

Ability of Immobilized Starter Cells and Metabolites to Suppress the Growth Rate and Aflatoxins Production by *Aspergillus flavus* in Butter

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Abstract: Antifungal activity of lactic acid bacteria (LAB)starter cultures, *Lactococcus lactis* ssp. *lactis* and *Leuconostoc mesenteroides* and their metabolites in single and mixed cultures were found to inhibit spoilage and aflatoxin production by *Aspergillus flavus* in butter ,and have potential as bio-preservative agents. Also, treating cream before churn with free cells culture proved to give the greatest antifungal control upon *A. flavus* growth and aflatoxin production; while the use of immobilized cells showed lower activity, then the immobilized metabolites of the mixed culture. In cream artificially contaminated with aflatoxin (B₁, B₂, G₁andG₂) treated with immobilized cells or immobilized metabolites of the mixed cultures revealed a reduction of the concentration of aflatoxins recovered from butter made from this cream. The study indicated that the use of lactic acid bacteria and their metabolites in cream or butter have the potential to be as food-grade bio-preservatives for extending the shelf-life of butter and combating the problem of moulds and associated toxins. [Journal of American Science 2010;6(7):131-138]. (ISSN: 1545-1003).

1. Introduction

Contamination of milk and milk products particularly butter by undesirable moulds is a major problem in Egypt and many other countries having similar warm climat .Since the butter usually is unsalted, bulk-packed and repacked, the conditions allow the growth of these moulds. *Aspergillus spp.*the common spoilage moulds generally grow faster, resist low water activity and tend to dominate spoilage in warmer climates. Many of these commonly occurring moulds can not only cause off flavor and discoloration, but also may produce highly toxic and extremely carcinogenic mycotoxins such as aflatoxins, trichothecenes, fumonisin, ochratoxin A and patulin (Schnurer & Magnusson, 2005). Aflatoxins have implicated into hepato-cellular carcinoma and acute hepatitis (Devries et al., 1990). Butter which is one of the popular varieties of dairy products with high nutritive value, and if moldy contaminated it might constitutes a public health hazard as well as economic losses throughout its deterioration (El-Diasty & Salem, 2007).The presence of moulds and yeasts in butter are objectionable, as they grow at a wide range of temperature and pH values and their count is used as an index of storability and sanitary quality of the product. Also, high lipid content and low water content make the butter more susceptible to spoilage by moulds than by bacteria (James and coulter, 1998). Moreover, many psychrotrophic strains of bacteria, yeasts and moulds have been implicated in spoilage of butter at temperatures below 5°C and some even below 0°C. (Rady and Badr, 2003).

.Mycotoxigenic moulds were isolated from Egyptian butter samples (El-Diasty &Salem, 2007).Also,Aflatoxins has been detected in butter samples(Aycicek et al., 2001and Aycicek et al.,2005).

On the other hand, some strains of lactic acid bacteria (LAB) such as *Lactococcus* ssp. and *Leuconostoc mesenteroides* that are already proven to be suitable as LAB starter cultures, were also found to produce metabolites with high antimicrobial activity . The application of their metabolites as biopreservatives in both fermented and non-fermented dairy products has become one of the important trends in food preservation. Furthermore, the use of cell-free metabolites of LAB cells offer the advantage of minimizing texture and flavor changes especially in non -fermented dairy products (Ghita et al., 2004).

On the other side of LAB beneficial usage, investigations revealed that some strains of LAB have been shown to inhibit both growth of mold (Batish et al., 1991& Cabo et al., 2002) and the production of mycotoxins (Coallier-Ascah & Idziak, 1985).Also, they could remove aflatoxins from aqueous solution (El-Nezami et al., 1998; Haskard et al., 2001), and binding with aflatoxin in the animal or human gut that was suggested as a method for controlling aflatoxin levels (Bueno et al., 2007).

Cell immobilization is a method of retaining microorganisms in a discrete location of a fermentation system and also allows the potential to harness the advantages of free culture systems while minimizing the disadvantages. In this regard,

immobilization of LAB has been shown to offer many advantages for biomass and metabolite production compared with free-cell systems such as high cell density, reuse of biocatalysts, improved resistance to contamination or bacteriophage attack, and physical and chemical protection of cells (Champagne et al., 1994). A popular method for bacterial immobilization is entrapment in gel materials, especially in seaweed gel materials such as carrageenan or alginate (Willaert & Baron 1996). Also, microencapsulation of some metabolites such as nisin has proven to be a powerful microbial inhibitor (Benech et al., 2003).

Therefore, the purpose of this study was to investigate the potential antifungal activity of some mesophilic starter cultures, and their immobilized cells and metabolites as specific bio-preservatives in order to protect butter against the development of moulds. Thus, the ultimate aim is extending the shelf-life of fermented and non-fermented butter and avoids the danger of aflatoxins.

