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In Vitro Anti-Mycotoxigenic and Anti-Aflatoxigenic Properties of Probiotic Bacteria: *Lactobacillus plantarum* and *L. paracasei*

A. Allameh[1*], F. Ani[2], M. Shams-Ghahfarokhi[3], M. Razzaghi-Abyaneh[4]

[1] *Department of Clinical Biochemistry, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Islamic Republic of Iran*
[2] *Nour Danesh Institute of Higher Education, Mimeh, Isfahan, Islamic Republic of Iran*
[3] *Department of Medical Mycology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Islamic Republic of Iran*
[4] *Department of Mycology, Pasture Institute of Iran, Tehran, Islamic Republic of Iran*

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Abstract

The present study was designed to examine the effects of *Lactobacillus plantaraum* and *L. paracasei* on the growth and aflatoxin-producing *ability of Aspergillus parasiticus.* In these experiments, direct and indirect interactions of two different probiotic lactobacilli with *A. parasiticus* was examined. A co-culture system which supported both fungal growth and probiotic bacteria showed the inhibitory actions of the lactobacilli on *A. parasiticus* growth. Disk Diffusion Assay was developed to examine the anti-fungal action of the probiotic cell free extract. The probiotic extracts were also applied in a micro-dilution assay to evaluate the anti-fungal and anti-aflatoxin properties of the probiotic extracts. The results showed that direct interaction of the bacteria with *A. parasiticus* in a spot-culture method (two-layer semi-solid/solid culture) caused a clear zone of inhibition of *A. parasiticus* growth. Fungal growth inhibition was found to be dependent on the concentration of the probiotic extracts added to *A. parasiticus* culture (micro dilution assay). The inhibitory effects of the probiotic extracts was further confirmed using Disc Diffusion Assay by showing that discs loaded with 50 or 100 µl of extracts resulted in a significant (4-5 folds) inhibition in *A. parasiticus* growth. The extract prepared from the probiotics inhibited the fungal growth and at higher levels. Accordingly, the probiotic extract (50% v/v) could significantly suppress aflatoxin levels in mycelia and fungal culture media. In conclusion, probiotic bacteria and their cell-free extracts produced under optimized condition exhibit anti-fungal and anti-mycotoxin effects provided that these bacteria undergo screening and selection prior to their application.

Keywords: Probiotic bacteria; Anti-fungal; Screening; Aflatoxin inhibition.

[*] Corresponding author; Tel: +982182883877; Fax: +982188006544; Email: allameha@modares.ac.ir

Introduction

Probiotics are resident normal flora of the intestinal tract and believed to play important roles in regulating proper intestinal function and digestion by balancing intestinal microflora. These beneficial microorganisms are widely used as live microbial dietary supplement and can help restoring intestinal microfloral balance [1-2].

There are a number of microorganisms with probiotic activity; lactic acid producing bacteria and bifidobacteria as well as *Saccharomyces* spp. and other organisms involved in fermentation are well known probiotics. Probiotics are beneficial in balancing between the harmful and protective intestinal microflora species and as the health benefits of probiotics increases, the application of these bacteria for treatment of gastrointestinal disorders also increases. Stimulation of immune system, enhancement of bowel mobility and modulation of inflammatory and allergic reactions are major biological functions described for probiotics. Probiotic bacteria can also influence the host by various mechanisms such as antimicrobial activity, enhancement of barrier function and immune-modulatory effects [3].

In recent years the market of probiotics continues to grow and human and animal food products containing probiotics is increasing. Recently it has been reported that enrichment of a fermented cereal beverage in Uganda substantially decreased the naturally occurring aflatoxins (AFB1, AFB2, AFG1 and AFG2) in the final beverage product [4]. The ability of probiotics bacteria to bind to aflatoxin B_1 (mycotoxin produced by *Aspergillus parasiticus*) has been studied. *In vitro* studies by El-Nezami and co-workers 1998 [5] showed that >80% of aflatoxin B_1 in solution binds to dairy strains of lactic acid bacteria. Similar *in vitro* evidences show the capacity of probiotic bacteria to bind to aflatoxins. AFB_1 is believed to predominantly bounds to a carbohydrate moiety on the surface of the bacteria [6].

