In vitro strain specific reducing of aflatoxin B1 by probiotic bacteria: a systematic review and meta-analysis

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ABSTRACT
This systematic review and meta-analysis aimed to investigate the capacity of probiotic bacteria for reducing of aflatoxin B1 as a food hazard. The results indicated that probiotic bacteria significantly decreased aflatoxin B1 by 45.99% (CI: 42.08%, 49.91%) also The rank order of binding subgroups were Lactobacillus 47.96% (CI: 43.51%, 52.40%), Bifidobacterium 43.95% (CI: 34.96%, 53.65%, I2 = 99.8%), Pediococcus 41.61% (CI: 31.49%, 51.74%, I2 = 99.3%), Lactococcus 33.56% (CI: 19.19%, 47.94%, I2 = 99.5%) and Enterococcus 27.14% (CI: 20.70%, 33.58%, I2 = 96.9%). Probiotic bacteria could be recommended as a biological tool for decreasing aflatoxin B1 in different safety approaches.

1. Introduction
Aflatoxins are the most distributed mycotoxins generated by toxigenic strains of Aspergillus such as A. flavus, A. parasiticus, and A. nomius (Sadeghi et al. 2019, Wacoo et al. 2020). Approximately 18 different forms of aflatoxin and its metabolite derivatives have been recognized. Aflatoxins have an unfavorable influence on humans and animal’s health due to their carcinogenic, mutagenic, teratogenic, and immunosuppressive properties (Bbosa et al. 2013). Aflatoxin B1 is the further most potent toxic that is categorized by the International Agency for Research on Cancer (IARC) as a cluster 1A (carcinogenic mediator) (Oatley et al. 2000, Herceg et al. 2013). This toxin has significantly impacted liver cells and might result in DNA damaging, mutation, abortion, and several toxic reactions (Bbosa et al. 2013). The aflatoxin’s producer fungi are environmentally widespread and able to contaminate food, feeds, and crops. So the most probable health risk and possible side effects occur in the population via consumption of aflatoxin-contaminated food. Concerning aflatoxins’ toxicity effect on humans, food products’ contamination poses a severe challenge in food safety (Klich 2007, Bbosa et al. 2013, Lili et al. 2018, Wacoo et al. 2020). The contamination of foods with aflatoxin B1 is one of the top ten hazards notified in annual reports from many countries by the Rapid Alert System for Food and Feed (RASFF) (Piglowski 2019).

Numerous studies were investigated about preventing/inhibiting aflatoxin production, reducing/detoxifying aflatoxin in contaminated products, and inhibiting the absorption of aflatoxin at the gastrointestinal tract (Afshar et al. 2020). Efforts have been prepared to eliminate toxins from contaminated sources or cut down into fewer toxic complexes (Zaki et al. 2012). There were different approaches to decrease aflatoxin bioavailability in foods. The most important techniques can be biological decontamination and chemical inactivation (Lili et al. 2018, Sadiq et al. 2019, Afshar et al. 2020). Based on consumer demands for safe foods and avoiding the chemical process, it must establish effective specified methods that are cost-effective, environment according, applicable, available, and safe.

In this regard, the biodegradation of aflatoxin using nonpathogenic microorganisms and their derivatives have been considered as a relevant strategy (Oliveira et al. 2013, Kim et al. 2017).

It is revealed that probiotic microorganisms be efficient in mycotoxins removals, mainly aflatoxins, and ochratoxins (Huang et al. 2017). Probiotics are live microorganisms with various health benefits generally recognized as safe (GRAS) (Food and Agriculture...
A wide variety of strains of bacteria and fungi originated from different sources are setting as probiotics. The most predominant probiotic bacteria strains used in food as an additive or supplement included *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *Leuconostoc*, *Pediococcus*, *Propionibacterium*, *Bacillus*, *Enterococcus*, and *Escherichia coli*. Also, some species of Yeast (*Saccharomyces* and *Candida*) and molds (*Aspergillus niger*, *Aspergillus oryzae*) are considered probiotics for adding to food and feed. Current investigation has displayed that specific strains of probiotic bacteria can eradicate aflatoxins in vitro, ex vivo, and in vivo (Turbic et al. 2002, Muthaiyan et al. 2011). The decontamination efficiency of aflatoxins was reported to significantly varied by different strains. Also, some experimental factors could affect on aflatoxin removal capacity of used probiotic microorganisms.