2. Material and Methods

2.1. Starter cultures

Reference test strains of *Lactococcus lactis* spp. *Lactis* CH-1 (culture A), *Lactococcus lactis* spp. *lactis* biovar. *diacetylactis* MB-1 (culture B) and *L. lactis* spp. *cremoris* CH-1 (culture C) were obtained from Chr. Hansens, Copenhagen, Denmark. *Leuconostoc mesenteroides* spp. *mesenteroides* B-1118 (D) was provided by Northern Regional Research laboratory (NRRL) Illinois, USA.

2.2. Fungal test strains

Nine strains of moulds were obtained from Standard Association of Australia 80 Arthur st., North Sydney, NSW : *Aspergillus parasiticus* NRRL 2999, : *A. parasiticus* NRRL 2669, *A. flavus* NRRL 3251, *A. flavus* NRRL 5906, *A. ochraceus* NRRL 3174, *Penicillium discolor* NRRL 1951, *Penicillium* spp., *Fusarium manifforme* NRRL 6322, *F. manifforme* NRRL 2284

2.3. Reference aflatoxin and fungal inocula

Pure reference Aflatoxins (B₁, B₂, G₁ and G₂) (in acetonitrile:benzene 98:2 v/v) were purchased from Sigma, Chemical Company, St. Louis, USA. The spore suspension of fungal inocula was prepared according to the method of Arumsharma et al., (1980) by growing the mold on Potato Dextrose Agar (PDA) (Oxoid, Basingstoke, UK), for 7 days at 28 °C. The spores were harvested by adding 10 mL sterile distilled water and subsequently diluted to a turbidity gave a spore suspension of ~ 10⁴ spores mL⁻¹ by plating, previously experimented.

2.4. Antifungal activity assay

The agar overlay method of Magnusson & Schnauzer (2001) was used to detect antifungal activity. The four LAB strains separately were inoculated as 2 cm long lines on Elliker's agar plates (Oxoid) and incubated at 30 °C for 48h. The plates were then overlaid with 10 ml each of malt extract soft agar (2% malt extract and 0.7% agar) (Oxoid) containing the moulds to be tested (~10⁴ fungal spores mL⁻¹). The plates were examined for the development of clear inhibition zones around the bacterial streak and the area of the zones was scored as follows : (-) no suppression; (+) no fungal growth on 0.1 to 3 % of the plate area per bacterial streak; (++) no fungal growth on 3 to 8 % of plate area per bacterial streak ; (+++) no fungal growth on > 8 % of plate area per bacterial streak.

2.5. Immobilization of the cells and metabolites:

Immobilization of the cells:

Cells were trapped in sodium alginate according to the procedure of Kilnkenberg et al., (2001). Elliker's broth media (Gibson et al., 1965) (500 ml) was inoculated with 5% (v/v) active culture of *L. lactis* spp. *lactis* and *lc. mesenteroides* (1:1) and incubated at 35 °C for 48 h. Cells were harvested aseptically by centrifugation (8400 ×g, 20 min, 4 °C). The pellets were suspended in dilution buffer to a concentration of 4 g dry weight cells L⁻¹. Resuspended cells were mixed with an equal volume of 4 % (w/v) sodium alginate, yielding a final cell concentration of 2 g dry weight cells L⁻¹, which was used in the experiments. The mixture of alginate and cells was added drop wise into a sterile solution of sodium chloride (0.2 mol L⁻¹) and calcium chloride (0.05 mol L⁻¹). Sodium chloride was used in the gelling solution to ensure a homogeneous polysaccharide concentration throughout the beads. To ensure complete gelling, the beads were stirred for at least 40 min in this solution

Immobilization of the metabolites:

The same was carried using following mixed LAB cultures (1:1) of A+D strains, A+B strains, C+ D strains and C+B strains.

After removing the cells by centrifugation as described above the supernatant was heat treated (80 °C for 10 min) to kill the remaining cells. The cell free supernatant (metabolites) was immobilized by mixing with equal volume of 4 % (w/v) sodium alginate solution and preceded as described for immobilization the cells (Kilnkenberg et al., 2001).

2.6. Effect of mesophilic starter culture on growth and toxin production by *A. flavus* in broth

A previously tested spore suspension of ~10⁴ spores mL⁻¹ of *A. flavus* NRRL5906 was used to

inoculate 5 flasks, each containing 200 mL Elliker's medium. Four flasks were inoculated with 2 % (v/v) of one of the mixed (1:1) cultures: A+D, A+B, C+D and C+B. The fifth flask was kept as a control (without starter). All flasks were incubated at 25 °C for 21 days. Weights of mycelia and aflatoxins production were determined at the end of incubation period.

Determinations of mycelial dry weights were obtained by drying filtered mycelium at 50°C for 12 h. (Davis et al., 1986).

