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Screening and characterization of a salt-tolerant aflatoxin B_1-degrading strain isolated from Doubanjiang, a Chinese typical red pepper paste

Chi ZHAO[1,2], Ling DONG[1], Fengju ZHANG[1], Yongliang LUO[3#], Zebo YANG[1#], Xiaoping ZHANG[2*], Zhihua LI[1*]

Abstract

Aflatoxin B_1 (AFB_1) is a carcinogenic mycotoxin present in many foods, posing a serious human health problem. Oriental high-salt fermented foods are highly susceptible to AFB_1 contamination due to their production process and condition. Therefore, it is necessary to isolate microbial resources that can remove AFB_1 in a high-salt condition. In the current study, forty-nine salt-tolerant microorganisms with the ability to remove AFB_1 were isolated from Doubanjiang, a Chinese typical high-salt red pepper paste. Among them, *Bacillus velezensis* AD8 had the highest removal rate of AFB_1. The AFB_1 removal activity of *B. velezensis* AD8 was mainly attributed to culture supernatant, and it was sensitive to sodium dodecyl sulfate (SDS) or/and proteinase-K treatment, which indicated that extracellular enzyme contributed to the degradation of AFB_1. The optimal incubation time, pH value and NaCl concentration of the initial medium for degradation of AFB_1 by the *B. velezensis* AD8 were investigated. In addition, the AFB_1 concentration had no significant effect on degradation activity. The addition of nitrogen source, Mg^{2+} and Mn^{2+} stimulated degradation rate, whereas addition of carbon source, Zn^{2+} and Fe^{2+} inhibited degradation. Thus, *B. velezensis* AD8 might be a potential candidate to degrade AFB_1 in high-salt food matrices.

Keywords: aflatoxin B_1 (AFB_1); biodegradation; salt tolerance; *Bacillus velezensis* AD8.

Practical Application: *B. velezensis* AD8 has great potential as a microbial resource to degrade AFB_1 in high-salt food matrices.

1 Introduction

Aflatoxins (AFs) are secondary metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus*, and are characterized by strong toxicity and wide distribution, especially AFB_1 (Marroquín-Cardona et al., 2014; Ismail et al., 2018). As the most toxic aflatoxin, AFB_1 has been classified as a first-level human carcinogen, which has caused serious safety problems worldwide and caused huge economic losses annually (Rocha et al., 2014; Pimpitak et al., 2020). The carcinogenic, teratogenic, mutagenic and immunosuppressive capabilities of AFB_1 necessitate research on the ways to control the buildup of the toxin in the food chain (Adebo et al., 2016a; Gonçalves et al., 2021).

Currently, the strategies to eliminate AFB_1 include prevention and treatment (Verheecke et al., 2016). The prevention strategy aims to prevent the growth of AFB_1 producing fungi in pre-harvest and post-harvest periods, including physical and chemical sterilization methods (Grace et al., 2015). However, few of these methods can totally inhibit the occurrence of all AFB_1 producing fungi; in addition, the methods may decrease nutritional value and affect flavor (Womack et al., 2014). The treatment strategy uses a variety of methods to decrease the quantity of existing AFB_1 (Verheecke et al., 2016). Among them, biological treatments is safe, economical and environmentally friendly, and minimize decreases in the quality of treated products (Mishra & Das, 2003). Therefore, the research on reducing AFB_1 by microorganisms has received considerable attention (Verheecke et al., 2016). Previous studies indicated that some microorganisms including *Lactobacillus*, *Pseudomonas*, *Bacillus*, *Rhodococcus*, *Aspergillus*, *Pleurotus* and *Streptomyces*, which are able to reduce AFB_1 have been isolated (Ismail et al., 2018; Guo et al., 2020b). Several microorganisms adsorb AFB_1 using cell wall, whereas others biosynthesize enzymes, including aflatoxin oxidase (AFO), laccase and Mn peroxidase (MnP) to attack different sites on AFB_1 molecule (Verheecke et al., 2016). However, limitations remain, including incomplete and inefficient degradation, non-adaptation to some specific food production conditions, especially for the traditional high-salt fermented foods in East Asia (soy sauce, soybean paste and broad bean paste) (Li et al., 2018b).

Manufacturing typical high-salt fermented foods in East Asia entails a special process to incorporate molds such as *Aspergillus oryzae* and *Mucor* and produce them in an open environment (Li et al., 2017, 2018b). Previous studies indicated that due to fermentation conditions and raw material contamination, pollution of *A. flavus* was inevitable (Zhang et al., 2020b). Therefore, there is an urgent need to develop a high-efficiency, environmentally friendly detoxification system that is suitable for high-salt fermentation conditions to deal with possible AFB_1 pollution in the production process.

