10.21608/avmj.2025.337244.1482 Assiut University website: www.aun.edu.eg

EFFECT OF PROBIOTICS AGAINST *E. COLI* AND *STAPH. AUREUS* IN CHILLED TILAPIA FISH FILLETS DURING REFRIGERATION STORAGE

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Received: 21 November 2024; **Accepted:** 30 December 2024

ABSTRACT

Getting knowledge of new technology developments to enhance food goods is becoming more popular. Nowadays, natural preservatives such as probiotics are preferred over chemical ones by all parties involved in food safety. Chemical preservatives have been shown to have numerous negative effects on food ingredients and human health. This study was conducted to investigate the antimicrobial effect of two probiotic strains (*Lactobacillus acidophilus* and *Bifidobacterium lactis*) alone against *Staph. aureus* and *E. coli* growth in chilled fresh tilapia fillet samples (that were previously irradiated with UVR to ensure that the samples were free of target microorganisms) during storage at 4°C for 8 days. The results showed that *Lactobacillus acidophilus* had almost the same effect as *Bifidobacterium lactis* in reducing *Staph. aureus*. However, *Bifidobacterium lactis* was more effective than Lactobacillus acidophilus in reducing *S. aureus* count. Moreover, the growth of *S. aureus* continued until the 6th day of storage, with complete inhibition done on the 8th day. In addition, *Bifidobacterium lactis* was more effective than *Lactobacillus acidophilus* in reducing *E. coli* count. Overall, *E. coli* was able to persist in the presence of both probiotics until the end of the experimental period. The maximum reduction in *E. coli* counts reached 0.806 log10cfu/g (47.17%) by using *Bifidobacterium lactis*. Therefore, it is recommended to use probiotics as one of the biological preservation systems for foods against *Staph. aureus* and *E. coli.*

Keywords: Tilapia fish fillets samples, Probiotics, *Staph. aureus, E. coli, Lactobacillus acidophilus, Bifidobacterium lactis*.

INTRODUCTION

Seafoods play a significant role in the human diet due to their high nutritive value and quality. Also, several marine products are directly linked to nutritional quality and the improvement of human health (Tacon and Metian 2018; Jayasekara *et al.,* 2020). Modern dietary trends over the past two decades have driven great attention to the aquaculture industry, which is now considered one of the main columns of global trade, to respond to an incredible rise

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in demand for fish and fish products on a global scale and to meet market needs (FAO 2020). Since the proliferation of microorganisms quickly changes the odor, flavor, color, and texture of fish products, quality losses of fish meat result, making fish products highly perishable food (Tavares *et al.,* 2021; Walayat *et al.,* 2023).

One of the most widely cultivated and economically significant fish species globally is tilapia (Arumugam *et al.,* 2023). The food industry is always searching for new ways to preserve food to prevent microbiological deterioration of perishable items such as fish fillets (Siddiqui *et al.,* 2024). To preserve and produce food of superior quality with an extended shelf life, several technologies have been developed in conjunction with intelligent packaging. Understanding antimicrobial potency against certain foodborne pathogens is a fundamental requirement for extending the shelf life and controlling food quality (Fadiji *et al.,* 2023).

Pathogens present a risk to customers, cause large financial losses, and reduce productivity when they are present in food products (Jhalka *et al.,* 2014). Water, vegetables, dairy products, and meat and animal products are all known to harbour *E. coli,* a human disease. It is identified as the causative agent of hemorrhagic colitis. Blood, cramps, stomach pain, fever, nausea, and vomiting are symptoms of diarrhoeal diseases associated with E. coli infections (Abongo and Momba, 2009). The use of probiotics as microbial preservatives has gained a lot of interest recently since consumers are becoming more conscious about artificial additives (Rameez *et al.,* 2024). Probiotics can reduce *Staph. aureus* and *E. coli* count, whereas *lactobacilli* have antibacterial properties. However, the growth of yeast, mould, or faecal coliforms was rarely inhibited by probiotics (Carvalho *et al.,* 2021). Accordingly, probiotic foods primarily contain lactic acid bacteria (LAB) and bifidobacteria (Ansari *et al.,* 2023). Because of its capacity to alter the human host system's defences against foodborne pathogens, LAB has attracted a lot of research lately. Because of this, these bacteria are currently being investigated for their prospective applications as an alternative to antibiotics in human medical treatments as well as a bio-preservative agent in the food and dairy industries (Rashed *et al.,* 2022).

Several modes of action are used by bacteriocins. Certain substances have the capacity to induce porosity in the target microorganism's cell membrane, hence augmenting its permeability. Additionally, these substances may prevent the production of the cell wall. Some can enter the bacterium's cytoplasm and release RNA or DNA. Only strains closely related to the generating organism can be inhibited by bacteriocins, which have a limited spectrum of inhibitory action. However, they can also inhibit a variety of Gram-positive microbes (Betancur-Hurtado *et al.,* 2022).

