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INHIBITION OF GROWTH AND AFLATOXIN PRODUCTION OF *Aspergillus flavus* by *Lactococcus lactis*

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ABSTRACT: ***This study was conducted to determine the effect of Lactococcus lactis on mold growth and aflatoxin production by Aspergillus. flavus in a liquid cultures and to explore the properties of the antifungal substances produced by L. lactis in the presence of A. flavus. A. flavus growing in the presence of L. lactis in Lablemco tryptone broth medium resulted in an inhibition of aflatoxin production without affecting the mold growth. Toxin production was inhibited by 89%, 85%, and 80% when L.lactis was grown for 16, 24, and 48 hr, respectively, prior to inoculation with A. flavus spores. When 16 hr L.lactis cultures were inoculated into a 24, 48, 72, 96, , 120, 144, and 168 hr cultures of A. flavus toxin production was reduced in the 24, 48, 72, and 96 hr of A. flavus cultures by 80%, 77%, 67%, and 62% respectively. When L.lactis and A. flavus were inoculated simultaneously, the total aflatoxin content decreasd by 77%. Concentrated L. lactis metabolites inhibited the production of aflatoxin completely. The drop in pH of the medium as a result of L. lactis growth was not the cause of the observed inhibition. Dialysates of the L. lactis metabolites inhibited aflatoxin production completely, indicating that the inhibitor was low-molecular-weight compound. Chloroform, methanol and ethyl acetate extraction, yielded extracts that inhibited aflatoxin production. The chloroform methanol extract were applied to G15 sephadex. One of the fractions showed an inhibitory compound. An additional peak was obsereved when an inhibitory fraction of sephadex chromatography was run on HPLC.***

Key words: ***Aflatoxin, Lactococcus Lactis, Inhibition, Aspergillus flavus***

INTRODUCTION

Aflatoxins are extremely carcinogenic, teratogenic and mutagenic (Godic and Vengust 2008) fungal secondary metabolites produced by *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* (Hong *et al.,* 2008, Hussain *et al.,* 2008). These toxins have harmful effects on humans, animals and crops that result in illnesses and economic losses (Giary *et a*l., 2007). According to the Food and Agriculture Organization of the United Nations (FAO), at least 25% of the world's food crops are contaminated with mycotoxins (Shetty and Jespersen 2006). Aflatoxins are found in various plant products including peanuts, copra and soya or in cereals such as wheat, maize and rice (Alborzi *et al*., 2006). Aflatoxins have received increased attention from the food industry and the general public for two

main reasons: (A) aflatoxins (particularly aflatoxin B_1) are not only toxic to humans and animals, but this also carcinogenic of all known natural compounds (Groopman *et al*., 1981). (B) the high incidence of aflatoxins in food and feed throughout the world (Ellis *et al*., 1991, Jelinek *et a.,l* 1989).

Because of the high toxicity to both humans and animals, several studies were done regarding removing aflatoxin by physical (Diaz *et al*., 2004, Gowda *et al*., 2007) and chemicals methods (Mishra and Das 2003). However, such treatments require expensive equipments and may result in losses of nutritional quality of treated commodities and undesirable health effects(Hong *et al*., 2008, Phillips *et al.,* 1994, Samarajeewa *et al*., 1990).

The disadvantages of physical and chemical degradation techniques have encouraged recent imphasis on biological degradation to improve the safety and nutritional quality of food for human and animal consumption (Alberts *et al*., 2006). It has been reported that many microorganisms, inculding bacteria, mold, and yeast are able to remove or degradate small amounts of aflatoxin in foods and feeds (Ciegler *et al.,* 1966, Karlovsky 1999), but biological detoxification of aflatoxin has not been established in practice. *Flavobacterium aurantiacum* has been used to remove aflatoxin from liquid medium and food products without the production of toxic by products (Ciegler 1966, Hao and Brackett 1988, Line and Brackett 1995).

Lactic acid bacteria (LAB) are found naturally in many food products such as dairy and meat products, and vegetables. LAB have a GRAS status (generally recognized as safe) (Schnurer and Magnusson 2005) and they are used in various food products because of their preservative potential and their effects on the organoleptic properties (Gourama and Bullerman, 1995). Numerous studies have clearly shown that LAB have beneficial health effects and inhibit the mutagenicity and carcinogenicity of a range of toxic compounds found in the human diet (Fuchs *et al*., 2008, Haskard *et al*., 2000).

Among the *Lactococci, Lactococcus lactis* species have been known to produce certain antimicrobial compounds which inhibit a wide variety of pathogenic and spoilage organisms (Reddy and Ranganathan 1983). The inhibitory substances reported to be produced by *L. lactis* species are mostly antibacterial in nature, however, in some cases these substances have also been found to be antifungal in nature (Gourama 1997, Luchesse and Harrigan 1990, Magakyan and Chuprina 1978, Weckach and Marth 1977). However, the number of published studies on antifungal LAB is relatively low.

This study was performed to determine the effect of *L. lactis* on mold growth and aflatoxin production by *A. flavus* in a liquid cultures and to further explore the properties of the antifungal substances produced by *L. lactis* in the presence of *A. flavus*.