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[1] *Institute of Agro-products Processing Science and Technology, Sichuan Academy of Agricultural Sciences, Chengdu, Sichuan, People's Republic of China*
[2] *College of Resources, Sichuan Agricultural University, Chengdu, Sichuan, People's Republic of China*
[3] *Agriculture, Rural and Forestry Bureau of Pidu District, 998 Wangcong Middle Rd, Chengdu 611730, Sichuan, People's Republic of China*
#The two authors provided samples of Doubanjiang and suggested experimental design.
*Corresponding author: lzh82@hotmail.com, zhangxiaopingphd@126.com

Thus, the purposes of the current study were to (1) isolate salt-tolerant microorganisms capable of removing AFB_1, (2) explore the action mode of selected strain to remove AFB_1, and (3) optimize culture conditions to maximize the removal of AFB_1. This study provided new microbial resources to reduce AFB_1 in the high-salt fermented food matrix.

2 Materials and methods

2.1 Chemicals and medium

AFB_1 standard was obtained from Pribolab Co., Ltd. (Qingdao, China). HPLC-grade methanol and acetonitrile were purchased from Fisher Scientific (Shanghai, China). Nutrient broth (NB) (peptone: 10 g/L, beef powder: 3 g/L, sodium chloride: 5 g/L) and agar (NB with 15 g/L agar) (NA), Yeast Extract peptone Dextrose (YPD) broth (yeast Extract: 10 g/L, peptone: 20 g/L, glucose: 20 g/L) and agar (YPD with 15 g/L agar) (YPDA) were used for screening and culture of microorganisms. Other analytical grade reagents were obtained from local chemical stores.

2.2 Isolation salt-tolerant microorganisms

Salt-tolerant microorganisms including bacteria and fungi were isolated from Doubanjiang (XIN HONG WANG FOOD Co., Ltd, Pixian County, Chengdu, China). Briefly, 2 g of Doubanjiang sample was homogenized in 9 mL of sterile saline, and the supernatant was diluted to 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} folds. 50 µL of dilution was spread on NA and YPDA media supplemented with 10% NaCl (W/V) and incubated at 37 °C (bacteria) or 30 °C (fungi) for 48 h. Single colonies were transferred to fresh NA and YPDA media with 10% NaCl (W/V), which was repeated thrice. All isolated strains were archived in glycerol and stored at -80 °C.

2.3 Molecular identification of isolates

DNA extraction of isolated strains was undertaken using FastDNA® Spin Kit for Soil (MP Biomedicals Co., Ltd. CA, USA) based on the manufacturer's instructions. Fungal 5.8S rDNA gene and two ribosomal internal transcribed spacer regions were amplified in EasyCycler (Analytik Jena Co., Ltd, Germany) using primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). Bacterial 16S rDNA gene region was amplified using primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-TACGGCTACCTTGTTACGACTT-3'). Total 50 µL reaction system contained 1 µL of each primer at a concentration of 2 µM, 2 µL of template, 25 µL of Taq PCR Master Mix 2X (Sangon Biotech Co., Ltd, Shanghai, China), and 21 µL of sterile water. Amplification was programmed as follows: 94 °C for 5 min, then 30 cycles at 94 °C for 30 s, 55 °C for 30 s and 72 °C for 2 min, followed by 72 °C for 5 min. Purification and sequencing were undertaken by Sangon Biotech Co., Ltd. (Shanghai, China). Sequences were analyzed using BioEdit software, and then BLAST search comparison present in NCBI GenBank (National Center for Biotechnology Information, 2021) was undertaken to obtain the closest species. Phylogenetic trees were constructed using the maximum-likelihood method in MEGA 5 (Tamura et al., 2011).

2.4 Evaluation of microbial removal of AFB_1

The rate of AFB_1 removal was evaluated using the previously described method (Xia et al., 2017). Briefly, pre-screened strains were inoculated into 30 mL of NB and YPD media for 24 h, and then 950 µL of culture and 50 µL of AFB_1 standard solution were mixed to make AFB_1 concentration reach 500 ppb, and placed in a shaker (37/30 °C,160 rpm) for 48 h in dark.

Residual AFB_1 was quantified using the high-performance liquid chromatography (HPLC) method based on the protocol described by Huang et al. (2017). Briefly, mixed culture was centrifuged (3 min, 12000 rpm, 4 °C) and filtered through a 0.22-micron membrane. Finally, AFB_1 content in the supernatant was determined on Agilent 1260 HPLC system (Agilent Corporation, USA) equipped with XDB-C18 column (250 x 3 mm i.d., 5 mm particle size, Agilent Corporation, USA). HPLC conditions were as follows: Injection volume was 20 µL and the mobile phase was methanol-water (1:1, v/v) with a flow rate of 1mL/min at 30 °C. AFB_1 was determined using an ultraviolet detector at 360 nm.

