The Effect of Probiotics on Staphylococcus Aureus and E. Coli in Minced Meat

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A B S T R A C T

Nowadays, all interested parties in the field of food safety are shifted to use natural food preservatives instead of chemical ones which proved to have many draw backs either on human health or food composition. The present study was conducted to study the effect of using two probiotic strains (*Lactobacillus acidophilus* and *Bifidobacteriumlactis*) individually on the growth and survival of some food-borne pathogens represented by *Staphylococcus aureus* and *Escherichia coli* experimentally inoculated separately into fresh minced beef, previously gamma irradiated using 5 KGy to be sure that samples were free from microorganisms under investigation during storage at 4°C.The obtained results revealed that the effect of *Lactobacillus acidophilus* on the reduction of *Staph.aureus* count was almost identical to the effect of *Bifidobacteriumlacti*s. Moreover, *Staph.aureus* growth persisted till the 6th day of storage, while the organism was completely inhibited at the 8th day of the experiment. *Bifidobacteriumlacti*s was more effective in reducing *E. coli* count through the 8 days of experimental study than *Lactobacillus acidophilus*. Overall, *E. coli* could persist till the end of the experimental period in the presence of both probiotics. The maximum reduction % of *E. coli* count reached 2.0 $\log_{10}$cfu/g (46.95%) in experimental samples using *Bifidobacteriumlacti*s.

Keywords: Minced meat, Probiotics, *Staph. aureus*, *E. coli*, *Lactobacillus acidophilus*, *Bifidobacteriumlacti*s and Radiation.

(http://www.bvmj.bu.edu.eg) **(BVMJ-34(1): 242-253, 2018)**

1. INTRODUCTION

Meat products are highly demanded due to their high biological value, reasonable price, agreeable taste and easily serving. On the other hand, these benefits come over the safety and quality of such food items because the vendors have lack information about the basic food safety rules and the principles of health culture. Unfortunately, meat products are subjected to contamination with several types of microorganisms from different sources during preparation, processing as the contamination occurs primarily from raw materials, grinding of meat which will spread exterior contamination essentially throughout the entire muscle mass, post processing handling, cross contamination and/or equipments, lack of refrigeration facilities, ambient temperatures above 20°C, lack of suitable transportation between the production

and marketing areas and improper storage temperature (Gibbons *et al.,* 2006).

Staphylococcal food-borne disease (SFD) is one of the most common food-borne diseases worldwide, resulting from the consumption of food that already contaminated by preformed *Staph. aureus* enterotoxins. Presence of pathogens in food products imposes potential hazard for consumers and causes grave economic loss and loss in human productivity (Jhalka *et al*., 2014).

E. coli is a human pathogen worldwide associated with meat and meat products, dairy products, vegetables and water. It is recognized as a bacterium causing hemorrhagic colitis. Diarrheal diseases linked to *E. coli* infections are characterized by blood, cramping, abdominal pain, fever, nausea, and vomiting (Abongo and Momba, 2009)

Food preservation is a continuous effort which aims either to eliminate or reduce the out-growth potential of spoilage and pathogenic microorganisms in foods. Until now, approaches to improve food safety have relied on chemical preservatives, antibiotics or through application of more drastic physical treatments using high temperature or refrigeration. Nevertheless, these methods have many drawbacks on the product quality (Rassoli, 2007).

Nowadays, consumers demand high quality, additive-free, safe, healthy, nutritious, vitamin-rich, minimally-processed, freshly taste and functional foods with extended shelf life and a natural or green image (Sarika *et al*., 2010). Applied research is ongoing to replace chemicals such as nitrite, sulfite, etc. by alternative means such as functional starter and/ or co-cultures for instance LAB to prolong the shelf-life of foods (De Vuyst, 2000)

The word "probiotic" comes from the Greek words "pro" and "biotic," meaning "for the life." Examples are LAB that are able to produce antimicrobial substances, sugar polymers, sweeteners, aromatic compounds, useful enzymes, or LAB with health-promoting properties, so called probiotic strains (Gregoria *et al*., 2013). This represents a way of replacing chemical additives by natural compounds, at the same time providing the consumer with new, attractive food products. The most commonly used probiotic microorganisms are *Lactobacillus* and *Bifidobacterium.* A major effort has been made to develop meat-based functional foods using strategies related to increasing the presence of beneficial compounds and limiting those with negative health implications (Carlos *et al*., 2015).