MATERIALS AND METHODS

Cultures:

L. lactis **(ATCC 11454) and** ***Aspergillus flavus*** **(V3734/10) were used for these experiments. The mold was grown on potato dextrose agar (PDA) slants at 30 ^{o}C for 5 days until well sporulated. Spores were harvested by washing slants with sterile 0.05% Tween 80 and the spores were loosened gently by brushing with a sterile inoculating loop. The spores were counted with a Hemacytometer and the suspension was adjusted to 10^{7} spores/ml^{-1}. An** ***L. lactis*** **frozen stock culture (-80 ^{o}C) was defrosted and transfers were made to 50 ml of Lablemco tryptone broth (LTB). After incubation for 16h at 30 ^{o}C, 0.1 ml of suspension was transferred to 50 ml LTB which was incubated for 48h at 30 ^{o}C. The latter suspension constituted the** ***L. lactis*** **working culture.**

Culture media:

Lablemco Tryptone Broth (LTB) containing 1% dextrose (ICN), 1% Yeast extract , 1% tryptone, 1% beef extract, 0.5% NaCl, 0.2% Na_2HPO_4 was used to grow both ***L. lactis*** **and** ***A. flavus.*** **The medium was sterilized at 121 ^{o}C for 15 min. When LTB medium at pH 4.5 was needed, the pH was adjusted with 5N HCl before sterilization.**

Experimental design:

For studies on competitive growth, several different experiments were done. In the first experiment, 50 ml of LTB broth was inoculated with ***L. lactis*** **suspension (ca. 10^{6} cfu) and incubated for different times e.g., 16, 24, 72, and 96hr at 30 ^{o}C before 1ml of spore suspension (10^{7}) was added. The incubation was then continued for 7 days at 30^{o}C. In the secound experiment 50 ml of LTB broth was first inoculated with 1 ml of mold spore suspension (10^{7}). A** ***L. lactis*** **suspension (10^{6}) was then injected into the mold culture after 24, 48, 72, 96, 120, 144, and 168hr. The incubation was then continued for 7 days at 30^{o}C. In the third experiment, 50 ml of LTB broth was inoculated either with 1 ml of mold spore suspension and 1 ml of** ***L. lactis*** **suspension simultaneously or with** ***L. lactis*** **followed by 16hr incubation before inoculating with the mold spore suspension.The incubation for both treatments continued for 7 days at 30^{o}C. Finally, 50 ml of LTB broth was adjusted to pH 4.5 and then inoculated with 1 ml mold spore to observe the effect of pH on aflatoxin production by** ***A. flavus*****.**

For each experiment ***A.flavus*** **was grown alone in LTB broth in order to use it as control for toxin production.**

In all experiments of aflatoxins B_1 and G_1, mycelial dry weight, and pH were determined.

Identification of bacterial inhibitory isolates:

1. Cell free supernatant from *L. lactis* :

one ml of *L. lactis* was defrosted and inoculated into 500 ml of LTB broth and incubated for 16h at 30°C. Culture was centrifuged (sorvall RC2B) at 4080 x G at 5°C for 10 min. The supernatant was collected and lyophilized (Thermovac). Various amounts of the lyophilized material were added to 50 ml of LTB broth, followed by inoculation with of *A.flavus* spores (10^7). The flasks were incubated for 7 days at 30°C. Ten g of lyophilized material in 10 ml water were also dialyzed overnight against. (MWCO : 1000, Spetrum) at 3°C. The dialyzate was lyophilized and the concentrated material assayed for aflatoxin inhibition as described above.

Once inhibition was detected experiment were repeated on large scale..Six flasks of 500 of sterile LTB broth were inoculated each with I ml *L. lactis* and incubated for 16h at 30°C. The medium free of culture was lyophilized (Vir Tis).

Extraction of antifungal compound(s):

If the *L. lactis* metabolites showed strong inhibition, three different extraction procedures of the antifungal compounds) were tested.

A. Chloroform methanol extraction:

The procedure which used to extract and purify the inhibitory compound(s) produced by *L. lactis* using chloroform and methanol are shown in Figure (1).

B. Methanol acetone extraction:

The procedure used was a modification of that described by (Shahani *et al*.,1977) figure (2). The powdery residue (10 g) obtained after lyophilization was disperersed in cold methanol (3-7°C) and stirred for one hour at 3-7 °C. The methanolic dispersion was centrifuged at 5000 x G at 5°C for 10 min to remove solid material and the supernatant was collected for further processing. The residue was extracted twice with cold material. The pooled methanol extract was concentrated in an evaporator and yield a yellow liquid residue. This residue was further extracted with cold acetone (3-7°C) and centrifuged to remove solids. The supernatant was saved and the residue was extracted twice with cold acetone. The pooled acetone extracts were concentrated. The methanol acetone extract was first assayed for inhibition of aflatoxin production by *A.flavus* and then applied to sephadex chromatography.

