ORIGINAL RESEARCH

Food Science & Nutrition

WILEY

In vitro activity of encapsulated lactic acid bacteria on aflatoxin production and growth of *Aspergillus* Spp

Rouhollah mohammadi[1] | Sepideh Abbaszadeh[1,2] | Aghil Sharifzadeh[3] |
Mojtaba Sepandi[1] | Maryam Taghdir[1] | Najmeh Youseftabar Miri[4] | Karim Parastouei[1]

[1]Health Research Center, Life style institute, Baqiyatallah University of Medical Sciences, Tehran, Iran

[2]Department of Nutrition and Food Hygiene, Faculty of Health, Baqiyatallah University of Medical Sciences, Tehran, Iran

[3]Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

[4]Department of Food Science, Faculty of Agriculture, Ferdowsi University, Mashhad, Iran

Correspondence
Sepideh Abbaszadeh, Baqiyatallah University of Medical Sciences, Tehran, Iran.
Email: abbaszade@ut.ac.ir

Aghil Sharifzadeh, University of Tehran, Iran.
Email: Asharifzadeh@ut.ac.ir

Abstract

This study aimed to investigate the potential ability of simultaneously used *L. acidophilus(LA-5)*, *L.rhamnosus(LGG)*, and *L.casei(LC-01)* in encapsulated (E) and nonencapsulated (NE) forms in mycelial growth of *Aspergillus* spp and aflatoxin production by *A. flavus*. In order to assess the zone of fungal growth inhibition by E and NE lactic acid bacteria, the agar well diffusion method was applied. Quantification of aflatoxin was performed using a high-performance liquid chromatography technique. Lactic acid bacteria exhibited high antifungal activity and significantly reduced AFB1, AFB2, AFG1, and AFG2 production in both E and NE forms compared to the control group. The percentage of reduction in total AFs production in treated samples with E and NE lactic acid bacteria was 94.1% and 95.5%, respectively. These results suggested that simultaneously used lactic acid bacteria in E and NE forms can prevent growth and decrease aflatoxin production of toxigenic aspergilla.

KEYWORDS
Aflatoxin, *Antifungal activity*, *Aspergillus*, Lactic acid bacteria

1 | INTRODUCTION

Fungal species are regarded as common contaminants of food, and they negatively affect the food quality and shelf-life. Around the world, fungal spoilage of food imposes vast economic losses annually. It has been stated that almost 5% –10% of food across the world and 50% of fruits and vegetables are lost yearly in tropical zones owing to fungal spoilage (Faizan et al., 2019). Many fungal species may produce mycotoxins (fungal toxic secondary metabolites), including aflatoxins, ochratoxins, and fumonisins, which lead to an extensive threat to food safety (Milicevic et al., 2016). In food products, Aspergillus is one of the most common mycotoxigenic fungal species, and aflatoxins (AFs), among mycotoxins, are the most toxic. The most carcinogenic AF is Aflatoxin B_1 (AFB$_1$) (Ahlberg et al., 2015), and it causes chronic and acute intoxication in human being (Shetty et al., 2007). AFs are potent

toxic secondary metabolites produced mainly by *A. flavus* and *A. parasiticus*. The food contamination with AFs causes severe financial losses and health problems (Furukawa & Sakuda, 2019). Aflatoxicosis is the acute form of exposure to AFs of severe hepatotoxicity cases; chronic exposure is associated with hepatocellular carcinoma, immunological suppression, and effects on nutritional status (Strosnider et al., 2006).

Chemical, biological, and physical methods such as oxidation, alkalization, medicinal plants, nanoparticles, and irradiation have been reported to eradicate, inactivate, and inhibit the growth of fungal spieces and the production of mycotoxins (Tola & Kebede, 2016). High costs, low nutritional value, undesirable organoleptic characteristics, and unknown health effects were the adverse points of the previous methods; however, some indigenous microorganisms, which traditionally are used in foods, have potential in the prevention of health adverse effects of mycotoxin (Bhat et al., 2010; Di Natale et al., 2009).