In vivo studies carried out in rats clearly show the protective effects of probiotics against aflatoxin toxicity [7]. Removal of aflatoxins by probiotic bacteria (*Berevibacillus laterosporus*) has also been reported in Japanese quail diet leading to detoxification of aflatoxins and inhibition of aflatoxicosis [8]. Very recently we reported that supplementation of *Lactobacillus plantarum* in broiler chicks for the entire period of breeding period (42 days) can alleviate aflatoxin related hepatotoxicity. Interestingly, the performance and the anti-oxidant factors were markedly improved in growing broilers given drinkable probiotics [9].

It has been reported that supplementation of human diet with a mixture of probiotics reduces the urinary excretion of AFB1-N7-guanine, a biomarker of biologically effective dose of exposure to aflatoxin B_1 [9]. It has also been reported that [10,11] supplementation of chicken fodder with probiotic reduced the aflatoxin B_1-related DNA damage (Comet assay).

Despite the reports showing anti-aflatoxigenic effects of probiotics, the mode of action of probiotic on aflatoxins is not well understood. A number of studies suggest that the mechanism of protection against AFB_1 is through binding of AFB_1 to cell wall constituents of probiotic bacteria [12].

The use of biological techniques for detoxification and/or elimination of mycotoxins from naturally contaminated grains and concentrated feed is important in reducing mycotoxins in human food chain. Hence considering the biological benefits of probiotics, identification of particular probiotic bacteria with anti-aflatoxigenic effects is promising for inhibition of the growth of aflatoxin- producing fungi and aflatoxin production.

Despite the studies carried out on the capability of probiotic bacteria to bind to aflatoxins, the mode of action of the probiotic bacteria and their cell-free products on the growth and aflatoxin production by the aflatoxigenic fungi is not fully understood. Hence development of specific assays could help screening of potential anti-fungal and anti-aflatoxin probiotic organisms. Also these assays could help optimization of the culture condition and supplementation of probiotics or their extracts for controlling aflatoxin-producing fungi.

Materials and Methods

Chemicals and reagents

Aflatoxin B_1 (AFB_1) was the product of Sigma Chemical Co (St. Louis, MO, USA). TLC plates and other solvents and reagents were the products of the E. Merck Company, Germany. MRS, SDA and PDA were purchased from the Ibresco, Tehran, Iran.

The L. plantaraum and L. paracasei

These bacteria were routinely cultivated on MRS agar for 24 h at 37º C. The isolated single colonies proved to be Gram-positive rod-shape bacteria were selected for inoculation into the MRS broth [13]. The growth rate was determined by inoculation of fresh cultured *lactobacillus* into flasks containing 100 ml of MRS broth, mixed and incubated at 37º C. The growth curve was determined as shown in Fig. 1 by comparing

the optical density at 650 nm of the culture at different time intervals (4, 12, 18 and 24 hours).

Preparation of cell-free extracts from L. plantarum and L. paracasei culture media.

Lyophilized cell-free extracted were prepared according to the method described by Saadatzadeh et al., [14] with modifications. Briefly, lactobacilli were grown in MRS broth at 37º C for 48 h. The cells were recovered by centrifugation (6000 g for 10 min at 4 ºC). The supernatant was removed and passed through a sterilized 0.22 filter unit. The filtrate was then concentrated using a freeze dryer at -70º C. The extracts were then divided into aliquots and stored in freezer for further use.

Aspergillus parasiticus NRRL-2999: Growth and aflatoxin production

The aflatoxin-producing ability of this strain was first verified on Sabouraud Dextrose Agar (SDA) media in Petri dish. Then the fungi were cultivated on Yeast Extract Sucrose (YES) agar which supports aflatoxin biosynthesis and the colonies were developed. The mycelia were used for slide culture and microscopic observation.

