Biotransformation and detoxification of aflatoxin B₁ by extracellular extract of *Cladosporium uredinicola*

Shuai Shao, Jun Cai*, Xin Du, ChangGao Wang, JianGuo Lin, and Jun Dai

Key Laboratory of Fermentation Engineering (Ministry of Education), Hubei Provincial Cooperative Innovation Center of Industrial Fermentation, Hubei University of Technology, Wuhan 430068, China

Received April 22, 2016
Revised August 30, 2016
Accepted September 12, 2016
Published online December 31, 2016

*Corresponding Author
Tel: +86-18971544678
Fax: +86-027-59750483
E-mail: caijun@mail.hbut.edu.cn

pISSN 1226-7708
eISSN 2092-6456

© KoSFoST and Springer 2016

Abstract Aflatoxin contamination of food and grain poses a serious economic and health problem globally. Aflatoxin B₁ (AFB₁) is extremely mutagenic and toxic as well as a potent carcinogen to both humans and livestock. In this study, the degradation of AFB₁ by extracellular extract of *Cladosporium uredinicola* was examined using high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), and liquid chromatography mass spectrometry. Within 24 h of incubation, AFB₁ was efficiently eliminated by the culture supernatant of *C. uredinicola* (84.5±5.7%) at 37°C; the elimination was proven to be enzymatic, and the enzyme was thermostable. The biotransformation products of AFB₁ detected by HPLC and TLC were proven to be the same compound. Analysis with LCMS showed that AFB₁ was bio-transformed to a structurally different compound (m/z=365 [M+Na]⁺), which is first reported. The cytotoxicity study to HeLa cells indicated that culture supernatant-treated AFB₁ is less toxic as compared with AFB₁.

Keywords: Aflatoxin B₁, biotransformation, *Cladosporium uredinicola*, culture supernatant, cytotoxicity

Introduction

Aflatoxins (AFs) are typically found as secondary metabolites of *Aspergillus parasiticus* and *Aspergillus flavus* (1). Among all aflatoxins, aflatoxin B₁ is the most toxic, mutagenic, and carcinogenic to both humans and livestock and is classified into group I as human carcinogen by the International Agency for Research on Cancer (2). Epidemiological dates have indicated that contamination of food with AFB₁ may be an important etiological factor in human liver cancer in several parts of Africa and Asia (3–5). Numerous chemical and physical methods for the detoxification of aflatoxin were reported but only a few of them (e.g., ammonia treatment) have been accepted for practical use (6). Both chemical and physical methods have disadvantages either because removal is not efficient or because of high costs or nutritional losses to the product (7). As an alternative, biological decontamination, being widely studied, seems to be attractive because it works under mild, environmentally friendly conditions (8).

Many microorganisms had been reported as being capable of removing AFB₁. These microorganisms contain yeasts, filamentous, fungi, and bacteria (9). Among them, probiotics were primarily studied on AFB₁-binding ability of their cell wall (10,11). Other microorganisms studied can degrade or transform AFB₁ to less toxic metabolites by attacking the carbonyl group, lactone, or difuran ring

of AFB₁ (12–16). Furthermore, some active enzymes responsible for the degradation of AFB₁ from microbes had been purified (17–20). In addition, some enzymes involved in lignin biodegradation (for example, manganese peroxidase and laccase from white-rot fungi) can degrade AFB₁ (21,22). Now, biological detoxification of AFB₁ has been extensively studied, and some methods have been applied in practice. For example, a non-toxicogenic *A. flavus* strain has been successfully applied to agriculture field to competitively exclude aflatoxin-producing *Aspergillus* species (23); the combined probiotics with aflatoxin B₁-degrading enzyme from *A. oryzae*, used as feed additive, can degrade AFB₁ and improve animal production (24); anaerobic solid fermentation of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* is able to completely biotransform aflatoxins in peanut meal (25).

