INSIGHTS-JOURNAL OF
HEALTH AND REHABILITATION

INSIGHTS-JHR
INSIGHTS-JOURNAL OF HEALTH AND REHABILITATION

INVITRO EVALUATION OF LACTOBACILLUS ACIDOPHILUS FOR ANTIFUNGAL ACTIVITY AND ITS IMPACT ON MAIZE NUTRITIONAL QUALITY

Original Research

Hafiz Muhammad Imran*[1], Mubbra Azam*[1], Anzalna Iqbal[2], Javeria Zahid[3], Mirza Muhammad Maroof Baig[4], Rameen Fatima[2], Tanzeela Iftikhar[5], Syed Faseeh ul Hassan Kazmi[6], Muhammad Ahmad Afzal[7], Nahl Jameel[5], Anam Tariq[2]

[1]Department of Animal Nutrition, Faculty of Veterinary and Animal Sciences, The Islamia University of Bahawalpur, Pakistan.
[2]Department of Biochemistry, Government College University Faisalabad, Faisalabad, Pakistan.
[3]Department of Zoology, The Islamia University of Bahawalpur, Pakistan.
[4]School of Biochemistry and Biotechnology, University of Punjab, Lahore, Pakistan.
[5]Institute of Microbiology, Government College University Faisalabad, Faisalabad, Pakistan.
[6]Department of Biotechnology, Kohsar University Murree, Pakistan.
[7]Department of Human Nutrition and Dietetics, Faculty of Medical Sciences, Government College University Faisalabad, Faisalabad, Pakistan.

Corresponding Author: Hafiz Muhammad Imran, Department of Animal Nutrition, Faculty of Veterinary and Animal Sciences, The Islamia University of Bahawalpur, Pakistan, imran07253@gmail.com

Mubbra Azam, Department of Animal Nutrition, Faculty of Veterinary and Animal Sciences, The Islamia University of Bahawalpur, Pakistan, mubarraazam173@gmail.com

Acknowledgement: The authors acknowledge the laboratory support and facilities provided by the respective host institutions.

Conflict of Interest: None

Grant Support & Financial Support: None

ABSTRACT

Background: Fungal contamination of food commodities is a persistent global concern, leading to spoilage, discoloration, nutrient degradation, and the production of toxic metabolites that pose serious risks to animal and human health. Among phytopathogenic fungi, *Aspergillus flavus* is particularly problematic due to its ability to colonize cereal grains and compromise both food safety and nutritional quality. In recent years, lactic acid bacteria have gained attention as eco-friendly alternatives to chemical preservatives because of their antimicrobial properties and their role in improving food quality and stability.

