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BIOACTIVITY OF *Brevibacterium linens* AGAINSET THE GROWTH AND AFLATOXINS PRODUCTION BY *Aspergillus flavus*

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ABSTRACT

The antimycotoxigenic of *Brevibacterium linens* (*B. linens*) against *Aspergillus flavus* (*A. flavus*) in solid and liquid media was studied. Aflatoxins B_1, B_2, G_1 and G_2 were detected in all tested media using TLC technique and quantitatively determined by HPLC. The obtained data revealed that *B. linens* reduced the growth of *A. flavus* when the two microorganisms were inoculated on nutrient agar medium at the same time (zero time) or when *B. linens* was grown for one day before *A. flavus* growth. While, complete inhibition of *A. flavus* growth was occurred in 2 and 3 days old culture of *B. linens*. Also, complete inhibition of aflatoxins production was occurred in *B. linens* culture at Zerotime and up to 3 days old.

Agitation during *B. linens* growth on nutrient broth medium is an important factor to prevent the fungal growth and imeresed production of the antifungal compound(s). The use of 100% of *B. linens* culture of 3 days old as a growth medium for *A. flavus* inhibited the fungal growth and sporulation.

The results indecated that *B. linens* has potential effect may be use as biological control against mold growth and mycotoxins production in foods and dairy products.

Keywords: *Brevibacterium linens, Aspergillus flavus*, Aflatoxins, Antimycotoxigenic, Antifungal, Bioactivity.

INTRODUCTION

Molds can grow in fermented products, cause spoilage and be extremely hazardous to human health through the production of mycotoxins. Scott (1989) has reviewed mycotoxigenic fungal contaminants of dairy products and their stability and penetration in cheese. Aflatoxins are secondary metabolites produced by *A. flavus* and several factors affect their biosynthesis such as biological, chemical and environmental (Ellis *et al.,* 1991). Aflatoxins have been demonstrated to be carcinogenic, teratogenic, mutagenic, hepatocellular carcinoma, acute hepatitis, Reye's syndrome, cirrhosis in malnourished children and kwashiorkor (Ellis *et al.,* 1991), primary liver cancer (IARC monographs, 1987) and other human cancer (Harison *et al.* 1993).

Microbial inactivation and fermentation have been studied for degradation and removal of aflatoxin (Hao *et al.* 1987). *Flavobacterium aurantiacum* and selected acid-producing moulds have successfully removed aflatoxin from liquid media. It was postulated that the reduction of aflatoxin levels was a result of acid production and subsequent transformation of aflatoxin B_1 to aflatoxin B_{2a} (Park, 1993).

Recently *B. linens* culture has been used as adjunct culture to accelerate ripening and flavor development of Cheddar and Ras cheese (Pattray& Fox, 1999 and Osman, 2002) and as inhibitor culture against the growth and sporulation of many different fungal species (Osman, 1999). The

current study aims to investigate the antimycotoxigenic of *B. linens* against *A. flavus*.

MATERIALS AND METHODS

Microorganisms

B. linens was isolated from Domiati cheese as described by (Osman, 1994). *A. flavus* was obtained from Mycotoxin Central Laboratory, National Research Center, Dokki, Cairo, Egypt.

B. linens and *A. flavus* were maintained on nutrient agar slants (LAB M) and sabouraud dextrose agar slants media, respectively and were monthly subcultured.

Preparation of *A. flavus* spore suspension:

A. flavus spore suspension (40 x 10^6 cfu ml^{-1}) was prepared as described by Osman (1999).

***B. linens* growth measurement:**

The growth of *B. linens* was measured by absorbance at 620 nm (Spectronic 20D, Milton Roy Company, USA) and the colonies were counted on nutrient agar medium after incubation at 30°C for 4 days.

