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Growth Pattern of Starter Cultures and Antifungal Activity of Some Bacteriocins and Inulin in Skim Milk Yoghurt

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ABSTRACT

The evaluation of the potentiality for *Lactobacillus acidophilus* and/or *Lactobacillus rhamnosus* bacteriocins with 3% inulin to inhibit *Aspergillus niger* and *Saccharomyces cerevisiae* growth in skim milk yoghurt was planned in this study. The manufactured yoghurt samples were examined for the starter cultures count, *Aspergillus niger and Saccharomyces cerevisiae* counts every 3 days till the appearance of the fungal spoilage. Results demonstrated that *Aspergillus niger* was not detected in (*L. acidophilus* bacteriocin and 3% inulin + *A. niger* 4 $\log_{10}$cfu/g) and (*L. rhamnosus* bacteriocin and 3% inulin + *A. niger* 4 $\log_{10}$cfu/g) yoghurt sample all over the storage period. *Saccharomyces cerevisiae* counts were decreased by the effect of both bacteriocins; Moreover, *L. acidophilus* bacteriocin showed higher antifungal activity than *L. rhamnosus* bacteriocin with 3% inulin. The current study proved that *Lactobacillus acidophilus* bacteriocin could be used as a natural antifungal agent in yoghurt

1. INTRODUCTION

Five to ten percent of the world's food production is lost due to fungal contamination (Pitt and Hocking, 2009). Fungi are responsible for the spoilage of various dairy products especially yoghurt which is considered as a good media for fungal growth as, most fungi can grow at wide pH range of 3 to 8 and can withstand low water activity levels 0.7 to 0.8 (Krue *et al*., 2004 and Gerez *et al*., 2013). Presence of molds and yeasts in milk and dairy products are undesirable even when found in few numbers resulting in objectionable changes that render the products of inferior quality (Abdel hameed, 2011).

Mold contamination may lead to the production of mycotoxin such as aflatoxins, which cause disease of man (Nwagu and Amadi, 2010). Food spoilage by molds causes extensive economic losses to the industry and may involve health risks to consumers due to both the toxicity and pathogenicity of some species (Gerez *et al*., 2010). Currently, the use of food-grade chemical antifungal agents has become increasingly unpopular with consumers, who look for foods that free from them (Gerez *et al*., 2010 and Wang *et al*., 2012).

In modern society, there is increasing demand for safe, healthy foods with high quality and preservatives-free products (Keenan *et al*., 2011 and Dinev *et al*., 2017). In this context, biopreservation, by using beneficial microorganisms and/or their metabolites to prevent spoilage, extend the shelf-life of foods with excellent technological properties without chemical preservatives, has attracted consumer interest (Wang *et al*., 2012 and Al-Haik *et al*., 2017).

Lactic acid bacteria produce a wide range of antimicrobial metabolites which include organic acids, diacetyl, hydrogen peroxide, antibiotics, exopolysaccharides and bacteriocins (Matei and Cornea, 2014 and Vatuiu and Popa, 2015).

Bacteriocin is one of the antimicrobial metabolites produced by the LAB. It defined as ribosomally synthesized, extracellularly released bioactive peptides, which have a bactericidal or bacteriostatic effect on other species, usually closely related organisms (Sari *et al*., 2018).

A lot of new information regarding the bacteriocins of *L. acidophilus* has emerged during the last few years, its bacteriocins belonging to class II

(Anjum *et al*., 2014). *L. rhamnosus* strain 68 was confirmed to produce a bacteriocin rhamnosin A, which was a small, heat-stable, and non lanthionine-containing peptide and therefore categorized as a class II bacteriocin (Dimitrijevic *et al*., 2009).

Inulin is considered the most used and effective in relation to many species of probiotics (Cardarelli *et al*., 2008). Inulin is not digested by host enzymes and reach the colon, where it affects on the intestinal microbiota and their metabolic activities and thus made health benefits to host as modulation of lipid metabolism, enhanced absorbability of calcium, improve the immunological system and modification of the bowel function (Marteau *et al*., 2011). Inulin is recognized as a natural food ingredient in all European Union and is Generally Recognized as Safe (GRAS) status in United States (Abed *et al*., 2016).

Inulin can be used for either their nutritional advantages or technological and functional properties, but they are often applied to offer a dual benefit: improving sensory properties, fat replacer, sugar replacer, emulsion, low calories and foam stabilizer (Franck, 2007; González-Herrera *et al*., 2015).

