International Journal of Drug Research in Clinics

Int J Drug Res Clin. 2025;3:e21
doi:10.34172/ijdrc.2025.e21
http://ijdrug.com

Original Article

Reduction of Aflatoxin B1 by Postbiotics Obtained From *Lactiplantibacillus plantarum* in Simulated Gastrointestinal Conditions

Vahid Asgharzadeh[1], Jalil Rashedi[2], Behroz Mahdavipoor[3], Mohammad Asgharzadeh[4*], Abdolhassan Kazemi[5], Seyyed Amin Seyyed Rezaei[1], Seyed Reza Moaddab[3], Mahdi Asghari Ozma[6*]

[1]Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran
[2]Tuberculosis and Lung Disease Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[3]Department of Laboratory Sciences, Faculty of Paramedicine, Tabriz University of Medical Sciences, Tabriz, Iran
[4]Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[5]Infectious and Tropical Disease Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[6]Cellular and Molecular Research Center, Qom University of Medical Sciences, Qom, Iran

Article History:
Received: October 1, 2025
Revised: October 10, 2025
Accepted: October 25, 2025
ePublished: November 24, 2025

***Corresponding Authors:**
Mahdi Asghari Ozma,
Email: asghariozma@tbzmed.ac.ir;
Mohammad Asgharzadeh,
Email: asgharzadehmo@yahoo.com

Abstract

Background: This study aimed to evaluate the anti-AFB1 effect of the postbiotics under simulated gastrointestinal conditions. Aflatoxin B1 (AFB1) is one of the most hazardous mycotoxins with strong carcinogenic and toxic effects, posing a serious threat to food safety and public health. To reduce mycotoxin contamination, the use of postbiotics obtained from probiotic bacteria, particularly *Lactiplantibacillus plantarum*, has emerged as a promising biotherapeutic strategy.

Methods: Postbiotics from *L. plantarum* were tested for AFB1 reduction under simulated GI conditions (gastric pH: 2.5, intestinal pH: 7.5). AFB1 levels were quantified by ELISA. In addition, metabolites were analyzed by gas chromatography–mass spectrometry, and cytotoxicity was assessed in HEK293 cells by methyl thiazole tetrazolium assay. Finally, the effects of pH (2–9) and temperature (25–100°C) on AFB1-reduction activity underwent evaluation.

Results: The postbiotic decreased AFB1 levels dose-dependently, ranging from 13% at 0.625 mg/mL to 86% at 10 mg/mL ($P<0.05$). Cell viability remained above 84% at all tested concentrations. Moreover, activity was maintained across a pH range of 2–9, with optimal reduction at a pH level of 7–7.5, and was most effective at physiological temperatures (25–37°C). Ultimately, a minor reduction in efficacy was observed under extreme pH and high temperatures.

Conclusion: Overall, *L. plantarum* postbiotics can effectively reduce AFB1 under simulated GI conditions while demonstrating low cytotoxicity. This suggests these postbiotics can be useful for improving food safety.

Keywords: Aflatoxin B1, *Lactiplantibacillus plantarum*, Postbiotics, Gastrointestinal simulation, Cytotoxicity

Please cite this article as follows: Asgharzadeh V, Rashedi J, Mahdavipoor B, Asgharzadeh M, Kazemi A, Seyyed Rezaei SA, et al. Reduction of aflatoxin B1 by postbiotics obtained from *Lactiplantibacillus plantarum* in simulated gastrointestinal conditions. Int J Drug Res Clin 2025;3:e21. doi:10.34172/ijdrc.2025.e21

Introduction

Aflatoxins, especially aflatoxin B1 (AFB1), are among the most potent and hazardous mycotoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus*. In addition, these secondary metabolites are highly toxic and carcinogenic, posing a major threat to human and animal health.[1] Further, AFB1 contamination in key crops (i.e., maize, peanuts, and tree nuts) causes a serious global food safety concern, particularly in regions such as Asia, which reports the highest contamination levels worldwide.[2] The detrimental effects of AFB1 affect multiple organs (e.g., the liver, kidneys, and immune system), leading to hepatic necrosis, anorexia, vomiting, and, in severe cases, liver cancer. Moreover, its immunosuppressive properties and effects on reproductive health underscore its public health significance.[3,4]

Physical, chemical, and biological approaches are considered conventional strategies for reducing AFB1 contamination. While physical treatments (e.g., heat and irradiation and chemical methods involving hydrolytic or oxidizing agents) can lower AFB1 levels, these techniques frequently compromise food quality and safety.[5] Consequently, biological methods, particularly those employing probiotics and their metabolites, have gained considerable attention as safer and more effective alternatives.[6]

Probiotics, especially lactic acid bacteria (LAB) like *Lactiplantibacillus plantarum*, have demonstrated substantial efficacy in reducing AFB1 levels.[7] The ability of LAB to mitigate AFB1 contamination is attributed to their cell wall components, including polysaccharides and peptidoglycans, which can bind toxins and prevent their absorption in the gastrointestinal (GI) tract.[8] Furthermore, LAB strains produce enzymes and metabolites that may alter the structure of AFB1, leading to reduced toxicity. [9] These metabolites, generally known as postbiotics, present an attractive approach to food safety, as they offer the benefits of probiotics without the challenges associated with storage, viability, and safety concerns.[10] Likewise, LABs are widely recognized for their health advantages, including immune regulation and enhancement of gut health.[11,12]

The GI tract plays a crucial role in AFB1 interaction, as digestive enzymes, bile salts, and microbiota can influence toxin bioavailability.[13] The in vitro simulation of the GI environment enables researchers to evaluate the ability of probiotics and postbiotics to decrease AFB1 under digestion-like conditions. These findings provide valuable insights into the potential of biological approaches for food safety and public health applications.[14] However, data on *L. plantarum*–derived postbiotics under simulated GI conditions remain limited; therefore, the present study aims to address this gap in research.