Antifungal activity of *B. linens* in solid medium:

The antifungal activity of *B. linens* on nutrient agar medium was examined according to Osman (1999). *B. linens* was subcultured twice in 100 ml nutrient broth (LAB M), incubated at 27°C with shaking (110 rpm) up to its late of logarithmic phase or stationary phase (48 hrs) and seeded in nutrient agar medium. Nutrient agar seeded medium (10^7-10^8 cfu ml^{-1}) was poured into sterile Petri dishes, allowed to solidify and incubated at 27°C.

A. flavus was grown on sabouraud dextrose agar at 27°C for 48 hrs. Blocks of agar were cut by sterile cork borer (5 mm) and placed centrally across the surface of seeded nutrient agar medium with *B. linens* at zero time, 1, 2 and 3 days old of *B. linens* culture and incubated up to 16 days at 27°C. The inoculated plates were examined daily for growth initiation, growth zone diameter (mm) and spore initiation. At the end of the experiment, the plates were autoclaved at 121°C/15 min to help the release of aflatoxin from mycelia (Tasi *et al.*, 1984) and kept at -18°C until aflatoxins determination.

Antifungal activity of whole *B. linens* culture in liquid medium:

B. linens was subcultured twice in 100 ml nutrient broth and incubated at 27°C in water bath with shaking (110 rpm) for 24 hrs. Nutrient broth (100 ml) was inoculated with 1% *B. linens* (second subculture, 25 x 10^8 cfu ml^{-1}) then incubated at 27°C with or without shaking (110 rpm) and inoculated with an agar block of *A. flavus* at zero time, 1, 2, 3 and 4 days old. 2 ml of antibiotic solution (500 mg each of chlortetracycline-HCl and chloramphenicol in diluted buffer phosphate; APHA, 1993)/ 100 ml culture was added to prevent the bacterial growth. The inoculated flasks were daily examined for fungal growth and spore initiation. Mycelium dry weight and pH values were determined at the end of the experiment, 16 days (Jenway, 3205), as well as, flasks showing fungal growth were autoclaved and kept at –18°C until aflatoxin determination.

In another experiment, flasks of nutrient broth (100 ml) were inoculated with an agar block of *A. flavus* and incubated at 27°C at zero time, 2 days (fungal growth initiation), 4 days (spore initiation) and 10 days then inoculated with (1%) *B. linens* (25

x 10^8 cfu ml^{-1}) and incubated at 27°C up to 16 days. At the end of the experiment (16 days) the pH values and aflatoxins were determined.

Antifungal activity of different ratios of *B. linens*:

100 ml of nutrient broth medium was inoculated with 1% *B. linens* and incubated at 27°C with shaking at 110 rpm. 25, 50 and 100% of *B. linens* culture of 1, 2, 3, 4 and 5 days old were mixed with nutrient broth medium with addition of 2 ml of antibiotic solution, inoculated with an agar block of *A. flavus* and incubated at 27°C up to 18 days. The inoculated flasks were daily examined for growth and spore initiation and at the end of the experiment for mycellium dry weights and pH values.

Mycelial dry weight:

The mycelial mats were collected by filtration through Whatman No. 1 filter paper, washed twice with distilled water, dried in an oven at 95°C until constant in weight and weighted.

Determination of pH value:

The pH value of the liquid cultures was measured by using a pH meter (PTI-15, Aqua Chemical Co., England).

Extraction and determination of aflatoxins:

Twenty-five ml of the tested media (3 replicates) were taken for aflatoxins extraction according to BF method (AOAC, 1995). The final extract of each samples was dissolved in 200 µl benzene- acetonitrile (98 + 2, v/v) for spotting on aluminum sheet TLC plates using toluene + ethylacetate + formic acid (6 + 3+ 1, **v/v/v**) as developing system followed by examination at 365nm UV lamp for aflatoxin detection. The residues of the positive samples were dried and derivatized by trifluoroacetic acid (AOAC, 1995), then 25 µl for each sample was injected into HPLC system under the following conditions:

HPLC system (Agilent) equipped with Quaternary pump model G1311A, Fluorescence detector model G1321A set at 360 nm and 440 nm excitation and emission wavelengths, autosampler model G 1329A and ODS (Zorbax) column (150 x 4.6 mm) was used for aflatoxins separation. The mobile phase methanol + water + acetonitrile (30 + 60 + 10, v/v/v) was isocratically used at flow rate 1ml/min. The obtained data were integrated and calculated by Chemstation software program.

