Saccharomyces cerevisiae and Probiotic Bacteria Potentially Inhibit Fumonisin B_1 Production in Vitro and in Vivo

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Abstract: The objective of the present study was to evaluate the efficacy of probiotic bacteria: *Lactobacillus rhamnosus* GG (LGG), *Lactobacillus rhamnosus* (LC705) and *Saccharomyces cerevisiae* (*S.cerevisiae*) to inhibit *Fusarium moniliform* (*F. moniliform*) growth *in vitro* and to eliminate fumonisin B_1 from body of mature rat *in vivo*. *S.cerevisiae*, LGG and LC 705 potentially inhibited *F. moniliform* growth and fumonisin B1 production in YES liquid media. The biologically active microorganisms (S.cerevisiae, LGG & LC705) had no toxic effects in rats when orally administered single doses of *S.cerevisiae* (10^{11} CFU ml^{-1}) and LGG & LC705 (10^9 CFU ml^{-1}). Moreover, daily treatments for 15 days with the three microorganisms in saline concomitant with FB1 in corn oil (5 mg/ml FB1), produced *by F. moniliform*, exhibited significant reduction in serum ALT, AST, GGT, creatinine, and BUN compared with the positive control group *(F. moniliform*). Blood glutathione (GSH) level significantly increased ($P< 0.05$) in groups treated with single-treatment of *S.cerevisiae*, LGG & LC705 or with fumonisin B_1 containing media. However, fumonisin B_1 - treatment severely depleted GSH level than other treatments. The best results found in *S.cerevisiae* > LGG > LC705 -YES media containing fumonisin B_1. The tested microorganisms are safely to use as food additives or preservative due to their antioxidant activity. Our study needs further continuation in this respect.
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1. Introduction

Fumonisins are a group of naturally occurring mycotoxins produced primarily by two fungi, *Fusarium verticillioides* and *F. proliferatum*, which frequently are found in corn. Fumonisins have been implicated in field cases of equine leukoencephalomalacia (Ross *et al.*, 1993), and porcine pulmonary edema (Osweiler *et al.*, 1992). Experimentally, fumonisins cause liver damage in all species studied to date, including pigs (Osweiler *et al.*, 1992), horses (Ross *et al.*, 1993), cattle (Osweiler *et al.*, 1993), sheep (Edrington *et al.*, 1995), rabbits (Gumprecht *et al.*, 1995; Ribeiro *et al.*, 2010), and rats (Suzuki *et al.*, 1995). Fumonisins also cause species-specific target-organ toxicity, such as in horses brain (Ross *et al.*, 1993), heart in pigs (Casteel *et al.*, 1994 ; Constable *et al.*, 2000; Smith *et al.*, 1999), kidney in sheep (Edrington *et al.*, 1995), rabbits (Gumprecht *et al.*, 1995; Laborde *et al.*, 1997), and rats (Direito *et al.*,2009; Suzuki *et al.*, 1995) as well as esophagus in rats and pigs (Casteel *et al.*, 1994). Epidemiologic data also has suggested an association between ingestion of contaminated corn with *F. verticillioides* and human esophageal cancer (Sydenham *et al.*, 1991). While administration of fumonisin has been fatal in a number of species, the cause of death has only been determined in pigs, where fumonisin causes acute left-sided heart failure and pulmonary edema consistent with sphingosine-mediated L-type calcium channel blockade of the heart and systemic vasculature (Constable *et al.*, 2000; Smith *et al.*, 1999 and 2000)

Probiotics are living microorganisms that when ingested may help to maintain the bacterial balance in the digestive tract of mammals, and may be included in the treatment of pathological conditions, such as diarrhoea, candidiasis, urinary infections, immune disorders, lactose intolerance, hypercholesterolemia, and food allergy (Shah, 2000; Mombelli and Gismondo,2000). They also have antigenotoxic effects; for example, species of Lactobacillus, Streptococcus, Lactococcus, and Bifidobacterium, have shown antimutagenicity in the Ames test, and their ability to decrease DNA damage in colon cells treated with N-methyl-N-nitro-N-nitrosoguanidine *in vitro* study (Pool- Zobel *et al.*,1996).

