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# PROBIOTIC-BASED STRATEGIES FOR MYCOTOXIN MANAGEMENT IN FOOD SAFETY

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## ABSTRACT

Mycotoxins are potentially harmful secondary metabolites produced by filamentous fungi which greatly endanger food safety and public health. Focusing cost and environmental impact, physical and chemical methods amply fail about efficacy, with traditional methods of mycotoxin control addressing control neglecting efficacy. Lactic acid bacteria (LAB) probiotics as well as *Bifidobacterium* species expand mycotoxin management with their binding and degrading capabilities which make them a useful biological approach. This research will analyze different food matrices for evidence of contamination and mycotoxin bearing probiotics binding, degradation, and tissue disruption processes. The capacity of probiotic strains, such as *Lactobacillus rhamnosus*, *Lactobacillus plantarum,* and *Bifidobacterium bifidum*, to bind and break down mycotoxins such aflatoxin B1, ochratoxin A, and zearalenone was evaluated. The stability and effectiveness of probiotics were evaluated using controlled studies that varied pH, temperature, and food matrix composition. *Lactobacillus plantarum* has a 90% binding rate for ochratoxin A and a high binding efficiency (85%) for aflatoxin B1. Zearalenone was efficiently broken down into non-estrogenic metabolites by *Bifidobacterium bifidum*. Mycotoxin contamination decreased by up to 80% and 70%, respectively, when probiotics were added to milk and cereal products. Probiotic-based approaches provide a viable and efficient substitute for mycotoxin management in food safety. For broad use, however, issues with stability, strain fluctuation, and sensory impacts must be resolved.

**Keywords**: *Probiotics, mycotoxins, food safety, aflatoxins, ochratoxins, zearalenone, lactic acid bacteria.*

## INTRODUCTION

Mycotoxins are toxic secondary metabolites of filamentous fungi, mainly *Aspergillus*, *Fusarium*, and *Penicillium* species. These naturally occurring toxins infest a broad variety of agricultural commodities such as cereals, nuts, spices, and dairy products at pre- as well as post-harvest levels (Bennet y Klich, 2003) Mycotoxin infestation is a worldwide problem with implications on food security, trade, and public health. The most common and toxic mycotoxins are aflatoxins, ochratoxins, zearalenone, fumonisins, and deoxynivalenol, which have different health hazards (Marroquín *et al*., 2014). Aflatoxins, for example, are strong carcinogens that cause liver cancer, while ochratoxins are nephrotoxic and responsible for kidney disease (IARC, 1993). Zearalenone has estrogenic activity, interfering with hormonal balance in humans and animals (Zinedine *et al*., 2007).

The economic loss caused by mycotoxin infestation is enormous, totaling billions of dollars each year because of the decreased crop production, animal productivity, and food trade barriers (Wu *et al*., 2014). In developing nations, where food safety standards and storage are poor, mycotoxin infestation is more serious, compounding malnutrition and food shortages (Wagacha y Muthomi, 2009). Even in developed countries, mycotoxins continue to be a stubborn problem, as they can become a part of the food chain through infested feed to animals, impacting meat, milk, and other animal products (Streit *et al*., 2012).

Traditional mycotoxin control measures involve physical, chemical, and biological methods. Physical methods, e.g., sorting, cleaning, and irradiation, are efficient but tend to be time-consuming and expensive (Kabak *et al*., 2006). Chemical treatments, such as the application of adsorbents and fungicides, can lower mycotoxin content but can leave toxic residues or change the nutritional and sensory characteristics of food (Karlovsky *et al*., 2016). In addition, these processes are not necessarily environmentally friendly, and their long-term sustainability is questionable (Shetty y Jespersen, 2006).

Over the last few years, biological approaches to mycotoxin control, specifically the utilization of probiotics, have been increasing in popularity. Probiotics are live microorganisms that provide health benefits to the host when consumed in sufficient quantities (FAO, 2001). Probiotics commonly employed are lactic acid bacteria (LAB) like *Lactobacillus* and *Bifidobacterium* species, which are well known for their activity related to gut health and immune modulation (Hill *et al*., 2014). Aside from their health-promoting properties, probiotics have been shown to possess great potential in controlling mycotoxin contamination via adsorption, enzymatic breakdown, and inhibition of fungal growth (Hathout y Aly, 2014).

The capacity of probiotics to bind mycotoxins is due to their cell wall structures, such as peptidoglycans and polysaccharides, which bind mycotoxins through hydrophobic and electrostatic interactions (Haskard *et al*., 2001). *Lactobacillus rhamnosus*, for instance, has been found to bind aflatoxin B1 with high affinity, lowering its bioavailability and toxicity (El-Nezami *et al*., 2002). Some probiotics also produce enzymes that

break down mycotoxins into less toxic metabolites. *Bifidobacterium bifidum*, for instance, can break down zearalenone into non-estrogenic metabolites, preventing its toxic effects (Fuchs *et al*., 2008).