Thus, this study aimed to determine how probiotics *Lactobacillus acidophilus* and *Bifidobacterium lactis* could enhance the bacterial safety of refrigerated tilapia fish fillets that had been inoculated with food-borne pathogenic bacteria, such as *Staph. aureus* and *E. coli*, and stored for eight days at 4°C.

MATERIALS AND METHODS

1. Collection and preparation of samples:

This experiment was performed in the Animal Health Research Institute's Damanhur lab. Six kilograms of fresh raw tilapia fish fillet samples were gathered from fish shops in the province of El Behera, which is close to Damanhur city. The samples were then securely transported to the laboratory in sterile polyethylene bags. In an hour, they will be placed in different boxes with cooling packs and kept at 4±1ºC until they are required for this research. First, sterile distilled water was used to wash and rinse the tilapia fish fillets. Next, a

sterile knife was used to cut the fillets into pieces that were roughly 5 cm by 5 cm in size. The parts were subjected to ultraviolet light (at 254 nm) for 30 minutes on each side while kept in sterile open Petri dishes (Valtierra-Rodriguez *et al.*, 2010).

The samples were divided into two groups, A and B, with the first group, A, inoculated by *Staph. aureus* 10^4cfu/g and the second group, B, inoculated by *E. coli* 10^4cfu/g, each group weighing 3 kg and each group subdivided into three subgroups (A1, A2, and A3) for group A and (B1, B2, and B3) for group B, respectively, (1 kg of each) (The first group's cut (A1 & B1), untreated tilapia fish fillets were kept in the refrigerator as control samples and the 2nd group (A2 & B2) was inoculated by *Lactobacillus acidophilus* 10^7cfu/g, while the third group (A3 & B3) was inoculated by *Bifidobacterium lactis* 10^7cfu/g). The experiment was carried out with 3 replicates, and the data were expressed as mean ± SE of 3 replicates.

2. Preparation of pathogenic strains:

Reference strains of *E. coli* NCTC 12241/ATCC® 25922 and *Staph. aureus* NCTC 10788/ATCC® 6538P were utilised (obtained from Becton Dickinson, France). The Food Hygiene Department of the Animal Health Research Institute in Dokki, Giza, Egypt, activated all strains. Every strain was cryopreserved and kept at -70°C in a cryoprotective vial with a preservative solution. Every strain's cryobead, or inoculum, was grown for an entire night at 35°C in tryptic soy broth. After that, cells were centrifuged at 8000 rpm for 10 minutes. The sediment that represented the cells was rinsed three times and re-suspended in sterile water containing 0.1% peptone before the supernatant was disposed of. The cells were diluted in peptone water that had been modified to provide 10^4 cfu/ml (4 log10 cfu/ml) of inoculum (Shehata-Amal *et al.*, 2013).

3. Preparation of LAB inoculum:

The origins of *Bifidobacterium lactis* and *Lactobacillus acidophilus* were the Australian Research Centre and Ch. Hansen's Lab in Denmark, respectively. Three consecutive subculturings on De-Man Regosa and Sharp medium (MRS) broth and agar at 37°C for a whole day were used to revive the cultures. The suspensions were centrifuged at 1,700 Xg for 15 minutes. After removing the supernatant, the bacterial pellets were washed twice with phosphate buffered saline (PBS; pH 7.3, 0.01 M). The concentration of *Bifidobacterium lactis* and *Lactobacillus acidophilus* was then adjusted to achieve the required inoculum level of 10^7cfu/ml (7 log10cfu /ml) (Maha *et al.*, 2015).

4. Sample inoculation:

The radiated tilapia fish fillet samples were split into two main sections. Group A received an inoculation of *Staph. aureus* to a final concentration of 10^4 cfu /g. The A1 group (control), while A2 and A3 received different inoculations of *Lactobacillus acidophilus* (10^7cfu/g) and *Bifidobacterium lactis* (10^7 cfu/g), respectively. After being subdivided into three equal groups (1 kg each). Group B was inoculated with *E. coli* to achieve a final concentration of 10^4 cfu/g. The group B1 (control) and B2 were inoculated with 10^7cfu/g *Lactobacillus acidophilus*, and B3 was inoculated with 10^7cfu/g *Bifidobacterium lactis*. At the zero-day, 2nd, 4th, 6th, and 8th days, counting the *E. coli* and *Staph. aureus* loads, analysis was done on all the groups. Additionally, on different days, triple sensory analyses of every trial were carried out (Shehata-Amal *et al.*, 2013).

5. Assessment of microbial growth:

A stomacher bag containing 25 grams of each material under investigation was aseptically filled with 225 millilitres of sterile peptone water (0.1%). After that, the mixture was aseptically serially diluted (APHA, 2001). Baird Parker agar plates were infected aseptically with one milliliter of each dilution, which was then spread out and incubated for 24 hours at 35ºC for the

Staph aureus and *E. coli* count on Eosin Methylene Blue (EMB) agar.

