Biodegradation of Ochratoxin A by Fungi Isolated from Grapes

LUÍS ABRUNHOSA, RITA SERRA, AND ARMANDO VENÂNCIO*
Centro de Engenharia Biológica-IBQF, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal

Ochratoxin A is a mycotoxin present in several food products for which levels should be reduced. Chemical, physical, and biological methods have been proposed for the detoxification of mycotoxins, biological methods being the more promising ones. In this report, filamentous fungi isolated from Portuguese grapes were assessed for ochratoxin A degradation capabilities. It was observed that 51 of the 76 tested strains, predominantly aspergillus species, were able to degrade more than 80% of ochratoxin A added to the culture medium and that the most potent species (more than 95% of initial amount) were the black aspergilli, A. clavatus, A. ochraceus, A. versicolor, and A. wentii. Other fungi frequently isolated from grapes, such as Alternaria, Botrytis, Cladosporium, and Penicillium, also showed significant degradation capabilities. It was observed that the compounds obtained from the degradation of ochratoxin A by black aspergilli and by A. ochraceus and A. wentii strains were different.

KEYWORDS: Alternaria; Aspergillus; Botrytis; Cladosporium; detoxification; grapes; ochratoxin A; Penicillium; wine

INTRODUCTION

Ochratoxin A is a 7-carboxy-5-chloro-8-hydroxy-3,4-dihydro-3R-methylisocoumarin, linked through its 7-carboxy group to l-β-phenylalanine by an amide bond (1). Ochratoxin A is a mycotoxic with nephrotoxic, hepatotoxic, teratogenic, carcinogenic, and immunosuppressive properties (2). Several fungi have been reported as ochratoxigenic, including Aspergillus aliusceaus, A. carbonarius, some strains of the A. niger aggregate, A. ochraceus, and Penicillium verrucosum. Others, such as Aspergillus albertensis, A. auricomus, A. avamori, A. foetidus, A. sclerotiorum, A. terreus, and A. wentii have also been reported as ochratoxin A producers, but their production is not yet well established (3, 4).

Ochratoxin A is frequently detected in food products, such as cereals (5), coffee (6), dried vine fruits (7), beer (8), and wine (9). In the particular case of wine, several efforts have been made to establish a correlation between ochratoxin A presence and fungi isolated from grapes. A. carbonarius and A. niger aggregate strains are now generally incriminated as primarily responsible for ochratoxin A production in grapes and derived products (10–12). It is generally accepted that ochratoxin A levels in food products need to be reduced as low as technologically possible (13). Several physical and chemical methods have been proposed for the removal of mycotoxins from foods and feeds (14). However, few of these have practical application. One example is the removal of ochratoxin A from wines by adsorption with fining agents. Studies showed that most of the fining agents have little effect on the removal of ochratoxin A and that in many cases the quality of wines is severely damaged (15, 16). Therefore, microbiological methods may present a good alternative in mycotoxin detoxification. The use of enzymes is far more convenient than the chemical or physical detoxification methods, since it is substrate specific (17).

Several reports of ochratoxin A biodegradation have been published. Cell cultures of wheat, maize, tomato, soybean, sweet potato tubers, and other plants completely transform ochratoxin A into a number of other products (18). Streptococcus salivarius subsp. thermophilus, Bifidobacterium bifidum, and yogurt bacteria have completely reduced ochratoxin A levels in milk samples containing 0.05 and 0.1 mg/L; Lactobacillus delbrueckii subsp. bulgaricus completely reduced ochratoxin A level in milk samples with 0.5 mg/L (19). Some strains of A. fumigatus, A. japonicus, and A. niger have also been reported to be able to degrade ochratoxin A to less toxic products such as ochratoxin α (20).

This study reports the capacity of ochratoxigenic and nonochratoxigenic filamentous fungi isolated from Portuguese grapes to degrade ochratoxin A to less toxic compounds.

MATERIALS AND METHODS

Filamentous Fungi. Seventy-six strains were selected among filamentous fungi isolated from Portuguese grapes, for which ochratoxinogenic ability was previously assessed according to the method of Bragulat et al. (10). Strains were set in three groups: 37 black aspergilli (A. carbonarius, A. ellipticus, A. foetidus, A. japonicus, and A. niger aggregate); 25 other Aspergillus strains; and 14 other genera. All the selected strains are preserved in the Micoteca da Universidade do Minho culture collection—MUM (21).

Media and Growth Conditions. Strains were first grown in malt extract agar (Blakeslee formula) for 7 days in the dark at 25 °C for inoculum generation. A spore suspension was collected and inoculated...
in 5 mL of yeast extract sucrose medium supplemented with ochratoxin A (YES-Difco + ochratoxin A: 2% of yeast extract from Difco, 15% of sucrose, and 1 µg/mL of ochratoxin A). A control for each strain was also inoculated in 5 mL of yeast extract sucrose medium (YES-Difco: 2% of yeast extract from Difco and 15% of sucrose). Inoculated flasks were incubated slanted to increase superficial area, for 6 days, in the dark, at 25 °C.