This study was conducted to investigate the effect of bacteriocins of *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* against *Aspergillus niger* and *Saccharomyces cerevisiae* growth in skim milk yoghurt with addition of inulin (3%).

2. MATERIALS AND METHODS

2.1. Cultures preparation

Both *Lactobacillus acidophilus* DSMZ 20079 *(L. acidophilus)* and *Lactobacillus rhamnosus* ATCC7469 *(L. rhamnosus)* was obtained from Cairo MIRCEN (Microbiological Resource Center), Faculty of Agriculture, Ain Shams University, Cairo, Egypt. Each one was activated in sterile MRS broth (de Man, Rogosa and Sharp) which obtained from Biolife, Italy incubated at 37°C overnight anaerobically in anaerobic jar under CO_2 and H_2 atmosphere (Gas Pak System) (Okuro et al., 2013).

2.2. Extraction of crude bacteriocins

Lactobacillus strains were separately subcultured in one liter MRS broth at 37°C for 16 hrs. They were heated in water bath at 100° C for 5 min to get rid of H_2O_2. The culture was centrifuged at 10.000 rpm for 15 minutes and the supernatant were collected. The culture was adjusted to pH 6.0 by 1N NaOH to inactivate antimicrobial activity of organic acids. Then the extract sterilized by using 0.45 µm pore size Seitz filter with single sheet to eliminate the possible presence of viable bacterial cells to obtain cell free crude bacteriocin (Sari et al., 2018).

2.3. Preparation of fungal strains

The strains of *Aspergillus niger* and *Saccharomyces cerevisiae* were obtained from Mycology Department, Animal Health Research Institute, Dokki, Egypt. The strain was activated in 9 ml yeast extract peptone dextrose broth (YPD) and incubated at 25°C for 48 hrs, to activate the strain till obtaining the concentration of 4 $\log_{10}$cfu/ml (Delavenne et al., 2015).

2.4. Yoghurt manufacture

Lyophilized mixed starter cultures containing *Lactobacillus bulgaricus (L. bulgaricus)* and *Streptococcus thermophilus* (1:1) were obtained from Cairo MIRCEN (Microbiological Resource Center), Faculty of Agriculture, Ain Shams University, Cairo, Egypt. Lyophilized mixed starter cultures were added to sterile 11% reconstituted skimmed milk powder then incubated at 37°C/24 hrs. The actived starter cultures were kept in refrigerator until use within 24 hrs (Badawi and El- Sonbaty, 1997).

Yoghurt was prepared as described by Nighswonger et al. (1996). Fresh raw mixed cow and buffalo milk (1:1) was obtained from the herd of Faculty of Veterinary Medicine, Benha University, Egypt then milk was subjected for skimming to obtain skim milk. Inulin was obtained from Baolingbao Biology Co., Ltd., Yucheng City, Shandong, China.

The bulk volume of milk was divided into 9 groups (1 L of each), yoghurt samples supplemented with 3% inulin then mixed well. Milk was heated to 85°C for 30 min then immediately cooled to 45°C and inoculated by the activated starter cultures (*L. bulgaricus* and *Streptococcus thermophilus*) as follows:

G1: 2% yoghurt starter cultures 1:1 (control).
G2: 2% yoghurt starter cultures 1:1 + *Aspergillus niger* (4 $\log_{10}$cfu/g).
G3: 1% yoghurt starter cultures 1:1 + 1% *L. acidophilus* bacteriocin + 3% inulin.
G4: 1% yoghurt starter cultures 1:1 + 1% *L. acidophilus* bacteriocin + 3% inulin + *Aspergillus niger* (4 $\log_{10}$cfu/g).
G5: 1% yoghurt starter cultures 1:1 + 1% *L. rhamnosus* bacteriocin + 3% inulin.
G6: 1% yoghurt starter cultures 1:1 + 1% *L. rhamnosus* bacteriocin + 3% inulin + *Aspergillus niger* (4 $\log_{10}$cfu/g).

G7: 2% yoghurt starter cultures 1:1 + *Saccharomyces cerevisiae* (4 $\log_{10}$cfu/g).
G8: 1% yoghurt starter cultures 1:1 + 1 % *L. acidophilus* bacteriocin + 3% inulin+ *Saccharomyces cerevisiae* (4 $\log_{10}$cfu/g).
G9: 1% yoghurt starter cultures 1:1 + 1% *L. rhamnosus* bacteriocin + 3% inulin+ *Saccharomyces cerevisiae* (4 $\log_{10}$cfu/g).

