Egyptian Journal of Animal Health

P-ISSN: 2735-4938 On Line-ISSN: 2735-4946
Journal homepage: https://ejah.journals.ekb.eg/

Impact of Probiotics on Some Food Poising Microorganisms In Milk Based Deserts

Hanan R. Ghanayem*, Naglaa A.A.*, Asmaa T. Talayea and Tamer M.H. El. Sharawey***

*Food Hygiene Department, Animal Health Research Institute, Tanta Branch, ARC.
**Microbiology, Animal Health Research Institute, Tanta Branch, ARC.

Received in 2/7/2023
Received in revised from 24/7/2023
Accepted in 7/8/2023

Keywords:

milk-based desserts
B. cereus
Staph. Aureus
PCR
Probiotic
L. acidophilus and B. lactis

ABSTRACT

Currently, the increased interest in the field of food safety are shifted toward using natural food preservatives instead of chemicals which may have some harmful effect either on human health or food constituents. A total of 60 milk-based desserts samples, rice pudding, Mahallabia, and custard (20 of each) were collected from different dairies and pastry shops in Tanta city, Egypt. The collected samples were examined for isolation and identification of *Staphylococcus aureus (Staph. aureus)* and *Bacillus cereus (B. cereus),* detection of virulence genes for both organisms using PCR and finally, studying the effect of two probiotics strains of *Lactobacillus acidophilus L. acidophilus*) and *Bividobacterium lactis* (*B.lactis*) on the survival of *Staph. aureus* and *B. cereus* in rice pudding during refrigerator storage for 14 days. The incidence of *Staph. aureus* was 40%, 20% and 10% in the examined rice pudding, Mahallabia, and custard samples, respectively. While the incidence of *B. cereus* was 60%, 45% and 30% in the same examined samples, respectively. The result of PCR for virulence genes revealed presence of *spa* and *coa* gens in all *Staph. aureus* isolates (100%) and revealed presence of *nhe* and *cytk* gens in all *B. cereus* isolates (100%). *Staph. aureus* and *B. cereus* counts were decreased in rice pudding samples significantly ($p<0.05$) at 3rd, 7thand10th day of storage in samples with added both probiotics strains. So, Rice pudding was considered suitable food for the delivery of probiotic micro-organisms, with sufficient viability and acceptable sensory characteristics.

INTRODUCTION

Recently, the development of food products has been directed toward producing healthier foods that have characteristics similar to those observed in fresh products, with increased nutritional and functional properties, and no chemical additives **(Normanno et al.**

*Corresponding author: Hanan R. Ghanayem, Tanta Provincial Lab., Animal Health Research Institute, Agricultural Research Center, Giza, Egypt
E-mail address:
DOI: 10.21608/ejah.2023.311947

2012).

Dairy desserts in Egypt are locally produced by traditional retail shops and the production takes place manually in stores. They are made from highly nutritive raw materials; milk and sugar and are easily spoiled by the multiplication of specific microbial contaminants **(Arakawa et al. 2008).**

Staphylococcus is facultative anaerobic round-shaped and Gram-positive cocci; which produces various virulence genes, toxins and aggressive enzymes. It can be found in numerous food products as well as raw milk and its products, it was responsible for a variety of symptoms and diseases, this micro-organism is known as one of the world's most important causes of disease epidemics associated with food consumption (**Zhao et al. 2020**). *Staph. aureus* is one of the most important pathogen in food poisoning, due to its wide spread and ability of many strains to synthesize enterotoxin. It causes gastroenteritis symptoms like nausea, vomiting, abdominal cramps and diarrhea **(Scherrer et al. 2004).**

Bacillus cereus is a Gram-positive, aerobic or facultative anaerobic spore-forming bacilli, commonly present in various natural environments and food ingredients **(Fiedoruk et al. 2017).** *Bacillus cereus* associated with two forms of gastrointestinal diseases. The emetic form of illness manifests in vomiting and nausea while, the diarrheal form mainly appears to manifest only in gastrointestinal symptoms such as watery diarrhea and abdominal cramps **(Chai et al. 2019).** The pathogenicity of *B. cereus* is caused by different toxins produced by this bacterium. The diarrheal type of food poisoning *B. cerus* is caused by enterotoxins such as non-hemolytic enterotoxin (NHE) and cytotoxin K (CytK) produced during vegetative growth in the small intestine (**Sergeev et al. 2005)**

PCR is a powerful molecular biological technique that was introduced to facilitate the detection of virulence factors by using DNA probes that detect specific virulence factors and also, a powerful diagnostic tool for the detection of pathogenic micro-organisms ***(*Malorny et al. 2003*)***

Food preservation is a continuous effort that aims to eliminate and reduce the out-growth of spoilage and pathogenic micro-organisms in foods. Probiotics are known as live micro-organisms that have alternative effect to antibiotics in the treatment of food borne diseases as it provide a health benefit to the host when administered in adequate amounts**. (Sanders et al. 2007).**

Probiotics are defined as live micro-organisms which when administered in adequate amounts (10^6-10^7cfu/g) confer a health benefit on the host" **(FAO/ WHO 2010).** Mainly, they are used as natural food preservatives and important factors for preventing diseases, controlling bacterial and fungal contamination, and improving human and animal health (**Lucatto et al. 2020**).

The viability of probiotic micro-organisms during processing and storage besides the acceptance by the consumers is the major criterion to deter-

mine the potency and the market prosperity of the probiotic product. Lactic acid bacteria are 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 micro-organisms**. (Alireza et al. 2016).**

There are numerous health benefits reportedfor *Bifidobacterium* spp. and *Lactobacillus* spp. that they faster recolonization of the intestinal microbiota after administration of antibiotics, treatment and prevention of diarrhea, alleviation of constipation, possible treatment of inflammatory bowel disease, reduction in lactose intolerance in some individuals, reduction in serum cholesterol level, increased resistance to microbial infections, impact on immune function, and potential role in cancer prevention **(Zavisic et al. 2012).**

Lactobacillus acidophilus is a probiotic micro-organism available in conventional food (milk, yoghurt and toddler formula) and dietary supplements. Antagonistic activity of *L. acidophilus* against food borne microorganisms such as *E. coli, Staph. aureus, L. monocytogenes* and *Cl. perfringens* has been previously reported **(Kasimoglu and Akgun 2004).**

Therefore, the objective of this study is isolation and identification of *Staph. aureus and B. cereus* from some milk based dessert samples, detection of virulence genes of these pathogens, followed by evaluation to the effect of probiotic *L. acidophilus and B. lactis* on sensory attributes and growth of *Staph. aureus and B. cereus* in rice pudding during storage for 14 days at 4^{oC}**.**

MATERIAL and METHODS:

1- Collection and handling of the samples:

A total of 60 milk-based desserts samples, rice pudding, Mahallabia, and custard (20 of each) collected from different dairies and pastry shops in Tanta city, Egypt. Each sample was obtained as sold to the public and transferred directly to the laboratory in ice box under complete aseptic condition without delay for further examination.

2. Isolation and identification of *Staph. aureus* and *B. cereus*:

The isolation and identification of *Staph. aureus* was performed according to **(APHA, 1992; Quinn et al. 2002)** using brain heart infusion broth and Baird Parker agar, Mannitol salt agar (Oxoid). Meanwhile , *B. cereus* was isolated and identified according to **(Procopet al. 2017; Sandra and Tallen 2012)** using brain heart infusion broth and polymyxin– pyruvate- egg yolk– mannitol-bromothymol blue agar (PEMBA, Oxoid) .

