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A systematic review to introduce the most effective postbiotics derived from probiotics for aflatoxin detoxification *in vitro*

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REVIEW

Abstract

The purpose of this study was to gather information about the percentage of aflatoxin decontamination by postbiotics and to find the most effective postbiotic. In this review article, studies were collected from databases. All the articles related to experimental studies were included in the study. It appeared that the postbiotics derived from *Lentilactobacillus kefiri, Lentilactobacillus kefiri, Lacticaseibacillus rhamnosum, Lacticaseibacillus rhamnosum* and *Pediococcus pentosaceus* probiotic bacteria were the most effective postbiotics with 97.22%, 95.27%, 86.2%, 81.4% and 91% inhibitory effect against aflatoxins B_1, B_2, G_1, G_2 and M_1, respectively. Therefore, postbiotics could be used as safe anti-aflatoxin agents in food products.

Keywords: aflatoxins, cell-free supernatant, killed probiotics, non-viable probiotics, postbiotics

Introduction

Aspergillus flavus and *Aspergillus parasiticus* produce aflatoxins (AFs) which have mutagenic, carcinogenic, hepatotoxic, immunosuppressive and teratogenic effects in humans and animals, and are responsible for the loss of crops and animal feed (Hernandez-Mendoza *et al.*, 2009a, 2009b). They are present in food products, such as corn, peanuts, beans, dried fruits and dairy products, including milk (Ambadoyiannis *et al.*, 2004; Dalié *et al.*, 2010). Owing to their high thermal stability, aflatoxins remain in raw food and are not destroyed by processing and cooking, and are trasferred to humans through food (Zain, 2011). Since mycotoxins have low molecular weight, they are promptly absorbed in the gastrointestinal tract (GIT) and can be discovered in blood and milk 15 min and 12 h after intake, respectively (Martins *et al.*, 2001). More than 20 metabolites of the AF group are known, among which the most important ones are B_1, B_2, G_1 and G_2 with different levels of toxicity (Alshannaq and Yu 2017; Laciakova *et al.*, 2008; Mahmood *et al.*, 2018). These terms are derived through fluorescence under ultraviolet (UV) rays, blue (B) or green (G), and comparative chromatographic migration patterns through thin layer chromatography (TLC) (Lizárraga-Paulín *et al.*, 2011; Vosough *et al.*, 2013). AFM_1 and AFM_2 are hydroxylated metabolites of AFB_1 and AFB_2. Approximately 0.3–6.2% of AFB_1 ingested by animals is metabolized to AFM_1 (Figure 1) (Afshar *et al.*, 2020; Battacone *et al.*, 2003; Kumar *et al.*, 2017; Oatley *et al.*, 2000).

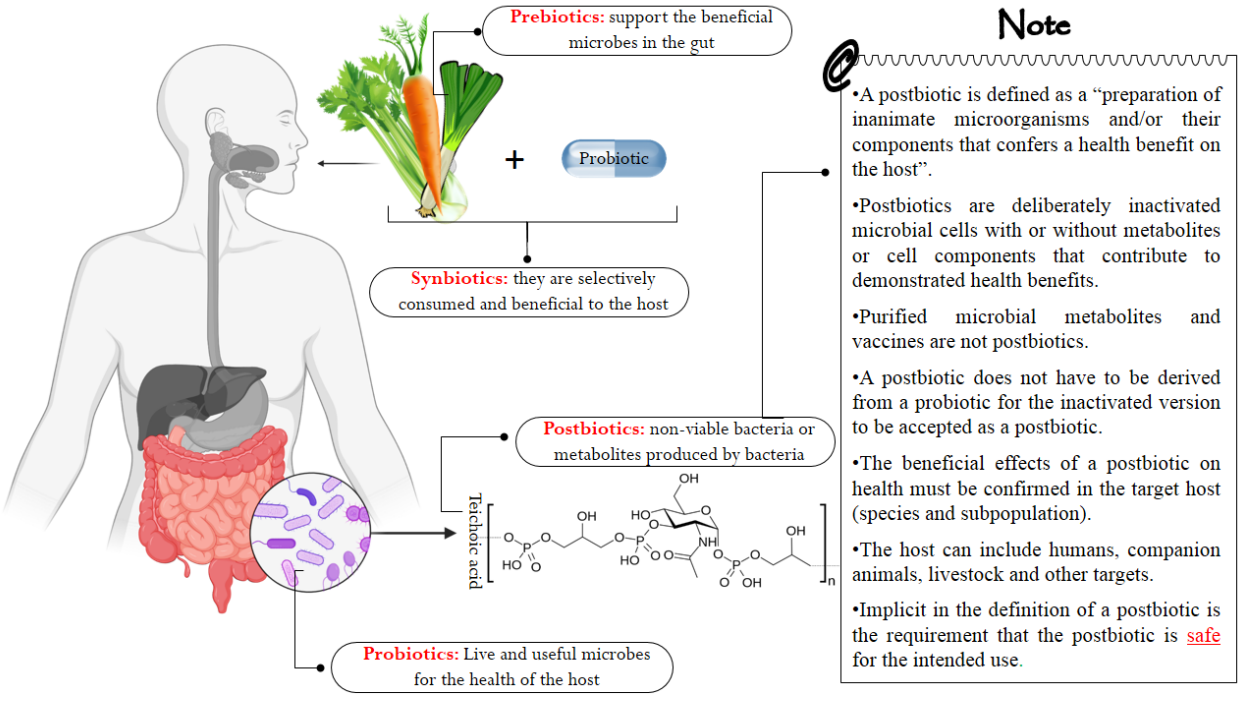

Figure 1. Sources of aflatoxin exposure and its flow in the food chain.

The order of acute and chronic toxicity of different aflatoxins is $AFB_1 > AFG_1 > AFB_2 > AFG_2$, where AFM_1 and AFM_2 are less toxic than their precursors (Ahmadi, 2020). Among these, AFB_1 showed the highest toxicity for animals and humans (Dhanasekaran *et al.*, 2011; Sklan *et al.*, 2001; Yu *et al.*, 2008). The International Agency for Research on Cancer (IARC) classified AFB1 and AFM1 as human carcinogens belonging to Group 1 and Group 2B, respectively (Caloni *et al.*, 2006; Darwish *et al.*, 2014; Marchese *et al.*, 2018). The Codex Alimentarius specifies maximum limit of aflatoxins as 15 µg/kg for almonds, hazelnuts, Brazil nuts, peanuts and pistachio nuts for further processing, and 10 µg/kg for almonds, Brazil nuts, hazelnuts and pistachio nuts for direct consumption and dried figs, with a maximum limit of AFM_1 as 0.5 µg/kg for milk. However, the maximum levels of aflatoxins in foods vary globally, depending on the type of product and also on the import/export regime (Food and Agriculture Organization (FAO), 2003; Pickova *et al.*, 2021). There are various approaches to reduce or remove mycotoxins, including physical (thermal inactivation, radiation, magnetic carbon absorption), chemical (treatment with acid, alkali, or oxidizing agent), and biological (microorganisms) treatments (Peng *et al.*, 2018). Among these methods, biological methods attracted a great deal of attention because of their high efficiency, low price, compatibility with the environment, and complete detoxification under favorable conditions (Adebo *et al.*, 2017; Armando *et al.*, 2012; Shetty and Jespersen, 2006). Among the biological methods, detoxification using postbiotics has gradually become the most appropriate biological detoxification method, because the use of microorganisms in food chains required a long process of national and international authorization (Zhou *et al.*, 2017; Zychowski *et al.*, 2013).

