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Aflatoxin B_1 decontamination by UV-mutated live and immobilized *Aspergillus niger*

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ABSTRACT

The decontamination of Aflatoxin B_1 (AFB_1) by immobilized cells of a new mutant strain, prepared on a base of HSCAS (hydrated sodium calcium aluminosilicate), was studied. Novel strains were induced by UV irradiation, from which 50 were screened according to their degradation efficacy on AFB_1, compared with the wild strain (FS-Z1). The FS-UV1 strain exhibited highest degradation efficacy, which was confirmed by 18SrDNA to be *Aspergillus niger*. The results indicate that both immobilized cells and this mutant strain which are incubated for 48 h at 30 °C, would considerably remediate AFB_1 in nutrient broth culture, by 95.32% and 82.43%, respectively. By the application of samples of contaminated cottonseed meal, with results of 93.46%~96.82%, the degradation rate was also validated. The results of Ames test indicate the mutagenic activity of treated AFB_1 is greatly abated, with treated controls. The Application of LC-q-TOFMS (liquid-chromatography, quadrupole, time-of-flight mass spectrometry) deduces the structure and molecular formulas of the degradation products. In the *vivo* study, the damages of photomicrographic evidence are decreased in kidney and liver and the serum biochemical parameters is improved, in response to preventative treatment with immobilized cells. This is the application of HSCAS-prepared, immobilized *A. niger* cells to degrade AFB_1 of contaminated samples. The investigation in this paper offers a novel path for economical, time-saving biodegradation of AFB_1 in foods and feeds.

1. Introduction

Aflatoxins (AFs) are highly-toxic secondary mycotoxins, generated in crops as secondary metabolites of *Aspergillus flavus* and *Aspergillus parasiticum* (Keller, Kantz, & Adams, 1994) fungi. For humans, AFB_1 (Aflatoxin B_1) is one of the most hazardous mycotoxins (Fink-Grernmels, 1999; Hussein & Brasel, 2001). AFB_1 may result in a loss of product nutritional value, organoleptic qualities, undesirable health effects, and higher equipment costs (Khayoon et al., 2010; McLean & Dutton, 1995; Mishra & Das, 2003). In recent years, many avenues of AFB_1 decontamination in food sources have been studied extensively. Inactivation of aflatoxin by physical and chemical methods has not been proved to be effective or economically feasible. Biological detoxification, on the other hand, offers an attractive alternative for elimination of toxins (Cao, Liu, Mo, Xie, & Yao, 2011). Beneficial microorganisms, such as bacteria and fungi, have been observed to substantially contribute to reduction of AFs in contaminated media (Tsai & Phillips, 1991). It has become clear in recent years that fungi play a central role in the degradation of AFB_1. Non-aspergilline fungi, including zygomycetous, ascomycetous, and basidiomycetous fungi (Alberts, Gelderblom, Botha, & van Zyl, 2009) and plant pathogens have been implicated in AFB_1 degradation. Reports on *Phaffia rhodozyma* and *Xanthophyllomyces dendrorhous* demonstrate that these microbes convert aflatoxins to nontoxic compounds (Péteri, Téren, Vágvölgyi, & Varga, 2007), *Rhizopus stolonifer*, *R. microsporus*, *R. homothallicus*(Varga, Péteri, Tábori, Téren, & Vágvölgyi, 2005), and Aspergillus species (Varga, Rigó, Tóth, Téren, & Kozakiewicz, 2003). Moreover, it has been reported that *Aspergillus niger* can intensively degrade AFB_1(Xu, Wang, Zhang, Yang, & Sun, 2013).

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Studies are attempting to improve the production of various wild-strain enzymes through mutagenesis. Recent approaches of obtaining reliable strains rely on physical and chemical treatment, in which UV radiation has been widely applied (Qi, Wang, Yang, Xia, & Yu, 2014; Zhang et al., 2014) to new strain production. It has become one of the most convenient and effective mutagenic agent. In many cases, mutated strains possess more desirable features than natural strains, being applied successfully to make ideal strains available in practice (Marzano, Gallo, & Altomare, 2013; Zayadan, Purton, Sadvakasova, Userbaeva, & Bolatkhan, 2014). Immobilization techniques have been established to overcome disadvantages of free cells, such as low mechanical strength, low cell density and difficulty in biomass separation from effluent. Immobilized microbial cells have a great potential for application in bioremediation (Mulla, Talwar, Bagewadi, Hoskeri, & Ninnekar, 2013; Núñez & Lema, 1987). Immobilized cells provide other advantages over freely-suspended cells, including simplification of separation from reaction mixtures, stability for repeated use, and avoidance of wash-out during continuous degradation processes (Mulla et al., 2013; Woodward, 1988). With the superior activity, density and stability, immobilized cells are capable of degrading more highly-concentrated aflatoxin than freely-suspended cells (Mulla et al., 2013; Woodward, 1988).

Through mutagenesis and production of immobilized cells, *A. niger* (wild) could be used to improve the degradation rate to AFB_1. *A. niger* was first exposed to UV irradiation, then the obtained mutants were screened according to their protein affinity with the natural isolate, and those which have the best capabilities of degrading AFB_1 would be further selected. Once being cultured for 48 h at 30 °C, immobilized *A. niger* exhibited severe degradation on AFB_1 throughout the observed degradation period.