The removal rate of AFB_1 was computed using the following Formula 1:

$$\left(1 - \textit{treatment group } AFB_1 \textit{ peak area} \;/\; \textit{Ck group } AFB_1 \textit{ peak area}\right) \times 100 \quad (1)$$

2.5 AFB_1 reducing ability of AD8 strain cells, cell lysates and culture supernatants

Protocol for determining degradation rate of AFB_1 by AD8 strain cells, cell lysate and supernatant was based on the protocol described by Rao et al. (2017). AD8 strain was pre-cultured in NB medium (160 rpm, 37 °C, 24 h). After 10 min centrifugation of liquid culture (8000 rpm at 4 °C), supernatant and cells were collected. Cells were washed thrice with phosphate buffer, and then resuspended in phosphate buffer. One portion of cell resuspension was directly mixed with AFB_1, whereas the other portion was disintegrated using an ultrasonicator (LiChen Corporation, Shanghai, China) wave before mixing. The removal rate was determined as previously described.

2.6 Effects of proteinase-K and SDS on the removal of AFB_1

Determination of effects of SDS and proteinase-K treatment on the supernatant of AD8 strain was based on the protocol described by Rao et al. (2017). The supernatant was treated with 1mg/mL proteinase K and/or 1% SDS at 30 °C for 6 h before mixing with AFB_1. The removal rate was determined as previously described.

2.7 Optimization of culture conditions for maximum degradation of AFB_1

Incubation time

AD8 strain was inoculated into 30 mL of NB liquid medium and cultured for 24 h (37 °C, 160 rpm) for activation. 100 µL of culture was then transferred to a fresh NB medium for 24 h. 950 µL of culture were mixed with 50 µL of AFB_1 standard solution

to attain AFB_1 concentration of 500 ppb, and placed in rotary shaker incubator (37 °C, 160 rpm) in dark. The degradation rate of AFB_1 was then analyzed at 24, 48, 72, 96, 120, and 144 h. Protocol for determination of AFB_1 was the same as the protocol described above.

pH value

AD8 strain was activated and inoculated into NB with initial pH adjusted to 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, and 8.5 with relevant sodium phosphate buffers (sodium hydroxide and phosphoric acid), and incubated in rotary shaker incubator (37 °C, 160 rpm) for 24 h. AD8 strain culture was then incubated with AFB_1 for 72 h. Protocol for determination of AFB_1 was the same as the protocol described above.

Metal ions

AD8 strain was activated and inoculated into NB liquid medium supplemented with 10 mM of Mg^{2+} ($MgSO_4$), Mn^{2+} ($MnSO_4$), Fe^{2+} ($FeSO_4$) and Zn^{2+} ($ZnSO_4$), and incubated in rotary shaker incubator (37 °C, 160 rpm) for 24 h. The culture was then incubated with AFB_1 for 72 h. Protocol for determination of AFB_1 was the same as the protocol described above.

Nutrients

AD8 strain was activated and inoculated into NB supplemented with 0.5 mg/mL nutrients (fructose, glucose, lactose, soluble starch, sucrose, beef extract, yeast powder, peptone, and tryptone) and incubated in a rotary shaker incubator (37 °C, 160 rpm) for 24 h. AD8 strain culture was then incubated with AFB_1 for 72 h, and subsequent procedures were the same as those described above.

NaCl concentration

AD8 strain was activated and inoculated into NB with initial salinities adjusted to 3%, 6%, 9%, 12% (w/v) with NaCl and incubated in a rotary shaker incubator (37 °C, 160 rpm) for 24 h. AD8 strain culture was then incubated with AFB_1 for 72 h, and subsequent procedures were the same as those described above.

AFB_1 concentration

AD8 strain was activated, and 950μL of culture was mixed with 50 μL of AFB_1 standard solution to attain AFB_1 concentration of 0.1, 0.2, 0.5, and 1ppm, and placed in a rotary shaker incubator (37 °C, 160 rpm) in dark. The method for AFB_1 determination was the same as the one described above.

2.8 Statistical analysis

The data were tested using analysis of variance (ANOVA) and Tukey's post hoc test in the IBM SPSS Statistics for Windows, Version 22.0 (IBM Corporation, Armonk, NY, USA). Differences were considered statistically significant at $P \leq 0.05$.