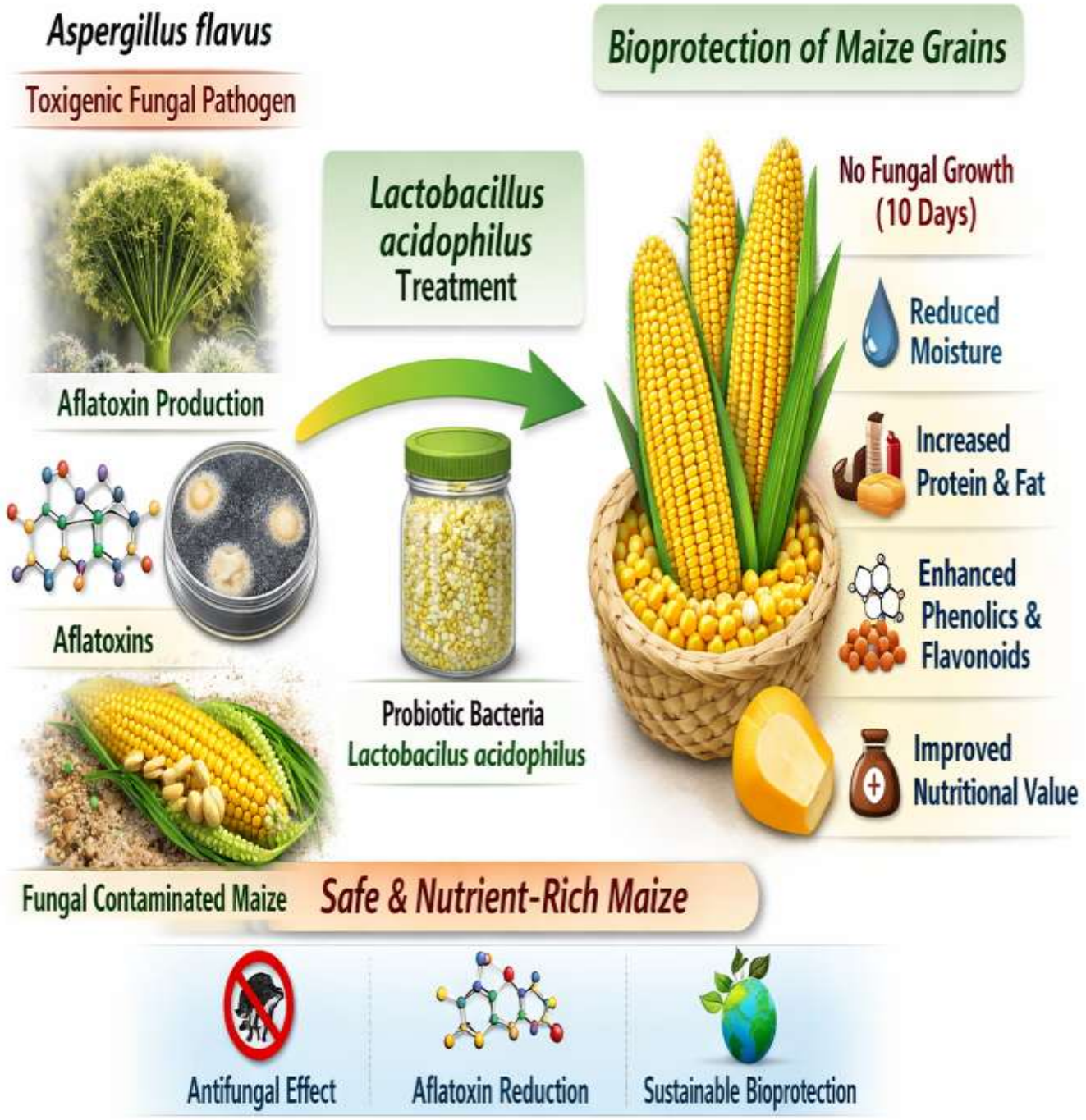
Objective: This study aimed to evaluate the antifungal and bioprotective potential of *Lactobacillus acidophilus* against *Aspergillus flavus* and to assess its impact on the nutritional and antioxidant profile of maize (*Zea mays* L.) grains.

Methods: The antifungal activity of *L. acidophilus* against *A. flavus* was assessed using an agar well diffusion assay. A bioprotection experiment was performed in which maize grains were treated with *L. acidophilus* culture and subsequently inoculated with *A. flavus*, followed by a 10-day observation period. Proximate analysis of maize grains was conducted to determine moisture, protein, fat, fiber, and ash contents. Total phenolic and flavonoid contents were quantified using spectrophotometric methods. All experiments were performed in triplicate and statistically analyzed.

Results: *Lactobacillus acidophilus* produced a clear inhibition zone of 30.54 ± 0.32 mm against *A. flavus*, while no inhibition was observed in the control. In the bioprotection assay, complete suppression of fungal growth was observed on treated maize grains after 10 days, whereas control grains showed visible fungal colonization. Proximate analysis revealed reduced moisture content (20.44 ± 0.45%) and increased protein (8.54 ± 0.87%), fat (2.54 ± 0.65%), fiber (16.87 ± 0.32%), and ash (12.54 ± 0.55%) contents in treated grains compared with controls. Total phenolic content increased to 100.65 mg GAE/g and flavonoid content to 93.44 mg quercetin equivalents/g following bacterial treatment.