In this study, we reported the biotransformation and detoxification of AFB₁ by *Cladosporium uredinicola* isolated from peanut shell. The properties of aflatoxin detoxification principle(s) were studied preliminarily, and the biotransformation products of AFB₁ were concentrated and studied.

Materials and Methods

Materials *C. uredinicola* used in this study was isolated from

peanut shell by using coumarin medium, which is initiated by Guan *et al.* (16) with slight modification (per liter): 1.0 g coumarin, 5.0 g $(\text{NH}_4)_2\text{SO}_4$, 2.5 g KH_2PO_4 , 1.0 g MgSO_4 , 0.5 g $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 0.1 g CaCl_2 , 20.0 g agar, and pH 6.5. *C. uredinicola* was collected in the China Center for Type Culture Collection (CCTCC) with the patent culture collection number: CCTCC M 2015181.

Aflatoxin B₁ purchased from Fermentek (Jerusalem, Israel) was dissolved in methanol (Fisher Scientific, Hampton, NH, USA) to a suitable concentration (20 µg/mL). High purity water used in experiment is obtained from a MilliQ purification system (Millipore, Bedford, MA, USA). Silica gel G thin-layer chromatography (TLC) plates were obtained from Branch of Qingdao Haiyang Chemical Co. (Qingdao, China). Other chemicals were obtained from chemical reagent services.

Transformation of AFB₁ by culture supernatant of *C. Uredinicola*

First, *C. uredinicola* was cultured in a potato dextrose medium (potato 200 g/L, dextrose 20 g/L) at 28°C with agitation at 180 rpm for 34 h in a shaker. Second, cells (11.25 mL) were transferred to a 250-mL flask containing 75 mL of Czapek-Dox liquid medium at 28°C with agitation at 180 rpm for 24 h. Then, mycelia were removed by centrifugation at $9,408 \times g$ for 10 min (3K15 centrifuge; Sigma-Aldrich, St. Louis, MO, USA), and the culture supernatant (extracellular extract) was used for the AFB₁ transformation experiment.

To determine whether the transformation is enzymatic, the effect of proteinase K (Vetec, Irvine, CA, USA; specific activity 30U/mg), SDS, and heat treatment on the AFB₁ transformation ability of culture supernatant of *C. uredinicola* was investigated. Culture supernatant of *C. uredinicola* was exposed to 1 mg/mL proteinase K for 6 h at 37°C; 1% SDS for 6 h at 37°C; boiling-water bath and sterilization at 110°C for 10 min respectively. After exposure, samples were centrifuged at $6,021 \times g$ for 10 min for removing precipitation emerged, and the supernatant was supplemented with 1 µg/mL AFB₁ with incubation at 37°C for 24 h. Sterile fermentation medium supplemented with AFB₁ was used as control (equal volume sterile water also added for the replacement of proteinase K and SDS). Then the residual AFB₁ was extracted and the AFB₁ biotransformation rate was determined.

Detection of residual AFB₁ Following incubation, residual AFB₁ of each sample was extracted three times with equal volume (1 mL) dichloromethane as described by Teniola *et al.* (26). The three dichloromethane extracts were combined and evaporated on a heat block at 50°C, and samples were dissolved in methanol, filtered through Millex-GV (0.22 µm; Durapore), and analyzed by high-performance liquid chromatography (HPLC). HPLC analyses were performed on a Shimadzu HPLC System (LC-20AD series; Shimadzu, Kyoto, Japan) using a guard column followed by a RP-C₁₈ (150×4.6 mm, 5 µm) HPLC column (Shimadzu). The mobile phase was methanol: acetonitrile: water (1:3:6, v/v/v) at a flow rate of 1 mL/min. AFB₁ was measured by UV detection (370 nm). The percentage of AFB₁ biotransformation was calculated using the following formula:

$$(1 - \text{residual quantities of AFB}_1 \text{ in treatment}) / \text{AFB}_1 \text{ quantities in control} \times 100\%.$$