3 Results and discussion

3.1 Isolation and identification of salt-tolerant microorganisms for AFB_1 removal

Doubanjiang is a high-salt fermented foods that is widely preferred by the Chinese people and is known as the soul of Sichuan cuisine (Li et al., 2016). It is made by fermenting broad bean (*Vicia faba L.*), red pepper (*Capsicum annuum L.*), salt, and wheat flour (*Triticum aestivum L.*), and microorganisms play an important role in the process (Li et al., 2017). In the current study, a total of 49 strains of salt-tolerant microorganisms were isolated from Doubanjiang using high-salt NA and YPDA medium (Figure 1). For bacteria, 34 strains belonging to *Bacillus* and *Staphylococcus* genera. At the species level, including *B. velezensis*, *Bacillus amyloliquefaciens*, *Bacillus licheniformis*, *Bacillus aryabhattai*, *Bacillus subtilis*, *Bacillus haynesii*, *Bacillus australimaris*, *Bacillus safensis* and *Staphylococcus devriesei*. For fungi, 15 strains were mainly hypertonic tolerant yeasts, including *Kodamaea ohmeri*, *Candida metapsilosis*, *Pichia manshurica*, *Pichia kudriavzevii*, *Hyphopichia burtonii*, *Candida parapsilosis*, *Debaryomyces prosopidis* and *Zygosaccharomyces rouxii*. Previous studies had reported the composition of complex microorganisms in Doubanjiang through the high-throughput sequencing method (Zhang et al., 2020a; Li et al., 2016). Compared with the studies above, Some genera like *Lactobacillus*, *Pseudomonas*, *Citrobacter*, *Tetragenococcus*, *Aspergillus*, *Cryptococcus*, were not isolated in the current study. The reason might be that lots of microorganisms cannot be cultivated in selective media until now (Yan et al., 2013). Similarly, Lu et al. (2020) established that *Bacillus sp.*, *Candida sp.* and *Zygosaccharomyces sp.* were the main microorganisms in the moromi-meju mixed fermentation phase of Doubanjiang by culture-dependent method, which was consistent with the current study.

Production of Doubanjiang is an open process, which leads increases chances of contamination by AFB_1. Results from a recent survey study indicated that the detection rate of AFB_1 in 929 Doubanjiang samples was 99.04%, and 6.8% of homemade Doubanjiang samples had higher AFB_1 content compared with Chinese national standards (Zhang et al., 2020b). In addition, Zhang et al. (2020a) established that AFB_1 content in the Doubanjiang fermentation process gradually decreased with time, and it was speculated that some of these microorganisms played a role in fermentation. Therefore, secondary screening was undertaken by the addition of AFB_1 (500ppb) in vitro to evaluate the removal activity of isolated salt-tolerant microorganisms from Doubanjiang. The results demonstrated that the removal rates of bacteria and fungi were in the range of 26.90%-68.32% and 0.33%-9.53%, respectively. Obviously, bacteria were the main contributor, especially *Bacillus*. Among them, *B. velezensis* AD8 had the highest removal rate of AFB_1 (68.32%) within 2 days among all isolated microorganisms (Figure 2). Therefore, the removal characteristics of AFB_1 by *B. velezensis* AD8 were further studied. The *B. velezensis* AD8 was deposited in the China Center of Industrial Culture Collection (CICC) with accession number 25120. The nucleotide sequences of isolated microorganisms were deposited in National Center for Biotechnology Information (OL873259-OL873273 and OL818243-OL818276).