Conclusion: The findings demonstrated that *Lactobacillus acidophilus* is an effective bioprotective agent against *Aspergillus flavus*, while simultaneously enhancing the nutritional and antioxidant quality of maize grains. This study highlights the promising application of probiotic-based biotechnology as a safe and sustainable strategy for improving cereal grain safety and value.

Keywords: Antifungal Agents, Aspergillus flavus, Lactic Acid Bacteria, Maize, Phenols, Probiotics, Zea mays.



INSIGHTS-JHR
INSIGHTS-JOURNAL OF HEALTH AND REHABILITATION

INTRODUCTION

Aspergillus is a filamentous fungus of major concern in food safety due to its ability to produce mycotoxins—secondary metabolites that frequently contaminate food and feed commodities and pose serious health risks to both animals and humans (1). Chronic exposure to these mycotoxins through contaminated diets has been associated with mutagenic, hepatotoxic, and immunosuppressive effects, contributing to a substantial global disease burden (2). Among the diverse species within this genus, *Aspergillus flavus*, *Aspergillus nomius*, and *Aspergillus parasiticus* are recognized as the principal producers of aflatoxins, with *A. flavus* considered the most aggressive and economically damaging due to its widespread distribution and high toxigenic potential (3,4). This species commonly contaminates major agricultural commodities, including maize, peanuts, and cottonseed, with aflatoxins B1 and B2 being the most prevalent and toxic forms encountered in the agricultural and food sectors (5). The persistence of these toxins not only threatens public health but also results in severe economic losses through reduced crop quality, trade restrictions, and food insecurity, particularly in low- and middle-income regions (6). Maize is one of the world's most important cereal crops, serving as a staple food for humans, a primary component of animal feed, and a key raw material for various industrial applications (7). Despite its central role in global food systems, maize production is highly vulnerable to fungal pathogens, with *A. flavus* representing a major challenge due to its capacity to reduce grain quality, compromise nutritional value, and significantly lower yield (8). Conventional control strategies rely heavily on synthetic preservatives and antifungal agents such as sorbates, nitrates, and benzoates; however, growing evidence indicates that prolonged exposure to these chemicals may pose adverse health effects, leading to regulatory restrictions and outright bans in several countries (9,10). This has intensified the demand for safer, sustainable, and cost-effective alternatives that can effectively control fungal contamination without compromising human health or environmental safety.

In this context, biological control strategies using beneficial microorganisms have gained increasing attention. Microorganisms with probiotic attributes offer a promising, eco-friendly approach to protecting cereal crops from fungal spoilage while potentially enhancing nutritional quality (11). Lactic acid bacteria (LAB), including *Lactobacillus plantarum* and *Lactobacillus fermentum*, are widely recognized as safe and have a long history of use in food fermentation and preservation (12). These bacteria act as natural preservatives and represent a cost-effective alternative to chemical fungicides. Their antifungal activity is largely attributed to the production of bioactive metabolites such as organic acids, hydrogen peroxide, fatty acids, and phenolic compounds, many of which inhibit fungal growth and mycotoxin production (13). Despite growing interest in LAB-mediated biocontrol, the antifungal potential of certain species, particularly *Lactobacillus acidophilus*, against *A. flavus* and its role in protecting maize grains remain insufficiently explored. Given this background, the present study is designed to address this gap by evaluating the in vitro antifungal activity of *Lactobacillus acidophilus* against *Aspergillus flavus* and assessing its potential to protect maize grains from fungal contamination while improving their nutritional quality. The objective of this work is to rationally investigate whether *L. acidophilus* can serve as a safe, effective, and sustainable biological alternative to chemical preservatives for controlling *A. flavus* in maize-based food systems.

