# Biodegradation of mycotoxins using postbiotics 40

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Developments in Applied Microbiology and Biotechnology

# Postbiotics

## Health and Industry

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CHAPTER

# 40 Biodegradation of mycotoxins using postbiotics

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## 1. Introduction to postbiotics in the food industry

### 1.1 Definition and characteristics of postbiotics

Postbiotics, a category of beneficial compounds originating from microorganisms or resulting from bacterial breakdown, confer health advantages to the host even after the microorganisms cease to exist. Coined from the Greek "post" (after) and "bios" (life), this term is part of the biotic family, including probiotics, prebiotics, and synbiotics (Vinderola et al., 2022). The term gained prominence in 2021 with ISAPP officially defining it, replacing earlier terms like nonviable probiotics, paraprobiotics, ghostbiotics, and heat-inactivated probiotics (Oberg et al., 2012). Also referred to as metabiotics, biogenic substances, or cell-free supernatants (CFS) metabolites (Cicenia et al., 2014; Oberg et al., 2012; Salminen et al., 2021; Tsilingiri et al., 2012), postbiotics comprise various soluble factors, such as enzymes, peptides, teichoic acids, and other bioactive molecules (Oberg et al., 2012; Tsilingiri et al., 2012).

Postbiotics possess distinct characteristics, including well-defined chemical structures, established safety parameters, and a prolonged shelf life. They manifest diverse physiological advantages, encompassing antiinflammatory, immunomodulatory, antiobesogenic, antihypertensive, hypocholesterolemic, antiproliferative, and antioxidant activities, despite the precise mechanisms remaining unclear (Shigwedha et al., 2014). Differentiation of postbiotics can be based on elemental composition or physiological functions, showcasing their favorable absorption, metabolism, distribution, and excretion capabilities. This suggests their ability to signal various organs and tissues, triggering diverse biological responses (Shenderov, 2013). Notably, postbiotics can emulate the health effects of probiotics without the need for the administration of live microorganisms (Tsilingiri et al., 2012).

Postbiotics offer a safer alternative to live probiotic bacteria, addressing concerns like bloating, flatulence, and potential health risks associated with probiotic use. Therefore, the practical applicability and functionality of postbiotics make them a valid and safer alternative, mitigating the risks associated with live probiotic bacteria (Doron & Snydman, 2015; Williams, 2010).

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### 1.2 Importance and potential applications in the food industry

In recent years, there has been a growing interest among researchers, manufacturers, and consumers in functional foods containing postbiotics. This trend is marked by a strong focus on understanding the mechanisms through which postbiotics exert their effects and creating innovative formulations for functional foods and preventive medications. This shift aligns with a broader societal movement toward a healthier lifestyle (Rad et al., 2020).

Fermentation plays a vital role in the food industry, producing postbiotics found in items like yogurt and pickled vegetables. These compounds enhance dietary supplements and improve fermented functional foods, serving as prophylactic agents in medical therapies. Unlike live probiotics, postbiotic quantities are easily controlled, ensuring production, and storage flexibility. This stability benefits food safety, taste, and nutritional profiles, giving a competitive edge. Postbiotics also find application in active packaging for extended food shelf life, offering innovative solutions. Clinical studies highlight their significance in digestion, absorption, metabolism, and distribution, solidifying their esteem in the food industry (Aghebati-Maleki et al., 2022; Hernández-Granados & Franco-Robles, 2020; Hosseini et al., 2022).

The surge in postbiotic popularity parallels the rising demand for healthier lifestyles and functional foods. Nisin, a postbiotic found in dairy and processed foods, enhances safety and shelf life (Huang et al., 2020). *Lactobacillus sakei* NRRL B-1917 supernatant enhances grilled beef safety (Beristain-Bauza et al., 2017). *Lactobacillus coryniformis* MXJ 32 acts as a bactericide in various foods, targeting *Staphylococcus aureus* and *Escherichia coli* for enhanced food safety (Lü et al., 2014). Pyrrolo and pyrazine-1,4-dione from *Lactobacillus salivarius* in ground beef and whole milk aid in biofilm removal by *Listeria monocytogenes*, contributing to food safety (Moradi et al., 2021). The bacteriocin-like inhibitor substance of *Lactobacillus plantarum* ST16P serves as a bioconservative in chicken breast, protecting against *Enterococcus faecium* for up to 7 days (da Silva et al., 2017).

In conclusion, the growing interest in postbiotics is driven by the pursuit of improved well-being and the desire for functional food options. Postbiotics offer stability, diverse applications, and potential benefits for food safety in modern, health-focused products.

## 2. Overview of mycotoxins in food

### 2.1 Definition and sources of mycotoxins

Mycotoxins are secondary metabolites of toxigenic fungi. They can contaminate a variety of food commodities and agricultural crops. According to the researchers, *Aspergillus*, *Fusarium*, and *Penicillium* are the major mycotoxin-producing fungi species. Different species of these fungi produce vary toxin types. More than 400 mycotoxins that are known, aflatoxins, fumonisins, ochratoxins, deoxynivalenol, zearalenone, and patulin are the most significant in terms of their commercial impact and risk to human and animal health (Richard, 2007).

Poor hygienic standards during transit and storage, high temperatures, high moisture content, and severe rains are the key factors for mycotoxin formation and their growth. Different countries frequently experience these situations. Due to the continent of growing population, there is a greater need for food substances to be stored. However, poor conditions for storage, shipping, and processing may promote fungal growth, which in turn causes the generation of mycotoxin and the contamination of foods and feedstuffs (Ramesh et al., 2003).

Mainly Africa and other regions of the world, the importance of food-borne mycotoxins cannot be overstated. Global attention has been drawn to the effects of such poisons on human health, animal productivity, and the economy (Darwish et al., 2014).