The mechanisms by which such toxin removal go on by probiotics have not yet been carefully considered. Some studies stated that probiotic microorganisms could bind to aflatoxin molecules via a physical adhesion in which the cell wall structure plays a crucial role in this phenomenon. The construction and conformation of cell walls have highly varied, even in genetically narrowly connected strains. Besides, the nature of the definite components of cell walls like glycoproteins for each strain could affect their capacity to bind aflatoxin (June and Dziewulski 2018, Afshar et al. 2020, Chlebicz and Slizewska 2020). Different chemical, physical and enzymatic actions were studied to determine the nature of binding sites in probiotics to aflatoxin B1 and other carcinogenic compounds (Mahmood Fashandi et al. 2018, Lili et al. 2018).

Regarding the incidence of aflatoxin B1 in food, controlling and reducing its levels in food using safe biological techniques such as probiotic-based ones has eligible benefits in public health guarantee. We aimed to perform a systematic review and meta-analysis to evaluate the efficiency of probiotic microorganisms for reducing aflatoxin B1.

### 2. Methods

The protocol of this study was registered in the center for Open Science Framework (OSF) database (http://www.osf.io/txga5, DOI:10.17605/OSF.IO/RCNGQ).

#### 2.1. Search strategy

To find potentially relevant studies for systematic review and meta-analysis, we searched articles in English references in Scopus, Web of Science, and PubMed databases from 1998 to 2020. The following set of keywords was used for the systematic search: (aflatoxin OR aflatoxin B1 OR aflatoxins) AND (probiotic bacteria OR lactic acid bacteria) AND (reduction OR detoxification OR degradation OR binding OR elimination). The reference lists of all relevant publications were manually searched to find potential additional articles (Figure 1).

#### 2.2. The proposed criteria for inclusion and exclusion of articles

Two researchers, after removing repetition articles in the EndNote X9 software (Thomson Reuters, New York, NY), independently reviewed the titles and abstracts of all articles retrieved and selected those meeting the following criteria: (1) reported change in aflatoxins B1 as the outcome of interest; (2) original interventional study that used viable probiotic bacteria to reduce aflatoxin B1; (3) interventional studies that were performed in laboratory media or food (4) full-text article available. Any discrepancy was resolved through consultation with a third researcher. The articles were excluded when they did not meet these criteria.

#### 2.3. Data extraction

Two authors independently and in duplicate extracted data from eligible studies. Any discrepancies were resolved through discussion to reach a consensus. The obtained data of each article (study characteristics) was as follows: first author's name, publication year, the species of bacteria, the origin of bacteria, the type of food/media, the percentage of aflatoxin B1 reduction/binding, standard deviation and sample size.

#### 2.4. Data synthesis and statistical analysis

The percent change and its standard error (SE) in aflatoxin B1 concentrations were considered as the effect size. Meta-analysis was conducted if at least two studies reported the effect size for the same outcome. Studies that reported percent change and its SE in aflatoxin B1 concentrations following probiotic bacteria usage, the effect size was included in the meta-analysis as reported. In studies that did not report direct SE, the SE was calculated by multiplying standard deviation (SD) by \(\sqrt{n}\). Study-specific results were pooled by using a random-effects model (DerSimonian and Laird 1986).
We assessed and reported heterogeneity quantitatively using the $I^2$ statistic and performed a $\chi^2$ test for homogeneity ($p_{\text{heterogeneity}} > 0.10$). Between-studies heterogeneity was explored via Cochrane’s Q test of heterogeneity and the $I^2$ statistic ($p < 0.05$) (Higgins et al. 2003). Potential publication bias was not checked due to a low number of studies included in the analyses ($n < 10$) (Shuster 2011). All analyses were conducted with Stata software, version 16 (StataCorp, College Station, USA). Statistical was considered as significant at $p$-values $< 0.05$.

