

Review Article

Harnessing Probiotics to Combat Aflatoxin: A Natural Approach to Food Safety: A Review

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Abstract

Aflatoxins, toxic secondary metabolites produced primarily by *Aspergillus flavus* and *Aspergillus parasiticus*, are among the most significant food safety challenges worldwide. These compounds are classified as Group 1 carcinogens by the International Agency for Research on Cancer (IARC) due to their association with liver cancer, immune suppression, and acute toxicity. Aflatoxin contamination commonly affects staple food items such as grains, nuts, and seeds, particularly in regions with warm and humid climates. The economic burden of aflatoxin contamination extends to reduced agricultural productivity and trade restrictions, necessitating innovative and effective mitigation strategies. This review investigates the potential of probiotics as a natural solution to counter aflatoxin contamination. Probiotics, including strains such as *Lactobacillus*, *Bifidobacterium*, and *Saccharomyces*, offer several mechanisms for reducing aflatoxin toxicity. These include bioadsorption, where probiotics bind aflatoxins and limit their bioavailability in the gastrointestinal tract; biotransformation, involving the enzymatic conversion of aflatoxins into less toxic metabolites; and competitive exclusion, which inhibits the growth of aflatoxin-producing molds. Additionally, probiotics contribute to immune modulation, enhancing the host's capacity to counteract aflatoxin exposure. Applications of probiotics in food systems and animal feed are promising. For instance, integrating probiotics into fermented foods has been shown to reduce aflatoxin concentrations significantly. Similarly, probiotics in livestock feed can decrease the absorption of aflatoxins, improving animal health and the safety of derived products. Despite these advances, challenges persist, including strain-specific efficacy, regulatory hurdles, and consumer acceptance of probiotic-enhanced food products. Future research should prioritize the development of innovative probiotic applications, such as bioencapsulation technologies to enhance stability and targeted delivery systems for maximizing their efficacy. Long-term clinical studies are needed to assess the sustainability and safety of probiotic interventions for aflatoxin detoxification. By addressing these challenges, probiotics could play a pivotal role in safeguarding public health, ensuring food safety, and enhancing the resilience of food systems globally.

Keywords

Aflatoxins, Probiotics, Food Safety, Mycotoxins, Food Processing, Health Risks, Fermentation

1. Introduction

Aflatoxins are toxic secondary metabolites produced by certain strains of molds, primarily *Aspergillus flavus* and *Aspergillus parasiticus*. These compounds are widely recog-

nized for their potent carcinogenic properties and are classified as Group 1 carcinogens by the International Agency for Research on Cancer (IARC) [62]. Aflatoxins can contaminate

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various agricultural products, including grains, nuts, and seeds, posing significant health risks to humans and livestock upon consumption [32]. The health impacts of aflatoxin exposure are severe, with potential outcomes ranging from acute toxicity to chronic diseases, including liver cancer and immune system suppression [23]. The economic burden associated with aflatoxin contamination extends beyond health implications, affecting agricultural productivity and international trade [34]. Effective strategies for mitigating aflatoxin contamination are essential for ensuring food safety and protecting public health.

Probiotics, defined as live microorganisms that confer health benefits to the host when administered in adequate amounts, present a promising natural solution to combat aflatoxin contamination [45]. Various strains of probiotics, including *Lactobacillus*, *Bifidobacterium*, and *Saccharomyces*, have demonstrated the ability to bind aflatoxins, transform them into less toxic forms, and enhance the host's immune response [4]. This review aims to explore the mechanisms by which probiotics can reduce aflatoxin levels and their potential applications in food safety.

2. Understanding Aflatoxins

2.1. Types of Aflatoxins

Aflatoxins are classified into several distinct types, each with varying levels of toxicity and occurrence:

Aflatoxin B1

Aflatoxin B1 is the most prevalent and toxic form of aflatoxin. It is commonly found in contaminated grains, nuts, and seeds. Due to its potent carcinogenic properties, it poses significant health risks to both humans and animals, leading to liver damage and increased cancer risk [1].

