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PROBIOTIC BACTERIA AS A TOOL TO PREVENT FUNGAL GROWTH AND AFLATOXINS PRODUCTION BY *Aspergillus parasiticus*

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ABSTRACT

Lactobacillus strains are used as probiotic bacteria in fermented dairy products and other fermented food products, whereas they are known to prolong the shelf life of these products. The inhibitory effect of cell free supernatant (CFS) of several *Lactobacillus* species on mycelium growth and the aflatoxin production by the aflatoxigenic strain of *Aspergillus parasiticus* was studied. A complete inhibition (100%) of mycelium growth and aflatoxin production was recorded when cell free supernatant of *Lactobacillus casei* was placed in a dialysis sac or in the medium without a dialysis sac or on the insertion of *A. parasiticus* after 16 hours with *L. casei*. Both *L. reuteri* and *L. gasseri* inhibited mycelium growth and aflatoxin production but to a lesser extent.

Cell free supernatant of *L. acidophilus* and *L. bulgaricus* showed the lowest effect on aflatoxin production as well as on mycelium growth. The inoculation of bacterial strains of *L. casei, L. gasseri* and *L. reuteri* before fungal growth inoculation by 16 hours caused inhibitory effects on mycelium growth and aflatoxin production. But these indications were not observed for the other treatments of *L. acidophilus* or *L. bulgaricus*.

The probiotic strains (*L. casei, L. reuteri*) with higher antifungal activity were used and incorporated in the manufacture of Ras cheese to replace the normal starters (*S. thermophilus, L. bulgaricus*), where they prevented the growth of fungi during the three month of the storage period.

In conclusion probiotic bacteria may be able to produce active substances that can inhibit aflatoxins production by *A. parasiticus* in most treatments used, and the inhibitory effect depends on the type of lactic acid and / or the treatment used.

Keywords: probiotic bacteria, *Aspergillus parasiticus*, fungal growth and aflatoxin production, Ras cheese.

INTRODUCTION

Ras cheese is the main Egyptian hard cheese that is rather similar to the Greek, Kefalotyri, whereas the name in both countries means, " head " (Dabiza and Fathi, 2003). Ras cheese is now the best-known hard cheese in Egypt and indeed through out the Arab world (Abou-Donia, 2002). The contamination of dairy products with undesirable moulds such as the genera *Aspergillus*, *Penicillium*, *Rhizopus*, *Fusarium* and *Mucor* is a serious and frequently disturbing problem in dairy industry. The potentially toxigenic species within the genera *Penicillium*, *Aspergillus* and *Fusarium* were mainly detected in cheeses (Montagna *et al*., 2004). Many molds find cheese an excellent medium for growth and they can become moldy during ripening, curing, refrigerated storage and also after the cutting and slicing with unclean equipment in shops or at home (Abu Sree, 1997). Mold growth on cheese is considered undesirable because of their growth imparted musty off-flavors to

the cheese. Certain species of these genera are able to proliferate and produce toxins at low storage temperatures. The presence of aflatoxins in dairy products may be the result of the contamination of milk caused by contamination of feeding stuff consumed by the cow, or direct fungal contamination of dairy products which may result in the formation of aflatoxins (Van Egmond, 1989). Aflatoxin M_1 was found in 89.47% of cheese samples collected from supermarkets during August in Bursa, Turkey (Oruc and Sonal, 2001).

Aflatoxins are a group of mycotoxins with mutagenic, carcinogenic and immunosuppressive properties (Eaton and Gallagher, 1994, IARC, 1993). They are produced by toxigenic *A. flavus* and *A. parasiticus* and *A. nomius* strains (Peltonen *et al.*, 2001). Aflatoxins have been found in many food and animal feeds (Wood, 1989) and their production in such commodities can be influenced by several factors, including temperature, water activity, pH, available nutrients and competitive growth of other micro-organisms (Ellis *et al.*, 1991). Decontamination of food and feed containing aflatoxins is an objective for improving the food and feed supply. Several techniques are used for the preservation of food and feeds by means of physical methods and several chemical additives (Farkas, 2001 and Davidson, 2001). A variety of physical, chemical and biological approaches have been reported to degrade aflatoxins (Park, 1993 and Piva *et al.*, 1995). The current methods are not very effective and require expensive equipment and may cause losses in the nutritional quality of treated commodities. In addition, the undesirable health effects of such treatments have not been fully evaluated (Phillips *et al.*, 1994).