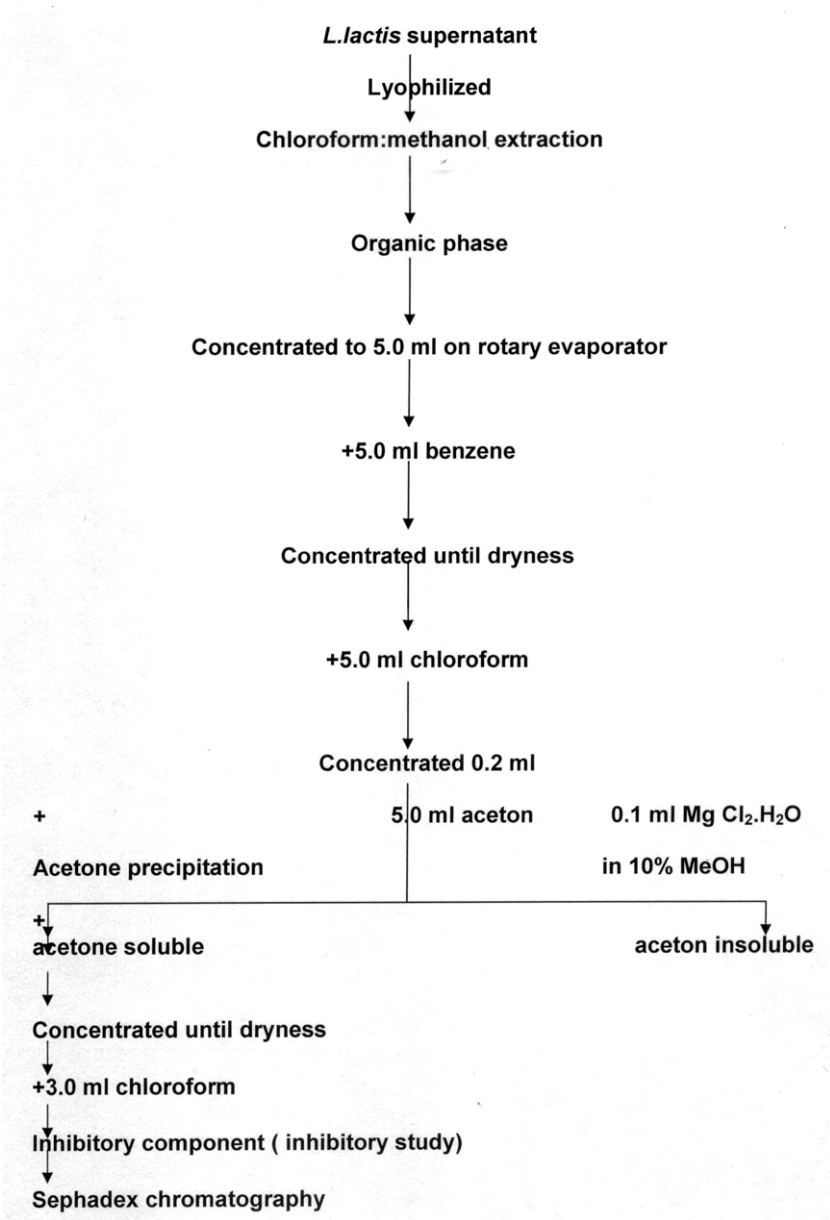

Figure 1: Steps in partial purification of antifungal compounds from *L.lactis* using chloroform and methanol extraction (Coallier-Ascah and Idziak, 1985).

C. Ethyl acetate extraction:

L.lactis **was grown in 500 ml sterile LTB broth for 48h at 30 ^{o}C. The culture was centrifuged and the supernatant acidified to pH 3 and extracted with ethyl acetate.**

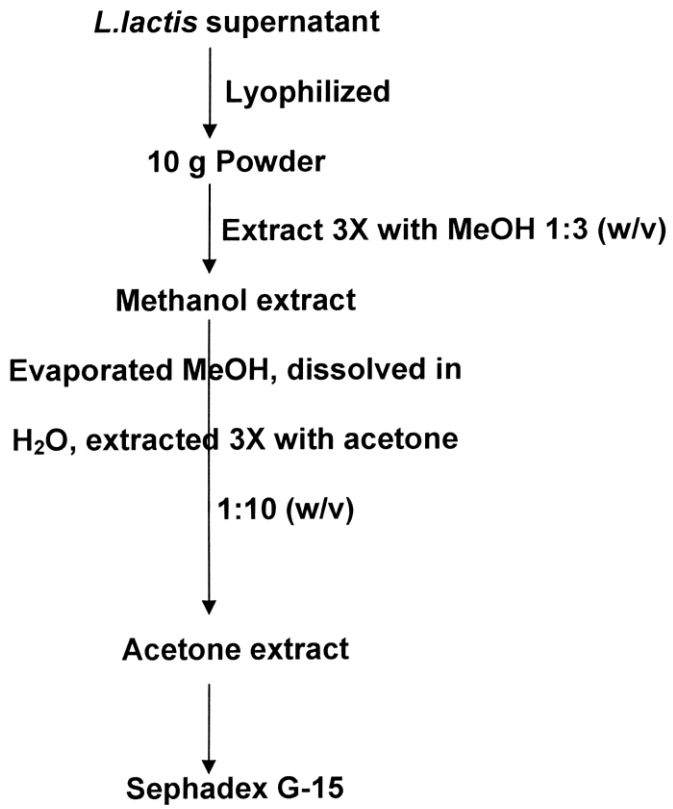

Figure 2: Steps in partial purification of antifungal compounds from *L.lactis* using methanol and aceton extraction (Shahani *et al*. 1977) .

The ethyl acetate extracts were evaporated till dryness and then resuspended in 5 ml of ethyl acetate. Part of the ethyl acetate extract was used for sephadex chromatography, and part to assay for inhibition production by *A.flavus.*

Uninoculated LTB broth was also extracted to see if the concentrated nutrients have any affect on the growth and aflatoxin production by *A.flavus.*

Chromatographic purification of antifungal compound(s):

1. Sephadex:

Two ml of each of the extracts, A, B, and C above were applied separately to18.5 cm x 2.5 cm sephadex G 15 column. The methanol acetone (B) was eluted with 0.05% N HCl; chloroform methanol (A) and ethyl acetate (C) extracts with methanol/water (1 : 1). Elute was monitored at 254 nm and 5 ml fractions collected, pooled, concentrated and inhibition activity assayed.

