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Screening of Antagonistic Activity of Probiotic Bacteria against Some Food-Borne Pathogens

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Abstract The objectives of this study were to assess the microbiological quality of Egyptian yoghurt and to investigate the antagonistic activity of some probiotic bacteria against some food borne pathogens *in vitro* and in yoghurt. The results indicated a poor microbiological quality of yoghurt, the microbiological parameters recorded higher values than admissible levels. Results from the agar spot test and well diffusion assay showed the capability of probiotic bacteria to inhibit the growth of *S. aureus*, *E. coli O157:H7* and *L. monocytogenes in vitro*. Yoghurt produced from milk artificially contaminated with *S. aureus* and *E. coli O157:H7* was used for studying the ability of *L. acidophilus La-5* and *B. longum ATCC15707* to inhibit the growth of such pathogens. During the refrigerated storage the counts of both pathogens decreased significantly in the probiotic yoghurt than control one. Survival of both *L. acidophilus La-5* and B. *longum ATCC15707* in yoghurt was satisfactory. **Practical applications:** The microbial contamination of yoghurt represents not only spoilage of the product but also constitutes a high risk to human health. Survival of probiotic bacteria in yoghurt was satisfactory, which indicate the suitability of yoghurt as a vehicle for probiotic bacteria. The probiotic bacteria have the ability to prevent the growth of *E. coli O157:H7*, S. *aureus and L.monocytogenes in vitro* and in yoghurt.

Keywords: *probiotics, S. aureus, E. coli O157:H7, yoghurt, quality*

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1. Introduction

Yoghurt is the most popular fermented dairy product consumed in Egypt. It is produced by fermentation of heat treated milk. Fermentation processes promote the development of essential and safe microflora, which play a vital role in preventing the outgrowth of pathogenic and spoilage microorganisms (Tesfaye et al., 2011).

"Probiotics" are defined as" live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO 2001). *Lactobacillus* and *Bifidobacterium* species are the most commonly used as probiotics. Dairy products have been used as carrier foods for probiotic bacteria. Among dairy products, fermented milks were the most used to deliver the probiotic bacteria (Bergamini et al., 2005). The consumption of such products has health promoting effects, including immunomodulation, alleviation of lactose intolerance, anti-tumor properties, reduction in cholesterol level and anti-infection properties (Kasimoglu and Akgun 2004).

In order to produce health benefits, a sufficient number of viable microorganisms must be present throughout the entire shelf life of the product. In this regard, minimum levels for probiotic bacteria in yoghurt ranging from 10^5-10^6 cfu mL^{-1} should be present (Dave and Shah 1997; Gueimonde et al., 2004). Therefore viability and activity of the probiotic bacteria in yoghurt are important considerations. Several studies reported varying viability of various probiotic bacteria in yoghurt (Klaver et al., 1993; Modler and Villa-Garcia 1993; Medina and Jordano 1994; Samona and Robinson 1994; Nighswonger et al., 1996; Shah et al., 1995; Dave and Shah 1997; Shah and Lankaputhra 1997; Canganella et al., 2000; Donkor et al., 2006).

Probiotic bacteria have been shown to possess antagonistic activity against food-borne disease agents such as *S. aureus*, *Salmonella spp.*, *E. coli*, *L. monocytogenes* and *Cl. Perfringens* (Kasimoglu and Akgun 2004; Karagozlu et al., 2007; Millette et al., 2007). This inhibition may primarily be due to the production of inhibitory compounds such as bacteriocins, hydrogen peroxide or organic acids as well as competitive adhesion to epithelium (Karagozlu et al., 2007).

Although fermented milks are generally considered to be safe, owing to its acidic nature, process failure and contaminated raw materials have resulted in their being involved in food- borne illness. During past decades, microorganisms such as *S. aureus, E. coli O157:H7, Salmonella spp., and L. monocytogenes* were reported as the most common food-borne pathogens that are present in many foods and able to survive in fermented milk products (Pazakova et al., 1997; Dineen et al., 1998 ; Gulmez and Guven 2003; Tsegaye and Ashenafi 2005; Karagozlu et al., 2007). Several studies were conducted to

determine the growth and survival of *S. aureus and E. coli O157: H7* in yoghurt (Canganella et al., 1998; Dineen et al., 1998; Zuniga-Estrada et al., 1999; Halawa and Abouzeid 2000; Bachrouri et al., 2002; Ogwaro et al., 2002). However, there is little information on the effects of probiotic bacteria on survival and activity of *S. aureus and E. coli O157:H7* in yoghurt (Massa et al., 1997; Kasimoglu and Akgun 2004).

