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Protective effect of *Devosia* sp. ANSB714 on growth performance, serum chemistry, immunity function and residues in kidneys of mice exposed to deoxynivalenol

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ABSTRACT

This study was conducted to investigate the toxic effects of deoxynivalenol (DON) and the ameliorating efficacy of *Devosia* sp. ANSB714 for the negative effects of DON on mice. In the experiment, 80 mice were randomly divided into 4 treatments: non-toxin control, toxin, non-toxin control + ANSB714 and toxin + ANSB714. During 28 days, the mice in treatment with 4.70 mg/kg DON only had significantly lower average daily gain as compared those with non-toxin control treatment ($P < 0.05$). Serum blood urea nitrogen, tumour necrosis factor-α and the residues of DON in kidneys in mice received the toxin diet were obviously higher than those with non-toxin control ($P < 0.05$). There were no significant differences ($P > 0.05$) between ANSB714 treatments and non-ANSB714 treatments on above parameters of mice. Adding ANSB714 to toxic diets could normalize deviant physiological effects of DON on mice.

1. Introduction

The trichothecene mycotoxins are a group of more than 200 structurally related sesquiterpenoid metabolites produced by foodborne and environmental fungi that are characterized by the tetracyclic 12, 13-epoxytrichothec-9-ene ring system (Grove, 2007). Toxicological effects associated with trichothecene mycotoxicosis in humans and animals include anorexia, gastroenteritis, emesis and hematological disorders (Pestka, 2007).

Deoxynivalenol (DON, vomitoxin) is a trichothecene commonly found in wheat, barley and corn that have been infected by the mold *Fusarium graminearum* (Schothorst and Egmond, 2004). The presence of mg per kg levels of DON in foods is of major human health concern worldwide (Pestka and Smolinski, 2005). All animal species evaluated to date are susceptible to DON according to the rank order of swine > mice > rats > poultry > ruminants (Rotter et al., 1996). Differences in metabolism, absorption, distribution, and elimination of DON among animal species have been suggested to account for this differential sensitivity (Pestka, 2007).

Biochemical and immunity function were tested as a reflection of the metabolism and inner organ status of animals. Researchers observed deteriorations in performance and immune function as well as changes in hematology and serum chemistry in broiler chicks fed diets containing 16–18 mg DON/kg from naturally contaminated wheat (Huff et al., 1986; Kubena et al., 1988, 1989; Harvey et al., 1991). Pronounced elevation in serum IgA and concurrent depressions in serum IgM and IgG were reported in mice fed purified deoxynivalenol (Forsell et al., 1986). An enhanced utilization of amino acids in pigs alleviated the impairment induced by DON stress in serum biochemistry and immune relevant cytokines

Abbreviations: DON, deoxynivalenol; HPLC, high performance liquid chromatography; ND, not detected; SE, standard error; ADFI, average daily feed intake; ADG, average daily gain; F/G, feed usage: body weight gain; TP, Total protein; ALB, albumin; BUN, blood urea nitrogen; CRE, creatinine; AST, aspartate amino transferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; IgA, ImmunoglobulinA; IgM, ImmunoglobulinM; IgG, ImmunoglobulinG; IL-1β, Interleukin-1β; IL-2, Interleukin-2; IL-6, Interleukin-6; TNF-α, tumour necrosis factor-α; PBS, phosphate buffered saline; FHB, Fusarium Head Blight; ELISA, enzyme-linked immunosorbent assay.

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in growing pigs (Wu et al., 2013).

Biological transformations, including the use of microorganisms, may provide an effective means to overcome the limitations of physical and chemical methods to manage this mycotoxin (Kabak et al., 2006). Currently, *Eubacterium* stain BBSH 797 is the only microorganism that has been used as a commercial feed additive for eliminating trichothecenes, including DON, after contaminated feed has been ingested by animals (He and Zhou, 2010). From one of 62 fish investigated, Guan et al. (2009) isolated a mixed microbial culture, designated as C133, which de-epoxidized DON. Yu et al. (2010) isolated one single colony from chicken digesta, *Bacillus* sp. LS100, which was able to completely transform DON to DOM-1 at 37 °C under anaerobic conditions. Regarding aerobic microorganisms, the Gram-negative soil bacterium E3-39 isolated in Japan belonging to the *Agrobacterium-Rhizobium* group (Shima et al., 1997) and a mixed culture D107 isolated in Germany (Völkl et al., 2004) were reported to oxidize DON to 3-keto-DON. The strain DDS-1 identified preliminarily as *Devosia* sp. DDS-1 could not only degrade DON in liquid medium, but also degrade DON in feedstuff (Xu et al., 2010). Moreover, Xu et al. (2013) reported that *Devosia* sp. DDS-1 can produce 3-AC-DON oxidase with good temperature and pH stability, and the enzyme requires metal ions as cofactor. Sato et al. (2012) isolated 13 aerobic DON-degrading bacteria from a variety of environmental samples. Of these 13 strains, nine belonged to the Gram-positive genus *Nocardioides* and the other four to the Gram-negative genus *Devosia*. The 3-epi-deoxynivalenol was the major DON metabolites produced by all strains.

