J. Microbiol. Biotechnol. (2016), 26(10), 1687–1695
http://dx.doi.org/10.4014/jmb.1606.06016

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Biodegradation of Ochratoxin A by *Aspergillus tubingensis* Isolated from Meju

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Received: June 9, 2016
Revised: July 29, 2016
Accepted: August 2, 2016

First published online
August 24, 2016

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pISSN 1017-7825, eISSN 1738-8872

Ochratoxin A (OTA), a mycotoxin, contaminates agricultural products and poses a serious threat to public health worldwide. Microbiological methods are known to be a promising approach for OTA biodegradation because physical and chemical methods have practical limitations. In the present study, a total of 130 fungal isolates obtained from 65 traditional Korean meju (a fermented starter for fermentation of soybeans) samples were examined for OTA-biodegradation activity using thin-layer chromatography. Two fungal isolates were selected for OTA-biodegradation activity and were identified as *Aspergillus tubingensis* M036 and M074 through sequence analysis of the beta-tubulin gene. After culturing both *A. tubingensis* isolates in Soytone-Czapek medium containing OTA (40 ng/ml), OTA-biodegradation activity was analyzed using high-performance liquid chromatography (HPLC). Both *A. tubingensis* strains degraded OTA by more than 95.0% after 14 days, and the HPLC analysis showed that the OTA biodegradation by the *A. tubingensis* strains led to the production of ochratoxin α, which is much less toxic than OTA. Moreover, crude enzymes from the cultures of *A. tubingensis* M036 and M074 led to OTA biodegradation of 97.5% and 91.3% at pH 5, and 80.3% and 75.3% at pH 7, respectively, in a buffer solution containing OTA (40 ng/ml) after 24 h. In addition, the OTA-biodegrading fungi did not exhibit OTA production activity. Our data suggest that *A. tubingensis* isolates and their enzymes have the potential for practical application to reduce levels of OTA in food and feed.

Keywords: Ochratoxin A, biodegradation, *Aspergilllus tubingensis*, meju

Introduction

Ochratoxin A (OTA) is a toxic secondary metabolite produced by several *Aspergillus* and *Penicillium* species found in contaminated feed and food products [2]. It has been documented that OTA consists of a chlorinated isocoumarin moiety linked to the carboxyl group of phenylalanine via an amide bond. In addition, OTA is hepatotoxic, nephrotoxic, carcinogenic, and immunosuppressive to animals and possibly to humans [30]. Agricultural products are known to be contaminated by ochratoxigenic fungi during harvest or storage. OTA poses a serious threat to human health and economies around the globe because it can be found in decaying vegetation, corn, storage grains, and other plant products such as flour, peas, peanuts, spices, and coffee beans [29]. The International Agency for Research on Cancer has classified OTA as a possible human carcinogen (group 2B) [13]. The Joint FAO/WHO Expert Committee on Food Additives suggested a provisional tolerable weekly intake of 100 ng/kg body weight (b.w.), corresponding to approximately 14 ng/kg b.w. per day [7], and the European Food Safety Authority proposed a tolerable weekly intake of 120 ng/kg b.w. for OTA [3]. In order to reduce the risk of consumer exposure to OTA, various detoxification strategies have been applied to prevent its occurrence in food and feed. In a study by Huwig *et al.* [12], adsorbents such as aluminosilicates, activated charcoals, and special polymers were mixed with

feed to eliminate mycotoxins. Although the use of pesticides during the production process can help minimize mycotoxin contamination, the long-term use of fungicides increases public concern about economic losses and food safety. It has also been proposed that OTA found in grains can be detoxified by O_3 treatment [15]. However, several of the physical and chemical methods that have been proposed for the detoxification of OTA have practical limitations, such as the loss of flavor and nutritional value of the food [21]. To overcome these challenges, microbiological methods may be the most promising approach for the biodegradation of OTA. Several microorganisms have been screened for OTA-biodegradation activity. One study by Rodriguez *et al*. [17] demonstrated that *Brevibacterium* sp. strains have the ability to completely degrade OTA at concentrations as high as 40 μg/ml, which is thousands times higher than the concentration of OTA commonly found in food. Similar studies have indicated that *Bacillus subtilis* CW 14 not only prevents OTA contamination but also degrades OTA in crops [23]. It was also shown that more than 95% of OTA was degraded by *Rhizopus* isolates within 16 days [28]. Similarly, a kinetic study on OTA in a synthetic grape juice medium has reported OTA detoxification by *A. niger* and *A. japonicus* [4]. In addition, mammalian enzyme carboxypeptidase A has the ability to cleave OTA [25].