Some microorganisms, especially probiotic bacteria, have received special attention because of their various beneficial effects, such as an easy, controllable, and safe method without loss of nutritional value to remove aflatoxins (Mahmood *et al.*, 2018). Along with the remarkable progress made in this field, the researchers got acquainted with a new concept of probiotics called postbiotics. The word "postbiotic" refers to bioactive compounds and does not match the definitions of prebiotics, synbiotics and probiotics. In mid-2021, the International Scientific Association for Probiotics and Prebiotics (ISAPP) provided a precise and well-considered definition of postbiotics, which is the "preparation of inanimate microorganisms and/or their components that confers a health benefit on the host" (Salminen *et al.*, 2021). According to the above definition, the term postbiotic refers to a set of macro and micro molecules, such as inactive microorganisms (cells that are not alive), parts of a cell (surface layer proteins, endo- and exo-polysaccharides, teichoic acid, muropeptides and cell-free supernatants [CFS]), or metabolites of a cell (bacteriocins, short-chain fatty acids [SCFA], organic acids and enzymes that are produced naturally by living probiotic cells during fermentation) (Figure 2) (Homayouni-Rad *et al.*, 2021).

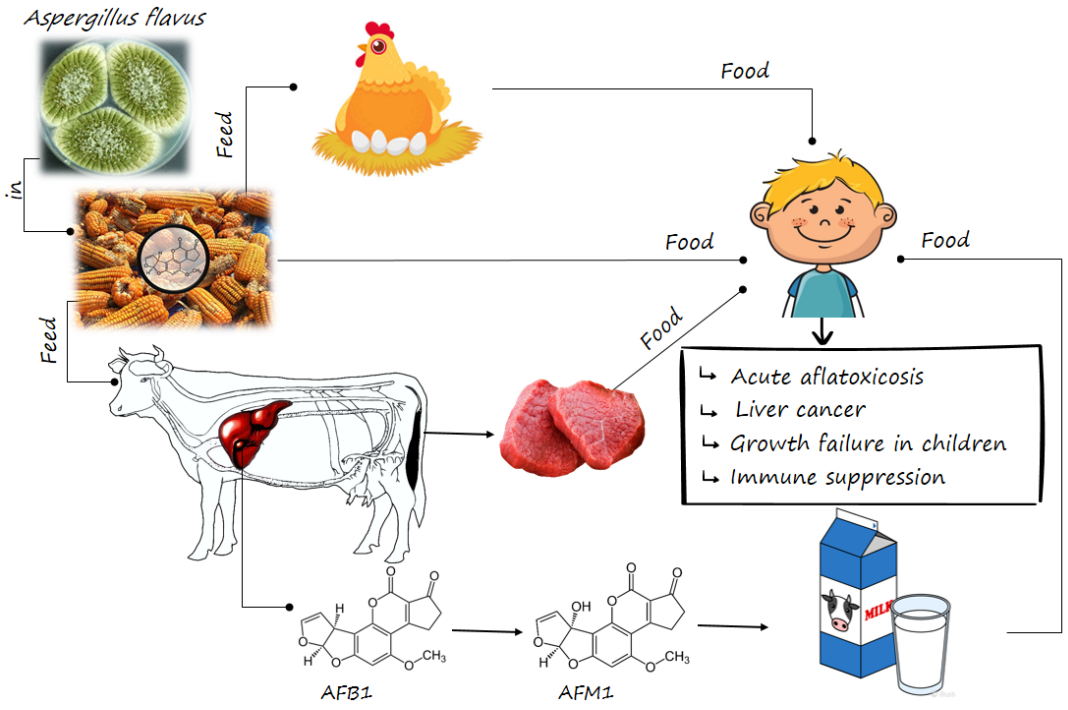

Figure 2. Definition of the family of probiotics, prebiotics, synbiotics and postbiotics (Vinderola *et al.*, 2022).

Terms such as parapsychobiotics, paraprobiotics, non-viable probiotics, heat-killed probiotics, ghost probiotics, meta-biotics, cell-free supernatant, biogenic, tyndallized probiotics, bacterial lysates, and postbiotics are also used (Homayouni-Rad *et al.*, 2021; Salminen *et al.*, 2021), although term postbiotic is used more commonly.

Probiotics are mostly lactic acid bacteria (Walhe *et al.*, 2022) and have many health benefits; however, because they are alive, they have limitations for use and treatment. Probiotic bacteria must maintain their viability even in inappropriate conditions, such as preparation of food, storage, processing, distribution as well as passing through the digestive system, so that they can perform their health-giving effects (Homayouni-Rad *et al.*, 2020; Rad *et al.*, 2020a). In spite of passing these challenges and survival, adverse reactions may occur, such as resistance gene transfer between other intestinal microbiota, different colonization patterns, presence of virulence factors in some probiotic species, preventing the colonization of other intestinal microbiota, meningitis, infectious endocarditis, urinary tract infections, and bacteremia, especially in infants, patients treated with antibiotics and have weak immunity (Abbasi *et al.*, 2021a, 2021b; Abriouel *et al.*, 2015; Kim *et al.*, 2018; Liong 2008).

Postbiotics have overcome these adverse effects and appeared as a suitable alternative to probiotics. In recent years, it has been shown that the viability of a probiotic is not necessarily to benefit from its favorable effects (Piqué *et al.*, 2019). It has been proven both *in vivo* and *in vitro* that the beneficial effects of probiotics and postbiotics include antibacterial, antiviral, antifungal, antioxidant, anti-proliferation, anti-mutation, anti-cancer, anti-obesity, anti-diabetic, anti-blood pressure, and immune system booster (Moradi *et al.*, 2021). In addition, postbiotics have unique features, such as safe origin, no toxic effect, low cost of preparation and transportation, and long shelf life (Homayouni-Rad *et al.*, 2021). Many results obtained from clinical studies indicated the suitability of digestion, absorption, metabolism and distribution of postbiotics (Asif *et al.*, 2023; Gao *et al.*, 2019; Shenderov 2013; Singh *et al.*, 2018), and these characteristics are suitable for the food and pharmaceutical industries (Aghebati-Maleki *et al.*, 2021).

Many studies were carried out to reduce the amount of aflatoxins by postbiotics in food model systems (Karazhiyan *et al.*, 2016; Nassar *et al.*, 2018) and culture media (Taheur *et al.*, 2019; Vosough *et al.*, 2014), but, so far, no study is discovered on the systematic review of articles for (1) determining the amount of aflatoxin reduction by postbiotics and (2) identifying and introducing the most effective postbiotic to reduce aflatoxins.

Materials and Methods

Search Strategy

This systematic review was based on the guidelines of the Preferred Reporting Items for Systematic Reviews

and Meta-Analyses (PRISMA), with the reports cited in this review being consistent (Moher *et al.*, 2015). In this study, the population, intervention, comparison and outcome (PICO) approach was used to identify terms.

