler, C., Broggini, G. A., 2012:** Assessment of the ochratoxin A production ability of *Aspergillus tubingensis*. *Food Addit. Contam. - Chem. Anal. Control Expo. Risk Assess.*, *29*, 9, 1450–1454. DOI: 10.1080/19440049.2012.698656

53. **Gherbawy, Y. A., Hamza, L. H. A., Maghraby, T. A., Shebany, Y. M., Abdelwahab, S. F., El-Dawy, E. G. A. M., 2025:** Characterization of *Aspergillus tubingensis* from grains in upper Egypt and their potential for ochratoxins production. *Food Biotechnol.*, 39, 1, 28–50. DOI: 10.1080/08905436.2025.2458242