Meju is a fermented starter for the production of Korean traditional fermented soybean products (doenjang, ganjang, and kochujang). Meju is often contaminated with OTA because of the growth of ochratoxigenic fungi. In the present study, two strains of OTA-biodegrading fungi were isolated from traditional Korean meju samples. The fungal strains were identified and examined for their OTA-biodegradation activities. The OTA biodegradation by crude enzymes prepared from the two fungal strains was also examined to determine whether these enzymes could be practically applied to reduce levels of OTA in food fermentation and/or feed production.

Materials and Methods

Meju Samples and Chemicals

A total of 65 meju samples were collected from 20 mid-western, 22 south-western, and 23 south-eastern Korean locations over the course of 2 years from March 1, 2013 to March 1, 2015.

The acetic acid, acetonitrile, and methanol used in the experiment were of HPLC grade and purchased from J. T. Baker (USA). Ultrapure water was prepared with the Milli-Q system (Millipore, USA). OTA (50 μg/ml) in benzene-acetic acid (at a ratio of 99:1 (v/v)) and carboxypeptidase A (E.C. 3.4.17.1) from bovine pancreas (CPA) were purchased from Sigma-Aldrich (USA). The OTA solution was evaporated under a gentle stream of N_2 at 50°C, dissolved in 5 ml of methanol, and used as the OTA stock solution. Ochratoxin α (OTα) was prepared using the enzymatic hydrolysis of OTA. The OTA stock solution (50 μg/ml in methanol) was mixed with 50% methanol and diluted with pure water to obtain 50 ng/ml OTA. Following this, 800 μl of OTA (50 ng/ml) was mixed with 200 μl of CPA, and they were reacted for 2 days at 25°C. HPLC was used to confirm that the OTA had completely converted to OTα, and the concentration of OTα in the enzymatic hydrolysis solution was considered to be equivalent to a 40 ng/ml OTA solution.

Screening of OTA-Biodegrading Fungi

A total of 130 fungal isolates from meju samples were used for the screening of OTA biodegradation as described previously [14]. Fungal culture in Soytone-Czapek (S-Cz) medium (3 g soytone, 1 g K_2HPO_4, 0.5 g KCl, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.01 g $FeSO_4 \cdot H_2O$, 30 g sucrose, and 1 L distilled water) supplemented with OTA was used for the screening of OTA-biodegrading fungi. A 500 μl fungal spore suspension (5×10^5 spores/ml) prepared from each PDA slant was inoculated into 100 ml of S-Cz medium spiked with 40 ng/ml OTA. The culture was incubated in the dark at 25°C with shaking at 100 rpm. After 7 and 14 days of incubation, the fungal culture broth was filtrated using Whatman No. 4 filter paper (USA) for quantitative analysis of OTA biodegradation. A 1 ml sample of the fungal culture filtrate and 3 ml of ethyl acetate were mixed using vortexing for 30 sec. The mixture was placed for 1 h at room temperature, and then the upper layer was transferred to a new glass vial. After 3 ml of ethyl acetate was added to the lower layer, the procedure above was repeated, and then the upper layer of this was combined with the first upper layer. The combined supernatants were evaporated under a gentle stream of N_2 at 50°C. The residue was dissolved in 50 μl of benzene-acetonitrile (98:2 (v/v)) for TLC analysis, from which 20 μl was spotted on a TLC plate (TLC Silica gel 60, 20 × 20 cm; Merck KGaA, Germany). Benzene-methanol-acetic acid (180:10:10 (v/v)) was used as a development solvent at room temperature. When the solvent reached 16 cm from the origin on a plate, the TLC plate was removed from the tank, dried at room temperature, and illuminated by UV light (λ = 365 nm) in a dark room.

Identification of OTA-Biodegrading Fungi

To identify the fungal isolates with OTA-biodegradation activity, morphological and molecular identifications were carried out. For the morphological identification of the fungal isolates, each isolate was inoculated onto a Czapek yeast autolysate (CYA) agar plate and incubated at 25°C for 5 days [18]. Morphological characteristics were examined in relation to their macro-/microscopic colony appearance. For the molecular identification of the fungal isolates, the DNA sequence analysis of the beta-tubulin gene was carried out according to the method described previously [10]. Fungal DNA was extracted from the mycelia cultured in potato dextrose broth (PDB; Difco, USA). A partial sequence of the β-tubulin gene

