TOXIGENIC MOULDS GROWING ON THE SURFACE OF TRADITIONAL CROATIAN HOUSEHOLD-PRODUCED DRY-FERMENTED SAUSAGES

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INTRODUCTION

Household production of dry-fermented sausages is characterised by the presence of wild-type moulds that spontaneously overgrow the product surface, mainly *Penicillium* and *Aspergillus* genera. Some of these moulds can produce mycotoxins that affect final products' quality and safety because they can be responsible for consumer acute & chronic toxicity (*1*). Out of mycotoxins that can be found in meat products, aflatoxin B_1 (AFB_1) and ochratoxin A (OTA) are of the greatest public health concern, while other mycotoxins, such as cyclopiazonic acid (CPA) and citrinin (CIT), haven't been thoroughly investigated yet (*2*). The aim of this study was to identified potentially toxigenic moulds from the surface of traditional Croatian household-produced dry-fermented sausages.

MATERIALS AND METHODS

Sausage samples

Thirty-five samples of four different types of dry-fermented sausages were produced by family farms seated in two different Croatian regions: eastern Slavonia (Kulenova Seka and Slavonian domestic sausage) (n = 17) and western Istria (Kosnica and Istrian-type sausage with additional ingredients) (n = 18).

Moulds' identification

Mould isolates were identified using a traditional method of depiction of macroscopic and microscopic morphological characteristics according to (*3*) and (*4*) and corroborated using a molecular polymerase chain reaction (PCR) method of beta-tubulin (*benA*) and calmodulin (*CaM*) loci sequencing using primer pairs BTa2a & BTa2b and CMD5 & CMD6, respectively (*5,6*). PCR reaction was performed using HotStarTaq Plus MasterMix Kit (Qiagen, Germany).

Purified PCR products were sent to Macrogen Inc. (Amsterdam, Netherlands). The obtained sequences were aligned using the Lasergene SeqManPro DNASTAR 13 (Madison, Wisconsin, USA) and then compared to those available from the GeneBank database using the BLAST algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

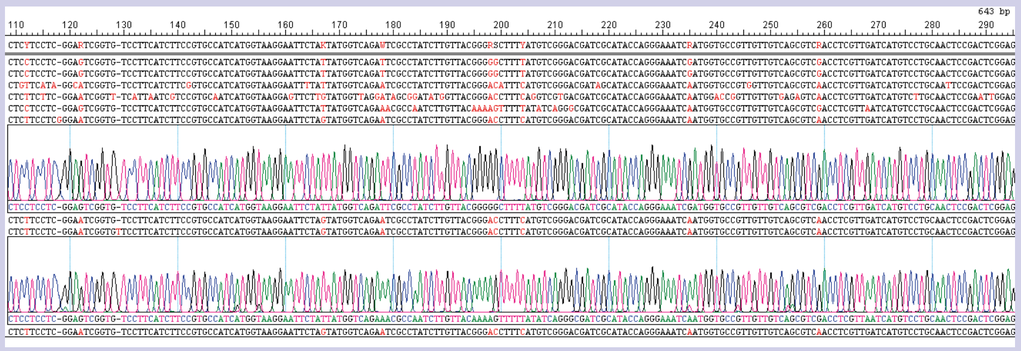

Figure 1. *Printscreen of DNA sequences in Lasergene DNASTAR 13 software*

RESULTS

Table 1. *Number of mould isolates from the surface of Croatian traditional dry- fermented sausages*

IDENTIFIED MOULD SPECIES	KOSNICA	ISTRIAN-TYPE SAUSAGE WITH ADDITIONAL INGREDIENTS	SLAVONIAN DOMESTIC SAUSAGE	KULENOVA SEKA
P. solitum	6	6	3	3
P. nalgiovense	4	10	7	4
P. citrinum	0	0	3	
P. commune	1	2	6	4
P.lanosum	1	0	0	0
P.brevicompactum	0	0	1	0
P. salamii	0	1	0	0
P.polonicum	0	0	0	1
Penicillium sp.	**12**	**19**	**20**	**12**
A.proliferans	1	0	0	0
A.pseudoglaucus	0	2	3	1
A.niger	0	0	1	0
A.flavus	0	0	2	1
Aspergillus sp.	**1**	**2**	**6**	**2**

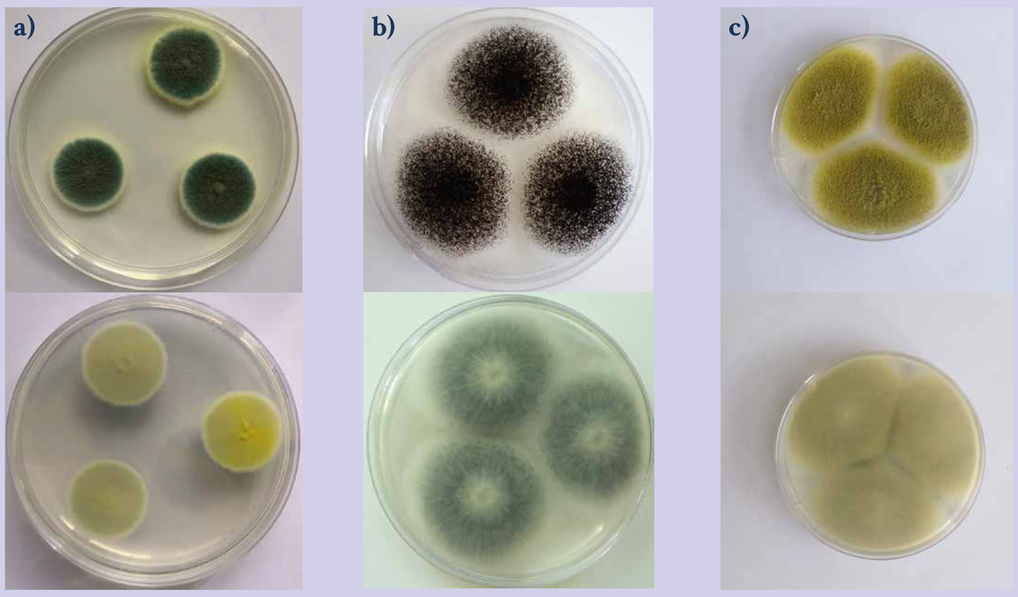

Figure 2. *Examples of identified moulds: a) Penicillium citrinum b) Aspergillus niger c) Aspergillus flavus on DG18 agar averse and reverse*

CONCLUSIONS

Moulds of the *Penicillium* genus were present in more isolates (85%) than those of the *Aspergillus* genus (15%) and exhibited a greater species diversity. Out of the identified mould species, four species are known as mycotoxin- producers, in specific *Penicillium commune* as CPA producer, *Penicillium citrinum* as CIT producer, *Aspergillus flavus* as aflatoxins and CPA producer and *Aspergillus niger* as OTA producer.

REFERENCES

1. Zadravec et al. (2020) *International Journal of Food Microbiology*, 317, 108459.
2. Zadravec et al. (2019) *Croatian Journal of Food Science and Technology*,2, 272-282.
3. Samson et al. (2004) Introduction to Food and Airborne Fungi. CBS, Utrecht.
4. Pitt and Hocking (2009) Fungi and Food Spoilage. Springer, New York.
5. Glass and Donaldson (1995) *Applied Enviromental Microbiology*, 61, 1323-1330.
6. Hong et al. (2006) *International Journal of Systematic and Evolutionary Microbiology*, 56, 477-486.

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HrZZ Croatian Science Foundation
TMP mould RISK Mycotoxins in traditional Croatian meat products