RESULTS AND DISCUSSION

Suppression of *A. flavus* growth and aflatoxins *B. linens*:

Fig. (1) showed that, *B. linens* culture of zero time and 1 day old reduced the growth of *A. flavus* compared with control, while, *B. linens* cultures of 2 and 3 days old completely inhibited fungal growth.

A. flavus growth initiation was occurred in control and zero time old *B. linens* culture. while, spore initiation occurred after 3 days for control and *B. linens* culture of zero time old. *A. flavus* spore initiation was completely inhibited in *B. linens* cultures of 1, 2 and 3 days old (Table 1). In this respect Osman (1999) reported that growth and/or sporulation of some isolates of *Alternaria* (*Alt.*) sp. 1, 18; *Alt. citri*; *A.* sp.; *A. flavus* 3a, 3b, 13; *A. niger* 4a,ab; *Cladosporium* sp.; *Mucor* sp. 7; *Penicillium* (*Pen.*) *digitatum*; *Pen. italicum* 8; *Rhizopus stolonifer*; *Trichoderma* sp. and *Fusarium* sp. 14 were inhibited by antifungal compound (s) produced by *B. linens*.

Aflatoxins production was completely inhibited (Table 1) when *A. flavus* was inoculated into *B. linens* culture of 0-3 days old, however, the *A.flavus* control produced 17.8, 8.3, 7.0 and 1.4 of aflatoxins B_1, B_2, G_1 and G_2, respectively. These results may be due to 1) *B. linens* could produce certain metabolites during growth phase that could prevent the mold growth and interfere with aflatoxin production, 2) bacterial acid production and subsequent transformation of aflatoxin B_1 to aflatoxin B_{2a} (Park, 1993), 3) this bacterium was able to degrade aflatoxins (Wiseman and Marth, 1981) or 4) adsorption of aflatoxins into the bacterial cell wall as a mechanism of this degradation (Doyle and Marth, 1978 and Masimango *et al.*, 1978).

Table (1): Growth initiation, spore initiation and aflatoxins production by *A. flavus* on nutrient agar medium seeded with *B. linens* at 27°C (average of 3 replicates).

Microorganisms and age (days)	Asp. flavus *(days)*		Aflatoxins production ng ml^{-1}				
	Growth onset	Spore onset	B_1	B_2	G_1	G_2	Total
A. flavus (control, without *B. linens*)	1	3	17.8 ± 1.1	8.3 ± 0.3	7.0 ± 0.2	1.4 ± 0.2	34.5 ± 1.8
A. flavus + *B. linens*(0.0 day)	1	3	ND[1]	ND	ND	ND	ND
A. flavus + *B. linens* (1 day)	2	-	ND	ND	ND	ND	ND
A. flavus + *B. linens* (2 days)	-	-	ND	ND	ND	ND	ND
A. flavus + *B. linens* (3 days)	-	-	ND	ND	ND	ND	ND

1. ND: not detected.

These results indicated that *B. linens* reduced the growth of *A. flavus* when the two microorganisms were inoculated on nutrient agar medium at the same time (zero time) or when *B. linens* was grown for one day before *A. flavus* growth. While, complete inhibition of both fungal growth and its aflatoxin production were occurred when *A. flavus* was inoculated into 2 and 3 days old of *B. linens* culture.

Suppression of *A. flavus* growth by *B. linens* under shaking conditions:

The initial count of *B. linens* was 54×10^5 cfu ml^{-1}, increased to 35×10^8 cfu ml^{-1} after 2 days and decreased to 58×10^7 cfu ml^{-1} after 4 days (Table 2).