Therefore, the present study was carried out to study the effect of both *Lactobacillus acidophilus* and *Bifidobacteriumlactis* probiotics on improving the bacterial safety of minced beef inoculated with food borne pathogenic bacteria including *Staph. aureus* and *E. coli* and stored at 4^{o}C.

2. MATERIALS AND METHODS

2.1. Collection and preparation of inoculated minced meat sample:

Raw minced meat sample (1200gm) was collected from supermarket and transported immediately to the laboratory in an ice box. Collected sample was prepared by packing in polyethylene package and sterilized by radiation by being exposed to Gamma radiation of 5 kGy dose (the source of Gamma irradiation was cobalt-60) at the National Center for Radiation Research and Technology (NCRRT) Nasr city, Cairo, Egypt, then divided into two equal portions, which packaged at separate bags (Nassif *et al*., 2015).

2.2. Preparation of pathogenic strains:

The pathogenic microorganisms used were *Staph. aureus* NCTC 10788/ ATCC® 6538P and *E. coli* NCTC 12241/ ATCC® 25922 reference strains (obtained from Becton Dickinson, France). All strains were activated in Food hygiene department - Animal Health Research Institute- Dokki, Giza, Egypt. Each strain was deep frozen stored in a cryo protective vial containing preservative solution at -70 ^{o}C. Cryo bead (inoculum) of each strain was cultivated in Tryptic Soy Broth overnight at 35°C. Then cells were centrifuged for 10 min at 8000 rpm. Supernatant was discarded, and the sediment represented the cells was washed three times and re-suspended in sterile 0.1 % peptone water. The cells were diluted in peptone water adjusted to obtain the desired inoculum level of 10^4cfu/ml (4 $\log_{10}$cfu/ml) (Shehata-Amal *et al.,* 2013).

2.3. Preparation of LAB inoculum:

Lactobacillus acidophilus was originally obtained from Ch. Hansen's Lab. (Denmark), and *Bifidobacteriumlactis* was obtained from Australian Research Center Australia, they were reactivated by three consecutive sub culturing on De- Man Regosa and Sharp medium (MRS) broth and agar at 37 °C for 24 hrs. The suspensions were centrifuged at 1.700 Xg for 15 minutes. The supernatant was discarded, and the bacterial pellets were washed twice with phosphate buffered saline (PBS; PH 7.3, 0.01 M) and the concentration of *Lactobacillus acidophilus* and *Bifidobacteriumlactis* was adjusted to obtain desired inoculum level of 10^7cfu/ml (7 $\log_{10}$cfu/ml) (Maha *et al.,* 2015).

2.4. Sample inoculation:

Samples of radiated minced meat were divided into two main portions, the first was inoculated with *Staph. aureus* to reach final concentration of 10^4cfu/g in examined minced meat, then sub divided into three groups, the 1st left as control, the 2ndwas inoculated with 10^7cfu/g *Lactobacillus acidophilus* (Group A), the 3rd was inoculated with 10^7cfu/g *Bifidobacteriumlactis* (Group B).The second one, was inoculated with *E.coli* to obtained a final concentration of 10^4cfu/g (4 $\log_{10}$cfu/g) then, sub divided into three equal groups (200 g of each); the 1stgroup was left as control, the 2nd(Group A)inoculated with 10^7cfu/g *Lactobacillus acidophilus*, while the 3rd(Group B) was inoculated with 10^7cfu/g *Bifidobacteriumlactis* (Shehata-Amal *et al*., 2013).

Analysis was conducted from all groups at zero day, 2nd, 4th, 6th and 8th days. Counting of *Staph. aureus* and *E. coli* load. All experiments were conducted in triplicate on separate days

2.5. Assessment of microbial growth:

It was applied according to APHA, 2001, where twenty-five grams of each examined sample was aseptically transferred into stomacher bag and blended with 225 ml sterile peptone water (0.1%), then serially diluted under aseptic condition. one ml of each dilution was aseptically inoculated and spreaded onto Baird parker agar plates, incubated at 35^{o}C for 24 hrs. for *Staph. aureus* count as well as Eosin Methylene blue (EMB) agar at 35^{o}C for 24 hrs for counting of *E. coli*.

2.6. Statistical Analysis:

A Handbook of Statistical analysis using SPSS (Ver. 20), according to Petrie and Watson (2013).

3. RESULTS:

Table (1): Effect of different used probiotics on of *Staph. aureus* count (log_{10}cfu/g) experimentally inoculated in radiated minced meat samples.