Then the samples of each group were mixed and put into sterile cups (100 ml) and incubated at 42°C until curd formation then kept at refrigerator at 4°C.

2.5. Microbiological examination of yoghurt

The yoghurt samples were examined microbiologically at appropriate intervals till the appearance of visible fungal spoilage. The yoghurt preparation and examinations were repeated three times.

2.5.1 Preparation of serial dilutions (APHA, 2001).

2.5.2 Enumeration of yoghurt starter culture bacteria

Lactobacillus bulgaricus and *Streptococcus thermophilus* were enumerated by pour plate method (Kodaka et al., 2005). *L. bulgaricus* was counted in MRS agar (pH 5.4) under aerobic incubation at 37°C for 48 hrs. While, *Streptococcus thermophilus* was counted on M17 agar at 42°C/48 hrs (Santo et al., 2012).

2.5.3 Enumeration of *Aspergillus niger* and *Saccharomyces cerevisiae* counts

From the already prepared serial dilutions, one ml was transferred into duplicate Petri dishes and thoroughly mixed with Sabouraud dextrose agar medium supplemented with chloramphenicol (0.01%) as described by IDF (1990). The plates were incubated at 25°C for 5-7 days.

2.6. Statistical analysis

Statistical analysis of the data was done using the analysis of variance in SPSS 16.0. Statistical comparisons were made by using one-way analysis of variance (ANOVA). The results were considered significantly different with $P<0.05$ as described by Clarke and Kempson (1997).

3. RESULTS AND DISCUSSION

3.1. Microbiological profile of yoghurt sample during storage

3.1.1. Yoghurt starter culture counts

The total counts of *L. bulgaricus* in G2 (yoghurt + *A. niger* 4 $\log_{10}$cfu/g), G3 (yoghurt with *L. acidophilus* bacteriocin and 3% inulin), were increased and reached a maximum count after 8 days of refrigerated storage. However, G4 (yoghurt with *L. acidophilus* bacteriocin and 3% inulin + *A. niger* 4 $\log_{10}$cfu/g), G5 (yoghurt with *L. rhamnosus* bacteriocin and 3% inulin), G6 (yoghurt with *L. rhamnosus* bacteriocin and 3% inulin + *A. niger* 4 $\log_{10}$cfu/g), G8 (yoghurt with *L. acidophilus* bacteriocin and 3% inulin + *Saccharomyces cerevisiae* 4 $\log_{10}$cfu/g) and G9 (yoghurt with *L. rhamnosus* bacteriocin and 3% inulin + *Saccharomyces cerevisiae* 4 $\log_{10}$cfu/g) reached maximum counts after 16 days, then the counts decreased gradually until the end of the shelf life (Table, 1).

Such reduction in the *L. bulgaricus* counts may be due to low storage temperature, the acidity produced by LAB growth and other metabolites such as bacteriocin against yoghurt starter cultures (Donker et al., 2007).

These results agreed with those obtained by Dave and shah (1997) who found that the viable counts of LAB gradually increased up to the 5th day, but their survival decrease gradually during 35 days of refrigerated storage.

L. bulgaricus in fermented skim milk prepared with 4% inulin showed stable count during 7 days of storage (Oliveria et al., 2011). The addition of inulin to milk increases the viability of *L. bulgaricus* during the storage of synbiotic yoghurt (Mazloomi et al., 2011). The viability percent of *L. bulgaricus* with inulin addition (4%) throughout 28 days' storage was 78.31% and 75.79% for non-fat control yoghurt samples (Paseephol and Sherkat, 2009).

There was a significance difference in the *L. bulgaricus* count between groups with inulin addition (G3, G4, G5, G6, G8 and G9) and groups without inulin (G1 and G7) they showed higher and stable counts throughout storage especially after 16 days of storage (Table, 1) ($p<0.05$). This may be due to inulin provided additional nutrients carbon and energy essential for promoting starter culture bacteria growth and that it protects cells from acid injury (Desai et al., 2004; Makras et al., 2005; Capela et al., 2006; Aryana et al., 2007; Donkor et al., 2007; Mayo et al., 2010).