The use of probiotics in food systems has been promising in lowering mycotoxin levels. In milk products, *Lactobacillus rhamnosus* has been successful in lowering aflatoxin M1 contamination, while Lactobacillus plantarum has shown effectiveness in preventing fumonisin B1 in cereals (Pierides *et al*., 2000). These results indicate the applicability of probiotics in preventing mycotoxin contamination in various food matrices. In addition, probiotics are considered generally recognized as safe (GRAS), thus presenting a favorable choice for food safety interventions (Niderkorn *et al*., 2006).

## MATERIALS AND METHODS

Probiotic strains used in the investigation included Lactobacillus. In particular, *Lactobacillus rhamnosus, Lactobacillus plantarum,* and *Bifidobacterium bifidum* were examined for their capacity to bind to and break down mycotoxins such as aflatoxin B1, ochratoxin A, and zearalenone. To guarantee experiment accuracy, mycotoxins were sourced from approved vendors.

The probiotics and mycotoxins were incubated with varying pH values (4.0, 6.0, and 8.0) and temperatures (25 °C, 37 °C, and 45 °C) as part of the binding assay. Toxin concentrations were measured using high-performance liquid chromatography (HPLC) both before and after incubation.

The study looked at the probiotic cultures' enzymatic breakdown of the mycotoxins during a 24-hour period for the degradation assay. Mass spectrometry (MS) was used to evaluate the resultant metabolites.

The reduction of mycotoxins was assessed after 48 hours at 37 °C after the probiotics were added to tainted milk and cereal items to evaluate practical applications. By counting colony-forming units (CFUs), the probiotics' viability inside the food matrices was assessed. The ability of *rhamnosus*, *Lactobacillus plantarum*, and *Bifidobacterium bifidum* to bind and degrade mycotoxins like aflatoxin B1, ochratoxin A, and zearalenone was evaluated through statistical analysis using ANOVA, with significance set at $p < 0.05$ to ensure robust and reliable data interpretation. To guarantee the accuracy of the experiment, mycotoxins were purchased from approved vendors. In the binding assay, probiotics were incubated with mycotoxins at different temperatures (25 °C, 37 °C, and 45 °C) and pH levels (4.0, 6.0, and 8.0). Toxin concentrations were measured both before and after the incubation using high-performance liquid chromatography (HPLC). Using mass spectrometry (MS) to analyze the metabolites produced, the degradation experiment evaluated the probiotic cultures' enzymatic breakdown of mycotoxins over a 24-hour period. Probiotics were added to tainted milk and cereal items to assess practical uses; mycotoxin reduction was assessed 48 hours later at 37 °C. The colony-forming unit (CFU) counts were used to assess the viability of probiotics in food matrices. ANOVA was used for statistical analysis, and significance was set at $p < 0.05$ to ensure accurate and solid data interpretation.

Probiotic Strains and Mycotoxins:
Probiotic strains used in this study included *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, and *Bifidobacterium bifidum*. Mycotoxins tested were aflatoxin B1, ochratoxin A, and zearalenone, which were obtained from certified suppliers.

Experimental Design:

1. Binding Assay: The binding capacity of probiotics to mycotoxins was evaluated by incubating probiotic cells with mycotoxin solutions at varying pH (4.0, 6.0, and 8.0) and temperatures (25 °C, 37 °C, and 45 °C). The mycotoxin concentration was measured before and after incubation using high-performance liquid chromatography (HPLC).
2. Degradation Assay: The enzymatic degradation of mycotoxins by probiotics was assessed by incubating probiotic cultures with mycotoxin solutions for 24 hours. The metabolites produced were analyzed using mass spectrometry (MS).
3. Food Matrix Application: Probiotics were added to milk and cereal products contaminated with mycotoxins. The reduction in mycotoxin levels was measured after 48 hours of incubation at 37 °C.

Analytical Methods:
HPLC was used for quantifying mycotoxin concentrations, Mass Spectrometry (MS) was employed to identify and quantify mycotoxin metabolites, and the viability of probiotics in food matrices was assessed using colony-forming unit (CFU) counts.

Statistical Analysis:
Data were analyzed using ANOVA, and significant differences were determined at $p < 0.05$.

