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Innovative application of postbiotics, parabiotics and encapsulated *Lactobacillus plantarum* RM1 and *Lactobacillus paracasei* KC39 for detoxification of aflatoxin M1 in milk powder

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Research Article

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Abstract

This study aimed to evaluate aflatoxin M_1 (AFM_1) level in milk powder and infant milk formulae, in addition to applying innovative methods for AFM_1 & AFB_1 detoxification. Fifty random samples of milk powder and infant formulae (25 of each) were collected from the Egyptian markets for assessing AFM_1 level using ELISA technique. Bioactive components comprising cell free supernatants (postbiotic), acid-dead cells (parabiotic) and the encapsulated-cells of *Lactobacillus plantarum* RM1 and *Lactobacillus paracasei* KC39 were evaluated for their antifungal activity against toxigenic mold strains and their impact on AFB_1 and AFM_1 reduction in reconstituted milk powder. AFM_1 concentration in unpacked milk powder was higher than that of packed samples and infant formulae, although these differences were not significant ($P > 0.05$). About 96.0, 29.4 and 25.0% of the tested infant formulae, unpacked, and packed milk powder were unacceptable in terms of the AFM_1 limit defined by Egyptian and European standards, while all samples were in accordance with the USA/FDA standard. All tested mycotoxigenic strains were sensitive to the different treatments of the probiotics with the highest sensitivity regarding *Fusarium* strain with *L. paracasei* KC39 compared to other genera. The degradation ratios of AFM_1 using the bioactives of the *L. paracasei* KC39 were higher than that of *L. plantarum* RM1 bioactives. Additionally, KC39 parabiotic manifested the best AFB_1 reduction (60.56%). In conclusion, the positive and highly significant relationship ($P < 0.05$) between these effective biocompounds mirrors their major detoxification role which gives a safe solution for AFs contamination issues in milk and milk products.

Milk and milk based-products, which are considered a principal part of sensitive age-group meals (infants, children, and elders), can be contaminated with aflatoxins (AFs), which are the most toxic and carcinogenic class of mycotoxins. AFs are produced by several *Aspergillus* species on tropical and subtropical agricultural commodities. Among AF types, aflatoxin B_1 (AFB_1), which is the most prevalent and toxic compound found in food and feed, is metabolized by cow-liver enzymes to the 4-hydroxy derivative (AFM_1), and secreted in milk (Mohammedi-Ameur *et al.*, 2020).

Health risks associated with the consumption of food contaminated with AFs are acute and chronic toxicity. AFB_1 can cause critical liver injury, liver cirrhosis and tumors, while AFM_1 is considered a major etiological factor for hepatocellular carcinoma, mutagenicity and teratogenicity in mammals. Recently, hepatocellular carcinoma associated with AF exposure has led to death (IARC, 2002). Furthermore, Food and Agriculture Organization (FAO) estimated that approximately 25% of feed and food are lost annually worldwide because of fungi and the associated mycotoxins (Moretti *et al.*, 2017).

Because of the high intake of milk powder by all age groups, the heavy consumption of milk-based infant formulae by sensitive age groups (infants and children) which is necessary for growth, the major toxicity of AFM_1 and the major economic losses (Ahmed *et al.*, 2020; GadAllah *et al.*, 2020), many countries have set a maximum permissible levels for AFM_1 in milk and dairy products which vary from 0.025 to 0.05 $\mu g\,kg^{-1}$ in Egypt, European Union, and USA (USA/FDA, 2005; EC European Commission Regulation, 1881/2006; ES Egyptian Standard, 7136/2010). Despite these regulatory control measures adopted for AFM_1, several studies proved its presence in milk and dairy products and their implication in human

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intoxication, which is mainly explained by its heat stability during the food processing. Therefore, it is tremendously important to control the quality of the animal feed in order to avoid contamination with AFB_1 and consequently bio transformation into AFM_1, in addition to adopting innovative methods for reducing or eliminating molds and aflatoxins from food and feed (Ahmed *et al.*, 2015). In this respect, prevention of fungal growth and the subsequent mycotoxin production in foods and feeds, as well as the detoxification of mycotoxins are the main proposed strategies. Chemical and physical methods are among the primary preventative methods. However, several health risks resulted from the extensive use, environmental problems, and lowering the food quality. Therefore, biological control is the safest and effective alternative for control the toxigenic fungi and their mycotoxins (Suresh *et al.*, 2020).

