USING OF SOME BIFIDOBACTERIA SPECIES AS BIOPRESERVATIVE CULTURES IN SOME DAIRY PRODUCTS

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ABSTRACT

The effect of using bifidobacteria spp. in the manufacturing of yoghurt and low salt soft cheese on the growth of *Escherichia coli* and *Staphylococcus aureus* was studied. When *B. bifidum* or *B. longum* was inoculated with these bacteria, the numbers of their population gradually decreased and disappeared in yoghurt on the 15th and 10th day of storage period at 4°C for *E. coli*, respectively. On the other hand, *Staph aureus* population was disappeared on the 10th and 7th day of storage period. Addition of *B. bifidum* or *B. longum* inhibited the growth of *E. coli* and *Staph aureus* in low salt soft cheese during storage at 4°C after 7 and 5 days of storage in cheese inoculated with *B. longum*, respectively. While both organisms couldn't be detected on the 7th day of storage in cheese inoculated with *B. bifidum*. The results of the current study indicated application of bifidobacteria spp. as biopreservative cultures in dairy products.

Key words: *Bifidobacteria, E. coli, Staph aureus, Biopreservative, Fermented milks*

INTRODUCTION

The use of probiotic culture to produce dairy products that claim health benefit as a part of the so called "functional foods" is a new trend in the dairy industry. The consumption of probiotic cultures positively affects the composition of gasterointestinal tract microflora and extends a range of host benefits which so far claimed to be; pathogens interference, immuno stimulation and immunomodulation, anticarcinogenic and antimutagenic activities, alleviation of symptoms of lactose intolerance, reduction in serum cholesterol, reduction in blood pressure, decrease incidence and duration of diarrhea, prevention of vaginitis and maintenance of mucosal integrity (Montes *et al.*, 1995; Klaenhammer and Kullen, 1999; Parodi, 1999).

Fermented milks are considered as one of the oldest dairy products consumed widely all over the world. They characterized by high nutritive value as they considered an excellent source of high quality protein, calcium, phosphorus, potassium, magnesium and vitamin B (Robinson, 1991; Piaia *et al.*, 2003). Fermented milks are generally fermented with a mixture of the two species, *Streptococcus salivarius subspp thermophilus* and *Lactobacillus delbrueckii subspp bulgaricus*. In recent years, fermented milks have become popular vehicles for incorporation of probiotic species (Guarner and Schaafsma, 1998). Therefore, bio-yoghurt and/or probiotic cheese that contains live probiotic bacteria with concentration of above 7 Log_{10} cfu/gm at the time of consumption in order to claim beneficial health effects (Duggan *et al.*, 2002). As bifidobacteria are the most important probiotic organisms that provide number of the above health benefits, they commonly used for preparation of new probiotic dairy products.

Bifidobacteria were grouped into 24 spp. but only 5 spp. of them (*B. bifidum, B. longum, B. infantis, B. breve* and *B. adolescentis*) have attracted attention in the dairy industry for the manufacture of functional fermented milk products (Tamime *et al.*, 1995). *B. bifidum and B. longum* characterized by a high intestinal colonization and acid resistance which make them useful for incorporation in fermented milk products (Kheadr *et al.*, 2002).

Many investigators detected food-borne spoilage and pathogens from the dairy products such as *B. cereus, B. subtilis, B. licheniformis, B. pumilus, B. coagulans, B. polymyxa, B. megatericum , Staph aureus, L. monocytogenes, E. coli, Salmonella spp., Shigella spp.* and *Yersinia pastis* (El-Zayat, 1988; El-Zayat *et al.*, 1990). The use of probiotic culture is extremely important to safe production of white pickled cheese. The traditional method of cheese manufacturing is not considered safe practice, since food-borne pathogens can survive in the absence of these cultures (Abdalla *et al.*, 1993). The antagonistic and biological

preservation of probiotic culture attributed to the inhibition of other microorganisms through competition for nutrient and/or by production of one or more antibacterial active metabolites which play an essential role in ensuring the safety and extending the shelf-life of fermented foods (Devlieghere *et al.*, 2004).

In sight of these facts, the aim of the current work is to study the incorporation of *B. bifidum* and *B. longum* in some fermented dairy products as biopreservative cultures against some pathogenic bacteria.