## 2.2 Regulatory guidelines for mycotoxin limits in food

The 21st-century imperative highlighted in the Mycotoxin report by the Council for Agricultural Science and Technology is to establish uniform international regulations addressing the contamination of food by mycotoxins (Wu, 2004). In 2002/2003, an international inquiry was conducted with the support of the Food and Agriculture Organization (FAO) to establish global standards and regulations pertaining to mycotoxins. The results of this inquiry revealed that by the end of 2003, more than 100 countries, collectively encompassing over 90% of the world's population, had implemented specific regulations or comprehensive guidelines concerning mycotoxins.

Because of these regulations and standardizing system, Asia, Europe, Africa, Latin America, and North America have controlled the mycotoxin levels in commodities and products in 2003 compared to 1995. However, the tolerance limits and regulatory requirements are different with the country because of the sampling and analytical methods. (Egmond & Jonker, 2003)

The allowable limits for various mycotoxins in foods and feedstuffs differ. Here are the permissible ranges:

- Aflatoxins B1, B2, G1, and G2 in foods: 0–40 ppb
- Ochratoxin A in food: 0–50 ppb
- Deoxynivalenol (DON) in food: 500–2000 ppb
- Zearalenone in food: 0–1000 ppb
- Patulin in foods: 0–50 ppb
- Fumonisins in food: 0–1000 ppb (Mazumder & Sasmal, 2001)

Table 40.1 **Health risks associated with mycotoxin contamination**.

# 3. Postbiotics and mycotoxin biodegradation

## 3.1 Role of postbiotics in mycotoxin degradation

Various methods aim to mitigate mycotoxin contamination in food and feed, broadly classified as physical, chemical, and biological treatments (Peng et al., 2018). Physical approaches, such as thermal inactivation and magnetic carbon absorption, deploy heat or magnetic materials to eliminate mycotoxins. Chemical treatments involve acids, alkalis, or oxidizing agents transforming mycotoxins into less harmful compounds. However, these methods often face challenges like biosafety risks, high costs, or limited efficacy. As a result, there's a growing preference for biological detoxification due to its efficiency, cost-effectiveness, and environmental compatibility (Adebo et al., 2017; Armando et al., 2012; Shetty & Jespersen, 2006). Biological treatments employ microorganisms like *Actinomycetales*, *Mycobacterium*, *Lactobacillus*, and Yeasts, which enzymatically degrade or modify mycotoxins, rendering them nontoxic (Peng et al., 2018; Rad et al., 2021). This method is increasingly recognized as optimal for mycotoxin removal (Zhou et al., 2017).

**Table 40.1 Responsible fungi to produce mycotoxins and their health impacts.**

| Mycotoxin type | Responsible fungi | Contaminated food commodities | Health impact | References |
|---|---|---|---|---|
| Aflatoxin(alatoxin B1, B2, G1, and G2 | *Aspergillus lavus*, *A. parasiticus*, *A. bombycis*, *A. ochraceus*, *A. nomius, and A. pseudotamari* | Rice, wheat, maize, figs, ground nuts, tree nuts, corn, spices, kernels, yam chips, cotton seeds | Carcinogenesis, hepatitis B diseases, suppress immune function, severe lesions of the liver in malnourished adults | (Klich et al., 2000) |
| Ochratoxin A | *Aspergillus*, *including A. alliaceus*, *A. auricomus*, *A. carbonarius*, *A. ochraceus*, *A. glaucus*, *A. melleus*, and *A. niger* | Milk, pork, cereals and cereal base processed foods | Acute nephrotoxicity, immunosuppressive actions, teratogenic effects in animal models | (Bayman et al., 2002; Darwish et al., 2014) |
| Fumonisins | *F. verticillioides* (formerly known as *F. moniliforme -Gibberella fujikuroi species complex)*, *F. proliferatum*, *F. nygamai*, *Alternaria alternata*, *A. niger* | Sorghum, maize and maize products | Leukoencephalomalacia (hole in the head syndrome) in equines and rabbit's pulmonary edema and hydrothorax in swine and hepatotoxic and carcinogenic effects and apoptosis in the livers of rats esophageal cancer | (Frisvad et al., 2007; Rheeder et al., 2002) |
| Patulin | *Penicillium patulum* | Apple juice and apple, grapes, pears, strawberries, nectarines, black mulberries, white mulberries, lingonberries, peaches, and plums | Inhibits the activity of numerous enzymes, immunosuppressive substance, convulsions, dyspnea, pulmonary congestion, edema, ulceration, hyperemia, and GI tract distension | (Erdoğan et al., 2018; Phillips, 1979; Ritieni, 2003) |
| Zearalenone | *F. graminearum*, *F. culmorum*, *F. equiseti*, and *F. crookwellense* | Cereals and cereal based food products | Premature puberty in girls, cervical cancer | (Darwish et al., 2014; Zinedine et al., 2007) |
| Deoxinivalenol | *F. graminearum* and *F. avenaceum* | Cereals, cereal base processed foods | Throat irritation, diarrhea, vomiting, blood in the stools, facial rash | (Darwish et al., 2014) |

Microbial detoxification, utilizing probiotic bacteria, is a viable biological method for mitigating mycotoxin contamination. Advancements in this field have introduced the concept of "postbiotics," particularly from lactic acid bacteria (LAB), which can bind to and neutralize mycotoxins, reducing their toxicity. Adding these postbiotics to contaminated food or feed presents a natural and safe approach to mycotoxin management in agriculture and food production, showcasing their potential in food safety. The process involves mycotoxin biodegradation, relying on mechanisms like epoxidation, hydroxylation, dehydrogenation, and reduction by extracellular or intracellular enzymes (Martínez et al., 2019; Salminen et al., 2021; Zhao et al., 2016).

## 3.2 Mechanisms of mycotoxin biodegradation by postbiotics

Microbes and nonpathogenic enzymes are crucial in biological detoxification, eliminating toxins and providing health benefits (Ebrahimi et al., 2021). Probiotics play a key role in mycotoxin removal, using biodegradation or biosorption mechanisms, with biodegradation being a more effective, long-term method. Specific strains hinder aflatoxin absorption by attaching them to cell walls, and probiotics produce compounds (organic acids, bacteriocins, and hydrogen peroxide) that suppress aflatoxin production and interfere with biosynthesis (Gerbaldo et al., 2012; Zhao et al., 2016).