2.7. Effect of immobilized metabolites of the mixed cultures on the growth of *A. flavus* on solid media

An agar overly method similar to that described above was used to determine the antifungal activity of immobilized metabolites. Immobilized metabolites of the mixed cultures: A+D cultures, A+B cultures, C+D cultures and C+B cultures, were laid separately on the middle line of the solid media containing $\sim 10^4$ cfu mL⁻¹ of *A. flavus* (NRRL 5906) spores. The plates were incubated at 28 °C for 7 days and assessed as described before.

2.8. Effect of starter culture, immobilized cells of starter culture and their immobilized metabolite on the growth and aflatoxins production of *A. flavus* in butter

Fresh milk was separated to obtain 30% fat cream. The cream was pasteurized and divided into two portions, one for the sensory evaluation and the other was inoculated with *A. flavus* (NRRL 5906) suspension to give an initial count of $\sim 10^4$ cfu g⁻¹. Each portion of cream was divided into 4 equal portions and treated as follows: Treatment 1 = inoculated with 2 % mixed starter culture of A+D cultures, Treatment 2 = inoculated with 2 % beads of immobilized mixed starter culture of A+D cultures Treatment 3 = inoculated with 5 % beads of immobilized metabolite of the mixed starter culture of A+D cultures and Treatment 4 = kept as control.

All samples of creams were aged for 72h at 7-10 °C, and then churned (without removing of the inoculated beads). The resulting butters (unsalted) were then distributed into covered plastic cups and stored at 7-10 °C for 60 days and analyzed for counts of the fungi and aflatoxin production.

2.9. Effect of immobilized starter culture and their metabolites on aflatoxins

Pasteurized 30% fat cream was spiked (12 µg mL⁻¹), with standard aflatoxins B₁, B₂, G₁ and G₂, each separately, and then divided into three portions. The first portion was inoculated with 2 % immobilized beads of the chosen mixed culture, the second with 5% immobilized metabolite of the mixed culture, the

third kept as control. All treatments were churned and the resulting butters stored at 10 °C and analyzed for toxins on day 0, 15 30 45 and 60.

2.10. Microbiological analysis

Ten grams of the prepared samples were transferred into a sterile flask containing 90 ml of warm sterile peptone water 1% (40±1 °C) to prepare a dilution of 10⁻¹ from which decimal dilutions up to 10⁻⁸ were prepared.

A. flavus (NRRL 5906) was counted on PDA (Oxoid) acidified to pH 3.5 with sterile lactic acid solution (10%). Plates were incubated at 25 °C for 4-5 days. Free cells of *L.lactis ssp. lactis* was counted on M17 agar (Oxoid). Plates were incubated at 30 °C for 48h. Free cells of *Lc.mesenteriodes* were counted on MRS agar (Oxoid) containing 30 µg mL⁻¹ of vancomycin (Levata-Jovanovic & Sandine, 1997). Plates were incubated at 30 °C for 48-72 h.

2.11. Extraction and determination of aflatoxin

Aflatoxins (B₁, B₂, G₁ and G₂) were analyzed according to A.O.A.C (2000) method. Essentially, 50 g test portion was weight into blender jar, then, 100 ml hexane and 250 ml methanol/water (55+45) were added and blended for 3 min at high speed. The blended sample was filtered and the filtrate transferred to a separating funnel. When the layers were separated, 20 ml of the lower layer was released into 150 ml beaker, heated on a hot plate at 60 °C till dryness, then cooled.

Toxins were detected qualitatively by thin layer chromatography (20×20 cm silica gel 60 aluminum sheets, Merck KGaA, Merck, Darmstadt, Germany) following the AOAC (2000) method. Quantitative detection of toxin was performed using high performance liquid chromatography (HPLC). The HPLC system comprised a Waters model 600 delivery system equipped with fluorescence detector model 470 and a Nova Pack (150×36 mm) C₁₈-column (Waters, Milford, MA, USA). A mobile phase of water/ methanol/acetonitrile (56:14:30, by vol.) was applied isocratically at used flow rate of 1 mL min⁻¹. Data were integrated and recorded using a Millennium Chromatography. Manger Software 2010 (Waters).

2.12. Sensory evaluation of butter

Sensory evaluation of butter as affected by immobilized starter, metabolites and free cells strains was carried out when fresh by trained 10 of panelist from the staff members of Dairy Science Department, National Research Centre, with score for flavor (50 points), body and texture (35 points) and appearance (15 points), according to Nelson and Trout (1956).

2.13. Statistical analysis

All experiments were repeated in triplicates and each analysis in duplicated and average results was tabulated. Data were statistically analyzed to calculate the least significant difference (LSD) at the 5% level of probability procedure according to Snedcor & Cochran (1980).