Methods

Bacterial Strain and Growth Conditions

In this study, *L. plantarum* was used as the probiotic strain, previously isolated from traditional Iranian dairy products and identified using standard 16S rRNA sequencing, confirming its similarity to reference *L. plantarum* strains. This strain was selected based on prior in vitro assessments. It was archived in the microbial strain repository of the Drug Applied Research Center, Tabriz University of Medical Sciences, and preserved at −80°C in de Man, Rogosa, and Sharpe (MRS) broth supplemented with 25% (v/v) glycerol until experimental use. For experimental procedures, the bacteria were cultured in an MRS broth (Merck, Germany) supplemented with 0.6% (w/v) yeast extract (Sigma-Aldrich, USA) and incubated at 37°C for 24 hours under anaerobic conditions (5% CO_2).

Preparation and Determination of Postbiotics Concentration

To prepare the postbiotic, the probiotic strain *L. plantarum* was initially cultured on MRS agar and then transferred into a Falcon tube with MRS broth (Merck, Germany) supplemented with 0.6% yeast extract (Sigma-Aldrich). Next, the culture was incubated at 37°C for 24 hours in a shaking CO_2 incubator. After incubation, the culture was centrifuged at 10,000 × g for 15 minutes at 4°C. The supernatant was aseptically collected and sterilized using a 0.22 µm filter (Millipore Inc., Billerica, USA) before immediate use in further studies. To determine the concentration of postbiotics, the filtered solution underwent lyophilization using a Christ Alpha 1-2 LDplus freeze dryer (Martin Christ, Germany) under controlled conditions: 48 hours at 0.04 mbar (-50°C), followed by 12 hours at 0.001 mbar (-80°C). Moreover, the dry weight of the lyophilized postbiotic was measured using an analytical balance, yielding approximately 10 mg/mL of the original solution. Additionally, the concentration of 10 mg/mL was selected as the maximum tested dose based on preliminary experiments, which demonstrated optimal AFB1 reduction without significant cytotoxicity. This concentration, which is also in line with previously reported effective postbiotic doses, was used for all subsequent experiments.[15,16]

Gas Chromatography–Mass Spectrometry Analysis of Postbiotic Metabolites

The GC-MS analysis of postbiotic metabolites was performed using Shimadzu QP-5050 equipment with a GC-17A gas chromatograph equipped with an HP-5 capillary column (phenylmethyl siloxane, 30 m, 0.25 mm internal diameter) and a mass spectrometer with a mass range of 50–600 m/z. In addition, helium was used as the carrier gas at a flow level of 1 mL/min with a 1:30 split ratio. The injector and detector temperatures were set at 250°C and 280°C, respectively. Further, the column temperature was programmed to increase linearly at 5°C/min, from 60°C to 250°C, and was maintained at this temperature for 10 minutes. Furthermore, retention indices were calculated based on the retention times of n-alkanes injected under identical chromatographic conditions. Eventually, GC-MS was utilized to identify and analyze the components by comparing the mass spectra with those in the Wiley (nl7) and Adams Library.[17]

Cell Viability Assay

The impact of different concentrations of postbiotics (0.625 mg/mL, 1.25 mg/mL, 2.5 mg/mL, 5 mg/mL, and 10 mg/mL) on cell viability was evaluated using the methyl thiazole tetrazolium (MTT) assay (Sigma-Aldrich) on the human embryonic kidney 293 (HEK293) cell line from the Razi Vaccine and Serum Research Institute, Iran. Cells were grown in RPMI 1640 medium (Gibco) supplemented with 10% fetal bovine serum in 96-well plates at a density of 7×10^3 cells per well, under 5% CO_2 at 95% air humidity until reaching 80% confluency. Moreover, the postbiotic solution was filtered through a 0.22 µm membrane before treatment. Additionally, HEK293 cells were treated in triplicate with 20 µL of postbiotic per well (total volume: 180 µL). Control cells received fresh growth medium without postbiotics. After 48 hours, 10 µL of the MTT solution (5 mg/mL) was added to each well, followed by a 4-hour incubation in a CO_2 incubator. The medium was removed, and 1% dimethyl sulfoxide was added to dissolve the formazan crystals. Absorbance was measured at 570 nm using an enzyme-linked immunosorbent assay (ELISA) plate reader (StatFax 2100). Finally, an ELISA

plate reader was employed to assess the formazan dye absorbance at 570 nm.[18,19] The following equation was used to ascertain the rate of cell viability:

Cell viability (%) = OD CFS treated/OD control × 100

Simulated Gastrointestinal Conditions

Simulated GI fluids were prepared to mimic the conditions of the human digestive tract. The simulated gastric fluid contained KCl (2.2 g/L), NaCl (6.2 g/L), $CaCl_2$ (0.22 g/L), $NaHCO_3$ (1.2 g/L), and pepsin (0.3%, ≥ 250 units/mg solid; Sigma-Aldrich), with the pH adjusted to 2.5 using 0.1 N hydrochloric acid (HCl) in order to replicate gastric acidity. Similarly, the simulated intestinal fluid included KCl (0.6 g/L), NaCl (5 g/L), $CaCl_2$ (0.3 g/L), bile salts (0.45%), and pancreatin (0.1%, 8 × USP specifications; Sigma-Aldrich), with the pH adjusted to 7.5 using 0.1 N sodium hydroxide (NaOH) to simulate the alkaline environment of the intestine. It should be noted that both suspensions were filtered through a 0.22 µm filter in order to ensure sterility before use in the experiments.[20,21]