A. parasiticus spores were produced after 5 days in culture on SDA. The spore suspension was prepared and the spores were counted using Neubauer slide. Three different concentrations of spore suspension were prepared in sterile phosphate buffer saline (PBS) for inoculation of fresh cultures.

Production of fungal spores and inoculation into broth

Flasks (50 ml) containing YES broth were inoculated with *A. parasiticus* spores (10^5/ml spores) and incubated at 28 ºC. At time intervals (24 and 48 h), mycelia and media were collected and separated for extraction and detection of aflatoxins produced. Four types of aflatoxins have been extracted and identified on TLC plates in both mycelia samples and the culture media (Fig. 2). Aflatoxin production was carried out on YES broth by inoculating *A. parasiticus* spores (10^5/ml media). Mycelia and media were recovered after 7 days and processed for aflatoxin extraction and estimation.

Extraction of aflatoxins from culture media and mycelia were done as described in our previous publication [15-16]. Briefly; fungal mycelia was ground in a mortar and pestle with the help of acid-washed sea sand (Sigma Chemical Co., Mo, USA). The chloroform extract was removed and stored at -20 °C for further use. Similarly, aflatoxins in culture media were extracted by chloroform. The chloroform extracts were then dehydrated by passing through the filter bed containing sodium sulfate. Thereafter the solvent (chloroform) was evaporated to dryness in a vacuum dryer (Heidolph, WB 2000, Germany). The samples were stored at -20 °C before separation on TLC plates pre-coated with silica gel with fluorescent indicator 254 nm on aluminum sheets (Fluka Chemie AG, Germany). The solvent system in the chromatography tank was chloroform: methanol (98:2 v/v). The Rf values obtained from aflatoxins (B1, B2, G1 and G2) in mycelia and culture media preparations were identified by comparing their Rf values with those obtained for aflatoxin standard (Sigma Chemical Co., Mo, USA) which was run on the same plates.

Interaction of the Lactobacilli with A. parasiticus in spot culture method

The spot culture technique was applied to investigate the inhibitory effects of *lactobacilli* on growth and aflatoxin production by *A. parasiticus* [17]. Two layer culture system was used to assess the direct effect of lactobacillus on growth rate of *A. parasiticus*. This assay was performed in plates (6.0 Cm diameter), the lower layer was prepared by MRS agar (1% agar) for lactobacillus growth. The lactobacilli were cultivated in wells. Each well was inoculated with either *L. plantarum* or *L. paracasei*. The upper layer consisted of YES agar (0.8% agar) suitable for *A. parasiticus* growth cultured. The culture condition was optimized at 32 ºC for 24 hours. The zone of growth inhibition of *A. parasiticus* was measured and considered as the inhibitory effects of *lactobacilli*.

Disc diffusion assay

The A. parasiticus NRRL-2999 was used for evaluating the antifungal susceptibility of cell-free extracts prepared from probiotic isolates. The assay was performed in discs (6.0 mm Dia., MAST Diagnostics) normally used for evaluation of antifungal agents. The disc diffusion method was carried out on Mueller Hinton agar (MHA) supplemented with 2% glucose and 0.5 µg/mL methylene blue.

Agar plates were swab inoculated with a suspension of *A. parasiticus* adjusted to the turbidity of a 0.5 McFarland standard. The plates were allowed to dry for 5 to 15 min and discs loaded with two levels (50 and 100 µl) of antifungal agents or probiotic extracts were placed on the agar surface. The plates were incubated for 24 h at 35 °C. The interpretive criteria for the disc test were based on zone diameter of growth inhibition determined following the guidelines described by Barry *et al.,* [18].

Effects of cell-free extract from lactobacillus culture media on A. parasiticus growth

Disc Diffusion Assay was used to examine the effect of the bacterial cell-free extract on *A. parasiticus* growth. The assay was performed by exposing fungal growth in YES agar to adsorbing discs containing 50 or 100 µl of culture media prepared from either *L. plantarum* or *L. paracasei*. The exposure time was adjusted to 24 and 48 hours after transferring the discs to the fungal culture. The discs containing extract from culture media without bacterial growth was also used and considered as control.