was amplified using two primers: Bt2a (5′-GGT AAC CAA ATC GGT GCT GCT TTC-3′), and Bt2b (5′-ACC CTC AGT GTA GTG ACC CTT GGC-3′) [11]. A polymerase chain reaction (PCR) was performed using the thermal cycler PC708 (ASTEC, Japan). The PCR was conducted in a 50 µl solution containing 5 µl of 10× PCR buffer, 3 µl of 2.5 mM deoxyribonucleotide triphosphate, 0.4 µl of each primer (100 pmol), 0.3 µl of Taq polymerase (Biofact, Korea), 39.9 µl of sterile deionized water, and 1 µl of DNA template. The conditions for the PCR were as follows: predenaturation for 4 min at 95°C; denaturation for 1 min at 95°C; annealing for 1 min at 60°C; extension for 2 min at 72°C (35 cycles of denaturation, annealing, and extension); and a post-extension for 7 min at 72°C. The amplified PCR products were sequenced by Biofact. The Basic Local Alignment Search Tool was used to find the local similarity between sequences from the fungal isolates and the strains from GenBank in the National Center for Biotechnology Information (NCBI) nucleotide database. Each sequence of beta-tubulin gene from the two *A. tubingensis* strains was used to determine the phylogenetic relationships with those of *A. tubingensis* type strains and closely related genera. A phylogenetic tree was constructed using a neighbor-joining tree in MEGA5 [27].

Time Course of Fungal Growth, pH, and OTA Biodegradation

A kinetic study of fungal growth, pH, and OTA biodegradation was carried out with the two *A. tubingensis* strains. Each fungal spore suspension (5×10^5 spores/ml) was inoculated into 100 ml of S-Cz medium spiked with OTA (40 ng/ml), followed by incubation in the dark at 25°C with shaking at 100 rpm for 14 days. The fungal dry cells were weighed, and the pH of the culture broth was measured with a pH meter (HI-2210; Hanna Instruments, USA). To measure fungal growth, the fungal culture was filtrated through pre-dried filter paper, and the mycelia on the filter paper were dried for 24 h at 80°C before weighing.

To assess the degradation of OTA, culture filtrates taken on the 7^{th} and 14^{th} days were analyzed qualitatively using HPLC. An OTA standard and samples taken from the culture broth were filtered through a 0.22 µm membrane filter (Sartorius, Germany) before HPLC analysis. A Dionex Ultimate 3000 UHPLC system (Thermo Scientific, USA) was used for OTA analysis. Separation was carried out using a Nova-Pack C_{18} column (4.6 mm × 250 mm, 5 µm; Waters, USA). The injection volume for the OTA standard and the samples was 50 µl. The mobile phase, acetonitrile-water-acetic acid (99:99:2 (v/v/v)), was pumped in at a constant flow rate of 0.8 ml/min, giving a total run time of 20 min. A fluorescence detector was used for determination of OTA at the excitation wavelength of 330 nm and emission wavelength of 460 nm.

All experiments for fungal growth, pH, and OTA biodegradation were carried out in triplicates, and the results are expressed as the mean ± standard error.

Effect of pH on OTA Stability

To determine the influence of pH on the stability of OTA in S-Cz medium, 50 ml of the broth was adjusted with 1 N HCl or 1 N NaOH so that the pH ranged from 1 to 10. The pH-adjusted S-Cz broth was supplemented with OTA (40 ng/ml) and incubated for 21 days at 25°C. The OTA concentration was analyzed by HPLC and compared with that of the control S-Cz broth (pH 7).

OTA Biodegradation by Crude Enzymes Prepared from the Two *A. tubingensis* Strains

To prepare the fungal crude enzymes, a 500 µl spore suspension (5×10^5 spores/ml) for each fungal isolate was inoculated into 250 ml of S-Cz medium and incubated for 14 days at 25°C with shaking at 100 rpm. Each fungal culture broth was filtered using Whatman No. 4 filter paper, and then 100 ml of cold acetone (−20°C) was added to 30 ml of the filtrate. The mixture was centrifuged at 10,000 ×*g* for 20 min at 4°C to obtain a precipitate, followed by resuspension in 2 ml of phosphate-buffered saline, pH 7.0, which was used as fungal crude enzymes. The crude enzymes were aseptically filtered using 0.45 µm sterile cellulose non-pyrogenic acetate membrane filters (Sartorius), and immediately used for OTA-degradation testing. To assay the OTA-biodegradation activity of each crude enzyme at pH 5 and pH 7, a 2 ml phosphate-citrate buffer solution (pH 5 and pH 7) supplemented with 40 ng/ml OTA was reacted with 0.5 ml of crude enzyme solution for 0, 1, 12, and 24 h at 25°C.

OTA Production Test for *A. tubingensis* Isolates

OTA production by the two *A. tubingensis* isolates was tested in S-Cz medium. Toxigenic *Aspergillus niger* KU028 (stock culture at Korea University) was used as a positive control strain. Each fungal spore suspension (5×10^5 spores/ml) was prepared with a 0.05% Tween 80 solution and inoculated into the S-Cz broth. After incubation for 14 days at 25°C with shaking at 100 rpm, the concentration of OTA in the culture broth was analyzed using HPLC.