The main objectives of this study were to assess the microbiological quality and safety of locally produced plain yoghurt in Beni-Suef governorate, Egypt, to investigate the possible antagonistic activity of selected probiotic bacteria against *S. aureus, E. coli O157:H7 and L. monocytogenes in vitro* and to investigate the effect of *L. acidophilus La-5* and B. *longum ATCC15707* on survival of *S. aureus and E. coli O157:H7* in yoghurt during the fermentation and refrigerated storage. Moreover, the viability of *L. acidophilus La-5* and B. *longum ATCC15707* over the duration of storage was investigated to assess the possible use of yoghurt as a carrier for probiotic bacteria.

2. Materials and Methods

2.1. Microbiological and Chemical Analysis of Yoghurt

Fifty plain yoghurt samples were randomly obtained from local markets in Beni-Suef city, Egypt. All samples were transported to the laboratory under refrigerated conditions (4°C ± 2°C) in retail packages within 1-2 h of collection and analyzed immediately upon arrival.

For microbial counts, 10 gm of yoghurt samples were homogenized with 90 mL of a 0.1% sterile peptone water (Oxoid, UK) for initial dilution. Decimal dilutions were prepared with the same diluent (9 mL) and appropriate dilutions were used to enumerate coliform, faecal coliform, *E. coli, S. aureus* and yeast and mold according to Roberts and Greenwood (2003).

Regarding the chemical analysis of yoghurt, titratable acidity (TA; as lactic acid%) of yoghurt samples was determined according to the method of Bradley et al., (1992).

2.2. *In Vitro* Antimicrobial Activity Assay

2.2.1. Bacterial Strains

Lactobacillus acidophilus La-5 and Bifidobacterium longum ATCC15707 were kindly provided by Canadian Research Institute for Food Safety (CRIFS, Canada). Strains of Lactobacillus plantarum (IFPL33, IFPL81, IFPL106, IFPL119, IFPL124, IFPL150, IFPL156, IFPL162, IFPL189, IFPL207, IFPL250, IFPL252, IFPL379 and IFPL935), S. aureus, L. monocytogenes ATCC5672 and E. coli O157:H7 were provided by Consejo Superior de Investigaciones Cientificas (CSIC-Madrid, Spain). Probiotic strains were propagated in de Man Rogosa and Sharpe (MRS) broth supplemented with 0.05% L-cysteine hydrochloride (Sigma) at 37°C for 24 h under an atmosphere of 5% CO_2 for L. acidophilus La-5, anaerobically for B. longum ATCC15707 and aerobically for the others. All pathogenic strains were propagated in 10 ml of Brain Heart infusion (BHI) broth (Oxoid) at 37°C for 24 h. Growth of bacterial cultures was routinely monitored by optical density determinations at 600 nm (OD_{600}).

2.2.2. Agar Spot Test

Antibacterial activity was investigated by an agar spot test, using a colony overlay assay according to Tejero-Sarinena et al. (2012). Overnight cultures (10^7-10^9 cfu mL^{-1}) of probiotic strains were spotted (5 ul) on the surface of MRS agar and incubated at 37°C for 24 h under appropriate conditions, to allow colonies to develop. Subsequently, the plates were overlaid with 10 mL of 0.7% (w/v) BHI agar at 45°C, previously inoculated with 100 ul (10^7-10^9 cfu mL^{-1}) of an overnight culture of the indicator pathogen strain. MRS agar plates without any probiotic spot were also poured with BHI agar containing indicator pathogenic strain as a control. All the plates were incubated aerobically at 37°C for 24 h. Zones of inhibition around the spots were examined and scored.

2.2.3. Well Diffusion Assay

Cell free culture supernatants (CFCS) were obtained by centrifugation of overnight culture (10^9 cfu mL^{-1}) of probiotic strains at 12000 xg for 10 min at 4°C. The pellet was discarded and the CFCS was neutralized to pH 6.2 with 1M NaOH and filter sterilized through 0.45 um pore size filter. Aliquots (30 uL) of the CFCS were place in wells (7 mm diameter), cut in cooled MRS agar plates. The supernatant was allowed to dry for 1 h inside the wells at room temperature. The plates were overlaid with 10 mL of 0.7% BHI agar at 45°C, previously inoculated with 100 ul of an overnight culture of the selected pathogen strain (~10^7 cfu mL^{-1}). The plates were left to solidify and then incubated at 37°C for 24h. Inhibition zones around the wells were measured in mm from the edge of the wells (Ayeni et al., 2009).