Detection of AFB₁ biotransformation products HPLC and TLC were used for detecting the biotransformation products of AFB₁ by the culture supernatant of *C. uredinicola*. For this purpose, the following samples were analyzed: (a) AFB₁ standard sample, (b) the culture supernatant, (c) the culture supernatant added with AFB₁ (1 µg/mL). After 24 h of incubation, the samples were processed as above and analyzed by HPLC and TLC. The condition of HPLC is same as that described before. TLC plates were developed in chloroform:acetone (85:15, v/v), where after AFB₁ and its biotransformation products on the plates were examined with excitation at 365 nm using a UV analyser (ZF7-C; Shanghai Kanghua Biochemical Analyser Product Co., Shanghai, China).

Whether the product detected by HPLC and that detected by TLC was same compound was also studied. The product detected by TLC was purified from TLC plates and detected by HPLC. First, the spot corresponding to product was scraped from TLC plates and milled to powder in a tube. Then, dichloromethane was added into the tube for dissolving the product from silica gel. After incubation at 37°C for 12 h in darkness, the silica gel was removed by filtering the mixture through Millex-GV (0.22 µm; Durapore), the filtrate was then evaporated on a heat block at 50°C, redissolved in methanol, and analyzed by HPLC. As negative control, silica gel on the TLC plate was processed as above. The sample that AFB₁ reacted with the culture supernatant was used as positive control. In addition, the spot corresponding to residue AFB₁ on TLC plate was scraped and experimented.

AFB₁ biotransformation product analysis To scale up the product of AFB₁, 20 mL reaction mixture was used and the concentration of AFB₁ was raised to 5 µg/mL. The reaction mixture was extracted twice with dichloromethane. The extract was evaporated, and the sample was redissolved in methanol. The concentrated sample was then analyzed by LCMS (1200 serial HPLC; 6420 Triple Quad LC/MS; Agilent, Santa Clara, CA, USA) using a guard column followed by a RP-C₁₈ (150×4.6 mm, 5 µm) HPLC column (Shimadzu) and methanol: acetonitrile: water (1:3:6, v/v/v) as mobile phase at a flow rate of 0.3 mL/min. Samples were measured by UV detection (370 nm).

AFB₁ concentration affecting AFB₁ biotransformation For obtaining the optimum AFB₁ concentration in the reaction mixture, the culture supernatant was added with 0.5, 1, 2, 3, 4, and 5 µg/mL AFB₁ and incubated at 37°C for 24 h. Following incubation, residual AFB₁ was extracted from the samples and analyzed by HPLC, and the transformation rate was calculated as the formula described above.

Cytotoxicity test for AFB₁ and culture supernatant-treated AFB₁ MTT (3-(4, 5-dimethylthiazol-2-yl) 2, 5-diphenyl tetrazolium bromide) assay to HeLa cells was conducted for the toxicity study of AFB₁ and

culture supernatant-treated AFB₁. Methanol and dichloromethane were evaporated from AFB₁ standard and culture supernatant-treated AFB₁ samples, respectively. Then, DMSO was added and the samples were diluted by DMEM containing 2% FBS to obtain a serial concentration of AFB₁ (2, 4, 6, 8, and 10 µg/mL). DMSO was used as control.

HeLa cells (10,000 cells per well) were seeded into each well of a 96-well culture plate (Corning Inc., Corning, NY, USA) and incubated at 37°C for 24 h under 5% CO₂ atmosphere. Then, the medium was replaced with fresh medium (DMEM containing 2% FBS) containing test samples as described above, and the plate was incubated at 37°C for 48 h. After incubation, cultural supernatants were replaced with MTT (5 mg/mL, 30 µL/well) and the plate was then incubated for 4 h at 37°C. Following incubation, the MTT reagent was replaced with DMSO and the absorbance at 490 nm was recorded using a microplate reader (BioTek Instruments Inc., Winooski, VT, USA). The cytotoxicity of test samples was determined using the following formula: cytotoxicity = $1 - \text{OD}_{490}$ of treated cells / OD_{490} of control cells.