Studies showed that Lactic acid bacteria (LAB) are suitable for preventing fungal growth and prolong the shelf-life of food products (Faizan et al., 2019; Dalié et al., 2010). Organic acids, diacetyl, fatty acids, bioactive antimycotic peptides, bacteriocins, carboxylic acids, lactones, hydrogen peroxide, reuterin, and alcohols are the reported antifungal compounds produced by several LABs (Crowley et al., 2013). Many elements have been proven to affect the viability of LAB in food, such as pH, storage temperature, together with processing conditions (Kim et al., 2008). After consumption, the viable LAB in food must survive the gastrointestinal ecosystem with estimated 0.3% bile salt concentration (Mainville et al., 2005; Shah, 2007). Consequently, it is essential to use a method to selectively protect these usefull organisms under adverse conditions without affecting their antifungal effects. Encapsulation is an operational protection of the LAB against these affected conditions (Nedovic et al., 2011). One of the most common microencapsulation techniques in the food industry is spray drying that engages with the atomization of a LAB suspension and coating material into a drying gas chamber, causing quick water evaporation. Numerous studies have reported the efficiency of this quick and less expensive method to protect microorganisms (Yonekura et al., 2014; Ilha et al., 2015; Rokka and Rantamäki, 2010; Meyer et al., 2019,; Assadpour & Jafari, 2019), which produces powders with tiny particle sizes and a smoother mouthfeel; this would allow the LAB addition to a broader range of foods (Yonekura et al., 2014). Whey proteins, as encapsulation biomaterial, increase the viability of the LAB in adverse conditions (Abd El-Salam and El-Shibiny, 2015). No studies have been conducted on the antifungal effects of spray-dried LAB and its effect on AF production; thus, this study aims to evaluate the effects of LAB encapsulation with spray drying on AF production and growth of Aspergillus spp.

2 | MATERIALS AND METHODS

2.1 | Microorganisms and culture conditions

L. acidophilus (LA-5), *L. rhamnosus* (LGG), and *L. casei* (LC-01) were bought from CHR-Hensen Co., Denmark. All these microorganisms were maintained in sterile 20% v/v glycerol at − 80°C before use. Bacterial colonies were kept at 4 ˚C on MRS agar plates (BioMerieux, France) for next steps. *A. niger* (PTCC 5,012), *A. flavus* (PTCC 5,004), and *A. parasiticus* (PTCC 5,286) as bread-spoiling fungi, were purchased from the Iranian Research Organization for Science and Technology (IROST) and applied as test cultures in antifungal assays. They were maintained on Potato Dextrose Agar (PDA, Oxoid, Hampshire, UK) plates at 25 ˚C for 72 hr (Figure 1).

2.1.1 | Encapsulation by spray drying

Commercial skim milk powder (Sigma-Aldrich, Steinhein, Germany) was used for the bacterial suspension preparation. Whey protein concentrate (WPC) (25%) (5.99% total solids and 35.28% protein) (Razavizadeh et al., 2014) and the inulin 10% (Santosa et al., 2019), as carrier agents, were provided in distilled water and sterilized for 15 min at 121°C. By centrifugation at 3,220 × g at 4°C for 10 min, the mixed bacterial cultures (LA-5, LGG, and LC-01 in equal proportions) were harvested, twice washed with sterile saline, and collected by centrifugation. The washed bacterial cells were resuspended in 5 ml sterile saline, and the mixed cell count was determined using the surface plate method in duplicate in MRS agar. The mixture ratio of LAB strain and the coating material was 1:9 (v/v). For encapsulation, 5 ml prepared bacterial was suspended in a sterilized premixture with 6% UHT skimmed milk. Then, the suspension was mixed at 37°C for 120 min. The process of spray drying was carried out based on a modified technique with lower temperatures and more cell viability (Fritzen-Freire et al., 2013; Bustamante et al., 2015). A mini spray dryer (B190, Buchi, Switzerland) was applied with a 120°C air inlet temperature. Prior to being applied, encapsulating agent solution with bacterial culture was maintained at room temperature under magnetic stirring. The compressor air pressure of 0.4 MPa and a drying airflow rate of 55 m3/ h were applied (Santosa et al., 2019). After spray drying, the final powder was placed in sterile dark flasks and kept at room temperature (Figure 2).

The microcapsules (1.0 g) achieved through spray drying were suspended with 9 ml sterile normal saline and mixed for 1 min, to release the cells. After adequate dilution, the numbers of entrapped
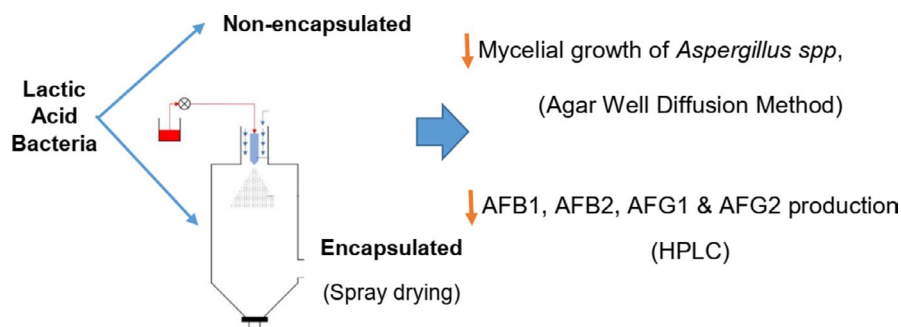

FIGURE 1 This study showed that Lactic acid bacteria exhibited antifungal activity and significantly reduced AFB1, AFB2, AFG1, and AFG2 production in both encapsulated and nonencapsulated forms compared to the control group. The results of this study suggested that simultaneously used lactic acid bacteria in E and NE forms can prevent growth and decrease aflatoxin production of toxigenic aspergilla

cells were measured by using surface plate count technique and encapsulation yield (EY) was computed through Equation 1:

EY (%) = (log N/ log N_0) ×100 [1]. Where N was the number of viable entrapped cells (CFU/ml) released from the encapsulates, and N_0 was the number of free viable cells (CFU/ml) before encapsulation.