Probiotics, defined as live microorganisms which, when administered in adequate amounts, confer a health benefit on the host, can be used for this purpose. Lactic acid bacteria (LAB) are on the top of these probiotic microorganisms because of their good safety history in food applications, linked to the production of several bioactive metabolites, the fact that they are generally recognized as safe, and their low production cost. Among the LAB used, *Bifidobacterium* and different Lactobacilli (*L. paracasei*, *L. plantarum*, *L. reuteri*, *L. amylovorus*, *L. rhamnosus*, and *L. fermentum*), act through the adsorption and binding of mycotoxins by their cell wall that contains polysaccharides, protein, and peptidoglycans (Badr *et al.*, 2020; Ren *et al.*, 2020). Viability of the LAB is affected by the gastrointestinal ecosystem following the consumption of food contained probiotics (Shah, 2007). Consequently, encapsulation is considered a functional solution for protection of the LAB under adverse conditions without influencing their antimicrobial effects. Use of whey protein as an encapsulation biomaterial increases the viability of these microorganisms under adverse conditions (Mohammadi *et al.*, 2021). However, only a limited number of studies have estimated the antifungal effects of the encapsulated LAB and its effect on AF production.

The functional properties of paraprobiotics (non-viable cells or the cellular extract) in AF control have been reported by many researchers who have recorded the higher binding efficiency of the nonviable cells compared to the viable cells because of a greater number of binding sites exhibited for AFs and the absence of undesirable fermentative changes in milk that are caused by viable cells (Zhang *et al.*, 2019). Variation in the stability of this form of binding suggested the use of a postbiotic as a promising alternative method for fungal growth inhibition and AF degradation (biological detoxification). These are defined as the metabolic byproducts secreted by live bacteria or released after bacterial lysis that have beneficial effects on the host. They include organic acids, short chain fatty acids, carbon dioxide, hydrogen peroxide, phenyllactic acid, and bioactive low molecular weight peptides, reuterin, diacetyl and bacteriocins and bacteriocin-like inhibitory substances. This innovative method is characterized by broad spectrum of target mycotoxins, low cost, minimal side effects regarding nutrients and the suitability for a wide range of liquid and solid foods (Moradi *et al.*, 2021).

Owing to the importance of the continued AFM_1 monitoring in milk products, especially those intended for infants, as well as the scarcity of reports covering the reduction control of AFM_1 using the encapsulated probiotics and acid treated cells (parabiotics) together with the absence of studies covering AFM_1 detoxification by the postbiotics, the present study was designed to evaluate AFM_1 levels in infant milk formulae and milk powder retailed in the Egyptian markets with assessing the degree of compatibility with the different standard regulations. As a practical solution, the study proposed the use of the bioactive compounds; postbiotic (cell free supernatant), parabiotic (acid treated probiotic) and the encapsulated cells of *L. plantarum* RM1 and *L. paracasei* KC39 for the reduction of AFM_1 & AFB_1 and the assessment of their antifungal effect against toxigenic mold strains.

Materials and methods

Collection of samples

A total number of fifty random samples of infant cow's milk-based formulae ($n = 25$) and full-fat milk powder ($n = 17$ unpacked and 8 packed) were collected from local markets in Cairo and Giza governorates, Egypt.

Determination of AFM_1 content

Prevalence of AFM_1 in the examined samples was carried out using indirect enzyme-linked immune-sorbent assay (ELISA) test kit, BioFront Technologies, Commonwealth Blvd., Tallahassee, USA according to (Sani *et al.*, 2012).

Reduction of the AFs using the bioactive compounds of Lactobacillus plantarum *RM1 and* Lactobacillus paracasei *KC39*

Preparation of the probiotic cell pellets

The new strains of *Lactobacillus plantarum* RM1 and *Lactobacillus paracasei* KC39, isolated from the fermented Rayeb and Karesh cheese, respectively by (Shehata *et al.*, 2018, 2019) were activated on MRS broth media at 37°C/24 h, then, transferred to Lab-fermenter (Jupiter stirred mini-fermenter 4L, Solaris Biotech., Porto Mantovano, Italy) containing MRS-broth and incubated at 37°C/24 h and the yield of each strain was separately centrifuged at 4100 *g*/5°C/30 min to obtain the cell-pellets.

