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Short communication

Toxin-producing fungi on feed grains and application of yeasts for their detoxification

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Abstract

The research deals with the contamination of feeding crops with fungi, producing mycotoxins and use of selected yeasts for detoxification. The thin-layer chromatography and ELISA methods were applied for the analysis of fungal secondary metabolites. The results showed a high rate of the grain contamination with fungi, mainly from genera *Penicillium, Fusarium, Aspergillus* and *Alternaria.* Some of the fungal strains produced 6-9 toxic compounds. The novelty of the study was the application of the yeast strains in detoxification of aflatoxins, zearalenon and deoxynivalenol in feed.

Key words: feeding crops, fungi, mycotoxins, biological detoxification, yeasts

Introduction

Contamination of feeding crops with toxic fungi is a serious ecological problem concerning quality and safety of production affecting animal health. The presence of some fungi in food or feed raw may indicate possible contamination with mycotoxins. For example, *Penicillium verrucosum* may indicate the presence of ochratoxin A (Lund and Frisvad 2003), *Fusarium* – deoxynivalenol (Karlovsky 2011). Various chemical and physical measures are used for the decontamination of grains (Selwet 2008). The aim of the research was to evaluate the contamination level of feeding crops with fungal species able to produce toxins and to search yeasts detoxifying mycotoxins in feed.

Materials and Methods

Overall 86 grain samples were taken in different regions of Lithuania during harvesting, storage and flour processing. Evaluation of grain surface contamination with fungi was performed using the serial dilution plating technique. Fungal species, frequently detected and possible toxin producers (18 strains), were examined for the toxin production. The thin-layer chromatography (solvents: toluene-ethyl acetate-formic acid – 5:4:1) and the ELISA methods were used to test for fungal secondary metabolites (Chu 1996, Samson et al. 2000). The ability of yeasts (10^4 CFU/mL) to detoxify aflatoxins, zearalenone (ZEN) and deoxynivalenol (DON) was studied using wheat flour and a composite fodder for the sucker pigs.

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Table 1. Amounts of ochratoxin and patulin produced by *Penicillium* on different substrata.

Fungi	Growth substrata	Ochratoxin (μg/kg)	Patulin (μg/kg)
Penicillium crustosum Thom	Malt agar	7.5	0
	Bran	11.5	0
	Wheat	23.0	0
	Oat	18.0	0
Penicillium griseofulvum Dierckx	Malt agar	5.3	10.0
	Bran	10.5	8.0
	Wheat	10.8	6.5
	Oat	14.5	7.0
Penicillium aurantiogriseum Dierckx	Malt agar	14.0	7.0
	Bran	9.8	10.1
	Wheat	20.1	4.1
	Oat	15.6	6.0

Table 2. Amounts of mycotoxins in feed before (a) and after (b) cultivation of yeast strains.

Variant of treatment	Amount of mycotoxins, mg/kg					
	Aflatoxins		Zearalenon		Deoxynivalenol	
	a	b	a	b	a	b
*Wf + *Saccharomyces cerevisiae* S.1.5(T)	traces	0	0.150	0.020	0.650	0.100
**Cf + *Saccharomyces cerevisiae* S.1.5 (T)	0.003	0	0.400	0	0.400	0.125
Wf + *Geotrichum fermentans* G.1	traces	0	0.150	0	0.650	0.100
Cf + *Geotrichum fermentans* G.1	0.003	0	0.400	0	0.400	0.130
Wf + *Metschnikowia pulcherrima* M.1	traces	0	0.150	0	0.650	0.120
Cf + *Metschnikowia pulcherrima* M.1	0.003	0	0.400	0	0.400	0.080
Wf + *Kluyveromyces marxianus* K.7.1 (T)	traces	0	0.150	0	0.650	0.050
Cf + *Kluyveromyces marxianus* K.7.1 (T)	0.003	0	0.400	0.030	0.400	0.100

* Wf – wheat flour, ** Cf – composite fodder for sucker pigs.

Results and Discussion

The study showed that the cereal grains were heavily contaminated by fungi (up to 7.5×10^5 CFU/g). The highest numbers of fungi were isolated under storage conditions. There were 24 genera and 81 species of fungi isolated from the grains. Fungi of the genera *Penicillium* (24%), *Fusarium* (18%) and *Aspergillus* (9%), class *Zygomycetes* (16%) and species containing melanin (*Demateaceae*) (14%) were the most common. It was reported that fungal growth and mycotoxin production are influenced by the presence of other contaminant moulds (Magan et al. 2003). Therefore, the evaluation of the dominant species on grains is important. *Alternaria alternata, Dreschlera sorokiniana, Fusarium. equiseti, F. graminearum, F. oxysporum, F. poae, Penicillium expansum, P. verrucosum, A. niger* and *A. oryzae* dominated on studied grains. In addition, fungi such as *Mucor racemosus*, *M. hiemalis*, *Rhizopus oryzae* and *R. stolonifer* were also frequent.

The studied fungi synthesized more than one toxin. The highest number of secondary metabolites was produced by *Alternaria alternata* (9 compounds; and tenuazonic acid, patulin, peritrem and cytochalazin were identified among them). The tested strains of *Penicillium* genus also produced 3-7 compounds (among them – patulin, citrinin, ochratoxin and cytochalazin). *Penicillium atramentosum, P. aurantiogriseum, P. expansum* and *P. spinulosum*

were found out to produce patulin. Meanwhile, *Penicillium viridicatum* synthesized ochratoxin (OTA). This toxin was also produced by *Aspergillus amstelodami*, whereas *A. flavus* produced kojic and cyclopiazonic acids. Strains of *Fusarium* produced mostly T-2 toxin and cytochalazin.

It was found out that while growing on different substrata, *Penicillium* produced various amounts of ochratoxin and patulin (Table 1). *P. crustosum* and *P. aurantiogriseum* produced the greatest amount of ochratoxin on wheat, whereas *P. griseofulvum* – on oat. The most intensive patulin synthesis was found in *P. aurantiogriseum* grown on bran.

Four selected yeast strains were used for the detoxification of mycotoxins in feed substrata (Table 2). The yeasts detoxified aflatoxins completely. *Geotrichum fermentans* and *Metschnikowia pulcherrima* detoxified ZEN. DON was detoxified by the yeasts in composite fodder better than in flour. The yeast strains selected may be a promising means to reduce or prevent the adverse effects of mycotoxins on animal health and production safety.

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