2.2 | Antifungal effect

After aerobic incubation for 48 hr at 37 °C, free mixed bacterial culture were cultured on MRS (Abbaszadeh et al., 2015). The agar well diffusion method was used to assess the inhibitory activity (Abbaszadeh et al., 2015). Each fungal suspension counted with Neubauer counting chamber and mixed with MRS agar (10^5 conidia/

ml), after cooled to 55°C, dispended on strile plates (10ml in each plate), and permitted to solidify. Wells with 5 mm diameters were then prepared with Pasteur pipettes in the center of each plate. 10 µl of warm MRS agar was poured in each well in order to cover the base of the wells. Then, 100 µl of a log-phase culture of the free mixed LAB bacteria (10^6 CFU/ml) was added to each well. Encapsulated LAB was suspended with saline and then added to each well. After 5-day incubation of the plates at 30 °C, the growth inhibition was defined by measuring the inhibition region surrounding each agar well. On two separate times, all experiments were repeated in duplicate on each occasion. The fungal inhibition of each group against tested fungi was computed through the following Equation 2:

FI (%) = (IR/GR) × 100; [2].

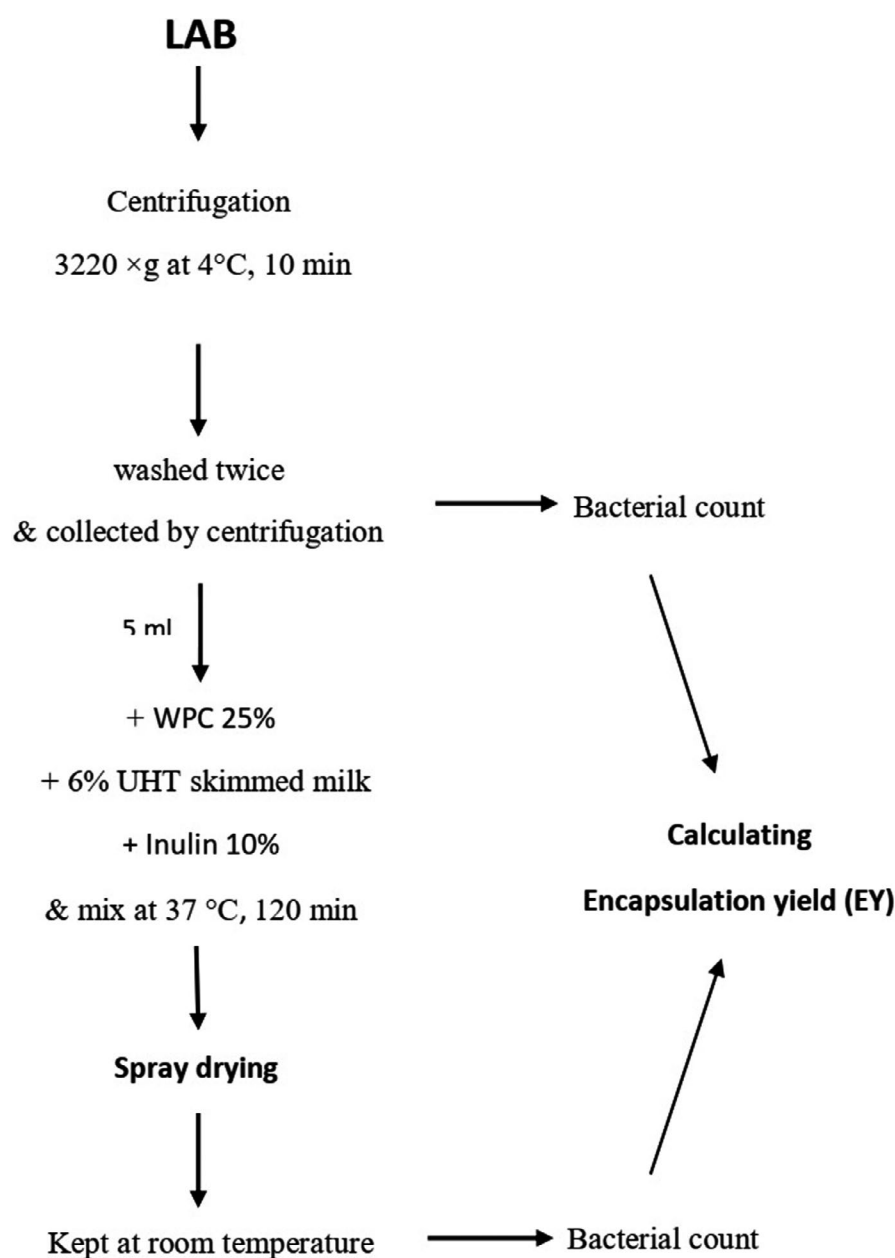FI: fungal inhibition; IR: inhibition radius; GR: growth radius.