Inhibitory effects of probiotic cell-free media on A. parasiticus growth: Micro-dilution assay

Micro-dilution assay was adopted to find out the inhibitory effects of different concentrations of the probiotic extracts on the mycelial growth of the *A. parasiticus*. This assay was carried out in 96 well plate by inoculation of *A. parasiticus* spores (10^4). Then different amount of the probiotic cell-free extract (0, 12.5, 25, 50, 75 and 100% v/v) was added to the well induplicate. The final volume of each well was adjusted to 200 µL. Briefly, a serial dilution of the probiotic cell-free extract was prepared by diluting the culture media as; 100, 75, 50, 25 and 12.5% (V/V). The plate was then incubated at 32 °C for 48 hours. At the end of the incubation period, the growth of mycelium was examined visually and the growth rate was compared with the controls and scored. All the assays were performed in duplicate and the growth rate has been evaluated in 2 separate wells for each treatment. The fungal growth in wells containing *A. parasiticus* in absence of the probiotic extracts were considered as positive control.

Effects of the cell-free extracts from lactobacilli on aflatoxin production

Culture media concentrate were prepared from either *L. plantarum* or *L. paracasei* and added to the fungal culture media in 50 ml capacity flasks. The final concentration of the lactobacilli culture concentrate was adjusted to 5-6% of the total volume and then incubated for 7 days at 28 ºC. The mycelia were separated from the culture media by filtration. Aflatoxins were extracted by using solvent extraction system both mycelia and media. Then the aflatoxins were separated on TLC plates and quantitated using standard samples on a scanner (TLC scanner, CAMAG, Switzerland) as described under section 2.3.1.

Statistical analysis

The statistical analysis of data was performed using ANOVA (SPSS Software Inc., Chicago, IL). A control group without treatment was considered for each group. Each experiment was independently carried out at least two times in duplicate. A **P**-value of less than 0.05 ($p<0.05$) was considered as statistically significant.

Results

Lactobacilli growth rate

As shown in Figure 1 the pattern of lactobacilli growth follows 3 distinct phases; log phase, lag phase and plateau phase during 24 hours culture in MRS culture media. The growth curve was identical for both

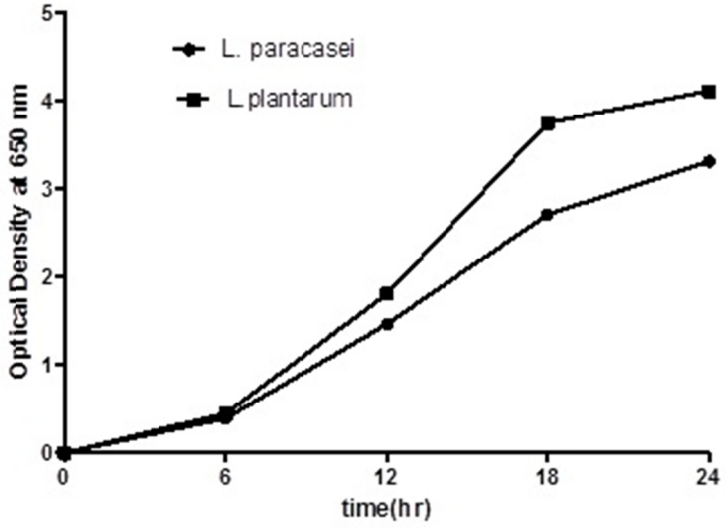

Figure 1. Growth curve of *Lactobacillus plantaraum* and *L. paracasei* in culture media.