Tested samples	Zero day	2nd day	4th day	6th day	8th day
Control	4.26±0.24	4.45"A"±0.26	4.9"A"±0.05	5.26"A"±0.24	4.88"A"±0.09
Group A*	4.26±0.24	3.82"a"±0.11	2.49"a"±0.2	1.72"a"±0.12	<1"a"
Group B*	4.26±0.24	3.65"a"±0.16	2.64"a"±0.3	1.49"a"±0.2	<1"a"

* Group A: samples treated with *Lactobacillus acidophilus.*
* Group B: samples contaminated with *Bifidobacteriumlactis*
* <1 log_{10}cfu/g was calculated by zero when applying statistical analysis.

Table (2): Reduction log_{10} count and % of *Staph. aureus* artificially inoculated in radiated minced meat samples treated with different used probiotics:

Tested samples		Zero day	2nd day	4th day	6th day	8th day
Group A	Log count	4.26±0.24	0.44	1.77	2.54	<1
	Reduction %	0.0%	10.33%	41.55	59.62	100%
Group B	Log count	4.26±0.24	0.61	1.62	2.77	<1
	Reduction %	0.0%	14.32	38.0	65.02	100%

* Group A: samples treated with *Lactobacillus acidophilus.*
* Group B: samples treated with *Bifidobacteriumlactis*

Table (3): Effect of different probiotics on *E. coli* count (10^4 log_{10}cfu/g) experimentally inoculated in radiated minced meat samples.

Tested samples	Zero day	2nd day	4th day	6th day	8th day
Control	4.26±0.24	3.9±0.05	4.49±0.2	5.42±0.39	6.25±0.52
Group A	4.26±0.24	3.86±0.07	3.69±0.09	3.32±0.15	3.1±0.17
Group B	4.26±0.24	3.77±0.07	3.1±0.17	2.73±0.05	2.26±0.24

* Group A: samples treated with *Lactobacillus acidophilus.*
* Group B: samples treated with *Bifidobacteriumlactis*

Table (4): Reduction log count and % of *E. coli* artificially inoculated in minced beef samples treated with different used probiotics:

Tested samples		Zero day	2nd day	4th day	6th day	8th day
Group A	Log count	4.26±0.24	0.4	0.57	0.94	1.16
	Reduction %	0.00	9.39	13.38	22.07	27.23
Group B	Log count	4.26±0.24	0.49	1.16	1.53	2.0
	Reduction %	0.00	11.5	27.23	35.92	46.95

*Group A: samples treated with *Lactobacillus acidophilus.*
*Group B: samples contaminated with *Bifidobacteriumlactis*.

4. DISCUSSION

Lowering the costs of bio preservation processes may be highly attractive, especially for small economies and developing countries, where food safety, wholesomeness, acceptability and overall quality, have become increasingly important and valued features to consumers even in developing countries (HolzapFel, 2002).

Lactic acid bacteria (LAB) are very important in converting of agricultural products into safe, delicious and shelf stable foods for human consumption. The preservative activity of LAB in foods that has a strong antagonistic effect against food spoilage and pathogenic microorganisms is mainly attributed to competitive exclusion for essential nutrients or adhesion sites of mucous cells, immune modulation, redox modification, accumulation of D-amino-acids and production of extracellular and diffusible antimicrobial metabolites, such as organic acids (lactic, propionic, formic and acetic acids), antifungal compounds (fatty acids or phenyl lactic acid), lysozymes, enzymes (proteases, amylases and lipases) and bacteriocins, which play an essential role in natural preservation (Yasillike *et al*., 2010). Besides ensuring safety, bacteriocin-producing LAB with their probiotic potentials could also be emerging as a means to develop functional meat products with desirable health benefits. Nevertheless, to be qualified as a candidate probiotic culture (Swetwiwathana and Visessanguan, 2015).

Lactic acid bacteria widely used in food preservation at refrigerator temperatures due to their ability to produce high amount of hydrogen peroxide and/or other antibacterial substances at refrigerator temperatures which inhibit food-borne pathogens and psychrophilic spoilage microorganisms (Alireza*et al.*, 2016). Since *Staph. aureus*, which is salt and nitrite tolerant, is also able to grow under anaerobic conditions, there is an increased risk that it will grow and produce toxins (Kaban and Kaya, 2006).