There is a great demand for novel strategies to prevent both the formation of aflatoxins in food and feed and the impact of aflatoxin contamination. Therefore biological decontamination using microorganisms is one of the well-known strategies for management of aflatoxins in food and feeds. Among the different potential decontaminating microorganisms, lactic acid bacteria represent a unique group, which is widely used in food fermentation and preservation (Shetty and Jespersen, 2006).

Lactic acid bacteria are found in many nutrient rich environment and occur naturally in various food products such as dairy and meat products, and vegetables (Carr *et al.*, 2002). They have traditionally been used as natural bio-preservatives of food and feed to extend shelf life and enhanced safety of foods obtained by using the natural or added microflora and their antimicrobial products (Ross *et al.*, 2002). Lactic acid bacteria play an essential role in the majority of food fermentation, and a wide variety of strains are routinely employed as starter cultures in the manufacture of dairy products. The protective effect of lactic acid bacteria against food mutagens such as heterocyclic amines, *N*-nitroso compounds and aflatoxins has been reported (Sreekuman and Hosono, 1998 and El-Nezami *et al.*, 2000).

The general public demands a reduced use of chemical preservatives or additives in food and feed and demands a stimulated research on antifungal lactic acid bacteria as bio-preservatives (Brul and Coote, 1999 and Schnurer and Magnusson, 2005). Therefore, the purpose of this investigation is to evaluate the role of some probiotic strains against

fungal growth and aflatoxin production by *A. parasiticus* on media, and also selecting the probiotic strains with the most antifungal activity to be applied in the manufacture of Ras cheese with antimycotytic properties.

MATERIALS AND METHODS

Cultures: -

A- Fungal strain: *Aspergillus parasiticus* was obtained from Agriculture Research Service, National Center for Agriculture, U. S. A.

B- Bacterial strains: *Lactobacillus acidophilus*, *L. bulgaricus* *L. casei*, *L. reuteri* and *S. thermophilus* were obtained from Denmark, and *L. gasseri* LA39 from Dr. T. Saito, Japan.

Media: -

DeMan Rogosa Sharpe (MRS) broth, Purchased from Fluka Biochemika and Potato Dextrose Agar (PDA) Purchased from DIFCO Laboratory, Detroit, U.S.A.

Aflatoxins standards:

Aflatoxins B_1, B_2, were purchased from Aldrich chemical Co., P. O. Box 355, Milw, WI 53201, 414-273-3850, U. S. A. while G_1 & G_2 were purchased from Sigma chemical company, P. O. Box 14508, St. Louis, MO 63178, U. S. A.

Preparation of inoculum: -

A strain of *A. parasiticus* was maintained on potato dextrose agar (PDA) slants for 7 to 10 days at 28 °C, after which the spore suspension was prepared, using saline solution containing 0.05% Tween 80 and gently dislodging the spores with a flamed wire loop. The spore suspension was then passed through sterile cheesecloth to remove mycelia debris as described by Bullerman *et al.* (1990).

Preparation of cell free supernatant (CFS):

Lactic acid bacteria were maintained and stored in sterile litmus milk. For each experiment, the species were transferred to 10-ml sterile modified MRS broth for regeneration by the method described by Bullerman *et al.* (1990). Cultures were transferred through 4 days by placing 0.1 ml of a 24-h culture to 10 ml of fresh MRS broth. After centrifuging at 445 × g for 10 minutes, the last 24-h culture was collected. The supernatant was separated from cell pellet and filtered through a 0.45μ sterile membrane filter to remove remaining cells. The cell pellet was washed twice with sterile 85% saline solution and recovered by centrifuging at 445 × g for 10 minutes.