Preparation of the Aflatoxin B1 Stock Solution

The AFB1 was obtained from Sigma-Aldrich (A6636) in a vial containing 1 mg of toxin powder. The working solution was prepared by dissolving the powder in acetonitrile. Residual acetonitrile was removed, if necessary, using a rotary evaporator at 50–60°C under reduced pressure. The residue was then reconstituted and diluted in phosphate-buffered saline (PBS, pH: 7.2) in order to achieve the final AFB1 concentration of 10 ppb (ng/mL). Finally, the prepared stock solution was stored in an amber glass jar at 4°C and protected from light until needed.[22,23]

pH and Thermal Stability Assessment of Postbiotics

The postbiotic stock solution (10 mg/mL) was prepared by dissolving lyophilized powder in sterile PBS (pH: 7.2). Next, aliquots were transferred to sterile microtubes for stability testing. For thermal stability, samples were incubated at 25°C, 37°C, 50°C, 75°C, or 100°C for 1 hour, 2 hours, or 5 hours, then cooled to room temperature. Concerning pH stability, samples were adjusted to a pH level of 2–9 using 1 N HCl or 1 N NaOH, incubated at 37°C for 2 hours, and neutralized to a pH level of 7.2 with sterile PBS. Moreover, AFB1-reduction activity was assessed by mixing 1 mL of 10 ppb AFB1 solution with 9 mL of each treated postbiotic sample, followed by incubation at 37°C for 2 hours. Residual AFB1 was quantified using a competitive ELISA kit (R-Biopharm, Darmstadt, Germany), and the reduction percentage was calculated relative to the initial concentration.[24]

Culturing the Samples in Simulated Gastrointestinal Conditions

The samples were treated under simulated GI conditions in two sequential steps, each performed in triplicate. To mimic stomach conditions, 1 mL of the postbiotic solution was added to 9 mL of simulated gastric fluid (pepsin/HCl, pH: 2.5) containing 10 ppb AFB1. After vortexing for 15 seconds, the mixture was incubated at 37°C for 2 hours. Next, 1 mL of the stomach fluid containing AFB1 and the postbiotic solution was transferred to 9 mL of simulated intestinal fluid (bile salts, pancreatin, and pH level of 7.5) and incubated under the same conditions. Afterward, the samples were centrifuged at 10,000 × g for 15 minutes to isolate any residual particles. Subsequently, the supernatant was collected, and the procedure was repeated to guarantee complete separation. The remaining AFB1 content in the supernatant was quantified using a competitive ELISA kit (R-Biopharm, Darmstadt, Germany). The results were compared with a control solution containing the same initial AFB1 concentration but lacking postbiotics. The reduction in AFB1 levels demonstrated the toxin-reduction capability of the postbiotic solution.[25]

Quantitative Analysis of Aflatoxin B1 Using Enzyme-Linked Immunosorbent Assay

A standard curve was prepared with AFB1 standards (0 ng/mL, 1 ng/mL, 5 ng/mL, 10 ng/mL, 20 ng/mL, and 50 ng/mL). Test samples were diluted as necessary to fall within the linear range of the curve. Additionally, duplicate wells of the antibody-coated microtiter plate received 50 µL of standard or diluted sample, followed by 50 µL of enzyme conjugate and 50 µL of anti-AFB1 antibody. Plates were mixed gently and incubated for 30 minutes at room temperature. Wells were then washed to remove unbound components. In addition, substrate/chromogen (100 µL) was added, and plates were incubated for 15 minutes at room temperature. Furthermore, the reaction was stopped with 100 µL of the stop solution, and absorbance was measured at 450 nm. Moreover, AFB1 concentrations were determined by interpolation from the standard curve. Ultimately, the reduction percentage was calculated relative to controls containing AFB1 without postbiotic.[26,27]

Statistical Analysis

The data are presented as means ± standard deviations (SD). Statistical analyses were performed using one-way analysis of variance in SPSS software (version 26), followed by Duncan's multiple range test to compare group means at a 95% confidence level ($P < 0.05$). All experiments were conducted in triplicate.

Results

Analysis of Metabolite Content in Postbiotics Using Gas Chromatography–Mass Spectrometry

GC–MS analysis revealed a heterogeneous metabolite profile in postbiotics derived from *L. plantarum*. The predominant compounds identified in this study included hydroxyacetone, butyric acid, lactic acid, acetic acid, 2-methylpiperidine, 2,3-butanediol, lauric acid, benzoic

acid, and 3-aminoisobutyric acid (Table 1).

Cell Viability

The results showed a dose-dependent effect, with cell viability remaining above 84% across all tested concentrations. At higher concentrations (2.5 mg/mL, 5 mg/mL, and 10 mg/mL), a statistically significant reduction was observed in cell viability compared with the untreated control (Figure 1).

Simulated Gastrointestinal Conditions and pH Stability Assessment of Postbiotics

The postbiotic maintained AFB1-reduction activity under simulated GI conditions, with no significant loss of efficacy. Incubation in simulated gastric (pH: 2.5) and intestinal (pH: 7.5) fluids resulted in a statistically significant decline in AFB1 levels compared with the control group. Across a pH range of 2–9, the postbiotic remained effective in toxin reduction, with the highest activity observed at pH levels of 7 and 7.5. A slight decrease in efficacy was also observed at the extreme pH values (pH: 2 and pH: 9) (Figure 2).