6. Sensory analysis:

Fifteen qualified panelists carried out the sensory analysis. They were instructed to use a 7-point hedonic scale to assess the uncooked fillets' appearance, flavor, aroma, texture (from firm to soft), and overall acceptability. Ruiz-Capillas and Moral (2001) deemed scores of less than 4 to be undesirable.

7. Statistical Analysis:

Three duplicate samples (n=3) were investigated for each attribute. The results were described using the mean and the standard deviation (SD) of the mean. One - Way ANOVA was used to compare the means using SPSS software version 17.0, followed by Duncan's Multiple Range Test (Duncan, 1955). $P < 0.05$ was regarded as significant when comparing mean differences using the least significant difference test.

RESULTS

Table 1: The mean rating for the sensory attributes of (group A) *Staph. aureus* count (log10cfu/g) in tilapia fish fillets that were radiated and refrigerated at 4°C after using various probiotics.

Descriptor	Sensory scores				
	Day 0	2nd	4th	6th	8th
1) Color					
Control Group A1	6.45±0.25c	5.85±0.64b	4.25±0.21a	3.45±0.87a	2.89±0.45d
Group A2 (inoculated by *Lactobacillus acidophilus*)	6.48±0.35a	6.38±0.11a	5.25±0.24a	3.92±0.24a	3.75±0.22a
Group A3 (inoculated by *Bifidobacterium lactis*)	6.52 ±0.15a	6.42±0.25b	5.32±0.52c	4.31±0.72d	3.95±0.85c
2) Odor					
Control Group A1	6.47±0.94a	6.22±0.66a	4.45±0.22a	3.27±0.54a	2.76±0.62a
Group A2 (inoculated by *Lactobacillus acidophilus*)	6.48±0.12a	6.34±0.52a	4.52±0.76b	3.85±0.21c	3.83±0.25d
Group A3 (inoculated by *Bifidobacterium lactis*)	6.49 ±0.35a	6.40±0.01a	5.25±0.25b	4.23±0.35c	3.94±0.55b
3) Texture					
Control Group A1	6.92±0.35d	6.26±0.32c	4.49±0.25a	3.33±0.33b	2.77±0.11a
Group A2 (inoculated by *Lactobacillus acidophilus*)	6.93±0.25a	6.38±0.57a	4.53±0.81b	3.87±0.66a	3.85±0.45c
Group A3 (inoculated by *Bifidobacterium lactis*)	6.95 ±0.44a	6.45±0.69a	5.29±0.32a	4.28±0.99a	3.96±0.42a
4) Overall Acceptability					
Control Group A1	6.66±0.52c	6.55±0.34a	4.34±0.23a	3.72±0.09b	2.79±0.05d
Group A2 (inoculated by *Lactobacillus acidophilus*)	6.69±0.43b	6.59±0.18c	5.52±0.86a	4.83±0.03a	3.81±0.04a
Group A3 (inoculated by *Bifidobacterium lactis*)	6.72 ±0.32a	6.65±0.71a	5.85±0.91a	4.92±0.01a	3.95±0.02a

Data expressed as mean ± SE of 3 replicates; values with different letters within the same row differed significantly at ($P<0.05$).

Table 2: The *E. Coli* count (log10cfu/g) in samples of radiated tilapia fish fillets after refrigeration at 4°C was measured using the mean sensory quality score of (group B) in response to various probiotic.

Descriptor	Sensory scores				
	Day 0	2nd	4th	6th	8th
1) Color					
Control Group B1	6.46±0.23a	5.84±0.53a	4.32±0.01d	3.49±0.09b	2.97±0.01c
Group B2 (inoculated by *Lactobacillus acidophilus*)	6.49±0.45c	6.35±0.65c	5.13±0.03d	3.85±0.02b	3.63±0.08a
Group B3 (inoculated by *Bifidobacterium lactis*)	6.53 ±0.25a	6.44±0.33a	5.31±0.05a	4.29±0.07a	3.90±0.02a
2) Odor					
Control Group B1	6.45±0.01a	6.29±0.25a	4.46±0.17b	3.08±0.03b	2.85±0.05c
Group B2 (inoculated by *Lactobacillus acidophilus*)	6.49±0.07d	6.35±0.45c	4.55±0.31a	3.22±0.10a	3.89±0.33b
Group B3 (inoculated by *Bifidobacterium lactis*)	6.50 ±0.05c	6.47±0.23a	5.11±0.11b	4.99±0.14a	3.90±0.04d
3) Texture					
Control Group B1	6.90±0.05a	6.29±0.54d	4.50±0.03a	3.45±0.62c	2.93±0.51b
Group B2 (inoculated by *Lactobacillus acidophilus*)	6.95±0.01a	6.42±0.98a	4.55±0.22a	3.75±0.02a	3.08±0.87a
Group B3 (inoculated by *Bifidobacterium lactis*)	6.99 ±0.08c	6.49±0.07b	5.31±0.25a	4.33±0.01d	3.85±0.31a
4) Overall Acceptability					
Control Group B1	6.70±0.01d	6.62±0.45c	4.31±0.01a	3.77±0.55b	2.84±0.96d
Group B2 (inoculated by *Lactobacillus acidophilus*)	6.79±0.35a	6.72±0.10a	5.50±0.02a	4.72±0.25a	3.85±0.05a
Group B3 (inoculated by *Bifidobacterium lactis*)	6.85 ±0.56a	6.78±0.92b	5.78±0.33a	4.86±0.35d	3.91±0.35c

Data expressed as mean ± SE of 3 replicates; Values with different letters within the same row differed significantly at (P<0.05).