A. flavus growth and sporulation were completely inhibited when inoculated into *B. linens* culture of zero - 4 days old at 27°C with shaking (110 rpm).

Mycelial dry weight of *A. flavus* and pH value of culture after 16 days were 2.91 mg ml^{-1} and 9.22, respectively (Table 2). Thus, growth of *A. flavus* was completely inhibited when inoculated into *B. linens* broth under shaking condition.

***A. flavus* growth in the presence of *B. linens* under static conditions:**

An agar block of *A. flavus* was inoculated into *B. linens* culture of zero, 1, 2 and 3 days old and incubated at 27°C. Growth of *A. flavus* and spore initiation was presented of occurred after 2 and 5 days incubation, respectively (Table 3). The pH values and mycelial dry weight of *A. flavus* inoculated into 0.0, 1, 2 and 3 days old cultures of *B. linens* were 8.63, 8.84, 8.84, 8.85 and 2.51, 2.73, 3.17 and 3.05 mg ml^{-1} respectively, however, aflatoxins production decreased when bacterial cultural age increased. The count of *B. linens* under agitation conditions was higher than that under static

conditions (e.g., 10^8 and 10^7 cfu ml^{-1} after 3 days, Tables 2-3), however mycelial dry weight under static conditions was higher than that under agitation conditions (e.g., 3.70 and 2.91 mg ml^{-1}). The most important point that aflatoxins production is completely inhibited under agitation conditions (Table 2) while it's still formed under static conditions. Thus, again, agitation during *B. linens* growth is an important factor to prevent the fungal growth and antifungal compound (s) production.

Table (2): Growth of *A. flavus* in the presence of *B. linens* under shaking conditions (average of 3 replicates).

Microorganisms and age (days)	Count of *B. linens* (cfu ml^{-1})	pH value	*A. flavus*			
			Growth onset (days)	Spore onset (days)	Mycelia dry weight (mg ml^{-1})	Aflatoxins[1] (ng ml^{-1})
A. flavus (control, without *B. linens*)	-	9.22± 0.12	1	-	2.91 ± 0.16	ND[2]
A. flavus + *B. linens* (0.0 day)	54 ± 5 x 10^5	7.00 ± 0.10	-	-	-	ND
A. flavus + *B. linens* (1 day)	25 ± 8 x 10^8	8.72 ± 0.20	-	-	-	ND
A. flavus + *B. linens* (2 days)	35 ± 9 x 10^8	8.95 ± 0.15	-	-	-	ND
A. flavus + *B. linens* (3 days)	22 ± 6 x 10^8	9.12 ± 0.20	-	-	-	ND
A. flavus + *B. linens* (4 days)	58 ± 5 x 10^7	9.12 ± 0.10	-	-	-	ND

1. B_1, B_2, G_1, G_2 and total. 2.ND: not detected.

***B.linens* growth in the presence of *A. flavus* under static conditions:**

Growth and spore initiation of control treatment occurred after 2 and 4-5 days, respectively (Table 4). The pH values of 0.0, 1, 2 and 3 days old of *A. flavus* culture were 8.63, 8.58, 8.61 and 8.73 respectively, while, mycelial dry weight was not affected by the addition of *B. linens* (e.g., 3.70, 2.51, 3.55, 3.78 and 3.71 mg ml^{-1}, respectively).

Aflatoxins (B_1, B_2, G_1, G_2 and total) of *A. flavus* culture (control treatment) were (38.4, 62.4, 27.4, 17.2 and 145.0 ng ml^{-1}, respectively) while, culturing two microorganisms together at zero time decreased aflatoxins production to 13.8, 9.0, 26.2, 5.1 and 54.1 ng ml^{-1}, respectively.