Effect of cell free supernatant of *Lactobacillus* species on fungal growth and aflatoxin production:

The CFS of *Lactobacillus* species was inserted individually in 10-ml sterile Lablemco tryptone broth (LTB) for *L. acidophilus* and *L. bulgaricus*, while Tryptone soy broth (TSB) was used for *L. casei*, *L. reuteri* and *L. gasseri* in a dialysis sac with molecular weight 12,000 and 14,000. Dialysis sacs were aseptically placed inside a 250-ml flask containing 50-ml sterile LTB for *L. acidophilus* and *L. bulgaricus*, while TSB was used for *L. casei*, *L. reuteri* and *L. gasseri*. Four treatments of this experiment were used as follows: CFS in dialysis sac was inoculated with 1ml (10^6) spore suspension

of *A. parasiticus* (T-1). The second treatment (T-2) CFS without dialysis sac was inoculated. The third treatment (T-3) CFS in dialysis sac and the fourth one (T-4) CFS without dialysis sac were maintained for 16 hours before spore suspension inoculation, then all treatments were incubated at 28°C ± 2°C for 8 days. Aflatoxin production and mycelium dry weight were estimated.

Analysis: -

1- Extraction of aflatoxins:

The cultures were extracted twice with chloroform, and then they were filtered through glass wool. After which the filtrates were transferred to a separating funnel. The lower chloroform layer was passed through anhydrous sodium sulphate. The extracts were finely dried under nitrogen. The extracts were stored in vials at –20 °C until the aflatoxin determination

2- Mycelium dry weight:

The mycelium mats were collected by filtration through Whatman No. 4 filter paper, washed twice with water and dried in an oven at 95°C until constant weight and weighed.

3- Determination of aflatoxins:

Spots of extracted samples and aflatoxin standards were applied on a (20 × 20cm) pre- coated aluminum sheets of silica gel 60 without fluorescent indicator (TLC), activated at 105 °C for 2 hours. Extracted samples were dissolved in benzene: acetonitrile (98:2 v/v). TLC plates were then developed in toluene: ethylacetate: 88% formic acid (60:30:10 v/v/v). After development, TLC plates were dried and exposed to long wavelength ultraviolet light for visual estimation as describes by (Gourama and Bullerman, 1995).

4- Apparatus:

A DESAGA CD 60 spectrodensitometer was used in assay with a reflectance mode at excitation wave length 360 nm, and emission wave length of 430 nm. This was carried out by Micro Analytical center, Faculty of Science, Cairo University.

5- Calculation of aflatoxins:

Aflatoxin concentration was calculated as ppb by the following equation according to (AOAC Methods, 1990).

$$\text{Aflatoxin (ppb)} = (B \times Y \times S \times V) / (Z \times X \times W)$$

Where:

B = area of aflatoxin peak in sample

Y = concentration of aflatoxin standard µg/ml

S = aflatoxin standard spotted µl

V = final dilution of sample extract µl

Z = area of aflatoxin peak in standard

X = sample extract spotted µl

W= g sample represented by final extract

Percentage of inhibition was calculated using the following equation:

$$\{100 - (\text{treatment} / \text{control} \times 100)\}$$

Effect of probiotic bacteria on total fungal count in Ras cheese

Probiotic and traditional Ras cheese were manufactured at Dairy Dept., Faculty of Agriculture, Ain Shams University according to the method

described by Abu Sree (1997). Traditional Ras cheese (control) was processed using the normal starters *S. thermophilus* and *L. bulgaricus* (1: 1 v/v). For probiotic Ras cheese, two strains were used *L. casei* and *L. reuteri* (1: 1 v/v).

The un-waxed traditional Ras cheese (control) and the probiotic Ras cheese were sprayed with *A. parasiticus* spore suspension (10^6). Cheese was ripened in a ripening cabinet (90% RH and 12 ± 2°C) for 90 days.

Microbiological analysis:

Samples of probiotic and control of hard cheese were analyzed after 30 days, 60 days and 90 days of ripening period. Cheese samples were prepared according to the method recommended by APHA (1985), and examined for *A. parasiticus* count according to ICMSF (1996).

