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RESEARCH ARTICLE

Isolation of *Bacillus velezensis* DZ013 and Its Protective Effects Against Aflatoxin B_1-Induced Hepatic Oxidative Stress and Inflammatory Injury in Chickens

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ABSTRACT

Aflatoxin B_1 (AFB_1) is classified as a hazardous mycotoxin, posing serious threats to public health and livestock production due to its detrimental effects on animal health and the integrity of the food supply chain. The *Bacillus* genus possesses strong adaptability, extensive enzyme secretion capabilities and probiotic characteristics, and demonstrates great potential in the degradation of mycotoxins. In the present study, we isolated a novel strain, *Bacillus velezensis* DZ013, from chicken feed, which exhibited significant activity in degrading aflatoxin B1. *In vivo* experiments demonstrated that dietary intervention of DZ013 significantly reduced serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, enhanced hepatic catalase (CAT) and superoxide dismutase (SOD) activities, and decreased malondialdehyde (MDA) concentration in AFB_1-exposed broilers. Furthermore, DZ013 supplementation considerably alleviated hepatic histopathological damage and attenuated the inflammatory response through activation of the *Nrf2* pathway and concurrently suppressed the *NF-κB* pathway. Collectively, our data reveal that DZ013 alleviates AFB1-triggered hepatic oxidative stress and injury by coordinately modulating the connection between the *Nrf2* and *NF-κB* pathways, promoting antioxidant capacity while inhibiting inflammation, thereby providing a theoretical basis for bacterial biodetoxification and novel AFB_1 management strategies in poultry.

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INTRODUCTION

Aflatoxins (AFs) are mycotoxins synthesized by *Aspergillus* species, with AFB1 exhibiting the highest toxicity among all known aflatoxin variants (Ostry *et al*., 2017). Due to its potent hepatotoxicity, carcinogenicity, and immunotoxicity, the International Agency for Research on Cancer (IARC) has classified AFB1 as a Group 1 carcinogen (Zhao *et al*., 2021). AFB1 is toxic to a broad spectrum of animal species, and poultry are particularly sensitive due to their metabolic characteristics (Cao *et al*., 2022). AFB1 exposure induces multi-systemic damage, resulting in organ injury, diminished production performance, and increased mortality. Therefore, efficient detoxification methods are urgently needed for the poultry industry.

The liver is the main organ responsible for detoxification and metabolic transformation, and it is also the key organ for the metabolism and transformation of AFB_1 (Jobe *et al*., 2023). AFB_1 is mainly absorbed in the intestine and carried to the liver via the portal vein, where CYP450 enzymes metabolize it into the highly reactive AFBO, leading to liver poisoning, hemorrhagic necrosis, fatty degeneration, and fibrosis (Wang *et al*., 2021). AFB1 contributes to liver damage via mitochondrial dysfunction, oxidative stress, and inflammation, with the latter two

mechanisms being especially critical (Moloi *et al*., 2024). Under oxidative stress, AFB_1 impairs *Nrf2* activity, which hampers the endogenous antioxidant response, leading to an excess of reactive oxygen species (ROS) that attack immune system components, causing structural and functional damage (Suzuki and Yamamoto, 2017). In the inflammatory response, AFB_1 could trigger excessive immune responses, stimulating excessive production of inflammatory factors by tissue cells, disrupting immune balance and exacerbating liver inflammation (Chen *et al*., 2019). Additionally, an increase in the concentration of AFB_1 would accelerate the build-up of lipids within liver tissues and trigger the programmed death of liver cells, which is known as apoptosis (Liu *et al*., 2020).

Conventional methods for aflatoxin control suffer from limitations including unstable efficacy, potential for secondary pollution, narrow application scope, and high cost. In recent years, microbial degradation has amassed significant research interest because of its advantages of being environmentally sound, safe, highly specific, and capable of complete detoxification (Khatoon *et al*., 2025). Microbial detoxification involves the enzymatic or metabolic breakdown of toxic moieties within mycotoxin structures by microorganisms or their secreted products, thereby transforming these compounds into substances with reduced or negligible toxicity (Cai *et al*., 2020). Numerous microorganisms, including *Bacillus*, *Pseudomonas*, *Lactobacillus*, and non-aflatoxigenic *Aspergillus* species, can degrade AFB_1. Notably, *Bacillus* species constitute over half of all currently known microorganisms capable of degrading AFB_1. *Bacillus* species are widely distributed and possess strong adaptability and the ability to secrete various enzymes, which facilitate the absorption of nutrients by animals and they are highly suitable as feed additives for poultry (Bampidis *et al*., 2022). Therefore, the focus of this study is to isolate strains with efficient degradation capabilities of AFB_1 and then apply the isolated strains to the chicken liver injury model caused by AFB_1 to investigate their protective effect on liver damage in broilers and the underlying mechanisms. This research establishes a conceptual framework for creating innovative biological detoxifiers and proposes a secure, effective, and sustainable approach to mitigating AFB_1-associated hepatic damage in poultry.

MATERIALS AND METHODS

Isolation and identification of strains: The bacterial strain was isolated from visibly non-moldy portions adjacent to moldy areas of chicken feed samples collected from a local poultry farm in Tianjin, China. These samples were then enriched in Luria-Bertani (LB) broth (Beyotime, Shanghai, China) for 24h at 37°C. The suspension was serially diluted and plated on *Bacillus cereus* selective differential agar (Coolaber, Beijing, China) at 37°C for 24h. Individual colonies were purified by repeated streaking. Putative *Bacillus* isolates were screened on medium containing 0.01% coumarin (OriLeaf, Shanghai, China). Selected isolates were cryopreserved in 60% glycerol at -80°C. A commercial kit was employed for the extraction of genomic DNA (Takara, Otsu, Japan). The 16S rDNA gene was amplified with primers (27F/1492R) and sequenced. BLAST alignment and phylogenetic analysis using MEGA 7.0 were performed.

***In vitro* inhibition potential of DZ013 strain:** Strain DZ013 was cultured in LB broth (Bio-way, Shanghai, China) at 37°C, 180rpm for 24h, then transferred (5% v/v) into fresh LB and incubated at 37°C (160rpm) for 48h to obtain fermentation culture.

For antagonism assays, *Aspergillus flavus* spore suspension ($7.8–8.2 \times 10^8$ CFU/mL) was prepared. A double-layer PDA plate with Oxford cups was used; 120μL fermentation culture was added to wells, with sterile saline as control. Plates were cultured at 36°C for 72h.

For AFB_1 degradation, 8mL fermentation broth was mixed with 2mL of 500ng/mL AFB_1 (final 100ng/mL), AFB_1 content was analyzed using the enzyme-linked immunosorbent assay kit (Shanghai Sangong Biotech, Beijing, China). To investigate the detoxification mechanism, the DZ013 fermentation broth was centrifuged at 8500×g (Temp: 4°C), and the complete fermentation broth, the cell lysate supernatant, and the bacterial cell suspension were compared. To determine the active components, the supernatant was treated with 1mg/mL of proteinase K at 37°C for 1 hour, or heated at 100°C for 30 minutes, or filtered (0.22microns) before being mixed with AFB_1.