MATERIALS and METHODS

1. Cultures preparation

1.1. Starter cultures

Yoghurt starter cultures contain *Streptococcus salivarius subspp thermophilus* and *Lactobacillus delbrueckii subspp bulgaricus* (1:1) were obtained from Chr. Hansen's Laboratories, Horsholm, Denmark and *Bifidobacterium bifidum* ATCC 15696, *Bifidobacterium longum* ATCC 15707 were obtained from the Egyptian Microbial Culture Collection at Cairo Microbiological Resources (MIRCEN), Faculty of Agriculture, Ain Shams University, Egypt. Yoghurt starter cultures were transferred into sterile skimmed milk, then incubated at 40°C for 24 h. Both bifidobacterial strains were reactivated by three consecutive subculturing on MRS broth supplemented with 0.05% (W/V) L-cystein-HCl, then incubated anaerobically at 37°C for 18-24 h without agitation (Hull and Robert, 1984).

1.2. Target bacteria

Escherichia coli and Staphylococcus aureus were previously isolated from examined yoghurt samples in Department of Food Technology, Faculty of Agriculture, Benha University, Egypt. They were propagated till obtain approximately 6 Log_{10} cfu/ml from each strain.

2. Antibacterial activity of Bifidobacterial species in some fermented milks

2.1. Bifidus yoghurt

Yoghurt was manufactured according to Robinson and Tamime (1994). Fresh cow's milk (3% fat), obtained from dairy farm, Faculty of Veterinary Medicine, Benha University, was heated at 90 °C for 20 min, cooled to 40 °C then portioned into two parts and inoculated with *E. coli* or *Staph aureus* at 6.65 and 6.75 Log_{10} cfu/ml, respectively. Each part of inoculated milk was divided into 3 groups and was cultured with:

A) Yoghurt starter cultures, *S. thermophilus* and *Lb. bulgaricus* (1:1) at a level of 2 % for preparation of standard (control) yoghurt (T1).
B) The mixed probiotic culture contained *B. bifidum, S. thermophilus* and *Lb. bulgaricus* (1:1:1) at a level of 2 % for preparation of bifidus yoghurt (T2).
C) The mixed probiotic culture contained *B. longum, S. thermophilus* and *Lb. bulgaricus* (1:1:1) at a level of 2 % for preparation of bifidus yoghurt (T3).

The inoculated milk was packaged into plastic cups and incubated at 40°C for 6 h and then stored at 4°C. *E. coli* and *Staph aureus* counts of the resultant yoghurt were examined at zero time then after 1, 3, 7, 10 and 15 days of cold storage at 4°C. Three trials were done for this experiment.

2.2. Probiotic Cheese

Low salt soft cheese was manufactured as described by Mehanna and Rashed (1990) and El-Sheikh *et al.* (2001) with slight modification. Reconstituted skim milk powder (<1.25% fat,< 32% protein and >53% lactose) was used for manufacture of cheese and NaCl (3%) were added to milk at 37°C.

The bulk volume was divided into 6 groups, the first portion was inoculated by *E. coli* (T1), the second portion was inoculated by *Staph aureus* (T2), The third portion was inoculated with *B. bifidum* and *E. coli* (T3), the fourth portion was inoculated with *B. bifidum* and *Staph aureus* (T4), the fifth portion was inoculated with *B. longum* and *E. coli* (T5) and the sixth portion was inoculated with *B. longum* and *Staph aureus* (T6). Inoculated milks were kept at 37°C till proper curd was obtained, then the curd was kept to drain for 18 h in a previously sterilized stainless steel frames lined with cheese cloth. The obtained cheese with their respective whey were packaged in pre-sterilized aluminum cups and tightly covered with aluminum foil paper then kept at refrigerator for 15 days. Cheese samples were microbiologically examined for the counting of *E. coli* and *Staph aureus* at zero time and after 1, 2, 3, 5, 7 and 15 days. The experiment was repeated in triplicates and average results for each group were tabulated.