Statistical analysis:

It was carried out by the completely randomized design in factorial arrangement (F-test). The least significant variance (L. S. D) was used for comparing treatment means (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Fungal growth (as dry mycelium weight) and aflatoxins production (as ppb) by *A. parasiticus* were accounted for after 8 days of incubation for the control and the treatments (T-1, T-2, T-3 and T-4) of all probiotic strains.

1-Fungal growth:

Behavior of *A. parasiticus* growth in the presence of *Lactobacillus* species was studied. This behavior changed depending on *Lactobacillus* species used and the type of treatments. Data in Table (1) revealed that treatment T-2 of both *L. acidophilus* and *L. bulgaricus* was highly effective in inhibiting fungal growth followed by treatments T-1, T-4 and T-3 in descending order. In regard no real significant variance ($P > 0.05$) between control and all treatments was recorded for both *L. acidophilus* and *L. bulgaricus*

Results showed that all treatments of *L. casei* completely inhibited the fungal growth (100%). On the other hand both *L. gasseri* and *L. reuteri* treatments T-3 and T-4 completely inhibited (100%) fungal growth. Reduction (61.54%) of the fungal growth was also recorded for T-2 of *L. gasseri*. Data revealed real significant variance ($P < 0.05$) between control and treatments T-2, T-3 and T-4 of *L. gasseri* and *L. reuteri*.

Data in Table (1) also showed that cell free supernatant of all tested strains incorporated in the flasks (T-2) was more active in inhibiting fungal growth than that incorporated in dialysis sac (T-1). On the other hand incorporation of bacteria used simultaneously with spore suspension of *A. parasiticus* was more effective in the inhibition of fungal growth than the incorporation of the bacteria 16 hours before spore suspension. The percentage of inhibition for *L. acidophilus* was 16.41%, 51.56% and *L. bulgaricus* 31.64%, 54.80% for treatments T-1 and T-2 respectively. In contrast *L. gasseri* and *L. reuteri* which were incorporated 16 hours before spore suspension (T-3 and T-4) completely inhibited (100%) the fungal growth and was more effective than the incorporation simultaneously (T-1 and T-2).

Table (1): Effect of different *Lactobacillus* species on fungal growth and percentage of inhibition

Treatments	*Lactobacillus* species									
	L. acidophilus		*L. bulgaricus*		*L. casei*		*L. gasseri*		*L. reuteri*	
	A	B	A	B	A	B	A	B	A	B
Control	256		177		50		65		85	
T-1	214	16.41%	121	31.64%	0*	100%	55	15.38%	43	49.41%
T-2	124	51.56%	80	54.80%	0*	100%	26*	61.54%	0*	100%
T-3	278		153	13.56%	0*	100%	0*	100%	0*	100%
T-4	224	12.50%	112	36.72%	0*	100%	0*	100%	0*	100%
L. S. D	-		-		6.74		20.78		24.38	

* indicates a significant variance ($P < 0.05$)
A = mg fungal growth
B = Percentage of inhibition

Fungal growth could be enhanced, retarded or remain unchanged as a result of another microorganism in the environment (El-Gendy and Marth, 1981), however the competitive growth is probably a factor in the inhibition of fungal growth (Bullerman *et al.*, 1990). Inhibition of fungal growth may be due to the antifungal activity of the compounds produced by lactic acid bacteria (Plockova *et al.*, 1997) such as organic acids (i.e. lactic and acetic acids) (De Muynck *et al.*, 2004). Similar results were reported that culture supernatant of *Lactobacillus* species has reduced fungal growth of *A. flavus*. The inhibition of fungal growth was probably due to inactivating the viability of spores (Xu *et al.*, 2003).