Preparation of RM1 and KC39 postbiotics

The cell-free supernatant (CFS) was prepared according to Shehata *et al.* (2018, 2019). The solution derived from *L. plantarum* RM1 and *L. paracasei* KC39 bacterial-pellet centrifugations were known as postbiotics. They were collected, purified and sterilized by an individual sterile-membrane (0.22 µm), then lyophilized by a Dura-Dry MP freeze-dryer (FTS System, USA) to yield dry-pure powder.

Preparation of RM1 and KC39 parabiotic

The treated probiotic cell-pellets were prepared according to El-Nezami *et al.* (1998) with modification. Hydrochloric acid (1 M) was used for acidification of the media that made the bacterial cells to die. The bacterial concentrations were 2.1×10^{11} and 1.7×10^{11} CFU ml^{-1} media for RM1 and KC39, respectively.

Preparation of the encapsulated bacterial cells

Bacterial strains were encapsulated by wall material consists of maltodextrin and whey protein (1:2), according to the method designated by Abdel-Razek *et al.* (2018) with bacterial cell

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concentrations of 2.1×10^{11} and 1.7×10^{11} CFU ml^{-1} for RM1 and KC39, respectively.

Determination of the antifungal activity of bacterial treatments

The antifungal activities of the postbiotics, parabiotics, and encapsulated probiotics were assessed against four toxigenic fungal strains (*A. flavus* ITEM 698, *A. parasiticus* ITEM 11, *Fusarium moniliforme* KF 488, and *Penicillium chrysogenum* ATCC 10106), which were obtained from ISPA, Bari, Italy using the agar well diffusion method according to Badr *et al.* (2020).

Estimation of the effect of postbiotics, parabiotics and the encapsulated probiotics on AFB_1 secretion in a liquid media

Impact of the bacterial treatments on AFB_1 reduction in a liquid media was done as described by Shehata *et al.* (2019) with modifications. The spores of *Aspergillus flavus* ITEM 698 were inoculated into yeast extract sucrose (YES) broth media at a concentration of 10^5 ml^{-1} and incubated for 18 h. Postbiotics, parabiotics, and the encapsulated cell pellets were inoculated separately (1 mg ml^{-1} media), then re-incubated (96 h/30°C). AFB_1 secreted in the liquid media was determined using ELISA method according to Sani *et al.* (2012). The reduction in fungal mycelial weight was compared to the control one and the antifungal efficacy (AE) was calculated as a percentage by the following equation:

$$AE\% = [(MFWc - MFWt)/ MFWc] \times 100$$

Where AE: is the antifungal efficacy of the treatment, MFWc: is the dried mycelia-weight of control fungal growth, MFWt: is the dried mycelia-weight of treated fungal growth.

The effect of postbiotics, parabiotics, and encapsulated probiotics on AFM_1 reduction

The reduction in AFM_1 concentration owing to each treatment was determined in a model of reconstituted milk powder according to Negera and Washe (2019) with some modification. AFM_1 residue was determined using ELISA according to Sani *et al.* (2012). Toxin inhibition was calculated as a ratio of inhibition by the following equation:

$$\%TRR = [Toc - Tot /Toc] \times 100$$

Where TRR: is the toxin reduction ratio achieved by the treated components, Toc: is the toxin concentration in the control buffer solution, Tot: is the toxin concentration in a buffer solution with the different treatments.

Statistical analysis

The data were statistically analyzed using SPSS Version 17.0 software. An independent sample *t*-test was conducted to compare the AFM_1 levels in infant milk and milk powder, *Mann–Whitney U* test was used when data were not normally distributed. Pearson's correlation coefficient (r) was used for assessing the impact of using postbiotics, parabiotics, and the encapsulated cells on the reduction of AFM_1 and AFB_1. Significance was set at P-value <0.05.