FIGURE 2 Flowchart for LAB encapsulation with spray drying method

2.3 | Fungal dry weight (FDW) determination

Based on the method of *Rasooli* and *Razzaghi-Abyaneh*, fungal dry weight was computed. The fungal mycelia were filtered through Whatman filter no. 1, and washed carefully with distilled water. The mycelial mats were located on preweighed Petri plates and allowed to dry for 6 hr at 60°C and then kept for 18 hr at 40°C. The petri plates containing dry mycelia were then weighted. This procedure was performed for both LAB treated and control groups.

2.4 | Effect on Aflatoxin production

The fungal spore suspension was provided with the method of Fan and Chen (1999). From PDA solid cultures in a sterile phosphate buffer solution with Tween 80 (0.05%), spores of *A. flavus* incubated at 25°C were collected. A flamed wire loop was used to loosen the spores. By filtration through sterile cheesecloth, mycelial debris was removed. It was diluted further to achieve a final spore suspension with about 1.5×10^7 spores/ml determined with a Neubauer counting chamber; for all experiments, the spread plate method on PDA plates was applied.

The effect of *lactobacilli* on AF production by *A. flavus* was detected through inoculating 1 ml bacterial suspension with 1 McFarland concentration (E and NE) in yeast extract sucrose broth (YESB) supplemented with fungal spores (10^7 spores/ml), followed by incubation for 10 days at 28°C and 100 rpm. Plain YESB media and YESB media supplemented with known fungal spores were also incubated as negative and positive controls, respectively, with no bacterial inoculation. Medium with lactobacilli and fungus, after incubation, was filtered through Whatman filter paper 1#, extracted and analyzed by HPLC, and then compared with controls (Huang et al., 2017).

2.5 | AFs extraction

AFs were extracted from YESB based on the AOAC standard technique with certain modifications. Concisely, the AFs were extracted from a 50 ml YESB with 300 ml of a solvent mixture of methanol, n-hexane (100 ml), and water (80:20,v/v) and mixing for 3 min vigorously in a high-speed blender. A fluted filter paper was used to filter the extract, and 10 ml of filtrate was diluted and mixed with a solution of phosphate buffer (1:6,v/v). The mixture was injected to an immune-affinity column with specific antibodies of AFs B1, B2, G1, and G2. The AFs were eluted with methanol from immune-affinity columns into acid-washed vials, and finally, 20 μl was applied into HPLC (Hassan & Habibi, 2011).

2.6 | Determination of AFs

The HPLC apparatus was applied to determine AFs. The HPLC setup comprised an Agilent 1,200 Series quaternary pump and a standard autosampler, using a A 1,200 series fluorescence detector with Agilent Technologies (Palo Alto, USA). To manage data, Agilent's ChemStation software was employed. Combinations of three reagents were involved in the mobile phase. AF level determination in the sample extracts was done by isocratic reversed-phase liquid chromatography with Symmetry® C18 column with a 5μm particle diameter, 100 A° pore diameter with matching guard column (Waters Corporation, Milford, USA) and a Kobra cell. For derivatization and fluorescence detector, excitation and emission wavelengths were 362 and 440 nm, respectively. Methanol, acetonitrile, and water (20:20:60 v/v) with 119 mg of potassium bromide, 100 μl of 65% Nitric acid were the mobile phase and filtered through a Millipore 0.22 μm membrane filter before use. At the same fluorescence emission wavelength in a single run, AFs elute in the order of G_2, G_1, B_2, and B_1 (Hassan & Habibi, 2011).

2.7 | Statistical analysis

The tests were carried out in triplicate. In this research, the collected data were expressed as mean ± standard deviation; they were subjected to a one-way analysis of variance (ANOVA) and dependent samples *t* test. Tukey's test was applied to perform multiple comparisons. Statistical significance was set at $p < .05$. SPSS Version 16.0.1 was applied to perform all analyses (SPSS Inc., Chicago, USA).

3 | RESULTS AND DISCUSSION

3.1 | Encapsulation yield (EY)

The average EY obtained in the current study was 96.8 ± 0.8% that is similar to those reported in previous studies using skim milk and cheese whey for spray drying of *L. paracasei* with 93.12% survival rate (Ilha et al., 2015). Lower EY results were found by Maciel et al., with an average EY of 76.58% for spray-dried *L. acidophilus* La-5 microcapsules with reconstituted sweet whey. According to previous studies, the survival of spray-dried LAB depends on the outlet temperature of spray dryer, the types and concentrations of the encapsulating agent, as well as considering that generally, cell culture from a stationary phase survived better than the log-phase culture (Corcoran et al., 2004). Although spray drying causes some physical injuries to the microcapsules, the bacterial cells release, and heat generation, as shown in this study, the spray-dried method with proper liquid feed solution can have an acceptable EY. Whey protein is a favorable biomaterial for probiotic encapsulation due to its effect on bacterial entrapment and prevention of cells overheat in high temperatures (Doherty et al., 2010).