Aflatoxin B1 Reduction

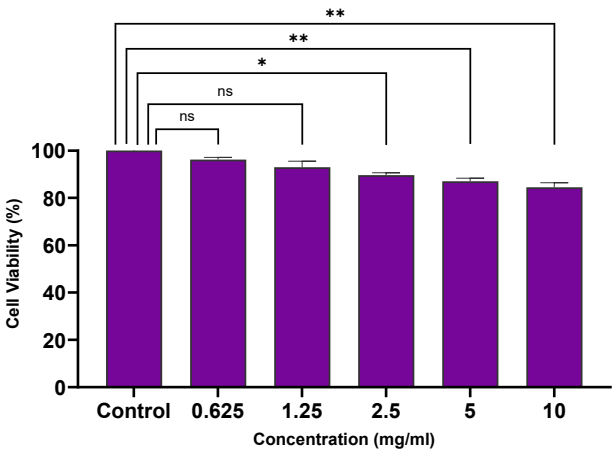

Figure 1. The Mean Percentage of Cell Viability After the MTT Assay for Different Concentrations (0.625 mg/mL, 1.25 mg/mL, 2.5 mg/mL, 5 mg/mL, and 10 mg/mL) of *L. plantarum* Postbiotic

Note. SD: Standard deviation; MTT: Methyl thiazole tetrazolium; *L. plantarum*: *Lactiplantibacillus plantarum*. Data are presented as means ± SD. $^{**}P<0.01$, $^{*}P<0.05$, ns: Not significant

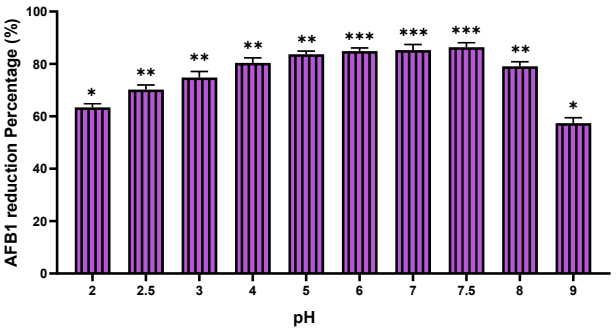

Figure 2. Aflatoxin B1 Reduction Percentages by Postbiotic (Concentration of 10 mg/mL) at Different pH Levels From 2 to 9

Note. $^{***}P<0.001$, $^{**}P<0.01$, $^{*}P<0.05$. Data are presented as means ± standard deviations

AFB1 reduction by probiotic-derived postbiotics under simulated GI conditions is shown in Figure 3. In all treatments, AFB1 reduction was significantly higher than that of the control group. Reduction percentages ranged from about 13% to 86%, with the highest and lowest reductions observed at 10 mg/mL and 0.625 mg/mL, respectively. The results demonstrated a clear dose-dependent pattern.

Thermal Stability Assessment of Postbiotics

The highest reduction rates were observed at 25°C and 37°C, with approximately 68% and 77% reductions, respectively, after 5 hours of incubation. Similar trends were found at shorter incubation times (1 hour and 2 hours). As the temperature increased, AFB1-reduction efficiency gradually decreased, reaching approximately 57% at 50°C, 35% at 75°C, and 13% at 100°C. At 100°C, the decrease was not statistically significant compared with other conditions (Figure 4).

Discussion

The potential health advantages of bioactive compounds,

Table 1. The Most Constituent Compounds Identified in the Postbiotics of *L. plantarum* Using GC-MS

No.	Compound
1	Hydroxyacetone
2	Butyric acid
3	Lactic acid
4	Acetic acid
5	2-methylpiperidine
6	2,3-butanediol
7	lauric acid
8	benzoic acid
9	3-aminoisobutyric acid
10	pyrrolo[1,2-a] pyrazine-1,4-dione
11	1, 4-diaza-2, 5-dioxo-3-isobutyl bicyclo [4.3.0] nonane

Note. *L. plantarum*: *Lactiplantibacillus plantarum*; GC-MS: Gas chromatography–mass spectrometry.

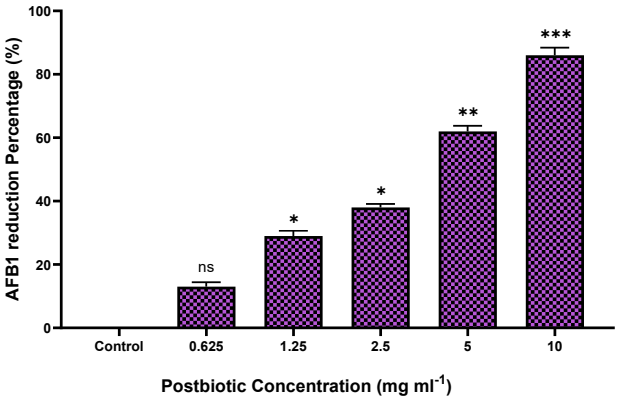

Figure 3. Reduction Percentage of AFB1 by Different Concentrations of Postbiotics

Note. AFB1: Aflatoxin B1. $^{***}P<0.001$, $^{**}P<0.01$, $^{*}P<0.05$, ns: Not significant. Data are presented as means ± standard deviations