Animal feeding and diet details: The animal experiment protocol was approved by the Animal Ethics Committee of Tianjin Agricultural University, China. 160 1-day-old male AA broilers were randomly divided into the control group, the AFB_1 group, the AFB_1 + DZ013 group, and the DZ013 group (40 birds in each group). From day 1 to day 7, the AFB1 group and the AFB_1 + DZ013 group were fed with feed containing 3.0mg/kg AFB_1 (Tao *et al*., 2022). The AFB_1-supplemented feed was prepared by dissolving the AFB_1 standard (purity ≥ 98%, Sigma-Aldrich, USA) in methanol, mixing the solution stepwise into the basal diet, and verifying the final concentration 3mg/kg by HPLC with fluorescence detection. From day 8 to day 21, all groups were fed with the basal diet, and the AFB_1 + DZ013 group and the DZ013 group were intragastrically administered 0.5mL of DZ013 suspension (6.2×10^8 CFU/mL) daily, while the control group and the AFB_1 group were intragastrically administered sterile LB medium. Throughout the 21-day experimental period, body weight was recorded individually on days 1 and 21. Feed intake was measured daily on a pen basis. Average daily gain (ADG) was calculated as (final body weight − initial body weight) / 21 days. Average daily feed intake (ADFI) was calculated as total feed consumed per pen divided by the number of broilers per pen and divided by 21 days. Feed conversion ratio (FCR) was calculated as ADFI / ADG.

Sample collection: At the end of the 21-day experimental period, 12 broiler chickens were selected from each group to collect blood. The blood was centrifuged at 3000×g, at 4°C for 15 minutes to isolate the serum, which was then stored at -80°C. Subsequently, the livers were taken out. One section was fixed in 4% paraformaldehyde for future histological study, while the other part was immediately stored at -80°C.

Serum biochemical parameters: Serum levels of AST, ALT, MDA, CAT, SOD, T-AOC, TNF-α, IL-6, and IL-10 were determined with commercial ELISA kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Histopathological assessment: Paraffin embedded liver tissues were prepared, sectioned at 5μm through microtome, and performed H&E staining. Then, the sections were observed under an optical microscope (Nikon, Japan).

RNA isolation and quantitative real-time (qRT) PCR analysis: Total RNA was extracted from liver tissues using the RNA extraction kit (Beyotime, Shanghai, China), and then cDNA was synthesized. qRT-PCR was performed using SYBR Green, with β-actin as internal reference. The $2^{-\Delta\Delta Ct}$ method was applied to calculate the fold change in mRNA expression relative to the control group.

Western blot analysis: After preparing the liver tissue lysate using RIPA buffer (Beyotime, Shanghai, China), the proteins were subjected to SDS-PAGE separation and blotted onto a PVDF membrane. Then, the primary antibodies (*p-NF-κB p65, Nrf2, HO-1, IκBα, β-actin*) and corresponding species-specific secondary antibodies were incubated. Finally, the bands were displayed by chemiluminescence and quantified using ImageJ.

Statistical analysis: Statistical comparisons were performed using one-way ANOVA implemented in GraphPad Prism. Results are expressed as mean±SD, with *P<0.05 indicating statistical significance.

RESULTS

Isolation and identification of strain DZ013: Four bacterial strains exhibiting typical *Bacillus* morphology were isolated from chicken feed, all of which were Gram-positive bacilli. Coumarin, as a structural analogue of AFB_1, was used as the sole carbon source in the culture medium for secondary screening. Among the isolates, strain DZ013 showed the most vigorous growth (Fig. 1A and 1B), and was therefore selected for further characterization. Based on 16S rDNA sequence analysis and phylogenetic reconstruction, strain DZ013 was closely affiliated with the *Bacillus velezensis* cluster, showing high sequence similarity (Fig. 1C and 1D). Collectively, morphological and molecular data identified strain DZ013 as *Bacillus velezensis*.

***In vitro* inhibition potential of DZ013:** The antagonistic activity of DZ013 against *Aspergillus flavus* was assessed using the flat-hole drilling method. As shown in Fig. 2A, DZ013 markedly inhibited fungal growth, resulting in ellipsoidal colonies, whereas the control group exhibited normal circular colonies.

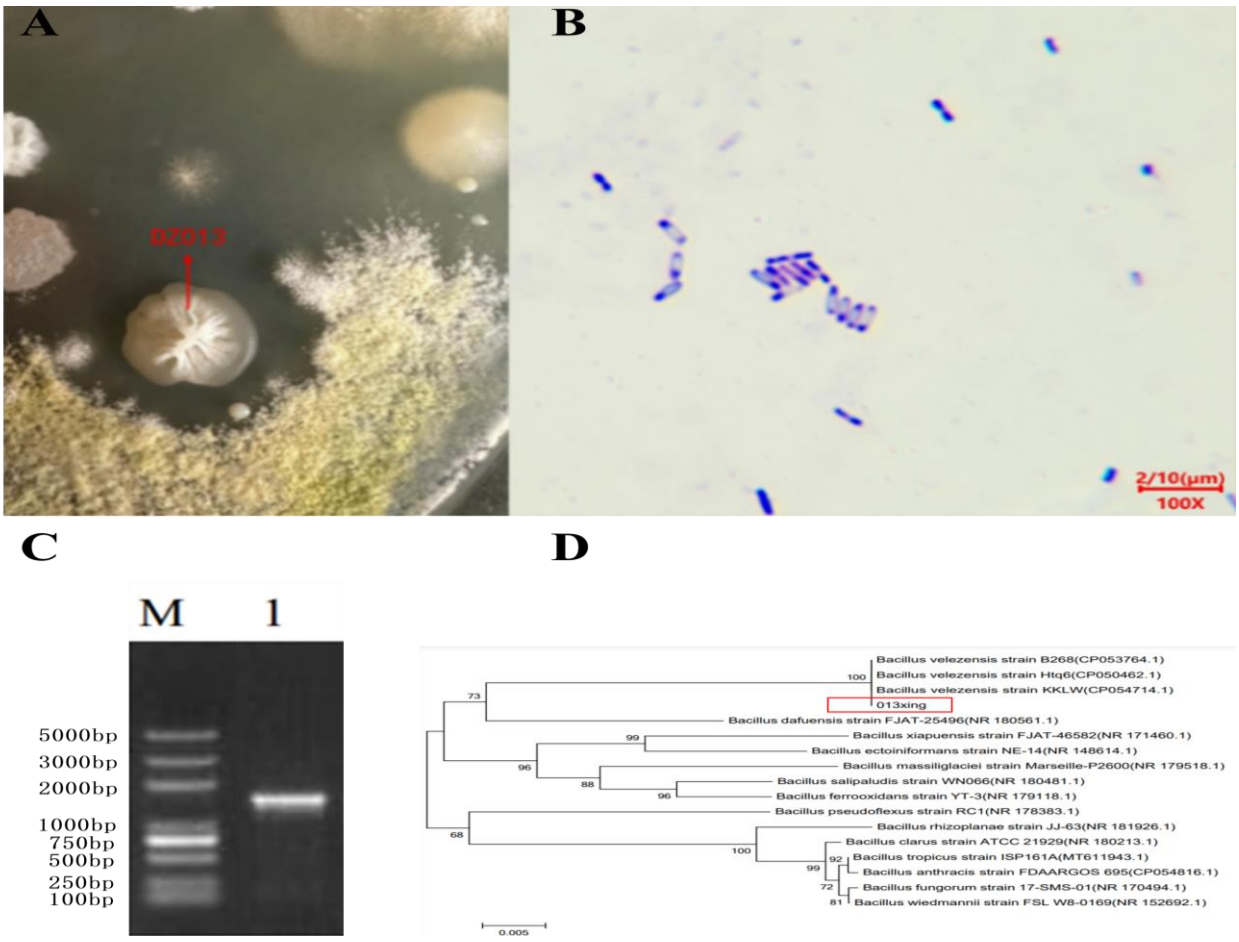


Fig. 1: Identification and Isolation of the AFB1-degrading bacterial strain DZ013. (A) Morphological characteristics of DZ013 colonies cultured on LB agar for a 24-hour period. (B) Microscopic image of DZ013 after Gram staining, showing Gram-positive rods. (C) Electrophoretic analysis of the 16S rDNA gene PCR amplicon derived from DZ013. Lane M: Marker indicating DNA molecular size; Lane 1: the approximately 1500 bp amplicon. (D) Neighbor-joining analysis of 16S rDNA sequences reveals the phylogenetic placement of strain DZ013 (highlighted in red) within the *Bacillus* clade. 0.005 substitutions per nucleotide site are shown by the scale bar.