2.3. Impact of Probiotic Bacteria on Growth and Survival of *S. aureus* and *E. coli O157:H7* in Yoghurt

Cow's milk was heated in a water bath at 90°C with agitation for 30 min then cooled to 45°C and inoculated with 2% (v/v) yoghurt starter cultures (Yoflex®, which contained S. thermophilus and L. delbrueckii ssp. bulgaricus, Chr. Hansen). Immediately following the addition of starter, the inoculated milk was divided into 5 portions. The first and second portions of milk were separately inoculated with L. acidophilus La-5 and B. longum ATCC15707 to give an initial inoculum of 4 × 108 and 4 × 108 cfu mL-1, respectively. Each portion was subdivided into two batches, one inoculated with S. aureus and the other inoculated with E. coli O157:H7 at rate of 3 × 108 and 2 × 108 cfu mL-1 respectively. Another two portions of milk were separately inoculated with 3 × 108 and 2 × 108 cfu mL-1 of S. aureus and E. coli O157:H7 respectively (control). The last portion was maintained without any further inoculation. All portions were incubated at 43°C until firm coagulum was formed (4-5 h). After fermentation infected and control yoghurts were stored at 4°C for 15 days. The samples were taken from milk after inoculation (0 h), at termination of fermentation (4 h) and daily during the storage period for chemical and microbiological evaluation.

The number of colony forming units (cfu/g) was determined by surface spread technique onto Sorbitol Mac Conkey agar and Eosin Methylene Blue agar for enumeration of E. coli O157:H7 and onto Baird Parker agar for enumeration of S. aureus. Plates were incubated at 37°C for 24 h and then counted for viable organisms (Roberts and Greenwood, 2003).

To enumerate L. acidophilus La-5 and B. longum ATCC15707, 0.1 mL of the serially diluted samples were pour-plated on MRS agar supplemented with 0.05% L-cysteine hydrochloride. Colonies on the plates were counted after 48 h of incubation at 37°C under appropriated conditions.

2.4. Statistical Analysis

SPSS pocket program for windows (version 16, 2007) was used for the statistical analysis. Values of different parameters were expressed as the mean ± standard error (SE). One-way analysis of variance (ANOVA) and Duncan's multiple range tests were used to determine significant differences in the measured attributes at $P < 0.05$.

3. Results and Discussion

Table 1. Microbial load of yoghurt samples (/g)

	No. of positive samples		Min	Max	Mean ± SE
	No	%			
Total coliform	37	74	3	1.1×10^3	$2.47 \times 10^2 \pm 5.69 \times 10$
Faecal coliform	24	48	3	1.1×10^3	$1.91 \times 10^2 \pm 5.15 \times 10$
E. coli	16	32	3	1.1×10^3	$9.29 \times 10 \pm 3.75 \times 10$
S. aureus	19	38	1×10^2	1.5×10^5	$1.73 \times 10^4 \pm 5.02 \times 10^3$
Yeast and Mold	42	84	4×10^2	3×10^5	$1.55 \times 10^4 \pm 8.68 \times 10^3$

Table 2. Results of Titratable acidity of examined yoghurt (%)

Number of samples	Min	Max	Mean ± SE
50	0.55	1.2	0.76 ± 0.018

Table 3. *In vitro* antimicrobial activity of probiotic strains (agar spot test)

Strain	*S. aureus*	*L. monocytogenes ATCC 5672*	*E. coli O157:H7*
L. acidophilus La-5	+++[a]	+++	+++
B. longum ATCC15707	++	++	++
L. plantarum IFPL150	++	++	++
L. plantarum IFPL (33, 81, 106, 119, 124, 156, 162, 189, 207, 250, 252, 379, 935)	+	+	+

[a]range of inhibition : +, diameter of inhibition zone 5 -10 mm; ++, 11-17 mm; +++, > 17 mm.