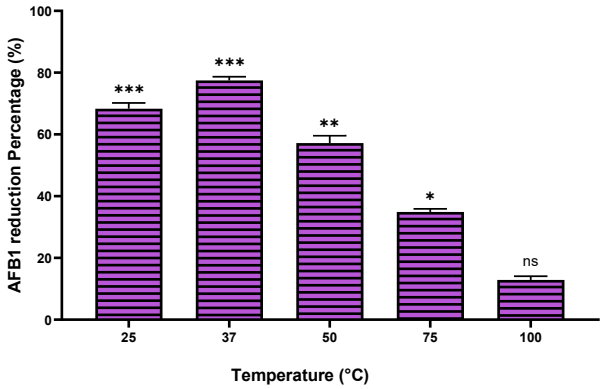

Figure 4. Aflatoxin B1 Reduction Percentages by Postbiotics (Concentration of 10 mg/mL) at Different Temperatures
Note. ***$P<0.001$, **$P<0.01$, *$P<0.05$, ns: Not significant. Data are presented as means ± standard deviations

including probiotics, prebiotics, and postbiotics, have attracted considerable scientific attention, especially for their ability to improve the balance of gut microbiota and general host health.[28-30] In addition, the durability and wide-ranging activity of postbiotics, which are non-viable microbial products or metabolites formed from probiotics, have made them promising agents.[31] Unlike live probiotics, postbiotics are resistant to environmental conditions, processing, and GI conditions, thus making them a practical and cost-effective alternative for such applications in the field of food safety or disease prevention.[32,33] Therefore, this study focused on the anti-AFB1 properties of *L. plantarum* postbiotics under simulated GI conditions. AFB1 is one of the most toxic and carcinogenic mycotoxins, considered a major global concern for global food safety.[34]

The GC-MS analysis confirmed that *L. plantarum*-derived postbiotics exhibit a highly diverse biochemical composition, consisting of various bioactive compounds (e.g., hydroxyacetone, butyric acid, lactic acid, acetic acid, 2-methylpiperidine, 2,3-butanediol, lauric acid, benzoic acid, and 3-aminoisobutyric acid). Among these substances, organic acids (e.g., lactic and acetic acids) are particularly noted for their role in neutralizing AFB1.[35] Lauric acid, known for its biosurfactant properties, may further enhance AFB1 reduction by disrupting biofilm formation and increasing its solubility in aqueous environments, thereby destabilizing the toxin.[36,37] This mechanism is consistent with the findings of previous studies, highlighting the effectiveness of fatty acid-derived postbiotics in reducing mycotoxin toxicity.[37]

Mycotoxin contamination, particularly AFB1, is a major food safety concern. Research has shown that lactic acid fermentation can effectively reduce AFB1 levels in foods. This approach offers promising solutions for reducing mycotoxin contamination in food.[38] Moreover, it may reduce the risk of aflatoxin in plant-based foods by decreasing the levels of free AFB1. The research demonstrated that conventional lactic acid fermentation, with *L. plantarum* as the starting culture, might be used to reduce the risk of AFB1 contamination in protein-rich plant-based meals.[39] Furthermore, another study, examining the interactions of mycotoxins throughout the GI system using an in vitro semi-dynamic model, revealed that AFB1 bioaccessibility increased by around 16% during the intestinal phase when concurrently exposed to other mycotoxins, resulting in notable alterations in intestinal cell viability and inflammation. The results further indicated that the presence of several mycotoxins may affect their bioaccessibility and toxicity inside the GI tract.[40] A study evaluated the efficacy of different postbiotics in mycotoxin reduction, concluding that specific postbiotics, particularly those derived from probiotic yeasts, display considerable potential in neutralizing mycotoxins such as AFB1, thereby enhancing food safety.[41]

The stability and efficacy of postbiotics under GI conditions play a crucial role in determining their potential as therapeutic agents. A key consideration is whether these bioactive compounds can maintain their functional properties throughout digestion and effectively exert their benefits in the host.[42] The observed stability of the postbiotic under GI conditions aligns with the results of previous research, highlighting the resilience of probiotics and postbiotics in such environments. Additionally, research on *L. plantarum* C88 revealed its capacity to mitigate AFB1 toxicity by augmenting antioxidant enzyme activity and diminishing AFB1 metabolites.[43] Furthermore, the ability of postbiotics to withstand the harsh effects of stomach acid, digestive enzymes, and bile suggests that they may facilitate direct absorption and exert their beneficial effects after consumption. These findings support the potential application of *L. plantarum*-derived postbiotics as a stable and effective strategy to reduce AFB1 contamination in the GI tract.[44]

Postbiotics have gained significant attention due to their antimicrobial properties and potential applications in food preservation and safety.[45] These bioactive compounds maintained AFB1-reduction activity across a wide pH range and at physiological to moderately elevated temperatures, making them effective in diverse environmental conditions. Their stability and efficacy in bioactive edible packaging systems offer a safer and more sustainable alternative to traditional preservatives, thus playing a growing role in the food industry.[46] In our study, the postbiotic maintained its AFB1-reduction activity under simulated GI conditions, with no significant degradation in gastric (pH: 2.5) and intestinal (pH: 7.5) fluids. It could also considerably reduce AFB1 levels ($P<0.05$). Across a pH range of 2–9, the postbiotic showed peak activity at a pH level of 7, with stable toxin reductions at pH levels of 2 and 9, although minor decreases in efficacy were observed at extreme pH values. In addition, in our study, the thermal stability test demonstrated that postbiotics noticeably reduced AFB1 at various temperatures, with the highest detoxifying efficacy observed at 37°C (77%) and 25°C (68%) after 5 hours of exposure. Similar results were observed at shorter

exposure times. As the temperature increased, AFB1 reduction efficiency decreased, with a reduction to 57% at 50°C, 35% at 75°C, and 13% at 100°C, where activity was negligible, indicating limited stability under extreme heat. These results are in line with those of previous studies, suggesting that postbiotics obtained from LAB are more effective at physiological temperatures, probably because bioactive metabolites are better preserved under these conditions.[47] These results suggest the potential of *L. plantarum* postbiotics for applications under varying GI conditions. Additionally, in one critical review on bioactive edible packaging applications, postbiotics were noted as metabolic products of probiotics that uniquely possess antimicrobial properties and maintain AFB1-reduction activity across a broad pH range and under physiological and moderate processing temperatures. This, therefore, supports the effectiveness of postbiotics under various environmental conditions for their potential use in food preservation and safety applications.[48]

Although the above results clearly demonstrate a dose-dependent reduction of AFB1 in experimental studies, they should be considered with caution. It should be noted that the current study is in vitro-based and cannot fully recapitulate complex in vivo GI conditions in situ, of which digestive enzymes, microbial interactions, or intestinal transit time may all possibly affect postbiotic activity. Moreover, the postbiotic and other food matrix components (e.g., proteins, lipids, and carbohydrates) and their interactions might influence AFB1-reduction capacity in actual food matrices. Thus, additional in vivo and food-based studies are necessary to validate the practical applicability of these postbiotics.