Also, aflatoxins of 2 days old *A. flavus* culture were (6.8, 1.0, 15.4, 5.8 and 29.0 ng ml^{-1}, respectively) while, toxins were not detected in 5 and 10 days old *A. flavus* culture.Wiseman and Marth (1981) found that addition of *Lactococcus lactis* ssp. *lactis* to 3 day old *A. parasiticus* culture stimulated mold growth and this effect could be due to the removal of acids and/or to the production of stimulatory metabolites by the bacteria (Barr, 1976).

Thus, when *B. linens* inoculated into *A. flavus* culture of 0.0 or 2 days old under static conditions, the fungal growth, sporulation and aflatoxins production were occurred. While, aflatoxins disappeared when *B. linens* inoculated into *A. flavus* cultures of 4 and 10 days old may be because of the ability of *B. linens* to decompose aflatoxins.

Effect of using different ratios of whole *B. linens* culture on the growth of *A. flavus*:

Table (5) showed that the use of 25 and 50% of *B. linens* culture at different cultural ages up to 5 days old had no effect on the growth and spore initiation and mycelial dry weight of *A. flavus*. While the use of 100% of *B. linens* culture as a growth medium for *A. flavus* inhibited fungal growth and sporulation up to 25 days of incubation at 27°C. These results indicated that *B. linens* culture had antifungal properties starting from first day old.

Table (3): Growth of *A. flavus* in the presence of *B. linens* under static conditions (average of 3 replicates).

Microorganisms and age (days)	Count of *B. linens* (cfu ml^{-1})	A. flavus (days)		pH value	Mycelia dry weight (mg ml^{-1})	Aflatoxins (ng ml^{-1})				
		Growth onset	Spore onset			B_1	B_2	G_1	G_2	Total
A. flavus (control, without *B. linens*)	-	2	5	8.71 ± 0.20	3.70 ± 0.02	38.4 ± 1.5	62.0 ± 2.4	27.4 ± 1.8	17.2 ± 0.8	145.0 ± 6.5
A. flavus + *B. linens* (0.0 day)	54 ± 7 x 10^5	2	5	8.63 ± 0.10	2.51 ± 0.06	13.8 ± 0.7	90.0 ± 4.0	26.2 ± 1.4	5.1 ± 0.2	135.1 ± 6.3
A. flavus + *B. linens* (1 day)	71 ± 5 x 10^6	2	5	8.84 ± 0.30	2.73 ± 0.28	33.3 ± 2.3	79.0 ± 2.0	10.7 ± 0.7	11.3 ± 0.3	134.3 ± 5.3
A. flavus + *B. linens* (2 days)	25 ± 7 x 10^7	2	5	8.84 ± 0.40	3.17 ± 0.45	11.0 ± 0.2	22.9 ± 0.1	19.1 ± 5.2	6.9 ± 0.1	59.9 ± 5.6
A. flavus + *B. linens* (3 days)	30 ± 4 x 10^7	2	5	8.85 ± 0.35	3.05 ± 0.04	34.5 ± 1.1	1.0 ± 0.5	12.4 ± 0.5	13.4 ± 0.2	61.3 ± 2.3

Table (4): Growth of *B. linens*[1] in the presence of *A. flavus* under static conditions (average of 3 replicates)

Microorganisms and age (days)	A. flavus(days)		A. flavus		Aflatoxins(ng ml^{-1})				
	Growth onset	Spore onset	pH value	Mycelia dry weight (mg ml^{-1})	B_1	B_2	G_1	G_2	Total
A. flavus (control, without *B. linens*)	2	4	8.71± 0.30	3.70± 0.02	38.4± 1.5	62.0± 2.4	27.4± 1.8	17.2± 0.8	145.0 ± 6.5
B. linens + *A. flavus* (zero time)	2	5	8.63 ± 0.10	2.51 ± 0.02	13.8 ± 0.3	9.0 ± 0.3	26.2± 0.3	5.1 ± 0.2	54.1 ± 1.2
B. linens + *A. flavus* (2 days)	2	5	8.58 ± 0.10	3.55 ± 0.09	6.8 ± 1.3	1.0 ± 0.1	15.4 ± 0.2	5.8 ± 0.5	29.0± 2.1
B. linens + *A. flavus* (4 days)	2	5	8.61 ± 0.30	3.78 ± 0.08	ND[2]	ND	ND	ND	ND
B. linens + *A. flavus* (10 days)	2	5	8.73± 0.20	3.71 ± 0.07	ND	ND	ND	ND	ND