Results

Isolation of Fungi from Meju and Screening of OTA-Biodegrading Fungi

A total of 130 fungal strains were isolated from the 65 meju samples and used for the screening of OTA biodegradation. The OTA-biodegradation activity of each fungal isolate was analyzed using TLC after each fungal isolate was cultured in S-Cz medium containing OTA. The extract from the uninoculated sample (control) produced an OTA spot with typical blue fluorescence. Of the 130 blue fluorescent spots, those for isolates M036 and M074 disappeared or were reduced when compared with the control (data not shown), indicating that these two fungal isolates cause OTA degradation. Isolate M036 was from a meju sample from mid-western Korea, and isolate M074 was from south-

eastern Korea. The reduction in blue fluorescence exhibited by the two fungal isolates was more significant in the fungal cultures after 14 days than those after 7 days. These two isolates were selected and used for fungal identification and further OTA-biodegradation testing.

Identification of OTA-Biodegrading Fungal Isolates

To identify fungal isolates with OTA-biodegradation activity, morphological and molecular identifications were carried out. The two OTA-biodegrading fungal species (M036 and M074) formed dark brown to black colonies on CYA plates, and their spores were black and powdery in texture. Examination under a microscope revealed that they had an unbranched stipe (conidiophore) with a swollen vesicle, phialides borne directly on the vesicle, and conidia radiating onto the phialides from the vesicle (Fig. 1). These results indicated that the two OTA-biodegrading fungal isolates belong to the *Aspergillus* genus. The beta-tubulin gene was thus selected for the molecular identification of the two isolates. The PCR product size of the partial beta-tubulin gene from *Aspergillus* M036 and M074 was 415 and 420 bp, respectively. The beta-tubulin gene sequences of the two *Aspergillus* strains exhibited a similarity of more than 99% with the sequence in the same region of *A. tubingensis* strains in the NCBI database. The taxonomic position of the OTA-biodegrading *Aspergillus* M036 and M074, along with representative strains of *Aspergillus* section Nigri, based on the beta-tubulin gene is shown in Fig. 2. The two *Aspergillus* strains are closely related to *A. tubingensis* CBS 134.48^{T}, a type strain of *A. tubingensis*, in the molecular phylogenetic tree. Based on these results, the two OTA-biodegrading *Aspergillus* isolates were identified as *Aspergillus tubingensis* M036 and *Aspergillus tubingensis* M074, respectively.

Time Course of Fungal Growth, pH, and OTA-Biodegradation Activity in the Two *A. tubingensis* Cultures

A. tubingensis M036 and M074 were cultured in S-Cz medium supplemented with 40 ng/ml OTA for 14 days, and the fungal growth, pH, and OTA biodegradation were investigated. The fungal dry cell weight (DCW) increased up to 10 days, with maximum growth maintained in the later phase. The maximum DCW of *A. tubingensis* M036 and M074 was 172 ± 6.0 and 167 ± 6.5 mg, respectively, on the 14th day (Fig. 3). The pH of the *A. tubingensis* M036 and M074 cultures decreased drastically from pH 7.0 to pH 2.4 ± 0.3 and 2.1 ± 0.2, respectively, during the first 3 days and then slowly decreased to pH 1.8 ± 0.2 and 1.6 ± 0.1, respectively, by the end of the 14 days (Fig. 3). OTA biodegradation and the conversion to OTα by *A. tubingensis* M036 and M074 were analyzed qualitatively using HPLC; the results are presented in Fig. 4. The content of OTA in the media fell from 40 ng/ml to 5.4 ± 2.5 ng/ml for *A. tubingensis* M036 and to 11.9 ± 1.4 ng/ml for M074 after 7 days, an OTA degradation rate of 86.5% and 70.2%, respectively. After