3. Results and Discussion

3.1. Screening for antifungal activity

Preliminary study for antifungal activity of the four strains of LAB, predominantly mesophilic starter strains against different nine strains of common butter spoilage moulds, as shown in Table, 1. The LAB strains tested showed a spectrum of activity against the majority of the tested moulds. Out of the tested cultures *Lc. mesenteroides* and *L. lactis* spp. *diacetylactis* had a broad antifungal inhibitory spectrum with activity against all of the tested moulds. The selection was for those showed strong antifungal activity against *A. flavus* and *A. parasiticus*, which were reported as two ubiquitous species frequently found to be contaminants of food and are major sources of aflatoxins (Rustom, 1997). These results are in complement to those found by Suzuki et al., (1991) who also recorded the antifungal activity of lactic acid bacteria and some *Leuconostoc* strains. Also, *L. lactis* spp. *lactis* was able to exert antifungal activity especially against *A. flavus*. The obtained results were in agreement to those reported by Batish et al., (1997), Kumar et al., (2007) who recorded strong broad spectrum of antifungal activity of some Lactic acid bacteria especially *L. lactis* spp. *lactis*.

3.2. Effect of immobilized metabolites of starter culture on the growth of *A. flavus* on solid media.

The immobilized metabolites in combinations pairs of the mixed starter cultures of *L. lactis* spp. *lactis* + *Lc. mesenteroides* (A+D) were found to be mostly active against the growth of *A. flavus*. Antifungal substances in the cell-free supernatants of some lactic acid bacteria have previously been reported (AbdAlla et al., 2005; Cabo et al., 2002; Kumer et al., 2007). Generally, the combined metabolites in the current study enhanced the antifungal activity against the moulds tested compared with the individual strains. Trend may be concomitant with Effat (2000) who reported that combination between pairs of antifungal substances from LAB was found to be the most active against tested moulds.

It is noteworthy that the presence of immobilized metabolites from the mixed culture A+D (*L. lactis* spp. *lactis* and *Lc. mesenteroides*) eliminated fungal

growth and consequently prevented aflatoxin formation.

As *L. lactis* spp. *lactis* and *Lc. mesenteroides*, showed the highest antifungal activity, as they were selected for further application for producing lactic acid and aroma.

3.3. Effect of mixed cream starter cultures on the growth and toxin production of *A. flavus* in broth medium.

It is clear from the results in Table 2, that significant differences were found between the effects of the mixed cultures and the control on aflatoxin production by *A. flavus*. The highest values of aflatoxin production (8.03, 6.48, 4.04 and 5.0 μgL^{-1} for B₁, B₂, G₁ and G₂, respectively) and mycelium weight (16.94 gL^{-1}) were recorded for the culture of *A. flavus* (NRRL 5906) with *L. lactis* spp. *cremoris* + *Lc. mesenteroides* (C+D); as no significant difference was given between this mixed culture and the control (with respect to *A. flavus*). In contrast, results for co-culture of *A. flavus* with *L. lactis* spp. *lactis* and *Lc. mesenteroides* showed that this LAB mixed culture prevented the production of aflatoxins (Fig. 1, Table 2), and there was a more pronounced decrease in the mycelium weight of *A. flavus* than with the other cultures (as determined at the end of the incubation period for all treatments).

These results are in agreement with those obtained by El-Gendy & Marth (1981) who observed the arrested growth of *Aspergillus* spp. even after 2 weeks at 15 °C in the presence of *L. lactis* spp. *lactis* and *Lactobacillus casei*. Also, Coallier-Ascah & Idziak (1985) recorded that *L. lactis* produced inhibitors during the logarithmic phase of growth which reduced the amount of aflatoxin production by *A. flavus*.

3.4. Inhibition of growth of *A. flavus* and aflatoxins production in butter

Results in Table 3 reveal that mixed culture of *L. lactis* spp. *lactis* and *Lc. mesenteroides* (treatment 1) was effectively used in, preventing aflatoxin G₁ and G₂ production by *A. flavus*, and significantly reducing the levels of the aflatoxin B₁ and B₂ in all treatment. In contrast, in the inoculated control butter sample the mould grew and increased in counts to reach more than 10⁷ cfu g⁻¹ (Fig. 2). Also, aflatoxins B₁, B₂, G₁ and G₂ were produced at concentrations between 4.42-2.76 $\mu\text{g kg}^{-1}$ at day 30 with complete deterioration of the control butter (Table 3). Moreover, both immobilized cells and metabolites (treatments 2&3) eliminated the growth of *A. flavus* (Fig. 2, a) and retarded the aflatoxin production (Table 3). Immobilized cells were more effective than immobilized metabolites in inhibiting the growth of the fungi and consequently, the

production of aflatoxins. Therefore, immobilized metabolites can be used as biopreservation of sweet butter. The results obtained are also in accordance with that reported by Hegazy et al., (2006).

Counts of *Lactococcus* spp. and *Leuconostoc* spp. in butter (Treatment 1 and 2), as shown in Fig. 2 b, c indicated that the counts of both strains increased slightly during the first month of storage, possibly attributed to high fat content in butter samples and then these counts decreased gradually thereafter.