1. Initial count 54 x 10^5 cfu ml^{-1}. 2. Not detected.

To elemente the lack of nutritive elements in growth medium Sterile concentrate solution of nutrient broth medium (to had the same formula of nutrient broth medium) was added to *B. linens* culture of 1, 2, 3, 4 and 5 days old, then inoculated with *A. flavus* (Table 6). The results indicated that fungal growth and spore initiation occurred only at 1 and 2 days old *B. linens* culture (e.g., growth initiated after 3 and 4 days and spore initiated after 10 and 11 days, respectively). While, mycelial dry weight of 1 and 2 days old culture supplemented with concentrate solution of nutrient broth medium was 3.95 and 4.00 mg ml^{-1}, respectively.

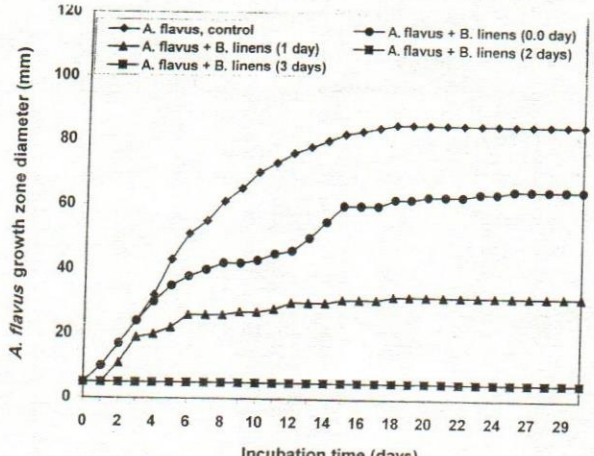

Fig. (1): Growth of *A. flavus* in the presence of *B. linens*

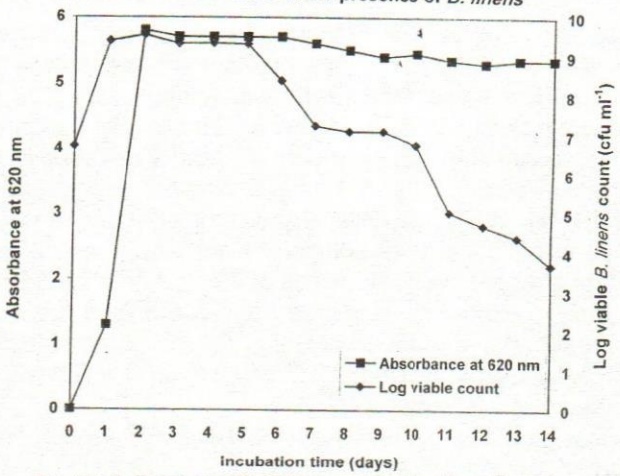

Fig. (2): *B. linens* growth curve on nutrient broth medium at 27°C/110 rpm

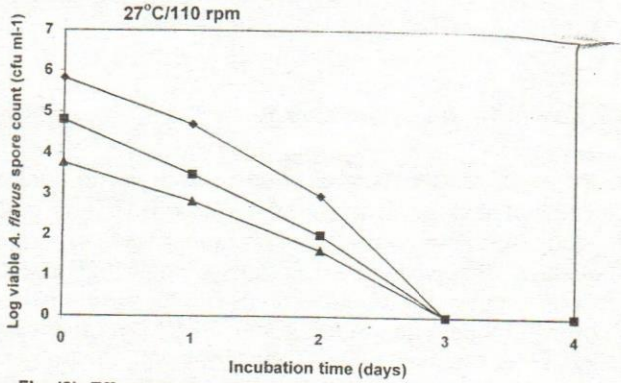

Fig. (3): Effect of initial *A. flavus* spore count on *B. linens* antifungal activity

Table (5): Effect of using different ratios of *B. linens* culture on the growth and sporulation of *A. flavus* (average of 3 replicates).

