*Assiut J. of Agric. Sci. 40 (Special Issue) (35-56)*

# INHIBITORY EFFECT OF SOME *Lactobacillus* SPECIES ON ACTIVITY AND AFLATOXIN PRODUCTION BY *Aspergillus parasiticus*

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**Abstract:** The inhibitory effect of cell free supernatants (CFS) from several *Lactobacillus* species on fungal growth and aflatoxin production by the aflatoxigenic strain *Aspergillus parasiticus* was studied. A complete inhibition (100%) of fungal growth and aflatoxin production was recorded when CFS of *L. casei* was placed in a dialysis sac or in the medium without a dialysis sac or by the insertion of *A. parasiticus* after 16 hours of the insertion of *L. casei*. Both *L. reuteri* and *L. gasseri* inhibited fungal growth and aflatoxin production, but to a lesser extent. CFS of *L. acidophilus* and *L. delbreukii* subsp. *bulgaricus* showed the lowest effect on aflatoxin production as well as on fungal growth. The inoculation of CFS of *L. casei, L. gasseri* and *L. reuteri* 16 hours before fungal growth, caused inhibitory effects on fungal growth and aflatoxin production, but these indications were not observed for the other treatments of *L. acidophilus* or *L. delbreukii* subsp. *bulgaricus*.

Regarding spore germination of *A. parasiticus* treated by the CFS of *Lactobacillus* species, it was noticed that *L. acidophilus* recorded the highest inhibitory effect on the germination of *A. parasiticus*, followed by *L. casei, L. reuteri,* and *L. gasseri*, while *L. delbreukii* subsp. *bulgaricus* showed the lowest effect. Scanning electron microscopy (SEM) was used to determine the microstructure changes in the conidiophores and spores after treatment with CFS of several *Lactobacillus* species, where the SEM micrograph showed the presence of great morphological deformation in the conidiophores shape and the number and shape of spores.

**Key words:** *Aspergillus parasiticus*; aflatoxin; antifungal; antiaflatoxigenic; spore germination; microstructure changes; scanning electron microscope.

## Introduction

Fungi are the most frequent cause of spoilage in about 5 – 10% of the food production worldwide, where they occasionally cause human disease. The potential mycotoxins produced from fungi are of particular concern. Aflatoxins are potent mycotoxins,

**Received on**: 19/5/2009 **Accepted for publication on**: 27/5/2009
**Referees**: Prof.Dr. Taha Abd El-Haleem Prof.Dr. Mohamed A. Mohran

produced as secondary metabolites by the toxigenic strains of *A. flavus* and *A. parasiticus* (Peltonen *et al.*, 2001). Aflatoxins are also potent carcinogenic, mutagenic, teratogenic and immunosuppressive mycotoxins, and have received worldwide attention due to their deleterious effects on human and animal health, as well as their importance in international food trade (Ellis *et al.*, 1991; IARC, 1993 and Eaton and Gallagher, 1994).

Even though a number of chemicals have been shown to inhibit aflatoxin production, treatments with such compounds are not effective enough; require expensive equipment and may cause loss in the nutritional quality of treated commodities. Such treatments may cause hazardous effects on human and animal health (Phillips *et al.*, 1994).

Currently, there is a strong debate about the safety aspects of chemical preservatives since they are considered responsible for many carcinogenic and teratogenic attributes as well as residual toxicity. For these reasons, consumers tend to be suspicious of chemical additives and thus the demand for natural and socially more acceptable preservatives has been intensified (Brul and Coote, 1999; Schnurer and Magnusson, 2005).

The exploration of naturally occurring antimicrobials for food preservation receives increasing attention due to consumer awareness of natural food products and a growing concern of microbial resistance towards conventional preservatives (Schuenzel and Harrison, 2002).

Lactic acid bacteria are found in many nutrient rich environments and occur naturally in various food products such as dairy and meat products, and vegetables (Carr *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. Numerous investigations indicated that lactic acid bacteria have beneficial health effects in humans (Ouwehand *et al.*, 2002; Saxelin *et al.*, 2005).

The protective effect of lactic acid bacteria against food mutagens such as heterocyclic aromatic amines, polycyclic aromatic hydrocarbons, reactive oxygen species and *N*-nitroso compounds have been reported (Sreekumen and Hosono, 1998; Knasmüller *et al.*, 2001; Stidl *et al.*, 2007).

Therefore the objective of this investigation was to evaluate the impacts of lactic acid bacteria on *A. parasiticus* mycelium dry weight, aflatoxin production, germination, and morphological changes in conidiophores and mold spores using scanning electron microscope.

## Materials and Methods

### Cultures: -

A- Fungal strain: *Aspergillus parasiticus* was obtained from Dr. S. W. Peterson- U.S. Dept. of Agriculture, Agriculture Research Service, National Centre for Agriculture, 1815 North University Street, Peoria, Illinois 61604-3999-0300, U. S. A.

B- Bacterial strains: *Lactobacillus acidophilus*, *L. casei*, *L. delbreukii* subsp *bulgaricus*, and *L. reuteri* were obtained from Chr. Hansen's lab. Denmark, while *L. gasseri* LA39 was kindly donated by Dr. T. Saito, Faculty of Biological Resource Science, Tohoku University, Japan.

### Media: -

DeMan Rogosa Sharpe (MRS) broth, purchased from Fluka Biochemika, potato dextrose agar (PDA) purchased from DIFCO Laboratory, Detroit, U.S.A, and tryptone soy broth (TSB) was purchased from Oxoid Ltd., Hampshire, England.

### 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.

### Dialysis sac:

Spectrum® Spectra/Por molecular porous membrane tubing, MWCO 12-14,000, recorder No. 25225-248 was obtained from VWR Scientific.