3.2 | Antifungal effect

Antifungal activity of encapsulated (E) and nonencapsulated (NE) LAB was examined on three important bread spoilage fungal species

with the well diffusion method (Figure 3). The amounts of FI showed that both E and NE bacteria inhibited fungal growth (Table 1). Although the antifungal activity of NE bacteria was a bit more than E, the differences were not significant ($p > .05$); it showed that spray drying encapsulation could not significantly reduce the LAB inhibitory effect. Although previous studies showed several LAB as potential bio-preservative agents with a broad antifungal spectrum (Faizan et al., 2019; Kabak & Var, 2004; Bueno et al., 2006), no studies conducted on the antifungal effect of spray-dried encapsulated LAB bacteria. Studies showed that LAB prevents the growth of fungi through producing metabolites, including organic acids, cyclic peptides, reuterin, and hydrogen peroxide (Sadiq et al., 2019). Guimaraes et al. (2018) reported 50% inhibition growth of *Penicillium nordicum* with LAB supernatant containing organic acids.

3.3 | Aflatoxin production

Various studies have shown that inhibiting the growth of mycotoxin-producing fungi is one of the best ways to prevent and reduce food contaminations with a mycotoxin (Blagojev et al., 2012). Along these lines, the AF binding ability of lactobacilli is a suitable biological way to prevent and control fungal food poisoning (Haskard et al., 2001; Peltonen et al., 2001). Previous investigations have shown that suspension inoculation containing a mixture of different species of lactobacillus inhibits mycotoxin biosynthesis in *Aspergillus* and *Penicillium* species (Gourama & Bullerman, 1995; Karunaratne et al., 1990). Thus far, no specific study has been performed concerning the effect of encapsulated LAB on their ability to inhibit AF production. In the

TABLE 1 Fungal inhibition (%) of encapsulated and nonencapsulated lactic acid bacteria using well diffusion method

	FI (%) of lactic acid bacteria	
	Encapsulated	Nonencapsulated
A. flavus	22.5 ± 1.4 [Aa]	26.1 ± 2.5 [Aa]
A. niger	23.6 ± 2.7 [Aa]	26.1 ± 0.9 [Aa]
A. parasiticus	26.1 ± 3.5 [Aa]	36.7 ± 3.8[Ab]

Note: Values are mean ± *SD*. Values with the same lowercase letter in the same column are not significantly different ($p > .05$). Values with the same capital letter in the same raw are not significantly different ($p > .05$).

present study, E and NE lactic acid bacteria presented the highest antifungal activity in the previous studies were simultaneously used against mycelium development and AF production by a toxigenic strain of *A. flavus* (Abbaszadeh et al., 2015). The mycelium production and the concentration of AFB1, AFB2, AFG1, AFG2, as well as total AFs detected in treated samples with E and NE-LAB, were analyzed (Table 2). According to the results, *L. rhamnosus* (LGG), *L. acidophilus* (LA-5), and *L. casei* (LC-01) significantly reduced AFB1, AFB2, AFG1, and AFG2 production in both E and NE forms compared to the control group ($p < .05$). The percentage of reduction in total AFs production in treated samples with E and NE-LAB was 94.1% and 95.5%, respectively. In agreement with the present work's results, previous studies demonstrated that lactobacilli inhibit AF production, as well as the growth of Aspergillus spp (Chang & Kim, 2007; Gerbaldo et al., 2012; Huang et al., 2017).

The percentage of reduction in AF production was 99.2% and 95% by *L. casei* and *Bacillus subtilis*, respectively (Chang & Kim, 2007; Kimura & Hirano, 1988). In the current study, in the NE group, the reduction in AF production was greater than the E group; however, this difference was not significant. Lactobacillus binding to AFs is a possible mechanism of AF reduction by lactobacillus (Chlebicz and Śliżewska, 2020, Hashemi & Amiri, 2020), and also, it is a reason for the greater effects of NE lactobacillus than that of encapsulated, although this difference is not significant, can be the decrease in binding due to encapsulation.

The encapsulated bacteria, after the treatment period, did not show any significant difference in mycelium dry weight of *A. flavus* compared to the NE and control group.

However, the NE group significantly decreased the mycelium dry weight of mycotic mass compared to the control group. The results of nonencapsulated bacteria are in line with the study of Chang in which *L. casei* inhibited the growth and mycelium dry weight *A. flavus* (Chang & Kim, 2007).