Conclusion

The postbiotics produced by *L. plantarum* demonstrated a significant, dose-dependent reduction in ELISA-detectable AFB1 levels under simulated GI conditions while exhibiting low intrinsic cytotoxicity. The stability of AFB1-reduction activity under physiological pH and temperature conditions highlights the practical relevance of these postbiotics as a potential food safety intervention. However, as this study was based on in vitro models and ELISA-based quantification, the observed reduction may primarily reflect AFB1 binding or reduced bioaccessibility rather than definitive chemical detoxification. Accordingly, further in vivo studies and advanced analytical investigations are required to confirm the mechanisms involved and validate real-world applications.

Ethics statement

This study was approved by the Local Ethics Committee of Tabriz University of Medical Sciences (IR.TBZMED.VCR.REC.1402.064).

Disclosure of funding source

This study was supported by the Biotechnology Research Center, Tabriz University of Medical Sciences (grant No. 70885).

Conflict of interests declaration

The authors declare they have no conflict of interests.

Acknowledgments

We would like to thank all collaborations by the staff of the Biotechnology Research Center, Tabriz University of Medical Sciences.

Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article.

Author contributions

Conceptualization: Mahdi Asghari Ozma
Data curation: Abdolhassan Kazemi
Formal analysis: Seyyed Amin Seyyed Rezaei
Funding acquisition: Mohammad Asgharzadeh
Investigation: Seyed Reza Moaddab
Methodology: Vahid Asgharzadeh
Project administration: Mohammad Asgharzadeh
Resources: Jalil Rashedi
Software: Behroz Mahdavipoor
Supervision: Mohammad Asgharzadeh
Validation: B.Mahdavipoor
Visualization: Jalil Rashedi
Writing–original draft: Vahid Asgharzadeh
Writing–review & editing: Mahdi Asghari Ozma

Consent for publication

Not applicable.

References

1. Fouad AM, Ruan D, El-Senousey HK, Chen W, Jiang S, Zheng C. Harmful Effects and Control Strategies of Aflatoxin B_1 Produced by Aspergillus flavus and Aspergillus parasiticus Strains on Poultry: Review. Toxins (Basel) 2019;11(3):176. doi:10.3390/toxins11030176
2. Pickova D, Ostry V, Malir F. A Recent Overview of Producers and Important Dietary Sources of Aflatoxins. Toxins (Basel) 2021;13(3):186. doi:10.3390/toxins13030186
3. Shabeer S, Asad S, Jamal A, Ali A. Aflatoxin Contamination, Its Impact and Management Strategies: An Updated Review. Toxins (Basel) 2022;14(5):307. doi:10.3390/toxins14050307
4. Chandra P. Aflatoxins: Food safety, human health hazards and their prevention. *Aflatoxins-Occurrence, Detoxification, Determination and Health Risks*: IntechOpen; 2021.
5. Rushing BR, Selim MI. Aflatoxin B1: A review on metabolism, toxicity, occurrence in food, occupational exposure, and detoxification methods. Food Chem Toxicol 2019;124:81–100. doi:10.1016/j.fct.2018.11.047
6. Yadav MK, Kumari I, Singh B, Sharma KK, Tiwari SK. Probiotics, prebiotics and synbiotics: Safe options for next-generation therapeutics. Appl Microbiol Biotechnol 2022;106(2):505–21. doi:10.1007/s00253-021-11646-8
7. Zhu Y, Xu Y, Yang Q. Antifungal properties and AFB(1) detoxification activity of a new strain of Lactobacillus plantarum. J Hazard Mater 2021;414:125569. doi:10.1016/j.jhazmat.2021.125569
8. Zhu FH, Chen XY, Hou LL, Dong JH, Liu HW, Zhu LQ, et al. Limosilactobacillus reuteri peptidoglycan alleviates aflatoxin B(1)-induced toxicity through adsorbing toxins and improving growth, antioxidant status, immunity and liver pathological changes in chicks. Br Poult Sci 2024;65(3):352–60. doi:10.1080/00071668.2024.2316228
9. Wang L, Huang Q, Wu J, Wu W, Jiang J, Yan H, et al. The metabolism and biotransformation of AFB(1): Key enzymes and pathways. Biochem Pharmacol 2022;199:115005. doi:10.1016/j.bcp.2022.115005