Aflatoxins, like Aflatoxins, can undergo decomposition via various mechanisms, such as epoxidation, hydroxylation, dehydrogenation, and reduction, with the specific pathway dependent on the facilitating agent (Martínez et al., 2019). Certain bacteria and fungi cells have demonstrated effectiveness in breaking down aflatoxins, mainly through microbial catabolic pathways and specific enzymes found in cell-free supernatants (CFS). These microorganisms modify the coumarin structure and cleave the difuran ring in the aflatoxin's chemical makeup. This specialization underscores the unique role of specific bacterial and fungal cells in aflatoxin degradation (Adebo et al., 2017; Chlebicz & Śliżewska, 2020; Dalié et al., 2010). Aflatoxins are temporarily absorbed in a biological process, forming an unstable complex based on absorption sites and affinity (Adebo et al., 2017; Ebrahimi et al., 2021). Nonviable bacteria can effectively eliminate aflatoxins by physically binding to cell wall carbohydrates (Bueno et al., 2007; Hernandez-Mendoza et al., 2009).

Probiotic bacteria, akin to other Gram-positive types, possess a well-structured cell wall comprising a peptidoglycan sacculus surrounding the cytoplasmic membrane, adorned with glycopolymers like teichoic acids, polysaccharides, and proteins (Chapot-Chartier & Kulakauskas, 2014). Extracellular polysaccharide (EPS), teichoic acid (TA), and peptidoglycan are key carbohydrate varieties in cell walls. Among them, peptidoglycan is the primary component responsible for AFB1 binding (Haskard et al., 2001, as shown by the rejection of exopolysaccharide (EPS) and minimal impact observed with teichoic acid in the binding process. Teichoic acids (TAs) serve distinct functions, categorized as Wall Teichoic Acids (WTAs) forming covalent attachments to peptidoglycan (PG), and Lipoteichoic Acids (LTAs) affixed to the cytoplasmic membrane via a glycolipid moiety (Chapot-Chartier & Kulakauskas, 2014; Lahtinen et al., 2004; Zhao et al., 2016).

Probiotic yeast cell walls, containing glucan and mannan, utilize various bonding mechanisms like ionic, hydrogen, and hydrophobic interactions to bind toxins (Al-Saad, 2018). Heating causes protein denaturation and Maillard reaction product formation, while acidity transforms polysaccharides into aldehydes, enhancing aflatoxin-binding efficiency in yeast (El-Nezami et al., 1998).

Keeping abreast of the latest scientific literature is crucial in the rapidly advancing field. Interdisciplinary research, encompassing microbiology, toxicology, and nutrition, is essential for gaining a comprehensive understanding of the complex mechanisms and implications associated with the biodegradation of mycotoxins by postbiotics.

## 3.3 Factors influencing the efficiency of mycotoxin biodegradation

Factors such as probiotic strain concentration, specificity, postbiotics, toxin levels, pH, duration, and cell wall components impact mycotoxin degradation (Ahlberg et al., 2015; Pfliegler et al., 2015). Creating optimal conditions for mycotoxin-degrading microorganisms is crucial, as microbial community composition and diversity significantly affect biodegradation efficiency, varying among different microorganisms.

Recent research challenges the traditional belief that bacterial viability is crucial for mycotoxin removal. Instead, there is a growing focus on “postbiotics,” as a safe and effective method. They have been shown to remove more mycotoxins than their live counterparts, without posing health risks (Sarlak et al., 2017; Vosough et al., 2014). This shift in perspective is particularly significant due to the difficulties live probiotics face in surviving the harsh stomach environment, potentially compromising their effectiveness in promoting gut health (Hamidi et al., 2013; Topcu et al., 2010).

In many instances, acid treatment has shown greater efficacy compared to heat treatment (Haskard et al., 2001; Rahaie et al., 2012). Various studies indicate that exposing bacteria to acid treatment substantially enhances their capacity to adhere to AFB1 (Aflatoxin B1) (El-Nezami et al., 1998; Peltonen et al., 2001).

Heat treatment of LAB results in an increased ability to bind Aflatoxin M1 (AFM1), attributed to modifications in cell surface properties and protein denaturation. This process potentially generates additional binding sites through Maillard reactions. The enhanced AFM1 binding capacity is observed in both phosphate-buffered saline (PBS) and milk, demonstrating efficiency comparable to that of live strains (Bovo et al., 2013; El-Nezami et al., 1998; Kabak & Var, 2008; Pierides et al., 2000).

Various mycotoxins, characterized by diverse structures and properties, display varying degrees of susceptibility to degradation. The presence of nutrients plays a vital role in sustaining the microorganisms responsible for mycotoxin biodegradation, and environments rich in nutrients can expedite the rates of degradation.

Acid treatment of LAB alters cell wall components, reducing peptidoglycan thickness and increasing hydrophobic binding sites. This promotes aflatoxin binding through enhanced hydrophobic interactions. Acid-treated LAB, despite a usual thick peptidoglycan layer, exhibits larger pores, potentially facilitating substance binding. Under acidic conditions, aflatoxins swiftly bind to cytoplasmic membrane components. This molecular transformation highlights acid treatment’s effects (Haskard et al., 2000; Hosseini, 2019). Cell-Free Supernatant (CFS) and LAB-derived postbiotics show promise in aflatoxin removal, supporting food safety (Fernandes et al., 2013). Controlled optimization of these processes is crucial for effective mycotoxin biodegradation strategies, vital for food safety and agriculture. Ongoing research aims to refine techniques addressing mycotoxin contamination across diverse settings.