3. Detection of virulence genes of *Staph. aureus* and *B. cereus*:-

DNA extraction: DNA was extracted from the isolated *Staph. aureus* and *B. cereus* using QIAamp DNA mini kit. It was applied on 3 random isolates. PCR Master Mix and cycling conditions of the primers during PCR was prepared according to Emerald Amp GT PCR mastermix (Takara) kit. Oligonucleotide

primers used in PCR have specific sequence and amplify a specific product (**Table A**)

Table A. Oligonucleotide primers sequences of virulence genes of *Staph. aureus and B. cereus*.

Bacteria	Target gene	Primers sequences	Amplified segment (bp)	Reference
Staph. aureus	*Spa*	TCA ACA AAG AAC AAC AAA ATG C GCT TTC GGT GCT TGA GAT TC	226 bp	Wada *et al*., 2010
	Coa	ATA GAG ATG CTG GTA CAG G GCT TCC GAT TGT TCG ATG C	Four different types of bands may be detected 350 bp 430 bp 570 bp 630 bp	Iyer & Kumosani, 2011
B. cereus	*Nhe*	AAG CIG CTC TTC GIA TTC ITI GTT GAA ATA AGC TGT GG	766 bp	Ehling-Schulz *et al*., 2006
	cytK	ACA GAT ATC GGI CAA AAT GC CAA GTI ACT TGA CCI GTT GC	421 bp	

4.Impact of probiotic bacteria on the growth and survival of *Staph. aureus* and *B. cereus* in vitro:

4.1. Preparation of bacterial strains:

Staph. aureus and *B. cereus* strains which isolated and identified from the proceeded samples (rice pudding, Mahallabia, and custard) then confirmed by PCR at Animal Health Research Institute (AHRI), Dokki, Egypt. The strains were adjusted at concentration of 10^7cfu/ml **(Kantachote and Charernjiratrakul 2008).**

Probiotic strains (*L. acidophilus* and *B. lactis*) were obtained from faculty of Agriculture-Ain Shams University, and adjusted to obtain desired inoculum level of 10^8cfu/ml (Maha et al. 2015).

4.2. Preparation of rice pudding:

The rice pudding was produced in amounts to obtain 3 kg of the final product. Using mixed ingredients (Buffalo's milk, rice, sucrose sugar, starch) by weight, heated 80-85 °C until the ingredients dissolved completely, and cooled to 40 °C in an water bath with continuous stirring.

4.3. Experimental trials:

As soon as the mixture reached the desired cooling temperature, the samples were divided into 12 equal groups (200g of each) and placed in aseptic polypropylene trays designed for disposable food packaging. The suspension of both probiotics (2ml for each group except C -ve) approximately 8 log cfu/ml in rice pudding was added at the beginning of the storage (1^{st}) day. Also the suspension of both *Staph. aureus*

and B. cereus (2ml for each group except C-ve) approximately 7 log cfu/ml were added. The inoculums of probiotic *Staph. aureus* and *B. cereus* strains were evenly distributed in the rice pudding each group separately by mixing with a sterile spatula for 50 second. Then cold and stored at 4±1°C for up to 14 days.

The samples divided into 12 equal groups (200gm each) arranged as follows:
C–ve: Control non inoculated either with *Staph. aureus, B. cereus or* probiotic strains.
Ca +ve: Inoculated with 10^7cfu/ml *Saph. aurus* strain.
Cb +ve: Inoculated with 10^7cfu/ml *B. cereus* strain.
GI : Inoculated with10^7cfu/ml *Staph. aureus* and 10^8cfu/ml *L. acidophilus.*
GII: Inoculated with both10^7cfu/ml *Staph. aureus* and10^8cfu/ml *B. lactis*.
GIII: Inoculated with 10^7cfu/ml *Staph. aureus* strain and mix of 10^8cfu/ml *L. acidophilus* and *B. lactis.*
GIV: Inoculated with 10^7cfu/ml *B. cereus* and10^8cfu/ml *L. acidophilus*.
GV: Inoculated with both10^7cfu/ml *B. cereus* and10^8cfu/ml *B. lactis*.
GVI: Inoculated with 10^7cfu/ml *B. cereus* and mix of10^8 cfu/ml *L. acidophilus* and *B. lactis*.
GVII: Inoculated with 10^8cfu/ml *L. acidophilus* only.
G VIII: Inoculated with 10^8cfu/ml *B. lactis* strain only.
GVIIII: Inoculated with 10^8cfu/ml of both *L. acidophilus* and *B. lactis* strains.

4.4-Determination of pH value:

The pH values of rice pudding was measured according to the method no. 981.12 of AOAC (2003) using a digital pH meter.

4.5-Microbiological Analysis:

L. acidophilus La-5 and *B. lactis* were counted by pour-plating 1 mL of each dilution in De-Man Regosa and Sharp medium (MRS) broth and agar, to which lithium chloride (0.2 g 100 mL^{-1}) (Merck, Darmstadt, Germany) and sodium propionate (0.3 g 100 mL^{-1}) (Sigma–Aldrich, St. Louis, MO, USA) solutions were added (LP-MRS agar). *L. acidophilus* after 2 days of aerobic incubation at 37°C for 48hours.B. lactis after 72 h of anaerobic incubation (Anaerobic System Anaerogen, Oxoid) at 37 °C **(Vinderola and Reinheimer 1999)** .

Staph. aureus count was determined for all trials throughout storage period according to **(ISO 2003).**
Enumeration of *B. cereus* was done according to (**Merzougui et al. 2014).**

4.6- Sensory evaluation:

Nine panelists, selected depending on their availability and willingness to participate in the study, from staff of Department of Food Hygiene Animal Health Research Institute, Tanta Branch. Evaluated the organoleptic characteristics of the rice pudding samples. The judges were asked to evaluate the coded samples (C -ve, GVII, GVIII and GVIIII, all of them from the same replicate), using a 9-point balanced hedonic scale (1: dislike extremely - 9: like extremely) based on color, texture, odor, and taste. The samples were presented at random digits and containing 20g of the product at 4 ± 1 °C. Water was provided to judges to clean their palates between samples (**Cardarelli et al. 2008).**

4.7. Statistical analysis:-

The data was statistically analyzed using one-way ANOVA of SPSS program for windows **SPSS Inc. Chicago, IL and USA (**Version 20)and Duncan's post hoc test with $p<0.05$ considered to be statistically significant**.**

RESULTS:-

Table 1. Incidence % of *Staph. aureus* and *B. cereus* isolated from examined milk based dessert samples (n=20)

milk based desserts	***Staph. aureus***		***B. cereus***	
	No.+ve	**%**	**No.+ve**	**%**
Rice pudding	8	40	12	60
Mahallabia	4	20	9	45
Custard	2	10	6	30