3. Results

3.1. Data description

Figure 1 shows the literature search and study selection process. The initial systematic search identified 491 potentially eligible publications. Of these, 17 studies were duplicates, and another 474 studies were screened based on the review of the title and abstract. Eventually, 64 studies were fully reviewed for inclusion in the systematic review. Finally, 31 studies provided sufficient information for the meta-analysis and were included in the analyses. Reasons for excluding studies are described in Figure 1. Besides, the summary of the data extracted from these articles has been presented in Supplementary Table 1. The 31 trials (161 study arms) were included in the analysis of the effect of probiotic bacteria on the binding of aflatoxin B1 (El-Nezami et al. 1998b, Oatley et al. 2000, Haskard et al. 2001, Peltonen et al. 2001, Turbic et al. 2002, Bueno et al. 2007, Khanafari et al. 2007, Fazeli et al. 2009, Hernandez-Mendoza et al. 2009, Topcu et al. 2010, Fernández-Juri et al. 2011, Pizzolitto et al. 2011, Bagherzadeh Kasmani et al. 2012, Hamidi et al. 2013, Sezer et al. 2013, Vosough et al. 2014, Serrano-Niño et al. 2015, Ghazvini et al. 2016, Singh et al. 2016, 2016.
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Drobna et al. 2017, Huang et al. 2017, Ma et al. 2017, Martinez et al. 2017, Alrabadi et al. 2018, Liew et al. 2018, Marrez et al. 2018, Kumara et al. 2019, Sadeghi et al. 2019, Chlebicz and Slizewska 2020, Wacoo et al. 2020). Distribution of studies included in the present review across different origin of considered bacteria were as follows: microbial culture collection (75.15%) > poultry (8.69%) > dairy product (5.59%) > fish feed (3.72%) > lamb and goatling stomach mucus (1.86%) > feces healthy dogs (1.24%) > milk (0.62%) ~ sour-dough (0.62%) ~ kefir grains (0.62%) ~ curd (0.62%) ~ Chinese traditional fermented food Tofu (0.62%) ~ yogurt (0.62%).

Regarding the used media to investigate the effect of probiotic bacteria on the binding of aflatoxin B1, it was observed that 94.4% of studies were performed in phosphate buffer saline (PBS) and 5.6% in De Man, Rogosa, and Sharpe agar (MRS). Sensitivity analyses of each sub-group (Lactococcus, Bifidobacterium, Enterococcus, and Pediococcus) were done after excluding each study from the principal analysis (Supplementary Tables 3–6).

3.1.1. Aflatoxin B1 reduction by probiotic bacteria strains

The rank order of bacteria genus used for binding of aflatoxin B1 based on the number of studies was Lactobacillus (28 articles) > Pediococcus (5 articles) > Lactococcus (4 articles) > Bifidobacterium (3 articles) ~ Enterococcus (3 articles). The results showed that probiotic bacteria significantly decreased aflatoxin B1 by 45.99% (CI: 42.08%, 49.91%), with high heterogeneity, \( I^2 = 100\%, P_{heterogeneity} < 0.001 \). The mean binding of Aflatoxin B1 in subgroups of probiotic bacteria was as follows:

The results showed that the genus Lactobacillus significantly reduced aflatoxin B1 by 47.96% (CI: 43.51%, 52.40%, Supplementary Table 2), with high heterogeneity, \( I^2 = 100\%, P_{heterogeneity} < 0.001 \). The rank order of aflatoxin B1 binding (%) by Lactobacillus species was as L. kefir (82%) > L. gasseri (67.7%) > L. thermophilus (65.7%) > L. paracasei (59.03%) > L. Lactis (59%) > L. pantheris (57.6%) > L. reuteri (56.34%) > L. rhamnosus (55.56%) > L. fermentum (55.23%) > L. casei (47.54%) > L. buchneri (46.8%) > L. acidophilus (45.38%) > L. plantarum (45.02%) > L. brevis (40.40%) > L. johnsonii (37.55%) > L. helveticus (32.23%) > L. salivarius (26.3%) > L. pentosus (17.4%) > L. Jun 6 (16.8%) > L. delbrueckii (16.45%) > L. L3LBS2 (12%) > L. mucosae (10.8%) > L. animalis (8.8%) >) > L. thermophiles (6.7%). The effect of Bifidobacterium on aflatoxin B1 binding was 44.31% (CI: 34.96%, 53.65%; \( I^2 = 99.8\%, P_{heterogeneity} < 0.001 \;\text{Figure 2(A)}\).

The obtained rank order regarding Bifidobacterium species was B. bifidum (73.25%) > B. animalis (45.7%) > B. JO3 (41%) > B. longum (37.25%) > B. CH4 (37%) > B. lactis (33.8%). The significant effect of the genus Pediococcus on aflatoxin B1 binding was 41.61% (CI: 31.49%, 51.74%; \( I^2 = 99.3\%, P_{heterogeneity} < 0.001 \;\text{Figure 2(B)}\).

The observed rank order between the Pediococcus bacteria species was P. pentosaceus (%8.73) > P. acidilactici (%30.31).

The analysis of the effect of the genus Lactococcus on aflatoxin B1 Binding showed binding by 33.56% (CI: 19.19%, 47.94%, Figure 2(C)), with high heterogeneity, \( I^2 = 99.5\%, P_{heterogeneity} < 0.001 \).