3. Bacteriological analysis

One gram of yoghurt was taken under aseptic conditions and used for bacteriological analysis. Also, cheese samples were homogenized with sodium citrate (2%). Ten fold serial dilutions were prepared and cultured under optimum conditions for each examined bacteria. From each prepared dilution, 0.1 ml was transferred and evently spread over a dry surface of EMB and Baird Parker plates for enumeration of *E. coli* and *Staph aureus*, respectively, and the inoculated plates were incubated at 37 °C for 24-48 h (APHA, 1992).

4. Statistical analysis

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).

RESULTS

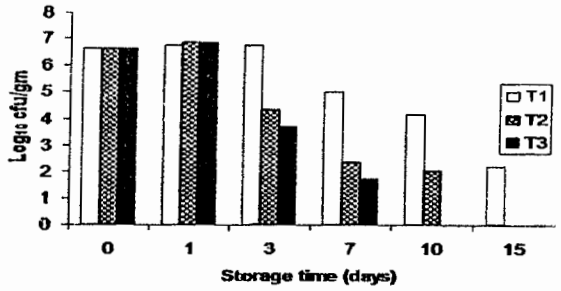

Figure 1

Viability of *E. coli* in bifidus yoghurt samples during refrigerated storage
T1: Yoghurt prepared with 2% yoghurt starter culture
T2: Yoghurt prepared with 2% *B. bifidum* + standard yoghurt starter cultures
T3: Yoghurt prepared with 2% *B. longum* + standard yoghurt starter cultures

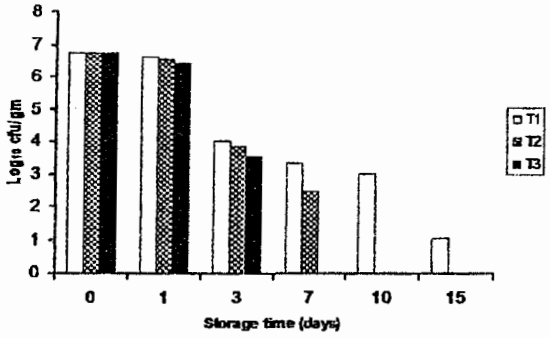

Figure 2

Viability of *Staph aureus* in bifidus yoghurt samples during refrigerated storage
T1: Yoghurt prepared with 2% standard yoghurt starter culture
T2: Yoghurt prepared with 2% *B. bifidum*+ standard yoghurt starter cultures
T3: Yoghurt prepared with 2% *B. longum* + standard yoghurt starter cultures

Table 1: Effect of bifidobactaria species on the growth of *E. coli* and *Staph aureus* in low salt soft cheese.

Storage time (days)	T1	T2	T3	T4	T5	T6
0	6.34 ± 0.18^{a1}	6.21 ± 0.14^{ab1}	6.34 ± 0.11^{ab2}	6.21 ± 0.20^{b1}	6.34 ± 0.10^{a2}	6.21 ± 0.13^{ab1}
1	6.38 ± 0.11^{a1}	6.48 ± 0.13^{a1}	6.37 ± 0.21^{b1}	6.24 ± 0.08^{c2}	6.34 ± 0.16^{c2}	6.00 ± 0.11^{ac1}
2	6.48 ± 0.08^{a3}	6.66 ± 0.09^{b1}	6.00 ± 0.14^{bc3}	5.65 ± 0.17^{bc2}	5.00 ± 0.16^{c3}	5.12 ± 0.15^{bc2}
3	6.54 ± 0.13^{a3}	6.75 ± 0.10^{b1}	4.21 ± 0.08^{b3}	4.35 ± 0.22^{ab1}	4.72 ± 0.20^{a1}	3.30 ± 0.09^{c2}
5	6.35 ± 0.2^{a2}	6.79 ± 0.15^{a2}	3.00 ± 0.10^{c2}	1.04 ± 0.15^{a3}	2.38 ± 0.13^{b1}	Nil
7	5.00 ± 0.09^{a2}	6.33 ± 0.11^{b2}	ND	ND	ND	ND
15	4.43 ± 0.14^{a1}	6.33 ± 0.11^{c1}	ND	ND	ND	ND

T1: Soft cheese inoculated with *E. coli* (control)
T2: Soft cheese inoculated with *Staph aureus* (Control)
T3: Soft cheese inoculated with *E. coli* and 8 Log_{10} cfu/ml of *B. bifidum*
T4: Soft cheese inoculated with *Staph aureus* and 8 Log_{10} cfu/ml of *B. bifidum*
T5: Soft cheese inoculated with *E. coli* and 8 Log_{10} cfu/ml of *B. longum*
T6: Soft cheese inoculated with *Staph aureus* and 8 Log_{10} cfu/ml of *B. longum*
ND: Not detected
abc Values in the same row having different superscripts differ significantly ($P < 0.05$).
123 Values in the same column having different superscripts differ significantly ($P < 0.05$).
The values indicated were the mean of three trials $\pm$ SD (Standard Deviation).

