MICROBIOLOGICAL DEGRADATION OF THE T-2 TOXIN AND DIACETOXYSCIRPENOL UNDER LABORATORY CONDITIONS

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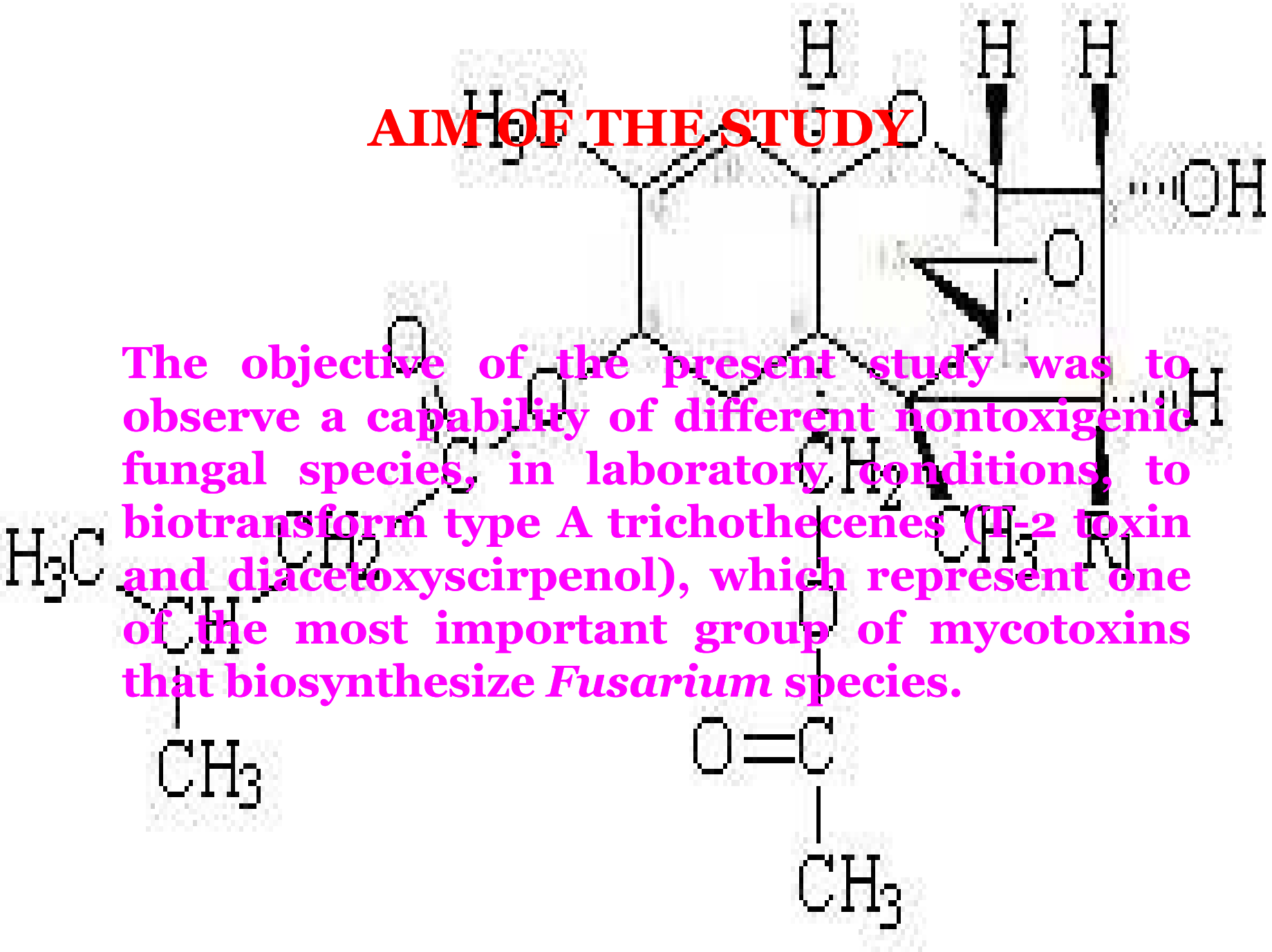
INTRODUCTION

Considering a daily increase of data on the mycotoxin presence in both, food and feed mixtures, a need to establish practical and economic procedures for mycotoxin detoxication has arisen.