# 4. Postbiotic strains for mycotoxin biodegradation

## 4.1 Selection and isolation of postbiotic strains with mycotoxin degradation potential

The process in which mycotoxins are broken down by microorganisms is known as mycotoxin biodegradation (AL-Nussairawi et al., 2020). This can happen naturally or on purpose using microorganisms that have been designed particularly to break down mycotoxins (Kim & Vujanovic, 2017). Bacteria, fungi, and yeasts are just a few of the microorganisms that can break down mycotoxins. These microorganisms break down mycotoxins via a range of processes, including enzymatic, chemical, and physical breakdown (Kim & Vujanovic, 2017). Biological degradation is the most common mechanism of mycotoxin degradation and that includes the biodegradation by microorganisms or their enzymes. Enzymes produced by microorganisms can break down mycotoxins into smaller, less toxic compounds (AL-Nussairawi et al., 2020). Chemical degradation is another mechanism of mycotoxin degradation. In this process, microorganisms use chemicals to break down mycotoxins. Ozonation can be applied as a chemical method. Physical degradation is the least common mechanism of mycotoxin degradation. In this process, microorganisms physically break down mycotoxins into smaller pieces (AL-Nussairawi et al., 2020). Sorting, extrusion, and application of adsorbents are physical techniques (AL-Nussairawi et al., 2020). Selecting and isolating postbiotic strains with mycotoxin biodegradation capabilities is a complex process that involves various steps to ensure the identification of effective and safe strains (Vanhoutte et al., 2016). Finding prospective sources of microorganisms with a history of mycotoxin degradation is the initial stage in this approach (AL-Nussairawi et al., 2020). These sources could consist of soil, plant matter, fermented foods, or even the gastrointestinal tract of animals with the capacity to degrade mycotoxin.

Collecting samples from the identified sources is the next stage (AL-Nussairawi et al., 2020). These samples should contain a variety of microbial species (Moradi et al., 2021). For an example, soil samples should be gathered from various sites if you're seeking for strains in the soil. The next step is to isolate microorganisms. In order to do this, dilute the samples that were taken and plate them on the right growth media. Depending on the bacteria strain, the degradation time varies (Ndiaye et al., 2022). This facilitates the isolation of certain microbial populations. More than one mycotoxin can reportedly be degraded by a variety of microorganisms (Ndiaye et al., 2022). Some kinds of LAB can break down multimycotoxin (Ndiaye et al., 2022).

Subsequently, create a screening method to evaluate an isolated strain's mycotoxin degradation capacity. Growing each strain in the presence of particular mycotoxins and checking for degradation byproducts could be involved in this. The degradation products can be examined using high-performance liquid chromatography (HPLC) or mass spectrometry (Moradi et al., 2021). Then perform the Selection of Effective Strains to find strains with a significant capacity for mycotoxin degradation. Next, give the chosen strains a variety of biochemical and molecular characteristics to help to identify them as a specific species or strain (Moradi et al., 2021). Additionally, do safety evaluations to make sure the strains don't hurt people, animals, or the environment (Moradi et al., 2021).

Then, to confirm the strains' capacity to degrade mycotoxin, test them in controlled settings or real-world situations. This may involve tracking the progress of mycotoxin reduction using samples of contaminated food. To improve the effectiveness of the chosen strains' mycotoxin degradation, further optimize their growth and fermentation characteristics (AL-Nussairawi et al., 2020). Once identified effective and safe strains, scale up their production through fermentation processes (Moradi et al., 2021). This involves optimizing the fermentation conditions for maximum postbiotic production. To reduce mycotoxin contamination in food, isolated postbiotic strains can be added to a variety of products, including probiotics, prebiotics, and synbiotics (Moradi et al., 2021). When creating goods using these postbiotic strains, make sure to follow safety and regulatory criteria.

However, there are some difficulties with mycotoxin biodegradation, such as the fact that it can be a slow process, that it sometimes fails to completely reduce the levels of all mycotoxins, and that it can be challenging to scale up for commercial usage.

## 4.2 Characterization and identification of effective postbiotic strains

Probiotic bacteria produce bioactive substances known as postbiotics during metabolic or fermentation activities (Liu et al., 2022). These substances include metabolites, proteins on cell surfaces, elements of cell walls, and other bioactive chemicals (Halász et al., 2009; Liu et al., 2022) They have similar potential health advantages as probiotics, including immunological regulation, anti-inflammatory actions, and gut health maintenance. For the development of specialized therapeutic applications, it is essential to identify and characterize efficient postbiotic strains.

When considering the identification of effective postbiotic strains, researchers frequently start by extracting and cultivating a variety of bacterial strains from various sources, such as environmental samples, fermented foods, and human gut bacteria (Halász et al., 2009). The selection of strains with the necessary metabolic capacities can be determined by metabolomics analysis (Morrison and Tom, 2016). For instance, strains with high short-chain fatty acid or antimicrobial peptide production levels can be given priority (Halász et al., 2009). Using cell-based assays, strains can be tested for particular bioactivities in vitro, such as antiinflammatory or immunemodulating properties. The impact of postbiotic strains on numerous health outcomes, such as gut health, immunological responses, and metabolic parameters, can be assessed using animal models, notably rodents. To verify the effects of postbiotic strains in humans, clinical trials are essential. Techniques like gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) are used to analyze the metabolic profile of postbiotic bacteria. These techniques allow for the identification and measurement of short-chain fatty acids (SCFAs), peptides, bioactive metabolites, and other substances produced during fermentation. This metabolite profiling is important in the characterization of postbiotic strains (Halász et al., 2009). It is possible to figure out the bioactive proteins that postbiotic bacteria produce by analyzing their proteomes. A complete set of proteins expressed by an organism is called a proteome. Proteomic methods based on mass spectrometry offer information on the identity and quantity of proteins involved in immune regulation, antiinflammatory responses, and other health-promoting activities. The genetic foundation of the bioactivity of postbiotic bacteria is revealed by analyzing their genomic and transcriptome profiles (Halász et al., 2009). Comparative genomics enables the discovery of distinctive gene clusters that generate certain advantageous metabolites.

