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Biological control of some types of mycotoxins by probiotic bacteria and yeast strains

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Abstract

Fungal and mycotoxin contamination of raw milk and dairy products are constitute a potential hazard to human health and food safety. Therefore, the study was designed to assess the potential use of antifungal compounds produced by probiotic bacteria and yeast strains for growth inhibition and subsequent aflatoxin (AFS) and ochratoxin (OTA) production from select strains of Aspergillus flavus (OK605586) and Penicillium nordicum (OK605551), respectively. The AFS and OTA residues were determined by Lateral Flow Immuno Assay (LFIA) after 14 days of incubation for strains, at 25 °C in YES medium, as a model for mycotoxins determination. According to the current investigation results, probiotic bacteria and yeast strains had a good ability to inhibit the growth of toxigenic fungi strains. There were noticeable decreases in mycotoxins production; Lactobacillus Plantarum exhibited the highest reduction figures of AFS and OTA production by A. flavus and P. nordicum, 64.51% and 87.21%, respectively.

Moreover, the combination of Lactobacillus acidophilus and Lactobacillus Plantarum reduced AFS and OTS concentrations from 35 ng/ml and 7.82 ng/ml to zero ng/ml, for both toxins after 14 days of incubation, with the highest reduction value, actually 100%. However, the same result trend was also observed in the case of probiotic yeasts, where Saccharomyces. Cerevisiae showed the best ability to reduce AFS and OTA concentrations by 83.15% and 75.6%, respectively, compared with Torulaspora delbrueckii strain. Additionally, the combination of S. cerevisiae and T. delbrueckii demonstrated the greatest reduction figure in AFS and OTA concentration, actually 100%.

Key words: *Aspergillus flavus, Penicillium nordicum, L. plantarum, L. acidophilus, S.cerevisiae* and *T. delbrueckii* .

INTRODUCTION

Mycotoxins produced by Aspergillus, Penicillium, Fusarium spp. are natural contaminants in food. However, mycotoxins are well known to cause toxicities to human and animals. These toxins possess detrimental effects including toxigenic, carcinogenic, mutagenic, teratogenic and immunosuppressive.

These genotoxic compounds target many organs like kidneys, liver and immune systems. Several types of mycotoxins attracted attention of many mycologists such as aflatoxins (AFs), Ochratoxin (OTs), trichothecenes and fumonisins where AFs and OTs are the most important (Reddy et al.,)[1]. On the other hand, Aspergilli and Penicillia among other genera of fungi such as fusaria, rhizopia and trichoderma are considered important producers of mycotoxins which are toxic secondary metabolites causing health hazards to animals and human beings (Reddy et al.)[1]

Therefore, several strategies have been applied to prevent mycotoxins production or to destroy, to inactivate, or to decrease their bioavailability in contaminated foods. Physical, chemical or biological methods are used to prevent or inactivate mycotoxins production. Thus, the scientific society tends to use biological approaches instead of chemical and physical methods, due to some disadvantages such as the loss of nutritional quality and unhealthy effects on humans (Marrez et al.)[2].

One of the most useful biological methods to reduce aflatoxins is the application of probiotic yeasts and bacteria in the diet.Therefore, this work aimed to evaluate the ability of probiotics to inhibit mycotoxigenic fungi growth and eliminated their mycotoxins.

MATERIALS AND METHODS

Microorganisms and culture conditions: A. Lactobacillus acidophilus (ATCC LA5®):

LA-5® comes from the collection of dairy cultures at Chr. Hansen. It is safe for human consumption; it has been granted QPS (Qualified Presumption of Safety) status from EFSA in Europe.

B. Lactobacillus plantarum (ATCC 14917):

Culture was stored at -20 ^{0}C; the bacteria were frozen in deep freezer. Cultures of LAB from individual vials were activated in deMan Rogosa Sharpe (MRS) broth for 20 h at 37^{0}C in a 5% CO2–95% ambient air atmosphere before use. **C. Yeast strains:**

Saccharomyces cerevisiae AZ-EG. BOUZA 37 accession number (MF597761) and Torulaspora delbrueckii. AZ EGY lychee (57) accession number (MF496102) were obtained from Dairy Department, Faculty of Agriculture, Al–Azhar University, Cairo, Egypt. Kit for lateral flow immuno assay (LFIA):

The quantitative analysis of aflatoxin and ochratoxin were determined by using a rapid detection method (strip kits) of Lateral Flow Immuno Assay (LFLA) as mentioned by (Mwanza, et al.)[3] with some modifications. The Kits were purchased from Sigma Company, Egypt. The results were determined operated with the Lateral Logic software.