N. B : % was calculated according to the total number of examined samples

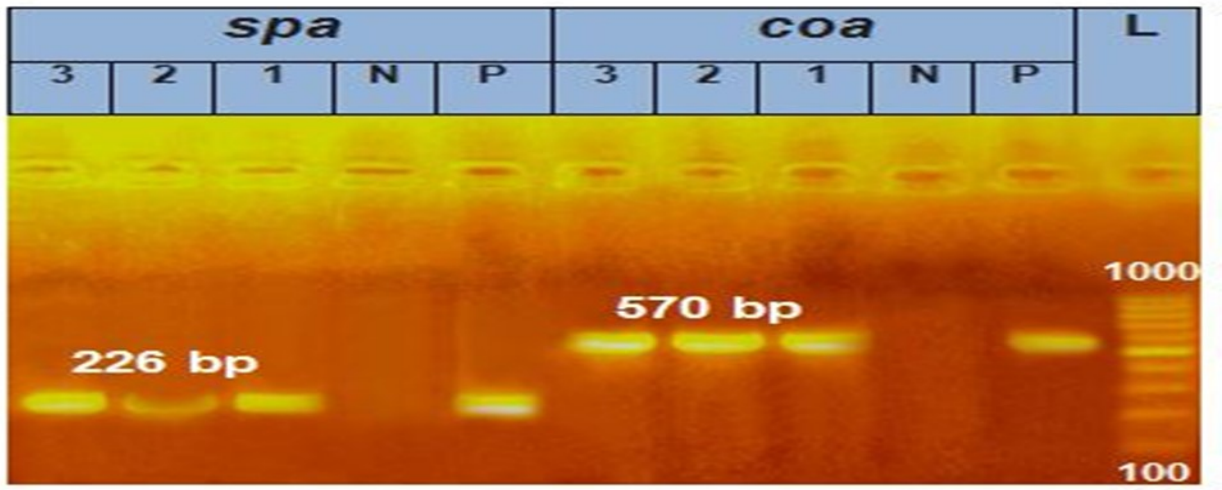

Figure (1): Gel electrophoresis pattern for detection of virulence genes of *Staph. aureus.*
Agarose gel electrophoresis showed amplification of *spa* gene of *Staph. aureus* at 226 bp and *coa* gene of *Staph. aureus* at 570 bp, l ane: 1, 2 and3 show positive amplification of *spa* and *coa* genes at 226 bp and 570 respectively L:ladder (100-1000bp) Pos: positive control; Neg: negative control.

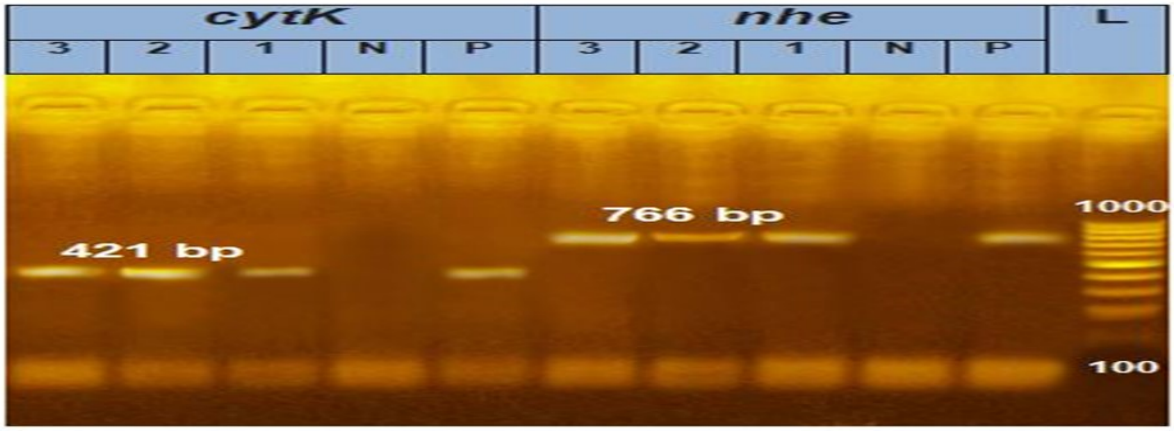

Figure (2):Gel electrophoresis pattern for detection of virulence genes of *B. cerus* figure .
Agarose gel electrophoresis showed amplification of *cytk* gene of *B. cereus* at 421bpand*nhe* gene of *B. cereus* at 766bp, lane: 1,2 and 3 show positive amplification of *cytk* and *nhe* genes at 421bp and 766, respectively. L:ladder (100-1000bp) Pos: positive control; Neg: negative control.

Table 2. Impact of Probiotic on survival of *Staph. aureus as* log cfu/g in Rice pudding during refrigeration storage.

Group Day	Zero day	3rd	7th	10th	14th
Ca+ve	6.83±0.08^{a}	6.96.±0.08^{a}	7.3±0.08^{a}	8.03±0.08^{a}	-
G I	6.73±0.1^{a}	5.53±0.1^{bc}	4.6±0.3^{c}	4±0.06^{c}	3.8±0.05^{ab}
G II	6.46±0.2^{a}	5.23±0.1^{c}	4.3±0.08^{d}	3.8±0.05^{d}	3.6±0.08^{b}
G III	6.64±0.1^{a}	5.8±0.08^{b}	4.9±0.05^{b}	4.4±0.08^{b}	3.9±0.05^{a}

Ca +ve – mean control +ve *Staph. aurus*
The values represent Mean ± SE of three experiments.
Means within a column followed by different letters are significantly different ($P < 0.05$).

Table 3. Impact of Probiotic on survival of *B. cereus* in Rice pudding during refrigeration storage

Groups Day	Zero day	3rd	7th	10th	14th
Cb +ve	6.56±0.1^{a}	6.53±0.06^{a}	6.63±0.08^{a}	7.53±0.1^{a}	-
G IV	6.13±0.08^{a}	5.56±0.14^{bc}	4.63.±0.08^{c}	4.1±0.06^{bc}	3.73±0.1^{bc}
GV	6.03±0.8^{a}	5.23±0.14^{c}	4.36.±0.03^{d}	3.6±0.2^{c}	3.46±0.2^{c}
G V1	6.32±0.1^{a}	5.73±0.08^{b}	4.9±0.05^{b}	4.4±0.08^{b}	4.03±0.08^{ab}

Cb +ve mean control +ve for *B. cereus*
The values represent Mean ± SE of three experiments.
Means within a column followed by different letters are significantly different (P < 0.05).

Table 4. Viability of probiotic in rice pudding during refrigeration storage.

Groups Day	Zero day	3rd	7th	10th	14th
G VII	7.6±0.05^{Aa}	7.5±0.05^{Aab}	7.5±0.05^{Aab}	7.33±0.08^{Abc}	7.2±0.03^{Ac}
G VIII	7.66±0.08^{Aa}	7.73.±0.1^{Aa}	7.53±0.06^{Aa}	7.26±0.03^{Ab}	7.16±0.03^{Ab}
G VIIII	7.5±0.08^{Aa}	7.56±0.1^{Aa}	7.43±0.03^{Aab}	7.2±0.05^{Ab}	7.3±0.05^{Aab}

The values represent Mean ± SE of three experiments.
Mean with capital letters in a column and small letters in a row differ significantly (P<0.

Table 5. Changes in sensory scores of rice pudding samples artificially inoculated with *probiotics L. acidophilus* and *B. lactis strains* during refrigeration storage at 4°cin comparison with control (c-ve)..