Table 3: *Staph. aureus* count (log10cfu/g) affected by different used probiotics in radiated tilapia fish fillets samples during refrigeration at 4°C (group A).

Chicken breast	*Staph. aureus* count (log_{10}cfu/g)				
	Day 0	2nd	4th	6th	8th
Control Group A1	4.24±0.22	4.47 ±0.35	4. 52±0.25	5.35±0.47	5. 56±0.24
Significant difference between group A1 and other groups (A2 and A3)	P>0.05	P<0.05	P<0.01	P<0.01	P<0.00
Group A2 (inoculated by *Lactobacillus acidophilus*)	4.24±0.22	3.85±0.33	2.45±0.62	1.83±0.45	<1
Group A3 (inoculated by *Bifidobacterium lactis*)	4.24±0.22	3.67±0.02	2.34±0.35	1.26 ±0.97	<1
Significant difference between group (A2 and A3)	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05

Data revealed as mean ± SD of 3 replicates; <1 log10cfu/g was calculated by zero when applying statistical analysis. ; P value refers to Statistical Significance difference value. No Significance difference at (P>0.05) and differed significantly at (P<0.05).

Table 4: Reduction log10 and % of *Staph. aureus* in radiated tilapia fish fillets after treated with different probiotics during refrigeration at 4°C**.**

Tested samples	*Reduction log10 (log_{10}cfu/g) and % of Staph. aureus*					
		Day 0	2nd	4th	6th	8th
Group A2 (inoculated by *Lactobacillus acidophilus*)	***Reduction log of Staph. aureus***	4.24±0.22	1.348	0.896	0.604	<1
	Reduction %	0.0%	9.2%	42.22%	56.84%	100 %
Group A3 (inoculated by *Bifidobacterium lactis*)	***Reduction log of Staph. aureus***	4.24±0.22	1.3	0.850	0.231	<1
	Reduction %	0.0%	13.44 %	44.81%	70.28%	100 %

Table 5: *E. coli* count (log10cfu/g) affected by different used probiotics in radiated tilapia fish fillets samples during refrigeration at 4°C (group B).

Tested samples	***E. coli* count (log_{10}cfu/g)**				
	Day 0	2nd	4th	6th	8th
Control Group B1	4.24±0.22	3.87±0.44	4.52±0.31	5.45±0.25	6.32±0.24
Significant difference between group B 1 and other groups (B 2 and B 3)	P>0.05	P<0.05	P<0.01	P<0.01	P<0.00
Group B2 (inoculated by *Lactobacillus acidophilus*)	4.24±0.22	3.82±0.23	3.65±0.04	3.29±0.54	3.07±0.23
Group B3 (inoculated by *Bifidobacterium lactis*)	4.24±0.22	3.72±0.65	3.09±0.22	2.69±0.02	2.24±0.05
Significant difference between group (B 2 and B 3)	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05

Data revealed as mean ± SD of 3 replicates; <1 log10cfu/g was calculated by zero when applying statistical analysis. ; P value refers to Statistical Significance difference value. No Significance difference at (P>0.05) and differed significantly at (P<0.05).

Table 6: Reduction log10 and % of *E. coli* in radiated tilapia fish fillets after treated with different probiotics during refrigeration at 4°C.

Tested samples	***Reduction log10* (log_{10}cfu/g) *and % of E. coli***					
		Day 0	2nd	4th	6th	8th
Group B2 (inoculated by *Lactobacillus acidophilus*)	***Reduction* Log *of E. coli***	4.24±0.22	1.34	1.29	1.19	1.12
	Reduction %	0.0%	9.9%	13.9%	22.4%	27.6%
Group B3 (inoculated by *Bifidobacterium lactis*)	***Reduction* Log *of E. coli***	4.24±0.22	1.31	1.13	0.989	0.806
	Reduction %	0.0%	12.26%	27.12%	36.56%	47.17%

DISCUSSION

Despite being a popular and healthful food item, fish can be perishable, making it challenging to keep it fresh (Prabhakar *et al.*, 2020). Even with refrigeration or freezing, this meal has a relatively limited shelf life (Xiaobao Nie *et al.*, 2022). Reducing the expenses of bio-preservation methods could be highly desirable, particularly for emerging economies and small businesses. In these areas, food safety, acceptability, wholesomeness, and general quality have grown in importance and are now sought-

after qualities by customers, even in developing countries (HolzapFel, 2002).