METHODS

This experimental laboratory-based study was designed to evaluate the antifungal potential of *Lactobacillus acidophilus* against *Aspergillus flavus* and to assess its bioprotective effect on maize grains, along with associated changes in nutritional quality. All experiments were conducted under controlled in vitro conditions. Broth and agar-based de Man, Rogosa and Sharpe (MRS) media, potato dextrose agar and broth, Folin–Ciocalteu reagent, and ethanol were procured from Sigma-Aldrich. Maize seeds used in the study were purchased from the local grain market of Faisalabad, Pakistan, and visually inspected to exclude visibly damaged or mold-contaminated grains prior to experimentation. The bacterial strain *Lactobacillus acidophilus* was obtained from the Institute of Microbiology, Government College University Faisalabad, Pakistan. The culture was routinely propagated in MRS broth at 35 °C for 24 h and preserved at 4 °C for short-term storage. The fungal strain *Aspergillus flavus* was acquired from the Fungal Culture Bank of the University of the Punjab, Pakistan. This strain was maintained on Vogel's medium and stored at 4 °C until further use. All microbial handling procedures were performed under aseptic conditions to minimize contamination and ensure experimental reliability. The in vitro antifungal activity of *L. acidophilus* against *A. flavus* was evaluated using the agar well diffusion assay. Briefly, freshly prepared *A. flavus* spore suspension was uniformly spread over potato dextrose agar plates. Wells of 7 mm diameter were aseptically punched into the agar, and 10 µL of actively growing *L. acidophilus* culture was dispensed into each well. MRS broth without bacterial inoculum served as the negative control. The plates were incubated at 30 °C for 72 h, after which zones of inhibition around the wells were measured to assess antifungal activity. For the bioprotection assay, *A. flavus* was cultivated in Vogel's broth at 30 °C for five days, and

INSIGHTS-JHR
INSIGHTS-JOURNAL OF HEALTH AND REHABILITATION

the spore concentration was adjusted to 1×10^8 spores/mL using standard hemocytometer counting techniques. Five grams of maize grains were soaked in 10 mL of *L. acidophilus* culture for 10 h to allow bacterial adherence and colonization. Treated grains were then transferred to sterile Petri dishes. Control grains were prepared similarly using sterile MRS broth instead of bacterial culture. Subsequently, 3 mL of *A. flavus* spore suspension was evenly distributed over both treated and control maize grains. The samples were incubated and visually monitored for fungal growth over a period of 10 days, following previously reported protocols (14).

To evaluate the impact of bacterial treatment on grain quality, proximate analysis of maize samples was performed in accordance with the standardized methods of the Association of Official Analytical Chemists, including determination of moisture, ash, crude protein, crude fat, and carbohydrate content (13). All analyses were conducted in triplicate to ensure accuracy and reproducibility. For the estimation of total phenolic and flavonoid contents, three grams of maize grains were finely ground and extracted with 20 mL of methanol. The mixture was centrifuged, and the resulting supernatant was used for further analysis. Total phenolic content was determined by adding 1 mL of Folin–Ciocalteu reagent to 0.5 mL of extract, followed by the addition of sodium carbonate. Ethanol served as the blank. The reaction mixture was incubated at room temperature for 1 h, and absorbance was recorded at 765 nm using a spectrophotometer (7). Flavonoid content was assessed by mixing 0.5 mL of extract with sodium nitrate, aluminum chloride, and 0.5 M sodium hydroxide, and absorbance was measured at 510 nm (11). Statistical analysis was performed using Minitab software. Results were expressed as mean ± standard error based on three independent replicates. One-way analysis of variance followed by Tukey's post hoc test was applied to determine statistically significant differences among treatments, with a p-value of <0.05 considered significant. As this study involved microbial strains and agricultural materials only, with no use of human or animal subjects, formal institutional review board approval and informed consent were not required. However, all experimental procedures adhered to standard laboratory biosafety and ethical research practices.