Aflatoxin B1 is the most toxic and prevalent form of aflatoxin, frequently found in contaminated grains, nuts, and seeds. Its potency as a carcinogen has led to its classification as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC) [28]. Chronic exposure to Aflatoxin B1 is associated with liver cancer and other serious health issues, including acute liver failure and immunosuppression [3]. Due to its widespread presence in agricultural products, Aflatoxin B1 poses a significant risk to public health and food safety.

Aflatoxin B2

Aflatoxin B2 is a less toxic variant compared to B1 but is still capable of causing harm. It is typically found alongside Aflatoxin B1 in contaminated food. While it is not as potent, Aflatoxin B2 can contribute to the overall toxicity in food products and is monitored for safety [6]. The presence of both B1 and B2 in food can complicate risk assessments and regulatory measure.

Aflatoxin G1 and G2

Aflatoxins G1 and G2 are primarily found in certain agricultural products, particularly in regions where specific molds

thrive. While they are not as commonly discussed as B1 and B2, they can still pose health risks and are monitored in food safety assessments [56]. Although they are less commonly discussed than Aflatoxins B1 and B2, they still pose health risks, particularly in animal feed. Aflatoxins G1 and G2 can lead to significant economic losses in agriculture due to their potential to contaminate food supplies and reduce marketability.

Aflatoxin M1

Aflatoxin M1 is a metabolite of Aflatoxin B1 and is commonly detected in the milk of lactating animals that have consumed contaminated feed. It poses a significant risk to consumers of dairy products, particularly in regions where dairy consumption is high [7]. detection of Aflatoxin M1 in dairy products is a critical concern for food safety authorities, as it can lead to the exposure of large populations to harmful levels of aflatoxins.

Understanding Mycotoxins and Their Impact

What are Mycotoxins?

Mycotoxins are toxic secondary metabolites produced by certain fungi, primarily found in agricultural products. These compounds are characterized by their low molecular weight and diverse chemical structures, which can range from simple heterocyclic rings to complex configurations with multiple-ring systems [61]. Mycotoxin production generally occurs when environmental conditions, such as temperature and humidity, favor fungal growth, impacting crops both before and after harvest as well as during processing and storage [13].

Aflatoxins: A Major Group of Mycotoxins

Overview

Aflatoxins are among the most studied mycotoxins, primarily produced by species of the *Aspergillus* genus, particularly *A. flavus* and *A. parasiticus*. The most significant aflatoxins include AFB1, AFB2, AFG1, and AFG2. Additionally, AFM1 and AFM2 are hydroxylated metabolites derived from AFB1 and AFB2, respectively, which can be found in dairy products derived from animals that consumed contaminated feed [16].

Health Risks

Aflatoxins pose serious health risks, particularly to the liver. Key health implications include:

Acute and Chronic Liver Disease: Prolonged exposure to aflatoxins can lead to severe liver damage and diseases such as cirrhosis and liver cancer [33].

Carcinogenic Effects: Aflatoxins are classified as Group 1 carcinogens by the International Agency for Research on Cancer (IARC), indicating sufficient evidence of their carcinogenicity in humans [7].

Immunosuppressive Effects: They can compromise both cellular and humoral immune responses, increasing susceptibility to infections [25].

Regulatory Standards for Aflatoxins

To protect public health, many countries have established regulations that specify maximum allowable levels of afla-

toxins in food products. The Codex Alimentarius provides recommendations for aflatoxin levels, typically ranging from 1 to 20 µg/kg, depending on the specific food type [10].

Methods for Detoxifying Mycotoxins

Various strategies are employed to mitigate the effects of mycotoxins, including:

Fungal Growth Prevention: Implementing good agricultural practices, such as crop rotation and proper storage conditions, to inhibit fungal development [8].

Detoxification Techniques: Utilizing chemical agents or biological methods, such as enzymatic treatments, to neutralize mycotoxins in contaminated food and feed [18].