Foods containing LAB exhibit a potent antimicrobial action against pathogenic bacteria and food deterioration. The main causes of this are immune modulation, redox modification, D-amino acid accumulation, competitive exclusion for necessary nutrients or mucous cell adhesion sites, and production of extracellular and diffusible antimicrobial metabolites, which are vital for natural preservation (Yasillike *et al.,* 2010).

The sensory evaluation of food is one of the most significant statistical techniques for precisely assessing the quality and acceptability by consumers of a certain food or food product. The treated tilapia fish fillets have superior sensory attributes and differ significantly from the untreated fish samples in their sensory properties. Texture, colour, and odour are essential because the sensory characteristics are thought to be the consumer's main evaluating variables for products and the obvious parts of their visual sense (Lazo *et al.,* 2017). The Egyptian Organisation for Standardization's Egyptian standard (EOS No. 3494 / 2020) states that the sensory evaluation of chilled fish fillets must preserve the species' inherent sensory qualities because there should be no alterations to the fish's chemical or microbiological characteristics beyond the permitted limits.

A study examining colour differences in food product quality found that water activity and microbial invasion lead protein, fat, and other important biomolecules to be downcast in their qualities (Masniyom, 2011). Nevertheless, it was found that no matter how different the treatments were made, the colour attributes were lost as the storage days passed. The colour parameters a* (redness–greenness) and b* (blueness–yellowness) showed significant changes ($P < 0.05$) between storage days during the trials (**Table 1 & 2**). Every two days during the eight days that the fish were stored at 4°C, the colour of the treated and untreated fish was measured. When the results of the fish fillet samples from the Control Group (GA1 & B1) were compared, it was discovered that the Lactobacillus acidophilus (GA2 & B2) and Bifidobacterium (GA3 & B3) infected Group A2 & B2. The colour characteristics were mostly retained in the longum samples.

Notably, during a study period of up to eight days, fish samples were inoculated with Bifidobacterium and Lactobacillus acidophilus (G A2 & B2). Longum (G A3 & B3) typically kept their original scent. In contrast to the control set of samples that were not treated, these samples retained most of their odour characteristics. However, adverse odour characteristics were seen in the GA2 & B2 and GA3 & B3 samples prior to the day 8 period of refrigerated storage (Table 1 & 2). The foul smell is caused by rancidity of the fat or putrefaction of the protein (Emborg *et al.,* 2005). Fish lose their original smell quickly because of microbial invasion, which starts quickly after the postmortem.

Surprisingly, after eight days of storage in (**Tables 1 & 2**). The samples were injected with both Bifidobacterium and Lactobacillus acidophilus (GA2 & B2). Longum (GA3 & B3) typically kept their original texture. As contrasted with those from other untreated control groups. Compared to the control group, the textural qualities were generally conserved in GA3 & B3 and GA2 & B2, respectively. On the other hand, all groups' samples had undesirable textural qualities prior to the eighth day of refrigeration. According to Arfat *et al.,* (2015), when the texture sensory score was lower than 4, it was in the unacceptable range and that the texture was of poor quality. Sankar *et al.,* (2008) claim that the soft texture that came from the texture quality deteriorating is caused by a range of microorganisms, primarily from bacterial species, which alter the structure of fish protein.

Regarding the acceptability generally (**Tables 1 & 2**). Samples from the GA2, B2,

& GA3, and B3 groups were inoculated with Bifidobacterium and Lactobacillus acidophilus (GA2 & B2). When compared to samples from the treatment groups (A2, B2 & A3, B3) and the control untreated group (A1 & B1), longum (A3 & B3), respectively, demonstrated the highest acceptability up to 8 days.