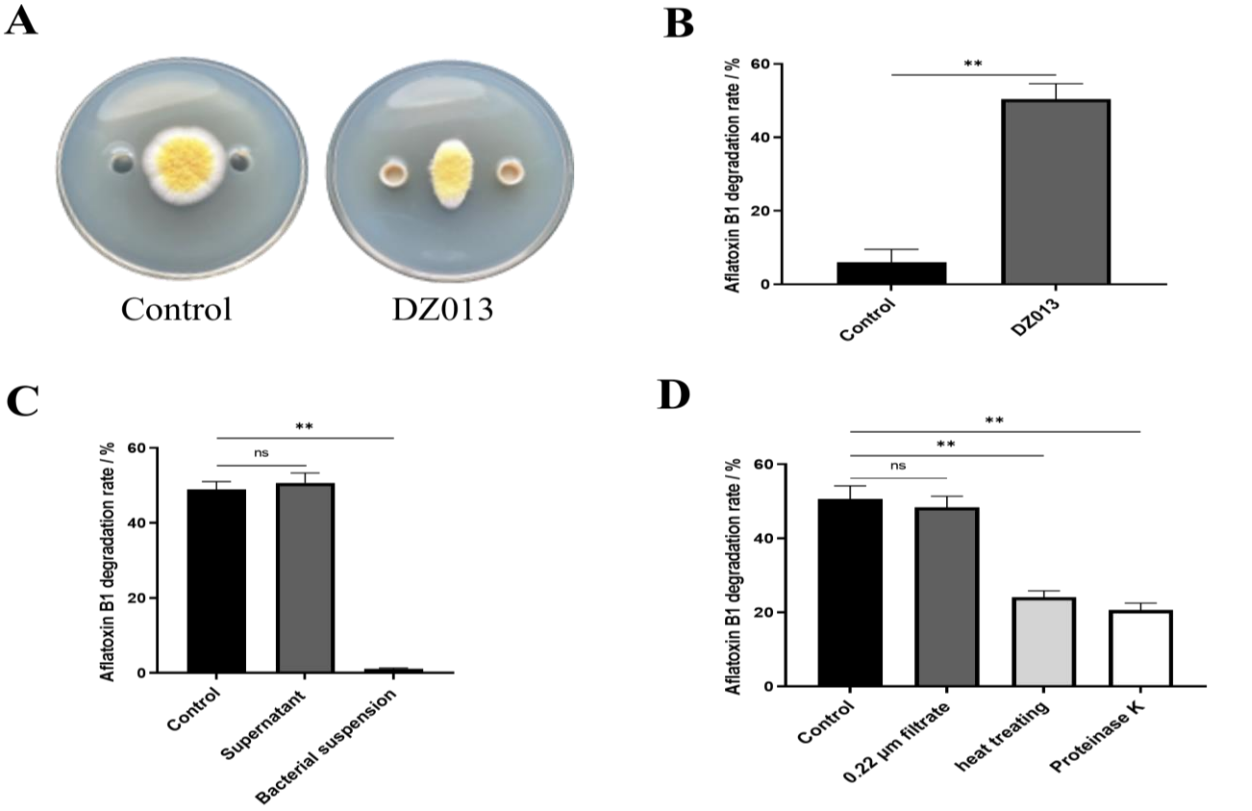


Fig. 2: *Bacillus velezensis* DZ013: Inhibition of *Aspergillus flavus*, Degradation of AFB1, and Identification of Active Components. (A) The growth-suppressing activity of strain DZ013 against *Aspergillus flavus*. (B) AFB_1 degradation rate after co-incubation with the fermentation broth of strain DZ013. (C) AFB_1 degradation rates of different bacterial fractions: whole fermentation culture, cell-free supernatant, and bacterial suspension. (D) Effects of 0.22 μm filtration, Proteinase K digestion, and heat treatment on its AFB_1 degradation activity. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared to the control group.

To evaluate the degradation of AFB_1, the fermentation broth of DZ013 was co-cultured with AFB1. The results indicated that the degradation rate in the DZ013 group was 50.45%, which was significantly higher than 5.95% as observed in the control group (Fig. 2B), suggesting that DZ013 had a good degradation effect on AFB_1. To identify the fraction responsible for degradation, various components of the fermentation culture were detected (Fig. 2C). It was found that the supernatant without cells (50.67%) and the complete fermentation broth (48.93%) showed comparable activity, while the activity of the washed cell suspension was significantly reduced ($P<0.01$), indicating that the degradation activity mainly occurred extracellular.

To further characterize the active components, the supernatant was subjected to various physicochemical treatments (Fig. 2D). After filtration through a 0.22μm membrane, 43.40% of the original degradation activity was retained. However, treatment with proteinase K reduced the activity to 20.65%, and heat treatment reduced it to 24.14%, both representing significant decreases compared with the untreated supernatant *($P<0.01$)*. These results indicate that the degradation effect of DZ013 on AFB_1 is exerted by a heat-labile, small-molecular-weight extracellular metabolite, likely of proteinaceous nature.

DZ013 alleviates AFB_1-induced growth retardation in chickens: As summarized in Table 1, dietary AFB_1 exposure significantly reduced FBW and ADG, while increasing the FCR in broilers when compared with the control group. However, supplementation with DZ013 significantly ameliorated these adverse effects, resulting in increased FBW and ADG and a decreased FCR relative to the AFB_1-alone group. These results indicate that *Bacillus velezensis* DZ013 could markedly mitigate the inhibitory effect on growth triggered by AFB_1 exposure in broilers.

Table 1: The influence of AFB_1 exposure and DZ013 administration on the productive parameters of broiler chickens

Items	Control	AFB_1	AFB_1+DZ013	DZ013
IBW(g)	55.49±2.88	54.46±3.34	55.99±3.56	56.74±3.01
FBW(g)	814.00±16.02	743.18±18.20**	785.04±14.13$^{\#\#}$	835.62±16.40
ADFI	57.30±4.06	56.86±4.14	57.74±3.74	58.16±3.60
ADG	36.30±2.28	32.22±1.82**	34.53±2.23$^{\#}$	37.20±2.10
FCR	1.58±0.12	1.76±0.14**	1.67±0.13$^{\#}$	1.56±0.11

All values are presented as mean±standard deviation (SD). Statistical significance is indicated as follows: $^{*}P<0.05$ and $^{**}P<0.01$ compared to the control group; $P<0.05$ and $^{\#\#}P<0.01$ compared to the AFB_1-treated group. Abbreviations: FCR, Feed Conversion Ratio; FBW, Final Body Weight; ADFI, Average Daily Feed Intake; ADG, Average Daily Gain; IBW, Initial Body Weight.

Histological analysis and serum biochemical results: As shown in Fig. 3A, the liver sections of both the control group and the DZ013 group showed normal liver structures, with intact tissue architecture, clear liver lobules, with large and round nuclei of liver cells. In contrast, livers from AFB_1-treated group showed marked pathological alterations, including hepatocyte swelling, lipid vacuolation of varying sizes, and disruption of lobular architecture, indicating severe hepatic injury. Importantly, adding DZ013 could greatly improve these histopathological alterations, indicating that DZ013 has strong liver-protective effects against liver damage caused by AFB_1.