2.HPLC:

The concentrated sephadex fractions inhibiting toxin production by A.flavus were diluted, filtered through a membrane filter with a pore size of 0.2 µm (Millipore, Fisher Sientific, Oshawa, ON, Canada) and passed through an Aminex HPX-87H 300mm x 7.8 mm column (Bio-Rad, Hercules, CA, USA) at a flow rate of 0.8 ml /min (0.05 N H2SO4). The eluate was monitored at 254 nm.

Analysis:

pH

The pH of the LTB culture was measured with bench-top (Fisher, model 210, USA).

Mycelial dry weight

The mycelium of the *A.flavus* culture was removed and washed three times with water. The mats were then placed in preweighed aluminum foil, dried in an oven at 100 ^{o}C for 24 hr, and cooled in a desiccator to constant weight.

Aflatoxin extraction and purification

Following incubating, aflatoxins were extracted from the LTB broth and purified using the method of Pons et al (1966). Aflatoxin extraction entailed macerating the mycelium in a mortar with a pestle and adding to the culture fluid. Fifty ml of chloroform were added and the resultant suspension heated in a water bath at 60 ^{o}C for 10 min. The mixture was transferred to a 250 ml separator funnel and the lower chloroform layer containing the aflatoxin was drained into a screw capped bottle. The extraction was repeated and the combined chloroform extract evaporated to dryness. The residue was then resuspended in 5 ml of chloroform in screw capped test tube. The aflatoxin samples were held at room temperature in the absence of light before chromatogram spotting separation.

Thin layer chromatography procedure

Standard 20 x 20 cm aluminum plates coated with silica gel (Whatman) were used in separating the aflatoxins. All plates were activated 30 min at 100^{o}C. Activated plates were developed were spotted with 5 µg of aflatoxin standards (B_1 and G_1). The spotted plates were developed in Toluene : Ethyl

acetate : Formic acid (60 : 30 : 10) (Pones 1966). The developed plate was air dried for 10 min, and then visualized under long wave UV (Chromato-VUE).

Aflatoxin standards

One ml of the aflatoxin standard (Sigma, 5 mg 0.5 ml^{-1}) was diluted to 25 ml in benzene : acetonitrite (98 : 2) in an aluminum foil wrapped volumetric flask. The standards were store at 5 ^{o}C.

Aflatoxin quantitation

The silica – resolved aflatoxins were quantified using a Turner model 111 Fluorometer (excitation filter no. 7-60 and transmission filter no. 48, 10x sensitivity setting) equipped with a thin layer chromatography automatic scanner. The chart recorder (Servo recorder model EU-20 B) was used to record fluorescent intensity at 100 millivolts. Spots on TLC plates were scanned in order of greatest mobility $B_1 \rightarrow G_1$. Peaks areas (peak x width at half peak height) were used in the calculation of toxin concentration using the formula developed by Pons *et al* (1966).

All experiments were done in triplicate; and each experiment repeated twice.

RESULTS AND DISCUSSION

Aflatoxin production by *A. flavus* in mono and mixed culture with *L.lactis*

Mold spores of *A. flavus* were inoculated into 10, 16, 24,48,72, and 96 hr *L.lactis* cultures in LTB broth. At the end of 7 days of mold growth, inhibition of toxin production was noted in all cases but the level of inhibition varied (Figure 3). Toxin production was inhibited by 89%, 85%, and 80% when *L.lactis* was grown for 16, 24, and 48 hr, respectively, prior to inoculation with *A. flavus* spores. However, the maximum inhibition was with 16 hr *L.lactis* cultures, a 16 hr pre-incubation of *L.lactis* followed by inoculation with *A. flavus* spores was followed in subsequent experiment. El-Nezami et al (1998) reported that the 24 hr old cultures of *Lactobacillus rhamnosus* GG and *Lactobacillus rhamnosus* LC-705 removed about 80% AFB_1 within 24 hr. In another study, El-Nezami *et al* (2002) found that both strains are most effectively bound B_1 than afltoxin B_2 , G_1 and G_2. While Line and Brackett (1995) reported that 24 hr cultures of *Flavobacterium aurantiacum* removed about 19% AFB_1 within 24 hr while 72 hr old cultures were the most effective and removed about 33% AFB_1 within 24 hr.

When 16 hr *L.lactis* cultures were inoculated into a 24, 48, 72, 96, , 120, 144, and 168 hr cultures of *A. flavus* (Figure 4) toxin production was reduced in the 24, 48, 72, and 96 hr of A. flavus cultures by 80%, 77%, 67%, and 62% respectively. With 120hr *A. flavus* culture, aflatoxin levels increased during the last three days of incubation.

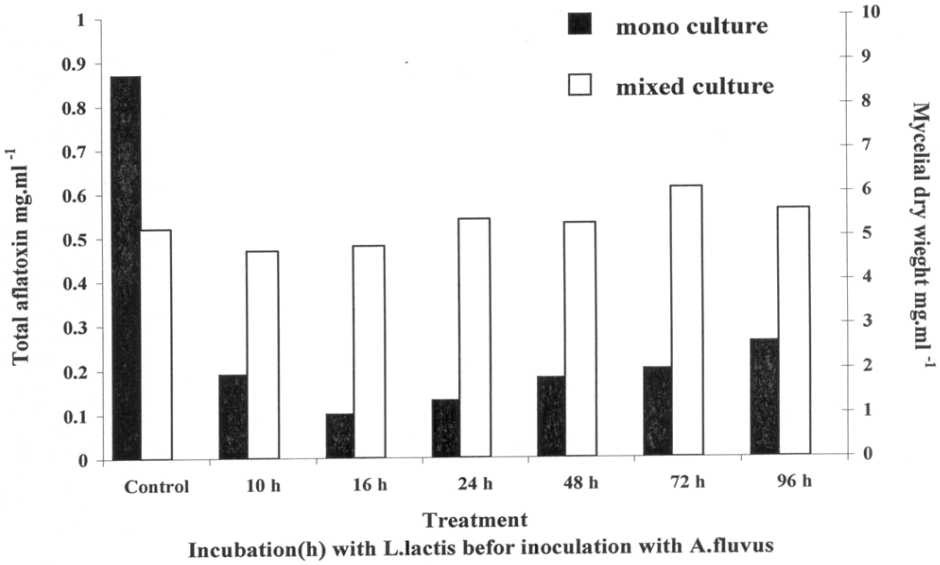

Fig:3: Total aflatoxin content in the mono and mixed cultures experiment at the end of the 7-day incubation period