Microorganisms, ratios and age (days)	pH value	*A. flavus* (days)		Mycelia dry weight (mg ml^{-1})
		Growth onset	Spore onset	
flavus (control, without *B. linens*)	8.51 ± 0.18	2	5	3.82 ± 0.02
A. flavus on:				
N. B[1] + 25% *B. linens* (1 day)	8.25 ± 0.12	2	5	2.60 ± 0.08
N. B + 25% *B. linens* (2 days)	8.27 ± 0.16	2	6	2.59 ± 0.20
N. B + 25% *B. linens* (3 days)	8.25 ± 0.11	2	6	2.58 ± 0.30
N. B + 25% *B. linens* (4 days)	8.30 ± 0.20	2	6	2.58 ± 0.07
N. B + 25% *B. linens* (5 days)	8.29 ± 0.10	2	6	2.49 ± 0.09
A. flavus on:				
N. B + 50% *B. linens* (1 day)	8.50 ± 0.30	3	5	2.60 ± 0.05
N. B + 50% *B. linens* (2 days)	8.55 ± 0.40	3	6	2.59 ± 0.10
N. B + 50% *B. linens* (3 days)	8.50 ± 0.30	3	6	2.57 ± 0.20
N. B + 50% *B. linens* (4 days)	8.45 ± 0.21	3	5	2.55 ± 0.07
N. B + 50% *B. linens* (5 days)	8.50 ± 0.10	3	6	2.40 ± 0.09
A. flavus on:				
100% *B. linens* (1 day)	8.72 ± 0.30	-[2]	-	-
100% *B. linens* (2 days)	8.95 ± 0.40	-	-	-
100% *B. linens* (3 days)	9.12 ± 0.20	-	-	-
100% *B. linens* (4 days)	9.05 ± 0.23	-	-	-
100% *B. linens* (5 days)	9.14 ± 0.12	-	-	-

1. N. B: Nutrient broth medium. 2. Negative results up to 25 days

To elemente the lack of nutritive elements in growth medium Sterile concentrate solution of nutrient broth medium (to had the same formula of nutrient broth medium) was added to *B. linens* culture of 1, 2, 3, 4 and 5 days old, then inoculated with *A. flavus* (Table 6). The results indicated that fungal growth and spore initiation occurred only at 1 and 2 days old *B. linens* culture (e.g., growth initiated after 3 and 4 days and spore initiated after 10 and 11 days, respectively). While, mycelial dry weight of 1 and 2 days old culture supplemented with concentrate solution of nutrient broth medium was 3.95 and 4.00 mg ml^{-1}, respectively. However, antifungal activity of *B. linens* culture of 3, 4 and 5 days old is still appeared up to the end of the experiment (25 days). Thus, antifungal compounds were produced at the stationary phase of *B. linens* culture of 3 days old (Fig. 2, 22 x 10^8 cfu ml^{-1}) which was incubated at 27°C with shaking at 110 rpm.

No fungal growth was observed in *B. linens* culture of 3 days old when inoculated with 10^4, 10^5 and 10^6 spore ml^{-1} (Fig. 3). Also, no fungal growth was noticed when 10 ml of the above culture was inoculated into 100 ml nutrient broth medium and incubated for 1 month at 27°C. These results mean that *B. linens* culture appeared fungicidal effect.