As depicted in Fig. 3B, AFB_1 exposure caused a marked increase in ALT and AST levels in the serum of broiler chickens relative to the control group, and these changes were significantly reversed with DZ013 supplementation ($P<0.05$). The results of the biochemical tests agreed with the histopathological observations, confirming that AFB_1 caused serious liver damage, which was effectively alleviated by DZ013 treatment.

Effect of DZ013 on antioxidant activity and inflammatory response: Compared to the control group, the AFB_1 group showed a significant increase in serum MDA concentration and decreased activities of SOD, CAT, and T-AOC. Alternatively, the addition of DZ013 effectively reversed these effects, indicating that DZ013 could reduce AFB_1-induced oxidative stress without disturbing the usual redox balance (Fig. 4A).

To evaluate the systemic inflammatory response, the levels of both pro- and anti-inflammatory cytokines in the serum were quantified, as shown in Fig. 4B. AFB_1 exposure induced a marked upregulation of the pro-inflammatory cytokines IL-6 and TNF-α, while concurrently reducing the level of the anti-inflammatory cytokine IL-10 *(P<0.01)*. Administration of DZ013 markedly counteracted these AFB_1-induced inflammatory alterations. These results demonstrated that DZ013 successfully attenuated the AFB_1-triggered inflammatory response by rebalancing the pro- and anti-inflammatory cytokine.

Influence of DZ013 on *Nrf2*, mRNA and protein levels: To elucidate the molecular mechanism underlying the antioxidant effects of DZ013, we examined the mRNA and protein expression of key components of the *Nrf2-Keap1* signaling pathway, including *Nrf2*, *Keap1*, *HO-1*, *and NQO*1 (Fig. 5). Relative to the control group, exposure to AFB_1 caused a downregulation in the levels of *Nrf2, NQO1* and *HO-1*, whereas it increased that of Keap1 *(P<0.05)*, as illustrated in Fig. 5A and 5B. In contrast, DZ013 significantly reversed these AFB_1-induced changes by promoting *Nrf2* expression, upregulating *HO-1* and *NQO1*, and downregulating *Keap1*, relative to the AFB_1-alone group (Fig. 5A and 5B). These findings indicate that the antioxidant efficacy of DZ013 is mediated, at least in part, through activation of the *Nrf2* signaling cascade and subsequent induction of cytoprotective gene expression.

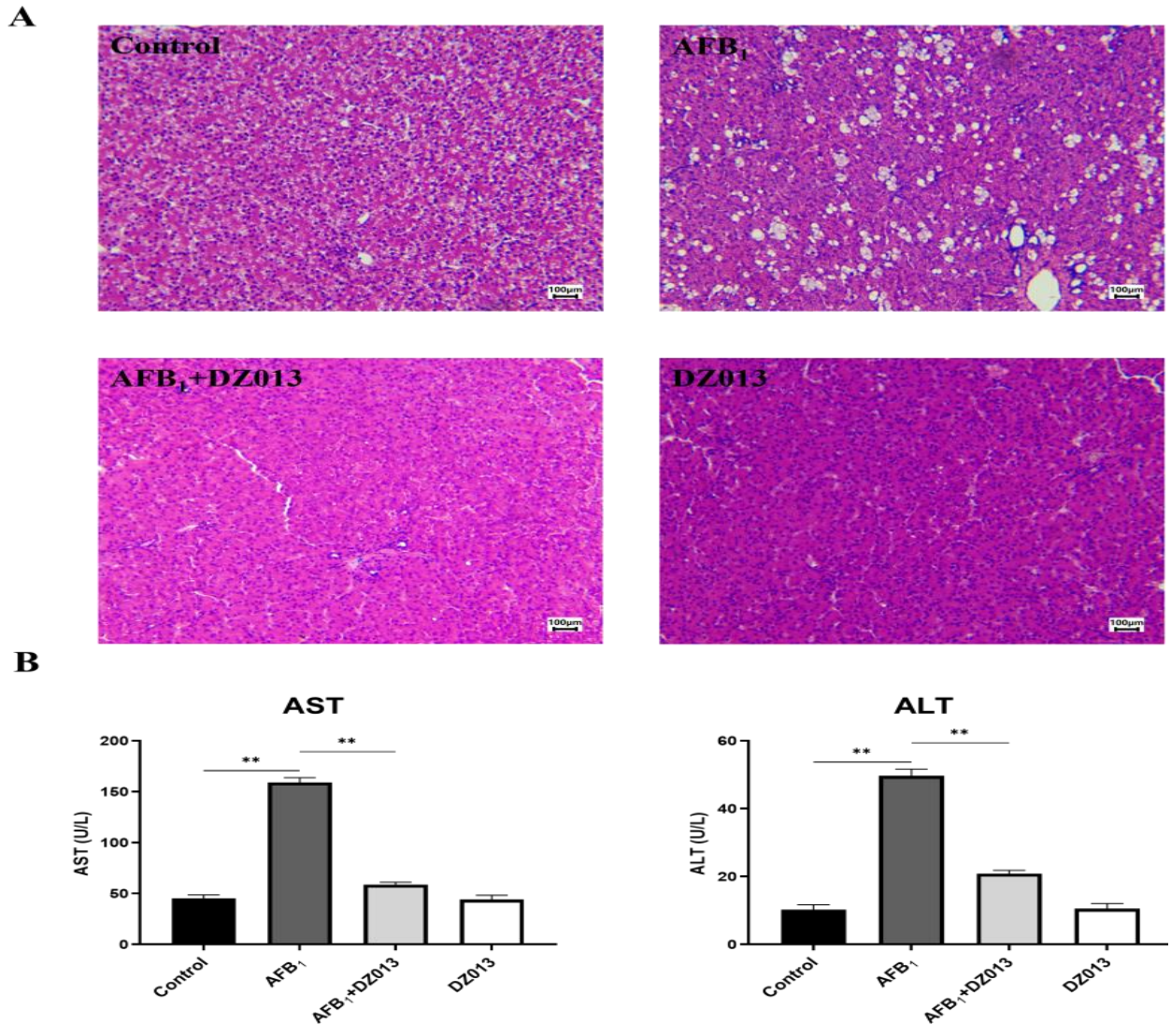


Fig. 3: Bacillus velezensis DZ013 mitigates hepatic damage in broilers caused by AFB_1. (A) Representative photomicrographs of liver sections from each group on day 21 (H&E staining; scale bar = 100 μm). (B) Serum ALT and AST concentrations. Values are means ± SD. Significant differences versus the control are denoted by * $P<0.05$ and ** $P<0.01$.