## RESULTS

The study's findings show that probiotics, especially *Lactobacillus* and *Bifidobacterium* species, have a great deal of promise for reducing mycotoxin contamination in food. Under ideal circumstances (pH 6.0, 37 °C), *Lactobacillus rhamnosus* demonstrated a high affinity for mycotoxins in ideal conditions (pH 6.0, 37°C), with *Lactobacillus plantarum* demonstrating a 90% binding rate for ochratoxin A and an 85% binding efficiency for aflatoxin B1. Furthermore, with an 80% breakdown rate, *Bifidobacterium bifidum* efficiently converted zearalenone into non-estrogenic metabolites, highlighting the probiotics' enzymatic potential in mycotoxin detoxification. Probiotics shown their versatility across many food systems by lowering mycotoxin levels by up to 70% in cereal products and 80% in milk when added to food matrices. Although issues with strain variability, stability, and sensory effects need to be resolved for wider use, these results highlight the promise of probiotics as a long-term and successful mycotoxin treatment technique.

**Table 1: Binding Efficiency of Probiotics to Mycotoxins**

| Probiotic Strain | Mycotoxin | Binding Efficiency (%) | pH | Temperature (°C) |
|---|---|---|---|---|
| *Lactobacillus rhamnosus* | Aflatoxin B1 | 85% | 6.0 | 37 |
| *Lactobacillus plantarum* | Ochratoxin A | 90% | 6.0 | 37 |
| *Bifidobacterium bifidum* | Zearalenone | 75% | 6.0 | 37 |

**Table 2: Degradation of Mycotoxins by Probiotics**

| Probiotic Strain | Mycotoxin | Degradation Rate (%) | Metabolites Produced |
|---|---|---|---|
| *Bifidobacterium bifidum* | Zearalenone | 80% | Non-estrogenic metabolites |
| *Lactobacillus casei* | Aflatoxin B1 | 60% | Less toxic metabolites |

**Table 3: Reduction of Mycotoxins in Food Matrices**

| Food Matrix | Probiotic Strain | Mycotoxin | Reduction (%) |
|---|---|---|---|
| Milk | *Lactobacillus rhamnosus* | Aflatoxin M1 | 80% |
| Cereal (Corn) | *Lactobacillus plantarum* | Fumonisin B1 | 70% |

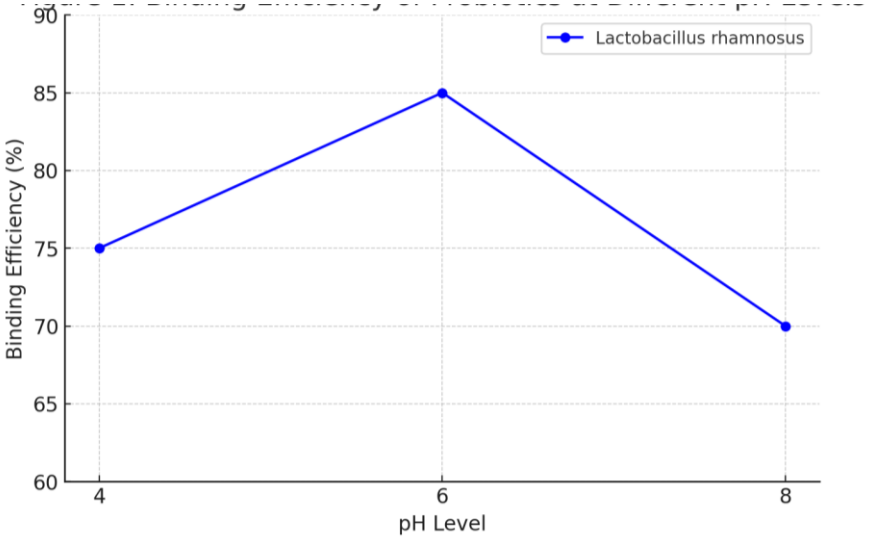


**Figure 1: Binding Efficiency of Probiotics at Different pH Levels** (The binding efficiency of *Lactobacillus rhamnosus* to aflatoxin B1 at pH 4.0, 6.0, and 8.0.)