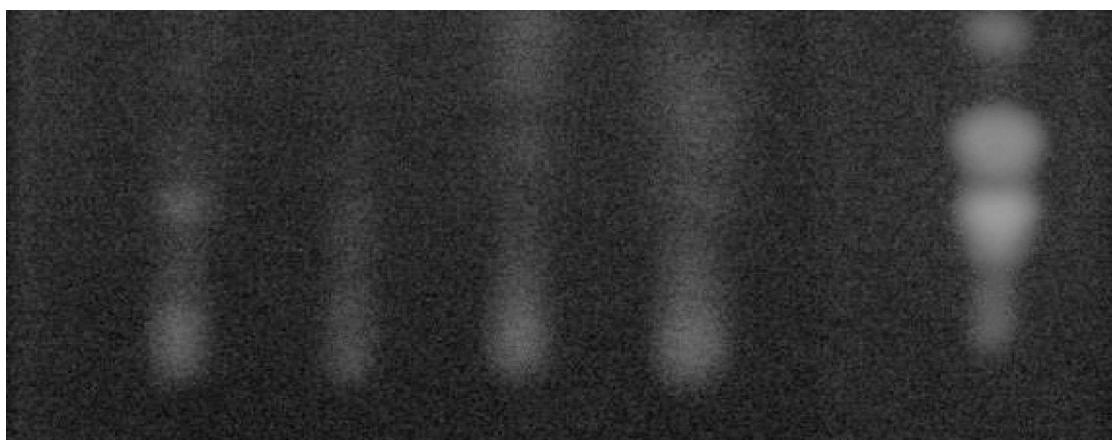
Concerning the sensory evaluation of the butter, the results showed that there were no difference in the flavor, body, texture and appearance of all butter samples and the panelist could not identify differences in mouth feel between butter with immobilized cultures and metabolites and control. Similar results were obtained by Hamayouni et al., (2008), and Ozer et al., (2009) who reported that addition of encapsulated culture had no significant effect on the sensory properties of some dairy products

3.5. Fate of Aflatoxins in the presence of immobilized starter cultures and their immobilized metabolites

Data regarding the percentage of the aflatoxins (B1, B2, G1 and G2) bounded in butter samples that had been made from cream artificially contaminated with 12 µg mL⁻¹ individually for each aflatoxin then stored for 60 days, are illustrated in Fig. 3. The data indicate that butter made with cream treated with immobilized starter culture cells, all the aflatoxins tested were bound at 90% of the original

concentration after storage for 60 days. In contrast, in butter made from cream treated with immobilized metabolites the levels of bounded aflatoxin were 45-56.3 % of the original concentration after 60 days storage. This could be attributed to the removal of the toxin which involves physical binding to bacterial cell walls or cell wall components (Peltonen et al., 2001). The binding of the toxins by immobilized metabolites may be due to the dead cells of the culture (or possibly dead cell material) remaining in the supernatant after the heat treatment, since dead cells of bacteria still display binding ability (Bueno et al., 2007).

Treating butter with immobilized cells was more effective in binding or removing aflatoxins, in agreement with El-Nezami et al., (1998), and Haskad et al., (2001) who recorded that certain strains of lactic acid bacteria removed aflatoxins from liquid media by physical binding. Also, binding aflatoxin by LAB allowed the detoxification without the need for the removal of the bacterial-toxin complex from the food, so, this method renders the toxin unavailable for absorption in the intestine. Moreover, aflatoxin bound by bacteria showed no toxicity effect on rats liver tissues of rats fed on the toxin complex (Metwally, et al, 2008).



Control A+B A+D C+B C+D

Fig. 1 Effect of mixed mesophilic starter culture on toxin production by *A. flavus* in broth medium detected by thin layer chromatography. A, *Lactococcus lactis* ssp. *lactis*; B, *Lactococcus lactis* ssp. *diacetylactis*; C, *Lactococcus lactis* ssp. *cremoris*; D, *Leuconostoc mesenteroides* ssp. *mesenteroides*