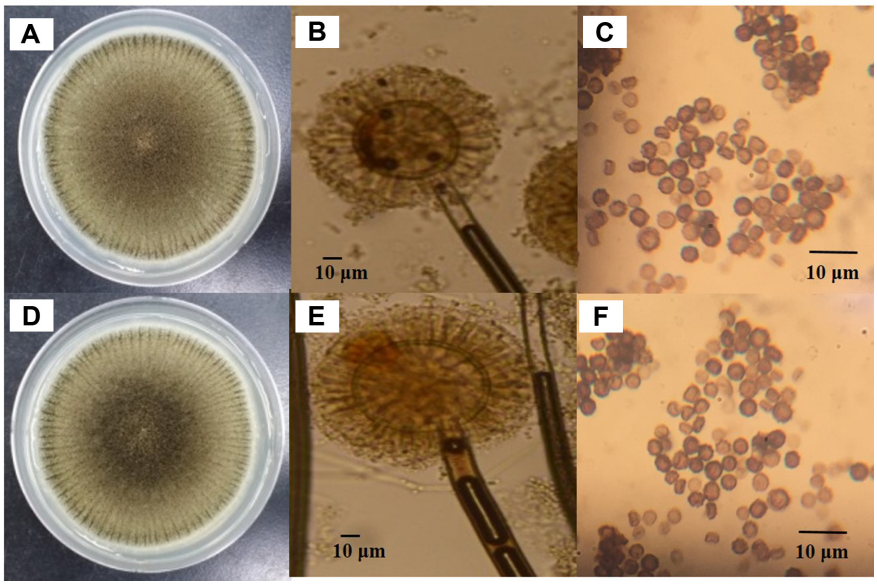

Fig. 1. Morphological characteristics of OTA-biodegrading fungi.

The fungal isolates M036 and M074 were inoculated onto CYA plates and incubated at 25°C for 5 days and examined under the microscope. (**A**) M036 colony, (**B**) M036 conidial head (×400), (**C**) M036 conidia (×1,000), (**D**) M074 colony, (**E**) M074 conidial head (×400), and (**F**) M074 conidia (×1,000). Scale bars 10 μm.

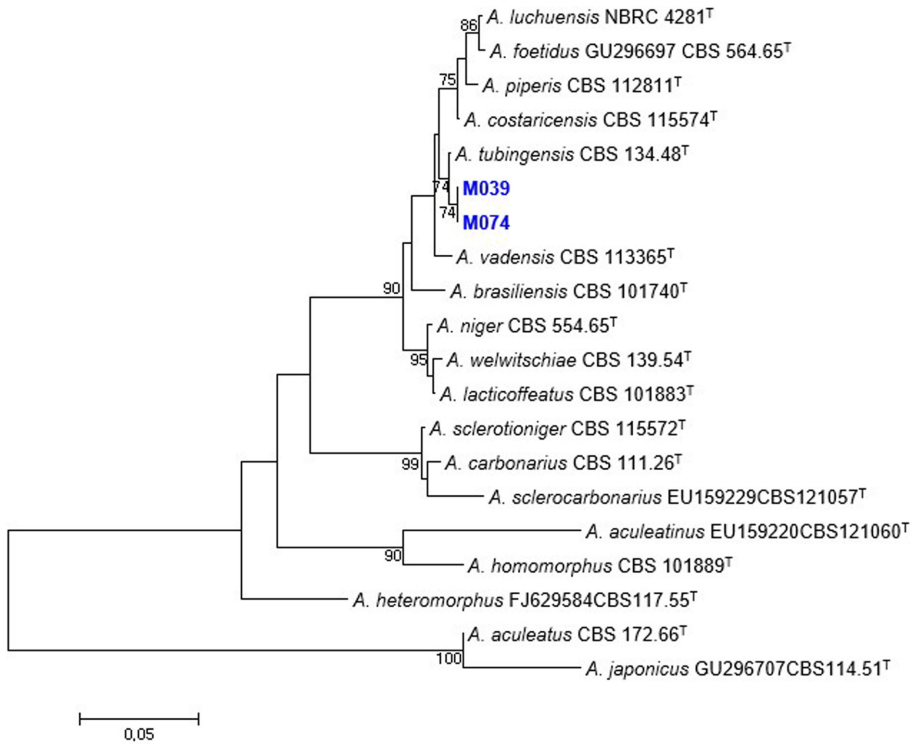

Fig. 2. Phylogenetic tree depicting the taxonomic position of the two *Aspergillus tubingensis* strains based on beta-tubulin.
Each sequence of the beta-tubulin gene from the M036 and M074 strains was used to determine their phylogenetic position. The phylogenetic tree was constructed using a neighbor-joining method. The "T" after the collection number indicates the type strain of the species.

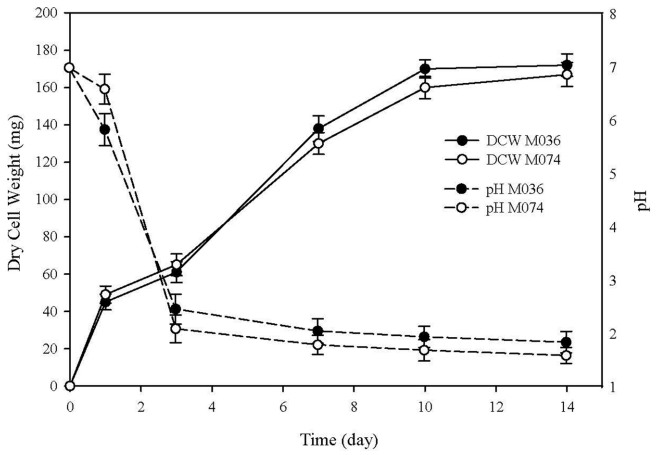

Fig. 3. Time course of fungal growth and pH change.
The dry cell weight (DCW) and pH of *Aspergillus tubingensis* M036 and M074 were observed over 14 days of incubation in S-Cz medium at 25°C. The DCW and pH were measured in triplicates. The results are expressed as the mean ± standard error.