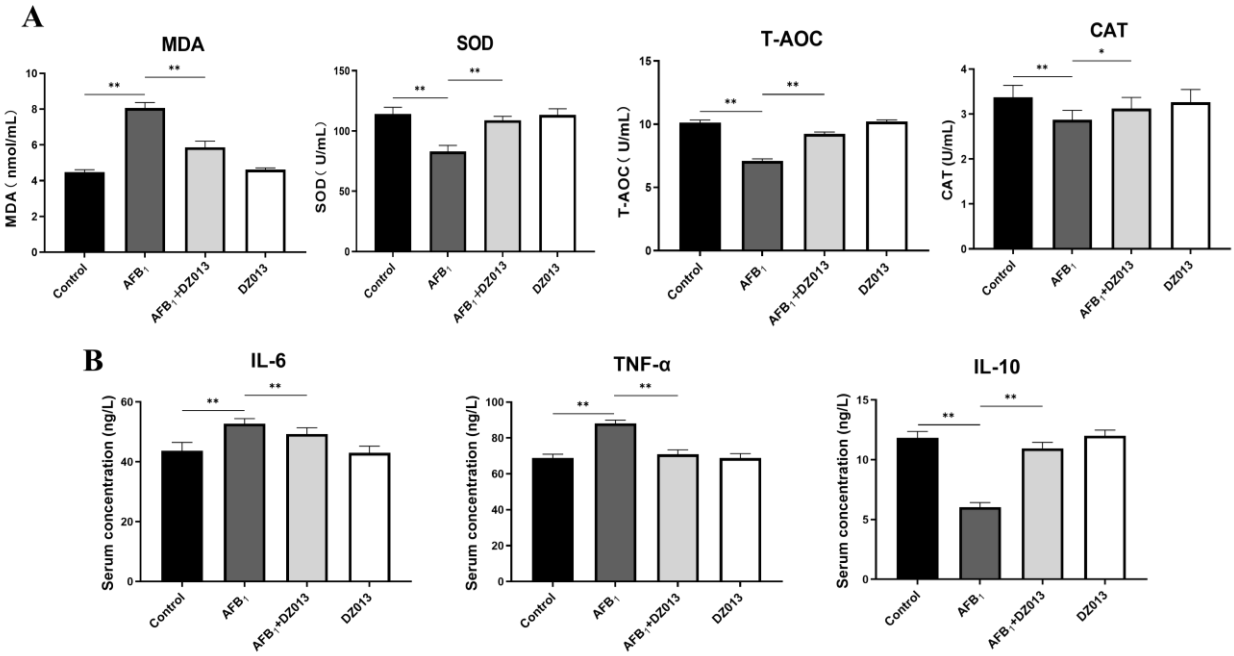


Fig. 4: *Bacillus velezensis* DZ013 exerts protective effects against AFB_1 in broilers via attenuation of oxidative stress and inflammation. (A) Serum concentrations of oxidative stress indicators: T-AOC, CAT, SOD and MDA. (B) Serum levels of inflammatory markers: TNF-α, IL-10 and IL-6. Values are means ± SD. Significant differences versus the control are denoted by * P<0.05 and ** P<0.01.

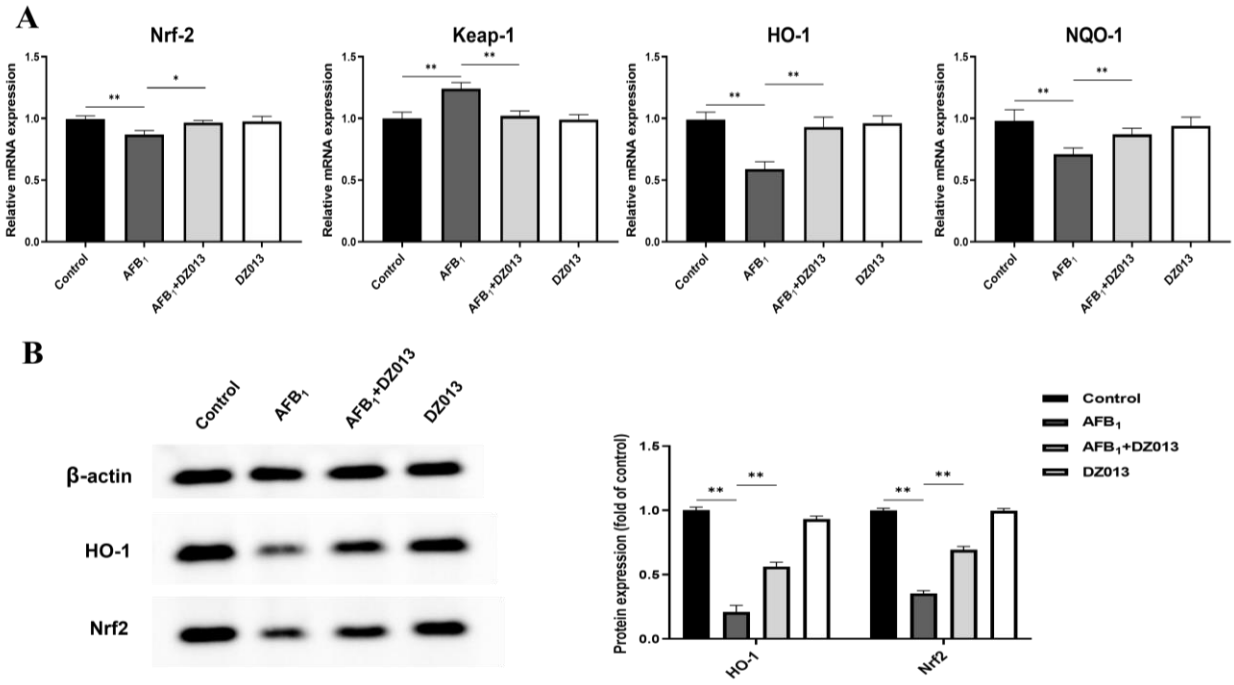


Fig. 5: *Bacillus velezensis* DZ013 activates the hepatic Nrf2 signaling cascade in broilers that were subjected to AFB_1 exposure. (A) The levels of mRNA relative expression of NQO1, HO-1, Keap1, and Nrf2 were determined by qRT-PCR. (B) Representative Western blot bands (upper panel) and quantitative analysis (lower panel) of HO-1 and Nrf2 protein expression. Gene and protein expression data were normalized to β-actin, which served as the internal reference/loading control. Values are means ± SD. Significant differences versus the control are denoted by * P<0.05 and ** P<0.01.

DZ013 Suppresses AFB_1-Triggered Activation of *NF-κB*: To determine whether the anti-inflammatory effect of DZ013 involves regulation of the *NF-κB* pathway, we measured the mRNA expression of *NF-κB p65, IκBα*, and the downstream inflammatory cytokine *IL-1β* in liver tissues (Fig. 6A). Additionally, we evaluated pathway activation at the protein level by measuring the *p-p65 (phosphorylation of NF-κB p65)* and the expression of *IκBα* via Western blot (Fig. 6B). AFB_1 exposure significantly activated the *NF-κB* pathway, as evidenced by upregulated mRNA expression of *NF-κB p65, IκBα*, and *IL-1β*, increased *p-p65* protein levels, and altered *IκBα* expression *(P<0.01)*. In contrast, DZ013 supplementation significantly suppressed this AFB_1-induced activation, reversing the AFB_1-induced upregulation of both *NF-κB p65* and *IκBα*. Collectively, our data demonstrate that the liver-protective action of *Bacillus velezensis* DZ013 operates, in part, by blocking the *NF-κB* signaling pathway, with a consequent reduction in pro-inflammatory mediator production.