RESULTS

The antifungal activity of *Lactobacillus acidophilus* against *Aspergillus flavus* was clearly demonstrated under in vitro conditions. The agar well diffusion assay revealed a pronounced inhibitory effect, with *L. acidophilus* producing a mean zone of inhibition of 30.54 ± 0.32 mm against *A. flavus*. In contrast, the negative control containing only MRS broth showed no measurable inhibition, confirming that the observed antifungal effect was attributable to the bacterial activity rather than the growth medium. The bioprotective efficacy of *L. acidophilus* was further evident during the maize grain protection assay. After 10 days of incubation, maize grains treated with MRS broth alone exhibited visible green sporulation characteristic of *A. flavus*, indicating active fungal colonization. In comparison, maize grains pretreated with *L. acidophilus* showed complete suppression of fungal growth throughout the observation period, demonstrating a strong protective effect of the bacterium against phytopathogenic contamination. Marked differences were observed in the nutritional composition of maize grains following bacterial treatment. Moisture content was reduced in *L. acidophilus*-treated grains (20.44 ± 0.45%) compared with MRS-treated grains (25.00 ± 0.55%). Conversely, protein content increased from 5.23 ± 0.76% in control grains to 8.54 ± 0.87% in treated grains. Fat content showed a notable rise from 0.54 ± 0.43% to 2.54 ± 0.65%, while fiber content increased from 14.55 ± 0.22% to 16.87 ± 0.32%. Ash content also showed a substantial enhancement, rising from 8.65 ± 0.44% in control grains to 12.54 ± 0.55% following *L. acidophilus* treatment, indicating improved mineral availability.

Analysis of bioactive compounds further supported the beneficial impact of bacterial treatment. Total phenolic content was higher in *L. acidophilus*-treated maize grains, reaching 100.65 mg gallic acid equivalents per gram, compared with 80.54 mg/g in MRS-treated grains. Similarly, flavonoid content increased markedly from 65.32 mg quercetin equivalents per gram in control grains to 93.44 mg/g in treated grains, reflecting a significant enhancement of antioxidant-related components. The biochemical and nutritional shifts observed in maize grains following *Lactobacillus acidophilus* treatment indicated a marked reduction in conditions that favor aflatoxin biosynthesis by *Aspergillus flavus*. Treated grains exhibited significantly lower moisture content alongside substantial increases in protein, fat, fiber, and ash contents compared with MRS-treated grains. Reduced moisture is a critical determinant in limiting fungal metabolic activity and mycotoxin production, while enhanced nutritional density and mineral content reflect improved grain integrity and reduced fungal degradation. The absence of visible fungal colonization in treated grains, coupled with elevated levels of phenolic and flavonoid compounds reported earlier, further supports suppression of toxigenic fungal metabolism. Collectively, these compositional changes strongly suggest a biologically mediated reduction in aflatoxin-producing potential in *L. acidophilus*-treated maize grains, aligning with the observed antifungal and bioprotective effects and reinforcing the role of this probiotic bacterium in mitigating *A. flavus*-associated toxigenic risk under the studied conditions.

Table 1: Proximate Analysis of MRS and *L acidophilus* Maize Grains

Parameters (%)	MRS treated grains	*L acidophilus* treated grains
Moisture	25±0.55[a]	20.44±0.45[b]
Protein	5.23±0.76[b]	8.54±0.87[a]
Fat	0.54±0.43[b]	2.54±0.65[a]
Fiber	14.55±0.22[b]	16.87±0.32[a]
Ash	8.65±0.44[b]	12.54±0.55[a]