## 4.3 Safety assessment of postbiotic strains for food applications

Postbiotics are the bioactive soluble chemicals that microbes release, and they exhibit a variety of bioactivities, such as compounds with antimicrobial properties (da Silva & Alexander, 2023). Certain

LAB types are considered as probiotics, and the postbiotic products they produce typically provide users with similar or complimentary health advantages (Moradi et al., 2020).

Food safety, assuring a lower growth of pathogens in food, and increasing consumer health are all made possible by the antibacterial compounds generated by probiotic bacteria, which are known as postbiotics (Vanhoutte et al., 2016). Food safety assures that there are no microbiological or chemical contaminants in the food that pose serious health risks (Vanhoutte et al., 2016). One of the most serious public health issues, foodborne illness results in several annual hospitalizations and fatalities and has long been a major contributor to the well-being of the neighborhood (Moradi et al., 2020). By removing or decreasing microbiological and chemical pollutants, food safety assurance is achieved during processing, distribution, and storage (Vanhoutte et al., 2016).

Postbiotics differ from probiotics in a few ways that make them valuable components (Ndiaye et al., 2022). Postbiotics have advantages over parent bacterial live cells, such as longer shelf lives, safe structures, no ability to spread antibiotic resistance, no production of biogenic amine, defined chemical composition and safety, ease of use and storage, stability in a wide range of pH and temperature, and broad-spectrum antimicrobial activity (Moradi et al., 2020). Postbiotics may be used in many food matrices with the goal of ensuring food safety. Utilizing particular bacteria and their antimicrobial metabolites, biopreservation is a cutting-edge method for increasing the shelf life of food and preventing microbial deterioration (Ndiaye et al., 2022). Postbiotics show a wide range of antimicrobial properties in vitro environments, including activity against spoilage microbes, which is very crucial for the food sector (Moradi et al., 2020).

There are many potential applications of postbiotics mixture in biopreservation of different food commodities including bio-preservation of meat and fish products, dairy products, vegetables, and bread (Vanhoutte et al., 2016). The nutritional value of meat, fish, and related items is greatly impacted by bacterial infection, which also leads to undesired organoleptic alterations and poses a risk to consumer health. Postbiotics, which can be applied directly to the product by coating or spraying, contain antimicrobial components for usage in meat and meat products. The type of meat and the postbiotics' nature determine how these antimicrobial compounds are applied. Also, in order to guarantee the safety of dairy products, postbiotics act as biocontrol agents. Without impacting sensory acceptability, the postbiotics significantly reduce the cheese's fungus population. When considering biopreservation of vegetables and bread, the shelf life of processed commercial bread and vegetables is increased by postbiotics from various strains of *Lactobacillus* (Vanhoutte et al., 2016).

There are many safety aspects of postbiotics. Consuming postbiotics has no known clinical or epidemiological hazards, and because there are no living bacteria present, there is no chance of infection (da Silva & Alexander, 2023). Postbiotics have the ability to reduce the acute liver damage caused by lipopolysaccharides. Also, postbiotics have the ability to boost liver antiinflammatory cytokine concentrations while decreasing hepatic inflammatory infiltration and serum indicators for liver injury. Some postbiotics can increase microbial activity in the gut, which can lead to increased weight gain, feed intake, nutritional intake, and nutrient digestibility.

When determining the safety of postbiotic strains, a variety of criteria are taken into account. They are the identity and characteristics of the strain, the production procedure, comprising the culture medium, the fermentation environment, the strain's intended application, such as a nutritional supplement or an ingredient in a food product and the intended audience, which can include young children or healthy adults. The evaluation of postbiotic strains for safety is a crucial step in making sure that these products are safe for customers to use.

## 5. Biodegradation of specific mycotoxins by postbiotics

### 5.1 Aflatoxins: Degradation mechanisms and postbiotic strains involved

Addressing Aflatoxin removal is crucial, and ongoing scientific and technological efforts aim to tackle this food safety and public health threat. Solutions for reducing mycotoxins in plant and animal foods include physical, chemical, and biological methods (Peng et al., 2018). The biological approach uses helpful microorganisms to break down mycotoxins in foods and feeds. This method is cost-effective, eco-friendly, and ensures complete detoxification under suitable conditions (Adebo et al., 2017; Armando et al., 2012; Shetty & Jespersen, 2006; Zhou et al., 2017; Zychowski et al., 2013).

Recent research has delved into the application of postbiotics to reduce aflatoxin levels in food models and culture media (Karazhiyan et al., 2016; Nassar et al., 2018). Nevertheless, until recently, there has been a lack of comprehensive reviews assessing the effectiveness of postbiotics in diminishing aflatoxin levels and identifying the most potent postbiotic agents for this purpose.

In the context of aflatoxin removal, biodegradation represents a prolonged and enduring process, which is facilitated by extracellular or intracellular enzymes (Zhao et al., 2016). The detoxification of aflatoxins in biological techniques involves microbial or enzymatic processes, with specific cells of bacteria and fungi playing a key role. Probiotics also contribute to aflatoxin reduction through the production of inhibitory metabolites and removal mechanisms (Afshar et al., 2020).

Probiotics facilitate the enzymatic breakdown of aflatoxins, transforming them into less harmful compounds through microbial catabolic pathways and specific enzymes. Aflatoxins, characterized by a double furan ring and coumarin structure, undergo alterations in the coumarin structure and difuran ring cleavage during microbial degradation (Adebo et al., 2017; Dalié et al., 2010). Studies suggest that probiotics generate metabolites, such as organic acids and bacteriocins, along with hydrogen peroxide, which hinder aflatoxin production and disrupt its biosynthesis (Gerbaldo et al., 2012). Additionally, probiotics exhibit the capacity to eliminate aflatoxins through biodegradation or biosorption mechanisms (Afshar et al., 2020).