Group Day		**C-ve**	**GVII**	**GVIII**	**GVIIII**
Zero	Color	9.00±0.53^{aA}	8.7±0.33^{aA}	8.7±0.32^{aA}	8.7±0.3^{aA}
	Oder	9^{aA}	8.6±0.3^{aA}	9^{aA}	9^{aA}
	Texture	9^{aA}	8.6±0.3^{aA}	8.6±0.3^{aA}	8.6±0.3^{aA}
	Taste	8.7±0.3^{aA}	8.6±0.2^{aA}	9^{aA}	8.6±0.2^{aA}
3rd day	Color	8.6±0.3^{aA}	8.3±0.3^{aA}	8.6±0.3^{Aa}	8.3±0.3^{aA}
	Oder	8.6±0.3^{aA}	8.6±0.3^{aA}	9^{aA}	8.6±0.3^{aA}
	Texture	9^{aA}	8.6±0.3^{aA}	8.6±0.3^{aA}	8.6±0.3^{Aa}
	Taste	8.3±0.3^{aA}	8.2±0.3^{aA}	8.7±0.3^{aA}	8.3±0.3^{aA}
7th day	Color	7.3±0.3^{bB}	8.±0.0^{aB}	8.3±0.3^{aB}	8.±0.3^{aB}
	Odor	7^{bB}	7.8±0.3^{aB}	8.3±0.3^{aB}	8^{aB}
	Texture	7.5.±0.3^{bB}	8.6±0.3^{aB}	8.3±0.3^{aB}	8.3±0.3^{aB}
	Taste	7.1±0.3^{Bb}	7.9±0.1^{aB}	8.3±0.3^{aB}	8^{aB}
10th day	Color	-	7.6±0.3^{aC}	8^{aC}	7.6±0.3^{aC}
	Oder	-	7.6±0.3^{abc}	8^{ac}	7.8±0.5^{ac}
	Texture	-	8.6±0.3^{aB}	8.3±0.3^{aB}	8.3±0.2^{aB}
	Taste	-	7.3±0.3^{bC}	8.±0.0^{aB}	7.5±0.3^{bC}
14th day	Color	-	7^{aD}	7.3±0.3^{aD}	7^{aD}
	Oder	-	6.9±0.3^{aD}	7.9±0.2^{aD}	7.3±0.3^{aD}
	Texture	-	7.5±0.3^{aC}	7.1±0.3^{aC}	7.2±0.4^{aC}
	Taste	-	6.5±0.4^{Db}	7.3±0.3^{aD}	6.6±0.3^{bD}

Mean ±SE with different capital letters in a column and small letters in a row differ significantly (P<0.05).

Table 6. pH of controls and inoculated groups with probiotics and bacterial strains in rice pudding during refrigerator storage at 4±1°C

Groups/ Day	**Zero day**	**3rd**	**7th**	**10th**	**14th**
C-ve	6.93±0.06^{a}	6.98±0.09^{a}	6.93±0.09^{a}	6.90±0.09^{a}	6.89±0.09^{a}
Ca+ve	6.87±0.09^{a}	7.05±0.02^{b}	6.97±0.01^{a}	6.46±0.01^{b}	6.80±0.01^{b}
G1	6.8±0.09^{a}	6.86±0.03^{c}	6.58±0.07^{c}	6.1±0.03^{c}	5.79±0.01^{e}
G11	6.85±0.09^{a}	6.94±0.09^{b}	6.85±0.02^{ab}	6.47±0.01^{b}	6.4±0.02^{c}
G111	6.94±0.01^{a}	6.94±0.06^{b}	6.69±0.11^{bc}	6.14±0.02^{c}	5.98±0.1^{d}
Groups/ Day	**Zero day**	**3rd**	**7th**	**10th**	**14th**
C-ve	6.93±0.06^{a}	6.98±0.09^{a}	6.93±0.09^{a}	6.90±0.09^{a}	6.89±0.09^{b}
Cb+ve	6.93±0.09^{a}	6.91±0.01^{a}	6.74±0.01^{ab}	6.55±0.09^{b}	6.98±0.02^{a}
G1V	6.85±0.01^{b}	6.79±0.09^{b}	6.71±0.04^{b}	5.98±0.02^{c}	5.43±0.01^{e}
GV	6.87±0.01^{b}	6.89±0.09^{ab}	6.73±0.06^{ab}	6.57±0.01^{b}	6.1±0.03^{c}
GV1	6.85±0.09^{b}	6.92±0.01^{ab}	6.76±0.04^{ab}	6.02.±0.03^{c}	5.78±0.01^{d}

Means within a column followed by different letters are significantly different ($P < 0.05$).

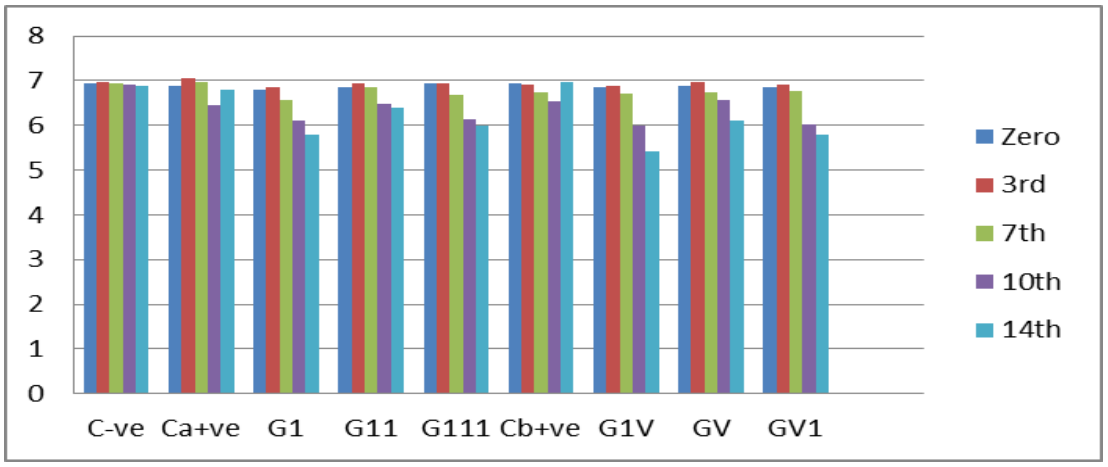

Figure (3):Change in pH of controls and inoculated groups with probiotics and bacterial strains in rice pudding during refrigeration storage at 4±1°C.

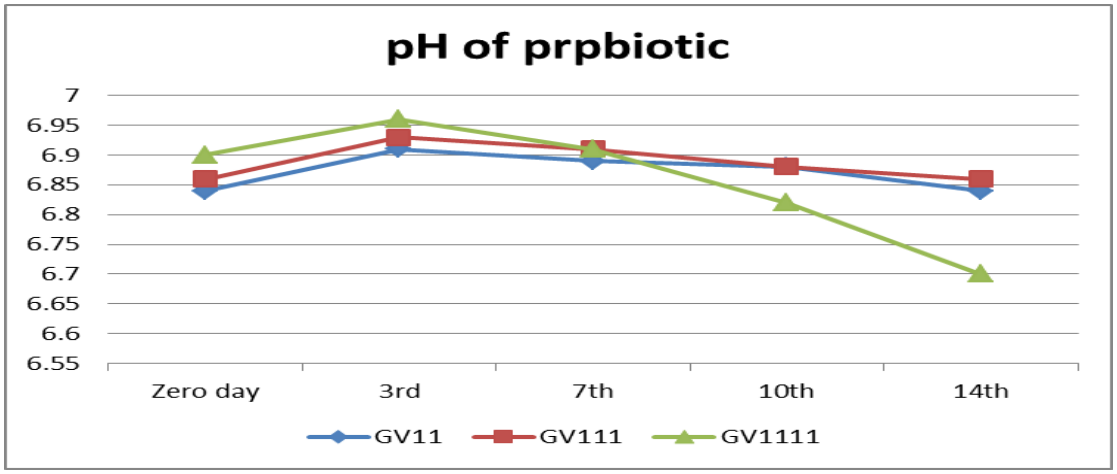

Figure (4): Change in pH of groups inoculated with probiotics strains only in rice pudding during refrigeration storage at 4±1°C.