Our study started on 01 September 2022 and continued up to 27 October 2022. Four electronic databases, including Web of Science, Google Scholar, PubMed and Scopus, were used from the beginning till 25 September 2022. The following keywords were used to search databases: "Probiotics" AND "Postbiotics" OR "Non-viable probiotics" OR "Parapsychobiotics" OR "Paraprobiotics" OR "Heat-killed probiotics" OR "Ghost probiotics" OR "Metabiotics" OR "Cell-free supernatant" OR "Biogenic" OR "Tyndallized probiotics" OR "Probiotic lysates" AND "Detoxification" AND "Aflatoxin." These words along with synonyms or Medical Subject Heading (MeSH) terms were added to the search terms.

Duplicate cases were excluded from the final selection of the study. As this review focused on published papers, gray literature found on Google Scholar was not included. After selecting relevant titles and abstracts, all texts of the selected articles were evaluated. A PRISMA flowchart was used to graphically represent the selection of final articles (Figure 3).

Inclusion and exclusion criteria

Articles were considered eligible if the full-text articles met the below-mentioned conditions:

1. Full text of articles must be accessible
2. Article must be written in English
3. Articles were peer-reviewed
4. Articles written about the detoxification of aflatoxin by postbiotics
5. Articles about the inhibitory effect of probiotic metabolites or killed probiotics against aflatoxin
6. *In vitro* articles
7. Articles that considered probiotic bacteria

Articles that did not use postbiotics as an inhibitory agent and dealt with other methods or that document *in vivo* conditions were excluded from the study. Furthermore, conference abstracts, letters to editors, and non-peer-reviewed studies were also excluded. In this study, two reviewers were used to include the articles. They screened all titles and abstracts independently to find eligible articles. Sometimes, if needed, the third referee was consulted to clear doubts, increase self-confidence and reduce errors. For example, if it was not specified in articles whether postbiotics or probiotics were used, a third reviewer was consulted for clarity.

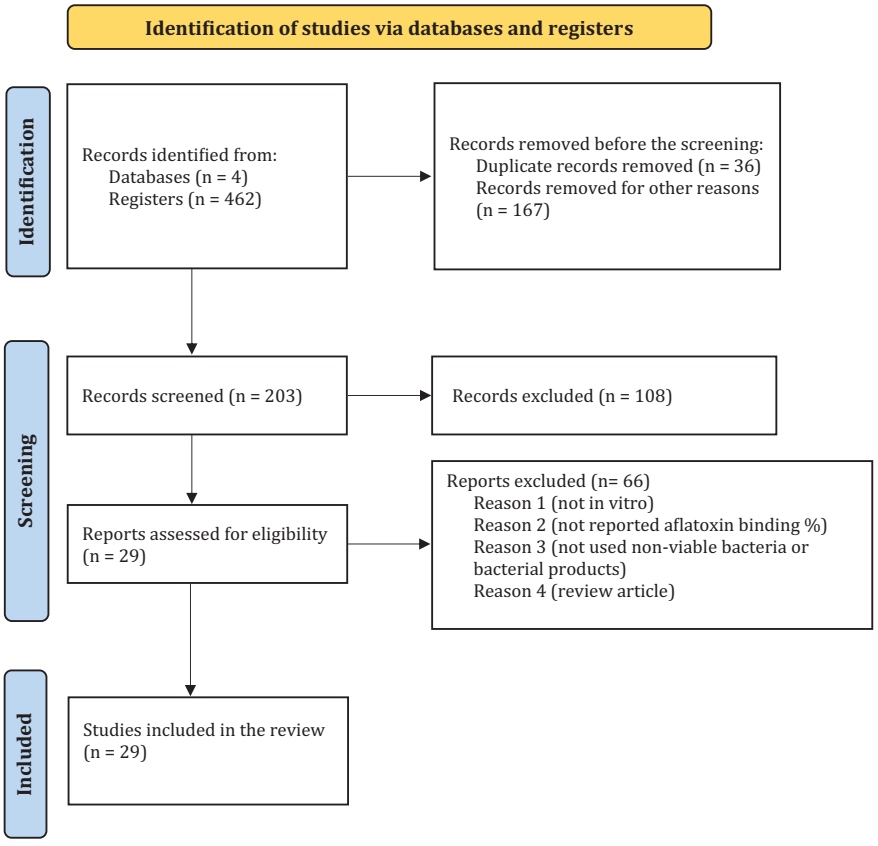

Figure 3. PRISMA flow chart of the systematic review search process.

Data extraction

Two reviewers independently extracted the data using a pre-designed table. The extracted data included article title, year of publication, probiotics type, type of aflatoxin, results, and other relevant findings. The extracted data were analyzed.

Quality assessment

Risk of bias assessment for each article included evaluation in the six areas of research logic: description of methodology, rationale of research, description of results, characterization and testing, overall conclusions, and description of discussion (Khizer *et al.*, 2021).

The current risk of bias framework was adapted from the Cochrane Handbook for Systematic Reviews of Interventions (Tarsilla, 2010). Owing to the lack of a suitable risk assessment tool in this particular field, namely food safety, the evaluation process of each included study was carried out independently by two members of the review team. The final decision was that once any disagreements were resolved in the process, a categorization and binding agreement would be reached.

At the end of the work, a systematic review checklist was used to ensure accuracy of work process (Page *et al.*, 2021).

Results and Discussion

Content extraction

The present paper considered a new field of research that focused on biological compounds derived from probiotics, with a limited number of available studies. The information for this study was extracted from 29 articles in line with the objectives of the present study. It should be noted that the results of this systematic review have limitations, as in some papers, it was not mentioned whether the strain in question was a probiotic or not.

Various factors, such as proving that LABs are probiotics, type of inactivation of microorganisms and extraction methods, and the exact nature of the consumed postbiotic (cell-free supernatant or cells killed by different physical and chemical treatments), were also considered. Therefore, in the future studies, it would be important to determine the aforementioned factors to obtain healthy and anti-toxic effects of the considered postbiotics.

Creation and isolation of postbiotics

As mentioned, probiotics must be alive to exert their health effects, but their viability has created limitations for use and treatment. In recent years, it has been shown that the viability of a probiotic is not related to its beneficial effects. Hence, the focus on the health benefits of microorganisms has shifted from live probiotics to postbiotics. According to different definitions, postbiotics include both nonviable bacteria and substances derived from bacterial metabolism. These side products provide specific physiological effects to host by providing more biological activity (Cicenia *et al.*, 2016), and because postbiotics do not contain live microorganisms, the risks associated with their consumption are minimized. Furthermore, postbiotics can be used in a controlled and standardized manner (Shigwedha, 2014). The problem of dose standardization, which is a significant issue in probiotic production, does not exist in the case of postbiotics. Production methods have been investigated by researchers to produce postbiotics in a controlled, standardized, and efficient manner to enable their analysis and use in food, pharmaceuticals and nutritional programs. Although there are no clear international regulatory standards for postbiotics, in some countries, postbiotics have been included in their national regulations and their products have entered public domain (Gelmetti *et al.*, 2022; Kiran *et al.*, 2023). The use of postbiotics derived from probiotics is a specific, effective, environment-friendly, cheap and safe strategy. Since postbiotics have unique features, such as specific chemical structure, safe and long shelf life, they can be used in a delivery system, such as medicine or beneficial foods, to achieve wellness goals, prevention and treatment of diseases (Shigwedha, 2014; Tomar *et al.*, 2015).

