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International Journal of Food Microbiology 99 (2005) 321–328

INTERNATIONAL JOURNAL OF Food Microbiology

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Degradation of ochratoxin A and other mycotoxins by *Rhizopus* isolates

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Received 27 August 2004; received in revised form 4 October 2004; accepted 12 October 2004

Abstract

Several filamentous fungi representing the genera *Rhizopus* and *Mucor* were examined for their ability to degrade ochratoxin A (OTA), aflatoxin B_1, zearalenone and patulin in a liquid medium. While none of the isolates exhibited aflatoxin degrading activity, ochratoxin A, zearalenone and patulin were decomposed by several isolates. Ochratoxin A was successfully degraded by *Rhizopus stolonifer*, *R. microsporus*, *R. homothallicus* and two *R. oryzae* isolates, and by four unidentified *Rhizopus* isolates. Kinetics of ochratoxin A detoxification of selected *Rhizopus* isolates was also examined. *Rhizopus* isolates were able to degrade more than 95% of ochratoxin A within 16 days. A *R. stolonifer* isolate could also effectively decompose ochratoxin A on moistened wheat. Further studies are in progress to identify the enzymes and genes responsible for ochratoxin detoxification and to transfer these genes to other *Rhizopus* isolates or microbes which could be used safely for decontamination of cereal products.

Keywords: Ochratoxin; Degradation; *Rhizopus*; Mycotoxins

1. Introduction

Mycotoxins are secondary metabolites of fungi, which may cause diseases in animals or humans. Mycotoxin contamination of agricultural products is a serious health hazard throughout the world. Although the prevention of mycotoxin contamination in the field is the main goal of agricultural and food industries, the contamination of various commodities with *Fusarium*, *Aspergillus*, *Alternaria* and *Penicillium* fungi and mycotoxins is unavoidable under certain environmental conditions. Mycotoxin production is dependent on a number of factors, e.g. water activity of the stored product, temperature, gas composition, the presence of chemical preservatives and microbial interactions. An integrated approach for controlling several of these

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doi:10.1016/j.ijfoodmicro.2004.10.034

factors could give much more effective control of deterioration without requiring extreme control of any one factor. Decontamination/detoxification procedures are useful in order to recuperate mycotoxin contaminated commodities. Several strategies are available for the detoxification of mycotoxins. These can be classified as physical, chemical and (micro)biological approaches (Varga and Tóth, in press). However, physical and chemical methods met with varying degrees of success. Microbes or their enzymes could be applied for mycotoxin detoxification; such biological approaches are now being widely studied (Sweeney and Dobson, 1998; Bata and Lásztity, 1999). For example, such biological method has been patented for fumonisin detoxification using an enzyme and its gene from *Exophiala spinifera* (Duvick et al., 1997; Blackwell et al., 1999). Besides, several microbes have been found to be able to degrade ochratoxin A (OTA) (Table 1).

In this study, several zygomycete fungi representing mainly the genus *Rhizopus* were examined for their ability to degrade aflatoxin B_1, zearalenone, patulin and OTA in a liquid medium. Kinetics of OTA degradation was examined in some selected isolates, and model experiments using moistened wheat as substrate have also been set up to examine the applicability of the isolates for OTA degradation in cereals.

Table 1
Microbes able to degrade ochratoxin A

Microbes or enzymes	Reference
Rumen microbes	Galtier and Alvinerie, 1976; Hult et al., 1976; Akiyama, 1997
Butyrivibrio fibrisolvens	Westlake et al., 1987
Lactobacillus, *Streptococcus*, *Bifidobacterium* sp.	Skrinjar et al., 1996
Acinetobacter sp.	Hwang and Draughon, 1994
Phenylobacterium sp.	Wegst and Lingens, 1983
Aspergillus niger, *A. fumigatus*	Varga et al., 2000
Aspergillus niger, *A. versicolor*, *A. wentii*, *A. ochraceus*	Abrunhosa et al., 2002
A. niger (lipase)	Stander et al., 2000
Pleurotus ostreatus	Engelhardt, 2002
Saccharomyces cerevisiae, *Lactobacillus* sp., *Bacillus subtilis*, *B. licheniformis*	Böhm et al., 2000
Carboxypeptidase A	Deberghes et al., 1995; Stander et al., 2001

2. Materials and methods

Altogether 55 zygomycetous isolates were screened for mycotoxin degradation (Table 2). The strains were maintained on malt extract agar slants.

2.1. Screening of *Rhizopus* isolates for mycotoxin degrading activities

The strains were grown in 2 ml of YES (2% yeast extract, 15% sucrose) medium containing either 7.5 μg ml^{-1} OTA, 3 μg ml^{-1} zearalenone, 1 μg ml^{-1} aflatoxin B_1 or 2.5 μg ml^{-1} patulin (Sigma). Test tubes were inoculated with dense spore suspensions of the strains and incubated at 25 °C for 10 days in the dark. Mycotoxins were extracted with 2 ml of dichloromethane. One milliliter of the organic phase was evaporated to dryness and dissolved in 100 μl dichloromethane. Twenty microliters of the extracts was spotted on thin layer chromatography (TLC) plates and chromatographed as described previously (Téren et al., 1996). The detection limit of this technique is 5 ng ml^{-1} (1 ng/spot) for OTA, and 250 ng ml^{-1} (50 ng/spot) for zearalenone and patulin. Experiments were repeated three times.

2.2. Kinetics of OTA degradation

For kinetic studies, 2 ml of liquid YES medium containing 7.5 μg ml^{-1} OTA was inoculated with 20 μl of spore suspensions (10^7 spores ml^{-1}) of selected *Rhizopus* isolates. The liquid cultures were grown for 2, 4, 6, 8, 10, 12, 14 and 16 days in triplicate. OTA was extracted with 2 ml of dichloromethane, the organic phase was transferred to a clean tube, vortexed with 2 ml of 1% $NaHCO_3$ and centrifuged. The aqueous phase was acidified to pH 2 and OTA was reextracted with an equal volume of dichloromethane. Aliquots (5–10 μl) of these extracts were applied to high performance TLC (HPTLC) plates, developed and OTA was identified as described previously (Varga et al., 1996). Kinetic studies were repeated three times.