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STUDY OF MICROBIAL CONTENT IN IMPORTED AND LOCAL DAIRY PRODUCTS (YOGHURT) FROM LOCALLY MARKETS IN BAGHDAD GOVERNORATE

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ABSTRACT

Yogurt or sour milk is thought to be an appropriate food source for a variety of microorganisms that may grow and reproduce in it from a variety of sources, some of which can lead to food poisoning, infection, or spoiling in humans. Thus, the purpose of this study was to assess the bacteriological content and identify any instances of Salmonella enterica, Staphylococcus aurous, Lactobacillus delbrueckii, Lactococcus lactis, and Lactobacillus acidophilus in addition to Escherichia coli bacteria. The percentage of pathogenic and beneficial bacteria present in milk, both locally produced and imported, is measured and compared to standard specifications for the number of permissible germs in milk. The yogurt is traded in stores and markets in Baghdad and its surrounding areas. The time frame for conducting this study was November 2021–March 2021. According to the study's findings, out of 100 samples of local milk, 23 samples included bacteria, and the amounts and kinds of both good and bad bacteria varied. My agencies are 18 positive samples of Staphylococcus aureus and 10 positive samples of Escherichia coli, respectively. In 12 positive Lactococcus lactis samples, 10 positive Lactobacillus delbrueckii samples, and 30 positive Lactobacillus acidophilus samples, Salmonella enterica germs were found. According to the results for 100 samples of imported yogurt, the following were the positive samples for microbial content, with varying levels of germs: Each sample tested positive for lactobacillus lactis, lactobacillus acidophilus, and lactobacillus delbrueckii. Their test results for the remaining microorganisms were positive. Escherichia coli is present in sample 12, whereas bacteria are present in sample 10. Sample 10 of Staphylococcus aurous tests positive for these microorganisms. The findings demonstrated that local milk is less prone to contamination than imported milk because it is produced in a manner that complies with health and safety regulations, shielding it from contamination. In addition to storage, the lack of preservatives, which slows down the decomposition of milk and increases the number of germs, and the transportation procedure from neighboring locations also contribute to the prevention of infection. Excellent and quick. Following the Gram stain characterisation of the isolates on MacConkey and blood agar media, Vitek was used to describe the isolates and all of the bacteria's DNA was recovered. Next, the isolates were added using the 16SRrna gene for molecular diagnosis. Sequencing and recording of isolates were done on the NCBI Global Genetics website.

INTRODUCTION

Milk products, especially yoghurt, play a vital role in our daily life (Das, Hasan and Parveen, 2015). Milk and dairy products are consumed daily by billions of people worldwide (Dogan and

Boor, 2003). Worldwide, the use of milk and dairy products is rising due to their superior nutritional value. Milk and dairy products, with their high nutritional content and beneficial characteristics, are a great growth substrate for a wide range of microorganisms.

Yogurt is the most popular dairy product in Iraq, consumed in large quantities. It is prepared from warm milk treated with a starting culture containing thermophilus-forming Streptococcus salivarius ssp. and Lactobacillus delbrueckii ssp. Bulgaricus (Clark S 2014).

Yogurt gives the body a variety of nutrients, including vitamins, minerals, and proteins.In addition, those with lactose intolerance can eat yogurt (Tamime AY 2007).

Minerals are necessary for many bodily functions. The biological, environmental, and nutritional state of animals determines the amount of various components found in milk and other dairy products. Additionally, the amount of minor and trace elements in dairy products is greatly influenced by geographic location, technical treatments, and feed material quality (Ibrahim K J. 2018).

Food-borne microbial illnesses cause over 20 million cases worldwide each year; A worldwide problem is milk and dairy products contaminated by microorganisms. Over the past twenty years, Salmonella entertica, Escherichia coli, Campylobacter jejuni, and Listeria monocytogenes have been the primary pathogens associated with the majority of infections spread by dairy products. One of the main economic issues facing the globe today is dairy product spoiling. meals with high microbial content and bacterial pathogens are often low-quality meals. Raw milk is the source of many of the microbial hazards connected to dairy products, such as butter, cheese, and yogurt. An infection such as Salmonella spp, or Escherichia coli might be among the live bacteria present in the alive animal, as could other pathogens like Staphylococcus aureus. Fecal contamination during the primary milk collection process might be the cause (Milk and Dairy Products in Human Nutrition, 2008). Additionally, it may get contaminated while being transported, stored, or manufactured. The dairy business still faces challenges in preventing contamination from spoilage bacteria and slowing the development of existing germs. As a result, The microbiological analysis of dairy and dairy products requires further attention. When making dairy products, it is stressed that proper hygiene precautions should be performed and the milk well drained. Additionally, from the standpoint of food safety, educating food workers about personal hygiene is crucial (Microbiology Handbook of Dairy Products, 2009).