Saccharomyces cerevisiae **(***S.cerevisiae*), in particular, has proven to benefit health in several ways

including stimulation of the growth of intestinal microflore in mammals; pH modulation in ruminants (which gives rise to an increase in the rate of celulitic bacteria), improvement of reproductive parameters in milk cows and fowls (fertility and fetal development), as well as reduction in the number of pathogenic microorganisms in monogastric animals (Dawson, 1993; Wallace, 1998). In addition, a study in mouse revealed that a component of the Sc cell wall (glucan) reduced the frequency of micronuclei induced by cyclophosphamide (Chovatovicova and Mavarova, 1992).

The aim of this study is to investigate the efficacy of probiotic bacteria (LGG and LC 705) and *Saccharomyces cerevisiae* to inhibit *F. moniliform* growth *in vitro* and to eliminate fumonisin B_1 from body of mature rat *in vivo*.

2. Material and Methods

Materials:

1. HPLC using Sep-pak silica cartridge C18 columns.
2. Freeze-dried powder of *Saccharomyces crevisiae* (baker's yeast strain) and lactic acid bacteria *(Lactobacillus rhamnosus* GG and *Lactobacillus rhamnosus* LC 705) were obtained from Valio Ltd. Helsinki, Finland.
3. Fumonisin and Cultures : (a) Potato dextrose agar (PDA) , (b) de`Mane- Regosa-Sharp (MRS), (c) Malt Extract Agar (MEA) and (d) Yeast extract-malt extract-sucrose broth medium were obtained from Sigma Chemical Company, P.O. box 145508, St., Lous, USA.
4. Diagnostic kits were purchased from Boehringer Mannheim GmbH Diagnostica, E.Merck, Postfach 4119, D-6100, Dramstadt, Germany. All other chemicals were of highest quality available and were obtained from commercial sources.
5. Animals: The study with Sprague Dawley rats was approved by the committee of ethics and biosecurity at NRC, Cairo, Egypt. The experiment was made using mature male rat weighing 120-130 gm b.wt., purchased from Animal House colony. Animals were divided into equal groups (six rats each) housed under standard environmental conditions (23 ± 1°C, 55 ± 5% humidity and a 12-h light: 12-h dark cycle) and maintained on a standard laboratory diet *ad libitum* with free access to water.

(1) In Vitro Study

(a) Preparation of *F.moniliform* spores and fumonisin B_1 extraction.

Cultures of *F.moniliform* were growing on potato dextrose agar (PDA) slants for 7 days at 25 °C (Bullerman,1986). The librated fumonisin B_1 were analyzed according to AOAC (2000) and quantified by HPLC technique (Sep-pak silica cartridge C18 columns) according to method's of Le Bars *et al.* (1994)

(b) Spores of Lactic acid Bacilli strains (*Lactobacillus rhamnosus* GG and *Lactobacillus rhamnosus* LC705) were cultured on MRS broth / agar at 37°C until a concentration of 10^9 bacteria / ml was obtained ;counting of viable bacteria was performed by both traditional plate counting and flow cytometry (FCM) method. Bacterial counts were expressed as colony-forming units (CFU) per ml media. Viability of bacterial populations was assessed by using SYTOX ® green nucleic acid stain(Molecular probes, S-7020)at 1 µM/10^6 -10^7 bacteria to detect non viable bacteria. A band pass filter of 525 nm was used to collect the emission for green SYTOX (El-Nezami *et al.*,1998).

(c) Preparation of yeast suspensions:

The concentration of 10^{11} viable cell/ gm was determined for the probiotic yea-Sacc^{1026} through twelve decimal dilutions made in saline solution. Organisms were seeded in Petri dishes containing Sabourad broth and incubated for 72 h at 25°C and then counted for viability (Tejada de Hernandez, 1985).

(d) Inhibition experiments:

Inhibition of mold growth in the presence of *S. cerevisiae* , *Lactobacillus rhamnosus* GG and *Lactobacillus rhamnosus* LC705 was performed on YES liquid media according to the method's of Bjornberg, and Schnurer (1993).