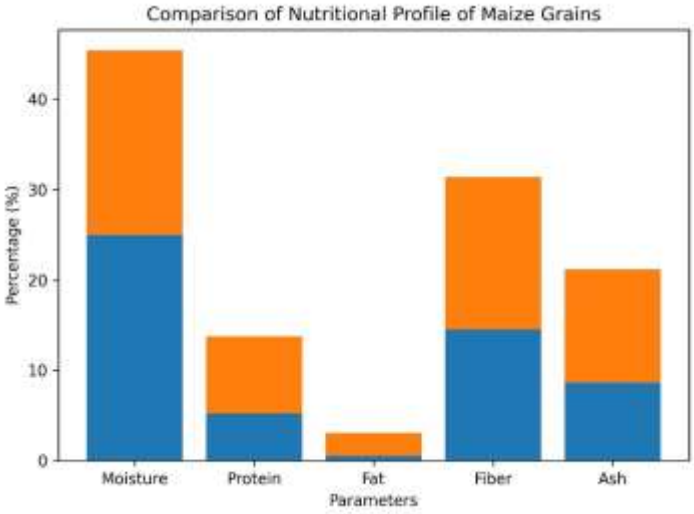


Figure 2 Comparison of Nutritional Profile of maize Grains

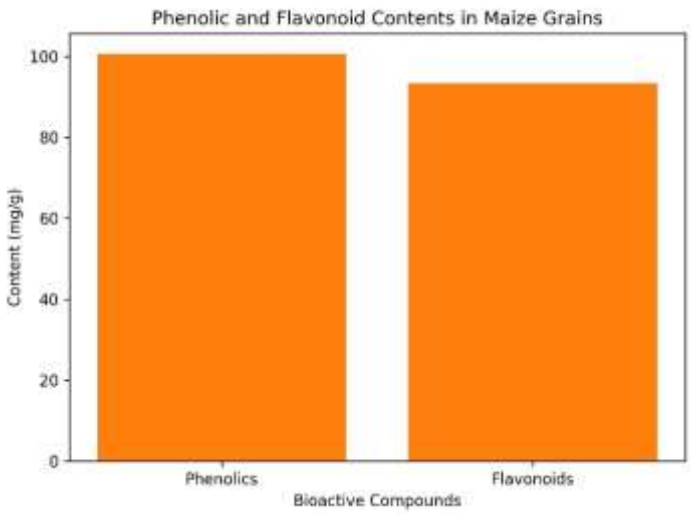


Figure 2 Phenolic and Flavonoid Contents in Maize Grains

DISCUSSION

Fungal infections in crop plants remain a major challenge for global food security, and their control has traditionally relied on synthetic chemical preservatives and fungicides. Although these agents are effective, increasing evidence of their adverse health and environmental effects has resulted in regulatory restrictions and bans in several regions, driving the search for safer and more sustainable alternatives (15). In this context, biological control strategies using beneficial microorganisms have emerged as a promising approach, particularly for postharvest protection of food commodities (16). The present findings demonstrated that *Lactobacillus acidophilus* exerted a strong inhibitory effect against *Aspergillus flavus*, supporting the growing body of evidence that lactic acid bacteria possess broad-spectrum antifungal activity. Comparable antifungal effects have previously been observed with other *Lactobacillus* species, reinforcing the concept that this functional trait is conserved across several members of the genus (17). The observed bioprotective effect of *L. acidophilus* on maize grains infected with *A. flavus* further substantiated its potential application as a natural preservative. The complete suppression of fungal growth in treated grains suggested that the bacterium created an unfavorable microenvironment for fungal proliferation. This protective effect is consistent with earlier reports in which lactic acid bacteria limited phytopathogenic fungi through the secretion of multiple antifungal metabolites, including organic acids, fatty acids, phenolic compounds, and proteinaceous substances (18). At the mechanistic level, these metabolites are known to disrupt fungal physiology by altering proton gradients, inducing oxidative stress through reactive oxygen species, inhibiting key metabolic enzymes, and causing membrane depolarization, ultimately leading to growth inhibition or cell death (19). The convergence of these mechanisms likely explains the robust antifungal performance observed in the present work. Beyond fungal inhibition, a notable strength of this study was the demonstration that *L. acidophilus* treatment improved the nutritional quality and antioxidant profile of maize grains. Increases in protein, fat, fiber, ash, phenolic, and flavonoid contents indicated not only protection from fungal degradation but also a potential enhancement of grain functional value. Similar nutritional improvements have been reported in maize and other cereals treated with probiotic bacteria, where bioactive

INSIGHTS-JHR
INSIGHTS-JOURNAL OF HEALTH AND REHABILITATION

compounds produced during bacterial metabolism contributed to nutrient enrichment and antioxidant preservation (20). Previous bioprotection studies using related lactic acid bacterial species against *A. flavus* in maize have also documented comparable increases in phenolic and flavonoid contents, supporting the reproducibility of these effects across different probiotic strains (21).