Table 4. *In vitro* antimicrobial activity of probiotic strains (well diffusion assay)

Strain	*S. aureus*	*L. monocytogenes ATCC 5672*	*E. coli O157:H7*
L. acidophilus La-5	+++[a]	+++	+++
B. longum ATCC15707	++	++	++
L. plantarum IFPL150	++	++	++
L. plantarum IFPL (33, 81, 106, 119, 124, 156, 162, 189, 207, 250, 252, 379, 935)	+	+	+

[a]range of inhibition : +, diameter of inhibition zone 4 -10 mm; ++, 10-20 mm; +++, > 20 mm.

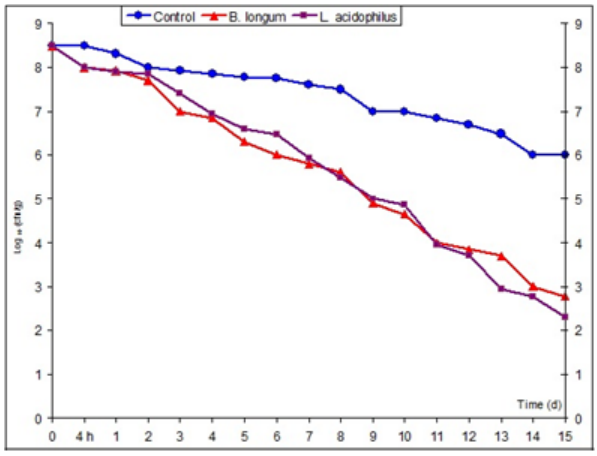

Figure 1. Behaviour of *S. aureus* during the fermentation and storage of probiotic yoghurt (L acidophilus La 5 and B longum ATCC 15707)

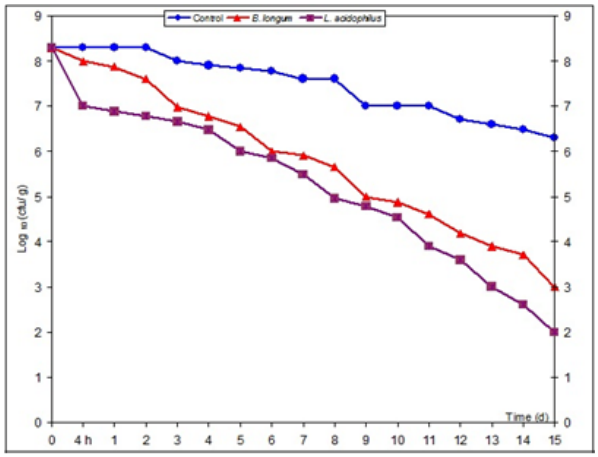

Figure 2. Behaviour of E. coli O157 during the fermentation and storage of probiotic yoghurt (L acidophilus La 5 and B longum ATCC 15707)

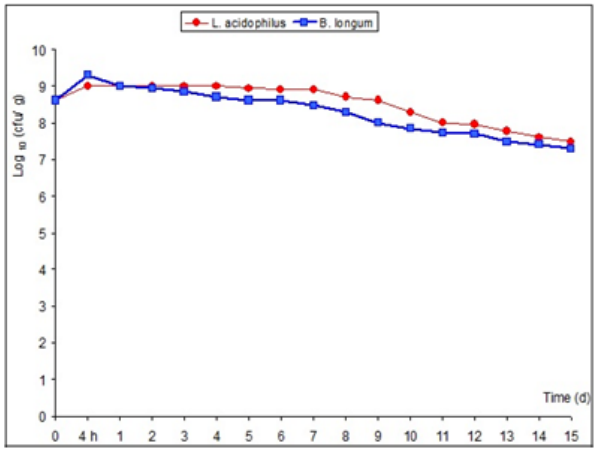

Figure 3. Survival of L acidophilus La 5 and B longum ATCC 15707 during the fermentation and storage of probiotic yoghurt