Table (1) showed higher count of *L. bulgaricus* in G7 than G1 (yoghurt control group) for 16 days of storage. These results came in agreement with those obtained by Lourens-Hattingh and Viljoen (2001) and Karaolis et al. (2013) whom found that the total LAB numbers (traditional yoghurt starter culture) were higher in yoghurt in the presence of *Saccharomyces boulardii* than control yoghurt samples without yeast during 28 days of storage.

Saccharomyces boulardii induces the growth and survival of LAB. The limited viability of LAB, as a result of their susceptibility to low pH, but it can

be reversed due *Saccharomyces boulardii*'s ability to metabolise organic acids. In commercial products, various probiotic lactobacilli and bifidobacteria show a decline in their viability during product's shelf life; therefore, the presence of *Saccharomyces boulardii* can contribute to better viability of these organisms into the yoghurt throughout its commercial life (Karaolis et al., 2013). This means that the yeast has a probiotic activity in combination with LAB to improve their viability over the storage period.

Groups with inulin showed higher and stable *Streptococcus thermophilus* counts than groups without inulin (Table, 2). The addition of inulin to milk increases the viability of *Streptococcus thermophilus* during the storage of synbiotic yoghurt (Mazloomi et al., 2011). While, the viability percent of *Streptococcus thermophilus* with 4% inulin throughout 28 days' storage were 97.35% and for non-fat control yoghurt sample was 96.74% (Paseephol and Sherkat, 2009).

Table (2) showed the mean count of *Streptococcus thermophilus* at zero time were ranged from 11.59±0.06 to 11.65±0.04 $\log_{10}$cfu/g among the all groups. The viability of *Streptococcus thermophilus* was decreased gradually till the end of shelf life of all yoghurt samples. This could be attributed to lactic acid production that made the environment unfavorable for the growth of streptococcus species (Güler-Akın et al., 2016). These results nearly similar to Saccaro et al. (2009); Kearney et al. (2011). Furthermore, there is no effect of inulin addition on *Streptococcus thermophilus* counts (Akin et al., 2007).

The *Streptococcus thermophilus* counts in G7 and G1 were 11.62±0.06 and 11.59±0.08 $\log_{10}$cfu/g at zero day; respectively. It decreased gradually till reach 8.29±0.01 and 8.05±0.10 $\log_{10}$cfu/g at 24th day of storage. G7 kept higher count than control group this may be due to probiotic activity of *Saccharomyces cerevisiae.* Incorporation of yeast showed a synergistic effect by enhancing growth and viable numbers of bacterial starter in yoghurt product (Niamah, 2017).

Saccharomyces cerevisiae as a potential probiotic can produce a variety of enzymes such as protease, cellulose, lipase and amylase (Fakruddin et al., 2017). Despite the inability to use lactose, yeast species can use glucose, galactose and organic acids as carbon source, which derived from the metabolism of lactic acid bacteria from lactose fermentation present in dairy products (Hattingh and Viljoen, 2001).

However, The *Streptococcus thermophilus* cells viability not affected in yoghurt prepared with mannan extracts from *Saccharomyces cerevisiae* in bio-yoghurt from buffalo milk for 28 days of storage (Al-Manhel and Niamah, 2017).

3.1.2. Enumeration of *Aspergillus niger* and *Saccharomyces cerevisiae* counts

Because the fungi are the most widespread food spoilage factors in fermented milk products and fungal resistance to chemical antifungal agents and other food preservatives, the use of antifungal LAB and bacteriocin as new antifungal agent as well as biopreservatives is very promising (Schnürer and Magnusson, 2005 and Lipińska et al., 2016).

Aspergillus niger can produce a variety of fungal metabolites, termed mycotoxins, depending upon growth conditions. The mycotoxins range from moderately to highly toxic. *Aspergillus niger* being the second most common pathogenic *aspergillus* spp. worldwide (Verweij and Brandt, 2007).

Aspergillus niger could be not detected in G4 (yoghurt with *L. acidophilus* bacteriocin and 3% inulin + *A. niger* 4 $\log_{10}$cfu/g) and G6 (yoghurt with *L. rhamnosus* bacteriocin and 3% inulin + *A. niger* 4 $\log_{10}$cfu/g) all over the storage period. While in G2 (yoghurt + *A. niger* 4 $\log_{10}$cfu/g) the *A. niger* count was 3.57±0.00 $\log_{10}$cfu/g at zero day and increased gradually to reach 5.94±0.20 $\log_{10}$cfu/g at 20th day of storage (Figure, 1).