Probiotic bacteria, similar to other Gram-positive bacteria, possess a complex cell wall structure composed of various macromolecules, including peptidoglycan sacculus, teichoic acids, and proteins associated with the peptidoglycan sacculus. These components play distinct roles in the cell wall (Chapot-Chartier & Kulakauskas, 2014). Cell wall peptidoglycan was the most likely carbohydrate component responsible for effective AFB1 binding. Teichoic acid was found to have a minimal effect on mycotoxin binding (Lahtinen et al., 2004; Zhao et al., 2016).

Despite advancements, comprehensive reviews on the efficacy of postbiotics and identification of potent agents are still lacking. Continued research in this domain holds promise for developing efficient strategies to combat aflatoxin contamination, safeguarding the food supply, and public health.

### 5.2 Ochratoxin A: Biodegradation pathways and postbiotic strains

Ochratoxin A is a prominent mycotoxin frequently found in food and feed, primarily associated with agricultural crops before and after harvesting. Various fungi, including *A. alliaceus*, *A. carbonarius*, *A. ochraceus*, *A. steynii*, *A. westerdijkiae*, *P. nordicum*, and *P. verrucosum*, contribute significantly to OTA contamination (Frisvad et al., 2016). Notably, *P. nordicum* is a major Ochratoxin A producer, commonly detected in specific cheeses and fermented meats. Conversely, *Aspergillus niger*, although prevalent, is less relevant due to the majority of isolates being nonochratoxigenic (Serra et al., 2006).

Biological detoxification, an increasingly acknowledged approach for eliminating Ochratoxin A form food and feed products, has gained significant attention due to its advantageous features. Microorganisms or enzymes play a crucial role in the detoxification of Ochratoxin A, either through degradation or adsorption mechanisms. Significant research has focused on exploring the biodegradation of Ochratoxin A, leading to the discovery of various microorganisms with potential for effectively degrading this mycotoxin (Wegst & Lingens, 1983).

Microorganisms can play a role in the degradation of Ochratoxin A through two distinct pathways. The first pathway entails the cleavage of the amide bond, resulting in the formation of nontoxic OTα and L-β-phenylalanine, thereby establishing a detoxification mechanism. The second pathway, which is currently less certain, explores the degradation of Ochratoxin A through lactone ring hydrolysis. Ongoing research is underway to assess the feasibility and efficacy of this process (Article, 1999).

The potential strategies for mitigating the harmful effects of Ochratoxin A, particularly in cases of food and feed contamination, lie in the pathways of microbial degradation. Several bacterial strains have shown the ability to degrade Ochratoxin A (Wegst & Lingens, 1983). Employing microorganisms like *Lactobacillus* and *Saccharomyces cerevisiae* is advantageous due to their long-standing use in the food industry and established probiotic qualities (Hwang & Draughon, 1994).

Furthermore, in addressing the high expenses linked with the purification of enzymes, the use of crude enzyme extracts emerges as a viable option for bioremediation in Ochratoxin-contaminated foods. This approach promotes safer food production through environmentally friendly processes (Abrunhosa et al., 2006; Stander et al., 2000). As research advances, these inventive methods show potential for mitigating the negative effects of Ochratoxin A, guaranteeing the safety of agricultural products and the health of consumers.

## 6. Applications of postbiotic-based mycotoxin detoxification

Mycotoxin contamination is a significant concern in agriculture as it poses a threat to both food safety and human health (Kokkonen et al., 2005). Owing to the limitations of traditional methods of mycotoxin mitigation, there is a growing need for innovative approaches to mycotoxin detoxification. One such approach is postbiotic-based mycotoxin detoxification, which is a promising and innovative method for eliminating mycotoxins from food and feed.

### 6.1 Mechanisms of postbiotic-based mycotoxin detoxification

Research has shown that certain postbiotics can function through various mechanisms in the detoxification of mycotoxins, including adsorption and binding, enzymatic transformation, and competitive exclusion (Chlebicz & Śliżewska, 2020). For example, postbiotic compounds derived from LAB have been demonstrated to adsorb mycotoxins, such as aflatoxins and deoxynivalenol, preventing their absorption in the gastrointestinal tract and promoting their elimination from the body (Gidey et al., 2020). Further research is needed to fully understand the potential of postbiotic-based mycotoxin detoxification and develop effective strategies for its implementation in agriculture and food production.

### 6.2 Postharvest treatment of mycotoxin-contaminated crops

The issue of mycotoxin contamination during postharvest storage is of significant concern within the food and agriculture industry, as it poses a critical and potential risk to food safety and results in substantial economic losses (El-Sayed et al., 2022; Kabak et al., 2006). Mycotoxins can penetrate the food chain either directly or indirectly through contaminated plant-based food components or the growth of toxic fungi on food. Those can form in corn, cereals, soybeans, sorghum, peanuts, and other food and feed crops in the field and during transport, as well as during postharvest storage.

Postbiotics can be introduced as a favorable postharvest treatment and technique to mitigate mycotoxin levels in crops with the aim of reducing existing concentrations or inhibiting potential growth. The application of postbiotics in postharvest treatment represents a proactive and sustainable approach to mycotoxin management. It not only addresses the immediate threat of mycotoxin contamination but also contributes to the long-term safety and quality of stored agricultural commodities.

### 6.3 Detoxification of mycotoxins during food processing

Mycotoxins are a significant concern in the food industry, particularly with regard to safeguarding public health, ensuring food safety, and maintaining the quality of food products. The control of mycotoxin contamination at each stage of food processing is crucial (Agriopoulou et al., 2020). However, mycotoxins pose a significant risk because of their heat stability during cooking and their limited removal by conventional food processing methods (Rao et al., 1982).

Incorporating postbiotics into food processing serves as a solution by acting as natural preservatives during fermentation. A study by Ibrahim et al. (2021) demonstrated that the use of LAB in the fermentation process not only provides health benefits but also extends shelf life and improves safety. Additionally, postbiotics generate organic acids such as lactic, acetic, and formic acids, effectively hindering the growth of pathogens like *Salmonella* and *E. coli*. This strategy aligns with the goal of reducing the risk of foodborne illnesses (Ibrahim et al., 2021; Ogunbanwo et al., 2003).