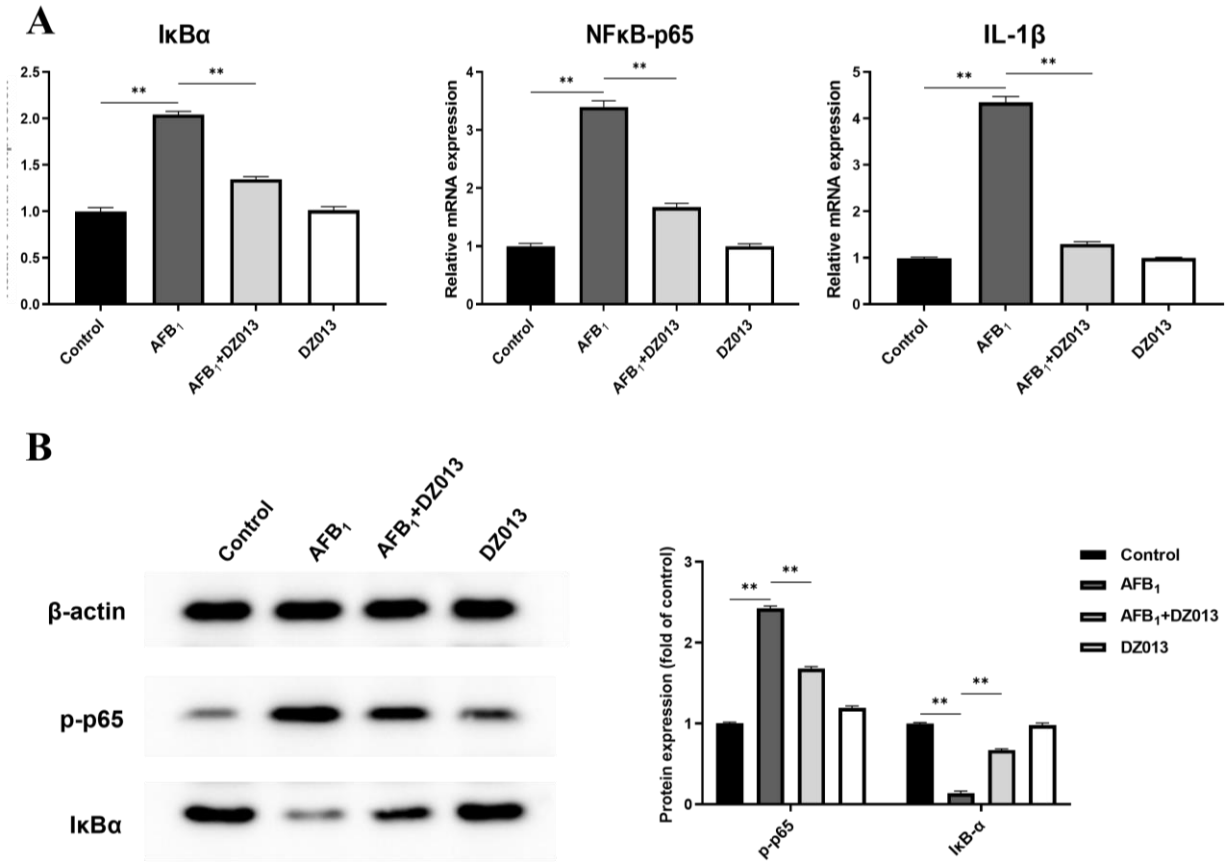


Fig. 6: Inhibition of AFB_1-induced hepatic NF-κB activation by Bacillus velezensis DZ013. (A) Relative messenger RNA expression levels of IκBα, IL-1β and NF-κB p65. (B) Representative immunoblot images (top) and corresponding densitometric analysis (bottom) depicting phosphorylated NF-κB p65 (p-p65) and IκBα protein levels. Gene and protein expression data were normalized to β-actin, which served as the internal reference/loading control. Values are means ± SD. Significant differences versus the control are denoted by * P<0.05 and ** P<0.01.

DISCUSSION

AFB1, a highly toxic mycotoxin, is categorized as a Group 1 human carcinogen by the IARC and ranks among the most hazardous and carcinogenic naturally occurring pollutants (Zhao *et al*., 2021). Its presence not only severely jeopardizes livestock health, leading to considerable economic impacts in animal husbandry but also represents a considerable risk to public health via its introduction into the human food supply through contaminated materials. Microbial detoxification has recently become a viable approach for managing mycotoxins because of its specificity and environmental friendliness. The degradation of AFB_1 has been effectively carried out by various microorganisms, including *Bacillus*, *fungi*, and *actinomycetes* (Magnoli *et al*., 2016; Huang *et al*., 2023). However, a lot of earlier screening projects focused mainly on how efficient degradation occurred, without considering the biological safety of the strains. Therefore, this research focused on the *Bacillus* genus, utilizing its recognized role as a probiotic, to identify strains capable of degrading AFB_1. The extraction of *Bacillus velezensis* DZ013 from commercial broiler feed, along with its shown ability to decompose AFB*1 in vitro* and *in vivo*, highlights its potential as a probiotic with functions of detoxification and health promotion.

Moreover, the distinctive spore structure of *Bacillus* allows it to endure extreme conditions like desiccation, elevated temperatures, and UV radiation. This characteristic guarantees that they could pass through the complex environment of the stomach and reach the intestines, where they have a beneficial impact, making them a great option for feed additives and functional supplements (Qin *et al*., 2022). *Bacillus velezensis,* a recently described member of the *Bacillus* genus, shares these beneficial traits and exhibits multiple functional properties, including antimicrobial activity, enzyme production, growth promotion, and immune enhancement, which make it an attractive probiotic candidate (Khalid *et al*., 2021). Additionally, they play a critical role in the degradation of mycotoxins. *Bacillus velezensis* could degrade mycotoxins through multiple mechanisms, such as strain E2 degrades OTA through enzymatic conversion and alkaline hydrolysis (Zhang *et al*., 2022), while the broad-spectrum strain IS-6 utilizes multiple enzymes including nudix hydrolase and carboxyl esterase to convert OTA into OTα (Jahan *et al*., 2023). Furthermore, the laccase-peroxidase system in strain B.26 degrades ZEN through hydroxylation or ring-opening reactions (Wei *et al*., 2025), and the aldehyde-ketone reductase present in the Vel-HNGD-F2 strain promotes DON detoxification by isomerizing it into the less toxic 3-isomer-DON (Liu *et al*., 2025). Compared with these strains, DZ013 exhibited a degradation rate of 50.45% against AFB_1, which is comparable to the rates reported for *B. velezensis* DY3108 (68.6%) and *B. subtilis* ZJ20 (40–65%) (Shu *et al*., 2018; Huang *et al*., 2023). This moderate-to-high degradation efficiency supports the

potential application of DZ013 as an effective AFB_1-detoxifying agent. It is noteworthy that the activity of degrading AFB_1 in *Bacillus velezensis* has been reported as extracellular and non-adsorptive, mainly present in the culture supernatant (Shu *et al*., 2018; Wu *et al*., 2022). The culture supernatant of the isolated strain DZ013 exhibits significant AFB_1 degradation activity, which is consistent with the previous research results. This finding aligns with reports on other degradative strains, such as *Bacillus subtilis* ZENL09 and *Brevundimonas vesicularis* ML17, which produce active substances of 25 kDa and 3 kDa, respectively (Peng *et al*., 2022; Xiang *et al*., 2024). Some research results also indicate that small peptides could mimic the structure of degrading enzymes and exert detoxification effects (Zhao *et al*., 2022). This provides a reasonable assumption about the properties of the active ingredients in DZ013 and offers a new perspective for our future research. The exact chemical composition of this metabolite and the detailed process of AFB_1 molecular degradation mediated by strain DZ013 still need to be further clarified.