DISCUSSION

1. Viability of *E. coli* and *Staph aureus* in bifidus yoghurt

The genus *Bifidobacterium* has been extensively studied because of its beneficial effects on health, especially the protection of the intestinal tract from microbial infection (Hamilton-Miller, 2003; Asahara *et al.*, 2004). Several mechanisms have been proposed to explain the efficacy of bifidobacteria in preventing infection. Current study showed that the initial count of *E. coli* increased at the first day of storage in all samples of yoghurt (T1, T2 and T3) from 6.65 Log_{10} cfu/g to be 6.74, 6.88 and 6.85 Log_{10} cfu/g, respectively. The count of *E. coli* decreased gradually in T2 and T3 samples on the 3rd day of storage with the mean values of 4.31 and 3.72 Log_{10} cfu/g, respectively. Then the reduction in bacterial population of T2 samples continued till 10th day of storage recording a mean count of 2.04 Log_{10} cfu/g and disappeared completely on the 15th and 10th day of storage in T2 and T3, respectively (Fig. 1).

While in control T1 sample, the count of *E. coli* was still high on the 3rd day of storage with a mean value of 6.74 Log_{10} cfu/g and began to decrease to be 5.00, 4.22 and 2.25 Log_{10} cfu/g at 7th, 10th and 15th day of storage, respectively (Fig. 1). The obtained data concluded that the number of *E. coli* decreased by the end of storage period of yoghurt. However, the best bactericidal effect was observed in T2 and T3 samples and the lower effect was belonging to T1 sample. Similar results were reported by Farghaly (2004); Amer *et al.* (2010); Abdel-Aziz (2011).

Acid tolerance of *E. coli* was recorded in many researches as Halawa and Abou Zeid (2000) who found that *E. coli* persisted for up to 25 days in manufactured yoghurt. Benjamin and Datta (1995) who concluded that no loss of viability of *E. coli O157:H7* at pH of 3.0 and 2.5 for 5 h. Moreover King *et al.* (2010) mentioned that *E. coli O157:H7* may have a greater ability to survive in more complex acidic environment such as those in the host and during food processing. It seems that the survival of *E. coli* in both low temperature and pH confirmed the implication of acidic food on some outbreaks due to *E. coli* infection (Sharp *et al.*, 1995). As shown in this study, application of *B. bifidum* and *B. longum* in yoghurt manufacturing can restrict *E. coli* growth which claim to overcome the severe outbreaks from *E. coli* infection.

Data for the behavior of inoculated *Staph aureus* in cold storage yoghurt have been shown in Fig. (2). The population of *Staph aureus* showed decreased count in all yoghurt samples from 6.75 Log_{10} cfu/g to be 6.62, 6.51 and 6.38 Log_{10} cfu/g in T1, T2 and T3 on the first day of storage, respectively. Then its counts became 4.00, 3.85 and 3.55 Log_{10} cfu/g, respectively on the 3rd day of storage. Disappearance of *Staph aureus* population was observed on the 7th day of storage in T3 sample followed by T2 sample on the 10th day of storage, while the microorganism remain viable on 15th day of storage in T1 sample. Similar results were obtained by Ahmed *et al.* (2002); Lengkey and Adriani (2009); Abdel-Aziz (2011). In addition, El-Shibiny *et al.* (2005) reported that bifidus yoghurt made from *B. bifidum* had almost similar flavor to standard yoghurt and both were very acceptable with good sensory characteristics which prevailed for 10 days of storage.