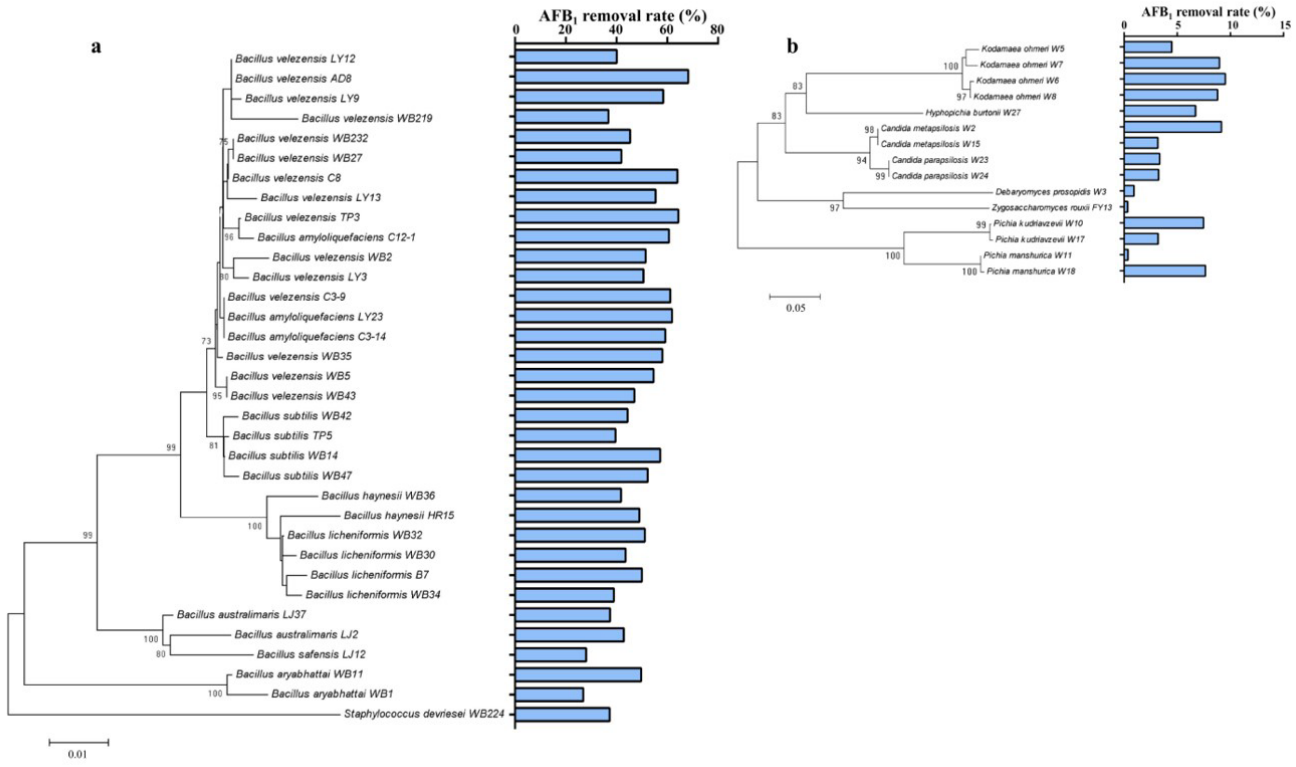

Figure 1. The AFB_1 removal rate of isolated microorganisms, and its phylogenetic trees based on (a) 16S rDNA and (b) ITS-5.8S rDNA sequences obtained through the maximum likelihood method. Numbers at branching points refer to bootstrap values (1000 re-samplings), and only bootstrap values over 70 are shown.

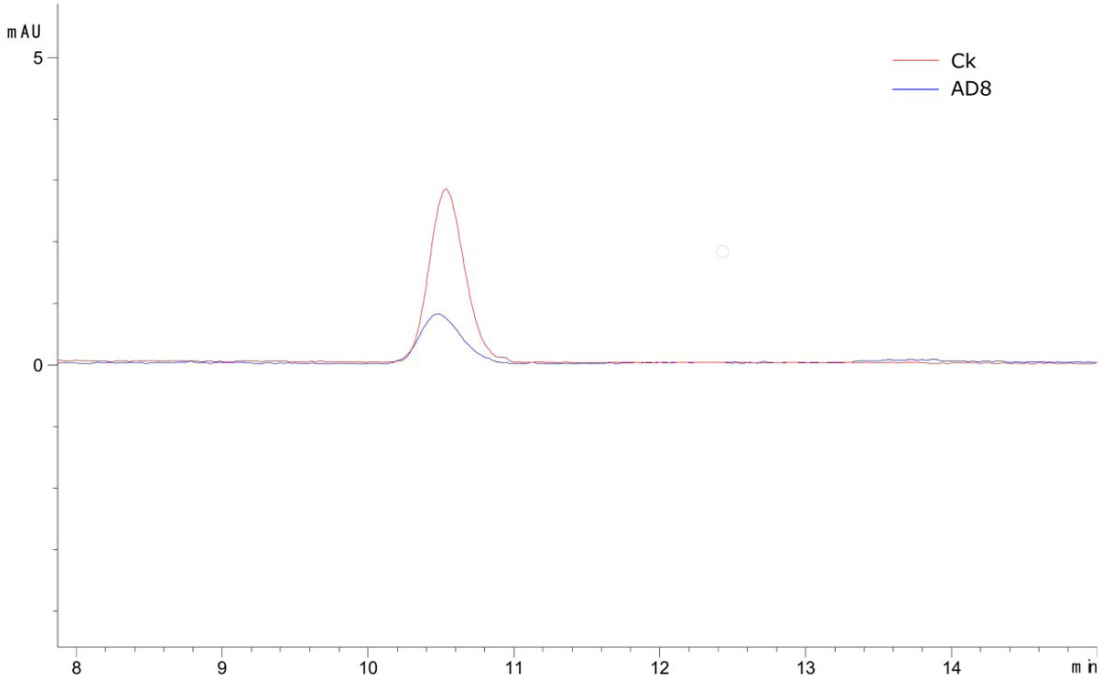

Figure 2. HPLC profile of AFB_1 removal in the control group (Ck) and *B. velezensis* AD8 (AD8) treatment group.