DISCUSSION

Dairy-based desserts are considered a good media for the growth of many microorganisms due to its high nutritive value and they are important means for transmission of different pathogens mainly in places where the hygienic measures are low **(Meyer-Broseta et al. 2003).**

The results showed in Table (1) indicated that the incidence of *Staph. aureus* was 40%, 20% and 10% in the examined rice pudding, Mahallabia, and custard samples, respectively. These results nearly similar to that obtained by **Hassan and Afifi (2016)** for the rice pudding and Mahallabia. While, **Eman and Saad (2016)** recorded higher incidence80%in examined rice pudding for CPS samples, but **Sotohy et al. (2022)** recorded lower incidence for rice pudding and Mahallabia .

The high contamination rate of dairy products with *Staph. aureus* is mainly due to the use of poor quality milk, environmental contamination as unclean hands of worker who either suffering from diseased or apparent healthy carriers, unsanitary production and marketing practices **(Araujo et al. 2002).**

While *B. cereus* incidence was 60%, 45%

and 30% in the examined rice pudding, Mahallabia, and custard samples, respectively. These results nearly similar to that of **Wallaa (2017)** who found that *B. cereus* incidence was 62%, 44% and 32% in the examined rice pudding, Mahallabia and custard samples. While**, Hassan and Afifi (2016**) recorded lower incidence for the rice pudding. Also, **Hussein et al. (2015)** recorded higher incidence for Mahallabia. Lower incidence for custard were recorded by **Van Netten et al. (1990)** .

Bacillus cereus has emerged as a major food borne pathogen during the last few decades and causes two types of illness through the elaboration of enterotoxins **(Jay 2005).** These different results were due to regional, seasonal, sampling and the degree of contamination that depends on the precautions observed during processing.

Staphylococcal infections are usually associated with the presence of virulence genes. The *spa* gene is mostly used for typing of *Staph. aureus*, encodes for protein A and the coagulase (*coa*) gene is another example of *Staph. aureus* virulence gene that is regarded as important, because it plays an essential role in the alliance with other genes to survive inside host cells and to invade its immune system **(Balaban and Rasooly, 2000).**

As showed in figure (1) PCR of virulence genes revealed presence of *spa* and *coa* gens in all *Staph. aureus* tested samples(100%). These results agreed with **Abdel-Tawab et al. (2016)** who established the presence of *spa* gene in nearly all of the isolates. Meanwhile, the results disagreed with that recorded by **Momtaz et al. (2013)** who reported that *spa* and *coa* were detected with 26.82% and 63.41%, respectively.

The pathogenecity of *B. cereus* is attributed to the production of two main toxin types (diarrheal and emetic toxins) that are responsible for emesis and diarrhea **(Schoeni and Wong 2005).**

The obtained results in Figure (2) regarded that all the examined *B. cereus* isolates were positively by PCR technique for virulence genes and revealed presence of *nhe* and *cytk* gens in all tested samples (100%), similar study conducted by **Cui et al. (2016)** who detected *nhe* by 100%, other findings reported by **Glasset et al. (2016)** who added the presence of *nhe* and *cytk* with 96% and 42%, respectively.

Milk and dairy products are one of the most important human needs because they rich in organic matter. Further, they are a suitable environment for the growth of various food borne pathogens and this indicates the necessity for milk hygiene and elimination of pathogenic micro-organisms and their products, including toxins **(Amelia et al. 2021).**

Probiotic bacteria affect many of microorganisms through changing the pH and glucose levels secreting enzymes and antimicrobial toxins, changing the expression of pathogenic genes and competition on food intake (**Kerry et al. 2018).** Since *Staph. aureus*, which is salt and nitrite tolerant, it also able to grow under anaerobic conditions, there is an increased risk that it will grow and produce toxins **(Kaban and Kaya 2006)**

Table (2) explained the effect of the two different probiotics on the growth pattern of *Staph. aureus* in experimentally inoculated rice pudding samples. At zero day, there were no significance difference between all examined groups (Ca+ve, GI, GII and GIII), from 2nd day till end of storage period, there were significantly difference ($p< 0.05$).*Staph. aureus counts* in rice pudding samples declined from (6.73±0.1 to 3.8±0.05) ,(6.46±0.2 to 3.6±0.08) and (6.64±0.1 to 3.9±0.05)log cfu/gin treated probiotics groupsG1,G II and G III respectively, when compared to (Ca +ve) during the storage period. So it was observed that *Staph. aureus* count was declined, when increasing probiotics concentrations to10^8cfu/g. These results agreed with previously reported by Nassif et al. (2015) who stated that *staph. aureus* count was decreased from6.48 at zero day to 3.52 $\log_{10}$cfu/g at the 9thday of storage. While **Hemmat et al. (2018)** found that *L. acidophilus* mitigate *staph. aureus* count from

4.26 at zero day of storage to 1.72 cfu/g at 6th-day of storage , and *B. lactis* also mitigate *staph. aureus* count from 4.26 at zero day to 1.49 log10cfu/g for at 6th day of storage, respectively. Also, **Eman and Saad. (2016)** reported that *Staph. aureus* count decreased significantly (p<0.05) at 3rd, 7thday of storage in comparison to zero day. The results indicated that *Staph. aureus* was reduced in all treatments especially group GII followed by group GI and then group G III, this agreed with **Kebary et al. (2005)** who found that all studied *Bifidobacteria* strains strongly inhibited the growth of *Staph. aureus*.

Probiotic are LAB which produces antimicrobial substances, such as organic acids, fatty free acids, ammonia, hydrogen-peroxide and bio surfactant. It also produces low-molecular-weight antibacterial peptide-bacteriocins that inhibit both gram positive and gram-negative enteric pathogens **(Gomes et al. 2012).**

Bacillus cereus is an important pathogenic organism for estimating the risk for food borne diseases. The actual disease symptoms, however, are caused by toxins, either produced during growth in the gut; enterotoxins or during growth in food emetic toxin (**Adams and Moss 2008).**

Table (3) showed that both *probiotic*s strains had inhibitory effect against *B. cereus* growth in rice pudding as lowering its count from (6.13±0.08 to 3.73±0.1), (6.03±0.8 to 3.46±0.2) and (6.32±0.1 to 4.03±0.08) log cfu/ g through 14 day of refrigeration storage in probiotics inoculated groups G IV, GV and GV1, respectively when compared to Cb +ve group. Moreover, *B. lactis* alone has better effect than combination of *L. acidophilus and B. lactis*. These results agreed with **Servin (2004) and Parada et al. (2007)** who revealed that the use of *Bifidobacterium lactis* BB-12 is profitable on hygienic quality of rice pudding due to the cumulative effects of antimicrobial agents such as hydrogen peroxide, bacteriocins and organic acids. To exert health benefits, probiotic bacteria must be viable and available in high concentrations, above 10^6 CFU/g product **(Shah. 2007).** Generally, the recorded data mentioned in table 2 and 3 agreed with **Berenice Arias et al. (2013)** who reported that LAB induce its antagonistic effects against pathogenic bacteria through its ability to produce bacteriocins and bacteriocins like substances which are narrow-spectrum proteinaceous toxins that serve to kill closely related bacteria.