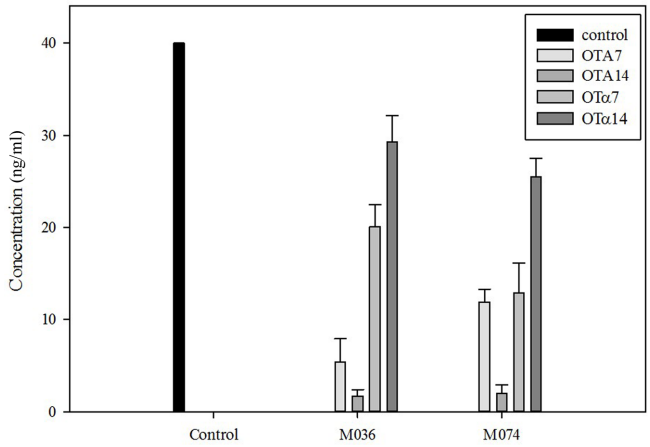

Fig. 4. OTA biodegradation and OTα production by *Aspergillus tubingensis* M036 and M074.
A. tubingensis M036 and M074 were incubated in S-Cz broth containing OTA (40 ng/ml) for 14 days at 25°C. The control was S-Cz broth containing OTA without fungal inoculation. The concentration of OTA and OTα was measured on the 7th and 14th days of incubation in triplicates. The results are expressed as the mean ± standard error.

14 days, the OTA concentration in the cultures of *A. tubingensis* M036 and M074 was 1.7 ± 0.7 and 2.0 ± 0.9 ng/ml, an OTA degradation rate of 95.8% and 95.0%, respectively. OTα production increased as OTA decreased for both *A. tubingensis* M036 and M074, with an OTα concentration of 20.08 ± 2.4 and 12.85 ± 3.3 ng/ml on the 7th day and 29.3 ± 2.9 and

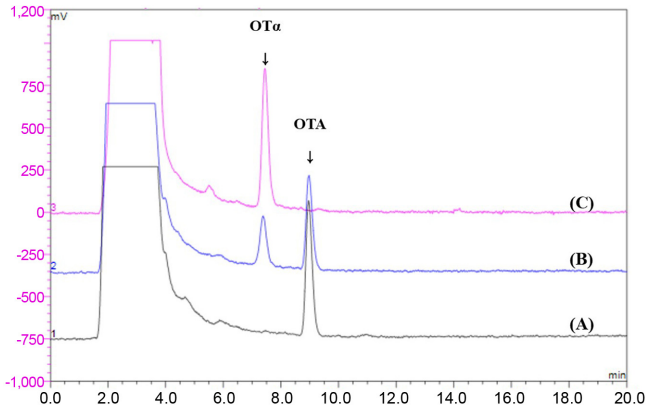

Fig. 5. HPLC analysis of OTA biodegradation by *Aspergillus tubingensis* M036 over 14 days.

A. tubingensis M036 was incubated in S-Cz broth containing OTA (40 ng/ml) for 14 days at 25°C. The peaks for OTA and OTα occurred at 9.0 and 7.4 min, respectively. (**A**) S-Cz broth spiked with OTA (control), (**B**) OTA and OTα concentration on the 7th day in S-Cz broth spiked with OTA, and (**C**) OTA and OTα concentration on the 14th day in S-Cz broth spiked with OTA.

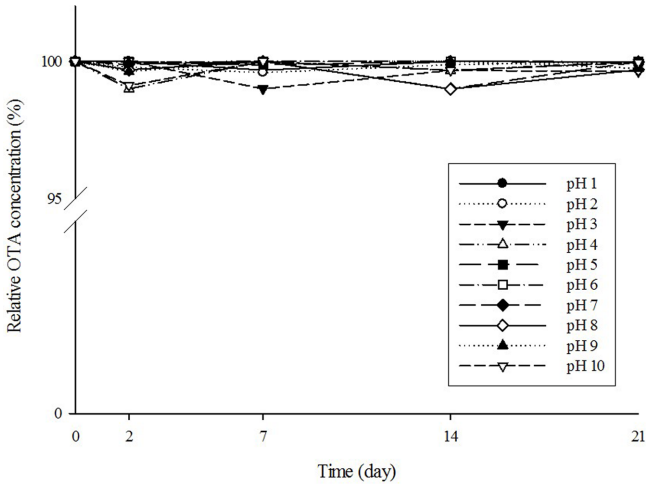

Fig. 6. Effect of pH on OTA stability.