Statistical analyses The experiment was conducted in three replicates, and the results were expressed as mean ± standard error. IBM SPSS statistics 19 was used for the one-way ANOVA analyses on response variable. A comparisons level of 5% was used and Duncan's

Table 1. Transformation of AFB₁ by culture supernatant of *C. uredinicola*, heat-treated supernatant, proteinase K treated supernatant and SDS treated supernatant

	AFB ₁ concentration (µg/mL)		Transformation rate (%)	<i>p</i>
	Initial	Final		
Control	1.0	0.9		
Culture supernatant	1.0	0.4	53.8±5.1	
Boiling-water bath	1.0	0.5	49.3±1.7	<0.334
Sterilization (110°C, 10 min)	1.0	0.8	18.1±1.4	<0.001
Proteinase K	1.0	0.6	31.8±3.4	<0.014
SDS	1.0	1.0	4.7±3.6	<0.001

Multiple Range Test was used to determine significant differences among means when significant differences were encountered.

Results and Discussion

Biotransformation of AFB₁ by *C. Uredinicola* Results in Table 1 show that proteinase K treatment reduced the ability of culture supernatant on AFB₁ transformation significantly ($p < 0.014$) and SDS

Fig. 1. Detection of AFB₁ and its products by HPLC (A) and TLC (B). HPLC: (a) 1 µg/mL AFB₁ standard sample. (b) the culture supernatant. (c) the culture supernatant added with AFB₁ standard sample (1 µg/mL), after 24 h of incubation. TLC: Lane 1: AFB₁ standard sample (50 ng). Lane 2: the culture supernatant added with the AFB₁ standard sample (1 µg/mL), after 24 h of incubation. Lane 3: the culture supernatant. Solvent system used: Chloroform:acetone=85:15.

Fig. 2. Detection of AFB₁ and P₂ purified from TLC plate by HPLC: (A) culture supernatant suspended with AFB₁; (B) AFB₁ purified from TLC plate; (C) P₂ purified from TLC plate; (D) silica gel of TLC plate. The condition of HPLC was same to that described in section 2.3, but the guard column was removed.

treatment almost destroyed the activity of transforming AFB₁ ($p < 0.001$). Proteinase K as a nonspecific proteinase can react with all proteins. The reduction on AFB₁ transformation rate may point to the transformation is enzymatic. SDS is an anionic surfactant that can cause the protein denaturation by destroying zymoprotein spatial structure. The loss of the ability of transforming AFB₁ also indicates the transformation of AFB₁ is enzymatic. However, the boil treatment group retained high AFB₁ transformation activity, which suggested that the active components may be thermostable. The degradation of AFB₁ by *flavobacterium aurantiacum* was proven to be enzymatic preliminarily by studying the effect of heat, proteinase K, and DNase I treatment on the AFB₁ degradation activity of crude intracellular protein extract (27). Alberts *et al.* (28) investigated whether the

degradation of AFB₁ is enzymatic by evaluating the stability of the extracellular extract of *Rhodococcus erythropolis* after proteinase K and SDS treatment. Large amount of DNA unlikely exist in extracellular fraction, so the reduction of AFB₁ by nonspecific bonding of DNA was not involved in this study; instead, heat, proteinase K and SDS treatment were chosen for investigation.

AFB₁ biotransformation product analysis HPLC detected a metabolite (named as P₁) generated by the culture supernatant from AFB₁ (Fig. 1A) with a retention time of 4.6 min, which is different from AFB₁ with a retention time of 7.8 min. TLC also detected a metabolite (named as P₂) under 365 nm with a much smaller R_f value than AFB₁ (Fig. 1B). Then, P₂ was purified by TLC and detected by HPLC (Fig. 2C). In Figure 2C, the peak with a retention time of 3.9 min corresponding to P₁ (Fig. 2A) is clear, but in the negative control (Fig. 2D), this peak is not found. The result indicated that P₁ was same compound as P₂. It is reported that the cleavage of the lactone ring of AFB₁ can result in nonfluorescence (29,30). The result of TLC indicated that the lactone ring of AFB₁ was intact, and the smaller retention time and R_f value suggested these two AFB₁ products (P₁ and P₂) were of much stronger polarity than AFB₁.