(2) In Vivo Study

Eight groups of normal rats (6 rats each) the first four groups (1, 2, 3, and 4) were orally administered *Lactobacillus rhamnosus* strain GG (LGG) , *rhamnosus* strain LC705 (LC 705) and *Saccharomyces cerevisiae (S.cerevisiae)* (10 ml / Kg b.wt.; 10^9 CFU/ 1 ml distilled water , while the control group (group 4) was given distilled water (10 ml /Kg b.wt.). The second four groups (5, 6, 7 and 8) were orally administrated fumonisin B_1 (10 mg /kg b.wt. in 10 ml corn oil) FB1 alone and /or in concomitant with LGG and LC 705, 10^{10} bacteria /ml and *S.cerevisiae* 100 µg cell/ ml water (as previously mentioned concentration). The number of rats in group 5 was 10 animals (fumonisin B_1 - treated rats) to avoid missing data in case of increase the mortality rate among this group (we choose random healthy a life 6 rats for blood samples.

After 15 days of the daily treatment; all animals were sacrificed and blood samples were collected from retro-orbital venus plexus in two test tubes : the first was heparinized test tube (imbedded in ice box) for determination of reduced glutathione (GSH) in whole blood by Ellman's method (1959) , the second test tube was plain for serum separation (1500 rpm for 15 min) to assess the following biochemical analysis: γ-glutamyl transferase (GGT) according to Rosalki *et. al*. (1970), aspartate and alanine aminotransferase (AST and ALT) activities and creatinine according to

the method of Thefeld *et al.*(1974), and BUN (Henry *et al.*,1974) using BioMerieu kits.

Statistical analysis: The obtained results analyzed by ANOVA (one or two-way) using Excel 2003 Microsoft Corp (11.5612.5606), Redmond, WA software package.

3. Results

Saccharomyces cerevisiae greatly reduced *F. moniliform* growth in YES media at concentration of 10^6 CFU/g from the first week of incubation. Meanwhile, probiotic bacteria did not alter *F. moniliform* -growth at the 2st week of incubation. Presence of FB1 in YES media resulted weak inhibitory effect.

It was clearly demonstrated that the addition of the tested biocontrol microorganisms to YES media containing *F. moniliform* all of them significantly inhibited FB1 production and mycelium growth in a variable degrees of inhibition. Fig.(1) showed that the production of fumonisin B_1 was inhibited by all studied biocontrol microorganisms, particularly, LGG was the most potent inhibitor for fumonisin B_1 production than *S.cerevisiae* or L705. The percentage of inhibition of fumonisin B_1 production was 92.88%, 89.00% and 78.64% for LGG, *S.cerevisiae* and LC 705 respectively, when compared with their respective controls (Fig. 1).

Dry weight of *F.moiliform* –mycelium also decreased significantly in YES media containing S.cerevisiae, LB GG or LB 705, the effect was 86.46%, 89.13% and 71.80%, respectively (Fig. 2).

It was found that *S.cerevisiae* and LGG were closely similar in their effect on fumonisin B_1 production and mycelium growth; in which they possess most effective agents than the effect of L705 in *F.moiliform* -YES media.

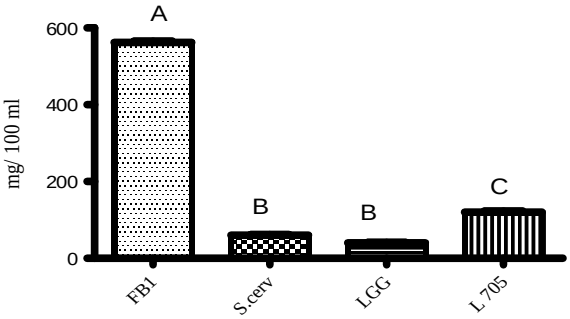

Fig. 1: Effect of S.cerv, LGGand L705 on FB1 production by *F. moniliform* in YES medium

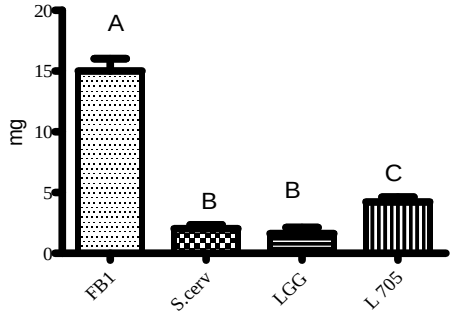

Fig. 2: Effect of S.cerv, LGG and L705 on *F. moniliform*- mycelium growth dry weight in YES medium

In vivo results:

Biochemical results (Table 1) showed the effect of different treatments on the liver enzymes (ALT, AST and GGT), kidney function tests (BUN and creatinine) and on GSH level in blood of all groups.