The antifungal activity appears to be stronger at lower pH range therefore; this may explain antifungal activity of *L. acidophilus* bacteriocin and *L. rhamnosus* bacteriocin in yoghurt acidic media (Rouse et al., 2008).

In this respect, *L. acidophilus* bacteriocin showed high antifungal activity against *A. niger* (Yang and Clausen, 2005; Yasmin et al., 2015; Lipińska et al., 2016). *L. acidophilus* is active against a variety of pathogenic and food spoilage molds and yeasts *Aspergillus, Fusarium, Mucor, Trichoderma, Candida* spp., etc. The antifungal activity of LAB may be attributed to the metabolic activity of acidification of the cytoplasm, which affects the proton motive force of the membrane, thus directly inhibiting fungal growth (Piper et al., 2001).

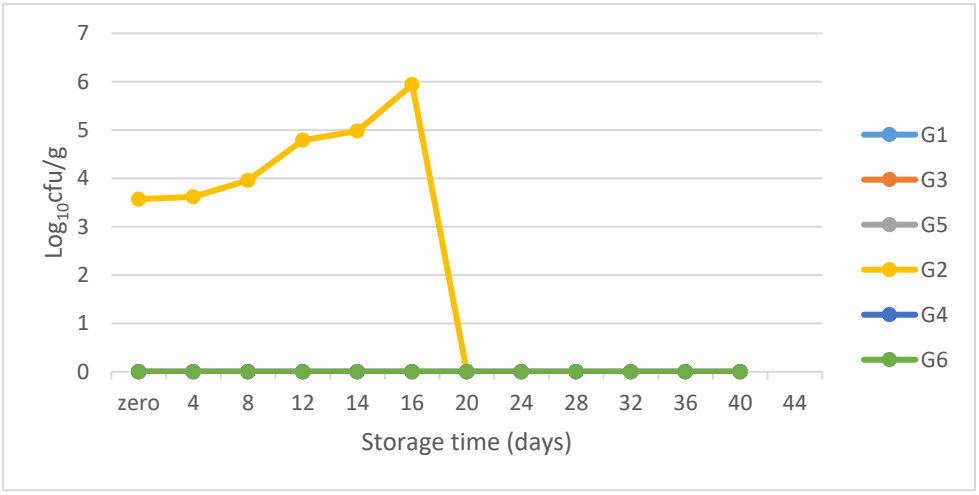

Figure (1) Count of *Aspergillus niger* (log_{10} cfu/g) for the examined yoghurt groups during their refrigeration storage (4°C).
G1: 2% yoghurt starter cultures 1:1 (control).
G2: 2% yoghurt starter cultures 1:1 + *Aspergillus niger* (4 log_{10}cfu/g).
G3: 1% yoghurt starter cultures 1:1 + 1% *Lactobacillus acidophilus bacteriocin* + 3% inulin.
G4: 1% yoghurt starter cultures 1:1 + 1% *Lactobacillus acidophilus* bacteriocin + 3% inulin + *Aspergillus niger* (4 log_{10}cfu/g).
G5: 1% yoghurt starter cultures 1:1 + 1% *Lactobacillus rhamnosus* bacteriocin + 3% inulin.
G6: 1% yoghurt starter cultures 1:1 + 1% *Lactobacillus rhamnosus* bacteriocin + 3% inulin + *Aspergillus niger* (4 log_{10}cfu/g).