Preparation of bacterial suspension:

Preparation of bacterial suspension was carried out according to (Zolfaghari, et a)l[4]. All bacteria were activated in De Man, Rogosa and Sharpe (MRS) broth and incubated at 37 °C for 24 h. Then, cell culture was centrifuged for 10 minutes at 3000 rpm and the supernatant was separated under sterile conditions. All cells were washed twice with phosphatebuffered saline (PBS). Finally, by using PBS at pH 7.2, its opacity was obtained by spectrophotometer over a 600 nm wavelength and absorption of about 1 equivalent to 1 × 10^{10} CFU/ml of the cell count of bacteria (Khanafari et al)[5].

Preparation of yeast suspension:

All probiotic yeasts were activated in the yeast mold broth (YMB) culture at 25 °C for 72 h. The cell culture was centrifuged for 10 minutes at 3000 rpm and the supernatant was removed under sterile conditions. Yeast cells were washed twice with PBS. Finally, by using PBS (pH 7.2), its opacity was obtained by spectrophotometer over a 600 nm wavelength and absorption of about 1.170 equivalent to 2 × 10^8 cells/ml of the cell count of yeast. But to preparation heat-treatment yeast, it is required to boile in 3 ml of PBS for 15 min (Ghofrani Tabari, et al) [6].

Probiotics Inhibition on the growth of (A. flavus and P. nordicum)and production mycotoxins(AFS and OTS).

Fifteen ml of YES medium, in a 250 ml-flasks, was inoculated by adding 1 ml of a suspension of spores (10^5 spores) of an toxigenic A. flavus and P. nordicum strains with or without 1ml of one of the inhibitory lactic acid bacteria strains (Lactobacillus. plantarum, Lactobacillus acidophilus) suspension (10^{10} cell), or Saccharomyces cerevisiae and Torulaspora delbrueckii (10^8

cell), with three replicates for each treatment. Individually, the flasks were incubated for 14 days at 25 °C. After the incubation period, the growth of the mycotoxingenic fungi A.flavus and P. nordicum in all flasks were visually examined.The extraction of myctoxins produced in the YES culture were determined according to the method Lateral flow Immuno Assay (LFIA) according (Mwanza, et al)[3]. The percentage of inhibition (fungal growth and mycotoxins (AFs and OTs) were calculated by using the following equation (treatment / control x100).

RESULTS AND DISCUSSION

The antimicrobial metabolites with antifungal effects from LAB additives for prevention of food spoilage by pathogenic microorganisms, use of these substances achieves an increasing demand for the production of safety foods, without any chemical additives.

So this study was designed to investigate the efficiency of using the Lactobacillus acidophillus and Lactobacillus plantarum isolated from dairy products on growth inhibition of Aspergillus flavus and Penicilium nordicum and consequently elimination of their aflatoxin production. Moreover, the study was also extended to evaluate their effect on aflatoxin biosynthetic pathways.

According to the findings in Tables (1and 2) and Figs (1 and 2), Aspergillus flavus and Penicillium nordicum strains produced aflatoxin and ochratoxin at concentrations of (35 and 7.82 ng/ml), respectively. However, when these strains were grown with Lactobacillus acidophilus or Lactobacillus plantarum, the Aflatoxin and ochratoxin concentrations decreased to (24.85 and 12.42 ng/ml) and (3.62 and1 ng/ml), respectively, with reduction rates of (29 and 64.51%) and (53.70 and 87.21%) for the two toxins, respectively. It was evident from the results optioned that Lactobacillus plantarum recorded a significantly lower of aflatoxin and ochratoxin production compared to Lactobacillus acidophilus, these results are agreement with those reported (Mohammadi et al and Møller et al)[7,8].

Also appeared from these results that when combination between Lactobacillus acidophilus and Lactobacillus plantarum with Aspergillus flavus or Penicillium nordicum, it was not able to produce toxin, as they recorded a decrease rate of up to 100% for aflatoxin and ochrotoxin.