Physical Treatments: Applying methods like heat treatment, ultraviolet light exposure, and ionizing radiation to reduce mycotoxin levels effectively [19].

Probiotics: A Promising Solution

Definition

Probiotics are live microorganisms that, when consumed in adequate amounts, confer health benefits to the host. The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) recognize probiotics for their positive effects on gut health and overall wellness [27].

How Probiotics Combat Mycotoxigenic Fungi

Probiotic bacteria can combat mycotoxigenic fungi through several mechanisms:

Competition for Resources: Probiotics compete with fungi for essential nutrients and physical space, thereby limiting fungal growth [29].

Production of Antifungal Compounds: They produce various metabolites that possess antifungal properties, inhibiting or killing pathogenic fungi [30].

Biofilm Formation: Probiotics can establish biofilms, which serve as protective barriers against fungal colonization [47].

Inducing Host Responses: Probiotics stimulate the host's immune system, enhancing resistance against fungal infections and promoting health [47].

Detoxification of Aflatoxins by Probiotics

Research has shown that probiotics can effectively detoxify aflatoxins through:

Biodegradation: This process involves the breakdown of aflatoxins into less harmful compounds, although some resulting metabolites may still pose risks.

Bio adsorption: Probiotics can bind aflatoxins, reducing their bioavailability and toxicity in the gastrointestinal tract, thus preventing their absorption into the bloodstream [31].

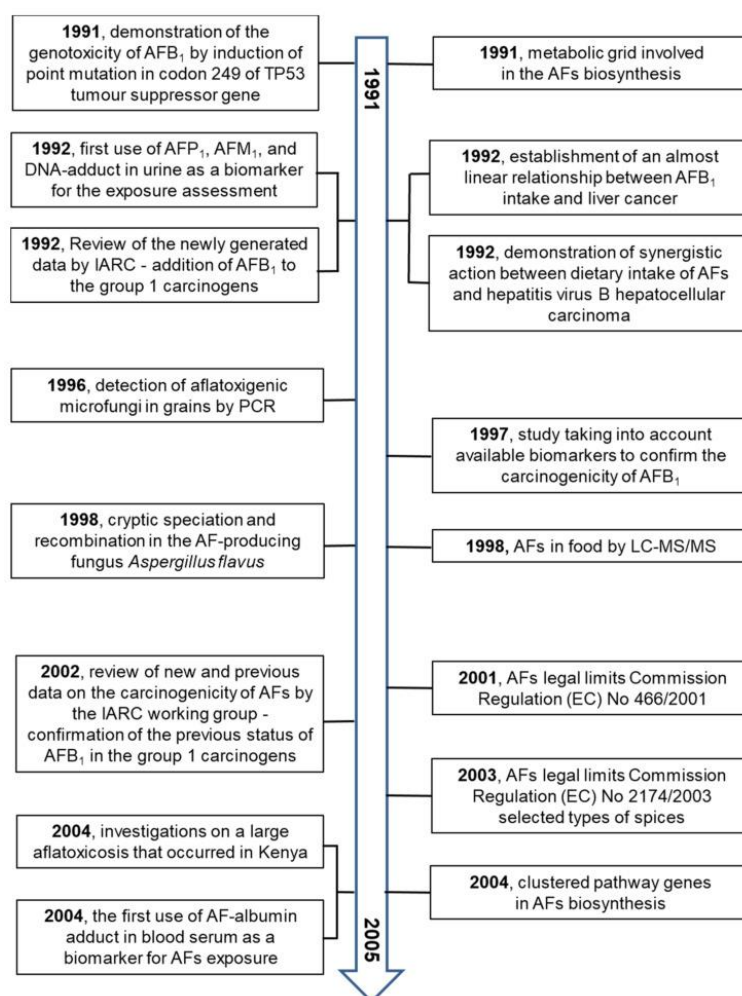


Figure 1. The milestones in aflatoxin research over the years 1961–2005.