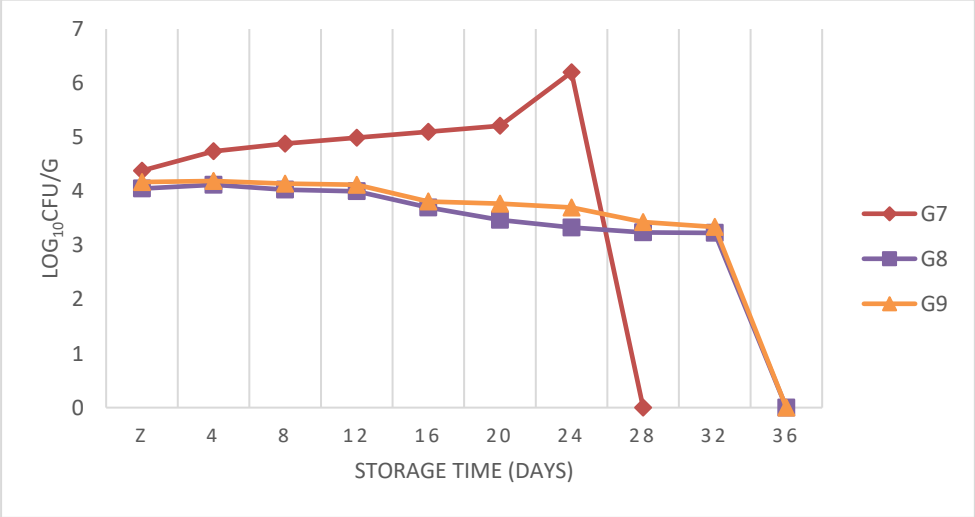

Figure (2) Count of *Saccharomyces cerevisiae* (log_{10} cfu/g) for the examined yoghurt groups during their refrigeration storage (4°C).
G1: 2% yoghurt starter cultures 1:1 (control).
G3: 1% yoghurt starter cultures 1:1 + 1% *Lactobacillus acidophilus* bacteriocin + 3% inulin.
G5: 1% yoghurt starter cultures 1:1 + 1% *Lactobacillus rhamnosus* bacteriocin + 3% inulin.
G7: 2% yoghurt starter cultures 1:1 + *Saccharomyces cerevisiae* (4 log_{10}cfu/g).
G8: 1% yoghurt starter cultures 1:1 + 1 % *Lactobacillus acidophilus* bacteriocin + 3% inulin+ *Saccharomyces cerevisiae* (4 log_{10}cfu/g)
G9: 1% yoghurt starter cultures 1:1 + 1% *Lactobacillus rhamnosus* bacteriocin + 3% inulin+ *Saccharomyces cerevisiae* (4 log_{10}cfu/g)

Table (1) Counts of *Lactobacillus delbrueckii subspp bulgaricus* (log_{10} cfu/g) for the examined yoghurt groups during their refrigeration storage (4°C).

Storage (days)	G1	G2	G3	G4	G5	G6	G7	G8	G9
Zero	12.92±0.09[a]	12.93±0.11[a]	12.91±0.13[a]	12.91±0.13 [a]	12.88±0.15[a]	12.89±0.14[a]	12.93±0.10[a]	12.90±0.13[a]	12.89±0.15[a]
4	13.65±0.06[a]	13.69±0.06[a]	13.63±0.12[a]	13.56±0.11[a]	13.58±0.13 [a]	13.60±0.12 [a]	13.62±0.09 [a]	13.59±0.12 [a]	13.58±0.12[a]
8	13.68±0.06[a]	13.74±0.04[a]	13.68±0.09[a]	13.65±0.09 [a]	13.62±0.13 [a]	13.64±0.11 [a]	13.68±0.07[a]	13.64±0.10[a]	13.64±0.09[a]
12	13.70±0.06[a]	13.74±0.06[a]	13.64±0.02[a]	13.71±0.07[a]	13.65±0.11[a]	13.69±0.08[a]	13.72±0.06 [a]	13.67±0.10[a]	13.67±0.09[a]
16	12.88±0.04[b]	13.64±0.09[a]	13.76±0.07[a]	13.74±0.07[a]	13.69±0.09 [a]	13.73±0.07[a]	12.97±0.07[b]	13.71±0.08 [a]	13.70±0.08[a]
20	11.96±0.02[a]	11.95±0.05[a]	12.04±0.03[a]	12.03±0.04[a]	12.03±0.03[a]	12.04±0.04[a]	11.78±0.06[b]	12.02±0.05[a]	12.01±0.04[a]
24	10.71±0.02[b]	S	11.62±0.08[a]	11.59±0.06[a]	11.62±0.09 [a]	11.61±0.03[a]	10.41±0.05[c]	11.53±0.08[a]	11.54±0.04 [a]
28	**S**	S	11.49±0.05[a]	11.51±0.07[a]	11.51±0.06 [a]	11.46±0.04[a]	S	11.40±0.08[a]	11.40±0.04[a]
32	**S**	**S**	10.36±0.07 [a]	S	10.43±0.04 [a]	**S**	S	10.28±0.08[a]	10.32±0.05[a]
36	**S**	**S**	10.27±0.07[a]	**S**	10.34±0.06[a]	**S**	**S**	**S**	S
40			9.13±0.13[a]		8.90±0.04[a]				
44			8.99±0.15[a]		8.01±0.04[a]				

Table (2) Counts of *Streptococcus thermophilus* (log_{10} cfu/g) for the examined yoghurt groups during their refrigerated storage (4°C).