The preservation of phenolic and flavonoid compounds observed in treated grains is particularly relevant, as fungal contamination is known to accelerate oxidative degradation of these metabolites. Phytopathogenic fungi secrete polyphenol oxidase enzymes that catalyze the conversion of phenolic compounds into quinones, a process that not only diminishes antioxidant capacity but also compromises overall food quality and nutritional value (22). This enzymatic oxidation has been shown to negatively affect flavonoids and other bioactive components, leading to measurable nutritional losses in contaminated food products (23). The suppression of fungal growth by *L. acidophilus* therefore likely limited polyphenol oxidase activity, indirectly preserving antioxidant compounds and contributing to the improved nutritional profile recorded in this study. Despite these encouraging findings, certain limitations should be acknowledged. The experimental design was confined to controlled laboratory conditions, which may not fully capture the complexity of field or storage environments where multiple biotic and abiotic factors influence fungal growth and mycotoxin production. Additionally, while strong antifungal and bioprotective effects were demonstrated, future studies could benefit from integrating advanced analytical techniques to further elucidate the dynamics of fungal metabolism and grain quality during prolonged storage. Expanding the investigation to include different maize varieties and storage conditions would also enhance the generalizability of the results. Overall, the findings provided compelling evidence that *Lactobacillus acidophilus* functioned as an effective bioprotective agent against *Aspergillus flavus*, while simultaneously enhancing the nutritional and antioxidant attributes of maize grains. These outcomes underscored the potential of probiotic-based strategies as safe, sustainable alternatives to chemical fungicides in cereal crop protection and highlighted a promising direction for future research and application in food safety and agricultural biotechnology.

CONCLUSION

This study demonstrated that *Lactobacillus acidophilus* possesses strong bioprotective capacity against *Aspergillus flavus* and effectively safeguarded maize grains from phytopathogenic fungal contamination. In addition to suppressing fungal growth, treatment with this beneficial bacterium contributed to meaningful improvements in the nutritional quality of maize and promoted the preservation of antioxidant-related bioactive compounds, including phenolics and flavonoids. These findings underscore the dual functional role of *L. acidophilus* as both a natural antifungal agent and a nutritional enhancer. Collectively, the results highlight its practical value as a sustainable, biotechnology-based alternative to chemical preservatives for improving maize grain safety, quality, and overall functional potential.

AUTHOR CONTRIBUTIONS

Author	Contribution
Hafiz Muhammad Imran*	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Mubbra Azam*	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Anzalna Iqbal	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Javeria Zahid	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published

Author	Contribution
Mirza Muhammad Maroof Baig	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Rameen Fatima	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published
Tanzeela Iftikhar	Contributed to study concept and Data collection Has given Final Approval of the version to be published
Syed Faseeh ul Hassan Kazmi	Writing - Review & Editing, Assistance with Data Curation
Muhammad Ahmad Afzal	Writing - Review & Editing, Assistance with Data Curation
Nahl Jameel	Writing - Review & Editing, Assistance with Data Curation
Anam Tariq	Writing - Review & Editing, Assistance with Data Curation

REFERENCES

1. Ahirwar, N. K., et al. (2020). Effective role of beneficial microbes in achieving the sustainable agriculture and eco-friendly environment development goals: a review. Front. Microbiol, 5: 111-123.

2. Ayaz, M., et al. (2023). Bacterial and fungal biocontrol agents for plant disease protection: Journey from lab to field, current status, challenges, and global perspectives. Molecules, 28(18): 6735.

3. El-Baky, N. A., & Amara, A. A. A. F. (2021). Recent approaches towards control of fungal diseases in plants: An updated review. Journal of Fungi, 7(11): 900.

4. Ito, S., et al. (2020). Chemical reactivities of ortho-quinones produced in living organisms: Fate of quinonoid products formed by tyrosinase and phenoloxidase action on phenols and catechols. International journal of molecular sciences, 21(17): 6080.

5. Jurczynski, Y., et al. (2024). A review of the most concerning chemical contaminants in drinking water for human health. Sustainability, 16(16): 7107.

6. Kraft, S., et al. (2021). Mold, mycotoxins and a dysregulated immune system: a combination of concern? International journal of molecular sciences, 22(22): 12269.