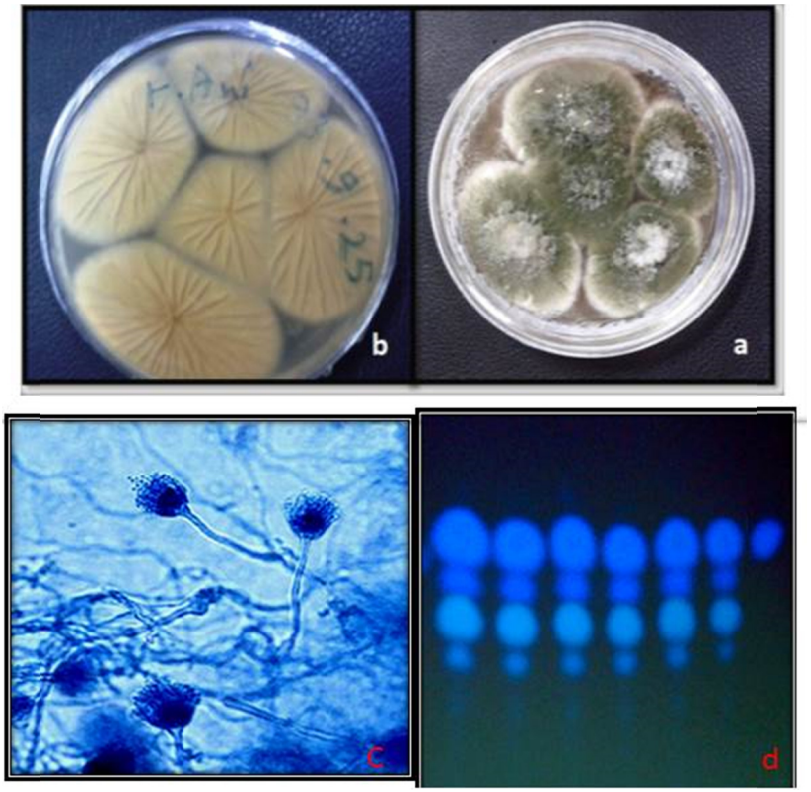

Figure 2. *Aspergillus parasiticus* growth and its aflatoxin production ability

A. parasiticus NRRL-2999 was routinely cultured on SDA as well as YES agar. Typical colonies were obtained after 24 hours of incubation at 28 ºC. Fungal colonies and mycelia obtained after 5 days were used for identification and characterization of the fungi. Part-a, and part-b show the *A. parasiticus* colonies cultured on YES agar. Part-c shows microscopic characteristics of the *A. parasiticus* mycelia. Part-d. Four naturally occurring aflatoxins (AFB1, AFB2, AFG1 and AFG2) detected in mycelia samples from *A. parasiticus* separated on TLC plate.

L. Paracasei and L. Plantaruam, the cell entered lag phase after 6 hours in culture and reached to maximum growth after 24 hours incubation at 37 ºC.

***Optimization of the A. parasiticus* growth and aflatoxin production**

A. parasiticus NRRL-2999 was cultured on SDA as well as YES agar and typical colonies were obtained after 24 hours of incubation at 28 ºC. Fungal colonies and mycelia obtained after 5 days showed typical morphological and microscopic characteristics (Fig. 2).

Inhibition of A. parasiticus growth in presence of lactobacillus (Spot culture technique)

The results of two-layer culture adopted for A. parasiticus and lactobacilli growth showed clear zones of growth inhibition of *A. parasiticus* around the *L. plantarum* and *L. paracasei.* As shown in Figure 3, the zone of growth inhibition was greater around the *L. plantarum* colony compared to that measured for *L. paracasei*.

Effects of cell-free extracts from L. plantarum and L. paracasei on A. parasiticus growth (micro-dilution

Figure 3. Direct interaction of lactic acid bacteria and *A. parasiticus NRRL-2999* in a two layer spot-culture technique.

(1), *L. plantarum* colony and (2) represents *L. paracasei* colony. In this two layer culture, the lower layer is MRS media (1% agar) to support Lactobacilli growth and upper layer is semi solid YES media containing 0.8% agar supports *A. parasiticus* growth. The culture condition was optimized to 32 ºC for 24 hours.