Effect of *A. flavus* initial spore count on the antifungal activity of *B. linens*:

The antibacterial effects of *B. linens* and their metabolites have been extensively investigated (Maisnier-Patin and Richard, 1995 and Siswanto *et al.*, 1996). In this study, it can be excluded that organic acids or hydrogen peroxide causes the inhibition observed where *B. linens* does not produce acids from carbohydrates when growing in peptone medium (Jones and Keddie, 1986) and the pH in the growth medium is ranged from 8.72 to 9.18 as well as *B. linens* is catalase positive. More research on the factors affecting on the antifungal activity of *B. linens* is needed. It would appear that *B. linens* have potential to be use as biological control agent in foods and dairy products to prevent mold growth and mycotoxins production.

Table (6) :Effect of using 100% of *B. linens* culture and concentrated nutrient broth (CNB) on the growth and sporulation of *A. flavus* (average of 3 replicates).

Microorganisms and age (days)	pH value	*A. flavus* (days)		Mycelia dry weight (mg ml^{-1})
		Growth onset	Spore onset	
flavus (control, without *B. linens*)	8.55 ± 0.22	2	5	3.91 ± 0.09
flavus + :				
B. linens (1 day)	8.60 ± 0.13	-[1]	-	-
B. linens (1 day) + CNB	8.68 ± 0.14	3	10	3.95 ± 0.08
A. flavus + :				
B. linens (2 days)	8.87 ± 0.16	-	-	-
B. linens (2 days) + CNB	8.95 ± 0.24	4	11	4.00 ± 0.09
A. flavus + :				
B. linens (3 days)	9.04 ± 0.23	-	-	-
B. linens (3 days) + CNB	8.84 ± 0.14	-	-	-
A. flavus + :				
B. linens (4 days)	9.15 ± 0.14	-	-	-
B. linens (4 days)+ CNB	9.10 ± 0.17	-	-	-
A. flavus + :				
B. linens (5 days)	9.18 ± 0.24	-	-	-
B. linens (5 days) + CNB	9.10 ± 0.15	-	-	-

1. Negative results up to 25 days.

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النشاط الحيوى لبكتريا *Brevibacterium linens*

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فى هذا البحث تم دراسة التأثير المضاد لبكتريا *B. linens* على نمو فطر *A. flavus* وإنتاجه للسموم الفطرية أثناء نموه على بيئات صلبة وسائلة وقدرت الافلاتوكسينات B_1, B_2, G_1, G_2 فى البيئات المختبرة باستخدام تكنيك TLC وكميا بواسطة HPLC .

ثبطت بكتريا *B. linens* نمو فطر *A. flavus* عند تلقيح كلا الميكروبين فى نفس الوقت على بيئة الاجار المغذى أو عند نمو بكتريا *B. linens* لمدة يوم قبل نمو فطر *A. flavus* . كما لوحظ تثبيط كامل لنمو الفطر عندما لقح فى مزرعة *B. linens* عمرها يومين وثلاثة أيام. بينما حدث منع كامل لإنتاج سموم الافلاتوكسينات عندما لقح فطر *A. flavus* فى نفس الوقت مع البكتريا أو عند نمو بكتريا *B. linens* لمدة من يوم إلى ثلاثة أيام قبل نمو فطر *A. flavus* .

كما أوضحت النتائج أن الرج أثناء نمو *B. linens* فى بيئة المرق المغذى عامل مهم لمنع نمو الفطر وزدادت من المركبات المضادة للفطريات وأن استخدام مزرعة *B. linens* عمر ثلاثة أيام كبيئة لنمو فطر *A. flavus* منعت نمو الفطر وتجرثمه وأظهرت المزرعة البكتيرية التى عمرها ثلاثة أيام تأثير قاتل لجراثيم الفطر.

أشارت النتائج لامكانية استخدام B. linens كعامل تحكم بيولوجي لمنع النمو الفطري وانتاج سموم الافلاتوكسينات في الأغذية والمنتجات اللبنية.