Results

The samples of milk powder and infant formulae were positive for AFM_1 contamination, with no statistically significant differences ($P > 0.05$) between them, although AFM_1-concentration in the packed milk powder sample (46.58 ± 7.69 ng kg^{-1}) was numerically lower than unpacked milk powder (88.78 ± 33.31 ng kg^{-1}) and infant formulae (78.83 ± 19.31 ng kg^{-1}) (Table 1).

According to the critical limit of AFM_1 depicted in different legislations, 96.0, 29.41, and 25.0% of the examined infant formulae, unpacked, and packed milk powder exceeded the Egyptian (ES Egyptian Standard, 7136/2010) and European regulations (EC European Commission Regulation, 1881/2006) which states a maximum limit of 25 and 50 ng kg^{-1} for infant formula and milk powder, respectively. All samples were acceptable within the USA/FDA regulation, which specifies a fixed maximum limit of 500 ng kg^{-1} for milk products and milk-based formulae (Table 2).

Data presented in Fig. 1 show that all mycotoxigenic fungi strains were sensitive to the different treatments of probiotics, with the highest sensitivity for *Fusarium* strain with *L. paracasei* KC39 compared to the other genera. Moreover, postbiotics exhibited the best antifungal activity against *F. moniliforme* (16.7 ± 1.3 and 23.7 ± 1.5 mm) for *L. plantarum RM1* and *L. paracasei* KC39, respectively.

Results of the bioactive compound application in yeast extract sucrose media containing a high producer strain of AFB_1 are shown in Table 3. Growth of *A. flavus* in the control and its secreted AFB_1 content were assessed as a reference used for comparing the different treatments. The obtained results demonstrated fungal-growth inhibition, with AFB_1 reduction ratio of 56.40, 50.27 and 38.60% for *L. plantarum* postbiotics, encapsulated cells and parabiotics, respectively. In relation to *L. paracasei*, the parabiotic KC39 achieved the highest reduction ratio (60.56%) of AFB_1 secretion followed by the encapsulated KC39 (52.73%), whilst postbiotic KC39 yielded a reduction percentage of 42.94%. These results potentially give a safe solution for AFB_1 contamination issues in milk and milk products.

The three RM1-bioactive compounds gave lower activities compared to those of the KC39-bacterial strain. It is important to refer to the postbiotics of KC39-strain as being the most effective, capable of a reduction of 89.8% from the applied models (Fig. 2).

The study revealed a very strong positive and significant correlation between both probiotic strains against -AFB_1 -AFM_1 in liquid media for CFBE and postbiotic ($r = 0.99$, $P < 0.05$). In contrast, only a weak correlation was found for encapsulated RM1-AFB_1 & -AFM1 ($r = 0.34$). Besides, the capsuled probiotic KC39 showed a moderate significant correlation with AFB_1 & -AFM_1 ($r = 0.58$, $P < 0.05$) and postbiotic of KC39-AFB_1 & -AFM_1 ($r = 0.58$, $P < 0.05$). The positive and highly significant relationship between these effective compounds with detoxification effect indicated that bioactive compounds play a major role in the detoxification effect of probiotic strains.

Discussion

Mycotoxins pose a serious health threat to humans and animals, and AFM_1 is the main mycotoxin found in milk and dairy products. This includes milk powder, which is used in the manufacture of many other milk products such as ice cream, cheese, evaporated milk and condensed milk, in addition to its use as

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Table 1. Prevalence of AFM_1 (ng/kg) in samples of dairy products

Product	No. of samples	Positive samples		Min.	Max.	Mean ± SE
		No.	%			
Infant formula	**25**	25	100	24.33	414.00	78.83 ± 19.31
Unpacked milk powder						
	17	17	100	25.67	478.00	88.78 ± 33.31
Packed milk powder	**8**	8	100	16.33	89.00	46.58 ± 7.69
All milk powder	**25**	25	100	16.33	478.00	75.28 ± 22.90

Aflatoxin M_1 was determined in nano-gram per kg using ELISA technique.
Mann–Whitney test was performed for comparing AFM_1 content in infant formulae and milk powder and between the packed and unpacked milk powder samples, showing non-significant differences between the different groups.