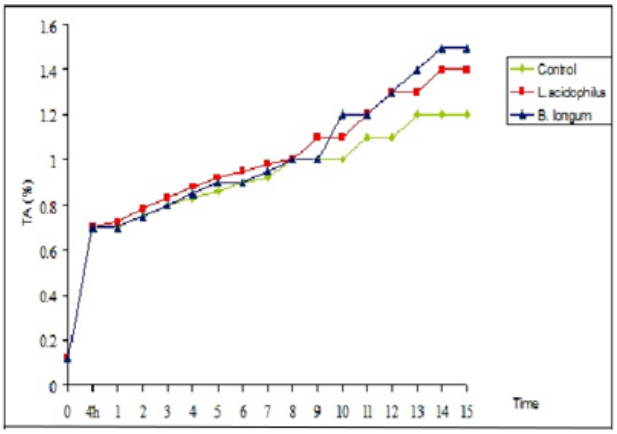

Figure 4. Changes in Titr atable acidity during the fermentation and storage of probiotic yoghurt

3.1. Microbiological and Chemical Analysis of Yoghurt

The microbiological quality of yoghurt is influenced by the initial flora of raw milk, the processing conditions and post-processing contamination. The presence of coliform bacteria in milk products generally provides an index of the hygienic standards of the product and its keeping quality. Results given in Table 1 show that the total coliforms and faecal coliforms could be detected in 74 and 48% of the examined yoghurt samples with a mean value of $2.47 \times 10^2 \pm 5.69 \times 10$ and $1.91 \times 10^2 \pm 5.15 \times 10$ MPN g^{-1}, respectively. Many reports dealing with the occurrence of coliforms in yoghurt have been accumulated. In those studies, various rates of coliforms were reported as 83.33, 82, 10, 46.7, 20 and 76% of examined yoghurt samples by Hassan (2003), El-Kasas (2004), El-Diasty and El-Kaseh (2009), El-Prince et al. (2010), Shafa (2012) and El-Malt et al., (2013) respectively.

According to the Egyptian Standards (2005), coliforms count of yoghurt should not exceed 10 cfu g^{-1}, 30 (60%) samples were found to be highly contaminated with coliforms over this limit. The existence of coliforms in yoghurt may not necessarily indicate a direct faecal contamination, but is more likely as an indicator of insufficient heat treatment of milk, use of unclean equipment, poor personnel hygiene, and water being contaminated or good manufacturing practice being not followed.

E. coli is an indicator of faecal contamination and the possibility of the presence of enteric pathogens. *E. coli* was isolated from 16 (32%) yoghurt samples with a mean count of $9.29 \times 10 \pm 3.75 \times 10$ MPN g^{-1} (Table 1). The contamination rate in yoghurt samples was generally higher than those reported by Hassan (2003), Abd Elaal (2008), Mezyed et al. (2008), El-prince et al. (2010) and Shafa (2012) as they found 26.67, 20, 20, 20 and 0% of their samples were contaminated by *E. coli* respectively. On the contrary levels of contamination as high as 47.6, 40 and 60% were reported by Aboul-Khier et al. (1985), El-Kasas (2004) and El-Malt et al. (2013), respectively.

All positive samples failed to comply with the Egyptian standards (2005), of freedom of 1 g of yoghurt from *E. coli.* Yoghurt can be easily contaminated by infected food handlers who practice poor personal hygiene or by water containing human discharges.

In the present study, 19 (38%) yoghurt samples were contaminated with *S. aureus* ranging from 10^2 to 1.5×10^5 cfu g^{-1}. All positive samples do not comply with the Egyptian standards (2005), of freedom of 1 g of yoghurt from *S. aureus* (Table 1). There are a number of studies concerning the incidence of *S. aureus* on yoghurt. Hassan (2003), Abd Elaal (2008), El-Shinawy et al., (2011) and El-Malt et al., (2013) reported 40, 28, 5 and 72% of the yoghurt samples examined in Egypt were contaminated with *S. aureus*. It obvious from the previous and the present data that yoghurt samples are frequently subjected to *S. aureus* contamination, which may indicate unhygienic handling and inadequate personal hygiene.

S. aureus is one of the most common causes of food poisoning in humans worldwide. Although all samples have lower counts of *S. aureus* than 10^6 - 10^8 cfu mL^{-1} levels that are regarded as significant for human food poisoning to occur (Kerouanton et al., 2007; Quinn et al., 2002), they still present a public health hazard. Therefore, the general hygienic practices aimed at minimizing bacterial contamination of yoghurt should be emphasized, as well as the growth of *S. aureus* must be prevented to avoid potential risk.

Neither the absence of *S. aureus* nor the presence of small numbers of organism can provide complete assurance that the yoghurt is safe, since conditions inimical to the survival of *S. aureus* may result in a diminished population or death of viable microbial cells, while sufficient toxins remains to elicit symptoms of staphylococcal food poisoning (Bennett and Monday, 2003).