Single treatment with probiotic bacteria (LGG and LC 705) showed non-significant changes in ALT, AST and GGT activities comparing with the control group. While, these enzymes significantly inhibited by the treatment with *S.cerevisiae* alone. BUN values did not altered than control values in groups treated with *S.cerevisiae* or with the two strains of probiotic bacteria. Whereas, there were significant decrease in creatinine and significant increase in GSH levels in these single treated groups when compared with the controls or the other treated groups (Table 1). Significantly elevation in amino-transferases activities in the group administered FB_1 alone; this elevation was normalized by co-administration with LGG, while the combined treatment with *S. cerevisiae* and LC705 meliorated the increased activities of transaminases toward the normal figures.

FB1-administration also affected the kidney functions; BUN and creatinine values elevated significantly ($P < 0.05$) than other treatments (Table 1). Combined treatments with *S.cerevisiae*, LGG and L705 concomitant with FB_1 resulted significant decrease ($P < 0.05$) in BUN values than FB_1-treatment alone but still increased than normal groups. Creatinine had similar results as obtained in BUN values, in which the combined treatments of probiotic bacteria (LGG & LC705) with FB_1 resulted a significant decrease in creatinine level than FB_1 alone; except the group treated with S.cerevisiae plus FB_1, its creatinine value was normalized showed non-significant changes than control group.

GSH level was depleted in rat administered FB_1 alone, and it was increased significantly by the combination with LGG, LC 705 and *S.cerevisiae* to become more than normal control values.

Table (1): Effect of different biologically active microorganisms on fumonisin B1 (FB1) in rats administered 10 mg/Kg.b.wt./PO of media containing FB1 alone and /or *Saccharomyces cerevisiae* (*S.cerevisiae*) , *Lactobacillus rhamnosus* GG (LGG) and *Lactobacillus rhamnosus* LC 705 (LC 705). (n= 6 rats / group, means ± SE of the means).

Parameters	Normal groups				FB1- treated groups			
	Control	LGG	LC 705	*S. cerevisiae*	FB1	F B1+ LGG	FB1+ LC 705	FB1+ *S.cerevisiae*
ALT IU/ml	36.64± 0.86 ^{A}	35.73 ± 0.81 ^{A}	34.55± 0.77 ^{AB}	32.67± 0.68 ^{B}	66.17± 1.48 ^{C}	39.27± 0.65 ^{D}	45.22± 0.98 ^{E}	42.15± 0.73 ^{F}
AST IU/ml	44.19 ± 0.65^{AD}	42.63± 1.32 ^{AB}	41.45 ± 0.87 ^{AB}	40.47 ± 1.12 ^{B}	71.56 ± 2.39^{c}	51.38 ± 1.27 ^{D}	49.82 ± 1.33 ^{D}	47.88 ± 1.57 ^{D}
GGT IU/ml	0.97 ± 0.018 ^{A}	0.93± 0.04 ^{A}	0.82 ± 0.023 ^{A}	0.85 ± 0.029 ^{A}	3.21 ± 0.16 ^{B}	1.52 ± 0.031 ^{C}	1.56 ± 0.040 ^{C}	1.28 ± 0.030 ^{D}
GSH mg/ dl	37.33 ± 0.78 ^{A}	40.67 ± 1.3^{BE}	42.43 ± 0.97 ^{BC}	43.67 ± 0.86 ^{C}	25.18 ± 0.73 ^{D}	39.73 ± 0.75 ^{E}	41.02± 0.87 ^{BE}	43.12 ± 0.81 ^{BC}
BUN mg/ dl	23.55 ± 1.04 ^{A}	22.47 ± 0.75 ^{A}	20.98 ± 0.61 ^{A}	21.52 ± 0.89 ^{A}	35.00 ± 1.10 ^{B}	29.28 ± 1.12 ^{C}	27.23 ± 0.47 ^{C}	28.52 ± 1.26 ^{C}
Creatinine mg/ dl	0.539± 0.025 ^{A}	0.525 ± 0.071 ^{B}	0.493 ± 0.019 ^{B}	0.485 ± 0.016 ^{B}	1.075 ± 0.091 ^{C}	0.729 ± 0.030 ^{D}	0.654 ± 0.042 ^{D}	0.518± 0.017 ^{A}