Table 1. Inhibitory effects of probiotic cell-free media on A. parasiticus growth: Micro-dilution assay

L. plantarum media (%)	Growth rate Relative % (% inhibition)	*L. paracasei* Media (%)	Growth rate Relative % (% inhibition)
0	100 (0)	0	100 (0)
12.5	85 (15)	12.5	95 (5)
25	70 (30)	25	90 (10)
50	65 (35)	50	85 (15)
75	55 (45)	75	75 (25)
100	50 (50)	100	65 (35)

This assay was carried out in 96 well plate. To each well *A. parasiticus* spores (10^4) in 100 µl of YES broth was added. Then different levels of the probiotic cell-free extract was added to achieve a total volume of 200 microliter. The following dilutions were prepared from each probiotic preparation.
A final dilution of each probiotic extract added to the culture media is; 100, 75, 50, 25 and 12.5% (V/V). The plate was incubated at 32 degree C for 48 hours. At the end of the incubation period, the growth of mycelium was examined visually and the growth rate was compared and scored. All the assays were performed in duplicate and the growth rate has been evaluated in 2 separate wells for each treatment.

Effects of cell-free extracts from L. plantarum and L. paracasei on A. parasiticus growth (micro-dilution assay)

In this assay the changes in fungal mycelial growth is based on visual examination. In this assay the cell free extract prepared from both the probiotic isolates resulted in a concentration-dependent inhibition in the fungal growth (Table 1). The anti-fungal effect of cell free extract from *L. plantarum* was relatively more as compared to that measured in presence of *L. paracasei*. The lowest level (12.5% v/v) of the *L. plantarum* extract caused about 15% retardation in the *A. parasiticus* growth. The fungal growth rate was reduced by approximately 50% and 30% when supplemented with 50% (1:1 v/v) of the cell-free extract from *L. plantarum* or *L. paracasei* resulted in about 50 and 35% inhibition in *A. parasiticus* growth.

Inhibitory effects of cell-free extract prepared from lactobacilli on A. parasiticus growth (Disc Diffusion Assay)

As shown in Figure 4, *the A. parasiticus* growth was significantly inhibited (4-5 folds) when treated for one time with discs containing either 50 or 100 µl of the cell-free extracts from *L. plantarum* or *L. paracasei*. The zone of inhibition in mycelia growth after 24 h in presence of the cell-free extracts from lactobacilli was significantly increased in presence of higher concentrations (100 µl) of the probiotic preparations. The assays performed for 24 h with the discs containing sterilized culture media (negative control) showed a minimum zone of inhibition. However, discs loaded with 50 or 100 µl of the cell-free extracts from *L. plantarum* or *L. paracasei* by average cause zones of >10 and > 17 mm respectively.

Influence of cell-free extract prepared from lactobacillus culture on aflatoxin production

As shown in Figure 5A and 5B, treatment of *A. parasiticus* in YES broth for 7 days with cell-free preparation from *L. plantarum* or *L. paracasei* produced no significant changes in total aflatoxins in mycelia as well as culture media by *A. parasiticus*.

Discussion

Removal and detoxification of mycotoxins in food and raw materials by physical methods and chemicals is often associated with disadvantages, particularly because of formation of toxic metabolites or residues in food products [16].

Various laboratory and large scale procedures have been developed for the elimination or detoxification of aflatoxins in food and feed commodities that are often associated with abrogation of aflatoxicosis in laboratory animals and in poultry [6, 7, 10]. Some studies show that enrichment of the poultry diet or drinking water with an appropriate probiotic can reduce aflatoxin levels [4] and its toxicity [9].

In vitro assays are considered as important tools to find out the possible mechanisms by which a single probiotic organism interacts with fungal growth and mycotoxin production. In the present study, assays were adopted to evaluate the efficiency of two lactobacilli bacteria and their cell-free extracts in challenging the growth and aflatoxin production by a potential aflatoxin producing fungi i.e, *A. parasiticus* strain.