4 | CONCLUSION

In the present study, antifungal and anti-aflatoxin effects of encapsulated LAB bacteria are tested for the first time, and results show that spray drying is a useful method for large-scale preparation of

FIGURE 3 Agar well diffusion assay for the determination of antifungal activity of LAB. Black line indicate the zone of inhibition (mm)

TABLE 2 Inhibitory effects of encapsulated and nonencapsulated lactic acid bacteria on aflatoxin production and mycelium dry weight (MDW)

Lactic Acid Bacteria	Aflatoxins (ppb)					MDW (mg/l)
	B1	B2	G1	G2	Total AFs	
Encapsulated bacteria	1,485 ± 18.4 [A]	994.2 ± 5.4 [A]	608.8 ± 11.5[A]	1,197 ± 4.2 [A]	4,285 ± 31.1 [A]	70.6 ± 0.6 [AB]
Nonencapsulated bacteria	1,202.5 ± 6.4 [B]	722.5 ± 17.7 [B]	414.5 ± 7.8 [B]	958 ± 4.2 [B]	3,297.5 ± 0.71 [B]	67.6 ± 0.6 [A]
Control (without bacteria)	38,599.5 ± 14.8 [C]	18,905 ± 63.6 [C]	9,474 ± 36.8 [C]	5,925.5 ± 7.8[C]	72,904 ± 19.8 [C]	72.6 ± 1.3 [B]

Note: Values are mean ± SD. Values with the same letter in the same column are not significantly different ($p > .05$).

encapsulated bacteria, aiming antifungal properties in food systems. This study is currently in progress to examine the effectiveness of such encapsulated bacteria on fungal contaminated bread.

ORCID

Sepideh Abbaszadeh (iD) https://orcid.org/0000-0003-2280-8687

REFERENCES

Abbaszadeh, S., Tavakoli, R., Sharifzadeh, A., & Shokri, H. (2015). LAB as functional probiotic isolates for inhibiting the growth of *Aspergillus flavus*, *A. parasiticus*, *A. niger* and *Penicillium chrysogenum*. *Journal De Mycologie Medicale*, 25(4), 263–267.

Abd El-Salam, M. H., & El-Shibiny, S. (2015). Preparation and properties of milk proteins-based encapsulated probiotics: A review. *Dairy Science and Technology*, 95, 393–412. https://doi.org/10.1007/s13594-015-0223-8

Ahlberg, S. H., Joutsjoki, V., & Korhonen, H. J. (2015). Potential of LAB in aflatoxin risk mitigation. *International Journal of Food Microbiology*, 207, 87–102.

Assadpour, E., & Jafari, S. M. (2019). Advances in Spray-Drying Encapsulation of Food Bioactive Ingredients: From Microcapsules to Nanocapsules. *Annual Review of Food Science and Technology*, 10(1), 103–131. https://doi.org/10.1146/annurev-food-032818-121641

Bhat, R., Rai, R., & Karim, A. (2010). Mycotoxins in food and feed: Present status and future concerns Compr. *Rev. Food Science Food Safety.*, 9, 57–81. https://doi.org/10.1111/j.1541-4337.2009.00094.x

Blagojev, N., Škrinjar, M., Vesković-Moračanin, S., & Šošo, V. (2012). Control of mould growth and mycotoxin production by lab metabolites. *Romanian Biotechnological Letters*, 17(3), 7219–7226.

Bueno, D. J., Silva, J. O., Oliver, G., & Gonzalez, S. N. (2006). Lactobacillus casei CRL 431 and Lactobacillus rhamnosus CRL 1224 as biological controls for Aspergillus flavus strains. *Journal of Food Protection*, 69(10), 2544–2548. https://doi.org/10.4315/0362-028X-69.10.2544

Bustamante, M., Villarroel, M., Rubilar, M., & Shene, C. (2015). *Lactobacillus acidophilus La-05* encapsulated by spray drying: Effect of mucilage and protein from flaxseed (*Linum usitatissimum* L.). *LWT- Food Science and Technology*, 62, 1162–1168. https://doi.org/10.1016/j.lwt.2015.02.017

Chang, I., & Kim, J. D. (2007). Inhibition of Aflatoxin Production of *Aspergillus flavus* by *Lactobacillus casei*. *Mycobiology*, 35(2), 76–81.

Chlebicz, A., & Śliżewska, K. (2020). In Vitro Detoxification of Aflatoxin B1, Deoxynivalenol, Fumonisins, T-2 Toxin and Zearalenone by Probiotic Bacteria from Genus Lactobacillus and *Saccharomyces cerevisiae* Yeast. *Probiotics and Antimicrobial Proteins*, 12, 289–301.

Corcoran, B. M., Ross, R. P., Fitzgerald, G. F., & Stanton, C. (2004). Comparative survival of probiotic lactobacilli spray-dried in the presence of prebiotic substances. *Journal of Apply Microbiology*, 96, 1024–1039. https://doi.org/10.1111/j.1365-2672.2004.02219.x

Crowley, S., Mahony, J., & van Sinderen, D. (2013). Current perspectives on antifungal LAB as natural bio-preservatives. *Trends in Food Science and Technology*, 33(2), 93–109.