The impact of the two distinct probiotics on the *Staph. aureus* growth pattern in samples of infected tilapia fish fillets was described in **Table (3)**. At day zero, all tested groups (A1, A2, and A3) reported 4.24 ± 0.22 log10cfu/g, with insignificant differences between them. The control group had a higher count (4.47 ± 0.35 log10cfu /g) on the 2nd day of storage than the other groups (A2 and A3) with a significant difference ($P < 0.05$). In contrast, there was an insignificant difference ($P > 0.05$) between group A2 (3.85 ± 0.33 log10cfu/g) and group A3 (3.67 ± 0.02 log10cfu/g). A highly significant difference ($P < 0.01$) was observed between the control group (4.52 ± 0.25 log10cfu /g) and both groups A2 (2.45 ± 0.62log10cfu /g) and A3 (2.34 ± 0.35 log10cfu/g), during the 4th day of storage, although there was no difference between group A2 and A3. Similarly, on the 6th day of storage, there was a highly significant difference ($P < 0.01$) between the control group (A1) (5.35 ± 0.47log10cfu /g) and both group A2 (1.83 ± 0.45 log10cfu /g) and A3 (1.26 ± 0.97 log10cfu/g), while there was an insignificant difference between groups A2 and A3. The control group (A1) (5.56 ± 0.24 log10cfu /g) and both Groups A2 and A3, which included (<1 log10cfu/g), had a significant statistical difference ($P < 0.00$) on the 8th day of the experiment. Nearly similar results regarding the effect of probiotics on the reduction of *Staph.aureus* counts were recorded by several investigators; Ibrahim, et al. (2018) and 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., Milani et al. (2003) reported that *Staph. aureus* growth was inhibited completely by addition of probiotics to chicken sausage. Probiotics inhibit the growth of *Staph. aureus* through the antibacterial metabolites of LAB, such as organic acids (which rapidly lower pH below 5.3), H2O2 (*Staph. aureus* is 2 to 10 times more sensitive to H2O2 than most LAB), Bacteriocins (which act better against Gram-positive bacteria than Gram-negative bacteria) and bacteriocin-like substances, may be the cause of the inhibition of Staph. aureus growth. (Batdorj et al. 2007). Different bacteriocins work in different ways. Some can stop the formation of the cell wall, while others can create holes in the target microorganism's cell membrane to increase its permeability. Some have the ability to reach the cytoplasm of the bacteria and release DNA or RNA, which stops a variety of microorganisms, including gram-positive and spore-forming ones, from growing. (Betancur-Hurtado et al., 2022).

The *Staph. aureus* count at day zero was shown in **Table (4)** along with the percentage growth rate reduction for Group (A1), which recorded 4.24±0.22 (0.0%) at zero-day, 1.348 (9.2%) at the 2nd day, 0.896 (42.22%) at the 4th day, and 0.604 (56.84%) at the 6th day. The growth of *Staph. aureus* was fully suppressed (<1 log10cfu /g) at a 100% decrease rate on the 8th day of the experiment. Conversely, *Staph. aureus* counts and reduction percentages for Group (A2) were 4.24±0.22 (0.0%), 1.3 (13.44%), 0.850 (44.81%), 0.231 (70.28%), and <1 log10 cfu/g with a 100% reduction rate at zero-day, 2nd, 4th, 6th, and 8th day of storage, respectively, in that order. According to Ibrahim *et al.* (2018), strains of lactobacillus may be able to slow down *Staph. aureus* proliferation and synthesis of enterotoxins in fermented sausage at 20°C. Regarding the impact of probiotics on the decrease in *Staph. aureus* counts, these results were almost the same.

The population of *Staph. aureus* was found to be less than 1 log10cfu/g in minced beef treated with 7 log10cfu/g probiotics (Kalalou *et al.,* 2004). In contrast, probiotic-treated control samples containing 4 log10cfu /g of

Staph. aureus was found to have 5 log10cfu /g after 7 days of storage. Furthermore, Kebary *et al.* (2005) discovered that every strain of Bifidobacteria they examined significantly impeded the growth of *Staph. aureus*. According to Shehata-Amal *et al.* (2013), the inhibitory impact of probiotic starter culture caused *Staph. aureus* to decrease in fermented sausage, while on the third day, the number of *Staph. aureus* increased by 1 log in the control group. During the storage period, Bahni and Dhar (2013) observed a significant ($P < 0.01$) decrease in the *staphylococci* count in the infected minced fish meat that had previously been treated with LAB. The *staphylococci* count decreased from 2.40 to 1.46 log10cfu/g. After 14 days in storage, the decrease was significant. According to Bomdespacho (2014), adding *Lactobacillus acidophilus* suppressed coagulase-positive *staphylococci*. Conversely, Sparo *et al.* (2013) found that, 48 hours after probiotic treatment, no viable *Staph. aureus* bacteria were found in ground beef meat. Furthermore, according to Nassif *et al.* (2015), the samples were totally spoilt on the eleventh day of storage, although the count of *Staph. aureus* dropped from 6.48 at day zero to 3.52 log10cfu/g on the ninth day.