Antioxidant capacity is a key indicator of an organism's health status. AFB_1 mainly causes damage to the health of broiler chickens by disrupting the normal oxidative stress state. This imbalance leads to an accumulation of ROS that overwhelms the endogenous antioxidant defense, resulting in oxidative damage. In response, the antioxidant system upregulates enzymes like SOD, CAT, and T-AOC to neutralize ROS and reduce lipid peroxidation end-products such as MDA, which protects against oxidative injury. (Nabi *et al*., 2022). Exposure to AFB_1 could induce the accumulation of MDA and ROS in the liver and regulate the expression of *Nrf2* and *HO-1*, ultimately damaging the liver's antioxidant capacity (Liu and Wang, 2016; Dong *et al*., 2024; Zhang *et al*., 2024). The *Keap1-Nrf2* signaling axis is the main endogenous system for resisting oxidative damage. It maintains the redox balance by regulating the expression of cellular antioxidant enzymes and detoxification enzymes, thereby protecting cells from damage induced by AFB_1 (Deshmukh *et al.,* 2017). Normally, *Nrf2* is associated with *Keap1* and remains within cytoplasm. Upon oxidative stress, *Keap1-Nrf2* dissociates, causing *Nrf2* to enter the nucleus. Subsequently, it upregulates the expression of *NQO1* and *HO-1*, helping to remove reactive oxygen species and promote the repair of damaged molecules, thereby providing protection against oxidative damage for the cells (Brasier, 2010; Tonelli *et al*., 2018). Accumulating evidence indicates that *Bacillus* species could mitigate oxidative liver damage by regulating the *Nrf2* pathway. For instance, *Bacillus licheniformis* and *Bacillus amyloliquefaciens* YN-BA4 can reduce chicken liver damage caused by AFB_1 through upregulating the *Nrf2* pathway, promoting antioxidant enzyme activity, and inhibiting MDA and ROS levels (Dong *et al*., 2024; Wang *et al*., 2025). The consistency between our findings and these previous reports strengthens the conclusion that activation of the *Nrf2* pathway is a common mechanism by which *Bacillus* species exert hepatoprotective effects against AFB1-induced oxidative stress. In this study, exposure to AFB_1 led to a significant increase in liver MDA levels, while reducing SOD and CAT activities and total antioxidant capacity (T-AOC). Intervening with *Bacillus velezensis* DZ013 effectively reversed these changes. Additionally, we found that DZ013 effectively increased the mRNA and protein levels of *Nrf2* and *HO-1* and inhibited *Keap1*, suggesting that DZ013 promotes the activation of the *Nrf2* pathway by reducing the inhibitory effect mediated by *Keap1*. These research results indicate that *Bacillus velezensis* DZ013 mainly alleviates liver damage caused by AFB_1 through the *Nrf2/Keap1* signaling pathway, thereby enhancing the antioxidant capacity. Notably, the upregulation of *HO-1* by DZ013 is particularly significant, as *HO-1* not only functions as an antioxidant enzyme but also exerts anti-inflammatory effects by inhibiting *NF-κB* nuclear translocation (Tonelli *et al*., 2018). This suggests that the protective effect of DZ013 may extend beyond antioxidant defense to encompass anti-inflammatory actions through *HO-1*-mediated suppression of *NF-κB* signaling.

In liver tissue, persistent inflammation could lead to liver damage, fibrosis, and carcinogenesis (Berasain *et al*., 2009). AFB_1 is a potent hepatotoxin that mainly induces liver inflammation by activating the NF-κB pathway. Under normal conditions, *NF-κB* is retained in the cytoplasm by binding to *IκB*. When stimulated by AFB_1, *IκB* undergoes phosphorylation and is degraded, releasing *NF-κB p50/p65* which then migrates to the nucleus and drives the expression of pro-inflammatory mediators (Zhou *et al*., 2016; Li *et al*., 2022). Studies have confirmed that AFB_1-induced liver damage in broilers is closely related to *NF-κB* activation (Ma *et al*., 2015; Liu *et al*., 2022), while certain species of *Bacillus* can alleviate inflammation by inhibiting the *NF-κB* pathway, for example, the extracellular polysaccharide of *Bacillus subtilis* can alleviate airway inflammation in mice (Zhang and Yi, 2022); *Bacillus coagulans* protects the intestine through the *TLR4/NF-κB* and *Nrf2* pathways (Wang *et al*., 2022), but the effect of these bacteria on counteracting AFB_1-induced liver inflammation through this mechanism has not been reported. In our study, AFB_1 exposure led to significant increases in the mRNA levels of *p65*, *IκBα*, and *IL-1β*, and also increased the protein expression of *p-p65* and *IκBα*. Intervening with DZ013 effectively reversed these changes, indicating that DZ013 can inhibit the *NF-κB* pathway to alleviate AFB_1-induced liver damage. However, there is a known interaction between *Nrf2* and the *NF-κB* pathway, and the observed *NF-κB* inhibition may be mediated by *Nrf2*-driven reactive oxygen species clearance or through anti-inflammatory products such as *HO-1* on *NF-κB* signal transduction feedback inhibition. Therefore, further research on the precise interaction between *Nrf2* and *NF-κB* is crucial for a comprehensive understanding of the liver protection mechanism of DZ013 and for strengthening its application in reducing the toxicity of AFB_1. Specifically, the observed inhibition of *NF-κB* by DZ013 may be mediated through two interconnected mechanisms: (1) direct suppression of *NF-κB* signaling components, and (2) indirect inhibition via *Nrf2/HO-1*-driven ROS clearance, which removes the upstream activators of *NF-κB*. This dual-layer regulation, wherein DZ013 simultaneously activates the *Nrf2* pathway and suppresses the *NFκB* pathway, likely underlies its potent hepatoprotective efficacy against AFB_1-induced liver injury.

Conclusions: In conclusion, our research successfully isolated *Bacillus velezensis* DZ013 from chicken feed. This bacterium can inhibit the growth of *Aspergillus flavus* and degrade AFB_1. Regarding *In vivo* experimentation, it could alleviate liver damage in broiler chickens caused by AFB_1 through enhancing the antioxidant defense system and inhibiting the inflammatory pathways. These effects are related to stimulating the *Nrf2*-mediated antioxidant system and simultaneously inhibiting the *NF-κB* pathway. These findings expand the application of the *Bacillus* genus in mold toxin protection and provide a new strategy for managing aflatoxin contamination. Our subsequent research will clarify the specific degradation pathways, isolate and identify active compounds, and evaluate the practical effectiveness of DZ013 in feed application and food safety intervention.

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REFERENCES

Bampidis V, Azimonti G, Bastos L, *et al*., 2022. Safety and efficacy of a feed additive consisting of *Bacillus velezensis* ATCC PTA-6737 (*Bacillus velezensis* PB6) for turkeys for fattening, turkeys reared for breeding, laying hens, minor poultry species for laying, piglets (weaned), weaned minor porcine species and sows (Kemin Europe NV). EFSA Journal 20(4): e07244.

Berasain C, Castillo J, Perugorria J, *et al.*, 2009. Inflammation and liver cancer: new molecular links. Annals of the New York Academy of Sciences 1155:206-21.

Brasier AR, 2010. The nuclear factor-B-interleukin-6 signalling pathway mediating vascular inflammation. Cardiovascular Research 86(2):211-8.

Cai M, Qian Y, Chen N, *et al*., 2020. Detoxification of aflatoxin B1 by Stenotrophomonas sp. CW117 and characterization the thermophilic degradation process. Environmental Pollution 261:114178.

Cao W, Yu P, Yang K, *et al*., 2022. Aflatoxin B1: metabolism, toxicology, and its involvement in oxidative stress and cancer development. Toxicology Mechanisms and Methods 32(6):395-419.

Chen Y, Li R, Chang Q, *et al*., 2019. *Lactobacillus bulgaricus* or *Lactobacillus rhamnosus* suppresses NF-κB signaling pathway and protects against AFB_1-induced hepatitis: A novel potential preventive strategy for aflatoxicosis? Toxins 11(1):17.

Deshmukh P, Unni S, Krishnappa G, *et al*., 2017. The Keap1–Nrf2 pathway: promising therapeutic target to counteract ROS-mediated damage in cancers and neurodegenerative diseases. Biophysical Reviews 9(1):41-56.

Dong W, Liu M, Liu B, *et al*., 2024. Isolation of *Bacillus licheniformis* and its protective effect on liver oxidative stress and apoptosis induced by aflatoxin B1. Poultry Science 103(10):104079.

Huang M, Guo J, Jia Y, *et al*., 2023. A *Bacillus subtilis* strain ZJ20 with AFB_1 detoxification ability: A comprehensive analysis. Biology 12(9):1195.