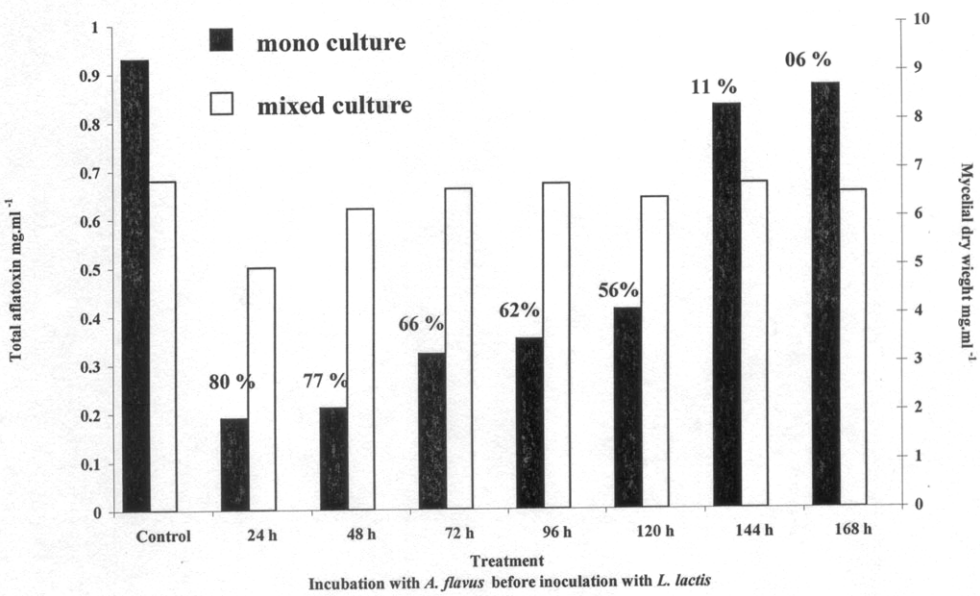

Fig. 4 : Total aflatoxin content in the mono and mixed cultures experiment at the end of the 7-day incubation period. L. lactis culture (16h) added to A. flavus culture

The decrease in the antifungal activity during the last three days of incubation may possibly due to exhaustion of available nutrients necessary for the production of antifungal compound (s). Enzymatic degradation of the antifungal compounds in the growth medium is another possibility. The inhibition was greater (82%) when *A. flavus* was inoculated into 16hr *L.lactis* cultures (Table 1).

Finally, when *L.lactis* and *A. flavus* were inoculated simultaneously, the total aflatoxin content decreasd by 77% to the control (Table 1)

Effect of *L.lactis* on growth of *A. flavus* and aflatoxin production

The growth of *A. flavus* (mycelial weight) was not affected by the presence of *L.lactis* in mixed cultures. Based on calculation of amounts of aflatoxins produced per unit of mycelial dry weight, it became apparent that the reduced toxin production was not a reflection of decreased growth of the mold. Thus two possibilities present themselves. 1) production of an inhibitor or 2) degradation of the formed toxin.

Effect of pH on aflatoxin production

The data on the influence of the pH on growth and production of antifungal substance by *L.lactis* are presented in the Table 1. The greatest amounts of growth of *A. flavus* in LTB occurred in medium adjusted to pH 4.5. The pH changes in LTB medium were similar in mono (*A. flavus*) and mixed (*L. lactis*) cultures (Table 2). In both instances, there was an initial decrease followed by an increase in pH. Similar finding were reported by Collier-Ascah and Idziak (1985), Davis et al (1966), and EL-Nezami et al (1998). Among the many factors that influence the growth and metabolism of microorganisms is the level of acid. During the fermentation of many foods, lactic acid is the primary acid produced. Coallier-Ascsh and Idziak (1985) reported that lactic acid was not inhibitory to aflatoxin biosynthesis. In another study, El-Gazzar et al (1987) found that lactic acid at pH 4.5 had no effect on mold growth and aflatoxin production. On the other hand, Karunaratne et al (1990) reported that the prevention of mould growth by *Lactobacillus* spp. was mainly due to a pH effect and microbial competition.

Luchese and Harrigan (1990) reported that the initial pH affected aflatoxin production and was linked to the culture medium used.

The increase in final pH is believed to be the result of high levels of nitrogen in the medium (Ciegler et al 1966) and for autolysis of fungal cells.

Wiseman and Marth (1980) did experiments similar to those described here but used *A. parasiticus* rather than *A. flavus*. In their study, *S. lactis* had considerably less impact on *A. parasiticus* than did *L.lactis,* on *A. flavus* as in our study.

Weckbach and Marth(1977) found that *A. parasiticus* could not complete effectively with *Rhizopus nigricans* and *Saccharomycis cerevisiae*, but dominated *Acetobacter aceti* and *Brevibacterium linens*.