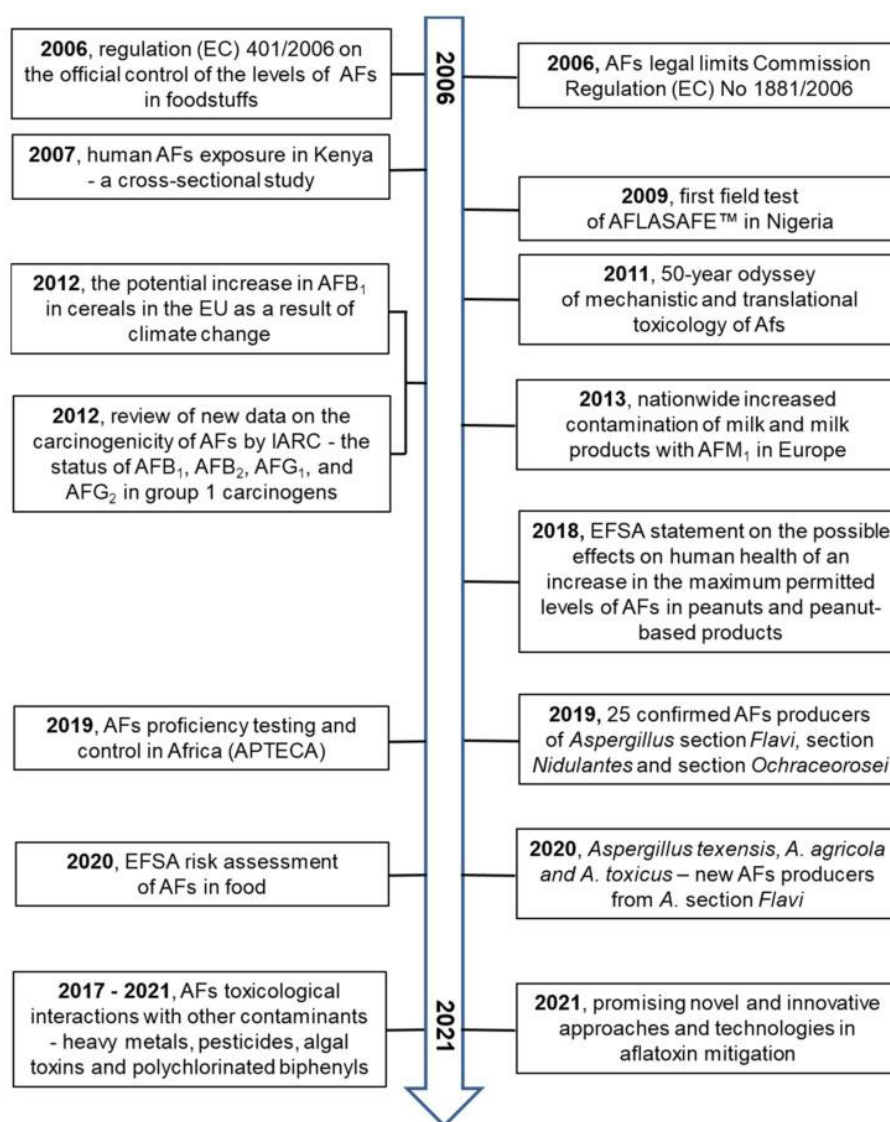


Figure 2. The milestones in aflatoxin research over the years 2006–2021.

2.2. Sources and Occurrence of Aflatoxin

Common Sources of Aflatoxins

Grains

Corn: One of the most common substrates for aflatoxin contamination. Aflatoxin levels can increase significantly during pre-harvest and post-harvest stages, particularly in warm and humid conditions [14].

Wheat: While less susceptible than corn, wheat can still be contaminated, especially during storage if conditions are favorable for mold growth [12].

Rice: Aflatoxin contamination in rice is less common but can occur, particularly in regions with high humidity [2].

Nuts

Peanuts: Peanuts are highly susceptible to aflatoxin contamination, especially during harvest and storage. Aflatoxins can develop in the field due to insect damage or in storage if moisture levels are not controlled [50].