3.2 The action mode of AFB_1 removal by B. velezensis AD8

Previous studies reported that adsorption and degradation are the main modes of AFB_1 biological control (Verheecke et al., 2016). To further explore the action mode of AFB_1 removal by *B. velezensis* AD8, we measured the removal rate of *B. velezensis* AD8 using the supernatant, cells and intracellular lysate. Findings showed that *B. velezensis* AD8 culture supernatant removed 77.49 ± 0.83% of AFB_1 (500ppb) after 72 h, whereas removal rates of cells and cell lysate were 3.36 ± 1.92% and 10.6 ± 0.67%, respectively (Figure 3). Results revealed that the supernatant was the main factor contributing to the removal of AFB_1. Further, the AFB_1 removal rates of the *B. velezensis* AD8 culture supernatant treated with SDS and proteinase-K were 8.33 ± 0.22% and 60.16 ± 2.25%, respectively (Figure 3). In the combined SDS–proteinase-K treatment, the degradation rate was 6.3 ± 0.25%. Taken together, the results suggested that the decrease in AFB_1 concentration was mainly due to an extracellular AFB_1 degrading enzyme instead of physical adsorption. Many researches had reported that some microorganisms can remove AFB_1 by adsorption, especially lactic acid bacteria (LAB). However, the microbiological adsorption mechanism is reversible in nature and is limited by the morphology and characteristics of the treating object (Huang et al., 2017; Verheecke et al., 2016). Compared with microbial adsorption, biodegradation of AFB_1 was considered to be a promising method due to its rapid and irreversible. Biodegradation of AFB_1 was mediated by enzymes synthesized by some microorganisms, such as aflatoxin oxidase (AFO), manganese peroxidase (Mnp) and laccase (Verheecke et al., 2016). Recently, the CotA laccase with highly thermostable from *B. licheniformis* had been reported to degrade AFB_1 into non-toxic aflatoxin Q_1 and epi-aflatoxin Q_1 (Guo et al., 2020a). Further researches are in progress to sequence the genome of *B. velezensis* AD8, and determine which genes are involved in the degradation process.

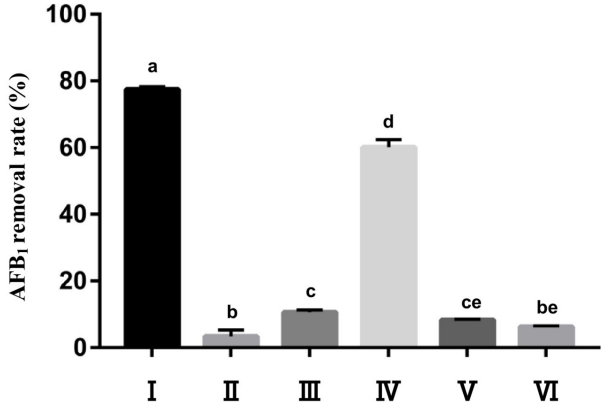

Figure 3. The action mode of AFB_1 removal by *B. velezensis* AD8 (**I**: supernatant of *B. velezensis* AD8 culture, **II**: cells of *B. velezensis* AD8, **III**: cell lysate of *B. velezensis* AD8, **IV**: supernatant of *B. velezensis* AD8 culture treated with proteinase K, **V**: supernatant of *B. velezensis* AD8 culture treated with SDS, **VI**: supernatant of *B. velezensis* AD8 culture treated with proteinase K plus SDS). Different letters among samples indicate significant differences ($p < 0.05$) by Tukey's post hoc test.

3.3 Characterization of B. velezensis AD8 AFB_1 degradation

The degradation ability of *B. velezensis* AD8 on AFB_1 under different culture conditions was investigated. The results showed that the *B. velezensis* AD8 degraded 62.06 ± 3.04% and 86.04 ± 0.46% of AFB_1 (500 ppb) when incubated for 24 and 144 h, respectively (Figure 4a). This suggested that *B. velezensis* AD8 degrade AFB_1 was a rapid and continuous manner. Similar results were obtained by Xia et al. (2017) and Alberts et al. (2006), who reported that *B. subtilis* JSW-1 and *Rhodococcus erythropolis* could degrade 67.2% and 66.8% of AFB_1 within 72 h, respectively.

In addition, the effect of initial medium pH ranging between 5 and 8.5 on the degradation rate of AFB_1 was explored (Figure 4b). Notably, initial medium pH had a significant impact on the ability of *B. velezensis* AD8 to degrade AFB_1. The degradation rate of AFB_1 was only 8.19 ± 3.73% and 21.05 ± 1.33% at pH 5 and 5.5, respectively. This was in contrast to Megalla & Hafez (1982), who reported that low pH (5-6) may contribute to the degradation of AFB_1. Our result showed that the degradation rate of *B. velezensis* AD8 to AFB_1 was the highest at 8.5 (80.77 ± 0.67%), which is similar to the researches by Rao et al. (2017) and Mwakinyali et al. (2019), who reported that high pH (>7) was suitable for AFB_1 degradation.