Table (4) revealed the change of viability of Probiotic in rice pudding during refrigeration storage for 14 day. The viable cell numbers of GVII, GVIII and GVIIII (7.6±0.05, 7.66±0.08 and 7.5±0.08 log cfu/g, at the 1st-day), respectively. Significantly decreased during 14 days of storage period (P<0.05). No significant differences observed between GVII, GVIII and GVIIII during zero, 3thand 7thdays of storage period (P>0.05).Satisfactory probiotic viability above 7 log cfu g^{-1} was found in the present study for *L. acidophilus and B.lactis*, which observed throughout the storage period. This observation is similar to that reported by **Abghari et al. (2011)** and nearly similar to **Ranadheera et al. (2013)** who found *L. acidophlus-5*count was ranged from 7.70 to 7.38 logcfu/g. While lower results recorded by **Cardarelli et al. (2008)** for the *L. acidophilus* strain, which were below 7 log cfu /g. So, results indicated that probiotics in rice puddings pioneered in the present study could have a potentiality to be used as carriers of *L. acidophilus* (LA-5) *and B. lactis* in food systems, as similar to the literature depending on viability and activity of probiotic bacteria incorporated into dairy desserts **(Aragon-Alegro et al. 2007).**

As observed in this study, that *B. lactis* populations was higher than those of *L. acidophilus*, and this agreed with **Pereira et al. (2010)**, nevertheless, the viability of *L. acidophilus* (97.1 %) was less affected than *B. lactis* (94.7%) at 14 day of storage due to the sensitivity of this strain to air during storage. Moreover, there seems to be a substantial influence of the food matrix on the viability of such micro-organisms in products.

Table (5) discussed the sensory properties, however, there were no significant differences

in (color, odor, texture and taste) for GVII, GVIII and GVIIII (P>0.05) compared with Control –ve at zero and 3rdday of storage, but at 7th day, showed significant difference (P<0.05) between c-ve and other probiotic treated groups. While there was no significant difference between probiotic treated groups from 7th until 14th of storage period. Control –ve was still acceptable until the 7th day. Use of probiotic bacteria showed the best score through the days (0, 3 and 7) of storage (taste, texture and odor). GVIII was favored for its taste, color and odor. However, GVII was preferred for its texture at 10 and 14 day of storage period, when compared to GVIII and GVIIII due to occurrence of heterogenic texture, while GVIII most preferred for its taste and odor, and this agreed with **MC Breaty et al. (2001)** who observed more extensive proteolysis and improved flavor in *B. lactis* BB-12 of samples during the storage. No abnormal odor in the examined probiotic rice pudding was detected and this agreed with **Tulay Ozcan et al. (2010) and Eman and Saad (2016).**

The presence of probiotic strains in the product improved its sensory characteristics. These micro-organisms might also be able to produce metabolites with antimicrobial activity, such as organic acids, alcohols, bacteriocins and reducing deterioration of product **(Buriti et al. 2012).**

PH is an important factor which can dramatically affect bacterial growth, probiotics spp. that able to tolerate a wide range of pH (1 -9) and grow well at acidic pH 1-5 **(Chowdhury et al. 2012).**

Table (6) and Fig. (3) Illustrated the pH values of the rice pudding samples on day one were very similar and no significant differences (P>0.05) were observed among different groups for pH. However, by the end of the storage period, pH reduced significantly (P<0.05) for both *Staph. aureus* and *B. cereus* bacteria during storage period as a result of acid production by probiotic bacteria, in compared to those of controls one (C-ve, Ca +ve and Cb +ve).The pH levels in probiotic rice pudding depended on the strain used where the pH values obtained in all *L. acidophilus* inoculated samples were significantly lower than those of *B. lactis* and controls samples, since *L. acidophilus* has higher acid formation ability. In general, the three trials studied showed increasing values of acidity and decreasing values of pH. This agreed with **Tulay Ozcan et al. (2010).**

Figure (4) Showed that during the storage period there was a small decrease but not significant in pH values was observed in G VII,G VIII and GVIIII groups*($p < 0.05$).* However, the changes of pH values did not affect the viability of the probiotic micro-organism strains Since, the pH values of the food samples remained close to neutrality, which is considered optimal for survival of probiotics in rice pudding and this agreed with **Homayouni et al. (2012).**

CONCLUSION:

Proper sanitation and hygiene during handling of rice pudding are important factors to protect the consumer health and prevent spoilage of the product. The results suggested that high levels of viable *L. acidophilus* and *B. lactis* in rice pudding is a good source for probiotic bacteria delivery with appreciated organoleptic quality and microbiological safety leading to good kinds for its future commercial production. *L. acidophilus and B. lactis* had antagonistic effect on *Staph. aureus* and *B. cereus* in rice pudding at refrigeration storage temperature. Moreover, *B. lactis* alone has better effect than combination of *L. acidophilus and B. lactis.*

REFERENCES:

Abdel-Tawab AA, El-Hofy, FI, Maarouf AA, Abbas SA. 2016. Molecular detection of some virulence genes of *S. aureus* isolated from mastitic Cows by PCR. Benha Vet. Med. J. 30(1): 238-245.

Abghari A, Sheikh-Zeinoddin M, Soleimanian Zad S. 2011. Non fermented ice cream as a carrier for Lactobacillus acidophilus and *Lactobacillu rhamnosus*. International Journal of Food Science and Technology 46(1): 84-92

Adams MR , Moss MO. 2008. Food Microbi-

ology. 3rd Ed. The Royal SociTY of Chemistry publishing, Cambridge, U.K.

Alireza G, Hrachya H, Andranik B. 2016. Elimination Of Pathogen Escherichia Coli O157: H7 In Ground Beef By A Newly Isolated Strain Of Lactobacillus Acidophilus During Storage At 5°C, Applied food biotechnology, 3 (3): 170-176

Amelia R, Philip K, Pratama YE, Purwati E. 2020. Characterization and probiotic potential of lactic acid bacteria isolated from dadiah sampled in West Sumatra. Food Science and Technology, (41):746-752.

APHA. "American Public Health Association . 1992. Standard Methods for the Examination of Dairy Product. 16th ed., New York.

AOAC "Association of Analytical Communities" 2003. Official methods of analysis of AOACInternational.B17th edition, 2nd revision. Gaithers burg, MD, USA.