2- Aflatoxin production:

Recorded results in Table (2) showed that all treatments of *L. acidophilus* caused complete inhibition (100%) of aflatoxin G_2 showing real significant variance ($P < 0.05$), whereas treatment T-1 reduced aflatoxin B_1 level by 23.74%. On the other hand stimulation effect of aflatoxin B_1 production was recorded for the treatments T-2, T-3 and T-4. Regarding aflatoxin B_2 treatments T-1 and T-2 caused the reduction of aflatoxin content by 45.76% and 19.04% respectively. In contrast treatments T-3 and T-4 caused the stimulation of aflatoxin production.

Table (2): Effect of *L. acidophilus* on aflatoxin production and percentage of inhibition

Lactobacillus species	Treatments	Aflatoxins (ppb)							
		B_1		B_2		G_1		G_2	
		A	B	A	B	A	B	A	B
L. acidophilus	Control	1529		625		1773		310	
	T-1	1166	23.74%	339	45.76%	424*	76.09%	0 *	100%
	T-2	2198	+	506	19.04%	0 *	100%	0 *	100%
	T-3	5066	+	656	+	292*	83.53%	0 *	100%
	T-4	6154	+	840	+	599*	66.22%	0 *	100%
	L. S. D.	-		-		0.141		0.074	

* Indicates a significant variance ($P < 0.05$)
A = Aflatoxin production (ppb)
B = Percentage of inhibition
\+ = Stimulation of aflatoxin production

Data presented in Table (3) indicated that, *L. bulgaricus* treatments T-1, T-2, T-3 and T-4 caused complete inhibition (100%) of aflatoxin G_2 production, whereas all treatments reduced aflatoxins B_1, B_2 and G_1, where the percentage of inhibition ranged between 1.92% to 83.06% depending on the aflatoxin type and treatments.

Data also indicated that *L. casei* treatments completely prevented and inhibited the production of all aflatoxin types. Real significant variance ($P < 0.05$) was recorded between control and all treatments for the two strains *L. bulgaricus* and *L. casei*.

Table (3): Effect of *L. bulgaricus* on aflatoxin production and percentage of inhibition

Lactobacillus species	Treatments	Aflatoxins (ppb)							
		B_1		B_2		G_1		G_2	
		A	B	A	B	A	B	A	B
L. bulgaricus	Control	2001		625		1110		310	
	T-1	836*	58.22%	613	1.92%	631*	43.15%	0 *	100%
	T-2	395*	80.26%	258*	58.72%	525*	52.70%	0 *	100%
	T-3	339*	83.06%	227*	63.68%	266*	76.04%	0 *	100%
	T-4	685*	65.77%	279*	55.36%	212*	80.90%	0 *	100%
	L. S. D.	0.449		0.153		0.153		0.047	

*** Indicates a significant variance ($P < 0.05$)**
A = Aflatoxin production (ppb)
B = Percentage of inhibition

Table (4) indicated that treatments T-3 and T-4 of the *L. gasseri* completely inhibited (100%) the production of aflatoxin types. It was also noticed that treatment T-2 completely inhibited (100%) aflatoxin B_2 and G_1 only showing real significant variance ($P < 0.05$). On the other hand treatment T-1 caused a slight stimulation of aflatoxin B_1 and G_1 production.

Table (4): Effect of *L. gasseri* on aflatoxin production and percentage of inhibition

Lactobacillus species	Treatments	Aflatoxins (ppb)							
		B_1		B_2		G_1		G_2	
		A	B	A	B	A	B	A	B
L. gasseri	Control	212		117		233		493	
	T-1	249	+	79	32.48%	300	+	490 *	0.61%
	T-2	236	+	0 *	100%	0 *	100%	84 *	82.96%
	T-3	0 *	100%	0 *	100%	0 *	100%	0 *	100%
	T-4	0 *	100%	0 *	100%	0 *	100%	0 *	100%
	L. S. D.	0.222		1.120		0.090		0.095	

*** Indicates a significant variance ($P < 0.05$)**
A = Aflatoxin production (ppb)
B = Percentage of inhibition
+ = Stimulation of aflatoxin production

Table (5) showed that treatments T-2, T-3 and T-4 *L. reuteri* caused complete inhibition (100%) of all types of aflatoxins, whereas treatment T-1 only reduced aflatoxin B_2, G_1 and G_2 production and stimulated aflatoxin B_1

production. In regard real significant variance ($P < 0.05$) was recorded between control and all treatments.