### Organism preparation:

Strains of *A. parasiticus* were 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 mycelial debris.

Lactic acid bacteria were maintained and stored in sterile litmus milk. For each experiment the species were transferred to 10-ml sterile modified de Man Rogosa Sharpe (MRS) broth for regeneration. The cultures were transferred three times for 4 days by placing 0.1 ml of a 24-h culture to 10 ml of fresh MRS broth. The last 24-h culture was collected and centrifuged at 445 × g for 10 min. The supernatant was separated from cell pellet and filtered through a 0.45μ sterile membrane filter to remove remaining cells. Cell pellets were washed twice with sterile 0.85% saline solution and recovered by centrifuging at 445 × g for 10 min. (Bullerman *et al.*, 1990).

**I- Effect of *Lactobacillus* species cell free supernatant on *A. parasiticus* growth and aflatoxin production:**

The CFS of *L. acidophilus* and *L. delbreukii* subsp. *bulgaricus, L. casei, L. reuteri* and *L. gasseri* were inserted individually in 10-ml sterile tryptone soy broth (TSB) 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 TSB. Four treatments had been investigated in the present study. 1 ml ($10^6$) spore suspension of *A. parasiticus* was inoculated in CFS of *Lactobacillus* species as follows: Directly in dialysis sac (T1) and without dialysis sac (T2), while in the other two treatments CFS was maintained for 16 hours before the fungal inoculation in dialysis sac (T3) and without dialysis sac (T4). Control flasks were only inoculated by 1 ml ($10^6$) spore suspension of *A. parasiticus*. 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 determination of aflatoxin.

**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:**

Extracted samples and aflatoxin standards were applied to a Waters (Milford, MA) HPLC equipped with a model 600 pump, and a model 474-fluorescence detector and Millennium 2010 software (Waters) was used to quantify aflatoxins. Separations were carried out at ambient temperature on Phenomenex 4μ ODS column, (250 x 4.6 mm). Aflatoxins were eluted with acetonitrile / methanol / water (1:3:6 v/v/v) as the mobile phase at a 1 ml/min flow rate. The detection wavelength for excitation and emission were set at 365 and 450 nm, respectively.

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 extracts µl

Z = area of aflatoxin peak in standard

X = sample extract injected µl

W= g sample represented by final extract

Percentage of inhibition was calculated using the following equation:

{100 – (Treatment / control x 100)}

**II- Effect of cell free supernatant of *Lactobacillus* species on spore germination of *Aspergillus parasiticus*:**

Each of the supernatant of *Lactobacillus* species was mixed with ($10^6$) of mold spore suspension in 2% malt extract. After homogenisation, 0.15 ml of each mixture was pipetted onto a sterile glass microscope slide and the mixture was retained within a rectangle delineated on the slide with molten paraffin wax.

The slides were incubated in moist Petri dishes at 25°C for 16 hours and spore germination was examined and calculated every 2 hours.

**III- Scanning electron microscope:**

Twenty-five milliliters of molten potato dextrose agar were transferred to Petri plates and allowed to solidify by refrigeration for 10 minutes. About 0.5 ml of *A. parasiticus* spore suspension was then transferred to the PDA plates and spread uniformly over the agar surface using a sterile bent glass rod. PDA plates were then dried at 37°C for 1 hour, after which wells of 0.6-mm diameter were cut. 100 µl cell free supernatants of lactic acid bacteria were then added into the wells. The plates were incubated at 28°C for 12-16 hours to facilitate the diffusion of the culture supernatant fluid into the agar medium. The plates were then inverted and incubated for 5 days.

An Electron probe micro analyzer (JXA – 840A) scanning electron microscope operating at 10 kV and at magnification range of 500 to 3500 was used.

**Statistical analysis:**

Statistical analysis of the effect of *Lactobacillus* species on fungal growth and aflatoxin production were carried out by the completely randomized design in factorial arrangement (*F*-test). The least significant difference (L. S. D) was used for comparing treatment means (Steel and Torrie, 1980).

**Results and Discussion**

**I- Effect of cell free supernatant of *Lactobacillus* species on *A. parasiticus* growth and aflatoxin production:**

Fungal growth and aflatoxins production by *A. parasiticus* were accounted for after 8 days of incubation for the control and the treatments (T1, T2, T3 and T4) of all *Lactobacillus* species. The results were the mean value of two replicates.

**Preparation for Scanning Electron Microscope (SEM):**

**1- Mycelium segments (1 $cm^3$)**

↓

**2-Added to 3% glutaraldehyde in 0.05M phosphate buffer (pH 6.8) at room temperature**

↓

**3-Chemical fixation was carried out using ethanol series and ending with absolute ethanol**

↓

**4-Dried in liquid carbon dioxide**

↓

**5-Placed in a freeze-drier**

↓

**6-Placed in Petri-dish (with a vial cap containing 4% osmium tetraoxide in water)**

↓

**7-Mycelium segments coated with 20:30 nm of 60:40 gold: palladium**

↓

**8-Scanning electron microscope**

**Fig.(1):**Diagram showing steps undertaken to prepare *A.parasiticus* for scanning electron microscope

Points 1 and 2 according to Mims *et al.* (1988); Lindroth *et al.* (1988) and Batish *et al.* (1990)

Points 3 to 7 according to Kale *et al.* (1996)

**1-Fungal growth:**

Behavior of *A. parasiticus* growth in the presence of CFS *Lactobacillus* species was studied. This behavior changed depending on the species of *Lactobacillus* and the type of treatments. Data in figure (2) reveal that treatment T2 of both *L. acidophilus* and *L. delbreukii* subsp. *bulgaricus* was highly effective in inhibiting fungal growth, followed by treatments T1, T4 and T3 in descending order, respectively. In this regard, no real significant variance ($P > 0.05$) between control and all treatments was recorded for both *L. acidophilus* and *L. delbreukii* subsp. *bulgaricus*

Results showed that all treatments of *L. casei* completely inhibited the fungal growth (100%), whereas both *L. gasseri* and *L. reuteri* treatments T3 and T4 completely inhibited (100%) fungal growth. On the other hand, reduction of the fungal growth at (61.54%) was also recorded for treatment T2 of *L. gasseri*. Data also revealed real significant variance ($P < 0.05$) between control and treatments T2, T3 and T4 of *L. gasseri* and *L. reuteri*.