2. Viability of *E. coli* and *Staph aureus* in low salt soft cheese

E. coli counts recorded in Table 1 showed slightly decrease during storage period for control cheese (T1). On the other hand, addition of *B. bifidum* to cheese milk (T3) decrease of *E. coli* counts was pronounced, whereas *E. coli* disappeared in cheeses made with addition of either *B. bifidum* (T3) or *B. longum* (T5) on the 7th day of storage. However, it remained in T1 up to 15 days of storage (Table 1). The above results are in agreement with that of Denkova et al. (2013) who indicated that the addition of *B. bifidum* to skimmed milk could reduce *E. coli* population from 9.1 Log_{10} cfu/ml at zero time to zero after 72 h from incubation.

Lactic acid bacteria have potential applications as biopreservatives in the food industry (O'Sullivan *et al.*, 2002). It inhibits the growth of some food spoilage and food-borne pathogens, such as *Listeria monocytogenes*, *Staphylococcus aureus*, *E. coli* and *Clostridium botulinum* (Cintas *et al.*, 1997).

Staph aureus has been incriminated in many outbreaks of food poisoning. Cases of enterotoxin, food poisoning caused by consumption of cheese heavily infected with *Staph aureus* were observed. So their presence in large numbers in cheese regard as public health hazard. The growth of *Staph aureus* was recorded along of storage period in control cheese samples (T2). Its population increased significantly from 6.21 Log_{10} cfu/ g at zero time to 6.79 Log_{10} cfu/g at the 5th day then lowered to 6.33 Log_{10} cfu/g on 15th day of storage (Table 1). This increase may be due to effect of salt that stimulate the growth of this bacterium (El-Zayat *et al.*, 2008). However, Ahmed (1990) reported that the count of *Staph aureus* was affected by starter cultures and salt concentration.

On the other hand, for cheese made with *B. bifidum* (T4) and *B. longum* (T6), the count of *Staph aureus* was reduced significantly from 6.21 Log_{10} cfu/g at zero time to 1.04 Log_{10} cfu/g and 3.30 Log_{10} cfu/g at 5th and 3rd days of storage, respectively. Then it was not detected on the 7th and 5th days of storage for T4 and T6, respectively (Table 1).

The current results are in agreement with that of Osman (1995), who reported that *Staph aureus* growth was inhibited by use of lactic starter culture during manufacturing of white soft cheese. Denkova *et al.* (2013) reported that *Staph aureus* is sensitive to *B. bifidum* when added to sterile skimmed milk and could reduce the population from 8 Log_{10} cfu/ml at zero time to be undetectable at 3rd day. El-Abd *et al.* (2003) studied the effect of some LAB on the properties of low salt Domiati cheese and found that spore-forming and Staphylococci in the resultant cheese were lower than that of control cheese as well as the treated cheese showed a clear improved aroma, taste and marked early full ripening. In the same context, El-Shibiny *et al.* (2005) reported that soft cheese made with addition of *B. bifidum* was of good, acceptable flavor, smooth body, easily cut and handled with extended shelf-life to 20 days.

In general, probiotic soft cheese with 3% salt was superior to the control cheese and this superiority has been shown from the ability of both bifidobacterial species to inhibit the growth of both *E. coli* and *Staph aureus*. In the same context, Sobeih *et al.* (2011) reported that low salt soft cheese (3% NaCl) with added *Lactobacillus acidophilus* culture at concentration of 3% had better organoleptic score, micrbiological quality and prolonged shelf-life (24 days) at refrigerated storage.

From the results achieved above, it was noticed that bifidobacteria had an inhibitory activity against *E. coli* and *Staph aureus* more than that of yoghurt culture bacteria and control soft cheese. This is may be due to the antimicrobial agents produced by bifidobacteria such as organic acids; mainly acetic and lactic acids (Bruno and Shah, 2002) and bacteriocins (Murad *et al.*, 2000; Shah, 2001). To date, some bacteriocins such as bifidin 1 (Cheikhyoussef *et al.*, 2010), bifidocin B (Yildirim and Jhonson, 1998; Yildirim *et al.*, 1999) and bacteriocin-like inhibitory substances (BLIS) (Collado *et al.*, 2005; Cheikhyoussef *et al.*, 2009) have been reported to be produced by bifidobacteria, and have an inhibitory activities against wide range of Gram-positive and Gram-negative bacteria.