ANOVA – one way

The different capital letters are significantly different at P< 0.05

4. Discussion

During the last decades, several studies have suggested that *Lactobacillus rhamnosus strain GG (LBGG)* and *L. rhamnosus strain LC-705 (*LB705) and yeast (*Sacharomyces cerevisiae*) are noted for their ability to bind mutagens *in vitro* studies (Hosono *et al*.,1990 and Yiannikouris *et al*., 2004).

In vitro study has shown that probiotic bacteria significantly reduced spores germination of *F.moniliform* and *Aspegillus flavus* (Smith,1978 and Nada *et al.*, 2010). Subsequently, they reduced mold growth and mycelial dry weight. These findings may due to the presence of aromatic compounds, such as organic acids, esters, alcohols, aldehyded, lactones and terpenes that produced by *Sacharomyces cerevisiae* (Janssens *et al.*,1992); and several antimicrobial compounds produced by probiotic bacteria which reduce fermentation products: lactic, acetic formic, propionic acids and hydrogen peroxide (Lindgren and Dobrogosz 1990).

In vivo study, the oxidative stress induced by aflatoxicosis administration was clearly observed as a model of food carcinogen (Abdel-Wahhab *et al.*, 1998 and Zhou *et al.*, 2001). Where mycotoxin (FB_1) toxicity appeared as a significant alteration in biochemical parameters and 20% deaths occurred among FB_1-treated group during the experimental period.

The explanation of this effect could be FB_1 biotransformation gives rise to various metabolites, it may covalently binds to DNA and to protein, which then alters enzymatic processes, such as glyconeogenesis, kreb's cycle, or fatty acid synthesis (Lesson *et al.*, 1995). Co-administration of FB1 with LGG, LC705, and *S.cerevisiae* diminished this oxidative damage.

The positive results are congruent with a study made in mice with the same probiotic for one week in concomitant with Ochratoxin A (OA) (Farag *et al.* 2010), where significant improvement were detected in weight level, probiotics counteracting the oxidative stress, prevent genotoxicity and spermatotoxic alterations induced by OA.

Madrigal-Santillan *et al.*(2006) studied the effect of *S.cerevisiae* (1x 10^8 live cells / g, 0.3% conc.) in mice fed FB_1 contaminated corn (0.4 and .08 mg /Kg) for 6 weeks. Authors found that *S.cerevisiae* improved the loss of weight gain and it had a potent adsorbent capacity without structural modification in FB_1 molecule. Recently, Nada *et al.*(2010) investigated that *S.cerevisiae* and probiotic lactic acid bacilli had protective effect against aflatoxicosis in rat when they orally administrated with aflatoxin B_1 for 15 days and ameliorated liver and kidney tissues from AFB_1toxicity.

Few literatures were dealed with the isolated cell wall of *S.cerevisiae in vitro* study, and proved the

presence of B-D-glucans in adequate percentage on the cell wall of *S.cerevisiae* spp. consequently, it has antioxidant activities, (Yiannikouris *et al.* 2003); Oliveira *et al.* (2009) and Sener *et al.* (2007) found that B-glucans reduced DNA-oxidative damage through scavenging of both •OH radicals and singlet oxygen species *in vitro* study.