METHODOLOGY

The **Sample collection: -** samples are transferred to the laboratory at a rate of time not exceeding 30 minutes, after which the samples are cultured on differential media for the purpose of diagnosing the most common bacteria present in milk. (February 2021 and March 2021) and included the areas (Al-Dora, Al-Bayaa, Al-Mansour, Al-Harithiya, Al-Ghazaliya, Al-Kadhimiyah, Al-Amiriya, Ur neighborhood, and Al-Baladiyat). Inside Baghdad Governorate.

Cultural characteristics: for detection the pathogenic bacteria, samples were cultured on macconkey and blood agar to differentiate the growth and colony characteristic and incubated in 37C for 24 hr. for detection the useful bacteria Serial dilutions of samples were made, from the last dilution; 0.1 ml was transferred to the poured MRS plates and incubated over night at 37˚C under anaerobic conditions using a gas generating kit. (Harrigan and MacCane, 1976).

Microscopically and biochemical tests: - Slides were made using a gram stain method according to (Atlas et al., 1995) and (Bergys manual), where the bacteria were examined to find out whether they were positive or negative for the gram stain, then they were cultured on MacConkey and the and blood agar media. For the purpose of processing, it to the next step, Yeast and bacteria can be identified using the Vitek identification method in order to move on to the next step. This test is

based on the microorganism's consumption of nutrients and metabolic processes; in order to pass, a specific amount of growth must be produced over a predetermined growth period of 18 to 70 hours. diagnosis by the molecular method PCR:

Polymerase chain Reaction PCR assay: The most common bacteria in yoghurt were selected and diagnosed using modern PCR methods (Vogelstein et al., 1979). 16SrRNA primer was designed for the purpose of diagnosis it. The DNA of each bacteria were isolated, Sequencing of the gene was carried out by the National Instrumentation Center for Environmental Management (NICEM), biotechnology lab (machine is a DNA sequencer 3730XL, Applied Biosystem); homology search was carried out using the Basic Local Alignment Search Tool (BLAST) program, which is available at the National Center Biotechnology Information (NCBI) and BioEdit program. afterwards add the primer and visualized on agarose gel electrophoresis by exposure to ultra violate light (302 nm) following Red Stain staining. (Sambrook et al., 1989).

The Primers Used in This Research

Table 1. The universal primer 16s RNA of gene of pathogenic bacteria

Primer	Sequence	Tm (°C)	GC (%)	Product size
Forward	5'- AGAGTTTGATCCTGGCTCAG- 3'	54.3	50.0	1250 base pair
Reverse	5'- GGTTACCTTGTTACGACTT- 3’	49.4	42.1	

Table 2: The optimum condition of detection the pathogenic bacteria

No.	Phase	Tm (°C)	Time	No. of cycle
1-	Initial Denaturation	94°C	3 min.	1 cycle
2-	Denaturation -2	94°C	45sec	35 cycle
3-	Annealing	60°C	45sec	
4-	Extension-1	72°C	1min	
5-	Extension -2	72°C	7 min.	1 cycle

Table (3) The primer of Lactobacillus sp. 16S rRNA gene and its optimum conditions designed in this study

Primer	Sequence	Tm (°C)	GC (%)	Product Size (bp)
Forward	5'-GCAAAGCGTTGTCGCGGATTTA-3'	60.86	50.00	894 base pair
Reverse	5'-GGCTTTGGGCATTGCAGACT-3'	58.86	55.00	

Table (4) Optimum condition of detection 16S rRNA gene

No.	Phase	Tm (°C)	Time	No. of Cycles
1	Initial Denaturation	95	5 min	35 cycles
2	Denaturation-2	95	45 sec	
3	Annealing	62	45 sec	
4	Extension-1	72	45 sec	

RESULTS AND DISCUSSION

The physiochemical characteristics of the yogurt made from cow milk vary depending on the production process, the source of the milk, the length of the fermentation process, and the starter that is introduced throughout the manufacturing process. The manufacturing process of the yogurt, which affected the incubation period, the source of milk based on the breed of cow and how they were fed, the storage method, and the storage temperature, were all responsible for the variations in physiochemical properties that were observed in the comparison across all categories (Awin Ibrahim Mohammed et al., 2021).