**Ochratoxin A Extraction.** After incubation, fungal colonies were removed from the remaining medium. The medium was acidified with 1 mL of 2 M HCl and extracted twice with 5 mL of chloroform. This removed ochratoxin A from the remaining medium. The medium was acidified with a Jasco FP-920 fluorescence detector (λex = 333 nm; λem = 460 nm) and a Borwin version 1.21.60 acquisition data system. The mobile phase consisted of a mixture of HPLC grade acetonitrile/water/acetic acid (99/99/2, v/v/v), filtered with a 0.2-μm GHP membrane filter (Gelman) and degassed. The mobile phase flow rate was set to 1.0 mL/min and the column temperature to 30 °C. The dried extracts were dissolved in 1 mL of mobile phase and filtered with a 0.45-μm Acrodisc GHP membrane filter (Gelman). Injections were done with a manual Knauer injection valve equipped with a 10-μL loop. A calibration curve, which was regularly checked, was prepared with three ochratoxin A standards (Sigma). Ochratoxin A was identified by comparing retention times of peak samples with standards. Quantification was done by measuring peak height and comparing it with the respective calibration curve. The ochratoxin A degradation percentage was calculated relative to the recovery mean value (85.9%) as the initial amount present in media.

Standards of ochratoxin A were prepared by acid or by enzymatic hydrolysis of ochratoxin A. Acid hydrolysis was performed as described by van der Merwe et al. (1). Enzymatic hydrolysis was performed with carboxypeptidase A (EC 3.4.17.1) from bovine pancreas (Sigma, type II-PMSF). Ochratoxin A was dissolved in 1 mL of buffered solution (0.025 M Tris-HCl, 0.5 M NaCl, pH 7.5) and 20 μL of enzyme solution was added. Total degradation was observed after 2 days of incubation at room temperature.

**RESULTS AND DISCUSSION**

As shown in Table 1, most of the tested strains degraded ochratoxin A while growing on the selected medium: 51 strains (67% of tested strains) were able to degrade more than 80% of ochratoxin A added to YES-Difco medium. These 51 strains belong to 13 different species from three genera: *Aspergillus* (10 species), *Cladosporium* (one species), and *Penicillium* (two species).

Most aspergilli extensively degraded ochratoxin A (>95%). Black aspergilli, *A. clavatus*, *A. ochraceus*, *A. versicolor*, and *A. wentii* were the species most effective in this regard. In a similar study, Varga and co-workers (20) have reported the degradation of ochratoxin A by some strains of *A. fumigatus*, *A. japonicus*, and *A. niger*. In our study we observed that other species also have ochratoxin A degradation capability. The differences observed between these two studies may be due to the ochratoxin A detection method employed by Varga et al.
Ochratoxin A Degradation by A. alliaceus, A. ochraceus, and A. wentii. The *Aspergillus alliaceus*, *A. ochraceus*, and *A. wentii* strains tested behaved differently from the black aspergilli. The two ochratoxigenic strains (one *A. alliaceus* and one *A. ochraceus*) produced large amounts of ochratoxin A. Therefore, the mycotoxin detected after the 6 days incubation exceeded the initial amount added. These strains either do not degrade ochratoxin A or do it at a slower rate than they produce it.

The nonochratoxigenic *A. ochraceus* (one strain) and *A. wentii* (four strains) degraded ochratoxin A in large amounts, yielding reaction products that are not produced during the degradation by the black aspergilli (Figure 1B). The degradation pathway of ochratoxin A in these two species differs from the one observed in the black aspergilli.

In this study, it is clearly observed that the fungi most frequently isolated from grapes, *Alternaria*, *Aspergillus*, *Botrytis*, *Cladosporium*, and *Penicillium* (10, 22), have significant ochratoxin A degradation capabilities, regardless of being ochratoxigenic or not. This capacity may be present in grapes and should be taken into account when trying to relate the ochratoxin A presence in grapes with their mycobiota. When analyzing the ochratoxin A degradation products, it was found that all the black aspergilli produced the same products and that *A. ochraceus* and *A. wentii* exhibited a different degradation pathway. Different degradation enzymes and pathways may be involved in the degradation of ochratoxin A by these species.

**SAFETY**

Ochratoxin A is a toxic compound that needs to be manipulated with care and with appropriate safety precautions. Decontamination procedures for laboratory wastes have been reported by the International Agency for Research on Cancer (IARC) (23) and were employed throughout this experimental work.

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**LITERATURE CITED**