REFERENCES

Abdalla, M.; Davidson, P. and Christen, L. (1993): Survival of selected pathogenic bacteria in white pickled cheese made with lactic acid bacteria or antimicrobials. J. Food Prot., 56, 11: 972-976.

Abdel-Aziz, M.M. (2011): The role of bifidobacteria in improvement of physical, chemical and microbiological quality of fermented dairy products. Ph.D Thesis, Fac. Vet. Med., Benha Univ.

Ahmed, A.H. (1990): Prevalence and survival of enteropathogenic *E. coli* in yoghurt. Assiut Vet. Med. J., 22, 44: 95-100.

Ahmed, A.; Aideid, H.A.; Sherif, M.M. and Wehba, H.H. (2002): Effect of bifidobacteria on some pathogenic bacteria in yoghurt and on serum cholesterol level in rats. J. Egypt. Vet. Med. Assoc., 62, 66: 43-56.

Amer, A.; Aiad, A. and Abd-Allah, M. (2010): Effect of yoghurt processing and ice-cream manufacture on viability of some food borne bacteria. Assiut Vet. Med. J., 56, 127: 108-119.

APHA (American Public Health Association) (1992): Compendium of Methods for the Microbiological Examination of Foods. 3rd ed., Public Health Association, Washington DC, USA.

Asahara, T.; Shimizu, K. and Nomoto, K. (2004): Probiotic bifidobacteria protect mice from lethal infection with Shiga toxin producing *Escherichia coli O157:H7*. Infect. Immun., 72, 4: 2240-2247.

Benjamin, M.M. and Datta, A.R. (1995): Acid tolerance of enterohemorrhagic *Escherichia coli*. J. Appl. Environ. Microbiol., 61: 1669-1672.

Bruno, F.A. and Shah, N.P. (2002): Inhibition of pathogenic and putrefactive microorganisms by bifidobacterium spp. Milchwiss., 57: 617-621.

Cheikhyoussef, A.; Pogori, N.; Chen, H.Q.; Tian, F.W.; Chen, W. and Tang, J. (2009): Antimicrobial activity and partial characterization of bacteriocin-like inhibitory substances (BLIS) produced by *Bifidobacterium infantis* BCRC 14602. Food Control, 20, 6: 553-559.

Cheikhyoussef, A.; Cheikhyoussef, N.; Chen, H.Q. ; Zhao, J.; Tang, J.; Zhang, H. and Chen, W. (2010): Bifidin 1- A new bacteriocin produced by *Bifidobacterium infantis* BCRC 14602: Purification and partial amino acid sequence. Food Control, 21, 6: 746-753.

Cintas, L.M.; Casaus, P. and Havarstein, L.S. (1997): Biochemical and genetic characterization of enterocin P, a novel sec-dependent bacteriocin from *Enterococcus faecium* P13 with a broad antimicrobial spectrum. Appl. Environ. Microbiol., 63, 11: 4321-4330.

Clarke, G.M. and Kempson, R.E. (1997): Introduction to the design and analysis of experiments. Arnold, a Member of the Holder Headline Group. 1st ed., London, UK.

Collado, M.C.; Hemandez, M. and Sanz, Y. (2005): Production of bacteriocin-like inhibitory compounds by human fecal bifidobacterium strains. Food Prot., 68, 5: 1034-1040.

Denkova, R.; Denkova, Z.; Yanakieva, V. and Blazheva, D. (2013): Antimicrobial activity of

probiotic lactobacilli, bifidobacteria and propionic acid bacteria, isolated from different sources. Microbial Pathogens and Strategies for Combating them: Science, Technology and Education (Méndez-Vilas, A. Ed.)

Devlieghere, F.; Vermeiren, L. and Debevere, J. (2004): New preservation technologies: possibilities and limitations. Int. Dairy J., 14, 4: 273-286.

Duggan, C.; Gannon, J. and Allan Walker, W. (2002): Protective nutrients and functional foods for the gaterointestinal tract. Am. J. Clin. Nutr., 75, 5: 789.

El-Abd, M.; Abd El-Fattah, A.; Osman, S. and Abd El-Kader, R. (2003): Effect of some lactic acid bacteria on the properties of low salt domiati cheese. Egypt. J. Dairy Sci., 31: 125-138.