In the current survey's findings, which were based on microbial plate counts, demonstrated that various types of bacteria are present in various sample types, particularly in terms of levels of ***Salmonella ssp , E. Coli and Staphylococcus aureus*** and ***Lactobacillus delbrueckii and Lactococcus lactis*** in imported and local Dairy Products (Yoghurt) in stores and markets in the city of Baghdad , By comparing the numbers of bacteria isolated from local and imported samples, based on their compared to what was specified in the Iraqi standard specification for dairy products No. 610, which was published in the Iraqi Facts Newspaper, Issue No. 3041 on April 15, 1985. This study was conducted from November 2021 to March 2021.

The results of the study in 100 samples of local yogurt showed the numbers and concentrations of germs varied, including: *Staphylococcus aurous*, 5 positive samples, *Escherichia coli*, with a ratio of 8 positive samples – 5 positive samples of *Salmonella* bacteria - *Lactococcus lactis*, 12 positive samples - *Lactobacillus delbrueckii*, 10 positive samples, Enterobacter cloca 2 positive samples, 12 positive samples of Lactobacillus acidophilus. As showed in table (5).

The total bacterial count of *staphylococcus spp ,E. coli* and *Salmonella ssp* ranged between 1.0×10^2 colony- to 2.1×10^3 colony forming units per milliliter (cfu/ml), The number of bacteria isolated in locally made yogurt is in the normal limits compared to what was specified in the Iraqi standard specification for dairy products No. 610, which was published in the Iraqi Facts Newspaper, Issue No. 3041 on April 15, 1985.

The results for 100 samples of imported yohurt showed that the positive samples for microbial content, with different numbers of bacteria, were as follows: *Lactobacillus delbrueckii* 10, *Lactococcus Lactis* 13. While 4 sample was positive for Staphylococcus aurous, Escherichia coli, with a ratio of 8 positive samples - give 3 positive of Salmonella, Lactobacillus acidophilus 10 positive, table (6).

The number of bacteria isolated from imported yoghurt ranged from ($0.35 \times {}^1 10$ (c.f.u/ml)), which is within the permissible limits according to the Iraqi standard specification for dairy products No. 610. The samples taken from the yoghurt showed that most of the samples were positively containing lactic bacteria. This result is natural since in the yoghurt industry these bacteria are added to the milk manufacturing for their benefit, additional types of *lactobacilli* bacteria may be added. The bacteria convert the sugar in milk, called lactose, to lactic acid, which thickens the milk and develops its distinctive tart flavor (Aswal et al., 2012).

All microorganisms, including harmful and non-pathogenic bacteria, were eliminated during the heat treatment process used to manufacture yogurts. Lastly, contamination with these species may occur throughout the production, processing, and packing stages. Every single microbe was inside the permeability limits. (Fernandez *et al*, 2000). (Frank *et al*,.2004).

After identifying the isolates on MacConkey medium and blood agar medium, and using a gram stain, the isolates were diagnosed using Vitek, and DNA was extracted from all bacteria and preserved until use. The isolates were then entered for molecular diagnosis using the 16SRrna gene fig (1). Sequencing was performed and the isolates were registered on the NCBI global genetics website.