In principle, there are several possibilities to avoid harmful effects of mycotoxin-contaminated food: contamination prevention - the development of resistant genotypes, inhibition of fungal development and mycotoxin biosynthesis, decontamination of biosynthesised mycotoxins by the application of physical and chemical methods and hindrance of mycotoxin adsorption in the digestive tract of animals.


In the case when mycotoxins are not adsorbed or are poorly adsorbed, the majority of physical and chemical methods of decontamination or the application of adsorbents are not sufficiently efficient, hence it is necessary to apply an alternative approach - enzymatic or microbiological degradation of toxins.
In the case of trichothecenes, 12,13-epoxide ring is responsible for their toxicity, hence a reductive deepoxidation caused by enzymes and/or living microorganisms has a significant loss of toxicity as a result.
Besides bacteria and yeasts, there are data showing that T-2 toxin and other trichothecenes can be detoxicated by different fungal species.

AIM OF THE STUDY



The objective of the present study was to observe a capability of different nontoxigenic fungal species, in laboratory conditions, to biotransform type A trichothecenes (T-2 toxin and diacetoxyscirpenol), which represent one of the most important group of mycotoxins that biosynthesize *Fusarium* species.

MATERIAL I METHODS

Microorganisms. Thirty isolates of nontoxigenic fungi belonging to species of *Aspergillus niger* van Tieghem (7), *Mucor* spp. (21) and *Syncephalastrum racemosus* (Cohn) Schroeter (2) were selected for test microorganisms. The majority of tested fungi originated from samples of feed and its components that were not mycotoxin-contaminated. The identification of fungi was done according to Samson and van Reenen-Hoekstra. Fungal cultures were maintained on potato dextrose agar (PDA) at 4-6 ºC. 4-6 º C.

Production of crude toxins. Crude T-2 toxin was produced from a liquid culture of *F. sporotrichioides* (M-1-1), while crude diacetoxyscirpenol (DAS) was produced from a liquid culture of *F. semitectum* (SL-B).

Filtration of liquid fungal cultures was performed after three-day cultivation in a fluid medium SPEK (saccharose 50 g L^{-1}, peptone-1 1 g L^{-1} and yeast extract 1 g L^{-1}; pH 6,2) on a rotary shaker (180 rpm) at room temperature (23-30 °C).

Crude extracts of T-2 toxin and DAS were produced by the use of ethyl acetate. After evaporation of ethyl acetate extracts to the dryness, dry residues of crude fusariotoxins were dissolved in 96% ethanol (1 mg mL^{-1}) and stored until used at 4-6 °C. Ethanol extracts of fusariotoxins were individually added to the test medium **VA** (Vogel's Medium N), immediately prior to its pouring into Petri dishes up to the final concentration of 0.02 mg mL^{-1}.

Mycotoxicological analyses. Discs (diameter 6 mm) were cut out of the central part of the fungal colony with a stainless steel borer and directly placed on thin layer chromatography (TLC) plates coated with Kieselgel G. Discs were afterward wetted with 10-20 µL of a chloroform-methanol (2:1, v/v) mixture. Several seconds later discs were carefully removed from the TCL plates. Discs with **VAT2** and **VADAS** media, with no test fungal culture (control), were simultaneously placed on the same TCL plates. Then, the extraction of controlled discs were done and chromatography plates were developed together with 5 µL of each working standard of tested mycotoxins (T-2 toxins and DAS) in a concentration of 0.01 µg kg^{-1}.

Thin layer chromatography was performed in a saturated tank of toulene-ethyl acetate- formic acid mixture (5:4:1, v/v/v).

RESULTS AND DISCUSSION

Table 1. Microbiological degradation of trichothecene A by means of the isolates of fungi of the group A. niger

No.	Origin	Designation	Mycotoxin degradation	
			T-2	DAS
1	Cob	L 1292/09	Yes	No
2	Cob	L D1/10-1	No	Yes
3	Cob	L D1/10-2	Yes	No
4	Cob	L D1/10-3	Yes	No
5	Cob	L 506/10	Yes	No
6	Feed mixture	L 47/10-1	Yes	No
7	Soil	Rb-gr/10	Yes	No

Isolates of fungi of the group *A. niger* mainly degraded T-2 toxin (6/7). *A. niger* isolates are also important from the aspects of biotransformation of other mycotoxins . It was determined that they can degrade ochratoxin A, as well as, zearalenone.