Data in figure (2) also shows that the cell free supernatants of all tested strains incorporated in the flasks (T2) was more active in inhibiting fungal growth than that incorporated in dialysis sac (T1). 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 inoculation.

The percentage of inhibition for *L. acidophilus* was 16.41%, 51.56% and *L. delbreukii* subsp. *bulgaricus* 31.64%, 54.80% for treatments T1 and T2, respectively. In contrast *L. gasseri* and *L. reuteri* when were incorporated 16 hours before spore suspension (T3 and T4) completely inhibited (100%) the fungal growth, and was more effective than the incorporation simultaneously (T1 and T2).

The antifungal activity of these lactic acid bacteria could not simply be assigned to the low pH, but most probably to the formation and secretion of antifungal organic metabolites. Since lactic acid bacteria have long been known to produce organic acids in their production medium (De Vuyst and Vandamme, 1994), these metabolites could be lactic acids (De Muynck *et al.*, 2004). Lactic acid bacteria also produce acetaldehyde, hydrogen peroxide, diacetyl, carbon dioxide, polysaccharides and bacteriocins (De Vuyst and Degeest, 1999; Rodríguez *et al.*, 2003).

El-Gendy and Marth (1980) reported that the addition of *Streptococcus lactic* plus *L. casei* to cultures of *Aspergillus* species retarded growth of these molds even after 2 weeks at 15°C. Presence of the lactic acid bacteria

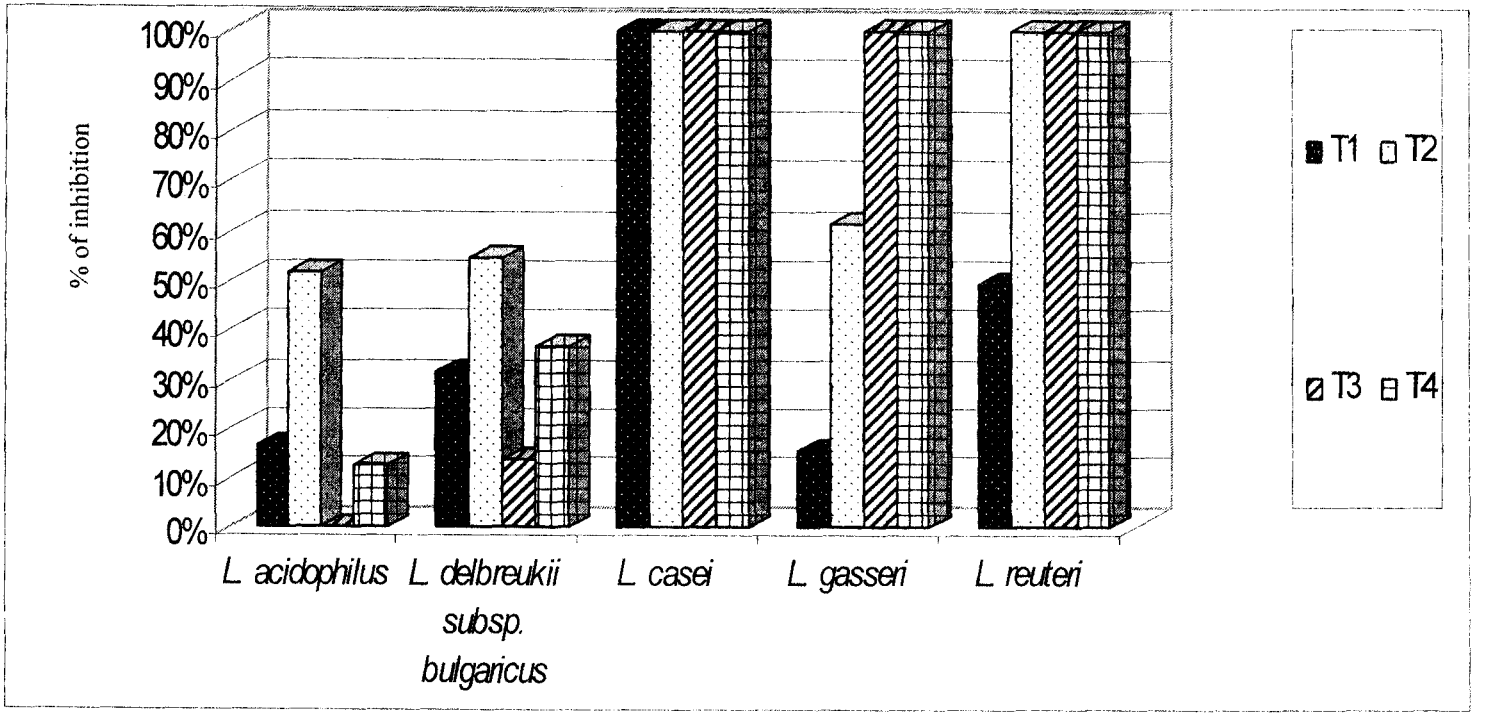


**Fig.(2):** Percentage of inhibition of *A. parasiticus* growth using different strains of *Lactobacillus* species

also caused appreciable inhibition of the growth of *Penicillium* species that were tested.