In this respect, Sangmanee and Hongpattarakere[9] stated that gene Omethyl transferase A (Omt-A) is one of the main 25 genes involved in the aflatoxin biosynthetic pathway in Aspergillus sp. This gene is capable of converting sterigmatocystin (ST) to Omethylsterigmatocystin(OMST) and Dihydrosterigmatocystin (DHST) to dihydroO-methylsterigmatocystin (DHOMST), while the absence of the Omt. A gene is the reason that Aspergillus sp. produces (ST) as the end product instead of aflatoxins (Price et al.)[10].

In addition, (Gomaa et al.)[11] stated that there was a vigorous reduction at transcriptional level of Omt- A gene observed in A. flavus that was treated by Lactobacillus spp. Ranged from 70 to 80%.

Moreover, the same authors reported that the concentrations of amino acids (Asparagine, glutamine, glycine, alanine, and leucine) of the antifungal compounds produced by Lactobacillus spp. exhibited great efficiency in controlling mycotoxigenic fungi growth.

As well as (Sangmanee and Hongpattarakere)[12]. Revealed that the mechanism of antifungal action of L. plantarum K35 supernatant causes damage to the cytoplasmic membrane and cell wall and consequent leakage of cytoplasmic

content, the formation of membrane-bound mitochondria and nuclei. vesicles followed by the destruction of

Table (1): Effect of probiotic bacteria on aflatoxin producing by Aspergillus flavus in vitro and quantification by (LFIA).

Treatments		Total of aflatoxin (AF/µg/ml)	% Reduction
Control	Aspergillus flavus	35	0
T1	Aspergillus flavus + Lactobacillus acidophilus	24.85	29
T2	Aspergillus flavus + Lactobacillus plantarum	12.42	64.51
T3	Aspergillus flavus + Lactobacillus acidophilus + Lactobacillus plantarum	0	100

Table (2) Effect of probiotic bacteria on ochratoxin producing by Penicillium nordicum in vitro and quantification by (LFIA).

Treatments		Total of ochratoxin (ng/ml)	% Reduction
Control	Penicillium nordicum	7.82	0
T1	Penicillium nordicum + Lactobacillus acidophilus	3.62	53.70
T2	Penicillium nordicum + Lactobacillus plantarum	1	87.21
T3	Penicillium nordicum + Lactobacillus acidophilus + Lactobacillus plantarum	0	100