However, few application of microbiological detoxification of DON to feeding animals has been reported. Recently, we have successfully isolated one single colony isolate ANSB714 from soil that demonstrated the ability to reduce DON in Lauria–Bertani medium. It showed an effective ability to degrade 97.34% DON for 24 h (containing 4.5×10^9 CFU/mL bacterial strain ANSB714 and 100 μg/mL DON). Using physiological, biochemical and 16S rRNA gene sequence analysis methods, the ANSB714 isolate was identified as *Devosia* sp. Acute toxicity tests (rat and mouse) and two mutagenicity tests (mouse sperm shape abnormality test and bone marrow micronucleus test) have proven that ANSB714 was low-toxic and safe. DON transformation by ANSB714 was also high in media containing corn steep liquid. ANSB714 maintained high transformation ability over a broad range of temperatures from 4 to 40 °C and pH values from 6.2 to 9.1 and no transformation occurred at pH levels below 6.2 (including at physiological stomach pH-values of 2–4) and above 9.2. However, the test was showed that the *Devosia* sp. ANSB714 had a high survival rate and tolerance in simulated gastric fluid and intestinal fluid (Huang and Adams, 2004).The evaluation of safety and efficiency of the bacterial isolate ANSB714 is very important prior to commercialization of an application. The safety evaluation of this isolate has been accomplished in mice. Therefore, it is critical to evaluate the efficiency of this isolate in animal production for assessing its application potential in feed industry.

2. Materials and methods

2.1. Chemicals

All reagents were of reagent-grade quality or better and purchased from Sigma Chemical Co. (St. Louis, MO), unless otherwise described. For the feeding study, DON was produced by *F. graminearum* 37,687 cultures obtained from the Agricultural Culture Collection of China and purified by silica gel chromatography (Clifford et al., 2003). Purity of DON was verified by a single HPLC peak at 218 nm. Concentrated toxin solutions were handled in a fume hood.

2.2. Microorganisms and culture

The strain, *Devosia* sp. ANSB714, was originally isolated from soil by our group. The ANSB714 was cultured in Luria-Bertani medium at 30 °C for 24 h using batch fermentation.

2.3. Animal trial and treatment

BALB/c male mice (3 weeks) were obtained from Vital River Laboratory Animal Technology Co., Ltd. in Beijing Animal handling was conducted in accordance with recommendations established by the National Institutes of Health and was approved by the China Agricultural University Institutional Animal Care and Use Committee. Mice were kept in environmentally protected transparent polypropylene cages with stainless steel wire tops and filter covers and acclimated for 1 week prior to onset of different experimental treatments. Cages were kept in class II ventilated cabinets for the duration of the experiment. Environmental conditions included temperature of 23–25 °C, relative humidity of 45–55%, and a 12-h light: dark cycle. Mice were housed in cages and provided with food and water *ad libitum*. A total of eighty, 4-week-old healthy BALB/c male mice were randomly divided into 4 groups (5 replicates per treatment and 4 mice per replicate) as follows: Non-toxin control, toxin, non-toxin control + ANSB714 and toxin + ANSB714. Composition of the experimental diets was based on NTP-2000 open formula meal diet (Zeigler Brothers, Inc., Gardners, PA). The concentrated DON solutions were added into the mice powder diets and mixed together to process into pellets diets of toxin and toxin + ANSB714 groups. The mice of the non-toxin control + ANSB714 and toxin + ANSB714 treatments were fed with the fermentation liquor of *Devosia* sp. ANSB714 (fermentation liquor of *Devosia* sp. ANSB714: sterilized water = 1:3, containing1.1×10^9 CFU/mL bacterial strain. Then the mixture 25 mL/day in drinking water bottle were fed to mice in the non-toxin control + ANSB714 and toxin + ANSB714 treatments.). Mice were fed with experimental diets for 4 weeks. Average daily gain (ADG), average daily feed intake (ADFI), feed usage: body weight gain (F/G) were calculated for each treatment in the experimental period.

2.4. Analysis of mycotoxin content in feed

The contents of the mycotoxins, including DON, zearalenone, aflatoxin and ochratoxin A, in the feed ingredients and formulated diets used in the study were determined using the appropriate methods of HPLC (Binder et al., 2007). The concentration of DON in toxin and toxin + ANSB714 was on average 4.70 mg/kg, while that of zearalenone was on average 21.94 μg/kg, the other mycotoxins were determined to be at concentrations below detection limits. Hardly any DON or other mycotoxins were found in the non-toxin control and non-toxin control + ANSB714 treatments. The concentrations of DON, zearalenone, aflatoxin and ochratoxin A were lower than Limit of Quantification (the LOQ of DON, zearalenone, aflatoxin and ochratoxin A were set at 0.06 mg/kg, 4 μg/kg, 0.1 μg/kg and 0.117 μg/kg, respectively) in the non-toxin control and non-toxin control + ANSB714 treatments.

2.5. Sample collection

After a 6-h fast, blood of mice was collected from orbital venous plexus. The serum was separated by centrifugation and stored in eppendorf tubes at −80 °C for further analysis. Mice were euthanized by cervical dislocation. The kidneys were dissected out immediately and washed with normal saline, dried on a filter paper. Organs were frozen at −80 °C.