On the other hand, many reports suggested that antifungal activity is a combination of organic acids such as lactic, acetic and phenyl-lactic acids (Laitila *et al.*, 2002) or bacteriocins (Effat, 2000) and low molecular weight antimicrobial agents and peptides (Strom *et al.*, 2002).

It could be concluded that the inhibition of fungi by lactic acid bacteria might be due to combination of acidity and microbial competition (Lavermicocca *et al.*, 2000). In agreement, Corsetti *et al.* (1998) determined that a mixture of acetic, caproic, formic, propionic, butyric, and *n*-valeric acids are responsible for the antifungal activity. Meanwhile Cabo *et al.* (2002) concluded that the un-dissociated acetic acid originates from the bacterial growth medium, and that the synergistic effect of the acetic acid present and the lactic acid produced was likely the main factor responsible for the antifungal properties of the selected bacteria. These results could explain some discrepancies in reports of the antifungal properties of lactic acid bacteria, since the role of acetic acid has not been considered in previous studies (De Muynck *et al.*, 2004).

**2- Aflatoxin production:**

The effect of different *Lactobacillus* species on aflatoxin production was also studied. Data in figure (3) indicates that all treatments of *L. acidophilus* caused complete inhibition (100%) of aflatoxin $G_2$, showing real significant variance ($P < 0.05$), whereas treatment T1 reduced aflatoxin $B_1$ level by 23.74%. On the other hand, stimulation effect of aflatoxin $B_1$ production was recorded for the treatments T2, T3 and T4. Regarding aflatoxin $B_2$ treatments T1 and T2 caused the reduction of aflatoxin content by 45.76% and 19.04% respectively. In contrast, treatments T3 and T4 caused the stimulation of aflatoxin production.

Data presented in figure (4) indicate that, *L. delbreukii* subsp. *bulgaricus* treatments T1, T2, T3 and T4 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. delbreukii* subsp. *bulgaricus* and *L. casei*.