Storage (days)	G1	G2	G3	G4	G5	G6	G7	G8	G9
Zero	11.59±0.08[a]	11.59±0.06[a]	11.64±0.05[a]	11.61±0.07[a]	11.65±0.04[a]	11.60±0.05[a]	11.62±0.06[a]	11.59±0.07[a]	11.59±0.06[a]
4	11.49±0.05[a]	11.55±0.03[a]	11.60±0.06[a]	11.56±0.05[a]	11.61±0.03[a]	11.56±0.05[a]	11.54±0.03[a]	11.56±0.07[a]	11.54±0.04[a]
8	11.38±0.02[b]	11.47±0.00[ab]	11.58±0.06[a]	11.47±0.02[ab]	11.56±0.02[a]	11.52±0.04[ab]	11.52±0.04[ab]	11.51±0.08[ab]	11.51±0.03[ab]
12	10.38±0.02[b]	10.49±0.01[a]	10.56±0.06[a]	10.50±0.02[a]	10.56±0.01[a]	10.52±0.02[a]	10.50±0.02[a]	10.50±0.03[a]	10.51±0.02[a]
16	9.32±0.03[c]	9.44±0.01[b]	10.44±0.07[a]	10.38±0.02[a]	10.44±0.00[a]	10.39±0.00[a]	9.45±0.02[b]	10.36±0.03 [a]	10.38±0.03[a]
20	8.12±0.04[c]	8.29±0.02[b]	9.39±0.07[a]	9.33±0.02[a]	9.37±0.00[a]	9.31±0.00[a]	8.28±0.02[b]	9.29±0.03[a]	9.27±0.02[a]
24	8.05±0.10[c]	S	9.37±0.05[a]	9.24±0.05[a]	9.27±0.03[a]	9.29±0.04[a]	8.29±0.01[b]	9.22±0.04[a]	9.21±0.01[a]
28	**S**	S	8.36±0.03[a]	8.25±0.05[bc]	8.27±0.02[bc]	8.32±0.01[ab]	S	8.22±0.00[c]	8.21±0.01[c]
32	**S**	**S**	7.28±0.04[a]	S	7.29±0.07[a]	**S**	S	7.18±0.03[a]	7.13±0.06[a]
36	**S**	**S**	7.22±0.04[a]	**S**	7.23±0.06[a]	**S**	**S**	**S**	S
40	**S**	**S**	7.11±0.09[a]	**S**	7.04±0.13[a]	**S**	**S**	**S**	S
44	**S**	**S**	6.94±0.19[a]	**S**	6.87±0.14[a]	**S**	**S**	**S**	S

G1: 2% yoghurt starter cultures 1:1 (control). G2: 2% yoghurt starter cultures 1:1 + *Aspergillus niger* (4 log_{10}cfu/g).
G3: 1% yoghurt starter cultures 1:1 + 1% *Lactobacillus acidophilus bacteriocin* + 3% inulin. G4: 1% yoghurt starter cultures 1:1 + 1% *Lactobacillus acidophilus* bacteriocin + 3% inulin + *Aspergillus niger* (4 log_{10}cfu/g). G5: 1% yoghurt starter cultures 1:1 + 1% *Lactobacillus rhamnosus* bacteriocin + 3% inulin.
G6: 1% yoghurt starter cultures 1:1 + 1% *Lactobacillus rhamnosus* bacteriocin + 3% inulin + *Aspergillus niger* (4 log_{10}cfu/g).
G7: 2% yoghurt starter cultures 1:1 + *Saccharomyces cerevisiae* (4 log_{10}cfu/g). G8: 1% yoghurt starter cultures 1:1 + 1 % *Lactobacillus acidophilus* bacteriocin + 3% inulin+ *Saccharomyces cerevisiae* (4 log_{10}cfu/g). G9: 1% yoghurt starter cultures 1:1 + 1% *Lactobacillus rhamnosus* bacteriocin + 3% inulin+ *Saccharomyces cerevisiae* (4 log_{10}cfu/g).
* The values indicated were the mean of three trials ± S.E (Standard Error).
[abc] Values in the same column having superscripts differ significantly ($p < 0.05$).
S: Spoiled sample