Dalié, D., Deschamps, A., & Richard-Forget, F. (2010). LAB − potential for control of mold growth and mycotoxins: A review. *Food Control*, 21, 370–380.

Di Natale, F., Gallo, M., & Nigro, M. (2009). Absorbents selection for aflatoxins removal in bovine milks. *Journal of Food Engineering*, 95, 186–191.

Doherty, S. B., Gee, V. L., Ross, R. P., Stanton, C., Fitzgerald, G. F., & Brodkorb, A. (2010). Efficacy of whey protein gel networks as potential viability-enhancing scaffolds form cell immobilization of *Lactobacillus rhamnosus GG*. *Journal of Microbiology Methods*, 80, 231–241.

Faizan, A. S., Bowen, Y., Fengwei, T., Jianxin, Z., Hao, Z., & Wei, C. (2019). LAB as Antifungal and Anti-Mycotoxigenic Agents: A Comprehensive Review. *Comprehensive Reviews in Food Science and Food Safety*, 18, 1403–1436.

Fan, J. J., & Chen, J. H. (1999). Inhibition of aflatoxin-producing fungi by welsh onion extracts. *Journal of Food Protection*, 62, 414–417. https://doi.org/10.4315/0362-028X-62.4.414

Fritzen-Freire, C. B., Prudêncio, E. S., Pinto, S. S., Muñoz, I. B., & Amboni, R. D. M. C. (2013). Effect of microencapsulation on survival of *Bifidobacterium BB-12* exposed to simulated gastrointestinal conditions and heat treatments. *LWT- Food Science and Technology*, 50(1), 39–44. https://doi.org/10.1016/j.lwt.2012.07.037

Furukawa, T., & Sakuda, S. (2019). Inhibition of Aflatoxin Production by Paraquat and External Superoxide Dismutase in *Aspergillus flavus*. *Toxins*, 11(2), 107. https://doi.org/10.3390/toxins11020107

Gerbaldo, G. A., Barberis, C., Pascual, L., Dalcero, A., & Barberis, L. (2012). Antifungal activity of two Lactobacillus strains with potential probiotic properties. *FEMS Microbiology Letters*, 332(1), 27–33. https://doi.org/10.1111/j.1574-6968.2012.02570.x

Gourama, H., & Bullerman, L. B. (1995). Inhibition of growth and aflatoxin production of *Aspergillus flavus* by Lactobacillus species. *Journal of Food Protection*, 58(11), 1249–1256. https://doi.org/10.4315/0362-028X-58.11.1249

Guimaraes, A., Venancio, A., & Abrunhosa, L. (2018). Antifungal effect of ˜ organic acids from LAB on Penicillium nordicum. *Food Additives and Contaminants: Part A*, 35(9), 1803–1818.

Hashemi, S. M., & Amiri, M. J. (2020). A comparative adsorption study of aflatoxin B1 and aflatoxin G1 in almond butter fermented by

Lactobacillus fermentum and Lactobacillus delbrueckii subsp. lactis. *LWT*, *128*, 109500. https://doi.org/10.1016/j.lwt.2020.109500

Haskard, C. A., El-Nezami, H. S., Kankaanpää, P. E., Salminen, S., & Ahokas, J. T. (2001). Surface binding of aflatoxin B1 by LAB. *Apply Environmental Microbiology*, *67*(7), 3086–3091.

Hassan, J., & Habibi, S. (2011). Reverse homogeneous liquid–liquid extraction as a miniaturized method for extraction of aflatoxins from pistachio and wheat and LC post-column derivatization-fluorescence detection. *Chromatographia*, *73*(9–10), 1005–1008. https://doi.org/10.1007/s10337-011-1981-y

Huang, L., Duan, C., Zhao, Y., Gao, L., Niu, C., Xu, J., & Li, S. (2017). Reduction of aflatoxin B1 toxicity by Lactobacillus plantarum C88: A potential probiotic strain isolated from Chinese traditional fermented food "tofu". *PLoS One*, *12*(1), e0170109. https://doi.org/10.1371/journal.pone.0170109

Ilha, E. C., Silva, T., Lorenz, J. G., Rocha, G. O., & Sant'Anna, E. S. (2015). *Lactobacillus paracasei* isolated from grape sourdough: Acid, bile, salt, and heat tolerance after spray drying with skim milk and cheese whey. *Europian Food Research Technology*, *240*, 977–984. https://doi.org/10.1007/s00217-014-2402-x

Kabak, B., & Var, I. (2004). The effect of Lactobacillus and Bifidobacterium strains on the growth and AFB (1) production of *Aspergillus flavus*. *Acta Alimentaria*, *33*(4), 371–376.

Karunaratne, A., Wezenberg, E., & Bullerman, L. B. (1990). Inhibition of mold growth and aflatoxin production by Lactobacillus spp. *Journal of Food Protection.*, *53*(3), 230–236.