Postbiotics, notably in fermented dairy like yogurt, show efficacy in detoxifying mycotoxins. LAB-derived compounds, like bacteriocins, are pivotal in hindering mycotoxin-producing mold growth, ensuring yogurt safety and quality (Bartkiene et al., 2022; Gänzle, 2015).

Fermented vegetables, such as sauerkraut and kimchi, benefit from the production of postbiotic metabolites, including organic acids and exopolysaccharides, which neutralize mycotoxins in fermented vegetable products. This enhances the safety and quality of traditional fermented vegetable dishes. Postbiotic-based detoxification also helps to address food security concerns by preserving more food for consumption (Capozzi et al., 2020; Xiang et al., 2019).

Compliance with regional food safety regulations is crucial for incorporating postbiotics in food processing. Varying guidelines, like those of the FDA and EFSA, necessitate manufacturers to substantiate the safety and efficacy of postbiotics, particularly in mycotoxin detoxification. Adherence to these regulations, supported by scientific evidence, is essential for approval and integration into food processing practices (European Parliament and the Council of the European Union, 2008).

Utilizing various food processing techniques to integrate postbiotics has proven to be an effective strategy for minimizing the presence of mycotoxins and improving the safety and quality of processed foods. The successful implementation of postbiotics in a range of food products serves as evidence of their efficacy. It is crucial to adhere to regulatory standards to ensure compliance with safety measures and safeguard the health of consumers.

### 6.4 Use of postbiotics as a preventive measure for mycotoxin contamination

Preventative measures in mycotoxin control are of utmost importance as these toxic compounds have the potential to contaminate crops and pose serious health risks to both humans and animals (Reddy et al., 2010).

Research findings and practical applications have highlighted the potential of postbiotics in preventing mycotoxin contamination in crops (Sadiq et al., 2019). For example, postbiotic compounds such as bacteriocins, generated by LAB, have been shown to inhibit the growth of mycotoxin-producing molds in various food products, including fermented dairy (Gänzle, 2015). This concept can be extended to agriculture, where postbiotics produced by beneficial microorganisms could potentially be applied to crops as biocontrol agents to prevent mycotoxin formation.

Implementing postbiotics in agriculture faces challenges such as optimizing delivery methods for effective crop application, requiring stable formulations. Understanding how postbiotics inhibit mycotoxin formation is crucial for developing targeted strategies. Regulatory and safety assessments are vital to prevent unintended consequences on crop quality and the environment (Hamad et al., 2022; Sadiq et al., 2019).

Further research is crucial to unlock the full potential of postbiotics in preventing mycotoxins in diverse crops. Exploring a wide range of postbiotics under various conditions is essential (Nazareth et al., 2019). Collaborative efforts among microbiologists, agronomists, and food scientists can drive innovative postbiotic strategies, ensuring a safer food supply.

Postbiotics provide advanced answers to combat mycotoxin pollution in the food and agriculture sector. Their substantial potential to improve food safety, quality, and public health requires continued research, development, and regulatory attention. Exploring various postbiotics and their uses brings us closer to ensuring a safer and more reliable food source.

## 7. Safety and regulatory aspects

### 7.1 Safety evaluation of postbiotic-treated food products

Food safety is a dynamic field, vital for public health, and governments are continually striving to improve it. Traditionally, preservatives were relied upon to minimize food spoilage and ensure safety (Barros et al., 2020).

The utilization of natural antimicrobial agents (AAs), particularly those derived from microorganisms such as LAB, has emerged as a noteworthy strategy for enhancing food safety. This approach allows manufacturers to shift away from synthetic additives, ensuring both food safety and the development of healthier food products. LAB strains, recognized as probiotics for their health benefits, produce bioactive soluble substances called postbiotics. These postbiotics possess diverse bioactivities, including antimicrobial properties, rendering them valuable in the food industry (Guimarães et al., 2020; O'Bryan et al., 2018).

Live probiotics, such as LAB strains, *Bifidobacterium* strains offer health benefits through gut colonization but can also impact food characteristics. However, incorporating probiotics into food can help combat pathogens through competition or the production of antimicrobial postbiotics. These postbiotics, derived from food-grade microorganisms, encompass soluble factors, cell compounds, and substances formed during microbial action on food. Additionally, "paraprobiotics" or "ghost probiotics" refer to nonviable microbial cells (Moradi et al., 2020).

Postbiotics contribute significantly to food safety by serving diverse functions, including bio preservation, packaging enhancement, and eradication of foodborne pathogen biofilms. They also play a role in breaking down harmful chemical contaminants like mycotoxins, pesticides, and biogenic amines. The efficacy in food systems relies on factors such as the chosen LAB strain, target microorganism or contaminant, concentration, application form, and food matrix features. Postbiotics, derived from these microorganisms, exhibit antimicrobial effects against pathogens and spoilage microorganisms, proving valuable in the food industry. These postbiotics encompass low-molecular-weight compounds like organic acids, $H_2O_2$, acetoin, and acetaldehyde, as well as high-molecular-weight substances such as bacteriocins and emerging agents like exopolysaccharides (EPS). Bacteriocins, heat-stable antibacterial peptides produced by LAB strains, offer safe applications in food preservation. Researchers are increasingly investigating postbiotic blends to enhance the safety and shelf life of diverse food products (Moradi et al., 2020).

Postbiotics effectively preserve perishable animal-based foods such as meat and fish by countering microbial contamination, extending shelf life, and maintaining freshness. Application methods include direct use, spraying, or coating, with enhanced effectiveness in refrigeration. Examples like marine-origin bacteria-derived postbiotics for fresh fish and divergicin M35-containing postbiotics for smoked salmon showcase their versatility. Bifidin bacteriocin from *Bifidobacterium lactis* benefits ground meat. Postbiotics also diminish virulent factors in foodborne pathogens, enhancing food safety (Koo et al., 2015). Efficacy hinges on compound type, concentration, and food matrix. Overall, postbiotics present a promising solution to microbial contamination challenges in meat and fish, elevating quality and safety (Pujato et al., 2014).