Table (5): Effect of *L. reuteri* on aflatoxin production and percentage of inhibition

Lactobacillus species	Treatments	Aflatoxins (ppb)							
		B_1		B_2		G_1		G_2	
		A	B	A	B	A	B	A	B
L. reuteri	Control	127		117		233		125	
	T-1	246	+	42*	64.10%	126*	81.97%	28	77.60%
	T-2	0 *	100%	0 *	100%	0 *	100%	0 *	100%
	T-3	0 *	100%	0 *	100%	0 *	100%	0 *	100%
	T-4	0 *	100%	0 *	100%	0 *	100%	0 *	100%
	L. S. D.	0.099		0.033		0.060		0.020	

*** Indicates a significant variance ($P < 0.05$)**
A = Aflatoxin production (ppb)
B = Percentage of inhibition
+ = Stimulation of aflatoxin production

Finally data in Table (6) revealed that cell free supernatants of *L. casei*, *L. gasseri* and *L. reuteri* incorporated in the flasks (T-2) were more effective in inhibiting aflatoxin production than those incorporated in dialysis sac (T-1). Whereas the incorporation of these bacteria 16 hours (T-3, T-4) before spore suspension of *A. parasiticus* completely inhibited aflatoxins and was more effective than the incorporation of bacteria simultaneously with spore suspension (T-1, T-2).

In contrast cell free supernatant of *L. acidophilus* incorporated in dialysis sac (T-1) was more active in inhibiting aflatoxin production than that incorporated in the flask (T-2). Results also revealed that incorporation of this bacterium simultaneously with spore suspension was more effective in inhibiting aflatoxin production than incorporating the bacteria 16 hours before spore suspension.

Table (6): Effect of different *Lactobacillus* species on total aflatoxin production and percentage of inhibition

Treatments	*L. acidophilus*		*L. bulgaricus*		*L. casei*		*L. gasseri*		*L. reuteri*	
	A	B	A	B	A	B	A	B	A	B
Control	4237		4046		1330		1055		602	
T-1	1929*	54.47%	2080*	48.59%	0 *	100%	1118	+	442 *	26.57%
T-2	2704	36.18%	1178*	70.88%	0 *	100%	320 *	69.66%	0 *	100%
T-3	6014	+	832*	79.43%	0 *	100%	0 *	100%	0 *	100%
T-4	7593	+	1176*	70.93%	0 *	100%	0 *	100%	0 *	100%
L. S. D.	1.81		0.854		0.496		0.174		0.218	

*** Indicates a significant variance ($P < 0.05$)**
A = Aflatoxin production (ppb)
B = Percentage of inhibition
+ = Stimulation of aflatoxins

Aflatoxin production was affected by the presence of lactic acid bacteria. There is more than one mechanism involved in degradation of aflatoxins. The inhibition of aflatoxins was probably due to low molecular weight bacterial metabolites, which may have interfered with the synthesis of aflatoxins (Gourama and Bullerman, 1995). Other investigators proposed that adsorption of aflatoxins to bacterial cell wall may be the mechanism of aflatoxin degradation by lactic acid bacteria (Shah and Wu, 1999). On the other hand the bacterial population and viability greatly affected the uptake of aflatoxins by cells (Line and Brackett, 1995).

Effect of probiotic bacteria on total fungal count in Ras cheese

Data in Table (7) revealed that fungal count in traditional Ras cheese was higher than that of probiotic Ras cheese. Data also showed that the number of fungal count highly varied. After 30 days of ripening total fungal count was 1.4×10^4 and 3×10^2 for traditional Ras cheese (control) and probiotic Ras cheese respectively. Fungal counts after 90 days of ripening were 1×10^5 and 4 x 10 for traditional Ras cheese (control) and probiotic Ras cheese respectively. From the previous results it could be demonstrated that probiotic bacteria incorporated in the Ras cheese had an effect and decreased the fungal growth in probiotic Ras cheese compared to the control. The possible explanation for the inhibition of growth and sporulation of moulds by lactic acid bacteria could be the facultative anaerobic conditions created by these bacteria in fermented food (Batish *et al.*, 1997).