Also, the results showed that Enterococcus significantly decreased aflatoxin B1 by 27.14% (CI: 20.70%, 33.58%, Figure 2(D)), with high heterogeneity, \( I^2 = 96.9\%, P_{heterogeneity} < 0.001 \). The observed rank order among the bacteria species was E. faecium (%28.36) > E. faecalis (%14.3).

3.1.2. Aflatoxin reduction in foods

Aflatoxin reduction using probiotic bacteria in various food are summarized in Table 1. The experiments were performed in different types of food such as dairy, nuts, cereals, meat, and water. Most studies were investigated aflatoxin removal using Lactobacillus, including L. acidophilus, L. acidophilus, L. delbrueckii, L. plantarum, L. casei, and L. brevis. Due to the use of different methods and conditions for reducing aflatoxin in various food matrices using probiotic bacteria, it is impossible to Poole the data or compare them. As it can be seen in Table 1 the reduction rate was significantly varied, ranging from 6.6 to 98.30% in reviewed studies.

4. Discussion

Up to current knowledge, this systematic review providing comprehensive information on the efficiency of probiotic bacteria strains in reducing aflatoxin B1. Based on the result, the mean of aflatoxin binding by probiotic bacteria was 45.99% (ranging from 0.9 to 100%) in used media in vitro. Also, the result suggested that the reduction of aflatoxin B1 was dependent on the species of probiotic bacteria. Lactobacillus found the highest reduction of aflatoxin B1 (100%), and a significant decrease was observed by Bifidobacterium, Pediococcus, Lactococcus, and Enterococcus species, respectively. The most used
Figure 2. Reduction of aflatoxin B1 by subgroups: (A) *Bifidobacterium*; (B) *Pediococcus*; (C) *Lactococcus*; (D) *Enterococcus*. B.: *Bifidobacterium*; P.: *Pediococcus*; L.: *Lactobacillus*; E.: *Enterococcus*; CI: confidence interval.
strains of probiotic bacteria for aflatoxin B1 reduction were including *L. rhamnosus*, *L. plantarum*, *L. acidophilus*, *L. brevis*, *L. fermentum*, and *L. casei*. The removal of aflatoxin in food products was also reviewed. The maximum reduction rates were different depending on the food products, the used method, the probiotic strains, and the experimental conditions.

### 4.1. The mechanism of aflatoxin reducing

The most proposed mechanism for aflatoxin interaction with probiotic bacteria strains is physical adsorption, known as binding. The release of aflatoxin occurs without any modification in the chemical structure of aflatoxin through the adsorption process. The binding phenomenon takes place between the cell wall of the microorganism and the aflatoxin molecule. This type of physical attachment involves non-covalent interaction such as Vader Waals, hydrophob, and hydrogen bonds (Oluwafemi et al. 2010, Pizzolitto et al. 2011, Afshar et al. 2020, Wacoo et al. 2020). The integrity of the cell wall of bacteria strains is vital for the adsorption of aflatoxin on the surface of microorganisms. Pizzolitto has described this relationship using adsorption isotherms and revealed that the number of adsorption sites in the microorganism and the affinity of aflatoxin molecules toward binding sites affected the adsorption of aflatoxin (Pizzolitto et al. 2011). According to the predicted model for physical adsorption of aflatoxin B1 using some probiotic bacteria by Bueno et al. the capacity of most strains to remove aflatoxin B1 is influenced by the number of binding sites per strain (M) and the equilibrium constant ($K_{eq}$) (Bueno et al. 2007). Based on the literature review, the binding of aflatoxin using probiotic microorganisms is fast and possibly reversible. However, some factors such as toxin and bacteria concentration directly influence the aflatoxin binding rate.

The other proposed mechanism for aflatoxin removal by probiotic bacteria is biodegradation. The biodegradation results in modification of aflatoxin structure and production of some possible metabolites (Ibitoye et al. 2020, Solis-Cruz et al. 2018). Since furan and lactone ring are the main component involved in toxic features of aflatoxin molecule, any split of them by a microorganism’s cell or via their enzymatic metabolites has a key role in detoxification (Ibitoye et al. 2020). However, some special cells of bacteria and fungi have been reported as an effective microorganism on aflatoxin degradation. Also, several

Table 1. Aflatoxin reduction using probiotic bacteria in various food.