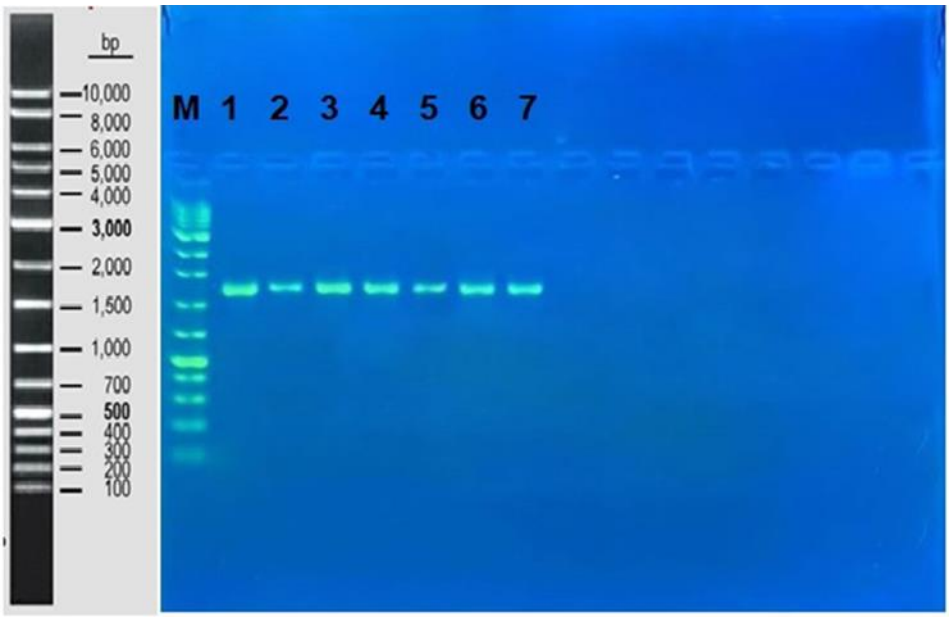


Figure (1) PCR product of the six bacterial isolate band size 1250 bp. The product was electrophoresis on 1.5% agarose at 5 volt/cm2. 1x TBE buffer for 1:30 hours. N: DNA ladder (100).