Dairy products have traditionally been carriers for probiotics, but various factors can negatively impact the viability of probiotic strains during processing and storage. As an alternative, postbiotics and individual postbiotic compounds have emerged with beneficial properties, serving as biocontrol agents to ensure the safety of dairy products. Some studies have highlighted the role of different bacteriocins and bacteriocin-producing LAB in controlling defects like cheese blowing (Bogovič Matijašić et al., 2007; Garnier et al., 2019).

However, the brownish color of postbiotics in MRS broth limits their use in many dairy products, except for those naturally colored brown or yellow, like chocolate milk. In dairy, individual postbiotic compounds are often preferred due to this limitation. Milk-based media are proposed as an alternative to synthetic MRS for industrial use. Studies have demonstrated that postbiotics from *Leuconostoc citreum* can effectively inhibit pathogens in milk. Nonetheless, it's essential to consider sensory aspects in dairy product applications, as postbiotics prepared in MRS can negatively affect sensory characteristics, particularly in white, liquid milk. Researchers are exploring alternative media and decolorization methods to address this issue. Postbiotics produced in low-heat milk and milk permeate have been shown to inhibit fungal spoilage in sour cream and semihard cheese without compromising sensory acceptance, offering a promising alternative to traditional antifungal compounds (Barros et al., 2020; Garnier et al., 2019).

Postbiotics also find applications in preserving fruits and vegetables by reducing microbial contamination. In vegetable decontamination and the prevention of foodborne pathogens in fruit juices, postbiotics emerge as secure alternatives to chlorine-based sanitizers (Tenea & Barrigas, 2018).

Postbiotics, particularly from antifungal-producing *Lactobacillus* strains like *Lactobacillus reuteri*, demonstrate effective antifungal properties. They successfully prevent mold in stored bread, enhancing its texture. These postbiotics inhibit fungal growth in diverse foods such as processed cheese, commercial bread, and tomato puree, extending their shelf life at various temperatures. These results highlight the broad applicability of postbiotics in improving the quality and safety of foods, particularly those susceptible to fungal contamination (Muhialdin et al., 2011).

Producing postbiotics in situ by LAB within food items presents a promising method to improve both the quality and safety of the products. Rather than directly incorporating postbiotics, researchers have explored the capability of LAB to generate postbiotics within the food matrix itself (Salas et al., 2019). For instance, research indicates that LAB can generate antifungal metabolites, such as organic acids, free fatty acids, and volatile compounds, in dairy products like sour cream, yogurt, cheese models, and semihard cheese (Costa et al., 2010).

Biofilm formation, a major concern in food safety, involves microorganisms attaching to surfaces, increasing the risk of contamination. LAB postbiotics have shown antimicrobial and antibiofilm properties. Biofilm formation occurs in five stages, and disrupting any of these stages can prevent it. Postbiotics, containing components like bacteriocins, organic acids, biosurfactants, and EPS, can interfere with different stages of biofilm formation. They can be used for both preventing and eliminating biofilms, making them a valuable tool in food safety (Ciandrini et al., 2016).

The interaction between postbiotics and food ingredients is complex. Some postbiotic metabolites, like exopolysaccharides (EPS), can inadvertently protect pathogens in food, posing a challenge to food safety. Additionally, the intricate relationship between postbiotics and food components may reduce their effectiveness (Hartmann et al., 2011).

However, there's an opportunity to create valuable postbiotic substances by adding specific ingredients to bacterial cultures or food. For instance, *Lactobacillus plantarum* strains can produce therapeutic compounds like 10-hydroxy-cis-12-octadecenoic acid (HYA) from linoleic acid. This could enhance the health benefits of food products. HYA has shown antimicrobial activity against *Helicobacter pylori* but requires further research on other pathogens and spoilage microorganisms (Matsui et al., 2017).

Probiotic food packaging using live bacteria faces challenges like reduced viability and uncertain antimicrobial activity. Postbiotics offer an alternative by being incorporated into packaging through coating, immobilization, direct inclusion, or lamination between layers. This approach ensures stability and controlled release, making it a practical choice for active packaging systems (Zabihollahi et al., 2020).

## 7.2 Challenges and future perspectives in postbiotic application for mycotoxin control

Postbiotics derived from acid-treated probiotics show significant effectiveness in mycotoxin removal. Despite these positive findings, challenges persist, necessitating a deeper understanding of detoxification processes and their optimization for practical industrial use. Bridging the gap between scientific research and large-scale implementation is a crucial hurdle to fully exploit these innovative approaches for enhanced nutrition and well-being. Extensive future research is vital to unravel the complex interactions among probiotics, prebiotics, synbiotics, and postbiotics, leading to more effective toxin prevention strategies and realizing their complete health benefits.

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# Postbiotics

## Edited by Dhanasekaran Dharumadurai and Prakash M. Halami

***Postbiotics: Health and Industry*** provides a detailed overview on the fundamentals, biological and therapeutic properties, safety, and application of postbiotics in health and industry.

This book is organized into five sections providing a comprehensive discussion of postbiotics. This book begins with coverage on the fundamentals of postbiotics which includes insights on probiotic microorganisms, postbiotics and host–microbe interaction, analysis, and characterization of postbiotics. The book goes on to delve into the different types of postbiotics and their mechanisms. The remaining portion of this book explores the various health, pharmaceutical, and industrial applications of postbiotics.

### Key Features

- Provides an overview on the separation, characterization, and identification of postbiotics from probiotic microbes
- Includes classes of postbiotics and their mechanisms of action
- Discusses the safety of postbiotics in humans and animals, the use of multiomics to understand the effect of postbiotics on human physiology, and analyzes the existing regulatory framework for postbiotics

### About the Editors

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