Table (1) explained the effect of the two different probiotics on the growth pattern of *Staph. aureus* in experimentally inoculated minced beef samples. At zero day, there were no significance difference between all examined groups (control, A and B), they recorded 4.26±0.24 $\log_{10}$cfu/g for each. At the 2nd day of storage, the control group had a higher count (4.45±0.26 $\log_{10}$cfu/g) resulted in presence of a significance difference ($P<0.05$) with the other two groups (A and B),

while there was no significance difference (P>0.05) between group A (3.82±0.11 log_{10}cfu/g) and group B (3.65±0.16 log_{10}cfu/g). At the 4th day of storage, the results showed the presence of highly significance difference (P<0.01) between control group (4.9±0.05 log_{10}cfu/g) and both of group A (2.49±0.2 log_{10}cfu/g) and B (2.64±0.3 log_{10}cfu/g), while the difference between Group A and B still not existed. At the 6th day of storage, the same as 4th day, there was a highly significance difference (P<0.01) between control group (5.26±0.24log_{10}cfu/g) and both group A (1.72±0.12 log_{10}cfu/g) and B (1.49±0.2 log_{10}cfu/g), While still no significant difference (P>0.05) between group A and B. At the 8th day of experiment, the significance difference was optimum (P<0.00) between control group (4.88±0.09 log_{10}cfu/g) and both of Group A and B which contained (<1 log_{10}cfu/g).

Table (2) revealed the log_{10}cfu/g of *Staph. aureus* count in zero time, in relation to its reduction % of growth rate in Group (A) which recorded 4.26±0.24 (0.0%) at zero time, 0.44 (10.33%) at the 2nd day, 1.77 (41.55%) at the 4th day, 2.54 (59.62%) at the 6th day. At the 8thday of the experimental time, *Staph. aureus* growth was inhibited completely (<1 log_{10}cfu/g) with 100% reduction rate. While for Group (B), *Staph. aureus* counts and reduction % were recorded 4.26±0.24 (0.0%), 0.61 (14.32%), 1.62 (38%), 2.77 (65.02 %) and <1 log_{10} cfu/g with 100% reduction rate at zero time, 2nd, 4th, 6th and 8th day of storage, respectively. Nearly similar results regarding the effect of probiotics on the reduction of *Staph.aureus* counts were recorded by several investigators; Sameshima*et al*. (1998) who found that *Lactobacillus* strains could be able to reduce the growth rate and enterotoxin production of *Staph. aureus* in fermented sausage at 20°C and 35°C., Milani *et al*. (2003) reported that *Staph. aureus* growth was inhibited completely by addition of probiotics to chicken sausage. Kalalou*et al*. (2004*)* noticed *that staph. aureus* population was reached to <1 log_{10}cfu/g in minced meat treated with 7 log_{10}cfu/g probiotics, while control non-treated samples with probiotics, which inoculated with 4 log_{10}cfu/g *Staph. aureus* have reached 5 log_{10}cfu/g during 7 days of storage. Moreover Kebary *et al.* (2005) found that all studied *Bifidobacteria* strains strongly inhibited the growth of *Staph. aureus*.

Kaban and Kaya (2006), Erkmen *et al*. (2009) and Shehata-Amal *et al.* (2013) found that *Staph. aureus* was reduced in number in fermented sausage due to the inhibitory effect of probiotic starter culture while the number of *Staph.aureus* increased by 1 log on the third day in control group.

Bahni, Dhar (2013) reported highly significant (p<0.01) reduction of staphylococci count, which decreased from 2.40 to 1.46 log_{10}cfu/g throughout the storage period and the reduction was significant after 14thday of storage in the inoculated minced fish meat previously treated with LAB. Bomdespacho (2014) stated that coagulase-positive staphylococci were inhibited by the addition of *Lactobacillus acidophilus.* In contrary, Reham, Amin (2012) found that growth of *Staphylococcus aureus* in minced meat samples stored at 4°C was completely inhibited after being treated with *Lactobacillus acidophilus* in the 3rd day of the experimental time. Also, Sparo *et al*. (2013) concluded that, no *Staph. aureus* viable bacteria were detected at 48 h in ground beef meat post-treated with probiotics. Moreover, Nassif *et al*. (2015) has been reported that count of *staph. aureus* was decreased from 6.48 at zero day till reach 3.52 log_{10}cfu/g at the 9th day of storage, while the samples completely spoiled at 11th day of storage.