The cumulative data sugget that growth and aflatoxin production by *A. flavus* or *A. paraciticus* can be inhanced, retarded or remain unchanged as a result of another microorganism in the environment.

Finally, visual observations done daily on the growth of the mould indicated that the decrease in aflatoxin production was associated with lack of sporulation.

Effect of concentrated bacterial metabolites on mold growth and aflatoxin production

Different amounts (100, 200, 300, 400, 500, and 600 mg) of concentrated bacterial metabolites from 16 hr *L.lactis* in LTB medium were tested for their inhibitory effect mold growth and aflatoxin production. These quantities of concentrated metabolites showed various degrees of aflatoxin inhibition (Table 3). The concentrated metabolites completely inhibited aflatoxin production by *A. flavus* at 600 mg. At 500 mg and below, the concentrated metabolites showed decrease inhibitory effect on the aflatoxin production. These different amounts of concentrated bacterial metabolites had no effect on mould growth of *A. flavus*. The final pH of these treatments was similar. The mold spores were observed in all treatments except that at 600 mg ml $^{-1}$ (highest inhibition percentage).

Effect of Dialysis

Dialysates (MW CO- 1000) of *L.lactis* culture medium yielded compound(s) that inhibited the toxin production by *A. flavus*. While mold growth, as measured by mycelial dry weight was not (Table 4). The inhibition of aflatoxin in this study was probably due to a low-molecular weight bacterial metabolite that diffused throwgh the dialysis membrane. This finding are consistent with the finding of Gourama and Bellerman (1995).

Purification of antifungal compounds(s) from *L.lactis*

A- Chloroform methanol extraction

The acetone component of the chloroform methanol extract of 16 hr incubation was added to *A. flavus* growing in LTB broth. There was no detectable amount of toxin produced. This extraction accomplished the removal of high molecular weight compounds from the LTB medium (Kates 1972).

B- Methanol Acetone extraction

The methanol acetone extract of *L.lactis* culture medium did not yield any compounds inhibiting toxin production by *A. flavus*.

C.Ethyl acetete extraction

This method of extraction of *L. lactis* culture medium yielded inhibiting compounds. The decrease in toxin production by *A. flavus* was greater than that of the other extraction solvent (A,B) however, this method did not eliminate high molecular weight compounds.

Chromatographic separation of antifungal compound(s)

A- Sephadex chromatography

The active material obtained from the chloroform methanol extraction of *L.lactis* culture medium gave 6 UV absorption fractions when eluted from sephadex G 15 (Figure 5). Fraction 4 showed an inhibitory compound (97%) compared to control (Table 5).

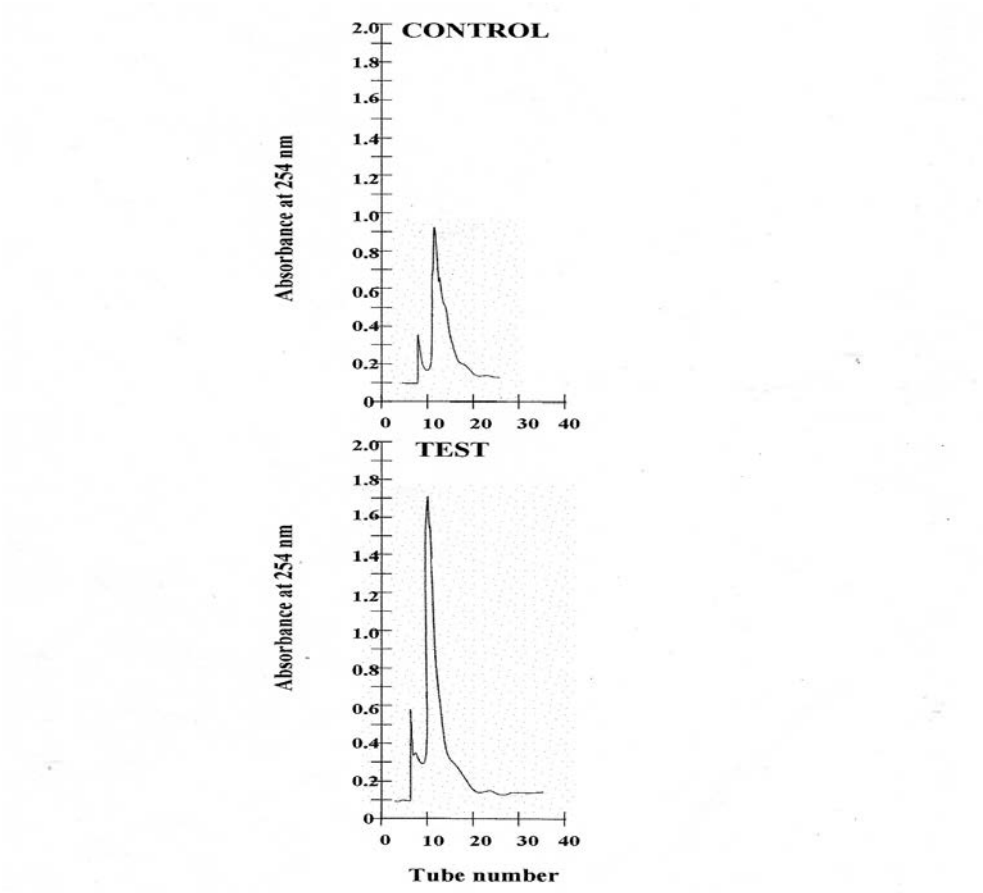

Figure 5:- Sephadex G15 of the antifungal material obtained from chloroform-methanol extraction

Table 5

The material obtained from the methanol acetone extraction of *L.lactis* culture medium gave 3 UV absorption fractions. None of these fractions were inhibitory.