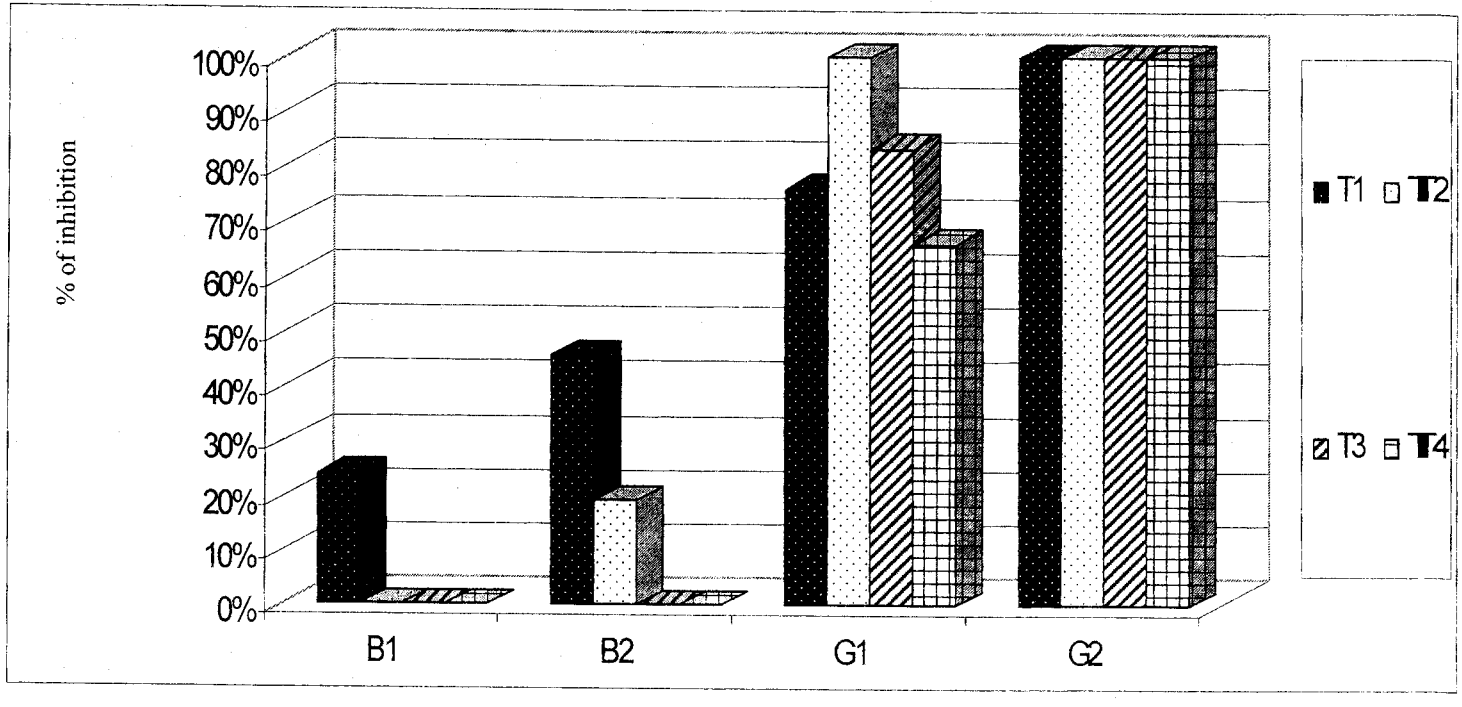


**Fig.(3):**Percentage of inhibition of aflatoxins production using *Lactobacillus acidophilus*

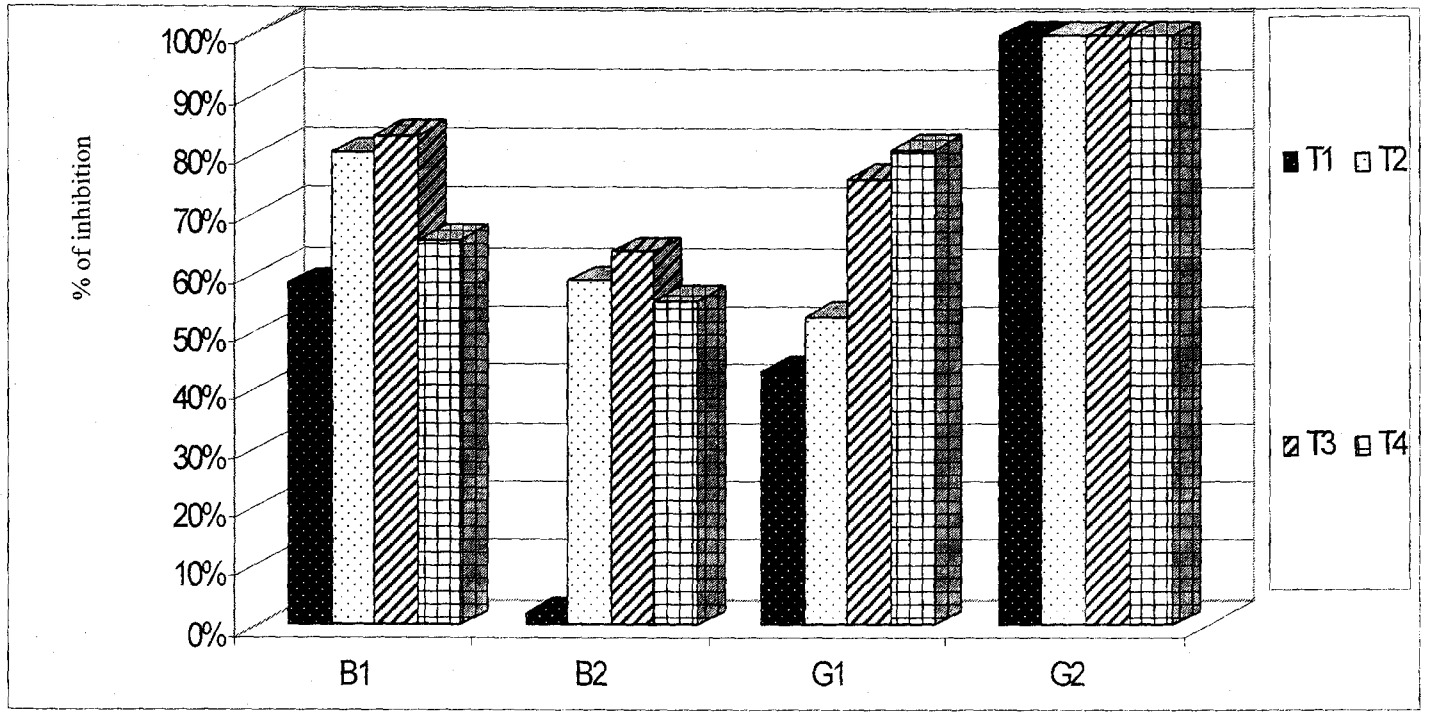


**Fig.(4):** Percentage of inhibition of aflatoxins production using *Lactobacillus delbreukii* subsp. *bulgaricus*

Results in figure (5) indicate that treatments T3 and T4 of the *L. gasseri* completely inhibited (100%) the production of aflatoxin types. It was also noticed that treatment T2 completely inhibited (100%) aflatoxin $B_2$ and $G_1$ only showing real significant variance ($P < 0.05$). On the other hand treatment T1 caused a slight stimulation of aflatoxin $B_1$ and $G_1$ production.

Figure (6) showed that treatments T2, T3 and T4 of *L. reuteri* caused complete inhibition (100%) of all types of aflatoxins, whereas treatment T1 only reduced aflatoxin $B_2$, $G_1$ and $G_2$ production, and stimulated aflatoxin $B_1$ production. In this regard, real significant variance ($P < 0.05$) was recorded between control and all treatments.

Finally data revealed that cell free supernatants of *L. casei*, *L. gasseri* and *L. reuteri* incorporated in the flasks treatment (T2) were more effective in inhibiting aflatoxin production than those incorporated in dialysis sac treatment (T1). Whereas the incorporation of these bacteria 16 hours (T3, T4) before spore suspension of *A. parasiticus* completely inhibited aflatoxins and was more effective than the incorporation of bacteria simultaneously with spore suspension (T1, T2).

In contrast, cell free supernatant of *L. acidophilus* incorporated in dialysis sac (T1) was more active in inhibiting aflatoxin production than that incorporated in the flask (T2). 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.

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)**.

**II- The effect of cell free supernatant *Lactobacillus* species on spore germination of *Aspergillus parasiticus*:**

Spore germination was enumerated during 16 hours. Data in Table (1) shows the behavior of mold spore germination during 16 hours of incubation with free cell supernatant of *Lactobacillus* species. The behavior of the spore