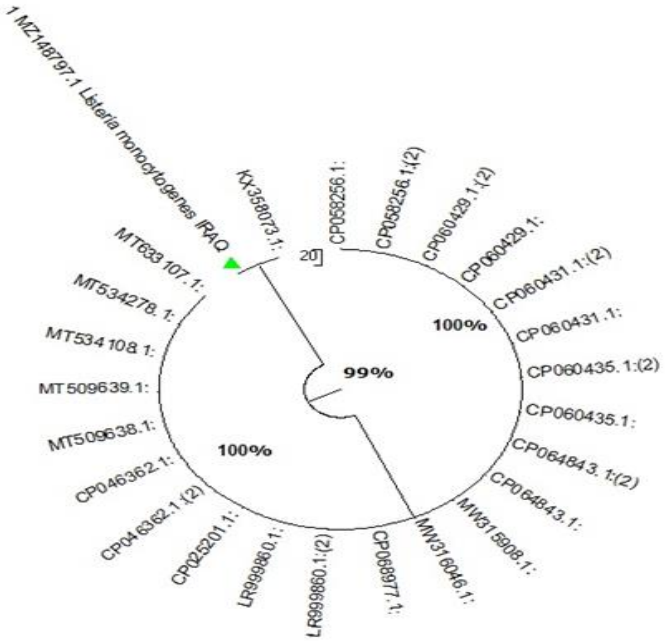


Figure (2) Phylogenetic tree analyses salmonella

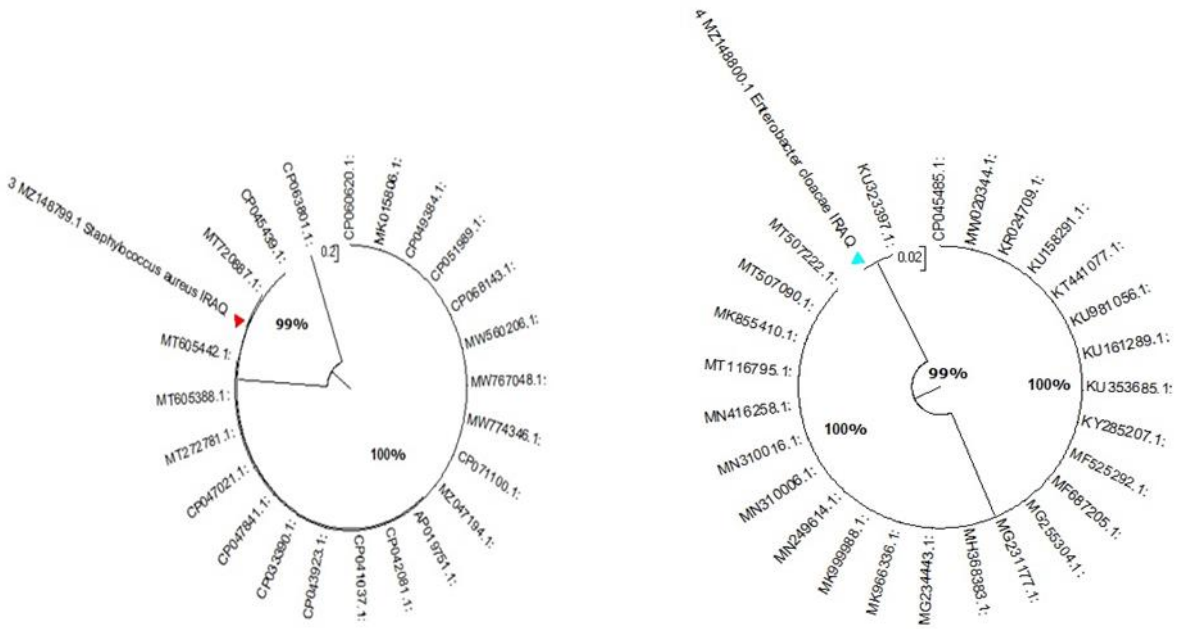


Figure (3) Phylogenetic tree analyses of : A-Staphylococcus aureus B-Enterobacter cloca

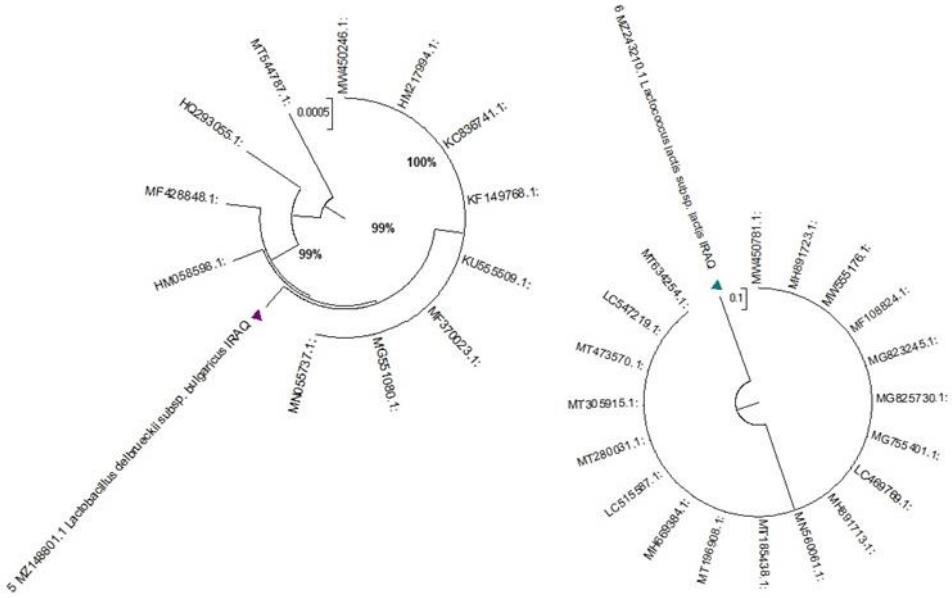
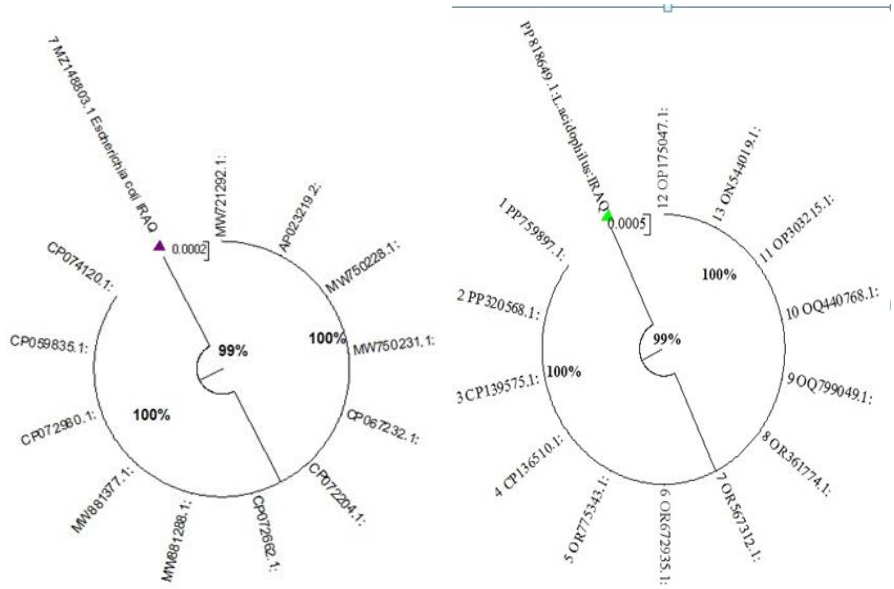