The effect of different probiotics on count of *E. coli* experimentally inoculated in radiated minced meat samples was cleared inTable (3) which revealed that at zero time, there were no significance differences between all examined groups (control, A and B) as all groups recorded almost the same *E. coli* count (4.26±0.24log_{10}cfu/g). Otherwise, there were no significance differences (P>0.05) between the three experimental groups. At the 2nd day of storage, the control non-treated group recorded *E. coli* count a little bit lower than at the zero time (3.9±0.05 log_{10}cfu/g) resulted in presence of a low significance difference (P<0.05) with other two groups (A and B), while there was no significance difference (P>0.05) between group A (3.86±0.07log_{10}cfu/g) and group B. (3.77±0.07 log_{10}cfu/g). At the 4th day of storage, the results showed the presence of highly significance difference (P<0.01) between Control group (4.49±0.2 log_{10}cfu/g) and both of group A (3.69±0.09 log_{10}cfu/g) and B (3.1±0.17 log_{10}cfu/g), while the difference between Group A and B didn't exist. At the 6th day of storage, the same as 4th day, the highly significance difference (P<0.01) was still persisted between control group (5.42±0.39log_{10}cfu/g) and both of group A (3.32±0.15 log_{10}cfu/g) and B (2.73±0.05 log_{10}cfu/g), while significance difference still didn't exist between group A and B (P>0.05). At the 8th day of experiment period, the significance difference was in optimum condition (P<0.00) between control group (6.25±0.52 log_{10}cfu/g), 3.1±0.17 and 2.26±0.24 for Group A and Group B, respectively.

Results illustrated in Table (4) showed the reduction log_{10}cfu/g of *E. coli* in treated groups, count in zero time, in relation to their reduction % of growth rate in Group (A) which recorded 4.26±0.24 (0.0%) at zero time, 0.4 (9.39%) at the 2nd day, 0.57 (13.38%) at the 4th day, 0.94 (22.07%) at the 6th day and 1.16 with reduction % represented 27.23% of *E. coli* count at the 8th day of the experiment. On the other hand, *E. coli* reduction log10 cfu/g and percentage for group B was recorded 4.26±0.24 (0%), 0.49 (11.5%), 1.16 (27.23%), 1.53 (35.92) and 2.0 (46.95%) at zero time, 2nd, 4th, 6th and 8th day of storage, respectively.

Bifidobacteria had more strong inhibitory activity than *L. acidophilus* towards Gram negative bacteria mainly, *Salmonella* spp. and *E. coli*. Probiotic LAB couldn't eliminate *E. coli* completely because the organism can resist acidic pH. LAB induce its antagonistic effects against *E. coli* through its ability to produce bacteriocins and bacteriocins like substances which are narrow-spectrum proteinaceous toxins that serve to kill closely related bacteria (Gordon and Obrien, 2006; Majeed *et al.*, 2011 and Berenice Arias *et al*., 2013).

Bacteriocins are not frequently active against Gram-negative bacteria. The lipopolysaccharide of the outer membrane of this classes of bacteria acts as a permeability barrier for the cell. It is responsible for preventing molecules from reaching the cytoplasmic membrane (Gao*et al*., 1999), this explained the cause of persistence of *E. coli* even in the presence of both *Lactobacillus acidophilu*s and *Bifidobacteriumlacti*s and didn't disappeared completely till the end of the experimental period as recorded in the present study. Moreover, similar results were recorded by Mindy *et al.* (1998) who stated that *Lactobacillus lactis* was able to reduce the number of *E. coli*O157:H7 in raw chicken breast meat stored at 7^{o}C for 7 days.

Pidcock *et al.* (2002) concluded that *Lactobacillus acidophilus* and *Bifidobacteriumlactis* may be used to increase the safety of Hungarian salami because these cultures gave strong inhibition of *E. coli* by more than 2.5 log units. Milani *et al*. (2003) found that addition of probiotics to chicken

sausage contained *E. coli* resulted in reduction of *E. coli* growth rate by 2 $\log_{10}$cfu/g.

In a study on vacuum-packaged fresh ground beef conducted by Smith *et al*. (2005) they found that the individual LAB isolates resulted in an average difference of 1.5 log cycles of *E. coli* O157:H7 after 12 days in ground beef stored at 5^0C. The authors also concluded that addition of LAB to raw ground beef stored at refrigeration temperatures may be an important intervention for controlling food borne pathogens. In this respect, Hutt (2006) concluded that *E. coli* was highly suppressed by *Bifidobacteriumlactis*. The same result obtained byMakras and De Vuyst (2006) who found that the maximum reduction of *E. coli* count reached 2.26 $\log_{10}$cfu/g (53.05%) in experimental samples using *Bifidobacteriumlacti*s. In addition, Aksu*et al.* (2008) found that *E. coli* O157:H7 which added to pasterma with protective probiotic culture showed approximately a 3-log cycle reduction at the end of the production.