Tree Nuts: Almonds, walnuts, and pistachios are also prone to contamination. The risk is heightened during warm weather and inadequate drying processes [37].

Seeds

Cottonseed: This is a significant source of aflatoxins, particularly in regions where cotton is grown extensively. Contamination can occur during growth, harvest, and storage [11].

Sunflower Seeds: Like cottonseed, sunflower seeds can be contaminated by aflatoxins, especially when stored improperly [5].

Environmental Factors Influencing Aflatoxin Production

Humidity: High humidity levels create an ideal environment for mold growth. Aflatoxin-producing molds thrive in moisture-rich conditions [41].

Temperature: Warm temperatures (between 25 °C and 30 °C) are conducive to the growth of aflatoxin-producing molds. Higher temperatures can exacerbate the problem, especially if combined with high humidity [24].

Improper Storage Conditions: Poor storage practices, such

as inadequate drying and ventilation, can lead to increased moisture levels in stored products, promoting mold growth and aflatoxin production [9].

Crop Stress: Factors such as drought or pest infestations can stress plants, making them more susceptible to mold infection and subsequent aflatoxin contamination [58].

3. Probiotics: A Natural Solution

3.1. Definition and Classification of Probiotics

Definition

Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host [26]. They are often referred to as "good" or "friendly" bacteria, primarily due to their role in promoting gut health and maintaining a balanced intestinal microbiota.

Classification of Probiotics

Probiotics can be classified into various genera and species, with several notable strains recognized for their health benefits:

Lactobacillus

Characteristics: This genus is known for its ability to ferment sugars into lactic acid, which helps lower the pH in the gut environment, inhibiting the growth of pathogenic bacteria [49]. *Lactobacillus* species are primarily found in fermented foods and the human gastrointestinal tract.

Common Species:

***Lactobacillus rhamnosus*:** Known for its ability to survive gastric acidity and bile salts, it is often used in the treatment of diarrhea and for enhancing gut health [48].

***Lactobacillus acidophilus*:** Commonly found in yogurt, this species is associated with lactose digestion and has been shown to improve symptoms of irritable bowel syndrome (IBS) [38].

***Lactobacillus plantarum*:** Known for its ability to produce antimicrobial substances, it helps in maintaining gut barrier function and modulating immune responses [17].

Bifidobacterium

Characteristics: This genus is predominantly found in the intestines of healthy infants and adults. *Bifidobacterium* species play a crucial role in maintaining gut microbiota balance and supporting immune function [21]. They are known for their prebiotic effects, promoting the growth of beneficial gut bacteria.

Common Species:

***Bifidobacterium bifidum*:** Often found in the intestines, it aids in digestion and has been linked to reduced gastrointestinal disorders [42].

***Bifidobacterium longum*:** This species is known for its ability to ferment a wide range of carbohydrates and has been shown to improve gut health and enhance immune responses [35].

***Bifidobacterium lactis*:** Frequently used in probiotic formulations, it has been studied for its role in improving gut

health and enhancing immune function [65].

Saccharomyces

Characteristics: This genus consists of yeasts, with *Saccharomyces boulardii* being the most studied probiotic yeast. It is renowned for its stability in the gastrointestinal tract and its potential to exert beneficial effects, particularly during antibiotic therapy [40].

Benefits: *Saccharomyces boulardii* is effective in managing antibiotic-associated diarrhea and has protective effects against pathogenic bacteria, contributing to a balanced gut microbiome [55].

3.2. Mechanisms of Aflatoxin Reduction

3.2.1. Binding and Sequestration

Probiotics can bind aflatoxins in the gut, preventing their absorption and reducing toxicity. Certain probiotic strains possess cell wall components that interact with aflatoxins, effectively sequestering them and limiting their bioavailability [43]. This binding action reduces the likelihood of aflatoxin entering the bloodstream, thereby minimizing its harmful effects.