The excellent antifungal effect of *L. acidophilus* is probably due to either organic acids or other pH-dependent antifungal compounds **(**De Muynck et al., 2004). In addition, the main antifungal metabolites are the organic acids, lactic, acetic and phenyllactic (Gerez et al., 2013). Other authors confirmed that *L. acidophilus* exerts biostatic effect on the fungal growth due to the accumulation of organic acids (Denkova et al., 2013). *L. acidophilus* strain is able to detoxify the mycotoxins (Al-Haik et al., 2017).

Lactobacillus rhamnosus was inhibited the fungal growth of all aflatoxicogenic strains and aflatoxin B_1 production was reduced 95.7-99.8%, this could be due to *L. rhamnosus* is a producer of secondary metabolites, such as organic acids, bacteriocins and hydrogen peroxide (Gerbaldo et al., 2012). *L. rhamnosus* was able to remove up to 80% of Aflatoxin-B from liquid media (El-Nezami et al., 1998).

The growth and survival pattern of *Saccharomyces cerevisiae* in yoghurt was presented in Figure (2). The count of *Saccharomyces cerevisiae* was gradually decreased from zero day till 32 days of storage in G8 (yoghurt with *L. acidophilus* bacteriocin and 3% inulin + *Saccharomyces cerevisiae* 4 $\log_{10}$cfu/g) and G9 (yoghurt with *L. rhamnosus* bacteriocin and 3% inulin + *Saccharomyces cerevisiae* 4 $\log_{10}$cfu/g). The mean count of *Saccharomyces cerevisiae* in G8 was 4.05±0.28 $\log_{10}$cfu/g at zero day and decreased to 3.23±0.26 $\log_{10}$cfu/g at 32nd day of storage. While, G9 was 4.17±0.32 $\log_{10}$cfu/g at zero day and decreased to 3.34±0.30 $\log_{10}$cfu/g at 32nd day of storage.

The count of *Saccharomyces cerevisiae* in G7 (yoghurt + *Saccharomyces cerevisiae* 4 $\log_{10}$cfu/g) was 4.38±0.27 $\log_{10}$cfu/g at zero day and increased gradually to reach 6.20±0.18 $\log_{10}$cfu/g at 24th day of storage (Figure, 2). The increasing count was attributed to carbon content and energy sources readily metabolized by *Saccharomyces cerevisiae* such as monosaccharides, lactic acid and galactose which available metabolites of lactic fermentation in yoghurt medium (Al-Manhel and Niamah, 2017).

The antimicrobial effect of bacteriocin may be attributed to colonizing protein peptides mechanism and/or direct inhibition of the growth of pathogens (Dobson et al., 2012). Bacteriocins can damage or kill target cells by binding to the cell envelope-associated mannose phosphotransferase system (Man-PTS) and subsequent formation of pores in the cell membrane. It can interfere with the cell wall enzyme production leading to inhibition of their virulence factors such as potency of protease production. It can kill their target cells by inhibition of gene expression and protein production (Cotter et al., 2013).

In the current study, *L. acidophilus* bacteriocin showed more powerful antifungal activity than *L. rhamnosus bacteriocin* in skim milk yoghurt.

4. CONCLUSION

In our hypothesis, the use of bacteriocin instead of chemical preservatives can be an alternative method to inhibit the growth of fungi in fermented dairy products.

Due to the low pH of yoghurt, it was more susceptible to fungal growth so, the present work deal with the effect of bacteriocins of *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* on the growth of *Aspergillus niger* and *Saccharomyces cerevisiae* inoculated by concentration of 4 $\log_{10}$cfu/g in skim milk yoghurt with 3% inulin.

The result of the study indicated that *Lactobacillus acidophilus* bacteriocin and *Lactobacillus rhamnosus* bacteriocin have antifungal activity but the better was *Lactobacillus acidophilus* bacteriocin. Moreover, *Aspergillus niger* was more susceptible to the bacteriocins than *Saccharomyces cerevisiae*.

The supplementation of inulin led to remarkably stimulation of *Lactobacillus bulgaricus*, and *Streptococcus thermophilus* growth.

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