Also, Hoyle et al. (2009) found that *E. coli* O157:H7 was reduced by 2 log cycles after 3 days of storage and by 3 log cycles after 5 days of storage. In addition, Lindqvist andLindblad(2009) reported 1 $\log_{10}$cfu/g reduction for *E.coli* in sausage stored at 8 ^{o}C for 21 days, while Tharmaraj and Shah (2009)stated that the inhibitory effect of all probiotic bacteria was weakest against *E. Coli* and strongest against *Staph. aureus* which was inhibited to a greater extent, this result agreed with that in the current study. Echeverry*et al.* (2010) recordedup to 3 log reduction of*E. coli* O157:H7 in meat products stored at 4.4°C for 14 or 21 days as compared with control samples. In addition, Hrachya*et al*. (2016) determined that the application of 1.4 x 10^7cfu/ml of *lactobacilli* to raw ground beef would result in 1 log reductions of *E. coli* O157:H7 during refrigerated storage at 5°C. Also, Alireza*et al*. (2016) reported reduction of *E. coli* O157:H7 by 1-2 log in ground beef stored at 5°C for 7 days in plastic vacuum bags depending on *L. acidophilus* ratio.

On contrary, Kalalou *et al*. (2004*)* stated that coliforms were reduced from 8 x 10^2 cfu/g to 10^2cfu/g after 24 hrs and to less than 1 cfu/g after 7 days storage of minced meat previously inoculated with 7$\log_{10}$cfu/g lactic acid bacteria (LAB). Moreover, Borowski *et al*. (2009) explained that (>=5.0 log) reduction of *E. coli* O157:H7 in examined ground and formed beef jerky previously inoculated with six commercially LAB containing cultures. Jofre *et al*. (2009) concluded that *E. coli* was unable to grow in experimentally inoculated slices of cooked ham, dry cured ham and marinated beef loin during storage at 4^oC in the presence of LAB. Also, Berenice Arias *et al*. (2013) mentioned that, *Lactobacillus acidophilus* and *Bifidobacterium*had the same antagonistic effect against *Escherichia coli* O157:H7. In addition, Sparo *et al*. (2013) through a comprehensive study, found that *E. coli* O157:H7growth was completely inhibited and the viable cells were not detected at 72 h in ground beef samples treated with probiotics.

While, Amin-Reham (2012) found that coliform count in ground beef treated with *L. acidophilus* was decreased from initial count of 6.72± 0.43 cfu/g to 6.0± 1.0 cfu/g in the first day then began to increase in the 2nd and 3rd day. Casaburi *et al*. (2016) reported no inhibitory effect of *Lactobacillus curvatus*54 M16 on tested Gram-negative bacteria. Moreover, Katie *et al.* (2017) noticed that the use of a commercial LAB intervention reduced STEC by 0.4 $\log_{10}$ cfu/cm^2 (P< 0.05) on intact beef strip loins during refrigeration storage.

These variations in reduction levels of different microorganisms upon using LAB may be attributed to many factors including: the initial count of pathogenic microorganism,

the concentration of the inoculum of used lactic acid bacteria, the ratio between the LAB and the pathogen which referred as LS: Pathogen ratio (the higher the ratio the greater the effect), the type of used probiotic or using mixed culture, the amount of lactic acid, bacteriocin and other antimicrobials produced by the different probiotic strains, the type of the nutritive medium or the food matrix used and the surrounding environment including temperature and pH.

5. CONCLUSSION:

The different probiotic strains (*L.acidophilus* and *B.lactis*) had antagonistic effect against *Staph.aureus* and *E.coli* in ground beefkept at refrigerator temperature.Moreover,*Lactobacillus acidophilus* and *Bifidobacteriumlacti*s had almost identical effect on the reduction of *Staph. aureus* count, while the organism was completely inhibited at the 8th day of the experiment.

*Bifidobacteriumlacti*s was more effective in reducing *E. coli* count through the 8 days of experimental study than*Lactobacillus acidophilus*. The maximum reduction % of *E. coli* count reached 2.0 $\log_{10}$cfu/g (48.26%) in experimental samples using *Bifidobacteriumlacti*s .

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