Table (5) illustrates how various probiotics affected the amount of *E. coli* that was experimentally inoculated in radiated tilapia fish fillet samples. At day zero, no significant difference was found between the examined groups, control B1, B2, and B3, as each group recorded nearly the same *E. coli* count (4.24 ± 0.22 log10cfu /g). A low significance difference ($P < 0.05$) was observed between the control non-treated group's *E. coli* count (3.87 ± 0.44 log10cfu /g) on the 2nd day of storage and the other two treated groups, B2 (3.82 ± 0.23 log10cfu /g) and group B3 (3.72 ± 0.65 log10cfu /g), while there was insignificant difference between group B2 and B3. A highly significant difference ($P < 0.01$) was observed between the control group (4.52 ± 0.31 log10cfu/g) and both group B2 (3.65 ± 0.04 log10cfu /g) and B3 (3.09 ± 0.22 log10cfu /g), at the 4th day of storage. There was no difference between groups B2 and B3. Similar to the 4th day, on the 6th day of storage, there was still a highly significant difference ($P < 0.01$) between the control group (5.45 ± 0.25 log10cfu /g) and both group B2 (3.29 ± 0.54 log10cfu /g) and B3 (2.69 ± 0.02 log10cfu/g), On the other hand, there was no significant difference between groups B2 and B3 ($P > 0.05$). On the 8th day of the experiment, there was a significant difference ($P < 0.00$) in the optimal condition between the control group (6.32 ± 0.24 log10cfu /g), group B2 and group B3 (3.07 ± 0.23 and 2.24 ± 0.05), respectively. These findings are consistent with the findings of (Gordon and Obrien, 2006; Majeed et al., 2011) who reported that Bifidobacteria had more strong inhibitory activity than *L. acidophilus* towards *E. coli*.These results also agree with Milani et al. (2003) who found that addition of probiotics to chicken sausage contained *E. coli* resulted in reduction of *E. coli* growth rate. Antibacterial properties of lactic acid strains have been demonstrated in relation to mineral elements. For example, the combination of copper and lactic acid has been shown to eradicate foodborne pathogens such as *E. coli* O157:H7. (Gyawali and Ibrahim, 2012). The antibacterial activity of probiotics against *E. coli* may be due to the compounds that LAB produces as organic acids, diacetyl, hydrogen peroxide, reuterin, and bacteriocins that lower the pH of the medium and enhance the permeability of the cell membrane. (Sharma et al., 2022).

The findings presented in **Table (6)** demonstrate the decrease in log10cfu/g of *E. coli* in the treated groups, measured at zero time, in correlation with their growth rate reduction percentage. Group (B2) recorded 4.24 ± 0.22 (0.0%) at day zero, 1.34 (9.9%) at the 2nd day, 1.29 (13.9%) at the 4th day, 1.19 (22.4%) at the 6th day, and 1.12 with reduction % representing 27.6% of the *E. coli* count at the eighth day of the experiment. Conversely, at zero-day, the 2nd, 4th, 6th, and 8th days of storage, respectively,

the *E. coli* reduction log10 cfu /g and percentage for group B3 were 4.24 ± 0.22 (0.0%), 1.31 (12.26%), 1.13 (27.12%), 0.989 (36.56%), and 0.806 (47.17%).

Gram-negative bacteria, primarily Salmonella spp. and *E. coli*, were more strongly inhibited by *bifidobacteria* than by *L. acidophilus*. *E. coli* is resistant to an acidic pH; therefore, probiotic LAB was unable to totally eradicate the bacteria. The ability of LAB to produce bacteriocins and bacteriocin-like substances, narrow-spectrum proteinaceous toxins that destroy closely related bacteria, allows it to exert antagonistic effects against E. coli (Berenice Arias *et al.,* 2013; Ibrahim *et al.,* 2018). A permeability barrier for the cell is provided by the lipopolysaccharide present in this kind of bacteria's outer membrane. This explains why *E. coli* remained persistent even in the presence of both *Lactobacillus acidophilus* and *Bifidobacterium lactis*, as documented in the current study, and didn't totally vanish until the end of the experimental period. According to Pidcock *et al.* (2002), *Lactobacillus acidophilus* and *Bifidobacterium lactis* cultures strongly inhibited *E. coli* by more than 2.5 log units, suggesting that they could be employed to boost the safety of Hungarian salami. According to Milani *et al.* (2003), adding probiotics to chicken sausage containing *E. coli* reduced the organism's growth rate by 2 log10cfu /g.

Hutt (2006) concluded that *Bifidobacterium lactis* significantly inhibited *E. coli* in this regard. A similar outcome was found by Makras and De Vuyst (2006), who reported that utilising *Bifidobacterium lactis*, the highest decline of *E. coli* count reached 2.26 log10cfu /g (53.05%). Furthermore, Aksu *et al.* (2008) found that after the production process, *E. coli* O157:H7 introduced to pasterma with protecting probiotic culture exhibited around a 3-log cycle reduction.

All probiotic bacteria had a stronger inhibitory impact on *Staph. aureus*, which was suppressed more than other bacteria, according to Tharmaraj and Shah (2009), and Lindqvist and Lindblad (2009) observed a reduction of 1 log10cfu /g for *E. coli* in milk that was kept at 8ºC for 21 days. These outcomes agreed with the current study's conclusions. In comparison to control samples, Echeverry *et al.,* (2010) found that beef products kept at 4.4°C for 14 or 21 days could reduce *E. coli* O157:H7 by up to 3 logarithmic units. Furthermore, Hrachya *et al.* (2016) found that adding 1.4 x 107cfu /ml of *lactobacilli* to raw ground beef will reduce the amount of *E. coli* O157:H7 by 1 log while being refrigerated at 5°C. Additionally, depending on the *L. acidophilus* ratio.