2. Experimental

2.1. Reagents, microorganism and animals

AFB_1 purchased from Arcos Organics Ltd., USA, and prepared stock standard solution of AFB_1 in methanol at 500 mg/mL, stored at −20 °C. From Huasheng Co., Yixing, China, HSCAS for this study was obtained, which powder was then diluted with 100 mL KH570 at 30 °C, and stirred at 120 rpm constantly overnight. After, the precipitate was washed three times in 75% ethanol, the cleaned product centrifuged at 8000 rpm for 15 min, then dried and sieved to less than 45 μm.

The China Center for Type Culture Collection (CCTCC) identified the strain preserved at our lab, named "FS-Z1." It was plated the strains (10^8 CFU/mL) in potato dextrose broth (PDB) medium, incubating for 72 h, at 30 °C and 150 rpm (Constant shaking incubator, Shanghai, China, ZHWY-2102C).

For this study, a total of 40 Sprague Dawley (SD) rats weighing 200–250 g were maintained, which were purchased from Slaccas Co., Shanghai. All animals were allowed to acclimate for a period of one week, and housed under artificial light and thermal control, free from any source of chemical contamination.

2.2. Preparation of mutant A. niger

FS-Z1 was cultured on potato dextrose agar (PDA) slants for 7 days at 30 ± 2 °C, until strong sporulation was observed. The harvested spores were suspended homogeneously in sterile, distilled water. Before performing experiments, it should be counted the spores with a hemocytometer and adjusted the concentration to 10^8 CFU/mL.

As mentioned, the mutants of *A. niger* were obtained through UV irradiation. FS-Z1 was incubated in potato dextrose broth (PDB) medium at 1: 5 (v: v) and 30 ± 2 °C, in a rotary shaker incubator (150 rpm) for 5 days. Next, 1 mL of the mycelial suspension (120–150 CFU/m L) was transferred to 90 mm Petri plates, which were (Achal, Savant, & Reddy, 2007) then exposed to 15 W germicidal UV lamp (254 nm), at a distance of 30 cm, over 4-min intervals from1-33 min. To target the optimal UV exposure time for a 10% survival rate, PDA medium was spread with 16 μL of irradiated mycelial suspension, thence incubating the plates, including a control, without UV at 30 °C for 48 h.

To develop the UV-induced mutants, the mycelial suspension was irradiated by UV for 17 min and plated in PDA medium under the above-given conditions. Randomly selected colonies (5–6) from each plate and transferred them into Casei medium. From these, colonies exhibiting the greatest ratio of colony diameter to full medium diameter were chosen, along with one low-ratio colony (FS) for further studies. Washing repeatedly with distilled water, and then cultured the selected strains in PDB for 48 h, then added AFB_1 (0.5 ppm) into the liquid culture in order to observe its degeneration rate by the various mutant strains. The mutant strain showing the highest ratio of degradation to AFB_1, in comparison to the wild type, was selected for subsequent experiments. It should be cultured the isolates six times to assure their mitotic stability.

2.3. Identification of the screened strain FS-UV1

Using PCR, and then amplified the18SrDNA of the final screened strain and obtained sequence by Sequencing Analysis 5.1 software (Applied Biosystems) and affination (http://blast.ncbi.nlm.nih.gov/Blast.cgi). FS-UV1was named and identified by the CCTCC.

2.4. Reduction of AFB_1 by FS-Z1 and FS-UV1 in liquid culture

Both the wild FS-Z1 and mutant FS-UV1 strains were cultured in PDB for 48 h by a rotary shaker incubator at 150 rpm and 30 °C. 100 μL of pre-inoculum was added to PDB (30 mL), containing AFB_1 (0.5 ppm), and incubated under the above conditions. At 2 h, 6 h, 12 h, 24 h, 48 h, assaying FS-Z1 and FS-UV1 degradation rates of AFB_1 by measuring the residual AFB_1.

2.5. Ames test

From Moltox, USA, the *Salmonella typhimurium* tester strains, TA97, TA98, TA99, TA100 and S9 (stocked in liquid nitrogen) were purchased. Tester strains were checked according to those requirements and characteristics stipulated by Maron (Maron & Ames, 1983). For the Ames carcinogenicity test, it extracted the culture product from FS-UV1 (cultured 48 h as above), once it had degraded AFB_1 for 2 h. For this test, the PDB medium was used as a negative control and the AFB_1 (0.2 μg/plate) as a positive control, proceeding in accordance with Méndez-Albores (Méndez-Albores,Arambula-Villa,Loarca-Piña,Castano-Tostado, & Moreno-Martínez, 2005).

2.6. Identification of degradation compounds

Samples both spiked and not spiked with AFB_1 were analyzed by liquid-interface chromatography, quadrupole time-of-flight, hybrid-tandem, mass spectrometry (LC-q-TOFMS). The extraction of residual AFB_1 from the samples proceeded as per Dan, Xu, and the operating conditions for LC-q-TOFMS accorded with Luo et al. (Luo, Wang, Wang, Wang, & Chen, 2013).

2.7. Preparation of immobilized cells and optimization of conditions

FTIR (Fourrier transform infra-red spectroscopy) and XRD (x-ray