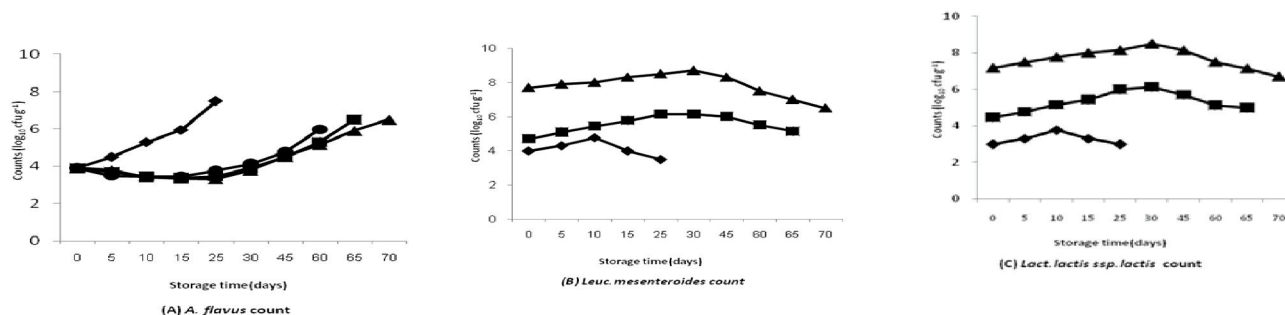


Fig.2. Changes in (A) *A.flavus*, (B) *Lc. mesenteroides* and *L. lactis ssp. Lactis* counts in treated butter during storage. (○), Treatment 1: inoculation with 2 % mixed starter culture A+D; (□), Treatment 2: inoculation with 2 % beads of immobilized mixed starter culture A+D; (△), Treatment 3: inoculation with 5% beads of immobilized metabolite of the mixed starter culture A+D; (◇), Treatment 4: no inoculation (control).

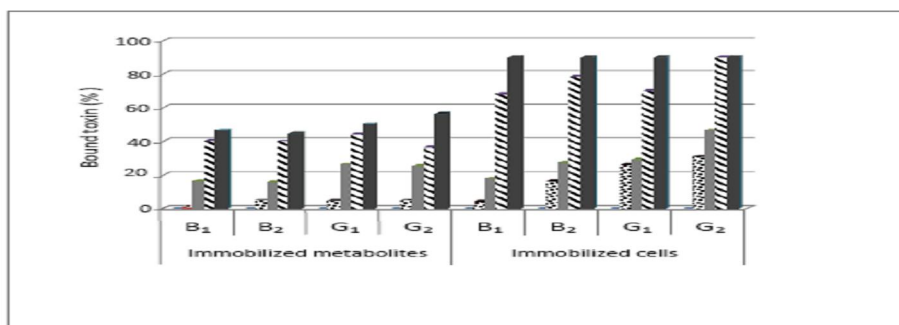


Fig. 3. Effect of immobilization of starter culture cells and their metabolites on aflatoxin binding in butter during storage: (□), day 1; (▨), day 15; (▩), day 30; (▧), day 45; (■), day 60. B₁, aflatoxin B₁; B₂, aflatoxin B₂; G₁, aflatoxin G₁; G₂, aflatoxin G₂.

Table 2.Effect of mixed starter culture on growth and toxin production by *A. flavus* in broth medium.

Cultures*	Aflatoxins ($\mu\text{g L}^{-1}$)**				Mycelium weight of <i>A. flavus</i> (g L^{-1})**
	B ₁	B ₂	G ₁	G ₂	
Control (<i>A. flavus</i>)	10.69 ^a	7.99 ^a	6.70 ^a	5.00 ^a	17.00 ^a
(A + B)mixed culture	4.15 ^c	3.60 ^b	Traces	Traces	14.02 ^{bc}
(A + D)mixed culture	0.00 ^d	0.00 ^c	0.00 ^c	0.00 ^c	12.35 ^c
(C + B) mixed culture	5.34 ^c	4.98 ^b	5.14 ^{ab}	2.11 ^b	15.32 ^b
(C + D) mixed culture	8.03 ^b	6.48 ^a	4.04 ^b	5.00 ^a	16.94 ^a

*Cultures are: A, *Lactococcus lactis ssp. lactis*; B, *Lactococcus lactis ssp. diacetylactis*; C, *Lactococcus lactis ssp. Cremoris*; D, *Leuconostoc mesenteroides ssp. mesenteroides*.

**Values within columns followed by the same superscript letters are not significantly different at 0.05%.

***LSD, least squares difference.

Table 3.Deter the production of aflatoxins from *A. flavus* NRRL 5906 by inoculation with mixed cultures of *Lactococcus lactis ssp. lactis* and *Leuconostoc mesenteroides ssp. mesenteroides* (A + D) and their immobilized cells and metabolites in butter stored at 10 °C.

Storage period (days)	Treatments ^{a,b}			
	Control	Treatment 1	Treatment 2	Treatment 3
	Aflatoxins ($\mu\text{g kg}^{-1}$)	Aflatoxins ($\mu\text{g kg}^{-1}$)	Aflatoxins ($\mu\text{g kg}^{-1}$)	Aflatoxins ($\mu\text{g kg}^{-1}$)

	B ₁	B ₂	G ₁	G ₂	B ₁	B ₂	G ₁	G ₂	B ₁	B ₂	G ₁	G ₂	B ₁	B ₂	G ₁	G ₂
5-25	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
30	4.1	3.0	4.4	2.7	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
35-55	5	8	2	6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
60	-	-	-	-	nd	nd	nd	nd	nd	nd	nd	nd	3.8	2.9	2.0	traces
65	-	-	-	-	nd	nd	nd	nd	3.7	2.8	traces	traces	6	4	5	-
70	-	-	-	-	1.6	1.9	nd	nd	3	0	-	-	-	-	-	-
					6	8										

reatments are: Control, mold, without LAB; Treatment 1, inoculation with 2 % mixed starter culture A+D; Treatment 2, inoculation with 2 % beads of immobilized mixed starter culture A+D; Treatment 3, inoculation with 5 % beads of immobilized metabolites of the mixed starter culture A+D.