The result for molds and yeasts in yoghurt are shown in Table 1. Counts for molds and yeasts ranged between 4×10^2 to 3×10^5 with an average count of $1.55\times 10^4 \pm 8.68 \times 10^3$ cfu g^{-1}. According to Egyptian standards (2005), 42 (84%) out of 50 samples tested were considered having unacceptable hygienic quality, since they were above the limit of 10 cfu g^{-1}. Nearly similar result was reported by Hassan (2003) who found 83.33% of the yoghurt samples tested was contaminated by molds and yeasts. In recent studies El-Diasty and El-Kaseh (2009), El-Shinawy et al., (2011) and El-Malt et al., (2013) reported that 50, 70 and 98% of the yoghurt samples tested were contaminated by molds and yeasts, respectively.

Contamination by molds and yeasts is one of the main limiting factors for the stability and the commercial value of yoghurt (Canganella et al., 1998). The presence of molds and yeasts in yoghurt may be attributed to contamination through air, contaminated packaging material (dust) or poor hygiene on the processing area. The presence of molds and yeasts in yoghurt are objectionable, as they grow at a wide range of temperature and pH values resulting surface discolouration, poor appearance, blowing, off-flavours and off-odours (ICMSF 2005). However, of even more serious concern is that molds are capable of producing toxic metabolites known as mycotoxins. Some of these toxins, such as aflatoxins, are known carcinogens (Bullerman, 1981).

Titratable acidity (TA) is normally used to estimate the freshness of milk and to monitor the production of lactic acid during fermentation. Results shown in Table 2 revealed that the TA ranged between 0.55 and 1.2% with a mean value of 0.76 ± 0.018%. Nearly similar TA was reported in previous study on small scale yoghurt by El-

Shinawy et al., (2011) stating that the mean value of TA was 0.75%. On the other hand, higher values of TA (1, 0.92 and 1.23%) were reported in earlier studies by Musaiger et al., (1998), Hassan (2003) and El-Kasas (2004) respectively. All samples surveyed had TA of the recommended ≤ 1.5% by Egyptian standards (2005). The development of acidity during fermentation is necessary for a well balanced aroma, texture and flavour of yoghurt as well as control the growth of harmful bacteria and some pathogens (Ozer and Kirmaci 2010).

Generally, Fermented milks, like the fresh milk from which they are produced, are liable to contamination. The microbial contamination of yoghurt at levels above the safety levels may be due to a certain extent to the use of traditional culture (starter) and manufacturing process carried out under very poor hygienic conditions.

3.2. Antimicrobial Activity of Probiotic Strains

All of the lactobacilli and *B. longum ATCC 15707* tested showed a zone of inhibition against all pathogens. *L. acidophilus La-5* showed the largest inhibition zone against all pathogens. *B. longum ATCC 15707* and *L. plantarum IFPL 150* showed strong inhibition activity against all pathogens. The remaining strains of *L. plantarum* tested showed the same degree of inhibition against all pathogens (Table 3).

The same probiotic strains were also tested by a well diffusion agar test, to study their ability to produce antimicrobial substances. We observed that the inhibitory effect against the pathogenic strains by a well diffusion assay were in accordance with the agar spot test (Table 4). The controls showed no inhibitory activity.

In vitro tests has proven useful for selecting isolates that have the ability to inhibit or compete with harmful bacteria and can provide an insight into possible mechanisms by which probiotics exert their antagonistic effects towards pathogens. The antagonistic activities demonstrated by probiotics may be due to the production of substances with antibacterial properties in particular: organic acids (e.g. lactic acid and acetic acid), hydrogen peroxide and bacteriocins (Tejero-Sariñena et al. 2012). They can also produce biosurfactants and other adhesion inhibitors and stimulate the immune response (Ayeni et al. 2009).

The potential of some Lactobacillus and Bifidobacterium spp. and there by products to control pathogens and spoilage microorganisms *in vitro* and in food systems has been evaluated by several researchers (Goktepe, 2006; Millette et al. 2007). The results found in those studies and present work demonstrate the capability of the selected probiotics to inhibit the growth of several pathogens such as *S. aureus*, *E. coli* O157:H7 and L. monocytogenes *in vitro* and the potential mechanism of action are specific to a particular strain (Tejero-Sariñena et al. 2012).