7. Kumar, A., et al. (2021). Aflatoxin contamination in food crops: causes, detection, and management: a review. Food Production, Processing and Nutrition, 3(1): 17.

8. Li, D., et al. (2025). Maize kernel nutritional quality—an old challenge for modern breeders. Planta, 261(2): 43.

9. Ma, M., et al. (2024). Antifungal mechanism of Lactiplantibacillus plantarum P10 against Aspergillus niger and its in-situ biopreservative application in Chinese steamed bread. Food Chemistry, 449: 139181.

10. Mahmoud, L. S. (2025). A summary of secondary metabolites and nanoparticles generated by filamentous fungi and their application in medicine. Bulletin of Faculty of Science, Zagazig University, 2024(4): 19-36.

11. Mahmoud, Y. A.-G., et al. (2025). Comprehensive review for aflatoxin detoxification with special attention to cold plasma treatment. Mycotoxin Research, 41(2): 277-300.

12. Meng, F.-B., et al. (2022). Effect of Lactobacillus plantarum and Lactobacillus acidophilus fermentation on antioxidant activity and metabolomic profiles of loquat juice. Lwt, 171: 114104.

13. Mesterhazy, A. (2024). Food safety aspects of breeding maize to multi-resistance against the major (Fusarium graminearum, F. verticillioides, Aspergillus flavus) and minor toxigenic fungi (Fusarium spp.) as well as to toxin accumulation, trends, and solutions—A review. Journal of Fungi, 10(1): 40.

14. Nleya, N., et al. (2021). Biodiversity of aflatoxigenic Aspergillus species in dairy feeds in Bulawayo, Zimbabwe. Frontiers in microbiology, 11: 599605.

15. Pintér, M., et al. (2025). Synthetic Food Preservatives Modulate Apoptotic Gene Expression in HepG2 Cells: Divergent Effects of Sodium Benzoate, Potassium Sorbate, and Sodium Metabisulfite. Nutrients, 17(21): 3466.

16. Salman, M., et al. (2022). Bioprotection of Zea mays L. from aflatoxigenic Aspergillus flavus by Loigolactobacillus coryniformis BCH-4. PloS one, 17(8): e0271269.

17. Salman, M., et al. (2020). In vitro antimicrobial and antioxidant activities of Lactobacillus coryniformis BCH-4 bioactive compounds and determination of their bioprotective effects on nutritional components of Maize (Zea mays L.). Molecules, 25(20): 4685.

18. Tariq, A., et al. (2025). Unveiling the potential of Loigolactobacillus coryniformis BCH-4 to ameliorate drought stress in Zea mays L. World Journal of Microbiology and Biotechnology, 41(2): 49.

19. Vajnai, Z., et al. (2025). Utilization of Aflatoxin-B1-Contaminated Corn by Yellow Mealworm Larvae for Common Carp Feed and Assessing Residual Frass Toxicity by Zebrafish Embryo Microinjection. International journal of molecular sciences, 26(20): 9851.

20. Wu L, Zhou J, Zhou A, Lei Y, Tang L, Hu S, et al. Lactobacillus acidophilus ameliorates cholestatic liver injury through inhibiting bile acid synthesis and promoting bile acid excretion. Gut Microbes. 2024;16(1):2390176.

21. Zhang J, Yang J, Luo J, Wu W, Luo H, Wei W, et al. Lactobacillus acidophilus potentiates oncolytic virotherapy through modulating gut microbiota homeostasis in hepatocellular carcinoma. Nat Commun. 2025;16(1):3315.

22. Lau HC, Zhang X, Ji F, Lin Y, Liang W, Li Q, et al. Lactobacillus acidophilus suppresses non-alcoholic fatty liver disease-associated hepatocellular carcinoma through producing valeric acid. EBioMedicine. 2024;100:104952.

23. Beghetti I, Panizza D, Lenzi J, Gori D, Martini S, Corvaglia L, et al. Probiotics for Preventing Necrotizing Enterocolitis in Preterm Infants: A Network Meta-Analysis. Nutrients. 2021;13(1).