^bnd, not detected.

(-) not tested

4. Conclusion

From the foregoing results, it can be concluded that some LAB strains had antifungal activity and thus have the potential for being effective as food-grade bio-preservatives to combat the problem of mold growth and aflatoxins in butter. Though, immobilization technology is prospective field for food safety and effectively controls the risk of contamination by *A. flavus*. Moreover, immobilized metabolites of some mesophilic starter can be used to extend shelf-life, limiting fungal spoilage and toxin formation, but this requires further investigation. This approach could be used in reducing the risk of mycotoxins contamination without producing off-flavor in sweet butter as a novel strategy in preventing the formation of aflatoxins in foods.

5. References:

1. AOAC. (2000). Official Method of Analysis of AOAC International, 25th ed. Maryland, USA: AOAC International.
2. AdbAlla, E.A.M., Aly, S., Saleh, E., Mary, S., & Hathout, A.S. (2005). The antimycotic effects of probiotic bacteria on the germination and ultra-structure of the aflatoxin fungi *Aspergillus parasiticus*. *Journal of Union Arab of Biologists, Cairo, 16B*, 33-48.
3. Arumsharma, A.G., Behere, S.R., & Padwal-Desal Nadharni, G.B. (1980). Influence of inoculum size of *Aspergillus parasiticus* spores of aflatoxin production. *Applied and Environmental Microbiology, 6*, 989-993.
4. Aycicek, H., Yarsan, E., Sarimehmetoglu, B., & Cakmak, O. (2002). Aflatoxin M1 in white cheese and butter consumed in Istanbul, Turkey. *Veterinary and Human Toxicology, 44*, 295-296.
5. Aycicek, H., Aksoy, A., & Saygi, S. (2005). Determination of aflatoxin levels in some dairy and food products which consumed in Ankara, Turkey. *Food Control, 16*, 263-266.
6. Batish, V.K., Lat, R., & Grover, S. (1991). Interaction of *Streptococcus lactis* subsp. *diacetylactis* DRC-1 with *Aspergillus parasiticus* and *A. fumigatus* in milk. *Cultured Dairy Products Journal, 26*, 13-14.
7. Benech, R.O., Kheadr, E.E., Lacroix, C., & Fliss, I. (2003). Impact of nisin producing culture and liposome-encapsulated nisin on ripening of lactobacillus added-Cheddar cheese. *Journal of Dairy Science, 86*, 1895.
8. Bueno, D.J., Casale, C.H., Pizzolitto, R.P., Salvano, M.A., & Oliver, G. (2007). Physical adsorption of aflatoxin B₁ by lactic acid bacteria and *Saccharomyces cerevisiae*: A theoretical model. *Journal of food Protection, 70*, 2148-2154.
9. Cabo, M.L., Braber, A.F., & Koenraad, P.M.F. (2002). Apparent antifungal activity of several lactic acid bacteria against *Penicillium discolors* is due to acetic acid in the medium. *Journal of Food Protection, 65*, 1309-1316.
10. Champagne, C. P., Lacroix, C., & Sodini-Gallot, I. (1994). Immobilized cell technology for the dairy industry. *Critical Reviews in Biotechnology, 14*, 109-134.
11. Coallier-Ascah, J., & Idziak, E.S. (1985), Interaction between *Streptococcus lactis* and *Aspergillus flavus* on production of aflatoxin. *Applied and Environmental Microbiology, 49*, 888-891.
12. Davis, N.D., Clark, E.M., Schrey, K.A. & Diener, U.L. (1986). In vitro growth of *Acremonium coenophialum*, an endophyte of toxic tall fescue grass. *Applied and Environmental Microbiology, 52*, 888-891.
13. Devries, H.R., Maxwell, S.M., & Hendrickse, R.G. (1990). Aflatoxin excretion in children with