3.3. Impact of Probiotic Bacteria on Growth and Survival of *S. aureus* and *E. coli* O157:H7 during Fermentation and Storage of Yoghurt

The selection of *L. acidophilus La-5* and *B. longum ATCC15707* for incorporation in yoghurt production was based on the results of a series of *in vitro* trails regarding their antimicrobial activities.

The counts of *S. aureus* and *E. coli O157:H7* in yoghurts made with probiotic and without probiotic strains culture, during manufacture and storage at 4°C are given in Figure 1 & Figure 2.

The behaviour of *S. aureus* and *E. coli O157:H7* during the fermentation and storage of yoghurt was similar. The viable population of the *S. aureus* and *E. coli O157:H7* in yoghurt declined as the storage period extended. The counts of *S. aureus* decreased significantly ($P < 0.05$) from 8.48 log cfu mL^{-1} to 2.3 and 2.78 log cfu g^{-1} for the yoghurt co-inoculated with *L. acidophilus La-5* and *B. longum ATCC15707* respectively, whereas they decreased to 6 log cfu/g in yoghurt made without probiotic strains. The counts of *E. coli O157:H7* decreased significantly ($P < 0.05$) from 8.3 log cfu mL^{-1} to 2 and 3 log cfu g^{-1} for the yoghurt co-inoculated with *L. acidophilus La-5* and *B. longum ATCC15707* respectively, while they decreased to 6.3 log cfu g^{-1} in yoghurt made without probiotic strains.

During the storage period, survival of *S. aureus* and *E. coli O157:H7* were significantly lower ($P < 0.05$) in yoghurt with probiotic compared to that without probiotic strains that indicates the antagonistic activity against both pathogens.

The behaviour of many pathogens of concern to food safety has been extensively investigated in traditional yoghurt. In previous studies, the cells of *E. coli O157:H7* and *S. aureus* have been shown to survive in yoghurt stored at 4°C from few days to several weeks (Canganella et al., 1998, Zuniga-Estrada et al., 1999, Halawa and Abouzeid 2000, Bachrouri et al., 2002 and Ogwaro et al., 2002). On the other hand, Dineen et al., (1998) showed that *E. coli O157:H7* was not recovered after the curd formation step in yoghurt manufactured with milk inoculated with 10^5 cfu mL^{-1}. However, there are a limited number of studies about the effect of probiotic bacteria on the behaviour of *S. aureus* and *E. coli* O157:H7 in yoghurt. Massa et al., (1997) reported a slight decrease in number of *E. coli O157:H7* after 7 d of storage at 4°C. The counts decreased from 7.08 to 5.32 log cfu mL^{-1} in traditional yoghurt, and from 7.38 to 5.41 log cfu mL^{-1} in "bifido" yoghurt. Kasimoglu and Akgun (2004) reported that 10^6 cfu g^{-1} of *E. coli O157:H7* were eliminated after 48 and 72 h in acidophilus and traditional yoghurt respectively. Al-Delaimy and Hamamdeh (2013) showed that *S. aureus* was inactivated after 12 and 9 d of storage at 4°C in traditional and probiotic yoghurt respectively. The different obtained by various workers might be due to differences in strain sensitivity to pH and temperature, type of starter cultures used, inoculums' level, acid adaptation conditions and properties of food system tested (Tsegaye and Ashenafi 2005; Hsin-Yi and Chou 2001)

The viability of probiotic bacteria in yoghurt must be kept sufficiently high to ensure that consumers receive health benefits. These benefits include the prevention of diarrhea, balancing of intestinal microflora, stimulation of the immune system, antitumor properties and alleviation of lactose intolerance (Goktepe, 2006). Of particular importance, is the capacity of probiotics to antagonize pathogens (Tejero-Sariñena et al. 2012).

In order to produce these benefits, the minimum level of probiotic bacteria in yoghurt has been suggested to be

10^5-10^6 viable cells per mL or g of product (Dave and Shah 1997; Gueimonde et al., 2004).