Aragon-Alegro LC, Alegro JHA, Cardarelli HR, Chiub MC, Saadb SMI. 2007. Potentially probiotic and synbiotic chocolate mousse. *LWT Food Sci. Technol., (*40): 669-675

Arakawa K, Kawai Y, Iioka HM, Tanioka M, Nishimura J, Kitazawa H, Tsurumi, K, Saito T. 2008. Microbial Community Analysis of Food-Spoilage Bacteria in Commercial Custard Creams Using Culture-Dependent and Independent Methods. J. Dairy Science, 91(8): 2938–2946

Araujo VS, Pagliares VA, Queiroz MLP, Freitas-Almeida AC. 2002. Occurrence of Staphylococcus and entero pathogens in soft cheese commercialized in the city of Rio de Janeiro, Brazil. J. Appl. Microbiol., (92):1172–117

Baines SK. 2013. Production of probiotic ice cream from goat's milk and effect of packaging materials on product quality. Small Ruminant Research 112(1/3): 112-174

Balaban N, Rasooly A. 2000. Staphylococcal enterotoxins', International Journal of Food Microbiology, 61(1): 1–10

Berenice Arias O, De la Luz Reyes M,Lilia Navarro V, Berenice Solis C, Mayra Márquez G, Gloria Sanchez S, Raúl Snell C, Raquel Zuñiga R. 2013. Antagonistic effect of probiotic strains against two pathogens: *Salmonella Typhimurium* and *E. coli* O157:H7 resistant to antibiotics, Vol. 11, Art. 5

Buriti FCA, Souza CHB, Saad SMI. 2012. Cheese as probiotic carrier: technological aspects and benefits Y.H. Hui (Ed.), Animal-based fermented food and beverage technology, CRC Press, Boca Raton (2012), pp. 749-784

Cardarelli HR, Aragon-Alegr, LC, Alegro JHA, De Castro IA, Saad SMI. 2008. Effect of inulin and Lactobacillus paracasei on sensory and instrumental texture properties of functional chocolate mousse. Journal of the Science of Food and Agriculture (88):1318- 1324

Chai SJ, O'Connor KA, Richardson LC, Tauxe RV. 2019. Incubation periods of enteric illnesses in food borne outbreaks, United States, 1998–2013. Epidemiol. Infect. 147:e285

Chowdhury A, Hossain N, Mostazir NJ, Fakruddin MD, Billah M. 2012. Screening of Lactobacillus spp. from Buffalo Yoghurt for Probiotic and Antibacterial Activity. J. Bacteriol. Parasitol., 3: 156

Cui Y, Liu Y, Liu X, Xia X, Ding S, Zhu K. 2016. Evaluation of the toxicity and toxic okinetics of cereulide from an emetic Bacillus cereus strain of milk origin. Toxins 8, 156–166

Ehling-Schulz M, Guinebretiere MH, Monthán A, Berge O, Fricker, M, Svensson B. 2006. Toxin gene profiling of enterotoxic and emetic Bacillus cereus. FEMS microbiology letters, 260(2): 232-240

Eman F. Abdel-Latif Saad MF. 2016. Effect of Bifidobacterium lactis on Quality of Rice Pudding as a Probiotic Food Carrier. Int. J. Curr. Microbiol . App. Sci 5 (8): 362-371

FAO/WHO. 2010. Codex standard for fermented milks (2nd ed.). Codex Stan 243-2003

Fiedoruk K, DrewnowskaJM,Daniluk T, Leszczynska K, Iwaniuk P, Swiecicka I. 2017. Ribosomal background of the *Bacillus cereus* group thermo types. *Sci. Rep.* 7, 46430; doi: 10. 1038 / srep4 6430

Gibson GR. 2002. Intestinal microflora research for the 21st Century. Bioscience Microflora, 20(4): 131-134

Glasset B, Herbin S, Guillier L, Cadel-Six S,

Vignaud ML, Grout J, Brisabois A. 2016. Bacillus cereus-induced food-borne outbreaks in France, 2007 to 2014: epidemiology and genetic characterisation. Eurosurveillance, 21(48):30413.

Gomes BC, Rodrigues MR, Winkelströter LK, Nomizo A. de Martinis EC. 2012."In vitro evaluation of the probiotic potential of bacteriocin producer *Lactobacillus sakei*1",Journal of Food Protection, (75):1083-1089

Hassan GM, Afifi SI. 2016. "Bacteriological Quality Assessment of Some Locally Manufactured Dairy Desserts Sold in Beni-Suef City, Egypt and Molecular Detection of Staphylococcus aureus Enterotoxin". Zagazig Vet. J., 44(2): 91-100

Hemmat IM, Reham AA, Khalid ST, Amira AE. 2018. The Effect of Probiotics on Staphylococcus aureus and E. Coli in Minced Meat .2 Benha Vetrinary Medical Journal, vol. 34, No. (1):242-253, March, 2018

Homayouni A, Azizi A, Javadi M, Mahadipour S, Ejtahed H. 2012. Factors influencing probiotic survival in ice cream: a review. International Journal of Dairy Science 7(1): 1-10.

Hussein MF, Sadek OA, EL Taher SG. 2015. "Occurrence of Bacillus cereus and Staphylococcus aureus organisms in some dairy desserts". Assiut Vet. Med. J., 61(145): 160 -165

ISO. International Standard Organization 2003. ISO standard DI 6888:2003 (E). Horizontal method for the enumeration of Coagulase Positive Staphylococci (Staphylococcus aureus and other species)

Iyer AP, Kumosani TA. 2011. PCR based detection of nosocomial infection causing MRSA (Methicillin resistant Staphylococcus aureus). In 2nd International Conference on Biotechnology and Food Science IPCBEE (Vol. 7).

Jay MJ, Loessner JM, Golden AD. 2005. Staphylococcal gastroenteritis. In: Modern Food Microbiology. 7th ed. Springer Science, New York, pp: 545-560.

Kaban G, Kaya M. 2006. Effect of starter culture on growth of *Staphylococcus aureus* Food Control;17(10):797-80

Kantachote D, Charernjiratrakul W. 2008. Selection of lactic acid bacteria from fermented plant beverages to use as inoculants for improving the quality of the finished product Pakistan J. Biol. Sci.,(7):1-8

Kasimoglu A, Akgun S. 2004. Survival of *Escherichia coli O157:H7* in the processing and post-processing stages of acidophilus Yoghurt. Int. J. Food Sci. Tech. (39): 563-568

Kebary KMK, Badawi, RM, Badran II, Hussein SA. 2005. Influence of some nutrients and bile salt on the production of antimicrobial agents by *Bifidobacteria.* Eg. J. of Dairy Sci.,33 (2): 157–170

Kerry RG, Patra JK, Gouda S, Park Y, Shin HS, Das G. 2018. Benefaction of probiotics for human health: a review. Journal of Food and Drug Analysis, 26(3): 927-939

Lucatto JN, Silva-Buzanello RA, Mendonça SNTG, Lazarotto TC, Sanchez JL, Bona E, Drunkler DA. 2020. Performance of different microbial cultures in potentially probiotic and prebiotic yoghurts from cow and goat milks. *International Journal of Dairy Technology*, 73(1): 144-156

Maha ME, Mahmoud E, Nagwa IMK, Mohamed KR. 2015. Studies on contamination of dairy products by aflatoxin M1 and its control by probiotics. J. Global Biosciences, 4(1): 1294-1312

Malorny B, Tassios PT, Radstrom P, Cook N, Wagner M, Hoorfar J. (2003. Standardization of diagnostic PCR for the detection of food borne pathogens. Inter. J. Food Microbiol., 1(25): 39-48

Martinho VJPD, Bartkiene E, Djekic I, Tarcea M, Barić C, Černelič-bizjak M, Szűcs V, Sarcona A, Ferreira V, Klava D. 2022. Determinants of economic motivations for food choice: Insights for the understanding of consumer behaviour. *Int. J. Food Sci. Nutr.* 2022, (*73*): 127–139

MC Brearty S, Ross RP, Fitzgerald GF, Collins JK, Wallace JM, Stanton C. 2001. Influence of two commercially available bifidobacteria cultures on Cheddar cheese quality. International Dairy Journal (11): 599-610

Mehrotra M, Wang G, Johnson WM. 2000. Multiplex PCR for detection of genes for

Staphylococcus aureus enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. Journal of clinical microbiology, 38(3): 1032-1035.