Our experiments was initiated with subculturing of *A. parasiticus* NRRL-2999, and adaptation of the culture condition to verify its ability to produce all the naturally occurring aflatoxins (Fig. 2). Besides, the growth curve of the two probiotic bacteria cultured in MRS medium was examined. As shown in Figure 1, the growth rate and cell population was more efficient in case of *L. plantarum* as compared to that of *L. paracasei*.

The first evidence of the inhibitory effects of the probiotic bacteria was observed in a two-layer spot culture technique which showed clear zones of

inhibition in *A. parasiticus* growth around the probiotic bacteria colonies (Fig. 3). Such interactions between the lactobacillus isolate with *A. flavus* showing the aflatoxin-binding capacity of probiotic lactobacillus with the fungi has been reported by others [19].

The anti-fungal action of the metabolites produced by the probiotics was further demonstrated using a disc diffusion assay by treatment of the *A. parasiticus* culture with the discs loaded with cell-free extract prepared from *L. plantarum* and *L. paracasei*. The data from this assay showed that treatment with the probiotic extract resulted in a time- and concentration-dependent inhibition in *A. parasiticus* growth rate as judged by the zone of inhibition (Fig. 4). The zone of inhibition surrounding the fungal colonies was over 17 mm in presence of discs loaded with 100 µl of the probiotic extracts. Relatively smaller zone of inhibition in 48 hours treatments compared to those treated for 24 hours could be justified by the instability of the probiotic active metabolites which are not yet identified.

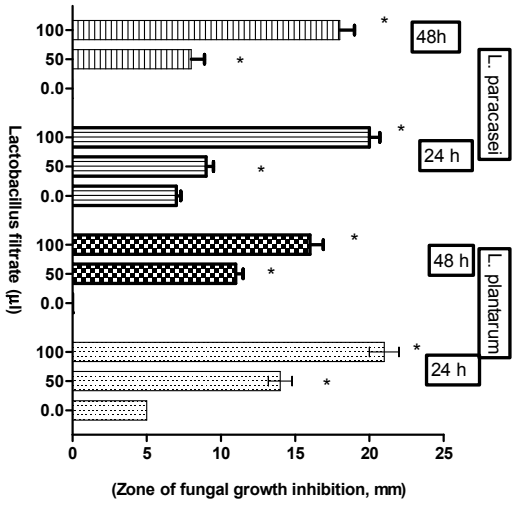

Figure 4. Comparison of the *A. parasiticus* growth treated with the cell-free extract prepared from the probiotic bacteria (Disc Diffusion Assay).

The discs were allowed to adsorb cell-free culture media prepared and lyophilized from *L. plantarum* or *L. paracasei* (50 or 100 µl) and placed on the culture plate for 24 or 48 hours before measuring the zone of inhibition. In this assay discs containing equal amounts of the extract prepared from sterilized culture media were also used and considered as control. * Sign indicates that the values are significantly different from respective control group ($P<0.05$).

It appears that the fungal growth tends to compensate the inhibitory action of the bacterial growth. The reasons for this phenomenon can be explained by knowing that active metabolites produced in lactobacilli media are diminished after 24 hours. Also resistance mechanisms by which *A. parasiticus* can gain towards lactobacillus preparation cannot be ruled out.

Further anti-fungal assays performed in microdilution assay performed in 96-well plate, revealed that the *A. parasiticus* growth was inhibited in presence of the cell free extract prepared from *L. plantarum* or *L. paracasei* (Table 1). This assay which is based on visual observation of the rate of *A. parasiticus* growth could clearly show the differences in the growth inhibition in presence of probiotic preparations. The rate of the fungal growth inhibition was decreased in presence of 12.5% (v/v) of the probiotic extract, which was further inhibited when the culture was supplemented with 25 and 50% of the probiotic preparation (Table 1).