Conversely, according to Kalalou *et al.* (2004), after seven days of storage of minced beef that had been previously injected with 7log10cfu /g of LAB, coliforms decreased from 8 x 10^2 cfu/g to 10^2 cfu/g and less than 1 cfu/g. Additionally, it was noted by Berenice Arias *et al.* (2013) that *Bifidobacterium* and *Lactobacillus acidophilus* both have antagonistic effects against *E. coli* O157:H7. Furthermore, in a thorough investigation, Sparo *et al.* (2013) discovered that in ground beef samples treated with probiotics, *E. coli* O157:H7 growth was totally repressed, and viable cells were not visible at 72 hours. However, Amin-Reham (2012) discovered that the coliform count in ground beef treated with L. acidophilus rose in the second and third days after initially decreasing from 6.72± 0.43 cfu/g to 6.0± 1.0 cfu/g in the first day. According to Casaburi *et al.* (2016), testing on Gram-negative bacteria revealed that Lactobacillus curvatus 54 M16 had no inhibitory impact. Furthermore, on intact beef strip loins stored under refrigeration, Katie *et al.,* (2017) found that the use of a commercial LAB intervention decreased STEC by 0.4 log10 cfu/cm^2 ($P < 0.05$).

CONCLUSION

The various probiotic strains (*B. lactis and L. acidophilus*) in tilapia fish fillet samples refrigerated showed antagonistic effects

against *E. coli* and *Staph. aureus*. Furthermore, the reduction of *Staph. aureus* count was nearly equal for *Lactobacillus acidophilus* and *Bifidobacterium lactis*, whereas the organism was entirely inhibited on the eighth day of the experiment. Over the course of the eight-day experimental investigation, *Bifidobacterium lactis* was more successful than *Lactobacillus acidophilus* in lowering the *E. coli* concentration. With *Bifidobacterium lactis*, the greatest reduction in *E. coli* counts percentage in experimental samples was 0.806 log10cfu/g (47.17%).

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تأثير البروبيوتيك ضد ميكروبي الايشريكية القولونية والمكور العنقودي الذهبي في شرائح أسماك البلطي المبردة أثناء الحفظ بالتبريد

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تطرح البروبيوتيك تطبيقات متنوعة لتعديل المكونات الغذائية لتحقيق الفوائد الصحية للبشر. هناك اتجاه متزايد للحصول على نظرة ثاقبة لتطورات التقنيات الجديدة لتحسين المنتجات الغذائية. في الآونة الأخيرة، أصبح جميع المعنيين بمجال سلامة الأغذية يفضلون استخدام المواد الحافظة الطبيعية بدلاً من المواد الكيميائية، والتي ثبت أن لها العديد من الأضرار على صحة الإنسان أو المكونات الغذائية. أجريت هذه الدراسة لمعرفة التأثير المضاد للميكروبات لسلالتين من المعززات الحيوية (لاكتوباسيلس اسيدوفيلاس و البيفيدوباكتيريوم لاكتس) لوحدهما ضد نمو وبقاء بعض مسببات الأمراض المنقولة بالغذاء المتمثلة في المكورات العنقودية الذهبية والإيشريكية القولونية في عينات شرائح البلطي الطازجة المبردة (التي سبق تشعيعها بالأشعة فوق البنفسجية للتأكد من خلو العينات من الكائنات الحية الدقيقة المستهدفة) أثناء التخزين عند درجة حرارة ٤ درجات مئوية لمدة ٨ أيام. أظهرت النتائج أن لاكتوباسيلس اسيدوفيلاس كان لها تقريبا نفس تأثير البيفيدوباكتيريوم لاكتس في تقليل عدد المكورات العنقودية الذهبية. ومع ذلك، كانت البيفيدوباكتيريوم لاكتس أكثر فعالية من لاكتوباسيلس اسيدوفيلاس في تقليل عدد المكورات العنقودية الذهبية خلال الدراسة التجريبية التي استمرت ٨ أيام. علاوة على ذلك، استمر نمو المكورات العنقودية الذهبية حتى اليوم السادس من التخزين، بينما تم تثبيط الكائن الحي تمامًا في اليوم الثامن من التجربة. بالإضافة إلى ذلك، كانت البيفيدوباكتيريوم لاكتس أكثر فعالية من لاكتوباسيلس اسيدوفيلاس في تقليل عدد والإيشريكية القولونية خلال الدراسة التجريبية التي استمرت ٨ أيام. وبشكل عام، تمكنت الإشريكية القولونية من الاستمرار في وجود كل من البروبيوتيك حتى نهاية الفترة التجريبية. أقصى انخفاض في أعداد الإيشريكية القولونية بلغ (٠,٨٠٦ log10cfu/g 47.17%) في العينات التجريبية باستخدام البيفيدوباكتيريوم لاكتس. ولذلك ينصح باستخدام البروبيوتيك كأحد أنظمة الحفظ البيولوجي للأغذية حيث انه اثبت فعاليته في القضاء علي بعض مسببات الأمراض المنقولة بالغذاء المتمثلة في المكورات العنقودية الذهبية والإيشريكية القولونية.