El-Sheikh, M.M.; Farrage, A.F.; Shahein, N.M. and El- Shibiny, S. (2001): Low fat Domiati cheese with particulate whey protein concentrate (PWPC). Egypt. J. Dairy Sci., 29: 331-342.

El-Shibiny, S.; Metwally, M.M.; Abd El-Gani, S.; Abd El-Fattah, A.M. and Okda, A.Y.M. (2005): Manufacture of some probiotic dairy products from ultrafiltered milk retentate. Egypt. J. Dairy Sci., 33: 215-227.

El-Zayat, A. (1988): The mode of action and cell destruction of disinfectants. Ph.D Thesis, Fac. Agric. Sci., Catholic Univ. Louvain, Belgium.

El-Zayat, A.; El-Safty, M.; El-Bagoury, E. and El-Backary, A. (1990): The microbial quality of raw materials and end products in Misr Milk and Food company. Egypt. J. Food Sci., 16: 151.

El-Zayat, A.; Ahmed, H.A.; El-Backary, A.S. and Osman, M.M. (2008): Using of lactobacillus isolates as biopreservative cultures in some dairy products. Egypt. J. Dairy Sci., 36: 13-30.

Farghaly, M.H. (2004): Studies on *Escherichia coli O157:H7* in milk and some milk products. Ph.D Thesis, Fac.Vet. Med., Assiut Univ.

Guarner, F. and Schaafsma, G.J. (1998): Probiotics. Int. J. Food Microbiol., 39, 3: 237-238.

Halawa, M.A. and Abou Zeid, A.M. (2000): Behavior of enterohemorrhagic *Escherichia coli* and enterotoxigenic *Staphylococcus aureus* in yoghurt and acidified milk. Vet. Med. J., Giza, 48, 2: 319-326.

Hamilton Miller, J.M. (2003): The role of probiotics in the treatment and prevention of *Helicobacter pylori infection.* Int. J. Antimicrob. Agents, 22, 4: 360-366.

Hull, P.R. and Robert, A.V. (1984): Differential enumeration of *Lactobacillus acidophilus* in yoghurt. Aust. J. Food Technol., 3: 160-164.

Kheadr, E.E.; Abd-El-Rahman, A.M. and El-Nemr, T.M. (2002): Survival of antimicrobial capacity of bifidobacteria and yoghurt bacteria during refrigerated storage of yoghurt made from lactose-hydrolyzed milk. Alex. J. Agric. Res., 47, 2: 81-91.

King, T.; Lucchini, S.; Hinton, J.C.D. and Gobius, K. (2010): Transcriptomic analysis of *Escherichia coli O157:H7* and K-12 cultures exposed to inorganic and organic acids in stationary phase reveals acidulant- and strain-specific acid tolerance responses. Appl. Environ. Microbiol., 76, 9: 6514-6528.

Klaenhammer, T.R. and Kullen, M.J. (1999): Selection and design of probiotics. Int. J. Food Microbiol., 50, 1-2: 45-57.

Lengkey, H.A.W and Adriani, L. (2009): Effects of milk fermented with *Lactobacillus acidophilus* and *bifidobacterium spp.* on lactic acid and acetic acid content and on *Staphylococcus aureus* and *Pseudomonas aeruginosa.* Biotechnol. Animal Husbandry, 25 (5-6): 719-724.

Mehanna, A.S. and Rashed, M.A. (1990): An attempt to improve the keeping quality of Tallaga cheese by using milk treated with carbon dioxide. Egypt. J. Dairy Sci., 18: 377-388.

Montes, R.G.; Bayless, T.M.; Saavedra, J.M. and Perman, J.A. (1995): Effects of milks inoculated with *Lactobacillus acidophilus* or a yoghurt starter culture in lactose-maldigesting children. J. Dairy Sci., 78, 8: 1657-1664.

Murad, H.A.; Fatma, A.F. and Zeinab, I.S. (2000): Bacteriocinogenic effect of *Bifidobacterium bifidum* and some strains of lactic acid bacteria on growth of *Staphylococcus aureus.* Minufiya J. Agric. Res., 25, 3: 631-647.