3.2.2. Biotransformation

Certain probiotic strains can metabolize aflatoxins into less harmful compounds through enzymatic processes. For example, specific *Lactobacillus* and *Bifidobacterium* species have been shown to possess enzymatic capabilities that transform aflatoxins into non-toxic metabolites, thereby reducing their overall toxicity [20]. This biotransformation is crucial for detoxifying aflatoxins in the gastrointestinal tract.

3.2.3. Competitive Exclusion

Probiotics inhibit the growth of aflatoxin-producing molds by competing for nutrients and space. By establishing a healthy microbiota in the gut or food matrices, probiotics can limit the colonization of pathogenic fungi, including those that produce aflatoxins [46]. This competitive exclusion plays a vital role in maintaining both gut health and food safety.

3.2.4. Immune Modulation

Probiotics enhance the host's immune response, helping to mitigate the adverse effects of aflatoxins. They can stimulate the production of immune cells and cytokines, which helps the body respond more effectively to toxin exposure [44]. Improved immune function can reduce the risk of aflatoxin-related health issues, such as liver damage and immune suppression.

3.2.5. Gut Health Improvement

A balanced gut microbiome supports detoxification processes and overall health. Probiotics contribute to maintaining a healthy gut environment, which is essential for efficient

detoxification mechanisms [51]. A well-functioning gut microbiome can enhance the metabolism and elimination of harmful substances, including aflatoxins.

4. Practical Applications

4.1. In Food Processing

Incorporating probiotics during fermentation and storage can effectively reduce aflatoxin levels in various food products. For instance, the addition of probiotics to fermented dairy products has been shown to significantly lower aflatoxin concentrations, improving food safety [36].

4.2. In Animal Feed

Adding probiotics to livestock feed can minimize aflatoxin absorption, promoting healthier animals and safer food products. Research indicates that probiotics can reduce aflatoxin toxicity in animals, leading to enhanced growth performance and immune responses [66].

4.3. Case Studies and Success Stories

Numerous studies have demonstrated the successful application of probiotics in reducing aflatoxin contamination in both food and feed. For example, a study by [54] showed significant reductions in aflatoxin B1 levels in poultry when probiotics were included in their diet.

5. Challenges and Considerations

5.1. Strain Selection

Selecting effective probiotic strains is crucial for achieving desired outcomes in aflatoxin reduction. Not all probiotics have the same capacity to bind or metabolize aflatoxins, making strain selection a key factor in their application [64].

5.2. Regulatory Standards

Adhering to regulatory guidelines is essential for the safe use of probiotics in food and feed industries. Regulations vary by region and product type, necessitating compliance with safety standards to ensure consumer protection [15].

5.3. Consumer Acceptance

Consumer awareness and acceptance of probiotics can influence their implementation in food products. Educational initiatives may be necessary to promote the benefits of probiotics and address any misconceptions [53].

6. Future Directions in Probiotic Research

The potential of probiotics to mitigate aflatoxin toxicity and enhance gut health presents numerous avenues for future research. This section outlines key areas for exploration, emphasizing innovative applications and the long-term effects of probiotics.

6.1. Innovative Probiotic Applications in Various Food Systems

Future research should explore the incorporation of probiotics into diverse food systems, focusing on the following aspects:

6.1.1. Functional Foods

Developing functional foods enriched with specific probiotic strains can address nutritional needs while providing health benefits, particularly in combating aflatoxin contamination. Research should investigate:

Targeted Strains: Identifying probiotic strains with proven efficacy in binding or detoxifying aflatoxins and integrating them into products ranging from beverages to snacks [52].

Consumer Acceptance: Conducting sensory evaluations and consumer studies to assess the acceptability of probiotic-enhanced functional foods [53].

6.1.2. Fermentation Processes

Probiotics can play a significant role in the fermentation of non-dairy products, including plant-based alternatives. Research opportunities include:

Strain Interactions: Studying the interactions between different probiotic strains during fermentation to optimize the production of beneficial metabolites and improve food safety [22].