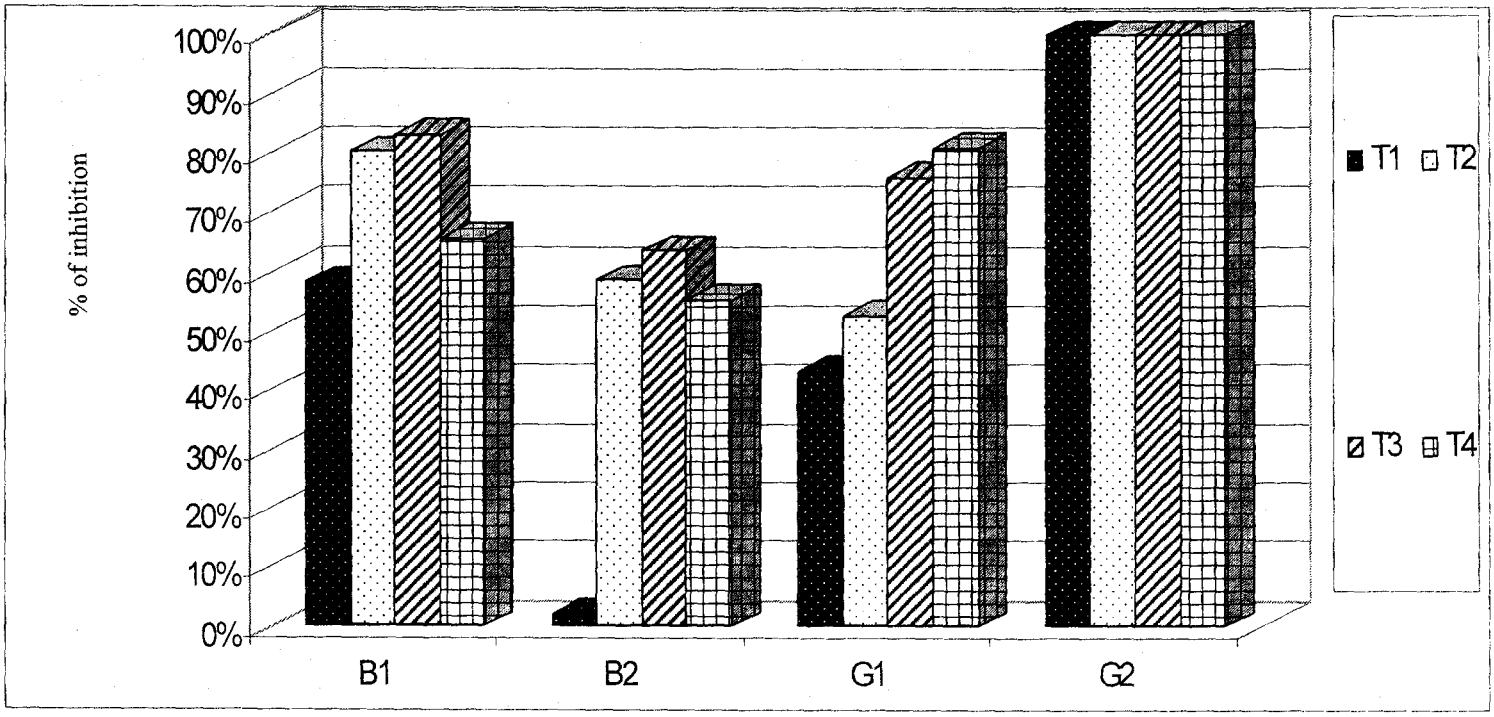


**Fig. (5):** Percentage of inhibition of aflatoxins production using *Lactobacillus gasseri*

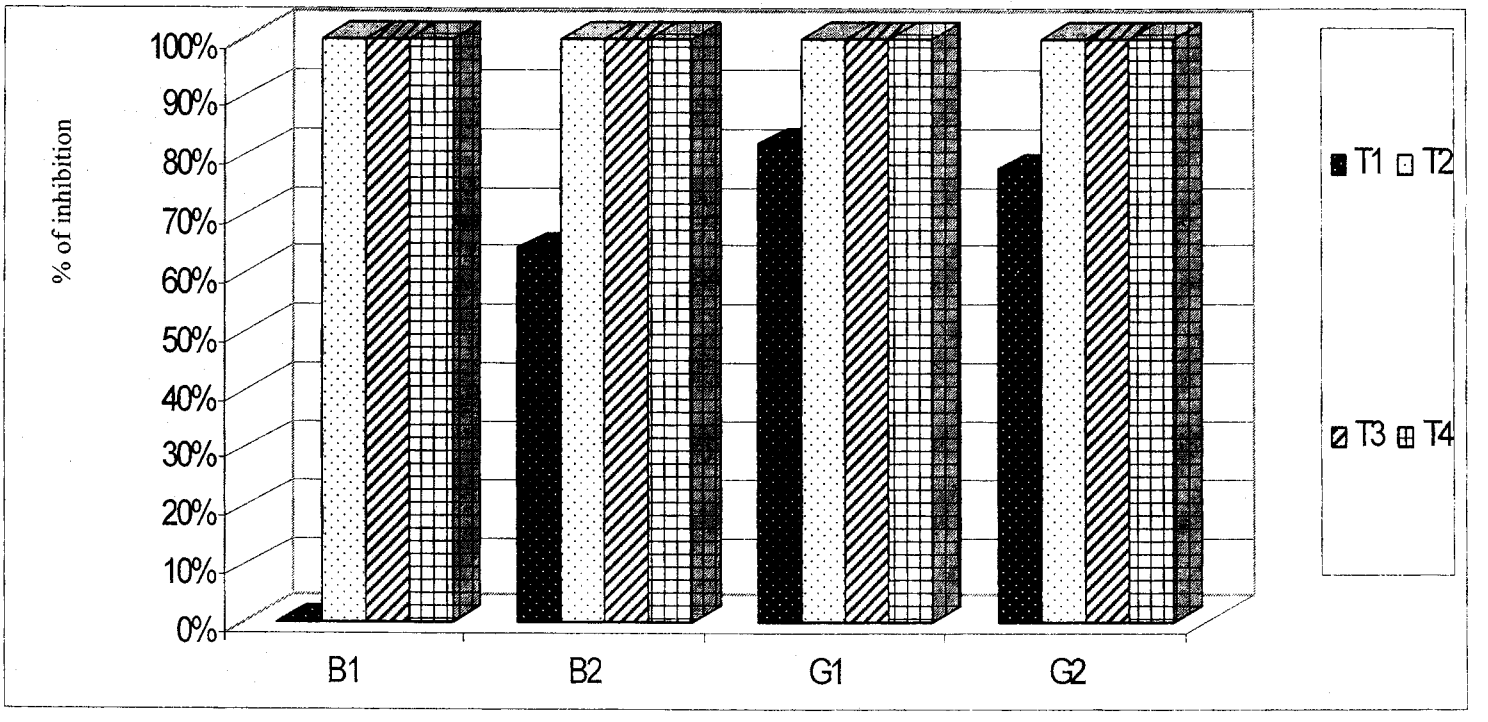


**Fig.(6):** Percentage of inhibition of aflatoxins production using *Lactobacillus reuteri*

germination was affected by the *Lactobacillus* species during the time of incubation whereas the germination of mold spore was inhibited. The CFS of *L. acidophilus*, *L. casei* and *L. gasseri* were the most effective on mold spore germination.

Data also show that the mean inhibition percentage of spore germination after 16 hours, whereas the highest inhibition (33.16%) of spore germination was caused by CFS of *L. acidophilus* followed by *L. casei*, *L. gasseri* and *L. reuteri* respectively, while the lowest inhibition (4.56%) of spore germination resulted by CFS of *L. delbreukii* subsp. *bulgaricus*.

**Table(1):** Percentage of inhibition of *Aspergillus parasiticus* spore germination using different species of *Lactobacillus*

| Incubation period (hours) | *Lactobacillus species* | | | | |
|---|---|---|---|---|---|
| | *L. acidophilus* * | *L. delbreukii* subsp. *bulgaricus* * | *L. casei* * | *L. gasseri* * | *L. reuteri* * |
| 0 | 100 | 100 | 100 | 100 | 100 |
| 2 | 69.98 | 54.96 | 67.05 | 52.60 | 40.93 |
| 4 | 66.12 | 55.41 | 62.51 | 58.73 | 45.35 |
| 6 | 71.98 | 50.10 | 44.74 | 69.54 | 44.23 |
| 8 | 45.40 | 40.48 | 33.60 | 30.56 | 27.98 |
| 10 | 43.19 | 27.60 | 34.53 | 23.75 | 27.37 |
| 12 | 13.49 | 11.97 | 19.49 | 11.17 | 10.00 |
| 14 | 21.37 | + | 15.88 | 7.15 | + |
| 16 | 33.16 | 4.56 | 27.25 | 18.88 | 11.11 |

*Indicates a significant variance ($P < 0.05$)

\+ Stimulation

Mean value of two replicates

Mold spore germination is a physiological reaction of dormant cells in response to changes in the environmental conditions such as nutrient penetration barriers, metabolic blocks, inhibitors, and low water content (Smith, 1978). Gottlieb (1978) and Griffin (1981) reported that germination of mold spores goes through three successive changes: the swelling, the emergence of germ tube and the elongation. El Gendy and Marth (1981) support our results, where they reported that the interaction between *A. parasiticus* and *L. casei* was due to some nutritional changes in the medium after the growth of *L. casei*. Results showed changes in the behavior of all *Lactobacillus* species concerning the effect of CFS on the mycelium dry