For structural characterization of the product of AFB₁, the sample was analyzed by LCMS analysis. AFB₁ was identified at $m/z=313.0$ for the protonated cation $[M+H]^+$, $m/z=335.0$ for the sodiated cation $[M+Na]^+$ and $m/z=351.0$ for the potassiated $[M+K]^+$ (Fig. 3A). Figure 3B showed that the AFB₁ biotransformation product has an m/z of $365[M+Na]^+$ and $380.9[M+K]^+$. *Dactylium dendroides* NRRL2575 (12), *Corynebacterium rubrum*, *A. niger*, *Trichoderma viride*, and *Mucor ambiguus* (13) can transform AFB₁ to aflatoxicol by the reduction of the C-3 keto with a molecular mass of 314. A $F_{420}H_2$ -dependent reductase from *Mycobacterium smegmatis* can reduce AFB₁ by α , β -unsaturated double bond between C-2 and C-6, but the product (molecular mass=314) is unstable and hydrolyzed spontaneously after an hour (31). The research of Wang *et al.* (21) suggests that AFB₁ can be first oxidized to AFB₁-8, 9-epoxide by manganese peroxidase (MnP) from *Phanerochaete sordida* and then hydrolyzed to AFB₁-8, 9-dihydrodiol (molecular mass=346). Recently, AFB₁ can be metabolized to three products by *Pseudomonas putida*: aflatoxin D₁ (molecular mass=286), aflatoxin D₂ (molecular mass=206), aflatoxin D₃ (molecular mass=149) (14). These AFB₁ biotransformation products of which the molecular mass was given are all we can find in literature. However, no one AFB₁ biotransformation product was reported with an m/z of $365[M+Na]^+$ described in this study.

Toxicity study of culture supernatant-treated AFB₁ Before toxicity study, an optimum biotransformation rate ($84.5 \pm 5.7\%$) was obtained when $0.5 \mu\text{g/mL}$ AFB₁ was reacted with the culture supernatant (Fig. 4), and the samples were used for next cytotoxicity study. The toxicity of biotransformed AFB₁ is essential to the application in practice in the future. As shown in Fig. 5, the cytotoxicity of culture supernatant-treated AFB₁ was much less than AFB₁ to HeLa cells. The

Fig. 3. LCMS analysis of AFB₁ (A) and its biotransformation product (B).

Fig. 4. Effects of AFB₁ concentration on the AFB₁ biotransformation rate.

Fig. 5. Cytotoxicity of AFB₁ and AFB₁ treated with culture supernatant.

cytotoxicity of AFB₁ increased rapidly along with the increase in AFB₁ concentration and reached 57.8% at 10 μg/mL. Compared with AFB₁,

the cytotoxicity of culture supernatant-treated AFB₁ increased much more slowly and 20.9% cytotoxicity was measured at 10 µg/mL, which is less than that of AFB₁ at 4 µg/mL.

C. uredinicola was mentioned as a hyperparasite of *Puccinia* sp. (32), but its AFs detoxification ability has never been reported. In the future, *C. uredinicola* can be applied in practice for its multifunction of resistance of the contamination of AFs and the biocontrol of rust species.

Acknowledgments We would like to acknowledge Key Laboratory of Fermentation Engineering (Ministry of Education), Hubei University of Technology for supporting this research, and Qin Huang for graphical support.

Disclosure The authors declare no conflict of interest.