The changes in the viable counts of *L. acidophilus La-5* and *B. longum ATCC15707* in yoghurt during manufacture and storage are given in Figure 3. Survival of both probiotics in yoghurt was satisfactory and the microbial counts remained stable with values around 8 log cfu g^{-1} throughout the storage period. Generally speaking, over 15 days storage at 4°C there was some decline in the numbers of *L. acidophilus La-5* and *B. longum ATCC15707*. Similar observations were reported by Shah et al., (1995), Dave and Shah (1997), Shah and Lankaputhra (1997), Canganella et al., (2000) and Gilliland et al., (2002). After 15 days of storage at 4°C, the yoghurt still contained ca. 7.48 and 7.3 log cfu g^{-1} of *L. acidophilus La-5* and *B. longum ATCC15707* respectively, thus satisfying the criteria for probiotic bacteria.

The viability of lactobacilli and bifidobacteria in fermented milks in the literature is variable. A poor survival for bifidobacteria in yoghurts was reported by Klaver et al., (1993) and Modler and Villa-Garcia (1993). In contrast, a satisfactory viability was reported by Medina and Jordano (1994) and Samona and Robinson (1994). Nighswonger et al., (1996) found that, although the viable counts of some of the L. *acidophilus* and *L. casei* used as adjuncts in yoghurt production declined significantly after storage, most probiotic populations were between 5 and 7 log cfu g^{-1} at the end of the products shelf life. Donkor et al., (2006) concluded that the ability of probiotic to survive in yoghurt was strain dependent. In addition L. acidophilus could survive in yoghurt at sufficient levels (> 10^6 cfu g^{-1}) for up to 28 days. Variation in the probiotic viability data among different authors may probably be attributed to strain variation, acid accumulation, interaction with starter cultures and storage condition.

The data suggest that yoghurt can be a suitable carrier food to supply consumers with lactobacilli and bifidobacterium having potential health and nutritional benefits. A similar observation was previously reported for *L. acidophilus* and *B. bifidum* by Kailasapathy and Rybka (1997), on the contrary Gilliland and Speck (1977) and Modler and Villa-Garcia (1993) considered yoghurt as unsuitable vehicle for *L. acidophilus* and bifidobacteria respectively. However, care should be used in strain selection to provide maximal survival during refrigerated storage of the yoghurt.

The acid production in yoghurt depends on the growing of microorganisms and their ability for fermentation of the lactose in milk. Yoghurt is manufactured by addition of a culture containing *S. thermophilus* and *L. delbrueckii ssp. Bulgaricus*. These organisms are active even at refrigerated temperatures and still can produce small amounts of lactic acid by fermentation of lactose resulting in noticeable TA increase (Shah et al. 1995). The TA increased over time from an initial 0.70% at termination of fermentation (4 h) to 1.2, 1.4 and 1.5% by 15 d of storage at 4°C, for control, *L. acidophilus La-5* and *B. longum ATCC15707* yoghurts respectively (Figure 4).

The concentration of lactic acid produced in control yoghurt was not significantly ($P > 0.05$) different than that in the probiotic yoghurts from day 1 until the end of storage period. A similar observation was previously reported by Donkor et al., (2006). This might imply that the inactivation of *S. aureus* and *E. coli O157:H7* in yoghurt during cold storage is not only governed by the acidity of the yoghurt but also by the presence of other inhibitory substances produced by the probiotic strains (Kasimoglu and Akgun 2004). Moreover, the lactic acid content appeared to have no effect on the viability of probiotic organisms (Akalin et al., 2004; Donkor et al., 2006).

4. Conclusion

Results of the study clearly indicate that the microbiological quality of locally produced plain yoghurt was inferior. The microbial contamination of yoghurt represents not only spoilage of the product but also indicate the presence of potential human pathogens. The presence of pathogenic bacteria as *E. coli* and *S. aureus* pose a risk for public health. Therefore, the hygienic standard needs to be strengthened during manufacture and storage to ensure production of safe, high quality yoghurt. The results obtained in this study also demonstrated the capability of the selected probiotic bacteria to inhibit the growth of *E. coli, S. aureus and L. monocytogenes in vitro*. Survival of both *L. acidophilus La-5* and *B. longum ATCC15707* in yoghurt was satisfactory as both bacteria remained viable at levels > 10^7 cfu g^{-1} after 15 d of storage at 4°C, which indicate that the yoghurt would be a suitable vehicle for probiotic bacteria. Both *L. acidophilus La-5* and *B. longum ATCC15707* have been shown to possess inhibitory activity toward the growth of *E. coli O157:H7* and *S. aureus* during the fermentation and storage of yoghurt.

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