weight and spore germination of *A. parasiticus*. In another meaning the maximum inhibition of mycelia dry weight did not coincide with the maximum inhibition of spore germination of *A. parasiticus*. This observation may be due to the presence of other active substances in the culture that might have affected the behavior of *A. parasiticus* as was previously reported by Wiseman and Marth (1981) and El Gendy and Marth (1981). Similar observations were recorded by Bullerman and Gourama (1995), who observed that actively growing *Lactobacillus* species cells totally inhibited the germination of *A. flavus* subsp. *parasiticus* spores, whereas culture supernatant broth from the mixture of *Lactobacillus* strains inhibited fungal growth, but did not destroy fungal spore viability. There is more than one possible explanation for this effect, the first is the presence of increased levels of acids produced by *Lactobacillus* species that caused the drop of the pH value, and the second is, the presence of volatile metabolites and enzymes produced by bacteria that could influence mycelia growth. Our observations are supported by Batish *et al.* (1989) and Plockova *et al.* (1997), who recorded that the antifungal activity of lactic acid bacteria are related to the acetic acid produced. Also Vendenbergh (1989) reported antifungal activity in the supernatant after the cultivation of several *Lactobacillus* strains in the media.

**III- Scanning electron microscope:**

*Aspergillus* conidiation is known to be the best model system for studying any morphological changes (Mims *et al.*, 1988; Timberlake, 1990). Data in Figure (7) show altered morphological changes in conidiophores and conidiospores as a result of the interaction of CFS of all *Lactobacillus* species and spore suspension of *A. parasiticus*, except *L. delbreukii* subsp. *bulgaricus*, which caused the lowest effect on the conidiophores

and conidiospores of *A. parasiticus*.

It could be indicated that these changes may be the result of the active substances in the CFS of different bacterial cultures, and that they may affect germination, mycelia growth and other physiological behavior such as aflatoxigenic capabilities as reported by Kale *et al.* (1996). Prade and Timberlake (1993) observed some of the developmental abnormalities in *A. nidulans* using mutant agents, whereas *A. nidulans* was similar to *A. parasiticus* (Mims *et al.*, 1988; Timberlake, 1990).