Figure (4) Phylogenetic tree analyses of:A- Lactobacillus delbrueckii B-Lactococcus lactis



A **B**

Figure (5) Phylogenetic tree analyses of : A- Escherichia coli. B- Lactobacillus acidophilus

For further identification of some general bacteria that found in yoghurt samples, PCR methods were used and each identified bacteria by PCR were recorded in NCBI as listed below:
Partial sequencing of the 16S ribosomal RNA gene from *Salmonella enterica* strain SMM939
ID for sequence: MT115788.1 Length: 602There is one match.
Range 1: Next Match Previous Match GenBankGraphics 71 to 560.

1- *Staphylococcus aureus* strain BVC Staph S50 16S ribosomal RNA gene, partial sequence Sequence
ID: MT720887.1Length: 797Number of Matches: 1
Range 1: 71 to 770GenBankGraphics Next Match Previous Match

2- *Enterobacter cloacae* strain S3 16S ribosomal RNA gene, partial sequence Sequence
ID: KU323397.1Length: 809Number of Matches: 1
Range 1: 71 to 770GenBankGraphics Next Match Previous Match

3- *Lactobacillus delbrueckii* subsp. *bulgaricus* strain MG515 16S ribosomal RNA gene, partial sequence
Sequence ID: MN055737.1Length: 1438Number of Matches: 1
Range 1: 211 to 1260GenBankGraphics Next Match Previous Match

4- *Lactococcus lactis* subsp. *lactis* strain SDCM 5128 16S ribosomal RNA gene, partial sequence
Sequence ID: MT634254.1Length: 1472Number of Matches: 1
Range 1: 224 to 1131GenBankGraphics Next Match Previous Match

5- *Escherichia coli* strain KA2 16S ribosomal RNA gene, partial sequence
Sequence ID: MW881377.1Length: 1396Number of Matches: 1
Range 1: 269 to 1178GenBankGraphics Next Match Previous Match.

6- Lactobacillus acidophilus strain MAA-IRAQ-25 16S ribosomal RNA gene, partial sequence GenBank: PP818649.1.

Table (5) percentage of bacterial distribution among locally yoghurt sample

Local Yoghurt/Type of Bacteria	No. of Total Samples	No. of Positive	%
Salmonella enterica	100	5	5%
Staphylococcus aureus	100	10	10%
Enterobacter cloacae	100	2	2%
Lactobacillus delbrueckii	100	10	10%
Lactococcus lactis	100	12	12%
Escherichia coli	100	8	8%
Lactobacillus acidophilus	100	12	12%

Table (6) percentage of bacterial distribution among imported yoghurt sample

Imported Yoghurt/Type of Bacteria	No. of Total Samples	No. of Positive	%
Salmonella enterica	100	3	3%
Staphylococcus aureus	100	4	4%
Enterobacter cloacae	100	8	8%
Lactobacillus delbrueckii	100	10	10%
Lactococcus lactis	100	13	13%
Escherichia coli	100	8	8%
Lactobacillus acidophilus	100	10	10%

The findings demonstrated that because local yogurt is produced and distributed to markets in a timely and healthful manner while adhering to safety and health regulations, it is less likely to be contaminated than imported milk. In addition to the lack of preservatives, which speed up the deterioration of the milk and increase the quantity of germs, the internal transit procedure also contributes to reduce the variation in its contamination.