In the food industry, postbiotics are produced using natural and laboratory (physical and chemical) methods. One of the most important methods to produce postbiotics in a natural form is the fermentation process. In this, prebiotic compounds are rendered to microorganisms involved in the fermentation process to produce postbiotics (Izuddin *et al.*, 2018). By using different laboratory methods, postbiotics are produced in a pure form with higher performance (Dunand *et al.*, 2019). To inactivate and destroy bacterial cells, physical methods, such as mechanical breaking, heat, gamma, or ultraviolet radiation, high hydrostatic pressure and ultrasound, or chemical methods, such as inactivation by acid, are used (Fan *et al.*, 2021). These methods change cellular structures and their physiological functions. Bacteria are destroyed by applying these methods and are no longer able to grow, but they still maintain their positive effects (de Almada *et al.*, 2016; Rad *et al.*, 2020b). In order to destroy and inactivate bacterial cells, extraction postbiotics by solvent and sonicator are also used (Amaretti *et al.*, 2013).

Table 1. Postbiotics derived from probiotics to eliminate the toxic effect of AFB_1, B_2, G_1, G_2 and M_1 in food model systems and culture media.

Probiotic strains	Treatment for obtaining postbiotics/type postbiotics	Medium kind	Probiotics concentration (CFU/mL)	Exposure time	AFB_1 removal (%)	References
L. rhamnosus (GG)	Heat-killed	Liquid media	-	4 h	81	El-Nezami *et al.*, 1998a
L. rhamnosus (LC-705)	Heat-killed	Liquid media	-	4 h	82	El-Nezami *et al.*, 1998b
L. rhamnosus (GG)	Acid-killed	Aqueous solution	1×10^9	-	75.52	Ghofrani *et al.*, 2018
L. rhamnosus (GG)	Cell wall	Aqueous solution	1×10^9	-	75.28	Ghofrani *et al.*, 2018
E. faecium (EF031)	Heat-killed	Aqueous solution	1×10^{10}	48 h	37.5	Topcu *et al.*, 2010
E. faecium (M74)	Heat-killed	Aqueous solution	1×10^{10}	24 h	30.5	Topcu *et al.*, 2010
L. plantarum	Heat-killed	Aqueous solution	1×10^{10}	48 h	60-70	Damayanti *et al.*, 2017
L. rhamnosus (GG)	Heat-killed	Cotton seed	1×10^9	24 h	47	Vosough *et al.*, 2013
L. rhamnosus (GG)	Acid-killed	Cotton seed	1×10^9	24 h	49	Vosough *et al.*, 2013
L. rhamnosus (GG)	Trypsin-treated	Aqueous solution	-	-	88	Lahtinen *et al.*, 2004
L. rhamnosus (GG)	α-Chymotrypsin-treated	Aqueous solution	-	-	56	Lahtinen *et al.*, 2004
L. rhamnosus (GG)	N-glycoproteinase F-treated	Aqueous solution	-	-	85	Lahtinen *et al.*, 2004
L. rhamnosus (GG)	SDS-treated	Aqueous solution	-	-	89	Lahtinen *et al.*, 2004
L. rhamnosus (GG)	Urea-treated	Aqueous solution	-	-	78	Lahtinen *et al.*, 2004
L. rhamnosus (GG)	$CaCl_2$-treated	Aqueous solution	-	-	49	Lahtinen *et al.*, 2004
L. rhamnosus (GG)	$MgCl_2$-treated	Aqueous solution	-	-	54	Lahtinen *et al.*, 2004
L. rhamnosus (GG)	EDTA-treated	Aqueous solution	-	-	49	Lahtinen *et al.*, 2004
L. rhamnosus (GG)	EGTA-treated	Aqueous solution	-	-	50	Lahtinen *et al.*, 2004
L. rhamnosus (GG)	Heat-killed	PBS	1×10^{10}	-	85	Haskard *et al.*, 2000
L. rhamnosus (GG)	Acid-killed	PBS	1×10^{10}	-	91	Haskard *et al.*, 2000
L. rhamnosus (GG)	Acid-killed	Yoghurt	2.1×10^9	24 h	77.9	Karazhiyan *et al.*, 2016
L. rhamnosus (GG)	Heat-killed	Yoghurt	2.1×10^9	24 h	72.5	Karazhiyan *et al.*, 2016
L. rhamnosus (GG)	Ultrasound-treated	Yoghurt	2.1×10^9	24 h	78.9	Karazhiyan *et al.*, 2016
L. plantarum (C88)	Heat-killed	Aqueous solution	1×10^{10}	-	50–60	Huang *et al.*, 2017
B. licheniformis (CFR1)	CFS[1]	PBS[2]	-	72 h	93.75	Rao *et al.*, 2017
E. faecium (MF4)	Heat-killed	PBS	1×10^8	24 h	70–80	Fernandez *et al.*, 2015
E. faecium (GJ40)	Heat-killed	PBS	1×10^9	24 h	80–90	Fernandez *et al.*, 2015

L. rhamnosus (GG)	Heat-killed	PBS	10^9	12 h	49	Vosough *et al.*, 2014
L. rhamnosus (GG)	Acid-killed	PBS	10^9	12 h	50	Vosough *et al.*, 2014
L. brevis	CFS	PBS	-	7 d	80–90	Taheur *et al.*, 2019
P.pentosaceus	CFS	PBS	-	7 d	30–40	Taheur *et al.*, 2019
L. kefiri	CFS	PBS	-	7 d	91.87	Taheur *et al.*, 2019
B. subtilis (ANSB060)	CFS	Aqueous solution	10^8	72 h	78.7	Gao *et al.*, 2011
L. reuteri (NRRL14171)	EPS	Aqueous solution	$1\text{-}3\times10^{10}$	4 h	<5	Hernandez-Mendoza *et al.*, 2009
L. reuteri (NRRL14171)	Protoplasts	Aqueous solution	$1\text{-}3\times10^{10}$	4 h	<10	Hernandez-Mendoza *et al.*, 2009
L. reuteri (NRRL14171)	Spheroplasts	Aqueous solution	$1\text{-}3\times10^{10}$	4 h	30–40	Hernandez-Mendoza *et al.*, 2009
L. reuteri (NRRL14171)	Bacterial cell wall	Aqueous solution	$1\text{-}3\times10^{10}$	4 h	60–70	Hernandez-Mendoza *et al.*, 2009
L. reuteri (NRRL14171)	Teichoic acid-deficient bacteria	Aqueous solution	$1\text{-}3\times10^{10}$	4 h	20	Hernandez-Mendoza *et al.*, 2009
L. reuteri (NRRL14171)	Bacteria treated with the polycation	Aqueous solution	$1\text{-}3\times10^{10}$	4 h	40–50	Hernandez-Mendoza *et al.*, 2009
L. casei (Shirota)	EPS[3]	Aqueous solution	$1\text{-}3\times10^{10}$	4 h	<10	Hernandez-Mendoza *et al.*, 2009
L. casei (Shirota)	Protoplasts	Aqueous solution	$1\text{-}3\times10^{10}$	4 h	10–20	Hernandez-Mendoza *et al.*, 2009
L. casei (Shirota)	Spheroplasts	Aqueous solution	$1\text{-}3\times10^{10}$	4 h	50–60	Hernandez-Mendoza *et al.*, 2009
L. casei (Shirota)	Bacterial cell wall	Aqueous solution	$1\text{-}3\times10^{10}$	4 h	60–70	Hernandez-Mendoza *et al.*, 2009
L. casei (Shirota)	Teichoic acid-deficient bacteria	Aqueous solution	$1\text{-}3\times10^{10}$	4 h	20–30	Hernandez-Mendoza *et al.*, 2009
L. casei (Shirota)	Bacteria treated with the polycation	Aqueous solution	$1\text{-}3\times10^{10}$	4 h	40–50	Hernandez-Mendoza *et al.*, 2009
L. rhamnosus (RC007)	CFS	GIT[4] simulation	1×10^8	48 h	80.40	Fochesato *et al.*, 2019
L. paracasei (KC39)	Acid-killed	Milk	-	-	69.56	Fahim *et al.*, 2021
L. acidophilus (ATCC 20552)	Heat-killed	PBS	10^8	1 h	73.8	Elsanhoty *et al.*, 2013
L. rhamnosus (TISTR 541)	Heat-killed	PBS	10^8	1 h	84.7	Elsanhoty *et al.*, 2013
L. sanfranciscensis (DSM20451)	Heat-killed	PBS	10^8	1 h	44.5	Elsanhoty *et al.*, 2013
B. angulatum (DSMZ 20098)	Heat-killed	PBS	10^8	1 h	69.5	Elsanhoty *et al.*, 2013
L. rhamnosus (GG)	Heat-killed	PBS	2×10^9	2 h	47.76	Balsini *et al.*, 2021
B.amyloliquefaciens (WF2020)	CFS	Liquid culture	-	96 h	37.16	Chen *et al.*, 2022b
L. kefiri	CFS	Liquid media	10^8	7 d	97.22	Taheura *et al.*, 2021
L. kunkeei	Heat-killed	PBS	-	-	54.56	Ebrahimi *et al.*, 2021
L. casei (Shirota)	Acid-killed	PBS	10^9	72 h	62	Ondiek *et al.*, 2022