Six fraction were obtained from the ethyl extracts of *L.lactis* culture, whereas only 5 fractions were collected from extracted LTB (Figure 6). These fraction have not, as yet, been assayed for inhibitory activity.

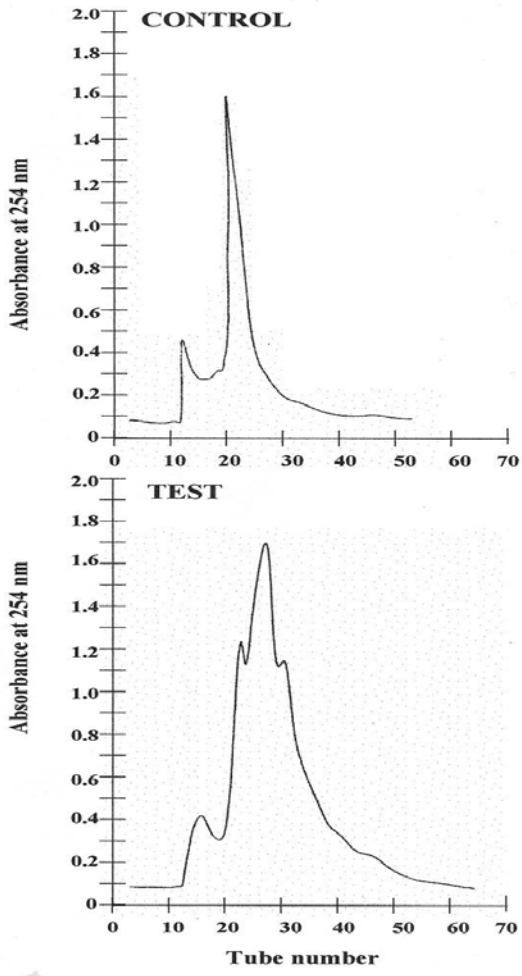

Figure 6 :- Sephadex G15 of the antifungal material obtained from ethyl acetate extraction

B- HPLC chromatography

Fraction 4 from sephadex G 15 separation of the chloroform methanol extract of *L.lactis* culture medium was run on HPLC. Compared with the HPLC profile of a similarly processed LTB extract, an additional peak was observed (Figure 7). There was insufficient material activity. Preparative HPLC or preparative thin layer chromatography may be tried to obtain more of inhibitory compound(s).

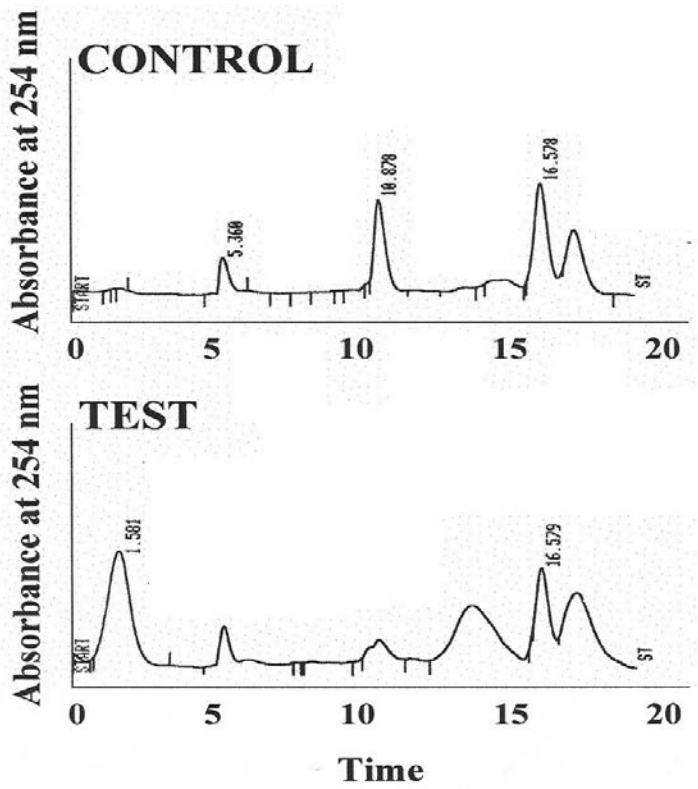

Figure 7 :- HPLC chromatogram of inhibition fraction (4) obtained from Sephadex G15

CONCLUSION

It is concluded that under the conditions of these experiments aflatoxin production was inhibited without affecting mold growth. The reduced pH was not the main reason for the inhibition. The inhibition was probably due to a low molecular weight metabolite(s) which may have interfered with the synthesis of aflatoxin. Chlorofrom methanol and ethyl acetate extraction of the L.lactis metabolites yielded extract that inhibited aflatoxin production. It appears from this study that the use of antifungal *L. lactis* have a great potential to be used in foods as a natural biological control agents to prevent aflatoxin production by molds.