References

- Maragos CM. Measurement of aflatoxins using capillary electrophoresis. pp. 51-58. In: Mycotoxin Protocols. Trucksess MW, Pohland AE (eds). Food and Drug Administration, Totowa, NJ, USA (2001)
- International Agency for Research on Cancer. Some naturally occurring substances: Food items and constituents, heterocyclic aromatic amines and mycotoxins. p. 599. In: IARC monograph on the evaluation of carcinogenic risk to humans. International Agency for Research on Cancer, Lyon, France (1993)
- Van Rensburg SJ, Cook-Mozaffari P, Van Schalkwyk DJ, Van der Watt JJ, Vincent TJ, Purchase IF. Hepatocellular carcinoma and dietary aflatoxin in Mozambique and Transkei. *Brit. J. Cancer* 51: 713-726 (1985)
- Autrup H, Seremet T, Wakhsii J, Wasunna A. Aflatoxin exposure measured by urinary excretion of aflatoxin B₁-guanine adduct and hepatitis B virus infection in areas with different liver cancer incidence in Kenya. *Cancer Res.* 47: 3430-3433 (1987)
- Ross RK, Yu MC, Henderson BE, Yuan JM, Qian GS, Tu JT, Gao YT, Wogan GN, Groopman JD. Urinary aflatoxin biomarkers and risk of hepatocellular carcinoma. *Lancet* 339: 943-946 (1992)
- Bata Á, Lásztity R. Detoxification of mycotoxin-contaminated food and feed by microorganisms. *Trends Food Sci. Tech.* 10: 223-228 (1999)
- Line JE, Brackett RE. Factors affecting aflatoxin B₁ removal by *Flavobacterium aurantiacum*. *J. Food Protect.* 58: 91-94 (1995)
- Wu Q, Jezkova A, Yuan Z, Pavlikova L, Dohnal V, Kuca K. Biological degradation of aflatoxins. *Drug Metab. Rev.* 41: 1-7 (2009)
- Fazeli MR, Hajimohammadali M, Moshkani A, Samadi N, Jamalifar H, Khoshayand MR, Vaghari E, Pouragahi S. Aflatoxin B₁ binding capacity of autochthonous strains of lactic acid bacteria. *J. Food Protect.* 72: 189-192 (2009)
- Jespersen L, Shetty PH. *Saccharomyces cerevisiae* and lactic acid bacteria as potential mycotoxin decontaminating agents. *Trends Food Sci. Tech.* 17: 48-55 (2006)
- Peltonen K, El-Nezami H. Aflatoxin b₁ binding by dairy strains of lactic acid bacteria and bifidobacteria. *J. Dairy Sci.* 84: 2152-2156 (2001)
- Detroy RW, Hesseltine CW. Aflatoxicol: Structure of a new transformation product of aflatoxin B₁. *Can. J. Biochem.* 48: 830-832 (1970)
- Mann R, Rehm HJ. Degradation products from aflatoxin B₁ by *Corynebacterium rubrum*, *Aspergillus niger*, *Trichoderma viride* and *Mucor ambiguus*. *Appl. Microbiol. Biot.* 2: 297-306 (1976)
- Samuel MS, Sivaramakrishna A, Mehta A. Degradation and detoxification of aflatoxin B₁ by *Pseudomonas putida*. *Int. Biodeter. Biodegr.* 86: 202-209 (2014)
- Liu DL, Yao DS, Liang R, Ma L, Cheng WQ, Gu LQ. Detoxification of aflatoxin B₁ by enzymes isolated from *Armillariella tabescens*. *Food Chem. Toxicol.* 36: 563-574 (1998)
- Guan S, Ji C, Zhou T, Li J, Ma Q, Niu T. Aflatoxin B₁ degradation by *Stenotrophomonas maltophilia* and other microbes selected using coumarin medium. *Int. J. Mol. Sci.* 9: 1489-1503 (2008)
- Motomura M, Toyomasu T, Mizuno K, Shinozawa T. Purification and characterization of an aflatoxin degradation enzyme from *Pleurotus ostreatus*. *Microbiol. Res.* 158: 237-242 (2003)