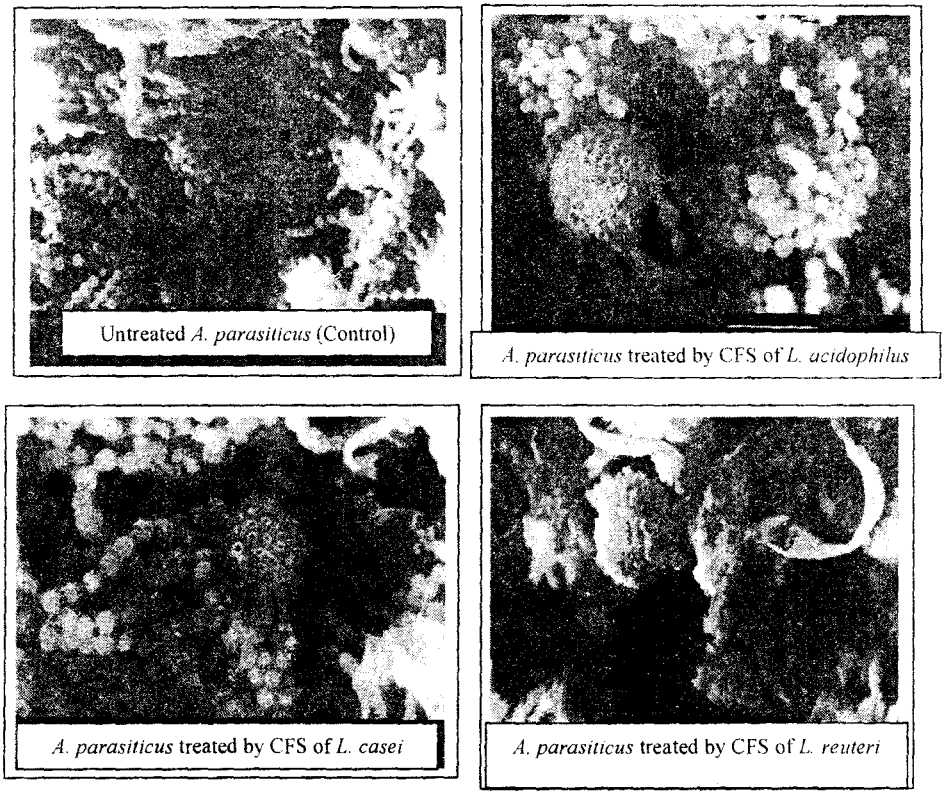


**Fig.(7):** Microstructure changes of *Aspergillus parasiticus* using different *Lactobacillus* species

**Conclusion:**

The effective substances produced by *Lactobacillus* species completely inhibited the growth of the aflatoxigenic *A. parasiticus* and actually prevented aflatoxin production, which could be considered a safe process for controlling aflatoxin contamination in different food products.

Suitable bacterial strains should be chosen as to ensure a good quality and a prolonged shelf life without aflatoxin and fungi contaminants. This effect was true for most treatments in this study and it seems that this inhibitory effect depends on the lactic acid bacteria species and / or the type of treatment used. The present findings suggested that the antifungal activity of the *Lactobacillus* species is likely due to different chemical substances produced by these bacteria. Such substances with antifungal and antiaflatoxigenic potentials need to be determined in future research.

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# التاثير المثبط لبعض أنواع اللاكتوباسيلس علي نشاط و إنتاج الأفلاتوكسين بواسطة أسبرجلس بارازيتكس

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أهتم البحث بدراسة التأثير المثبط للراشح الناتج من الخلية البكترية لعدد من انواع *Lactobacillus* علي نمو و إنتاج الأفلاتوكسين بواسطة فطر *A. parasiticus* . و أظهرت الدراسة أن *L. casei* حقق تثبيطا تاما (100%) لنمو الفطر و إنتاج الأفلاتوكسين عند إضافة الراشح الناتج من خلاياه داخل الـ Dialysis sac أو بدون أو بإضافة الفطر بعد 16 ساعة من إضافة البكتريا. كما أظهرت النتائج أن بكتريا *L. reuteri* و *L. gasseri* ثبطت نمو الفطر وإنتاج الأفلاتوكسين و لكن بنسبة أقل. و قد أظهرت النتائج أن الراشح الناتج من خلايا لكل من الـ *L. acidophilus* و *L. delbreukii subsp. bulgaricus* كان اقل تأثيرا علي إنتاج الأفلاتوكسين و كذلك علي نمو الفطر. كما أوضحت النتائج أن تلقيح سلالات البكتريا التالية *L. casei* و *L. gasseri* و *L. reuteri* قبل التلقيح بالفطر بمدة 16 ساعة يؤدي إلى زيادة التأثير المثبط علي نمو الفطر و إنتاج الأفلاتوكسين، إلا انه لم يتم ملاحظة هذه الدلالات بالنسبة للمعاملات الأخرى الخاصة بكل من الـ *L. acidophilus* و *L. delbreukii subsp. bulgaricus*.

و فيما يتعلق بانبات جراثيم الفطر *A. parasiticus* التي عولجت بالراشح الناتج من الخلايا البكترية لأنواع الـ*Lactobacillus* لوحظ ان الـ *L. acidophilus* قد سجل اعلي تاثير مثبط علي انبات جراثيم الفطر يليه *L. casei* و *L. gasseri* و *L. reuteri* . في حين كان *L. delbreukii subsp. bulgaricus* الأقل تاثيرا. و قد استخدم الميكروسكوب الالكتروني لتحديد التغيرات المجهرية في الحامل الكونيدي و جراثيم الفطر بعد المعالجة بالراشح الناتج من الخلايا البكترية لعدد من أنواع الـ*Lactobacillus* حيث اظهرت الصورة وجود تشوهات مورفولوجية عديدة في شكل الحامل الكونيدي و في عدد و شكل الجراثيم.