Live *Sacharomyces cerevisiae* has been used as food supplement (due to its high content of vitamins, particularly those of the vitamin B group, minerals, proteins and enzymes), and it is employed as a biotherapeutic or probiotic agent for re-equilibration of the intestinal flora. Some clinical studies have shown that these preparations are effective for the treatment of chronic diarrhea, especially those cases associated with parenteral nutrition and/ or super infection with *Clostridium difficile* (Bleichner *et al.*, 1997; Guslandi *et al.*, 2000). *Sacharomyces cerevisiae* therapeutic effects is attributed to its release of a 54-kDa protease that causes cleavage of *C. difficile* toxins A and B and diminishes their capacity for binding to receptors located on the human colonic brush-border membrane (Castagliuolo *et al.*, 1999) , this hypothesis may support our results in preventing fumonisins in the current experimental study.

Reduced glutathione (GSH) is the main component of endogenous non-protein sulfhydryl pool that scavenges free radicals in the cytoplasm (Ross 1988, Shaw *et al.*,1990). Because of their exposed sulfhydryl groups, non-protein sulfhydryls bind a variety of electrophilic radicals and metabolites that may be damaging to cells (Szabo *et al.*, 1992). In fact, antioxidants maintain the concentration of reduced GSH, which may restore the cellular defense mechanisms, block lipid peroxidation, and thus protect against the oxidative tissue damage (Toklu *et al.*, 2006).

B-Glucans are glucose polymers found in the cell walls of yeast, fungi, and cereal plants. The beneficial effects on the immune system and the lack of toxic or adverse effects (Vetvicka, 2001 and Sener *et al.*, 2007), had focused the studies on B-glucan molecule.

Currently, B -glucans are accepted to be one of the most powerful immune response modifiers (Brown and Gordon, 2003); it inhibits tumor development, enhances defense against bacterial, viral, fungal, parasitic challenge (Onderdonk *et al.*,1992; Kernoddle *et al.*, 1998). B–glucans activates macrophages (Cleary *et al.*, 1999; Vetvicka and Yvin, 2004). It induces production of cytokines (Soltys and Quinn, 1999; Engstad *et al.*, 2002); nitric oxide (NO), arachidonic acid metabolites (Ljungman *et al.*, 1998) and increases hematopioesis. As well as, B-glucans exerts radio-protective effects, improves wound healing by inducing the macrophage release of wound growth factors (Wei *et al.*, 2002) and lower serum lipids (Nicolosi *et al.*, 1999). Several mechanisms were proposed for the protective effect of B-glucan, one of them is related to antioxidant capacity of the molecule (Babincova *et al.*, 2002; Krizkova *et al.*, 2003; Sener *et al.*, 2005). Yiannikouris *et al.*, (2003 and 2004) and Young *et al.*,(2000) studied the adsorption capacity of yeast cell wall and the role of various B-D-glucan types in the efficacy of Zearalenone adsorption and thought to elucidate some of adsorption mechanisms *in vitro* studies with Zearalenone .

The potential function of these specific strains may be due to the capacity to reduce the carcinogenic or toxic effect of food carcinogens by binding to them or metabolically transforming them into toxic carcinogenic degradation products (Zhou *et al.*, 2001).

Our results indicated such effect by selected strains of *S.cerevisiae* and their ability to remove FB_1 from contaminated culture media. *S.cerevisiae* could greatly prevent FB_1 toxicity and improve liver and kidney markers; enhance GSH synthesis. *S.cerevisiae* is noted for its ability to bind mutagens (Hosono *et al.*, 1990). El-Nezami *et al.*, (1998) found that the maximum of $2X10^9$ CFU/ml was required for significant of aflatoxins removal (99%) by LGG and LC705 in vitro study.

Similar results in another strain (*Bifidobacteria*) were reported by Oatley *et al.* (2000) , where authors found *Bifidobacteria* bound to 25% - 60% of the FB1 added to the media.

Our study was in agreement with hypothesis that *S.cerevisiae* and lactic acid bacilli colonies could to diminished FB_1 (a model of food carcinogens) and protect experimental animals from its oxidative stress (Nada *et al.*, 2010) , in which *S. cerevisiae* administration concomitant with FB_1 gives us the best results than that with probiotic bacteria .

Conclusion, our results improved that, the tested probiotic bacteria and yeast had no toxic effects with their free radical scavenging and antioxidant properties, seems to be a highly promising agent in preventing food contamination with *F.moniliform* and greatly inhibit toxin production *in vitro* as well as they succeeded to protect various tissues against FB1-induced oxidative damage .

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