| Probiotic strain | Food product | Aflatoxin | Maximum aflatoxin reduction (%) | References |
|------------------|--------------|-----------|----------------------------------|------------|
| Kefir grains (*Lactobacillus plantarum, Lactobacillus casei, and S. cerevisiae*) | Pistachio nuts | AFB1 | 96 | (Ansari et al. 2016) |
| *Lactobacillus acidophilus, Lactobacillus acidophilus* | Yogurt | AFB1 | 64.56–96.58 | (Mosallaie et al. 2020) |
| *Bifidobacterium BB-12* | Coffee beans | AFB1 | 8.24 | (Florina et al. 2018) |
| Mixture of the probiotic strains | Peanut | AF | 66.7 | (Silva et al. 2015) |
| *Lactobacillus delbrueckii* mix with *S. cerevisiae UFMG 905* | Sorghum | AFB1 | 6.6 | (Padmaja and Periyar Selvam 2016) |
| *Lactobacillus delbrueckii* | Meat products | AF | 88 | (Ibrahim et al. 2018) |
| *Lactobacillus acidophilus* | Maize product (Ogi) | AFB1 | 60 | (Okeke et al. 2015) |
| Mixed culture of *Streptococcus lactis* and *Lactobacillus delbrueckii* | Maize meal | AFB1 | 75 | (Mokomen et al. 2006) |
| *Lactobacillus acidophilus, Lactobacillus brevis, Lactobacillus plantarum,* *Lactobacillus casei,* *Lactobacillus delbrueckii* | Sorghum-millet beverages | AFB1 | 19.3–69.4 | (Byakika et al. 2019) |
| *Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus plantarum,* *Streptococcus thermophiles,* *Bifidobacterium angulatum* | Water | AFB1 | 8.9–21.6 | (Elsanholy et al. 2016) |

AF: aflatoxin; AFT: aflatoxin total; AFB1: aflatoxin B1.
enzymatic metabolites of microorganisms, such as intracellular and extracellular enzymes, were introduced as effective compounds for aflatoxin degradation. In this mechanism, the aflatoxin might convert into non-toxic or less toxic compounds (Chlebicz and Slizewska 2020).

Some researchers have been notified that the cell wall component had a leading part in physical attachment to aflatoxin because non-viable probiotic bacteria also revealed significant binding capacity. Therefore, the binding of aflatoxin to probiotic bacteria is not dependent on cell activity (Pizzolitto et al. 2012, Ghazvini et al. 2016). It is frequently stated in studies that carbohydrates and protein constituents of the strains cell wall play the primary role in the attachment to aflatoxin B1. In this regard, the peptidoglycan and teichoic acid section in the cell wall appear to contribute to binding (Abdolshahi et al. 2018). Haskard et al proposed that aflatoxins may bind to probiotics like lactic acid bacteria’s cell exterior by hydrophobic communication (Haskard et al. 2001, Afshar et al. 2020).

However, aflatoxin binding by probiotic bacteria might be reversible depending on the strain and used treatment condition. It was reported that some aflatoxin-microorganism complexes may not be stable and release the aflatoxin (Elsanhoty et al. 2016, Liew et al. 2018, Wacoo et al. 2020). A study by Kabak observed that up to 39.8% of bounded aflatoxins was reversed in a simulated digestive model (Kabak and Ozbey 2012).

Most studies indicated that probiotic bacteria can detoxify aflatoxin B1 via a binding mechanism. Many species of Lactobacillus, including L. plantarum, L. brevis, L. fermentum, L. rhamnosus, L. acidophilus, and L. lactis, also, some species of Bifidobacterium, Enterococcus, and Pediococcus have been cited that have aflatoxin binding capacity in different foods (Khanafari et al. 2007, Elsanhoty et al. 2013, Hamad et al. 2017, Huang et al. 2017, Ma et al. 2017, Bartkiene et al. 2018, Florina et al. 2018, Marrez et al. 2018). However, the aflatoxin B1 reduction using probiotic bacteria through the binding or degradation mechanism could be considered an effective method for providing solutions to combat this food safety challenge.