Table 2. Acceptability of the samples in relation to AFM_1 content with the various regulation standards

Examined samples	Exceeding EC and ES regulations			Exceeding USA/FDA regulation		
	Permissible limit (ng/kg)	No.	%	Permissible limit (ng/kg)	No.	%
Infant milk formulae ($n = 25$)	25	24	96.00	500	0	0.0
Unpacked milk powder ($n = 17$)	50	5	29.41	500	0	0.0
Packed milk powder ($n = 8$)		2	25.00		0	0.0
All milk powders ($n = 25$)		7	28.00		0	0.0

EC: European Regulations (EC European Commission Regulation, 1881/2006), ES: Egyptian Standard (ES Egyptian Standard 7136/2010).

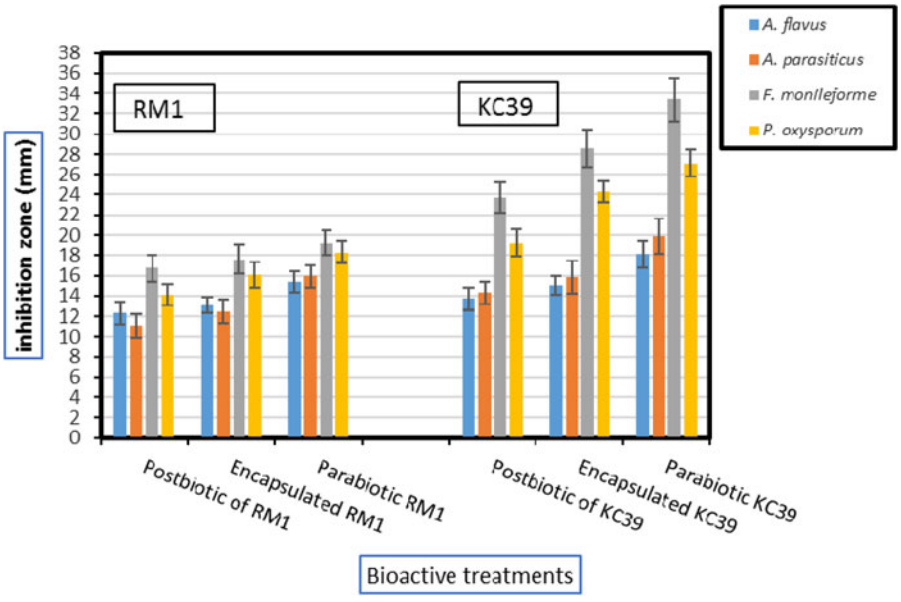

Fig. 1. Antifungal impacts of probiotic components against four toxigenic mold strains. *Antifungal impacts were represented as diameter of the inhibition zone (millimeter) of fungal growth regarding each component treatment.

an ingredient in many bakery products, processed meats, and soups (Ahmed *et al.*, 2015). Therefore, its contamination with AFM_1 means contamination of all downstream products. The problem is exacerbated since neither heat treatment nor mild acidic conditions have been reported to cause any substantial reduction in AFM_1 content. Moreover, infant milk formulae constitute the main meal for some newborns and infants during the first months of life, so it should be AFM_1 free (Meucci *et al.*, 2009).

The current study records a high rate of occurrence of AFM_1 in the tested samples of infant formulae and milk powder, whether packed or unpacked. AFM_1 is a metabolite, not a regular secretion by fungi, which might explain the lack of packed/unpacked difference. These outcomes are similar to those reported by Murshed (2020) and may relate to lack of awareness, insufficient regulatory infrastructure, and/or the absence of proper storage conditions for the animal feed, which resulted in contamination with AFB_1 that bio transformed into AFM_1 and was secreted in milk (Mohammedi-Ameur *et al.*, 2020).