The outcome of the probiotic treatments of the toxigenic fungi may vary depending on the experimental condition. In this line, El-Gendy and Marth [20] showed that simultaneous and consecutive

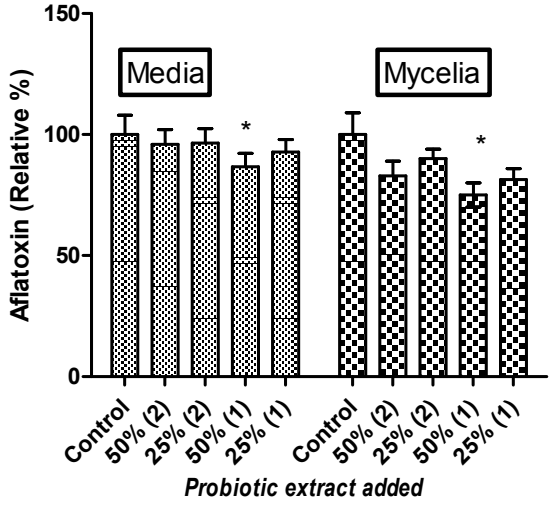

Figure 5. The effects of culture media concentrate prepared from lactobacilli on aflatoxin levels by *A. parasiticus* (Intra- and extracellular).

Experimental details are as described under Materials and methods section. * Sign indicates that the values are significantly different from respective control group ($P<0.05$). #1, is assigned to the samples prepared from *L. plantarum* and #2 is assigned to samples from *L. paracasei*. The amount (%) of the cell-free extracts are as indicated as volume/volume in the culture media.

inoculation of *L. casei ATCC 393* and *Aspergillus parasiticus NRRL 2999* (inoculation of *L. casei* three days prior to the fungal inoculation) in LAPT broth for 10 days at 28°C inhibited the fungal weight after consecutive inoculation. Also studies by Munoz et el., [21] showed that inhibition of *A. nomius* VSC after consecutive inoculation was significantly higher compared to the samples collected after simultaneous inoculation. It is of noteworthy that in the current study, the same culture media was used for simultaneous and consecutive growth which resembles the natural condition.

After showing the anti-fungal effects of the probiotics, it was assumed that the probiotic extracts could interfere in aflatoxin biosynthesis by these fungi. The treatments of *A. parasiticus* culture with probiotic extracts, under defined condition could reduce aflatoxin levels in mycelia as well as secreted aflatoxins (Fig. 5). Supplementation of the *A. parasiticus* culture with 50% (1:1 v/v) of *L. plantarum* for 24 hours resulted in a statistically significant reduction in aflatoxin levels in both mycelia and culture media. However, under similar condition, there was small changes in aflatoxin levels in the fungal growth treated with the extract prepared from *L. paracasei*. The superiority of *L. plantarum* over *L. paracasei* in aflatoxin inhibition is in agreement with results of the direct interaction of the two probiotics with the fungal growth. This difference could also be partially due to the better growth performance of *L. plantarum*.

The *in vitro* assays on anti-aflatoxin effects of the probiotics are in agreement with *in vivo* mechanism of action of *L. plantarum C88* for increasing fecal AFB1 excretion and reversing deficits in antioxidant defense systems [22]. The protective role of probiotics against aflatoxin-induced toxicity and mutagenicity could also be linked to the activities of phase-I and phase-II xenobiotic metabolizing enzymes [23].

Conclusion

A direct interaction of selected lactic acid probiotic bacterium with a potential aflatoxin-producing *A. parasiticus* can inhibit the fungal growth and aflatoxin production. The inhibitory effect of the probiotic bacteria could be mainly assigned to the active components of the cell free extract prepared from the probiotics which can efficiently inhibit the fungal growth and aflatoxin production by *A. parasiticus.* It is suggested that the use of probiotic bacteria and their cell-free extracts against aflatoxin-producing fungi needs screening and optimization prior to their possible supplementation in food and feed.

Highlights

- Lactic acid bacteria can directly interact with the fungi to inhibit mycelia growth of toxigenic *A. parasiticus*.
- Cell free extracts from lactic acid bacteria can efficiently inhibit *A. parasiticus* growth and aflatoxin synthesis.
- Inhibitory effects of probiotics on aflatoxin-producing fungi requires screening and optimization of culture system.

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