(*continues*)

Table 1. (Continued)

Probiotic strains	Treatment for obtaining postbiotics/type postbiotics	Medium type	Probiotics concentration (CFU/mL)	Time	AFB_2 removal (%)	References
L. brevis	CFS	PBS	-	7 d	60–70	Taheur *et al.*, 2019
P. pentosaceus	CFS	PBS	-	7 d	30–40	Taheur *et al.*, 2019
L. kefiri	CFS	PBS	-	7 d	95.27	Taheur *et al.*, 2019
L. acidophilus (ATCC 20552)	Heat-killed	PBS	108	1 h	69.9	Elsanhoty *et al.*, 2013
L. rhamnosus (TISTR 541)	Heat-killed	PBS	108	1 h	80.1	Elsanhoty *et al.*, 2013
L. sanfranciscensis (DSM20451)	Heat-killed	PBS	108	1 h	35.4	Elsanhoty *et al.*, 2013
B. angulatum (DSMZ 20098)	Heat-killed	PBS	108	1 h	62.9	Elsanhoty *et al.*, 2013
L. kefiri	CFS	Liquid media	108	7 d	95.27	Taheura *et al.*, 2021
L. kefiri	Heat-killed	PBS	-	-	39.57	Ebrahimi *et al.*, 2021
B. subtilis (ANSB060)	CFS	Aqueous solution	10^8	72 h	61	Gao *et al.*, 2011
L. acidophilus (ATCC 20552)	Heat-killed	PBS	108	1 h	71.7	Elsanhoty *et al.*, 2013
L. rhamnosus (TISTR 541)	Heat-killed	PBS	108	1 h	86.2	Elsanhoty *et al.*, 2013
L. sanfranciscensis (DSM20451)	Heat-killed	PBS	108	1 h	41.4	Elsanhoty *et al.*, 2013
B. angulatum (DSMZ 20098)	Heat-killed	PBS	108	1 h	66.8	Elsanhoty *et al.*, 2013
L. kefiri	Heat-killed	PBS	-	-	42.48	Ebrahimi *et al.*, 2021
L. acidophilus (ATCC 20552)	Heat-killed	PBS	10^8	1 h	67.9	Elsanhoty *et al.*, 2013
L. rhamnosus (TISTR 541)	Heat-killed	PBS	10^8	1 h	81.4	Elsanhoty *et al.*, 2013
L. sanfranciscensis (DSM20451)	Heat-killed	PBS	10^8	1 h	33.4	Elsanhoty *et al.*, 2013
B. angulatum (DSMZ 20098)	Heat-killed	PBS	10^8	1 h	61.7	Elsanhoty *et al.*, 2013
L. kefiri	Heat-killed	PBS	-	-	20.52	Ebrahimi *et al.*, 2021

B. subtilis (ANSB060)	CFS	Aqueous solution	10^8	72 h	75	Gao *et al.*, 2011
E. faecium	Heat-killed	Milk	7	28 d	78.4	Sarlak *et al.*, 2017
L. acidophilus (ATCC 20552)	Heat-killed	PBS	-	24 h	43.9	Elsanhoty *et al.*, 2014
L. rhamnosus (TISTR 541)	Heat-killed	PBS	-	24 h	46.2	Elsanhoty *et al.*, 2014
L.plantrium	Heat-killed	PBS	-	24 h	41.3	Elsanhoty *et al.*, 2014
B. angulatum (DSMZ 20098)	Heat-killed	PBS	-	24 h	46.4	Elsanhoty *et al.*, 2014
P. acidilactici (RC005)	CFS	Milk	-	48 h	22	Martinez *et al.*, 2019
P. pentosaceus (RC006)	CFS	Milk	-	48 h	91	Martinez *et al.*, 2019
L. rhamnosus (RC007)	CFS	Milk	-	48 h	28	Martinez *et al.*, 2019
K. marxianus (VM003)	CFS	Milk	-	48 h	100	Martinez *et al.*, 2019
L. Plantarum	Heat-killed	PBS	1×10^9	72 h	51.7	Abdelmotilib*et al.*, 2018
L. acidophilus	Heat-killed	PBS	1×10^9	72 h	67.92	Abdelmotilib*et al.*, 2018
B. bifidum	Heat-killed	PBS	1×10^9	72 h	70.62	Abdelmotilib*et al.*, 2018
K. lactis	Heat-killed	PBS	1×10^9	72 h	69.14	Abdelmotilib*et al.*, 2018
CPYS-NV[5]	Heat-killed	PBS	1×10^9	72 h	87.92	Abdelmotilib*et al.*, 2018
L. rhamnosus (ATCC 7469)	Heat-killed	UHT milk	10^{10}	24 h	12.3	Nassar *et al.*, 2018)
L. acidophilus (DSMZ 20079)	Heat-killed	UHT milk	10^{10}	24 h	27.3	Nassar *et al.*, 2018
L. rhamnosus (ATCC 7469)	Bacteriocin	UHT milk	10^{10}	24 h	77.3	Nassar *et al.*, 2018
L. acidophilus (DSMZ 20079)	Bacteriocin	UHT milk	10^{10}	24 h	72.6	Nassar *et al.*, 2018
L. paracasei (108)	Heat-killed	PBS	10^8–10^9	24 h	78.7	Cruz *et al.*, 2020
L. plantarum (49)	Heat-killed	PBS	10^8–10^9	24 h	76.6	Cruz *et al.*, 2020
L. fermentum (111)	Heat-killed	PBS	10^8–10^9	24 h	78.3	Cruz *et al.*, 2020

[1] Cell-free supernatant
[2] Phosphate-buffered salin
[3] Exopolysaccharides
[4] Gastrointestinal tract
[5] Combination of nonviable strains (*B. bifidum* + *L. acidophilus* + *L. Plantarum* + *S.* cerevisiae + *k. lactis*)

After this, steps such as centrifugation, dialysis and freeze-drying are also used to increase the efficiency of production and storage of these compounds (Amaretti *et al.*, 2013). Despite the existence of various techniques to obtain postbiotics, it is essential to optimize culture environments and bacterial growth conditions along with using new methods of extraction and identification. After achieving optimal production conditions on a laboratory scale, optimization in industry is recommended to maximize production. Finally, appropriate dose and frequency of use are determined.