Nutritional Profile Enhancement: Evaluating how probiotic fermentation can enhance the nutritional profile of plant-based foods, particularly in terms of digestibility and nutrient bioavailability [17].

6.1.3. Bioencapsulation

Bioencapsulation techniques can protect probiotics during food processing, improving their stability and viability [63]. Future research should focus on:

Encapsulation Materials: Exploring various materials (e.g., alginate, chitosan) for encapsulating probiotics to enhance their survival through processing and storage [57].

Release Mechanisms: Investigating controlled release mechanisms that can ensure probiotics are delivered effectively to the gut, where they can exert their detoxifying effects [39].

6.1.4. Probiotic Delivery Systems

The development of advanced delivery systems can significantly enhance the efficacy of probiotics:

Nanotechnology: Researching the application of nanotechnology for the encapsulation and delivery of probiotics to improve their survival rate in harsh gastrointestinal environments [66].

Microencapsulation Techniques: Evaluating microencapsulation methods to protect probiotics from environmental stressors, thus ensuring their viability until consumption [20].

6.2. Long-Term Effects of Probiotics on Gut Health and Aflatoxin Detoxification

Understanding the long-term implications of probiotic consumption is critical for assessing their role in gut health and aflatoxin detoxification. Key research areas include:

6.2.1. Clinical Trials

Conducting longitudinal clinical trials can provide valuable insights into the health benefits of probiotics:

Study Design: Designing randomized controlled trials to evaluate the long-term effects of specific probiotic strains on gut microbiota composition and function, particularly about aflatoxin exposure [44].

Health Outcomes: Assessing various health outcomes, including gastrointestinal health, immune function, and biomarkers of aflatoxin exposure [40].

6.2.2. Mechanistic Studies

Delving deeper into the mechanisms of probiotic action regarding aflatoxin detoxification is essential:

Molecular Interactions: Investigating the molecular interactions between probiotics and aflatoxins to elucidate how probiotics facilitate detoxification at the biochemical level [59].

Metabolite Analysis: Analyzing the metabolites produced by probiotics during aflatoxin detoxification to identify non-toxic derivatives and their potential health benefits [39].

6.2.3. Population Studies

Researching the effects of probiotics in diverse populations can help generalize findings:

High-Risk Groups: Focusing on populations with high exposure to aflatoxins, such as those in agricultural regions, to evaluate the effectiveness of probiotics in reducing health risks [62].

Cultural Dietary Practices: Investigating how cultural dietary practices may influence the efficacy of probiotics in different ethnic groups [53].

6.2.4. Sustainability and Safety

Assessing the long-term safety and sustainability of probi-

otics in food systems is crucial:

Safety Profiling: Conducting safety assessments of prolonged probiotic use, particularly in vulnerable populations such as children, the elderly, and immunocompromised individuals [15].

Impact on Microbiota: Studying the long-term effects of probiotic consumption on the gut microbiota to ensure that they do not disrupt the natural microbial balance [60].

7. Conclusion

The presence of aflatoxins in food supplies poses a significant threat to public health, necessitating effective mitigation strategies. Probiotics represent a promising natural approach to combat aflatoxin contamination through mechanisms such as binding, biotransformation, and competitive exclusion. Their incorporation into food processing and animal feed can enhance safety and reduce health risks associated with aflatoxins. However, challenges remain, including strain selection, regulatory compliance, and consumer acceptance. Future research should focus on innovative probiotic applications, long-term health effects, and detailed mechanisms of detoxification. Advancing our understanding of probiotics in this context is crucial for protecting public health and ensuring safer food systems.

Abbreviations

(IARC)	International Agency for Research on Cancer
(IBS)	Irritable Bowel Syndrome
AFB1	Aflatoxin B1
AFB2	Aflatoxin B2
AFG1	Aflatoxin G1
AFG2	Aflatoxin G2
AFM1	Aflatoxin M1
AFM2	Aflatoxin M2
(WHO)	World Health Organization
IBS	Irritable Bowel Syndrome

Conflicts of Interest

The authors declare no conflicts of interest.

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