With regard to the effect of additional nutrients on the degradation activity of *B. velezensis* AD8, it was shown in Figure 4c. The addition of different carbon sources to the initial medium led to varying degrees of decline in the degradation ability of *B. velezensis* AD8 on AFB_1. Furthermore, the degradation rates of AFB_1 after adding lactose, soluble starch, fructose, sucrose and glucose were 66.37 ± 1.04%, 65.64 ± 3.26%, 56.07 ± 3.29%, 56.07 ± 2.41%, and 43.93 ± 9.48%, respectively. However, the addition of nitrogen source to the initial medium stimulated the degradation rate of AFB_1 by *B. velezensis* AD8. The addition of beef extract, yeast extract, peptone and tryptone resulted in the degradation rate of AFB_1 to 74.85 ± 0.13%, 78.51 ± 0.96%, 73.9 ± 0.38%, and 80.56 ± 0.13%, respectively. Carbon is the key nutrient for microbial growth and energy metabolism, and nitrogen is an important component of proteins and nucleic acids (Wang et al., 2015). This result was in contrast to the findings of Mwakinyali et al. (2019), who reported that adding carbon or nitrogen can increase the degradation rate of *Myroides odoratimimus* strain 3J2MO on AFB_1. This may be due to the strain-specific differences. Considering the molecular structure of AFB_1 is carbon in nature, so microorganisms that can use it as a carbon source can also degrade it (Wang et al., 2018). It is speculated that when the carbon source is sufficient, especially glucose, the utilization rate of AFB_1 as a carbon source may be affected.

Effects of metal ions were shown in Figure 4d. Findings of the current study established that Mg^{2+} and Mn^{2+} stimulated degradation of AFB_1 by *B. velezensis* AD8 at degradation rates of 87.35 ± 0.13% and 83.41 ± 0.33%, respectively. However, the addition of Zn^{2+} and Fe^{2+} led to inhibition of degradation at rates of 13.30 ± 0.63% and 69.08 ± 1%, respectively. Similarly, previous studies showed that the degradation rate of AFB_1 increased with Mg^{2+}, whereas the addition of Zn^{2+} and Fe^{2+} decreased the degradation rate of AFB_1 (Rao et al., 2017; Mwakinyali et al., 2019).