It is important to note that postbiotics, unlike probiotics, do not add new species to intestinal microbiota, but like prebiotics, they develop the existing probiotics in the digestive system (Homayouni-Rad *et al.*, 2020, 2021). Therefore, it is necessary to carry out a comprehensive investigation for safety, optimization of different extraction methods, identification of the chemical composition of postbiotics, and the physiological role of postbiotics in the biological system to facilitate and use postbiotic compounds in food formulations.

The mechanism of action of postbiotics on the detoxification of aflatoxins

In the biological strategy, detoxification is done through a microbe or non-pathogenic enzymes and/or their catabolic pathways not only to reduce/remove but also to suppress toxins (Afshar *et al.*, 2020). One of these non-pathogenic microbes is probiotics, considered as safe (GRAS) and having health benefits (Afshar *et al.*, 2020). Probiotics can counteract the toxic effects of aflatoxins by the following three mechanisms: (1) Possibility of probiotics competing with aflatoxigenic mold strains to occupy space or an ecological niche, use available nutrients, and reduce the biosynthesis of aflatoxins (probiotics), (2) degradation of aflatoxins by enzymes (postbiotics), and (3) bind aflatoxins to the cell wall of probiotic strains and prevent its intestinal absorption (postbiotics) (Zhao *et al.*, 2016). In general, postbiotics can be classified into different groups based on their biological structure (lipid, protein, carbohydrate, etc.) (Thorakkattu *et al.*, 2022). Different types of postbiotics and their action in detoxification of aflatoxins are shown in Table 2.

Some studies reported that probiotics can produce metabolites, such as organic acids, bacteriocins and even hydrogen peroxide, that inhibit production of aflatoxins and also interfere in the biosynthesis of aflatoxins (Gerbaldo *et al.*, 2012; Gourama and Bullerman 1995).

Probiotics can even remove the produced aflatoxins using biodegradation or biosorption mechanisms (Afshar *et al.*, 2020). For removal of aflatoxins, biodegradation is a permanent process but takes a longer period than biological absorption. Biodegradation is done by extracellular or intracellular enzymes (Zhao *et al.*, 2016). Aflatoxins are decomposed by different mechanisms, such as epoxidation, hydroxylation, dehydrogenation, and reduction, depending on the decomposing agent (Martinez *et al.*, 2019). Double furan ring and coumarin structure are common in the chemical structure of aflatoxins (Adebo *et al.*, 2017; Dalié *et al.*, 2010). During microbial degradation, microbial catabolic pathways and specific enzymes present in cell-free supernatant influence coumarin conformational changes and difuran ring cleavage in aflatoxins (Adebo *et al.*, 2017; Dalié *et al.*, 2010).

Degradation or enzymatic conversion is the biological breakdown of mycotoxins into less or nontoxic compounds; only certain cells of bacteria and fungi are effective in this change (Chlebicz and Śliżewska 2020). In biodegradation, aflatoxins are converted into other undesirable metabolites, such as aflatoxicol (AFL), which is possibly harmful to health (Ji *et al.*, 2016). Therefore, it appears that biological absorption is a more suitable method than biological degradation.

Biological absorption is a short-term process, and the formed complex is unstable and depends on the number of absorption sites and the affinity of aflatoxins to these sites (Adebo *et al.*, 2017; Afshar *et al.*, 2020; Luo *et al.*, 2018). In biological absorption, an unspecified physical connection to the cell wall takes place, and aflatoxins are removed by physical adhesion. Aflatoxins bind to the carbohydrate components of the cell wall. Covalent bindings

Table 2. Different types of postbiotics and their action in detoxification of aflatoxins.

Different types of postbiotics	Method of detoxification of aflatoxin	References
Supernatant without cells	Degradation	Chen *et al.*, 2022a
Exopolysaccharide	Binding, adsorption	Taheur *et al.*, 2017
Cell wall parts (cell surface proteins, teichoic acids and peptidoglycan)	Binding, adsorption	Khosravi-Darani *et al.*, 2020; Liew *et al.*, 2018; Zolfaghari *et al.*, 2020
Short-chain fatty acids	Degradation	Śliżewska and Smulikowska, 2011
Vitamins	-	-
Cell lysate	Binding, adsorption	Lahtinen *et al.*, 2004

or metabolic degradation is not used in this mechanism. Therefore, nonviable bacteria also can bind and remove aflatoxin (Bueno *et al.*, 2007; Hernandez-Mendoza *et al.*, 2010).

Probiotic bacteria, similar to other Gram-positive bacteria, have a complex arrangement of macromolecules. The wall with a special structure contains compounds such as peptidoglycan (PG) sacculus that surround cytoplasmic membrane and other glycopolymers, such as teichoic acids (TAs) or polysaccharides (PSs) and proteins that decorate PG. Each of these compounds has a different function, for example, the TA unit is divided into two groups: (1) wall teichoic acids (WTAs), which are covalently linked to the PG molecule and (2) lipoteichoic acids (LTAs), which are anchored in cytoplasmic membrane with a glycolipid moiety (Chapot-Chartier and Kulakauskas 2014). WTA composition may constitute half of the total dry weight of the cell wall in certain bacterial species and has high hydrophobicity (Chapot-Chartier and Kulakauskas 2014; Swoboda *et al.*, 2010).

Exopolysaccharide) EPS(, teichoic or lipoteichoic acid, and peptidoglycan are the three main forms of carbohydrates present in the cell wall. As reported by Lahtinen (2004), there was no evidence of the involvement of EPS, cell wall proteins, Ca^{2+}, or Mg^{2+} in AFB_1 binding. The study confirmed that EPS is rejected as a possible component of binding AFB1, and cell wall peptidoglycan appeared as the most likely carbohydrate component effective in the binding process (Lahtinen *et al.*, 2004). Teichoic acid also has a little effect on binding of mycotoxin (Zhao *et al.*, 2016).

Aflatoxins bind to bacterial cell wall through weak non-covalent interactions, which occur with hydrophobic pockets on its surfaces, such as peptidoglycan and other cell wall polysaccharides, or their compounds that are strongly associated with bacterial peptidoglycan (Figure 4) (Fouad *et al.*, 2021). These non-covalent interactions include van der Waals interaction, electrostatic interactions, and hydrogen bonds. Van der Waals interaction was weaker, compared to hydrogen bonds, but since their large numbers maintain the stability of molecular structure, they play a major role in AFB_1 binding (Fouad *et al.*, 2021). Hydrogen bonds account for a small fraction of binding energy; however, hydrogen bonding may be important in the binding of aflatoxins (Haskard *et al.*, 2001; Lahtinen *et al.*, 2004).