- Kwashiorkor or marasmic Kwashiorkor: a clinical investigation. *Mycopathology*, 110, 1-9.
14. Effat, B.A. (2000). Antifungal substances from some lactic acid bacteria and Propionibacteria for use as food preservatives. *Journal of Agriculture science Mansoura University*, 25, 6291-6304.
 15. El-Diasty, E.M., & Salem, R.M., (2007). Incidence of lipolytic and proteolytic fungi in some milk products and their public health significance. *Journal of Applied Science Research*, 3, 1684-1688.
 16. El-Gendy, S.M., & Marth, E.H. (1981). Growth and aflatoxin production by *Aspergillus parasiticus* in the presence of *Lactobacillus casei*. *Journal of Food Protection*, 44, 211-212.
 17. El-Nezami, H., Kankaanpaa, P., Salminen, S., & Ahokas, J. (1998). Ability of dairy strains of lactic acid bacteria to bind common food carcinogen aflatoxin B₁. *Food Chemistry and Toxicology*, 36, 321-326.
 18. Ghita, E.I., Girgis E., Abd El-Fattah, E.S., & Badran, S.M. (2004). Biopreservative effect of the metabolites of some Lactic acid bacteria on cream and low fat butter. *Egyptian Journal of Dairy Science*, 31, 221-236.
 19. Gibson, C.A., Landerkin, G.B., & Morse, P.M. (1965). Survival of strains of lactic streptococci during frozen storage. *Journal of Dairy Research*, 32, 151-155
 20. Homayouni, A., Azizi, A., Ehsani M.R., Yarmand M.S., Razavi S.H. (2008). Effect of microencapsulation and resistant starch on the probiotic survival and sensory properties of synbiotic ice cream. *Food Chemistry* 111, 50-55
 21. Haskard, C.A., El-Nezami, H.S., Kankaanbaa, P.S., Salminen, S., & Ahokas, J.T. (2001). Surface binding of aflatoxin B₁ by lactic acid bacteria. *Applied and Environmental Microbiology*, 67, 3086-3091.
 22. Hegazy, E.M., Sadek, Z.I., & El-Shafei, K. (2006). Elimination the risk of aflatoxins production by *Aspergillus parasiticus* NRRL2999 by using some starter cultures. *Journal Union Arab Biology, Cairo, Microbiology and Viruses*, 15B, 15-40.
 23. Klinkenberg, G., Lystad, K.Q., Levine, W., & Dyrset, N. (2001). pH-controlled cell release and biomass distribution of alginate-immobilized *Lactococcus lactis* subsp. *lactis*. *Journal of Applied Microbiology*, 91, 705-714.
 24. Kumar, C.N., Narayanan, R., Kavitha, N., & Dhanalakshmi, B. (2007). Antifungal property of lactic acid bacteria. *Egyptian Journal of Dairy Science*, 35, 141-145.
 25. Ozer, B., Kimaci, H.A., Senel, I., Atamer, A., Hayaloglu, A. (2009). Improving the viability of *Bifidobacterium bifidum* BB-12 and *Lactobacillus acidophilus* LA-5 in white-brined cheese by microencapsulation. *International Dairy Journal* 19, 22-29
 26. Levata-Jovnnovic, M., & Sandine, W.E. (1997). A method to use *Leuconostoc mesenteroides* ssp. *cremoris* 9140 to improve milk fermentations. *Journal of Dairy Science*, 11, 11-19.
 27. Magnusson, J., & Schnurer, J. (2001). *Lactobacillus coryniformis* subsp. *coryniformis* strain Si3 produces a broad-spectrum proteinaceous antifungal compound. *Applied and Environmental Microbiology*, 67, 1-5.
 28. Metwally, M.; Abd Allah, EL S., Farag A. H., Sanaa M.A. B., & Nouh, A. (2008). Factors affecting lactic acid bacteria adsorption of aflatoxin and the effect of adsorbed toxin on rats' liver tissues. *Journal of Agriculture. Science. Mansoura University*, 33, 5187-5201.
 29. Nelson, J.A. and Trout, G.M. (1956). Judging dairy products. 4th Ed. The Olsen publishing Co., Milwaukee Wis. 53212.
 30. Peltonen, K., El-Nezami, H., Haskard, C., Ahokas, J., & Salminen, S. (2001). Aflatoxin B₁ binding by dairy strains of lactic acid bacteria and bifidobacteria. *Journal of Dairy Science*, 84, 2152-2156.
 31. Rustom, I.Y.S. (1997). Aflatoxin in food and feed: Occurrence, legislation and inactivation by physical methods. *Food Chemistry*, 59, 57-67.
 32. Rady, A.H & Bader, H.M. (2003). Keeping the quality of cows' butter by irradiation
 33. *Grasasy Aceites*, 54, 410-418.
 34. Schnurer, J., & Magnusson, J. (2005). Antifungal lactic acid bacteria as bio-preservation. *Trends in Food Science and Technology*, 16, 70-78.
 35. Snedcor, G.W., & Cochran, G.W. (1980). Statistical methods 6th edn. Iowa, USA: *University Press*.
 36. Suzuki, I., Nomura, M., & Morichi, T. (1991). Isolation of lactic acid bacteria which suppress mould growth and show antifungal action. *Milchwissenschaft*, 46, 635-639.
 37. Willaert, R., & Baron, G. (1996). Gel entrapment and micro-encapsulation: Methods, applications and engineering principles. *Review of Chemistry Engineering*, 12 N, 1-2.

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