Jahan I, Tai B, Ma J, *et al*., 2023. Identification of a novel *Bacillus velezensis* IS-6 nudix hydrolase Nh-9 involved in ochratoxin A detoxification by transcriptomic profiling and functional verification. Journal of Agricultural and Food Chemistry 71(26):10155-10168.

Jobe M, Mthiyane N, Dludla P, *et al*., 2023. Pathological Role of Oxidative Stress in Aflatoxin-Induced Toxicity in Different Experimental Models and Protective Effect of Phytochemicals: A Review. Molecules 28(14):5369.

Khalid F, Khalid A, Zhen Y, *et al*., 2021. Potential of *Bacillus velezensis* as a probiotic in animal feed: A review. Journal of Microbiology 59(7):627-633.

Khatoon A, Abidin Z, Ali A, *et al*., 2025. Managing mycotoxins in animal/poultry feed through innovative control strategies: a review. Kafkas Universitesi Veteriner Fakultesi Dergisi 31 (5): 581-602.

Li M, Liu P, Wang B, *et al*., 2022. Inhibition of nuclear factor kappa B as a therapeutic target for lung cancer. Alternative Therapies in Health and Medicine 28(1):44-51.

Liu J, Li P, Li X, *et al*., 2025. Expression, characterization, and application of an aldo-keto reductase mined from *Bacillus velezensis* Vel-HNGD-F2 for deoxynivalenol biodegradation. Food and Chemical Toxicology 196:115159.

Liu S, Kang W, Mao X, *et al*., 2022. Melatonin mitigates aflatoxin B1-induced liver injury via modulation of gut microbiota/intestinal FXR/liver TLR4 signaling axis in mice. Journal of Pineal Research, 73(2):e12812.

Liu X, Kumar S, Zhao X, *et al*., 2020. AFB1 Induced Transcriptional Regulation Related to Apoptosis and Lipid Metabolism in Liver of Chicken. Toxins 12(5):290.

Liu Y, and Wang W, 2016. Aflatoxin B1 impairs mitochondrial functions, activates ROS generation, induces apoptosis and involves Nrf2 signal pathway in primary broiler hepatocytes. Animal Science Journal 87(12):1490-1500.

Ma Q, Li Y, Fan Y, *et al*., 2015. Molecular Mechanisms of Lipoic Acid Protection against Aflatoxin B1-Induced Liver Oxidative Damage and Inflammatory Responses in Broilers. Toxins 7(12):5435-47.

Magnoli P, Rodriguez C, Poloni V. L., *et al*., 2016. Novel yeast isolated from broilers' feedstuff, gut and faeces as aflatoxin B(1) adsorbents. Journal of Applied Microbiology 121(6):1766-1776.

Moloi T P, Ziqubu K, Ndlovu Z, *et al*. 2024. Aflatoxin B1-induced hepatotoxicity through mitochondrial dysfunction, oxidative stress, and inflammation as central pathological mechanisms: A review of experimental evidence. Toxicology 509:153983.

Nabi F, Tao W, Ye R, *et al*., 2022. Penthorum Chinense Pursh extract alleviates aflatoxin B1-induced liver injury and oxidative stress through mitochondrial pathways in broilers. Frontiers in Veterinary Science 9:822259.

Ostry V, Malir F, Toman J, *et al*., 2017. Mycotoxins as human carcinogens—the IARC Monographs classification. Mycotoxin Research 33(1):65-73.

Peng M, Zhao Z, and Liang Z, 2022. Biodegradation of ochratoxin A and ochratoxin B by *Brevundimonas naejangsanensis* isolated from soil. Food Control 133:108611.

Qin Y, Angelini L, and Chai Y, 2022. *Bacillus subtilis* cell differentiation, biofilm formation and environmental prevalence. Microorganisms 10(6):1108.

Shu X, Wang Y, Zhou Q, *et al*., 2018. Biological degradation of aflatoxin B1 by cell-free extracts of *Bacillus velezensis* DY3108 with broad pH stability and excellent thermostability. Toxins 10(8):330.

Suzuki T and Yamamoto M, 2017. Stress-sensing mechanisms and the physiological roles of the Keap1–Nrf2 system during cellular stress. Journal of Biological Chemistry 292(41):16817-16824.

Tonelli C, Chio C and Tuveson A, 2018. Transcriptional Regulation by Nrf2. Antioxidants & Redox Signaling 29(17):1727-1745.

Tao W, Zhu W, Nabi F, *et al*., 2022, Penthorum chinense Pursh compound flavonoids supplementation alleviates Aflatoxin B1-induced liver injury via modulation of intestinal barrier and gut microbiota in broiler. Ecotoxicology and Environmental Safety 255:114805.

Wang J, Zhao Y, Zhou Y, *et al*., 2025. Inhibition of aflatoxin B1-induced murine hepatocyte pyroptosis by *Bacillus amyloliquefaciens* by activation of the Nrf2/HO-1 pathway. Ecotoxicology and Environmental Safety 302:118688.

Wang X, He Y, Tian J, *et al*., 2021. Ferulic acid prevents aflatoxin B1-induced liver injury in rats via inhibiting cytochrome P450 enzyme, activating Nrf2/GST pathway and regulating mitochondrial pathway. Ecotoxicology and Environmental Safety 224:112624.

Wang Y, Lin J, Cheng Z, *et al*., 2022. *Bacillus coagulans* TL3 inhibits LPS-induced caecum damage in rat by regulating the TLR4/MyD88/NF-κB and Nrf2 signal pathways and modulating intestinal microflora. Oxidative Medicine and Cellular Longevity 2022:5463290.

Wei Z, Zhang X, Shi L, *et al*., 2025. Functional characterization and mechanism of two zearalenone-degrading enzymes from *Bacillus velezensis* B.26. Journal of Agricultural and Food Chemistry 73(23):14629-14640.

Wu Q, Li H, Wang S, *et al*., 2022. Differential expression of genes related to growth and aflatoxin synthesis in *Aspergillus* flavus when inhibited by *Bacillus velezensis* strain B2. Foods 11(22):3620.

Xiang M, Liu P, Zhang H, *et al*., 2024. In vitro degradation of zearalenone by culture supernatant of *Bacillus subtilis*. Food and Bioprocess Technology 17(8):2206-2215.

Zhang J, Sun X, Chai X, *et al*., 2024. Curcumin mitigates oxidative damage in broiler liver and ileum caused by aflatoxin B1-contaminated feed through Nrf2 signaling pathway, Animals 14(3):409.

Zhang L and Yi H, 2022. An exopolysaccharide from*Bacillus subtilis* alleviates airway inflammatory responses via the NF-κB and STAT6 pathways in asthmatic mice. Bioscience Reports 42(1):BSR20212461.

Zhang Y, Li Z, Lu Y, *et al*., 2022. Characterization of *Bacillus velezensis* E2 with abilities to degrade ochratoxin A and biocontrol against *Aspergillus* westerdijkiae fc-1. Toxicon 216:125-131.

Zhao L, Zhang L, Xu Z *et al*., 2021. Occurrence of Aflatoxin B1, deoxynivalenol and zearalenone in feeds in China during 2018–2020. Journal of Animal Science and Biotechnology, 12(1):74.

Zhao Z, Zhang Z, Zhang H, *et al*., 2022. Small peptides in the detection of mycotoxins and their potential applications in mycotoxin removal. Toxins 14(11):795.

Zhou Q, Liu S, Yua D, *et al*., 2016. Therapeutic effect of total saponins from Dioscorea nipponica makino on gouty arthritis based on the NF-κB signal pathway: An In vitro study. Pharmacognosy Magazine 12(47):235-40.