With the two parameters mentioned below, the efficiency of a microorganism as an aflatoxin binder can be determined using the following formula:

$$\text{Efficiency} = (M \times Keq),$$

where M is the number of binding sites in the cell wall of the microorganism, which is different in each microorganism, and Keq is the equilibrium constant of the reaction (Bueno *et al.*, 2007).

According to the above-mentioned formula, differences in the structure of the cell wall and cell membrane of bacteria lead to differences between strains in the absorption of aflatoxins (Kosztik *et al.*, 2020; Pop *et al.*, 2022; Serrano-Niño *et al.*, 2013).

However, it seems that more studies are needed to identify the precise structures of the cell wall responsible for

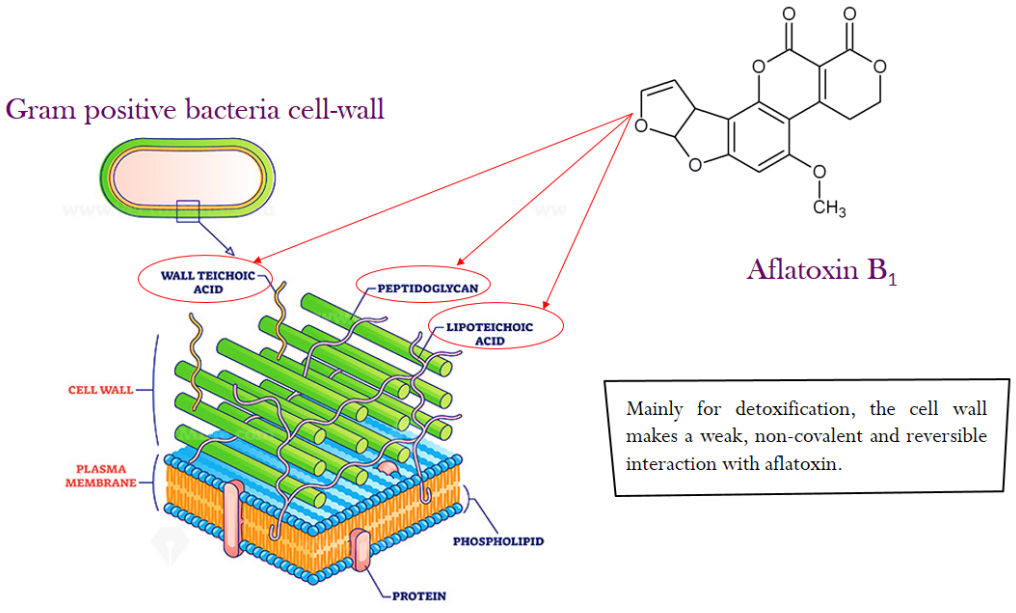

Figure 4. Schematic mechanism of aflatoxin detoxification in the cell wall of Gram-positive bacteria, such as probiotics.

binding mechanism. Therefore, after recognizing the involved components, their functions are defined *in vivo* experiments and can be used as a method to detoxify food and feed (Lahtinen *et al.*, 2004).

Necessary conditions to increase the removal of aflatoxins in postbiotics

In addition to the strain, some experimental factors also affect the capacity of aflatoxin removal by probiotics. Since the viability of probiotics at low pH conditions is reduced by passing through the stomach, the ability of nonviable probiotics to detoxify aflatoxins is an important topic to explore (Hamidi *et al.*, 2013; Topcu *et al.*, 2010). It has been shown that bacterial viability is not a prerequisite for aflatoxin detoxification (Sarlak *et al.*, 2017). Using killed probiotics is a safe method, because according to the literature, nonviable bacteria can remove more aflatoxin than live bacteria without causing any problems to humans (Vosough *et al.*, 2013). Bovo *et al.* (2013), Kabak and Var (2008) and Pierides *et al.* (2000) found that by inactivating the lactic acid bacteria by heat, their ability to remove AFM_1 significantly increases in PBS and milk, compared to live bacteria. El-Nezami *et al.* (1998a) reported that heat-treated dairy strains had the same removal capacity as live strains. It appears that by applying heat treatment, polysaccharides and peptidoglycans are strongly affected, resulting in denaturation of proteins, and the hydrophobic nature of the surface increases, or Maillard reaction products are formed. These changes cause aflatoxins to bind to bacterial cell wall and plasmatic membrane components, which are not available if the cell wall remains intact (Haskard *et al.*, 2001).

Acid treatment has also been shown to significantly increase the ability of bacteria to bind to AFB_1 (El-Nezami *et al.*, 1998a; Peltonen *et al.*, 2001). Haskard *et al.* (2001) also stated that in most cases, acid treatment is more effective than heat treatment. Several studies showed that acid treatment of yeast or LAB had the highest aflatoxin absorption, compared to live and heat-treated microorganisms (Haskard *et al.*, 2001; Hegazy *et al.*, 2011; Rahaei *et al.*, 2010). A study conducted in 1998 by El-Nezami and colleagues (1998a) stated that acid pretreatment increases the binding ability of nonviable LABs. Acidic pretreatment may lead to the cleavage of amine bonds in peptides and proteins, yielding peptides and amino acids (El-Nezami, *et al.*, 1998a). Haskard *et al.* (2001) reported that acid treatment may affect cell wall components, such as peptidoglycan and polysaccharide, releasing monomers and further fragmentation into aldehydes after breaking glycosidic bonds. Haskard *et al.* (2000) pointed out that acid-treated LAB may show more hydrophobic binding sites for aflatoxins due to protein denaturation, thus expecting hydrophobic interactions. Although the peptidoglycan layer in probiotics is very thick, the acid may reduce thickness and cross-links, leading to larger and more pores being available (Moghaddam *et al.*, 2019). Finally, aflatoxins are easily and quickly bound by the components of cytoplasmic membrane under acidic conditions (Bejaoui *et al.*, 2004).

Pretreatments of probiotic bacteria increases the efficiency of probiotics as adsorbents of aflatoxins because they cause protein denaturation, release some components and increase pore size, and, probably, affect change in charge distribution and hydrophobic nature of bacterial surface (Ahlberg *et al.*, 2015; Karazhiyan *et al.*, 2016). As a result, it allows aflatoxins to bind to the cell wall or its associated components because the available aflatoxin binding sites are increased (El-Nezami *et al.*, 2002). Haskard *et al.* (2000) expected salts to affect the surface charge of the bacteria, followed by electrostatic interactions, and the effect of salt addition was greater for nonviable cells, as demonstrated by Balsini *et al.* (2021).

In one study, treatment with sodium dodecyl sulfate caused a significant increase in the ability of the studied strain to bind AFB_1. Under sodium dodecyl sulfate, the protein was denatured and the cell wall composed of peptidoglycan was separated (Maragos, 2008).

Treatments such as divalent cations (Ca^{2+} and Mg^{2+}), chelators (EDTA and EGTA) as well as ultrasound and enzyme treatments, including various proteases, were ineffective to bind aflatoxins (Lahtinen *et al.*, 2004).