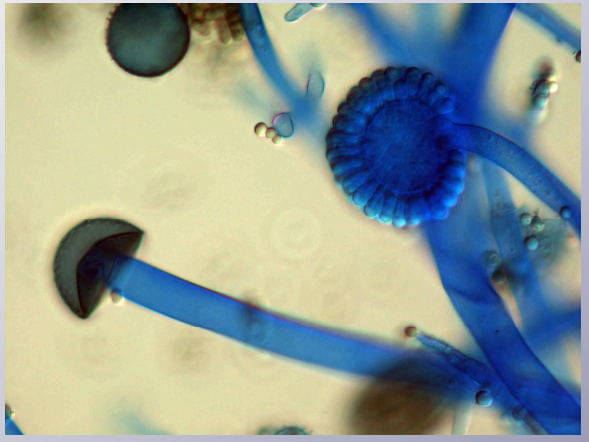
Mucor circinelloides* f. *circinelloides **van Tieghem (1/1) and *M. hiemalis* f. *hiemalis* (Wehmer) Schipper (1/2) isolates degraded exclusively DAS, while the *M. racemosus* f. *racemosus* Fresenius (1/1) isolate and the majority of unidentified species of the genus *Mucor* (11/17) biotransformed both type A trichothecenes (*Table 2*). Although El-Sharkawy and Abbas state that only the species *M. mucedo* (L.) Fries degrades T-2 toxin, it is obvious that other species of this genus of the phylum Zygomicota. According to literature data, beside stated fusriotoxins, the representatives of the genus *Mucor* can degrade one of the most toxic mycotoxins (aflatoxin B_1), which can be found as a natural contaminant of crops, and thereby of food and feed mixture.**

Table 2. Microbiological degradation of type A trichothecenes by the means of isolates of the fungus Mucor spp.

No.	Origin	Species (No. of isolates)	No. of isolates that degrade	
			T-2	DAS
1	Feed mixture	*M. circinelloides f. circinelloides (1)*	0	1
2		*Mucor hiemalis f. hiemalis (1)*	0	0
3	Sunflower meal	*Mucor hiemalis f. hiemalis (1)*	0	1
4		*Mucor racemosus f. racemosus (1)*	1	1
5		*Mucor sp. (2)*	2	1
6	Intraco	*Mucor sp. (1)*	1	0
7	Cob	*Mucor sp. (2)*	2	2
8	Feed mixture	*Mucor sp. (8)*	6	8
9	Maize grain	*Mucor sp. (2)*	0	1

Table 3. Microbiological degradation of type A trichothecenes by the means of isolates Syncephalastrum racemosus

No.	Origin	Designation	Mycotoxin degradation	
			T-2	DAS
1	Intraco	L 1288/09	Yes	No
2	Intraco	L 1307/09	Yes	No



There are not data in available literature on the ability of *S. racemosus* to decontaminate mycotoxins, but it is shown that it is able to biotransform immunosuppressive agent rapamycin that is given to patients after organ transplantations. Therefore, it is especially interesting our finding that this fungus has the ability to degrade T-2 toxin.

CONCLUSION

Under test laboratory conditions, residues of T-2 toxin were not determined in 70% of cases. At the same time, DAS residues were not detected in 53.3% cases.

Tested isolates of fungi belonging to the group *A. niger* biotransformed one or other type A trichothecenes, while *S. racemosus* biotransformed only T-2 toxin.

In the greatest number of cases (52.4%), isolates of fungi of the genus *Mucor* detoxicated both fusariotoxins.

Obtained results require the continuation of the initiated studies, because biological detoxication of food and feed is an approach that will gain on its importance with the aim to decrease food contamination and prevent occurrence of a health risk related to fusariotoxins and other mycotoxins (aflatoxin B_1, ochratoxin A).

ACKNOWLEDGEMENTS

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