10. Ozma MA, Fadaee M, Hosseini HM, Ataee MH, Mirhosseini SA. A Critical Review of Postbiotics as Promising Novel Therapeutic Agents for Clostridial Infections. Probiotics Antimicrob Proteins 2025;17(2):656–67. doi:10.1007/s12602-024-10406-0
11. Asghari Ozma M, Mahmoodzadeh Hosseini H, Ataee MH, Mirhosseini SA. Evaluating the antibacterial, antibiofilm, and anti-toxigenic effects of postbiotics from lactic acid bacteria on Clostridium difficile. Iran J Microbiol 2024;16(4):497–508. doi:10.18502/ijm.v16i4.16309
12. Ozma MA, Ghotaslou R, Asgharzadeh M, Abbasi A, Rezaee MA, Kafil HS. Cytotoxicity assessment and antimicrobial effects of cell-free supernatants from probiotic lactic acid bacteria and yeast against multi-drug resistant Escherichia coli. Lett Appl Microbiol 2024;77(9):ovae084. doi:10.1093/lambio/ovae084
13. Guerre P. Mycotoxin and Gut Microbiota Interactions. Toxins (Basel) 2020;12(12):769. doi:10.3390/toxins12120769
14. Feng P, Ye Z, Kakade A, Virk AK, Li X, Liu P. A Review on Gut Remediation of Selected Environmental Contaminants: Possible Roles of Probiotics and Gut Microbiota. Nutrients 2018;11(1):22. doi:10.3390/nu11010022
15. Buahom J, Siripornadulsil S, Sukon P, Sooksawat T, Siripornadulsil W. Survivability of freeze- and spray-dried probiotics and their effects on the growth and health performance of broilers. Vet World 2023;16(9):1849–65. doi:10.14202/vetworld.2023.1849-1865
16. Saarela M, Virkajärvi I, Alakomi H-L, Sigvart-Mattila P, Mättö J. Stability and functionality of freeze-dried probiotic Bifidobacterium cells during storage in juice and milk. International Dairy Journal 2006;16(12):1477–82. doi:10.1016/j.idairyj.2005.12.007
17. Chang HM, Foo HL, Loh TC, Lim ETC, Abdul Mutalib NE. Comparative Studies of Inhibitory and Antioxidant Activities, and Organic Acids Compositions of Postbiotics Produced by Probiotic Lactiplantibacillus plantarum Strains Isolated From Malaysian Foods. Front Vet Sci 2020;7:602280. doi:10.3389/fvets.2020.602280
18. Siyadatpanah A, Norouzi R, Mirzaei F, Haghirosadat BF, Nissapatorn V, Mitsuwan W, et al. Green synthesis of nano-liposomes containing Bunium persicum and Trachyspermum ammi essential oils against Trichomonas vaginalis. J Microbiol Immunol Infect 2023;56(1):150–62. doi:10.1016/j.jmii.2022.06.006
19. Paula-Ramos L, da Rocha Santos CE, Camargo Reis Mello D, Nishiama Theodoro L, De Oliveira FE, Back Brito GN, et al. Klebsiella pneumoniae Planktonic and Biofilm Reduction by Different Plant Extracts: In Vitro Study. ScientificWorldJournal 2016;2016:3521413. doi:10.1155/2016/3521413
20. Fernández de Palencia P, López P, Corbí AL, Peláez C, Requena T. Probiotic strains: survival under simulated gastrointestinal conditions, in vitro adhesion to Caco-2 cells and effect on cytokine secretion. European Food Research and Technology 2008;227(5):1475–84. doi:10.1007/s00217-008-0870-6
21. Xin M, Zhao M, Tian J, Li B. Guidelines for in vitro simulated digestion and absorption of food. Food Frontiers 2023;4(1):524–32. doi:10.1002/fft2.186
22. Zinedine A, Faid M, Benlemlih M. In vitro reduction of aflatoxin B1 by strains of lactic acid bacteria isolated from Moroccan sourdough bread. Int J Agri Biol 2005;7(1):67–70.
23. Li S, Li X, Liu X, Zhang Q, Fang J, Li X, et al. Stability Evaluation of Aflatoxin B(1) Solution Certified Reference Material via Ultra-High Performance Liquid Chromatography Coupled with High-Resolution Mass Spectrometry. ACS Omega 2022;7(44):40548–57. doi:10.1021/acsomega.2c05829
24. Chen G, Fang Q, Liao Z, Xu C, Liang Z, Liu T, et al. Detoxification of Aflatoxin B1 by a Potential Probiotic Bacillus amyloliquefaciens WF2020. Front Microbiol 2022;13:891091. doi:10.3389/fmicb.2022.891091
25. Zolfaghari H, Khezerlou A, Ehsani A, Yari Khosroushahi A. Detoxification of Aflatoxin B1 by Probiotic Yeasts and Bacteria Isolated From Dairy Products of Iran. Adv Pharm Bull 2020;10(3):482–7. doi:10.34172/apb.2020.060
26. El-kest MM, El-Hariri M, Khafaga N, Refai MK. Studies on contamination of dairy products by aflatoxin M1 and its control by probiotics. J Glob Biosci 2015;4(1):1294-312.
27. Horváth E, Pusztahelyi T, Adácsi C, Tanyi E, Pócsi I. Optimization and Validation of ELISA for Aflatoxin B1 Detection in Fermented Forages and Feeds. Scientifica (Cairo) 2022;2022:6059880. doi:10.1155/2022/6059880
28. Ozma MA, Moaddab SR, Asgharzadeh M, Kamounah FS, Abbasi A, Alileh NF, et al. Proteomic Alterations and Antimicrobial Effects of Postbiotics from Probiotic Lactic Acid Bacteria and Yeast Against Multidrug-Resistant Salmonella Typhimurium. Curr Microbiol 2025;83(2):98. doi:10.1007/s00284-025-04690-4
29. Mohammadpour D, Asadollahi A, Ozma MA, Mehramuz B, Ganbarov K, Kafil HS. Application of Probiotics and Postbiotics in Neurological Disorders. Curr Pharm Biotechnol 2025. doi:10.2174/0113892010336767250128072101
30. Nezhadi J, Rezaee MA, Ozma MA, Ganbarov K, Kafil HS. Gut Microbiota Exchange in Domestic Animals and Rural-urban People Axis. Curr Pharm Biotechnol 2024;25(7):825–37. doi:10.2174/0113892010261535230920062107
31. Lalezadeh A, Fadaee M, Saedi S, Nezhadi J, Ozma MA, Ahmadi S, et al. A Critical Review on the Potential of Inactivated Bacteria in Counteracting Human Pathogens. Curr Microbiol 2025;82(7):295. doi:10.1007/s00284-025-04282-2
32. Saedi S, Nezhadi J, Soleimani RA, et al. Effects of Postbiotics Derived from Lactobacillus plantarum and Bifidobacterium bifidum on Biofilm Formation and Virulence Gene Expression of Enterococcus faecalis. Jundishapur Journal of Microbiology 2025;18(10):e1656942025. doi:10.5812/jjm-165694
33. Abbasi A, Pourjafar H, Ozma MA, Taghizadeh M, Hosseini H. Enhancing raw trout fillet quality with Lactiplantibacillus plantarum postbiotics and potassium sorbate. Lett Appl Microbiol 2025;78(5):ovaf054. doi:10.1093/lambio/ovaf054
34. Pożarska A, Karpiesiuk K, Kozera W, Czarnik U, Dąbrowski M, Zielonka Ł. AFB1 Toxicity in Human Food and Animal Feed Consumption: A Review of Experimental Treatments and Preventive Measures. Int J Mol Sci 2024;25(10):5305. doi:10.3390/ijms25105305
35. Guimarães A, Santiago A, Teixeira JA, Venâncio A, Abrunhosa L. Anti-aflatoxigenic effect of organic acids produced by Lactobacillus plantarum. Int J Food Microbiol 2018;264:31–8. doi:10.1016/j.ijfoodmicro.2017.10.025
36. Mishra B, Mishra AK, Kumar S, Mandal SK, Nsv L, Kumar V, et al. Antifungal Metabolites as Food Bio-Preservative: Innovation, Outlook, and Challenges. Metabolites 2021;12(1):12. doi:10.3390/metabo12010012
37. Guimarães A, Venâncio A. The Potential of Fatty Acids and Their Derivatives as Antifungal Agents: A Review. Toxins (Basel) 2022;14(3):188. doi:10.3390/toxins14030188
38. Ahlberg SH, Joutsjoki V, Korhonen HJ. Potential of lactic acid bacteria in aflatoxin risk mitigation. Int J Food Microbiol 2015;207:87–102. doi:10.1016/j.ijfoodmicro.2015.04.042
39. Rämö S, Kahala M, Joutsjoki V. Aflatoxin B1 Binding by Lactic Acid Bacteria in Protein-Rich Plant Material Fermentation. Applied Sciences 2022;12(24):12769. doi:10.3390/app122412769
40. Sobral MMC, Gonçalves T, Martins ZE, Bäuerl C, Cortés-Macías E, Collado MC, et al. Mycotoxin Interactions along the Gastrointestinal Tract: In Vitro Semi-Dynamic Digestion and Static Colonic Fermentation of a Contaminated Meal. Toxins (Basel) 2022;14(1):28. doi:10.3390/toxins14010028
41. Zamanpour S, Noori SMA, Yancheshmeh BS, Afshari A,