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تثبيط إنتاج سموم ألأفلاتوكسين ونمو فطر *Aspergillus flavus* بواسطة بكتيريا *Lactococcus lactis*

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الملخص العربي

يهدف هذا البحث الي دراسة تأثير بكتيريا *Lactococcus lactis* على انتاج سموم ألأفلاتوكسين وعلى نمو فطر *Aspergillus flavus* في بيئات سائلة وكذلك التعرف على خواص المادة المضادة للفطريات و التي تم انتاجها بواسطة *L.lactis* في وجود الفطر. وجد أن نمو فطر *Aspergillus flavus* في وجود بكتيريا *Lactococcus lactis* في بيئة Lablmco Trypton Broth Medium أدى الى تثبيط انتاج سموم ألأفلاتوكسين بدون تأثيره على نمو الفطر. انتاج سموم ألأفلاتوكسين تم تثبيطها بنسبة ٨٩% ، ٨٥% ، ٨٠% عند استخدام بكتيريا *L. lactis* والتي تم تنميتها لمدة ١٦، ٢٤، ٤٨ ساعة على التوالي قبل تلقيحها بجراثيم الفطر. بكتيريا *L. lactis* التي تم تنميتها لمدة ١٦ ساعة والتي تم تلقيحها بجراثيم الفطر التي سبق تنميتها لمده ٢٤، ٤٨، ٧٢، ٩٦، ١٢٠، ١٤٤، ١٦٨ ساعة أدت الي خفض انتاج سموم ألأفلاتوكسين في البيئات التي تم تلقيحها بالفطر وتنميتها لمده ٢٤ ، ٤٨ ، ٧٢ ،٩٦ ساعة بنسبة ٨٠% ، ٧٧% ، ٦٧% ، ٦٢% على التوالي. عندما تم تلقيح البيئة ببكتيريا *L. lactis* وفطر *A. flavus* في نفس الوقت، أدى ذلك الي انخفاض في انتاج سموم ألأفلاتوكسين بنسبة ٧٧%. تبين من النتائج أن نواتج تمثيل *L. lactis* هي المسئولة عن تثبيط انتاج ألأفلاتوكسين كليا، بينما ألأنخفاض في pH البيئة كنتيجة لنمو *L. lactis* لم تسبب تثبيط لأنتاج ألأفلاتوكسين ، وبأجراء عملية فصل بألأنتشار الغشائي لنواتج عمليات التمثيل بواسطه بكتيريا *L. Lactis* والتي أدت الي تثبيط كلي لسموم ألأفلاتوكسين أمكن التعرف أن هذه المثبطات ذات وزن جزيئي

منخفض. المستخلص المتحصل عليه من عملية ألأستخلاص بواسطة الكلوروفورم و الميثانول و خلات ألايثايل أدى الي تثبيط انتاج سموم ألأفلاتوكسين.

L.lactis

L.lactis

L.lactis

L.lactis

Table (1): Mycelial dry weights and aflatoxin B_1 and G_1 production by A. flavus in mono and mixed culture

Culture	Mycelial Dry**** Weight (mg.ml^{-1})	Aflatoxin (ug. ml^{-1})****			Inhibition %	Final pH*
		B1	G1	Total		
Flavus (Control)	5.0	0.9	0.1	1.0	------	8.1
*L. lactis*** + *A. flavus*	4.8	0.11	0.06	0.17	82	7.9
*L. lactis**** + *A. flavus*	4.9	0.15	0.06	0.21	77	7.8
A. flavus (pH 4.5)	6.1	1.3	0.9	2.2	------	8.2

*Initial pH6.0
**Inoculated with *L.lactis*, incubated 16h and then inoculated with *A.flavus*
***Inoculated simultaneously with both organisms
****Values represent the mean of three replicates

Table (2): The pH values of mono and mixed cultures during the 7 days of incubation

Days of incubation	Culture	
	A. flavus	A. flavus + L. lactis
	pH	pH
1	4.5	4.5
2	4.6	4.6
3	5.4	5.0
4	7.2	7.0
5	7.5	7.5
6	7.7	7.7
7	8.0	7.85

Values represent the mean of three replicates

Table (3): Mycelial dry weights and aflatoxin B_1 and G_1 production in the concentrated bacterial metabolite (L.lactis)

Treatment ($mg.ml^{-1}$)	Mycelial Dry Weight ($mg.ml^{-1}$)	Aflatoxin (ug.ml-1)			Inhibition %	Final pH
		B1	G1	Total		
100	5.1	0.58	0.9	1.48	17	8.1
200	5.0	0.49	0.5	0.99	45	8.1
300	4.8	0.45	0.3	0.75	47	8.2
400	4.8	0.41	0.4	0.81	55	8.3
500	5.4	0.39	0.1	0.49	72	8.2
600	4.7	0.1	0.1	0.1	94	7.9
Control	4.9	1.0	0.8	1.8	-----	8.1

*Values represent the mean of three replicates

Table (4): Mycelial dry weights and aflatoxin B_1 and G_1 production in the concentrated bacterial metabolite after dialysis

Treatment ($mg.ml^{-1}$)	Mycelial Dry Weight ($mg.ml^{-1}$)	Aflatoxin (ug.ml-1)			Inhibition %	Final pH
		B1	G1	Total		
0.00	5.1	1.2	0.09	1.29	-----	8.1
25	4.7	0.1	0.03	0.13	90	7.9
50	4.5	0.0	0.0	0.0	100	7.8

Values represent the mean of three replicates

Table (5): Activity assay of collected fractions obtained after sephadex separation

Fractions	Sephadex of chloroform methanol extraction			Sephadex of methanol aceton extraction		
	Totalaflatoxin mg.ml-1 Test	Control	Inhibition %	Totalaflatoxin mg.ml-1 Test	Control	Inhibition %
1	0.74	0.8	26	1.0	0.9	0.0
2	0.91	0.9	9	0.95	0.8	6.0
3	0.50	0.9	50	0.52	0.9	49
4	0.03	0.8	97	-----	-----	-----
5	0.82	0.9	8	------	-----	-----
6	1.00	0.9	0.0	-----	-----	-----
Control	1.00	-----	-----	1.01	-----	-----

Values represent the mean of three replicates