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Table 3. Impact of the bioactive components of two probiotic strains on AFB_1 secretion in the liquid media

Treatments	Mycelia weight	% AE	AFB_1 (ng/ml)	Reduction ratio (%)
Control	3.74 ± 0.15	0.00	468.00 ± 7.24	0.00
L. plantarum RM1				
Postbiotic of RM1	2.27 ± 0.08	39.30	211.90 ± 4.27	56.40
Encapsulated RM1	2.59 ± 0.11	30.75	241.70 ± 4.98	50.27
Parabiotic RM1	2.91 ± 0.04	22.19	298.40 ± 5.36	38.60
L. paracasei KC39				
Postbiotic of KC39	2.47 ± 0.41	33.95	277.30 ± 4.41	42.94
Encapsulated KC39	2.16 ± 0.23	42.24	229.70 ± 5.08	52.73
Parabiotic KC 39	1.86 ± 0.37	50.26	191.70 ± 4.66	60.56

Results are expressed as means ± SE. AE: the antifungal efficacy of the treatment. Mycelia weight represents the mycelia weight of *A. flavus* ITEM 698 in the broth media.

The risk posed by AFM_1 may potentially extend to increased cancer incidence. For instance, AFM_1 increases risk of liver cirrhosis which in turn is a risk factor for hepatocellular hepatoma. Morsy *et al.* (2018) reported a doubling of hepatocellular carcinoma (HCC) over the past decade in Egypt but did not attempt to relate this to AFM_1. Nevertheless, the confirmed toxic and carcinogenic impacts of AFM_1 lead the international agency for research on cancer (IARC) to change its classification from possibly carcinogenic to carcinogenic to humans (IARC, 2002). Carcinogenicity of AFM_1 is influenced by the duration and level of exposure, which may be increased by frequent milk consumption (Ahlberg *et al.*, 2018). Accordingly, many countries have issued strict regulations concerning the maximum permissible AFM_1 levels in milk and dairy foods to protect the consumers from their public health effects. The elimination of risk sources represents a major challenge not only in relation to contaminated consumer products, but also to animal feeds which ultimately constitute the main source of AFM_1 in milk and milk products. Consequently, the main way for avoiding AFM_1 presence in milk is to prevent cattle from feeding contaminated rations, through adopting good feeding practices, having good storage conditions and applying good manufacturing practices.

Where contamination of dairy products is concerned, investigators search to solve this problem using natural components, including probiotic bacterial strains, which have been suggested to bind and inactivate aflatoxins. The binding ratio is affected by heat or acid treatment of the bacterial cells (El-Nezami *et al.*, 2000; Badr *et al.*, 2020). Recently, researchers referred to the bioactive components of the probiotic strains such as *L. plantarum* RM1 and *L. paracasei* KC39 basically as a new type of bacteriocin which could have a significant role in aflatoxin degradation (Shehata *et al.*, 2018, 2019).

An alternative approach is to inhibit the growth of mycotoxin-producing fungi (Ahmed *et al.*, 2021). Lactic acid bacteria (LAB) have been shown by several studies to be a suitable solution for preventing the fungal growth and prolonging the shelf-life of food owing to the produced antifungal compounds, such as organic acids, diacetyl, fatty acids, bioactive antimycotic peptides, bacteriocins, carboxylic acids, lactones, hydrogen peroxide, reuterin and alcohols (Faizan *et al.*, 2019). However, there are only a few reports concerning the antifungal effect of the bacterial bioactives, postbiotics, parabiotics and encapsulated LAB. By comparing the antifungal impact of these compounds we were able to show that, in general, parabiotics derived from LAB are the most effective treatment in exhibiting fungal growth suppression, followed by the encapsulated cells, then the postbiotics. The encapsulated cells of *L. paracasei* KC39 (isolated from the Egyptian traditional fermented milk 'Laban rayeb' and Karesh cheese by Shehata *et al.*, 2018, 2019) were particularly effective at inhibiting the mycelia of the four toxigenic mold strains that we tested. Moreover, *Fusarium moniliforme* was the most sensitive strain for the tested bioactives.

These results agree with those reported by Russo *et al.* (2017), whilst lower activity against the fungal growth of *A. flavus*, *A. niger* and *A. parasiticus* by the encapsulated *L. casei* (LC-01) was recorded by Mohammadi *et al.* (2021).