Yeasts have been shown to have a mechanism similar to probiotic bacteria in binding AFB_1 as a tool for detoxification (Shetty and Jespersen, 2006; Wu *et al.*, 2009). The cell walls of probiotic yeasts (e.g., *Saccharomyces cerevisiae*) also contain polysaccharides, such as glucan and mannan, which provide different mechanisms to bind with toxins (ionic, hydrogen, and hydrophobic bonds) (Saeidi and Safari, 2010). In probiotic yeasts, heat causes the denaturation of proteins or the formation of Maillard reaction products in the cell wall. By dissolving some of the mannoproteins in the cell wall, the permeability of the wall increases and leads to increased access to the hidden places of the cell wall as observed in probiotic bacteria. In addition, acid conditions act by affecting polysaccharides, converting monomers and breaking them into aldehydes, and some of the reactions may be intracellular in these acidic conditions (Karazhiyan *et al.*, 2016; Oliveira *et al.*, 2013; Rahaie *et al.*, 2010). As a result, aflatoxin are easily and rapidly bound by yeasts under acidic or thermal treatment (Zhong *et al.*, 2022).

Thermal treatments are the main and most common methods for preparing postbiotics, and these include

pasteurization, sterilization, Ohmic heating, radio frequency heating and microwave heating. The most common deactivation mechanism of these methods is the thermal effect, but due to different modes of heat generation, they show different deactivation efficiencies. Thermal treatments are low-cost, simple, and common methods for microbial inactivation, but they can cause irreparable damage to some physiologically active substances in microbial cells, such as proteins and polypeptides. Therefore, it is necessary to use more efficient and practical microbial inactivation methods, such as nonthermal treatments (Zhong *et al.*, 2022). In general, heat treatments mainly damage the cell membrane, break the nucleic acid, inactivate essential enzymes, denature protein and polymerizes such as the ribosome. However, the choice of temperature and time for postbiotic preparation depends on the type of microorganism, culture conditions, growth stage, PH and water activity (Zhong *et al.*, 2022). Some microorganisms inactivated by heat treatment showed various beneficial effects for aflatoxin removal (Abdelmotilib *et al.*, 2018; Montaseri *et al.*, 2014).

Removal and release of aflatoxin is a fast and reversible physical process without any chemical change in aflatoxins. The amount of toxin that is removed depends on various factors, such as probiotic/postbiotic strain concentration, treatment time, amount of cell wall components obtained from viable or nonviable bacteria, toxin characteristics, such as toxin concentration and toxin type, and pH of the environment (Ahlberg *et al.*, 2015; Pfliegler *et al.*, 2015). For example, it appears that 10^8–10^9 CFU/mL of viable or nonviable probiotic LAB are required to achieve a 50% aflatoxin removal (Bovo *et al.*, 2013; El-Nezami *et al.*, 1998a). Maximum and minimum binding capacity was observed at 10^{10} CFU/mL and 10^7 CFU/mL, respectively (Ismail *et al.*, 2017). Maximum binding occurs within the first few minutes of the reaction. The quick connection process is between 0 and 4 h. When the maximum amount of aflatoxins was removed by probiotics strains, no further reduction occurred. Lee *et al.* (2003) reported that more the aflatoxin is adsorbed by bacterial cells, more the time the adsorbed aflatoxin molecules would remain on the cell surface of bacteria (Lee *et al.*, 2003). In some studies, it was shown that the mixture of probiotics, bacteria or bacteria with yeast had a better effect in reducing aflatoxins (Florina *et al.*, 2018; Mokoena *et al.*, 2006; Okeke *et al.*, 2015). Owing to different food matrices, physicochemical characteristics, and treatment conditions, the rate of aflatoxin reduction is different.

Probiotic strains or postbiotics can bind with aflatoxins and form a probiotic–aflatoxin or postbiotic–aflatoxin complex. The stability of probiotic/postbiotic and aflatoxin complexes depends on strain, treatment and environmental conditions. Since the binding of aflatoxin to probiotics/postbiotics is weak and partially reversible (Peltonen *et al.*, 2001; Pizzolitto *et al.*, 2012; Topcu *et al.*, 2010), *in vivo* experiments appear as necessary, because aflatoxins may be released from the gastrointestinal tract, resulting in negative health consequences. The possibility of reversibility is 27.8–94.4%, depending on the strain (Moghaddam *et al.*, 2019). It is determined that LAB shows a significant adhesion to intestinal cells, which lose this characteristic by binding to aflatoxins, so the bacteria–aflatoxin complex is quickly excreted in the digestive system (Gratz *et al.*, 2010).

As mentioned in the detoxification of aflatoxins by probiotics/postbiotics, the process depends on the strain, irrespective of viable or nonviable probiotic (Topcu *et al.*, 2010). The studies conducted on the anti-aflatoxin effect of postbiotics included B_1, B_2, G_1, and G_2, the most effective of which are listed in Table 3.

Summarizing the previous table, postbiotics derived from probiotics can remove aflatoxins with a mean of 80%. Based on our review, studies have been done more on AFB_1, followed by AFM_1, probably because of the greater importance of these two aflatoxins. Strain *L. rhamnosus* (GG) has been mostly used in the literature, and studies have obtained different results, which could be due to food matrix, type of medium, physicochemical characteristics, and different treatment conditions. Among the food items, milk and dairy products are used maximum.

As already reported, after comparing the studies, we concluded that the acid-treated strain was more effective than the heat-treated strain. We also found that cell-free supernatant was very effective in removing aflatoxins. In fact, cell-free supernatant contains active ingredients

Table 3. The most effective postbiotics.

Probiotic strains	Type of postbiotics	Type of AF	AF removal (%)	References
L. kefiri (MH107106)	CFS	B_1	97.22	Taheura *et al.*, 2021
L. kefiri (MH107106)	CFS	B_2	95.27	Taheura *et al.*, 2021
L. rhamnosus (TISTR 541)	Heat-killed	G_1	86.2	Elsanhoty *et al.*, 2013
L. rhamnosus (TISTR 541)	Heat-killed	G_2	81.4	Elsanhoty *et al.*, 2013
P. pentosaceus (RC006)	CFS	M_1	91	Martinez *et al.*, 2019

and bacteriocin (Tenea and Barrigas, 2018), and probably the presence of several compounds makes it effective. It seems that the simultaneous use of several postbiotics derived from different strains can remove aflatoxins in large amounts, resulting in food safety. The result obtained from this study supports the development of future studies for the formulation of biological additives that could be used in functional foods.

Probiotics, prebiotics, synbiotics and postbiotics are novel concepts. However, biological detoxification technology is currently far from complete, and the determination and purification of metabolites is incomplete in many cases. In the future studies, the determination of various factors, such as the proof of LAB being probiotic, types of inactivation of microorganisms and extraction methods, and the exact nature of postbiotic (cell-free supernatant or cells killed by different physical and chemical treatments), to identify health effects and toxicity are essential. More studies are necessary to accurately identify and understand complex relationships between these concepts; this would help to understand the pathway of their mechanism of action. In addition, more research is needed to reveal the dose, time of detoxification, and usage of new preparations to maximize prevention and beneficial effects to reduce toxins on humans. In addition, *in vivo*, systematic review studies are also needed to support the health claims of postbiotic functional foods. At the same time, a big challenge exists, such as transferring scientific knowledge to business applications, which are a bridge between science and industry. Therefore, it would take a long time to develop biological additives to realize large-scale industrialization (Saeidi and Safari, 2010).

Author contributions

S. Zamanpour and B. Shokrollahi Yancheshmeh independently searched databases and extracted data individually. Any disagreement was resolved by consulting SM Noori, M. Hashemi and A. Afshari. Finally, the full text was written by S. Zamanpour, B. Shokrollahi Yancheshmeh, M. Hashemi and A. Afshari. Text editing was done by SM Noori.

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Conflict of Interest

There was no conflict of interest between authors.

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