4.2. Practical factors on aflatoxin B1 reducing by probiotic strains

4.2.1. Strain-specification
One of the main indexes that affect the interaction among the probiotic bacteria and aflatoxin is referred to probiotic strain specification. We found that the rate of aflatoxin B1 binding by probiotic bacteria was significantly strain-specific. Many different species of bacteria from different origins were used in studies to assess the aflatoxin binding capacity. Through the included studies, it is observed that various result was reported for aflatoxin B1 reduction percentage by the same probiotic bacteria. In some studies, high rates of aflatoxin B1 reduction were reported by L. plantarum, L. fermentum, B. bifidum, L. casei, L. acidophilus, L. kefiri, L. rhamnosus ranging from 80 to 100% (El-Nezami et al. 1998a, Khanafari et al. 2007, Pizzolitto et al. 2012, Ghazvini et al. 2016, Liew et al. 2018, Kumara et al. 2019, Ben Taheur et al. 2020). However, in other studies, a low reduction percentage was reported for L. plantarum APW2112A (0.9%), L. plantarum APW1434B (10.7%), and L. fermentum 27A (11.4%), and L. rhamnosus APW2288B (13.7%) (Peltonen et al. 2001, Wacoo et al. 2020). Wacoo et al. indicated that the amount of aflatoxin B1 bound to Lactobacillus species originated from the Gut Microbiota was increased by a rise in cell density. Moreover, some species have been showing higher aflatoxin B1 removal at low density regarding cell surface binding sites (Wacoo et al. 2020).

In research, the binding of aflatoxin B1 by Enterococcus faecium strains MF4 was reported as 42%, whereas for Enterococcus faecium strains GJ40 was 27% (Fernández-Juri et al. 2011). In this context, very different results for aflatoxin B1 reduction (41.1% and 5.6%) were observed between two species of Lactococcus strain, L. lactis ssp. cremoris ARH74 and L. lactis ssp. cremoris MK4 in Peltonen study (Peltonen et al. 2001). In Pediococcus genus, different reduction content was obtained for aflatoxin B1 among Pediococcus acidilactici species (Ma et al. 2017, Martinez et al. 2017). In vitro experiments emphasize that different cell wall structures and the number of the binding site of each strain of probiotic bacteria have a key role in aflatoxin B1 binding efficiency. Some unique strains of probiotic bacteria originated from dairy products reported as efficient aflatoxin B1 binders. It was reported that Lactobacillus spp., Lactococcus spp., and Bifidobacterium spp. from Kefir grains abled to bind 82% of aflatoxin B1 (Taheur et al. 2017).

It has been reported that co-culture of probiotic strains sometimes be effective in aflatoxin removal. Even though bacteria and their bacteriocins are sufficient alone, their detoxification efficiency was amplified when they were used together. It has been reported that L. lactis and L. plantarum were incubated in the mixed group had a meaningfully improved
capacity to bind toxins compared to use alone. (Sezer et al. 2013).

4.2.2. Incubation temperature

The Incubation temperature can be strongly influenced by the aflatoxin detoxification proficiency of probiotic bacteria. El-Nezami et al. proposed that 37°C was the best temperature for binding of aflatoxin B1 by Lactobacillus rhamnosus GG and Lactobacillus rhamnosus LC-705 (El-Nezami et al. 1998a). Rahaie et al. shown that the surface attachment efficiency of Lactobacillus to aflatoxins could improve by heat treatment (Rahaie et al. 2012). However, thermal treatment of probiotic bacteria seems to increase their binding level to aflatoxins. Not only is the bacterial cell wall available in this way, but other wall components can also contribute to this process. Although, the binding to aflatoxins may be reversible in untreated cells (Kuharić et al. 2018). Lee et al. identified that temperature dealing could cause a decline in the hydrophobicity and enhance the adsorption affinity. Thus hydrophobic interactions take part in the adsorption of aflatoxin B1 (Lee et al. 2003).

The previous appeared more dependable as heat treatment changed the bacteria’s surface possessions somewhat than exposing new adsorption positions (Haskard et al. 2001).

Many studies investigated the effect of viable and heat-killed probiotic bacteria on aflatoxin B1 binding rate. A study by Huang et al. founded that both viable and heat-killed of different L. plantarum had aflatoxin B1 reduction efficiency (20–60%) (Huang et al. 2017).