Raw milk is the source of many of the microbial hazards connected to dairy products, such as butter, cheese, and yogurt. Post-process contamination, which is brought on by decaying bacteria that obstruct the growth of organisms that survive, is still a problem for the dairy industry. Consequently, the microbiological investigation of milk and dairy products requires more attention. It is important to keep in mind that while making dairy products, milk needs to be completely pasteurized and that the right sanitary procedures need to be taken. From the perspective of food safety, it is also critical to train food workers on personal cleanliness. (Pal, et al., 2016).

Since regular yogurt generally has a pH of between 4.4 and 4.8, which is slightly acidic, long-term storage can raise the pH towards basicity, which can facilitate the growth of undesirable microscopic organisms. This is one additional way that yogurt's acidity helps explain why it can stop tiny creatures from growing.

CONCLUSION

The contamination of yogurt may not be the result of poor manufacturing, but there are other factors mentioned in the discussion of the results, and these results have a significant role in the contamination of yogurt to a very large extent. In addition to the method of transportation and storage, it also has a role in destroying yogurt or contaminating it with pathogens.

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المستخلص

يُعتبر اللبن الرائب (الزبادي) وسطاً غذائياً مناسباً لنمو وتكاثر أنواع مختلفة من الأحياء الدقيقة التي قد تصل إليه من مصادر عديدة والتي قد يكون بعضها من مسببات الفساد أو العدوى والتسمم الغذائي للإنسان؛ لذلك استهدفت هذه الدراسة تقييم المحتوى البكتريولوجي والكشف عن وجود بكتيريا *Escherichia coli* *Salmonella enterica* - *Staphylococcus aurous* - *Lactobacillus delbrueckii*- *Lactococcus lactis* -*Lactobacillus acidophilus*-.

في اللبن الرائب المتداول المحلي والمستورد في المحلات واسواق مدينة بغداد وضواحيها، ونسبة تواجد الأنواع المذكورة من البكتيريا الممرضة والنافعة في اللبن وكذلك مقارنة النتائج التي تم الحصول عليها بالمواصفات القياسية لاعداد الجراثيم المسموح بها. أُجريت هذه الدراسة في الفترة من شهر نوفمبر لسنة 2021 ف إلى شهر مارس لسنة 2021 .اظهرت نتائج الدراسة في 100 عينة من اللبن المحلي وجود الجراثيم في 23 عينة واختلفت اعداد وتراكيز البكتيريا الضارة والنافعة وكالاتي:Staphylococcus aureus عينة 18موجبة بنسبة Escherichia coli 18عينة موجبة - 10 عينة موجبة من جراثيم Salmonella - Lactococcus lactis 12 عينة موجبة -Lactobacillus delbrueckii 10 و Lactobacillus acidophilus 30عينات موجبة. وقد اظهرت النتائج ل100 عينة من اللبن الزبادي المستورد ان العينات الموجبة للمحتوى الميكروبي مع اختلاف اعداد الجراثيم كالاتي: فقد كانت جميع العينات موجبة لكل من الانواع من الجراثيم.

Lactobacillus acidophilus, Lactobacillus delbrueckii and Lactobacillus lactis موجبة. اما باقي الجراثيم فقد كانت موجبة وكالاتي

Staphylococcus aurous عينة 10موجبة *Escherichia coli* و عينة 12 موجبة - 10 عينة من جراثيم *Salmonella enterica* .

ان النتائج اظهرت ان اللبن المحلي اقل عرضة للتلوث من المستورد وذلك كونه غير معرض للتلوث عند تصنيعه بطرق تلتزم لمعايير الصحه والسلامة وكذلك تلعب عملية النقل من المناطق القريبة دورا في منع تلوثه بالاضافة الى عدم وجود المواد الحافضة يبطئ من فساد اللبن وزيادة اعداد الجراثيم بالاضافة الى الخزن الجيد والسريع. بعد تشخيص العزلات على وسط MacConkey ووسط أجار الدم وباستخدام صبغة جرام، تم تشخيص العزلات باستخدام Vitek وتم استخلاص DNA من جميع البكتيريا. بعد ذلك تم إدخال العزلات للتشخيص الجزيئي باستخدام الجين 16SRrna . تم إجراء التسلسل وتسجيل العزلات على موقع علم الوراثة العالمي NCBI.