Hashemi M. A Systematic Review to Introduce the Most Effective Postbiotic Derived from Probiotics for Aflatoxin Detoxifications. Preprint. Posted online February 23, 2023. Research Square PPR620778. doi:10.21203/rs.3.rs-2596093/v1

42. Blazheva D, Mihaylova D, Averina OV, Slavchev A, Brazkova M, Poluektova EU, et al. Antioxidant Potential of Probiotics and Postbiotics: A Biotechnological Approach to Improving Their Stability. Russian Journal of Genetics 2022;58(9):1036–50. doi:10.1134/S1022795422090058
43. Huang L, Duan C, Zhao Y, Gao L, Niu C, Xu J, et al. Reduction of Aflatoxin B1 Toxicity by Lactobacillus plantarum C88: A Potential Probiotic Strain Isolated from Chinese Traditional Fermented Food "Tofu". PLoS One 2017;12(1):e0170109. doi:10.1371/journal.pone.0170109
44. Liang B, Xing D. The Current and Future Perspectives of Postbiotics. Probiotics Antimicrob Proteins 2023;15(6):1626–43. doi:10.1007/s12602-023-10045-x
45. Isaac-Bamgboye FJ, Mgbechidinma CL, Onyeaka H, Isaac-Bamgboye IT, Chukwugozie DC. Exploring the Potential of Postbiotics for Food Safety and Human Health Improvement. J Nutr Metab 2024;2024:1868161. doi:10.1155/2024/1868161
46. Chaudhary A, Prajapati N, Prajapati A, Singh S, Joshi M, Prajapati D, et al. Postbiotic emissaries: a comprehensive review on the bioprospecting and production of bioactive compounds by Enterococcus species. International Journal of Food Science and Technology 2024;59(10):6769–82. doi:10.1111/ijfs.17431
47. Akman PK, Kutlu G, Tornuk F. Development and characterization of a novel sodium alginate based active film supplemented with Lactiplantibacillus plantarum postbiotic. Int J Biol Macromol 2023;244:125240. doi:10.1016/j.ijbiomac.2023.125240
48. Ramazanidoroh F, Hosseininezhad M, Shahrampour D, Wu X. Edible Packaging as a Functional Carrier of Prebiotics, Probiotics, and Postbiotics to Boost Food Safety, Quality, and Shelf Life. Probiotics Antimicrob Proteins 2024;16(4):1327–47. doi:10.1007/s12602-023-10110-5