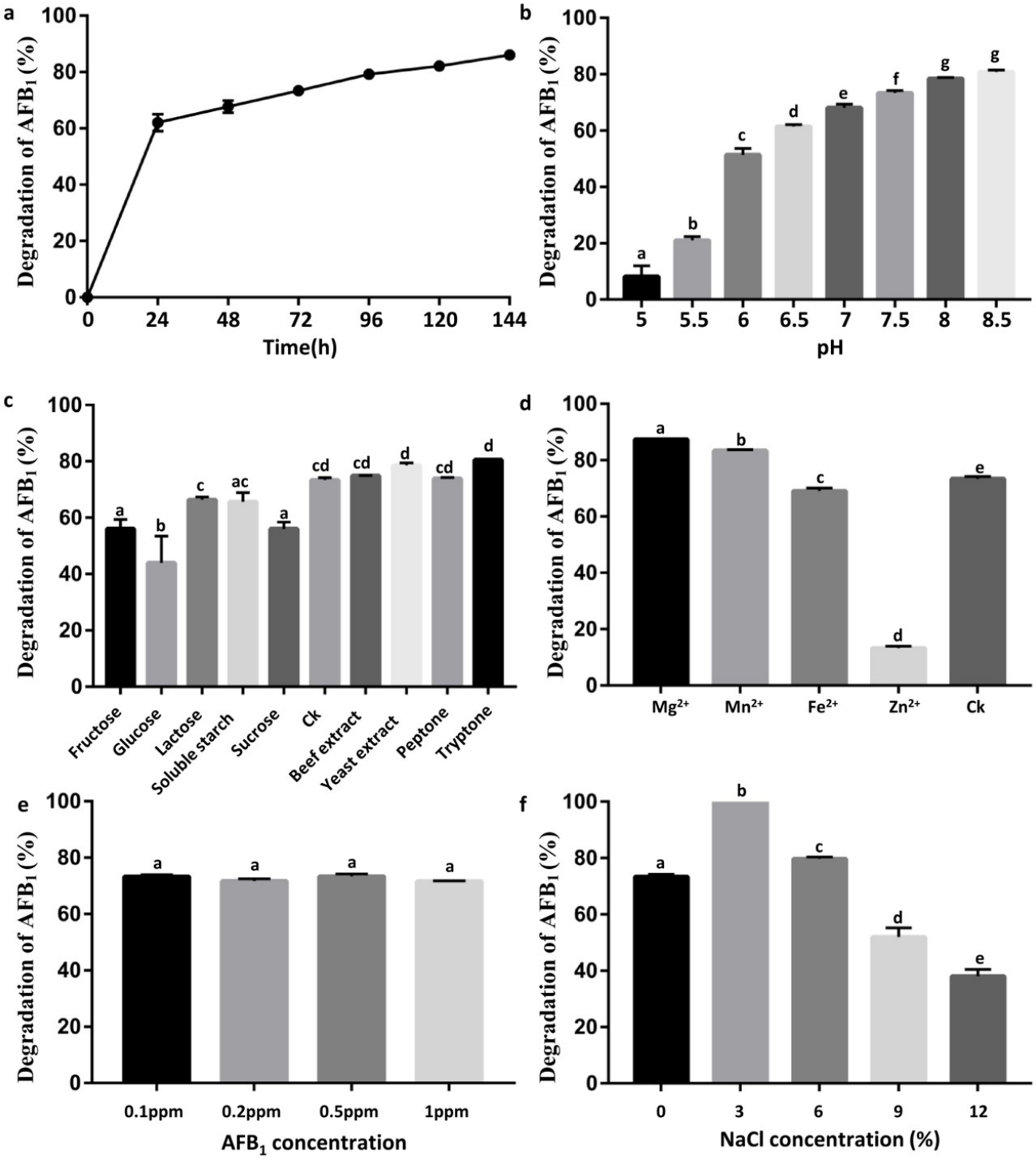

Figure 4. Effects of incubation time (a), pH (b), carbon and nitrogen (c), metal ion (d), AFB_1 concentration (e) and NaCl concentration (f) on the degradation of AFB_1 by *B. velezensis* AD8. Different letters indicate significant differences ($p < 0.05$) by Tukey's post hoc test.

Moreover, it was notable that all AFB_1 concentrations showed relatively stable degradation rates of 73.32 ± 0.63%, 71.67 ± 0.84%, 73.39 ± 0.83% and 71.6 ± 0.22% for 0.1 ppm, 0.2 ppm, 0.5 ppm and 1 ppm concentrations, respectively after 72 h (Figure 4e). Previous studies had shown that the removal rate of AFB_1 decreases as the concentration of AFB_1 increases (Li et al., 2018b). By contrast, *B. velezensis* AD8 had high efficiency and stable degradation rate and was not influenced by the concentration of AFB_1.

It had previously been reported that some microorganisms that cannot tolerate high-salt conditions can degrade AFB_1 (Adebo et al., 2016b; Eshelli et al., 2015). Therefore, the performance of AFB_1 degradation microorganisms under high-salt

conditions is also worthy of attention. The salt tolerance of *B. velezensis* AD8 had been demonstrated. Thus, the degradation ability in different concentrations of NaCl conditions were also explored in the current study (Figure 4f). Results showed that the degradation rate reached 100% at the salt content of 3% (W/V), whereas at salt contents of 6%, 9% and 12% (W/V), degradation rates were 79.67 ± 0.7%, 51.97 ± 3.2% and 38.01 ± 2.4%, respectively. Li et al. (2018a) found that *Tetragenococcus halophilus* CGMCC 3792 can degrade AFB_1 in salt condition. Comparatively, *B. velezensis* AD8 has a higher degradation ability than *T.halophilus* CGMCC 3792. The degradation rate of *B. velezensis* AD8 can reach 38.01 ± 2.41% even in a 12% salt concentration condition. Hence, *B. velezensis* AD8 has the potential to be applied to a high-salt food matrix.

4 Conclusions

In summary, 49 salt-tolerant microorganisms with the ability to degrade AFB_1 were isolated from Doubanjiang. *Bacillus* is the main contributor to the degradation of AFB_1 during fermentation of Doubanjiang. Among the isolated microorganisms, *B. velezensis* AD8 had the highest degradation ability, with degradation rates of 68.32% within 48 h (AFB_1 concentration: 500 ppb). The maximum degradation rates of *B. velezensis* AD8 were 86.04 ± 0.46% at 144 h incubation, 80.77 ± 0.67% at pH 8.5, and 100% at 3% Nacl (W/V). Most strikingly, the *B. velezensis* AD8 degraded 38.01 ± 2.41% of AFB_1 even at a concentration of 12% NaCl (W/V). Moreover, the concentration of AFB_1 did not influence the degradation rate by *B. velezensis* AD8. Addition of nitrogen, Mg^{2+}, and Mn^{2+} increased the degradation rate, whereas addition of carbon, Zn^{2+} and Fe^{2+} decreased the degradation rate. Thus, *B. velezensis* AD8 might be a potential candidate to degrade AFB_1 in high-salt food matrices.

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