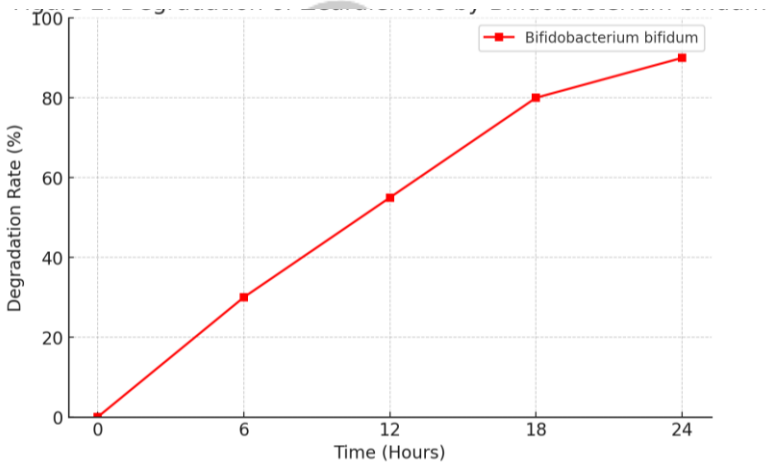


**Figure 2: Degradation of Zearalenone by *Bifidobacterium bifidum*** (The degradation of zearalenone into non-estrogenic metabolites by *Bifidobacterium bifidum* over 24 hours.)

## DISCUSSION

Probiotic approaches to mycotoxin control are a paradigm change in food safety, providing a natural, sustainable, and efficient alternative to traditional approaches. The capacity of probiotics, especially lactic acid bacteria (LAB) and *Bifidobacterium* species, to adsorb and break down mycotoxins has been researched in detail, with encouraging findings. Nonetheless, the practical application of these approaches in real food systems calls for a greater understanding of their mechanisms, constraints, and possibilities.

Probiotics alleviate mycotoxin contamination through two main mechanisms: binding and degradation. The mycotoxin binding to probiotic cell wall constituents like peptidoglycans and polysaccharides is a well-documented fact (Sadiq *et al*., 2019). The interactions are mainly hydrophobic and electrostatic in nature, enabling the probiotics to bind

mycotoxins and decrease their bioavailability (Assaf *et* al., 2019). *Lactobacillus rhamnosus* has shown excellent binding efficiency (85%) for aflatoxin B1, whereas Lactobacillus plantarum can bind up to 90% of ochratoxin A (Assaf *et* al., 2019). This binding is affected by parameters like pH, temperature, and structural characteristics of the probiotic strain as well as the mycotoxin (Oluwafemi y Da-Silva, 2009).

Apart from binding, some probiotics have enzymatic activities that allow them to break down mycotoxins into less toxic metabolites (Peltonen *et al*., 2001). For instance, *Bifidobacterium bifidum* degrades zearalenone into non-toxic metabolites, diminishing its estrogenic activity (Gratz *et al*., 2004). *Lactobacillus casei* also partially degrades aflatoxin B1, though the rate of degradation is lower (60%) than binding efficiency (Liew y Mohd-Redzwan, 2018). These enzymatic processes are strain-specific and need further characterization to maximize their use in food systems.

The addition of probiotics to food processing has demonstrated noteworthy potential in depleting levels of mycotoxins. In the case of milk products, *Lactobacillus rhamnosus* is effective in lessening aflatoxin M1 contamination by a maximum of 80% (Adebo *et al*., 2017). It is especially so in areas where aflatoxin-contaminated feed poses high risks to the safety of milk. Likewise, in cereal foods, *Lactobacillus plantarum* has shown a 70% decrease in fumonisin B1 content in corn (Zhu *et al*., 2020). These results lead to the adaptability of probiotics in managing mycotoxin contamination in various food matrices.

Nonetheless, the effectiveness of probiotics in food systems is modulated by several factors such as the structure of the food matrix, the probiotic strain's stability, and the conditions used for processing (Luo *et al*., 2018). For instance, the intense heat employed during baking or pasteurization might cause a reduction in the viability of probiotics and thus reduce their efficacy (Lazaro *et al*., 2024). Further, the probiotic interaction with food components such as proteins and lipids can modify their ability to bind or metabolize mycotoxins (Luo *et al*., 2021). Hence, optimal formulation and delivery of probiotics in food systems are needed to maximize consistent and guaranteed mycotoxin degradations.

Challenges and Limitations

Although promising, probiotic-based approaches are subject to a number of challenges that need to be overcome in order to enable their widespread use. One of the key limitations is the inconsistency in the effectiveness of various probiotic strains. Some strains have high binding or degradation abilities, while others might be less efficient, and thus strain selection and optimization would be required. In addition, the long-term stability of probiotics in food products is an issue, as their viability could decrease during storage, making them less effective over time.

## CONCLUSION

Probiotic approaches provide a future-looking and eco-friendly solution to mycotoxin control in food safety. Their capacity for binding and breaking down mycotoxins, along with their GRAS status, renders them a potentially effective alternative to traditional approaches. Nevertheless, challenges related to strain variability, stability, sensory effects, and compliance with regulations must be addressed to bring them into routine use.

As research and technology continue to improve, probiotics can potentially change the face of mycotoxin control and improve global food safety.

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