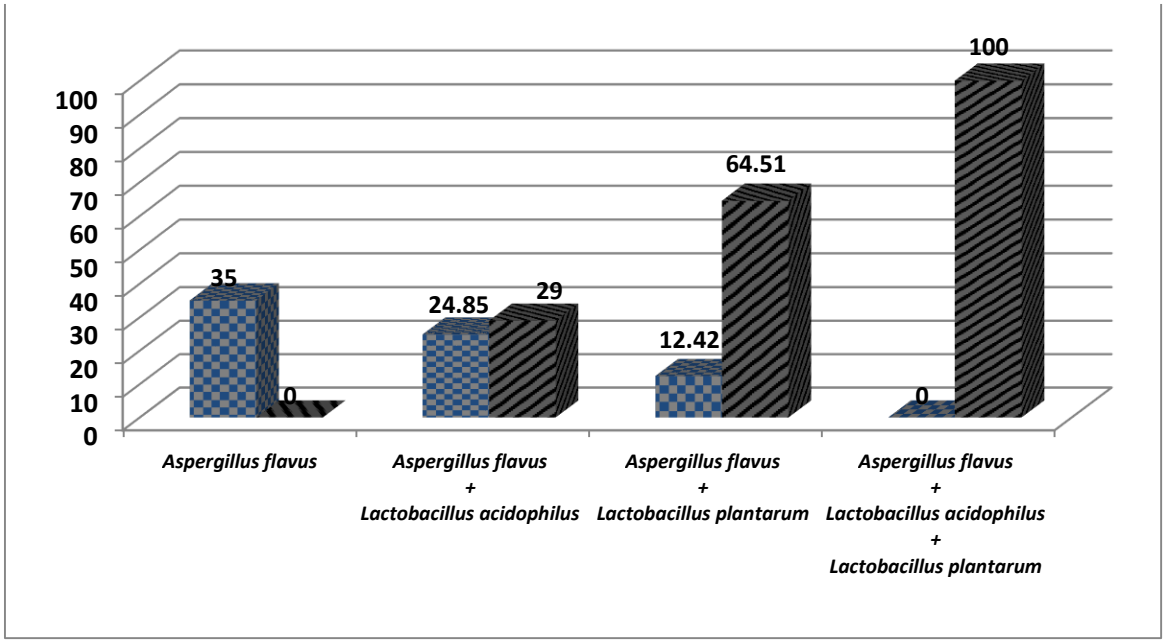


Figure (1): Effect of probiotic bacteria on aflatoxin producing by Aspergillus flavus in vitro and quantification by LFIA.

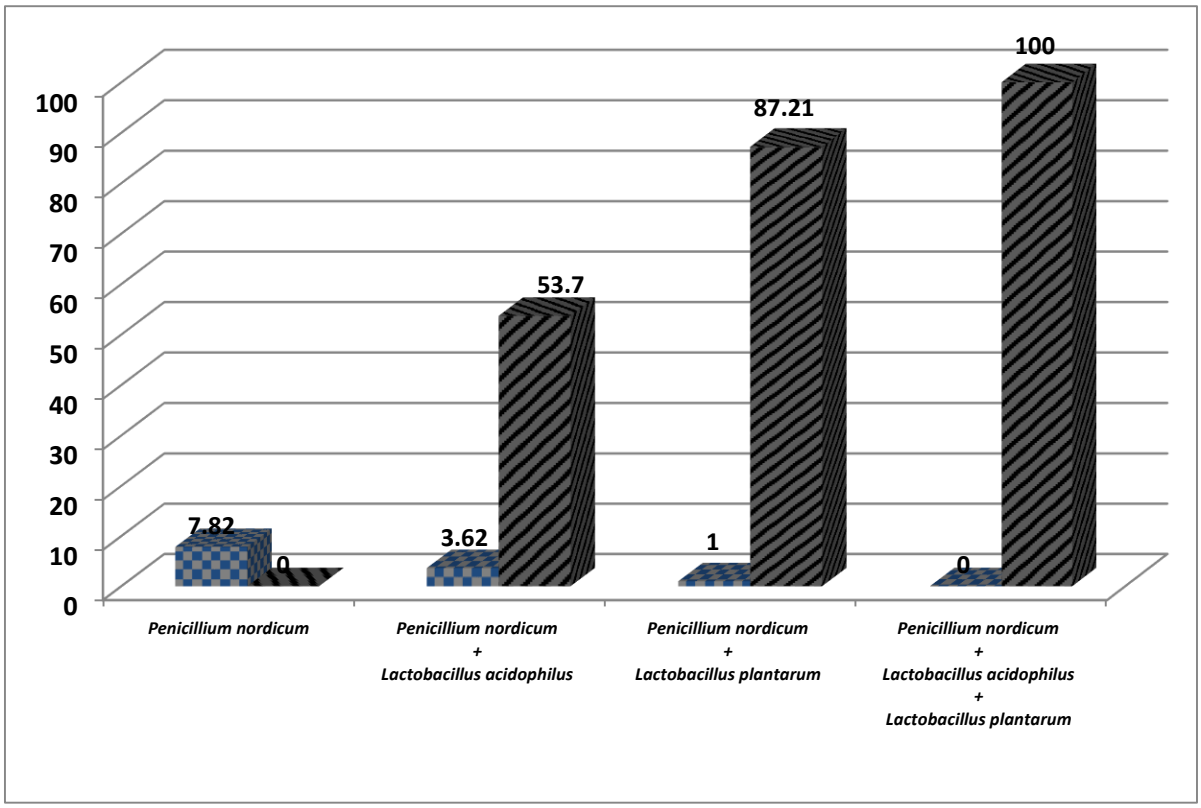


Figure (2): Effect of probiotic bacteria on ochratoxin producing by Penicillium nordicum in vitro and quantification by LFIA

From the data obtained it was found that Saccharomyces.cerevisiae, when grown with Aspergillus flavus in a liquid medium, had a good effect on reducing the level of aflatoxin producing at a concentration of (3.85 ng/ml), with a reduction of 83.15%, compared to the control, where the concentration of aflatoxin was (22.86 ng/ml). While Saccharomyces cerevisiae was grown with Penicillium nordicum, significantly decreased the incidence of ochratoxin, reaching 75.6%.