Merzougui SL, khider M. Grosset N. 2014. Prevalence, PFGE typing, and antibiotic resistance of *Bacillus cereus* group isolated from food in Morocco *Foodborne Pathog Dis*2014 11 145

Meyer-Broseta S, Diot A, Bastian S,Riviere G, Cerf O. 2003. Estimation of low bacterial concentration: Listeria monocytogenes in raw milk‖. Int. J. Food Microbiol., 80(1): 1-15

Momtaz H, Dehkordi FS, Rahimi E, Asgarifar A, Momeni M. 2013. Virulence genes and antimicrobial resistance profiles of Staphylococcus aureus isolated from chicken meat in Isfahan province, Iran, Journal of Applied Poultry Research, (22): 913–921.

Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA. 2007. Manual of clinical microbiology, 9th Ed. American Society of Microbiology Press, Washington D.C., USA.

Nassif MRM, Azza SMA, Mona MMA. 2015. Bio-Preservation of Minced Beef Meat as A recent Technology, Egypt. J, Agric. Res. 93, 4(A): 1-9.

Normanno G, La Salandra G, Dambrosio A, Quaglia NC, Corrente M, Parisi A, Santagada G, Firinu A, Crisetti E, Ceiano GV. 2012. Occurrence, characterization and antimicrobial resistanc of enterotoxigenic *Staphylococcus aureus* isolated from meat and dairy products. Int. J. Food Microbiol., (115): 290-296.

Parada JL, Ricoy Caron C, Medeiros ABP, Soccol CR. 2007. Bacteriocins from lactic acid bacteria: Purification, properties and use as biopreservatives. Brazilian Archives of Biology and Technology (50): 512-542

Pereira LC, Souza CHB, Behrens JH, Saad SMI. 2010. *Lactobacillus acidophilus* and *Bifdobacterium* sp. in co-culture improve sensory acceptance of potentially probiotic petit-suissecheese. ActaAlimentaria 39(3): 265-276

Procop G, Church D, Hall G, Janda W, Koneman E ,Schreckenberger P, Woods G. 2017. Aerobic and facultative gram positive bacilli‖. In Koneman's Color Atlas and textbook of Diagnostic Microbiology. 7 th ed. Lippincott Williams and Wilkins Company, Philadelphia, USA.

Quinn PJ, Carter ME, Markey B, Carter GR. 2002. Clinical Veterinary Microbiology –Bacterial causes of bovine mastitis, 8th edition.Mosby, Internal Ltd, London

Ranadheera CS, Evans CA, Adams MC, Baines SK. 2013. Production of probiotic ice cream from goat's milk and effect of packaging materials on product quality. Small Ruminat Research 112(1/3): 112-174

Sambrook J, Fritscgh EF. Mentiates. 1989. Molecular coloning. A laboratory manual. Vol!., Cold spring Harbor Laboratotry press, New York

Sanders ME, Gibson GR, Gill H, Guarner F. 2007. Probiotics: considerations for human health. Nutr. Rev.,(61): 91–99. Council for Agricultural Science and Technology (CAST), 36 (2007)

Sandra, M,Tallen, T. (2012). Efficient isolation and identification of Bacillus cereus Group. Journal of AOAC International, 95(2):446-451

Scherrer D, Corti S, Muehlherr JE, Zweifel C, Stephan R. 2004. Phenotypic and genotypic characteristics of *Staphylococcus aureus* isolates from raw bulk-tank milk samples of goats and sheep. Vet. Microbiol., (101):101-107

Schoeni JL, AC, Wong AC. 2005. Bacillus cereus food poisoning and its toxins of Food Protection, 68 (2005),636-648

Sergeev N, Distler M, Vargas M, Chizhikov V, Herold KE, Rasooly A. 2005. Microarray analysis of Bacillus cereus group virulence factors. J Microbiol Meth (65):488–502.

Servin AL. 2004. Antagonastic activities of Lactobacilli and Bifidobacteria against microbial pathogens, FEMS Microbiology Reviews 28, 405-440.PMID: 15374659

Shah NP. 2007. Functional cultures and health benefts. International Dairy Journal 17(11): 1262-1277

Sotohy A, Sotohy 1, Samia S, Abdel Naby Hanan S, Rania E. 2022. Microbiological Quality Assessment of Dairy Desserts Sold in New Valley Governorate NVVJ., Vol. 2, Issue (1), 2022. ISSN (Print) 2786-0272 ISSN (Online) 2786-0280International Dairy Journal 17(11): 1262-1277

Souza CHB, Saad SMI. 2009. Viability of *Lactobacillus acidophilus* La-5 added solely or in co-culture with a yoghurt starter culture and implications on physico-chemical and related properties of minas fresh cheese during storage. LWT - Food Science and Technology 42(2): 633- 640

Tulay Ozcan L, Yilmaz-Ersan A, Akpinar-Bayizit O, Irmak S, Pinar A. 2010. Viability of Lactobacillus acidophilus LA-5 and Bifidobacterium bifidum BB-12 in Rice Puddin. Uludag University, Department of Food Engineering, 16059 Gorukle, Bursa-Turkiye Received - Prispjelo: 10.02.2010. Accepted - Prihvaćeno: 24.05.201

Van Netten P, van De Moosdijk A, Van Hoensel P, Mossel DAA, Perales I. 1990. Psychrotrophic strains of Bacillus cereus producing enterotoxin. Journal of Applied Microbiology, 69(1): 73-79.

Vinderola CG, Reinheimer JA. 1999. Culture media for the enumeration of Bifidobacterium bifidum and Lactobacillus acidophilus in the presence of yoghurt bacteria. International dairy journal, 9(8): 497-505.

Wada M, khagvadorj E, Bian L, Wang C, Chiba Y, Nagata S, Shimizu T, Yamashiro Y, Asahara T, Nomoto K. 2010. Quantitative reverse transcription-PCR assay for the rapid detection of methicillin-resistant Staphylococcus aureus. Journal of Applied Microbiology (108):779–788

WF, A. 2018. Occurrence of Bacillus cereus in some milk-based desserts. Assiut Veterinary Medical Journal, 64(156): 41-46.

WHO 2006. World Health organization. Department of communicable diseases surveillance and response.

Zavisic G, Petricevic S, Radulovic Z, Begovic J, Golic N, Topisirovic L, Strahinic I. 2012. Probiotic features of two oral *Lactobacillus* isolates. Braz J Microbiol.(43) : 418 – 428

Zhao J, Niu C, Du, S, Liu, C, Zheng F, Wang J, Li, Q. 2020. Reduction of biogenic amines formation during soybean paste fermentation by using Staphylococcus carnosus M43 and Pediococcus acidilactici M28 as starter culture. LWT, 133, 109917.