Bacteriocins, organic acids, enzymes, alcohols, and low-molecular-mass substances are the main metabolites responsible for the antimicrobial action of LAB. These bioactive materials were reported to affect the aflatoxigenic fungal growth with subsequent reduction of their AF secretion (Zhao *et al.*, 2017; Ren *et al.*, 2020). Other studies demonstrated that lactobacilli inhibited AF production, as well as the growth of Aspergillus spp. (Huang *et al.*, 2017). RM1 postbiotics were most effective in reducing AFB_1 production, followed by the encapsulated cell then the parabiotics, but for KC39, in contrast, the ordering was parabiotics > encapsulated > postbiotics.

The reductions we obtained are lower than that reported by Mohammadi *et al.* (2021), who revealed that the encapsulated *L. casei* (LC-01) reduced AFB_1 almost completely (99.2%). *Lactobacillus* binding to AFs is the possible mechanism for AFB_1 reduction (Hashemi and Amiri, 2020). This was confirmed by Ben Taheur *et al.* (2020) who illustrated the reduction through the binding of AFs to the bacterial cell wall, particularly the glucomanann component which showed better capability to bind AFs whether bacteria were viable or killed (by acid or heat).

Various studies revealed the higher AF binding efficiency of nonviable cells compared to viable (Elsanhoty *et al.*, 2014). Our study revealed that acid dead KC39 (parabiotics) achieved the best AFB_1 reduction, which agrees with Haskard *et al.* (2001) who showed that acid, in particular, and heat treatment have a significant positive impact on the reduction of AFB_1 by *L. plantarum* and *L. casei*. Azab *et al.* (2001) observed that AFB_1 removal by *L. acidophilus*, *L. casei*, *L. helveticus* and *L. bulgaricus* was 43.1–87.0% for the acid treatment, which is lower than we observed. These results may perhaps be explained by the effect of acid on the cell wall. Through breaking the glycosidic linkages between polysaccharides or increasing hydrolysis of the proteins into smaller peptides and amino acids, the cell wall thickness is reduced and pore size increased (via decreasing the cross linkages). These changes expose more microbial cell sites for AF binding and inactivation (Haskard *et al.*, 2001).

It is also possible that bioactive components in the bacterial growth media could convert AFs to less toxic material. Such bacterial bioactive metabolites, or postbiotics, have been categorized into four distinct groups comprising micro-molecular organics, amino acids, antibiotics, and enzymes (Ren *et al.*, 2020). The reduction percentage of AFB_1 by RM1 postbiotics was nearly

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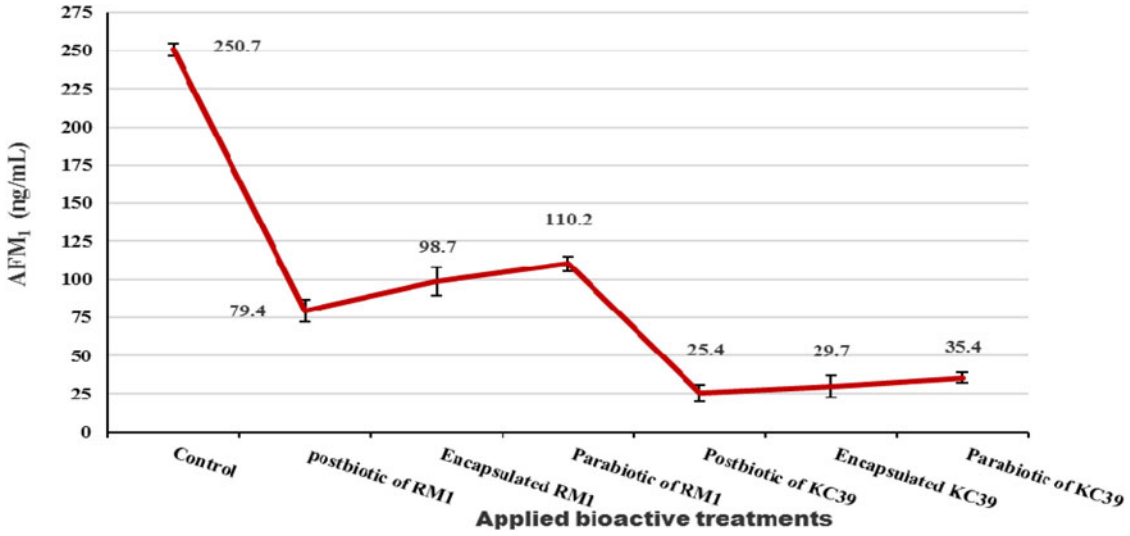