Regarding this,(Kim et al.)[13] suggested using the yeast Saccharomyces cerevisiae in a high-throughput bioassay to find phenolic compounds for control of the aflatoxigenic fungus Aspergillus flavus.

According to the findings, the production of aflatoxin and ochratoxin were reduced by an average of 81.67% and 62.4%, respectively, when Torulaspora delbrueckii, was added to Aspergillus flavus or Penicillium nordicum in the toxicity medium. Additionally, when Aspergillus flavus or Penicillium nordicum were combined with the probiotic yeasts (Saccharomyces cerevisiae and Torulaspora delbrueckii), they completely lost the ability to produce aflatoxin or ochratoxin at a rate of 100%.

Table (3): Effect of probiotic yeast on aflatoxin producing by Aspergillus flavus in vitro and quantification by (LFIA).

Treatments		Total of aflatoxin (AF/ng/ml)	% Reduction
Control	Aspergillus flavus	22.86	0
T1	Aspergillus flavus + Saccharomyces. Cerevisiae	3.85	83.15
T2	Aspergillus flavus + Torulaspora. delbrueckii	4.19	81.67

T3	Aspergillus flavus + Saccharomyces. cerevisiae + Torulaspora. delbrueckii	0	100

Table (4): Effect of probiotic yeast on ochratoxin producing by Penicillium nordicum in vitro and quantification by (LFIA).

Treatments		Total of Ochratoxin (OTA/ng/ml)	% Reduction
Control	Penicillium nordicum	5	0
T1	Penicillium nordicum + Saccharomyces. Cerevisiae	1.22	75.6
T2	Penicillium nordicum + Torulaspora. Delbrueckii	1.88	62.4
T3	Penicillium nordicum + Saccharomyces. cerevisiae + Torulaspora. delbrueckii	0	100