The pH of S-Cz broth was adjusted to range from 1 to 10 using 1 N HCl or 1 N NaOH. The broth was then spiked with 40 ng/ml OTA and incubated for 21 days at 25°C.

25.5 ± 2.0 ng/ml on the 14th day, respectively. Fig. 5 displays representative chromatograms for OTA biodegradation and OTα production by *A. tubingensis* M036.

Effect of pH on OTA Stability

The two OTA-biodegrading *A. tubingensis* strains exhibited a drop in pH to 2.1–2.4 in culture within the first 3 days. In order to investigate whether OTA was degraded as a result of this pH change, the stability of OTA in S-Cz broth of differing pH (pH 1 to 10) was measured. For all of these broth, OTA (40 ng/ml) remained steady for 21 days at room temperature (Fig. 6), which suggests that OTA is stable in the pH range of 1 to 10. This indicates that OTA degradation in the cultures of the two *A. tubingensis* strains was not due to an acidic pH.

OTA Biodegradation by Crude Enzymes

Crude enzymes produced from the fungal cultures after incubation for 14 days were used to examine OTA biodegradation at pH 5 and 7. The enzyme activity for OTA biodegradation at pH 5 was higher than that at pH 7. Fig. 7 presents the decrease in OTA over time and the corresponding increase in OTα at pH 5 and 7. The crude enzyme drastically decreased OTA within 1 h. At pH 5, the initial OTA level (40 ng/ml) was reduced to 4.3 ± 1.5 and 6.5 ± 1.6 ng/ml after 1 h and to 2.5 ± 0.6 and 3.5 ± 0.7 ng/ml after 24 h by crude enzymes from *A. tubingensis* M036 and M074, respectively, representing a 95.7% and 83.8% decrease after 1 h, and a 97.5% and 91.3% decrease after 24 h. At pH 7, the initial OTA level (40 ng/ml) was reduced to 11.8 ± 1.8 and 14.2 ± 1.5 ng/ml after 1 h and 7.9 ± 1.1 and 9.9 ± 1.0 ng/ml after 24 h by crude enzymes from *A. tubingensis* M036 and M074, respectively (OTA decrease: 70.5% and 64.5% after 1 h, and 80.3% and 75.3% after 24 h, respectively).

OTA Production Test of *A. tubingensis* Isolates

It has been reported that certain species of aspergilli produce OTA, including *A. ochraceus*, *A. niger*, *A. westerdijkiae*, and *A. tamari* [6, 9, 19]. Recently, Medina *et al.* [16] reported that *A. tubingensis* also produces OTA. *A. tubingensis* M036 and M074 were cultured in S-Cz broth for 14 days to examine whether OTA was produced, and then the concentration of OTA was measured using HPLC. *A. niger* KU028 was also cultured using the same medium to act as a representative OTA-producing strain. The OTA peak produced by the toxigenic *A. niger* KU028 in the HPLC analysis occurred at 9.0 min, whereas an OTA peak was not detected in the *A. tubingensis* M036 and M074 cultures (Fig. 8). This indicates that *A. tubingensis* M036 and M074, isolated from meju samples, were not OTA producers.

Discussion

The objective of this study was to investigate OTA-biodegradation activity by *A. tubingensis* strains isolated from traditional Korean meju. Food contamination by

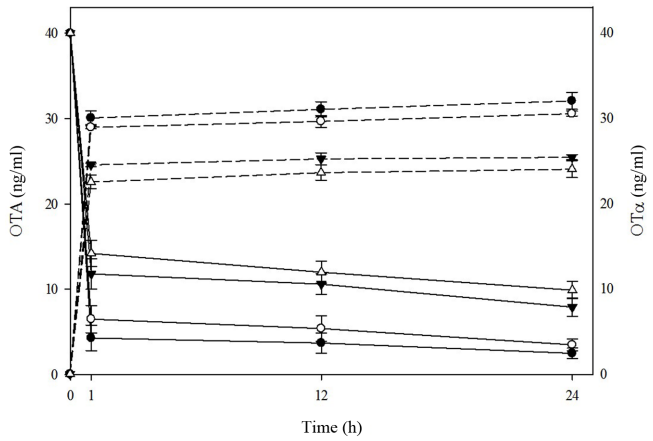

Fig. 7. OTA biodegradation and OTα production by crude enzymes obtained from *Aspergillus tubingensis* M036 and M074.