Both probiotic bacteria exerted antimycotic effect, this very beneficial safety property is very important since Ras cheese surface is usually contaminated with fungi and there is always a risk of aflatoxin production in the cheese. This also saved us the trouble of spraying the surface of cheese with antimycotic chemicals.

Table (7): Total fungal count (cfu /gm) of traditional and probiotic Ras cheese

Type of cheese	Ripening period (Days)		
	30	60	90
Traditional Ras cheese (control)	1.4×10^4	1.9×10^5	1×10^5
Probiotic Ras cheese	3×10^2	4 x 10	4 x 10

Conclusion

The probiotic strains *L. casei, L. reuteri* and *L. gasseri* were able to produce antifungal substances and were also able to inhibit aflatoxin production by *A. parasiticus*. From the effect exerted by these *Lactobacillus* species on fungal growth and aflatoxin production it would appear that they have the potential to be used as biological control agents in food to prevent fungal growth, where there is a strong indication that some inhibitory components are protein in nature. The antifungal and the antimycotoxigenic potential of *Lactobacillus* cultures have commercial applications and could be of great significance to both industry and consumers. Further investigations are needed to answer the behavior of these *Lactobacillus* strains and to purify and identify these compounds.

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استخدام البكتريا الحيوية كوسيلة لمنع النمو الفطري و إنتاج الأفلاتوكسين

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*** قسم سموم و ملوثات الغذاء – المركز القومي للبحوث**

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تستخدم سلالات الـ *Lactobacillus* كبكتريا حيوية في منتجات الألبان المتخمرة و كذلك في منتجات غذائية متخمرة أخرى، حيث من المعلوم إنها تطيل العمر الزمني لحفظ هذه المنتجات. و قد تم دراسة التأثير المثبط للراشح الناتج من الخلية البكترية (CFS) علي نمو الفطريات و إنتاج السموم الفطرية بواسطة *A. parasiticus* لعدد من الـ*Lactobacillus* . و قد تم تسجيل تثبيط تام (١٠٠%) لنمو الفطريات و إنتاج السموم عند إضافة الراشح الناتج من الخلية البكترية *L. Casei* داخل الـ Dialysis sac أو بدون أو بإضافة الفطر بعد ١٦ ساعة من إضافة البكتريا. كما أظهرت النتائج أن بكتريا *L reuteri* و *L. gasseri* ثبطت نمو الفطريات و السموم الفطرية.

و قد أظهرت النتائج أن الراشح الناتج من الخلية لكل من الـ *L. acidophilus* و *L. bulgaricus* اقل تأثيرا علي إنتاج السموم الفطرية و كذلك علي نمو الفطريات. كما أوضحت النتائج أن تلقيح سلالات البكتريا التالية *L. casei* و *L. gasseri* و *L. reuteri* قبل التلقيح بالفطر بمدة ١٦ ساعة يؤدي إلى زيادة تأثير التثبيط علي نمو الفطريات و إنتاج السموم الفطرية، إلا انه لم يتم ملاحظة هذه الدلالات بالنسبة للمعاملات الأخرى الخاصة بكل من الـ *L. acidophilus* و *L. bulgaricus*.

استخدمت السلالات الحيوية (*L. casei, L. reuteri*) ذات النشاط القوي المانع للنمو الفطري و أدخلت في صناعة الجبن الرأس لتحل محل البادئات الطبيعية (*S. thermophilus, L. bulgaricus*) حيث منعت نمو الفطر خلال فترة التخزين.

و في النهاية يمكن استنتاج أن البكتريا الحيوية تستطيع إنتاج مواد فعالة يمكنها تثبيط إنتاج السموم الفطرية بواسطة *A. parasiticus* في غالبية المعاملات المستخدمة و أن تأثير التثبيط يعتمد علي نوع البكتريا و/أو المعاملات المستخدمة.