4.2.3. Incubation time

Contact time between aflatoxin B1 and used probiotic bacteria is a noticeable factor in the attachment process between them. It is more emphasized in studies that aflatoxin B1 binding is a fast process, and the main part of it be removed from media at the first time of incubation by different strains. The rapid binding process varies from 0 to 4 h, but it is noteworthy that longer incubation times do not lead to further binding, especially in studies of Lactobacillus and Bifidobacteria (Serrano-Nino et al. 2013). By increasing the contact time from 1 min to 5 h, no significant difference was obtained in aflatoxin removal in PBS by probiotic bacteria (L. fermentum subsp. cellobiosus 408, L. casei 1, and L. acidophilus P22) and probiotic yeast (S. cerevisiae) in a study by Pizzolitto et al (Pizzolitto et al. 2011). In an in vitro assay, Kumara et al. found that four isolates of L. fermentum were able to bind aflatoxin B1 from media ranging from 66 to 85.2% at 2 h incubation. They observed that the binding saturation time was 2 h in a period of 0–7 h incubation (Kumara et al. 2019). Since the aflatoxin binding process did not take more time, it seems the cell wall of used microorganisms contributes to aflatoxin capture and separation from media. In a study on aflatoxin B1 reduction in milk by selected probiotic bacteria, it is reported that L. acidophilus could reduce 80% of aflatoxin B1 during 24 h whereas L. plantarum reduces 85% of aflatoxin B1 after 1 week (Marrez et al. 2018). In a similar study, all tested Lactobacillus strains showed more binding ability than extending contact time (0 to 24 h) in a proportional manner except L. mucosae, which had lower activity at high time. Also, the highest aflatoxin B1 reduction was obtained by L. ruteri KO4b (66.7%) and L. plantarum KG4 (59.4%) after 24 h incubation time (Drobné et al. 2017). The assessment of Lactobacillus rhamnosus ability for aflatoxin reduction showed it could bind varied content of aflatoxins at different incubation periods (Alrabadi et al. 2018). In most studies, it is confirmed that the incubation time is adequate up to a time in which maximum removal of aflatoxin has occurred via strains, and after that, there is no more reduction rate.

4.2.4. Initial microbial and aflatoxin B1 concentration

The amount of aflatoxin B1 removal is cell concentration-dependent. El-Nezami et al. indicated that L. rhamnosus GG and L. rhamnosus C705 could considerably remove aflatoxin at a bacterial concentration upper than 2 × 10^9 and 2 × 10^10 CFU/mL in which 99% and 87% of aflatoxin B1 were eliminated, respectively (El-Nezami et al. 1998a). Conferring to Kabak, some probiotic strains of Lactobacillus including L. acidophilus, L. acidophilus, L. acidophilus, B. bifidum, B. bifidum, and L. rhamnosus attached to aflatoxin at a bacterial concentration greater than 10^8 CFU/mL (Kabak and Var 2008). In similar studies also different bacteria showed different binding capacities directly related to the initial concentration. The maximum binding capacity was observed at 10^10 CFU/mL, and the minimum binding capacity was observed at 10^7 CFU/mL. In this group of probiotic strains, Lactobacillus helveticus and Lactobacillus plantarum showed the highest inhibitory effect. Also, Lactobacillus bulgaricus exposes more binding surfaces to aflatoxin (Ismail et al. 2017, Ahlberg et al. 2015). Other authors have been mentioned a direct correlation between cell number of strains and bound aflatoxin B1. Although at a definite bacterial concentration, any increase does not result in more binding of the present aflatoxin B1 in
used media. It seems an equilibrium condition developed among binding sites in the microorganism and unbound aflatoxin (El-Nezami et al. 1998b, Pizzolitto et al. 2011, Chlebicz and Slizewska 2020).

Besides, the efficiency of aflatoxin B1 reduction depends on its concentration in experiments. It is indicated that at low aflatoxin B1 content, the reduction follows a linear trend and changed to plateau at higher aflatoxin B1 levels. In real the amount of removed aflatoxin B1 by probiotics would increase by increasing its concentration. Still, regarding the limited number of binding sites in microorganisms, the final reduction rate did not increase necessarily (Pizzolitto et al. 2011, Singh et al. 2016, Rao et al. 2019). Impact of various aflatoxin B1 concentrations (from 0 to 10 µg/ml) and bacterial concentration (from 10^8 to 4 × 10^8 CFU/ml) on toxin detoxifying by L. acidophilus Po22 and L. fermentum subsp. cellobiosus 408 showed that aflatoxin B1 removal directly depended on present toxin and bacterial content and followed a saturable trend (Bueno et al. 2007). So, the saturation point must be noticed in all studies on aflatoxin B1 removal by microorganisms.