Kim, S.-J., Cho, S. Y., Kim, S. H., Song, O.-J., Shin, I I.-S., Cha, D. S., & Park, H. J. (2008). Effect of microencapsulation on viability and other characteristics in *Lactobacillus acidophilus* ATCC 43121. *LWT –. Food Science and Technology*, *41*, 493–500. https://doi.org/10.1016/j.lwt.2007.03.025

Kimura, N., & Hirano, S. (1988). Inhibitory strains of *Bacillus subtilis* for growth and aflatoxin production of aflatoxigenic fungi. *Agricultural and Biological Chemistry*, *52*(5), 1173–1179. https://doi.org/10.1271/bbb1961.52.1173

Mainville, I., Arcand, Y., & Farnworth, E. R. (2005). A dynamic model that simulates the human upper gastrointestinal tract for the study of probiotics. *International Journal of Food Microbiology*, *99*, 287–296. https://doi.org/10.1016/j.ijfoodmicro.2004.08.020

Meyer, T. A., Bondegaard, P. W., Guerra, P., Christfort, J. F., Kamguyan, K., & Nielsen, L. H. (2019). *Preparation and storage of bacteria used for a novel probiotic delivery system*. Abstract from Probiota.

Milicevic, D., Nastasijevic, I., & Petrovic, Z. (2016). Mycotoxin in the food supply chain implications for public health program. *Journal of Environmental Science and Health Part C: Environmental Carcinogenesis & Ecotoxicology Reviews*, *34*(4), 293–319. https://doi.org/10.1080/10590501.2016.1236607

Nedovic, V., Kalusevic, A., Manojlovic, V., Levic, S., & Bugarski, B. (2011). An overview of encapsulation technologies for food applications. *Procedia Food Science*, *1*, 1806–1815.

Peltonen, K., El-Nezami, H., Haskard, C., Ahokas, J., & Salminen, S. (2001). Aflatoxin B1 binding by dairy strains of LAB and bifidobacteria. *Journal of Dairy Science*, *84*(10), 2152–2156.

Razavizadeh, B. M., Mohammadi, F. K., & Azizi, S. N. (2014). Comparative study on properties of rice bran oil microcapsules prepared by spray drying and freeze drying. *JRIFST*, *3*(2), 97–114.

Rokka, S., & Rantamäki, P. (2010). Protecting probiotic bacteria by microencapsulation: Challenges for industrial applications. *Eur Food Research Technology*, *231*, 1–12.

Sadiq, F. A., Yan, B., Tian, F., Zhao, J., Zhang, H., & Chen, W. (2019). LAB as Antifungal and Anti-Mycotoxigenic Agents: A Comprehensive Review. *Comprehensive Reviews in Food Science and Food Safety*, *18*(5), 1403–1436.

Santosa, D. X., Casazza, A. A., Aliakbariana, B., Bedani, R., Isay Saad, S. M., & Perego, P. (2019). Improved probiotic survival to in vitro gastrointestinal stress in a mousse containing *Lactobacillus acidophilus La-5* microencapsulated with inulin by spray drying. *LWT - Food Science and Technology*, *99*, 404–410. https://doi.org/10.1016/j.lwt.2018.10.010

Shah, N. P. (2007). Functional cultures and health benefts. *International Dairy Journal*, *17*, 1262–1277.

Shetty, H. P., Hald, B., & Jespersen, L. (2007). Surface binding of aflatoxin B1 by *Saccharomyces cerevisiae* strains with potential decontaminating abilities in indigenous fermented foods. *International Journal of Food Microbiology*, *113*, 41–46. https://doi.org/10.1016/j.ijfoodmicro.2006.07.013

Strosnider, H., Azziz-Baumgartner, E., Banziger, M., Bhat, R. V., Breiman, R., Brune, M.-N., DeCock, K., Dilley, A., Groopman, J., Hell, K., Henry, S. H., Jeffers, D., Jolly, C., Jolly, P., Kibata, G. N., Lewis, L., Liu, X., Luber, G., McCoy, L., … Wilson, D. (2006). Workgroup report: Public health strategies for reducing aflatoxin exposure in developing countries. *Environmental Health Perspectives*, *114*, 1898–1903. https://doi.org/10.1289/ehp.9302

Tola, M., & Kebede, B. (2016). Occurrence, importance and control of mycotoxins: A review. *Cogent Food & Agriculture*, *2*, 1–12. https://doi.org/10.1080/23311932.2016.1191103

Yonekura, L., Sun, H., Soukoulis, C., & Fisk, I. (2014). Microencapsulation of *Lactobacillus acidophilus* NCIMB 701748 in matrices containing soluble fibre by spray drying: Technological characterization, storage stability and survival after in vitro digestion. *Journal of Functional Foods*, *6*, 205–214. https://doi.org/10.1016/j.jff.2013.10.008