Fig. 2. Reduction control of AFM_1 in reconstituted milk powder using the bioactive components of the two probiotic strains. *The data expressed as means of AFM_1 concentration.

similar to cell free supernatants of *B. subtilis* that provided 60% AFB_1 degradation in the study conducted by Suresh *et al.* (2020), who explained the degradation mechanism *via* enzymes, or other bioactive components expressed by *B. subtilis* rather than the toxin binding. Regarding AFB_1 reduction in the broth media by RM1 postbiotic, presence of protein with a new molecular weight within the RM1 postbiotics previously reported by Shehata *et al.* (2018) might be the main cause. This was in accordance with the theory provided by Ren *et al.* (2020). The inhibition recorded by KC39 postbiotic could be related to the presence of organic acids (lactic, phenyl lactic, hydroxyl-phenyl lactic, and indole lactic acid), previously identified by GC-MS, in addition to the purified bacteriocin which is a completely novel active peptide identified as bacteriocin KC39 by Shehata *et al.* (2019).

Regarding AFM_1 degradation, we observed that KC39 bioactives are more effective than their corresponding RM1 bioactive. KC39 postbiotics were the most effective bioactive at reducing AFM_1, followed by the encapsulated-KC39 then KC39-parabiotics. These results are in accordance with those recorded by Russo *et al.* (2017) who referred to the bacterial cell-free supernatant components as a main reason for aflatoxin degradation. The effective role of KC39 and RM1 postbiotics could be explained by the same theory illustrated before with AFB_1 (Ren *et al.*, 2020).

The results of KC39 parabiotics on AFM_1 are higher than that recorded by Assaf *et al.* (2017) who reported a reduction of 63% by *L. rhamnosus* GG in PBS after heat treatment, and Muaz and Riaz (2021) who reported that acid treated *L. paracasei* (10^8 CFU g^{-1}) successfully reduced AFM_1 in milk spiked with 0.2 µg l^{-1} to 47, and 62% AFM_1 removal against 10^9 CFU g^{-1}. The ability of the dead cells to remove AFs has been suggested through the formation of a non-covalent complex by the components of the bacterial cell wall (Shetty *et al.*, 2007). Moreover, the protein denaturation by the acid and heat treatments results in formation of hydrophobic surfaces which further act as binding sites for aflatoxins (Elsanhoty *et al.*, 2014).

Finally, postbiotics which are defined as soluble metabolites released by food-grade microorganisms during the growth and fermentation are rich in high and low molecular weight biologically active metabolites. There are still gaps concerning these substances. Postbiotics are suggested as superior to probiotics because of their defined chemical composition, safety, ease of use and storage, stability in a broad range of temperature and pH and their broad-spectrum antimicrobial activity. Moreover, they are a rich source of bacteriocin and bacteriocin-like inhibitory substances with antagonistic activity on major foodborne pathogens (Moradi *et al.*, 2021). Furthermore, they will provide a safely practical application through the manufacturing of infant formulae and milk powder *via* re-regulating the AF levels to be in the acceptable range with an increment of safety properties.

In conclusion, aflatoxins are a major hazard that could threaten food safety and dairy production. Degradation of AFs is vital to maintain the safety of foods and feeds. Contamination of infant formulae and milk powder with AFM_1 poses a health risk to specific groups (infants and the elderly, and our samples did exhibit a high incidence of AFM_1 contamination. As a novel solution, postbiotics, parabiotics, and the encapsulated *L. plantarum* RM1 and *L. paracasei* KC39 were evaluated. They had good antifungal impact against four toxigenic fungal strains. Moreover, these bacterial products were able to reduce the AFB_1 level, as well as AFM_1 in a simulated milk powder model. KC39 was more effective for AFM_1 reduction than RM1. We propose that bacterial bioactives could be applied as a solution to limit aflatoxin contamination in dairy products, particularly those directed to the sensitive age groups.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S002202992100090X

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