4.2.5. pH
Probiotic bacteria should have the ability to grow in low acidity such as stomach conditions and be resistant toward bile salts. It has been found the influence of pH on aflatoxins’ decontamination utilizing probiotic bacteria. Probiotic Lactic acid bacteria strains have been shown to have the highest AFB1 uptake at pH 2 and concentrations close to 0.05% of bile salts (Asurmendi et al. 2020). Experiments on Lactobacillus casei have been shown to neutralize aflatoxin toxicity in the gastrointestinal tract (Huang et al. 2017, Liew et al. 2018). The evaluation of the sensitivity of probiotic bacteria strains isolated from Kefir grains into pH showed that the bound complex of aflatoxin and selected bacteria were more stable at pH 7 and 8 also were sensitive to pH 3 (Taheur et al. 2017). Removal of aflatoxin B1 via Enterococcus faecium strains was highest at pH 7 comparing pH 3, 4, 5, 6. The E. faecium M74 and E. faecium EF031 showed a maximum reduction rate of 28.6 and 31.9%, respectively, at pH 7 (Topcu et al. 2010).

4.2.6. Aflatoxin reduction in food by probiotic bacteria
The removal of aflatoxin from food has been investigated using different techniques such as irradiation, fumigation, thermal processing (Oliveira et al. 2013, Al-Ruwaili et al. 2018, Emadi et al. 2021). Concerning food safety, bio-based strategies interested more researchers to apply biological treatments for aflatoxin reduction in food (Byakika et al. 2019). The use of probiotics had considerable attention regarding their potential to eliminate aflatoxins (Karlovsky et al. 2016, Florina et al. 2018, Sadiq et al. 2019). The reduction of aflatoxin using probiotic bacteria in food products has been investigated by directly adding strains to food or fermentation. Since the probiotic bacteria are edible and recognized safe, they can add to food a food ingredient. The aflatoxin reduction rate would differ due to different food matrix, physicochemical characteristics, and treatment conditions.

Kefir grains containing probiotic microorganisms, including Lactobacillus plantarum, Lactobacillus casei, and S. cerevisiae have been utilized for aflatoxin B1 reduction in pistachio. The maximum aflatoxin B1 reduction (96%) was obtained for 20% Kefir grains at 6 h incubation time and 30°C (Ansari et al. 2016). Several studies have focused on using efficient probiotic starters for detoxifying food by fermentation process. In a study, probiotic lactic acid bacteria starter (L. plantarum MNC 21, W. confusa MNC 20, and L. lactis MNC 24) were used to decrease aflatoxin B1 in a fermented sorghum-millet beverage. The finding indicates that used starter with aflatoxin binding capacity (69.4%) can be considered for enhancing the safety of cereal fermented product (Byakika et al. 2019). Some probiotic bacteria, including Bifidobacterium angulatum, Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus plantarum, Streptococcus thermophilus were assessed for evaluating their binding to aflatoxin B1 in contaminated water samples. This research illustrated that Removal AFB1 depended on the strain and used pH in which high reduction (21.6%) was attained at neutral range of pH (Elsanhuty et al. 2016). Besides, some authors have been reported that the application of mixed strains of probiotics, even bacteria or bacteria plus yeast showed a better reduction rate in aflatoxin level in food (Mokoena et al. 2006, Florina et al. 2018, Okeke et al. 2018). Co-culture of probiotic bacteria (Lactobacillus delbrueckii UFV H2b20) and probiotic yeast strain (S. cerevisiae var. boulardii, S. cerevisiae UFMG 905) in peanut grain indicated that the best reduction of aflatoxin percentage of 96.1% was observed with S. boulardii plus L. delbrueckii (Silva et al. 2015). In numerous reports, researchers have attempted different probiotic-based processing to detoxify aflatoxin B1 from food products that could reduce its content to a tolerable level.
5. Conclusion
The present meta-analysis of experimental studies illustrated that the in vitro aflatoxin B1 binding efficiency of the probiotic bacteria was strain-dependent. The probiotic strains of Lactic acid bacteria were the most attempted probiotic microorganisms for reducing aflatoxin B1 in reviewed studies. The higher binding was associated with Lactobacillus followed by Bifidobacterium, Pediococcus, Lactococcus, and Enterococcus species. The aflatoxin reduction rates are influenced by variables, including incubation temperature, contact time, initial microbial concentration, initial aflatoxin concentration, and pH. The present study showed that probiotic bacteria were an efficient candidate to control aflatoxin B1 in various food products. The fermentation processing of food using starters containing probiotic bacteria, especially using a mixture of probiotic strains, is a helpful method for masking aflatoxin B1 in the food product. Since Aflatoxin B1 is one of the leading food safety concerns worldwide, probiotic bacteria could use a friendly bio-strategy to reduce their content to a safe level.

Disclosure statement
No potential conflict of interest was reported by the author(s).

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