O'Sullivan, L.; Ross, R.P. and Hill, C. (2002): Potential of bacteriocin-producing lactic acid bacteria for improvements in food safety and quality. Biochimie J., 84, 5-6: 593-604.

Osman, E. (1995): Behavior of *Staphylococcous aureus* in Turkish feta cheese during manufacture and ripening. J. Food Prot., 58, 11: 1201-1205.

Parodi, P.W. (1999): The role of intestinal bacteria in the causation and prevention of cancer: modulation by diet and probiotics. Aust. J. Dairy Technol., 54, 2: 103-121.

Piaia, M.; Antoine, J.M.; Mateos-Guardia, J.A.; Leplingard, A. and Lenoir-Wijnkoop, I. (2003): Assessment of the benifits of live yoghurt: Methods and markers for in vivo studies of the physiological effects of yoghurt cultures. Microbiol. Ecol. Health Dis., 15, 2-3: 79-87.

Robinson, R. (1991): Therapeutic Properties of Fermented Milks. Elsevier Science Publishers Ltd, London.

Robinson, R.K. and Tamime, A.Y. (1994): Manufacture of Yogurt and other fermented milks. In: Modern Dairy Technology, Advances in Milk Products. Robinson, R.K.

(Ed.). London, Elsevier Applied Science. pp.: 1-48.

Shah, N.P. (2001): Functional foods from probiotics and prebiotics. J. Food Technol., 55, 11: 46-53.

Sharp, J.C.M.; Reilly, W.J.; Coia, J.E.; Curnow, J. and Synge, B.A. (1995): Escherichia coli O157:H7 infection in epidemiological overview, 1984-1994. PHLS Microbiol. Digest., 12: 134-140.

Sobeih, A.M.; E.M. Ibrahim, E.M. and Elbarbary, H.A. (2011): Effect of Lactobacillus acidophilus on shelf-life of low salted cheese. Assuit Vet. Med. J., 57: 22-36.

Tamime, A.Y.; Marshall, V.E. and Robinson, R.K. (1995): Microbiological and technological aspects of milks fermented by bifidobacteria. J. Dairy Res., 62, 1: 151-187.

Yildirim, Z. and Jhonson, M.G. (1998): Characterization and antimicrobial spectrum of bifidocin B, a bacteriocin produced by *Bifidobacterium bifidum* NCFB. J. Food Prot., 61, 5: 47-51.

Yildirim, Z.; Winters, D.K. and Johnson, M.G. (1999): Purification of amino acid sequence and mode of action of bifidocin B produced by *Bifidobacterium bifidum* NCFB. J. Appl. Microbiol., 86, 1: 45-54.

استخدام بعض عزلات من البايفيدو بكتيريا كمزارع حفظ حيوية لبعض منتجات الألبان

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تم دراسة تأثير استخدام بعض الأنواع من البكتيريا التابعة لجنس البايفيدو بكتيريا أثناء تصنيع الزبادى والجبن الطري ذو الملح المنخفض على نمو نوعين من البكتيريا غير المرغوبة وهما الايشريشيا كولاي والمكورات العنقودية الذهبية. عندما لقحت *B. bifidum* أو *B. longum* مع البكتيريا غير المرغوبة في اللبن الزبادى حدث انخفاض تدريجى فى أعداد البكتيريا غير المرغوبة واختفت تماما في الزبادى عند اليوم العاشر والسابع من فترة التخزين عند ٤ درجة مئوية بالنسبة لميكروب الايشريشيا كولاي، على التوالي. من ناحية أخرى، اختفى ميكروب المكورات العنقودية في اليوم السابع والخامس من فترة التخزين، على التوالي. وقد أدى إضافة *B. bifidum* أو *B. longum* للبن المعد لصناعة الجبن الطرى الى تثبيط نمو الايشريشيا كولاي والمكورات العنقودية الذهبية بعد ٧ أيام من التخزين عند ٤ درجة مئوية و ٥ أيام لميكروب المكورات العنقودية في الجبن المضاف معه *B. longum* وأشارت نتائج الدراسة الحالية الى امكانية تطبيق واستخدام مزارع البايفيدو بكتيريا كمزارع حفظ حيوية عند تصنيع منتجات الألبان.