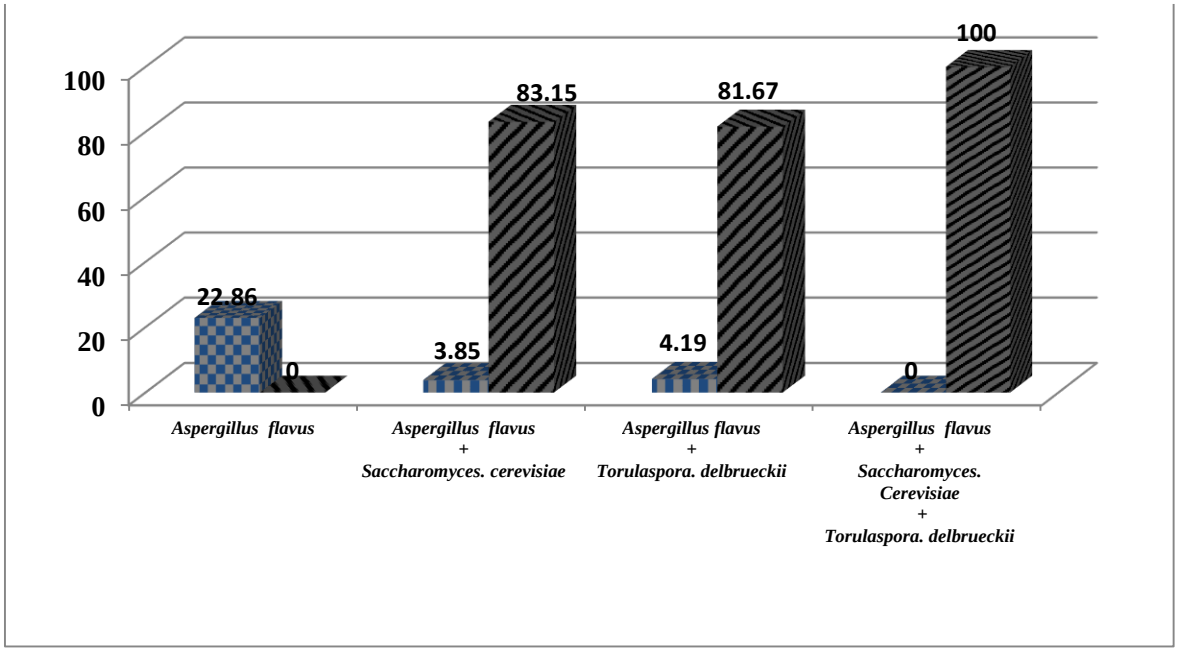
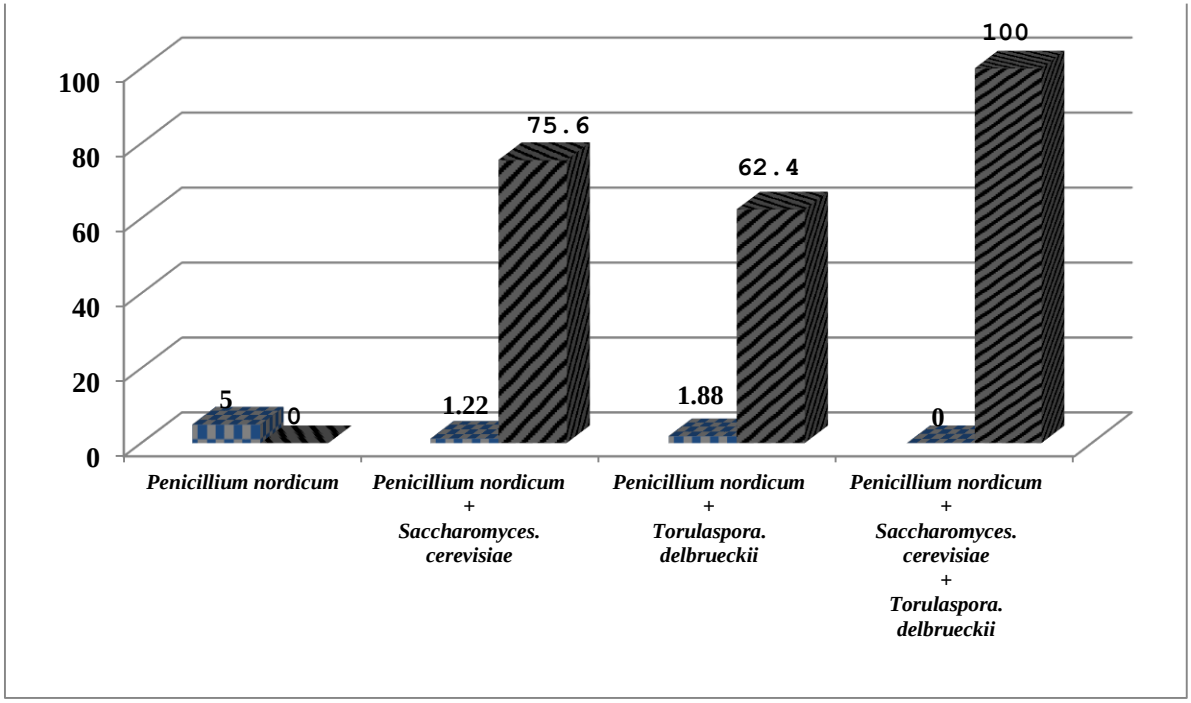


Figure (3): Effect of probiotic yeast on aflatoxin producing by Aspergillus flavus in vitro and quantification by LFIA.



Figure(4): Effect of probiotic yeast on ochratoxin producing by Penicillium nordicum in vitro and quantification by LFIA.

CONCLUSIONS

An efficient method of biocontrol of Aspergillus flavus and Penicillium nordicum toxins production, particularly in edible foods like dairy products with high nutritional and economic values, is the use of

probiotic bacteria and/or yeast strains to inhibit growth and subsequent aflatoxin (AFs) and/or ochratoxin (OTs) production. The current experiments have demonstrated the combination of L. acidophilus and L. plantarum had great potential capacity against A. flavus, P. nordicum growth, aflatoxin and ochratoxin generation in YES medium. The highest reduction in aflatoxin (AFS) and/or ochratoxin (OTA) concentration were also seen when S. cerevisiae and T. delbrueckii were combined. Additional research is needed to understand how this strains will be used commercially in the dairy field.

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