A. tubingensis M036 and M074 were cultured in S-Cz broth for 14 days at 25°C, from which crude enzymes were produced. Each crude enzyme was reacted with 40 ng/ml OTA in phosphate-citrate buffer solutions (pH 5 and 7) for 0, 1, 12, and 24 h, respectively. OTA concentration at pH 5: *A. tubingensis* M036 (—●—), *A. tubingensis* M074 (—○—); OTA concentration at pH 7: *A. tubingensis* M036 (—▼—), *A. tubingensis* M074 (—△—); OTα concentration at pH 5: *A. tubingensis* M036 (— -●- —), *A. tubingensis* M074 (— -○- —); OTα concentration at pH 7: *A. tubingensis* M036 (— -▼- —), *A. tubingensis* M074 (— -△- —).

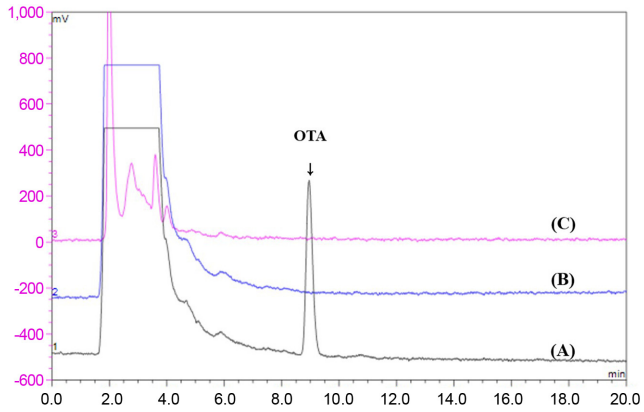

Fig. 8. OTA production test for *Aspergillus tubingensis* M036 and M074.

A. tubingensis M036 and M074 were incubated in S-Cz medium for 14 days at 25°C. *A. niger* KU028 was used as an OTA producer (control). The peak of OTA occurred at 9.0 min. (**A**) *A. niger* KU028, (**B**) *A. tubingensis* M036, and (**C**) *A. tubingensis* M074.

mycotoxins poses a serious health threat worldwide. Despite attempts to eliminate mycotoxins using physical and chemical methods, biological mycotoxin detoxification is thought to be a more practical solution that can be used to reduce the levels of mycotoxins in agricultural products. It has been shown that microorganisms are able to degrade mycotoxins in food and feed [2]. The reduction of OTA by *Lactobacillus* and *Streptococcus* species has been observed in milk [24]. OTA has been converted to OTα and phenylalanine by *Lactobacillus vitulinus* isolated from rumen fluid [20]. In addition, *Bacillus amyloliquefaciens* ASAG1 was able to degrade OTA efficiently in grain depot-stored maize [5]. Similar studies have clearly demonstrated that *Cupriavidus basilensis* Őr16 efficiently degrades OTA without producing toxic random (adventitious) metabolites [8]. A similar observation confirmed that *A. niger* can decompose OTA to OTα [1]. With a view to the industrial application of *A. tubingensis* in the biological control of OTA, kinetic studies were carried out in liquid culture media. OTA in culture broth incubated with *A. tubingensis* M036 and M074 was degraded by more than 95.0% within 14 days. It has been reported that OTα is produced by the carboxypeptidase enzyme [1]. This study demonstrated that crude enzymes obtained from the two *A. tubingensis* isolates hydrolyzed OTA to OTα, indicating the possible existence of extracellular enzymes. The crude enzymes converted more than 91% and 75% of the OTA present in the reaction assay to OTα at pH 5 and 7, respectively. This activity is sufficient to significantly reduce the levels of OTA during soybean fermentation because the fermentation process takes more than 6 months to complete. Alternatively, S-Cz medium could also have favored the production of extracellular enzymes, thereby increasing the degradation activities of *A. tubingensis*. Furthermore, the relative stability of OTA at various pH levels supports the assertion that OTA-biodegradation activity is not due to the pH drop caused by the presence of organic acids produced by the fungi.

It has been reported that 3%–10% of the *A. niger* strains produce OTA under favorable conditions [22]. In addition, it has been shown that OTA can be produced by *A. tubingensis* under laboratory conditions [16, 26]. In this study, *A. tubingensis* M036 and M074 isolated from meju did not produce OTA (Fig. 8), which indicates the two *A. tubingensis* strains can be used in food and feed production. Although researchers have previously reported OTA-biodegradation activity by certain microorganisms, including *A. niger*, biological elimination of OTA by *A. tubingensis* has not been reported to the best of our knowledge. In conclusion, *A. tubingensis* can be used as a starter culture for meju to decompose OTA that has contaminated the fermented product. In addition, the enzymes from the isolates could

be applied directly to food and feed to eliminate OTA. Thus, the isolation and purification of OTA degradation enzymes from *A. tubingensis* would be valuable, and further work on the purification, identification, and characterization of these enzymes is currently in progress.

Acknowledgments

This research was supported by research grants from the Korea Food and Drug Administration.

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