- Liu DL, Yao DS, Liang YQ, Zhou TH, Song YP, Zhao L, Ma L. Production, purification, and characterization of an intracellular aflatoxin-detoxifying enzyme from *Armillariella tabescens* (E-20). *Food Chem. Toxicol.* 39: 461-466 (2001)
- Cao H, Liu D, Mo X, Xie C, Yao D. A fungal enzyme with the ability of aflatoxin B₁ conversion: Purification and ESI-MS/MS identification. *Microbiol. Res.* 166: 475-483 (2011)
- Zhao LH, Guan S, Gao X, Ma QG, Lei YP, Bai XM, Ji C. Preparation, purification and characteristics of an aflatoxin degradation enzyme from *Myxococcus fulvus* ANSM068. *J. Appl. Microbiol.* 110: 147-155 (2011)
- Wang J, Ogata M, Hirai H, Kawagishi H. Detoxification of aflatoxin B₁ by manganese peroxidase from the white-rot fungus *Phanerochaete sordida* YK-624. *FEMS Microbiol. Lett.* 314: 164-169 (2011)
- Alberts JF, Gelderblom WCA, Botha A, Van Zyl WH. Degradation of aflatoxin B₁ by fungal laccase enzymes. *Int. J. Food Microbiol.* 135: 47-52 (2009)
- Ehrlich KC, Cotty PJ. An isolate of *Aspergillus flavus* used to reduce aflatoxin contamination in cottonseed has a defective polyketide synthase gene. *Appl. Microbiol. Biot.* 65: 473-478 (2004)
- Zuo RY, Chang J, Yin QQ, Wang P, Yang YR, Wang X, Wang GQ, Zheng QH. Effect of the combined probiotics with aflatoxin B₁-degrading enzyme on aflatoxin detoxification, broiler production performance and hepatic enzyme expression. *Food Chem. Toxicol.* 59: 470-475 (2013)
- Chen Y, Kong Q, Chi C, Shan S. Biotransformation of aflatoxin B₁ and aflatoxin G₁ in peanut meal by anaerobic solid fermentation of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. *Int. J. Food Microbiol.* 211: 1-5 (2015)
- Teniola, OD, Addo PA, Brost IM, Färber P, Jany KD, Alberts JF, Van Zyl WH, Steyn PS, Holzapfel WH. Degradation of aflatoxin B₁ by cell-free extracts of *Rhodococcus erythropolis* and *Mycobacterium fluoranthenorans* sp. nov. DSM44556T. *Int. J. Food Microbiol.* 105: 111-117 (2005)
- Smiley RD, Draughon FA. Preliminary evidence that degradation of aflatoxin B₁ by *flavobacterium aurantiacum* is enzymatic. *J. Food Protect.* 63: 415-418 (2000)
- Alberts JF, Engelbrecht Y, Steyn PS, Holzapfel WH, Van Zyl WH. Biological degradation of aflatoxin B₁ by *Rhodococcus erythropolis* cultures. *Int. J. Food Microbiol.* 109: 121-126 (2006)
- Lee LS, Dunn JJ, De Lucca AJ, Ciegler A. Role of lactone ring of aflatoxin B₁ in toxicity and mutagenicity. *Experientia* 37: 16-17 (1981)
- Parker WA, Melnick D. Absence of aflatoxin from refined vegetable oils. *J. Am. Oil Chem. Soc.* 43: 635-638 (1966)
- Taylor MC, Jackson CJ, Tattersall DB, French N, Peat TS, Newman J, Briggs LJ, Lalalakar GV, Campbell PM, Scott C, Russell RJ, Oakshott JG. Identification and characterization of two families of F₄₂₀H₂-dependent reductases from mycobacteria that catalyse aflatoxin degradation. *Mol. Microbiol.* 78: 561-575 (2010)
- Barros ST, Oliveira NT, Bastos STG, Maia LC. Hyperparasitism of *Cladosporium uredinicola* over *Puccinia puta* on the host *Ipomoea fistulosa*. *Mycologist* 13: 23-24 (1999)