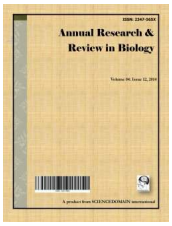

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# Improve Hygienic Quality of Egyptian Karish Cheese Employing Isolated Antagonistic *Lactobacilli* Strains

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***Authors' contributions***

*This work was designed and carried out in collaboration between all authors. Author AMGD wrote the first draft of the manuscript and performed the statistical analysis. Authors MGA and AMGD managed the analyses of the study and the literature searches. Author EHEA project PI managed funding of the study. All authors read and approved the final manuscript.*

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## ABSTRACT

Good health is one of the sustainable development goals. This study aims to improve hygienic quality of Karish Egyptian traditional cheese, using two wild antagonistic *Lactobacilli* isolates used as individual starter cultures; KP623 (*Lb. plantarum*) and KP654 (*Lb. delbrueckii* subsp. *lactis*) isolated from Karish cheese to keep autochthonous properties and function as bio-preservatives to extend shelf life. Collected Karish cheese samples were micro-biologically analyzed. Two isolates; KP623 and KP654 were selected for application out of thirty-seven *Lactobacilli* lactic acid bacteria (LAB) isolated strains and identified via 16S rRNA approach. Collected Karish samples reflected their inferior quality containing high counts of coliform, *Staphylococcus* spp., yeasts and molds (5.18, 2.51 and 4.95 $Log_{10}$ CFU $g^{-1}$ respectively). Employing the two antagonistic isolates enhanced both microbial quality and organoleptic properties. Results encourage recommending the two *Lactobacilli* strains as starter cultures for safe products avoiding human illness and economic losses.

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*Keywords: Lactobacillus spp.; wild strains; hygienic quality; Karish cheese; 16S rRnA.*

The sequence data of the two isolated LAB strains in this article have been deposited to the GenBank Data Library under the accession numbers KX378140 (KP623) and KX378141 (KP654).

## 1. INTRODUCTION

Food safety is about producing, handling, storing and preparing food in such a way as to prevent infection and contamination in the food production chain, and to help ensure that food quality is maintained to promote good health [1]. There are many documented evidences which demonstrated that disturbance of intestinal microbiota is linked to the risk of developing infectious, inflammatory and allergic diseases [2]. About 90% of the Karish cheese produced using primitive methods in the rural districts in Egypt; this traditional method affords many opportunities for microbial contamination. It is generally made from raw milk often of poor bacteriological quality, under unsatisfactory conditions, and finally, the product is sold uncovered without a container. Therefore, there is high risk of contamination and can be considered as a good medium for the growth of different types of spoilage and pathogenic microorganisms. Undesirable pathogenic bacteria microbial groups e.g., Coliform, *Staphylococcus, Salmonella* and *Escherichia coli*, were detected in traditional Karish samples, in addition to yeasts and molds [3,4].

Increasing demands by consumers for natural and chemical-free products has led the food industry to search for novel and alternative strategies for food bio-preservation. The genus *Lactobacillus* is essential to modern food technologies, because of increasing interest in beneficial effects that actively promoted the use of *Lactobacilli* especially for their potential to replace antibiotic growth promoters [5]. Attempts have been tried to add defined Lactic acid bacteria (LAB) as a starter culture in Karish cheese seeking for safety with conservation of Karish cheese properties [6]. Wild LAB strains are considered the renewable source of promising cultures in technological field as they lead to development in fermentation industry [7]. *Lactobacillus* is used as a starter culture in various food fermentations contributing to organoleptic properties, flavor and texture in addition to antagonistic activity to control the growth of the food borne pathogens [8].

The aim of this work was to evaluate and improve the hygienic quality of Karish cheese. Enhancing microbial quality was achieved through employing two wild antagonistic LAB *Lactobacilli* strains individually as starter cultures; KP623 (*Lb. plantarum*) and KP654 (*Lb. delbrueckii* subsp. *lactis*) isolated from Karish cheese, that allowed working on two parallel lines, allow milk heat treatment while keeping cheese autochthonous properties and selecting antagonistic promising strains qualified to function as bio-preservatives targeting extended shelf life.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection and Microbial Quality Assessment

Fifteen samples made of raw milk of artisanal Egyptian Karish cheese were collected from different districts, Alexandria Governorate, Egypt. The samples were kept in sterilized cups, stored refrigerated at 4ºC until analyzed fresh within two days.

Microbial quality of collected samples was assessed. For the total count the plate count agar (PCA) of Biolife (Italy) was used, for 48 h at 37ºC. Members of LAB group were counted on MRS agar (Biolife), for 48 h at 37$^{o}$C. Undesirable microbial contaminants were screened on selective media; *Staphylococci* spp. was enumerated on Staph 110 media (Biolife) for 48 h at 37$^{o}$C and coliforms were counted on Violet Red Bile agar (Biolife) at 37ºC for 20 h. The enumeration of yeasts and moulds was on Potato Dextrose agar (Biolife) acidified media for 7 days at 37ºC. The conventional diluting pouring plate technique was followed as described by Marth [9]. The results represented in colony forming unit $Log_{10}$CFU $g^{-1}$ of cheese sample. Same analysis was held for Karish cheese treatments for comparison.

### 2.2 Isolation and 16S rRNA Identification of *Lactobacilli* Strains

The samples were enriched in reconstituted skim milk RSM (12.5%) inoculated in selective MRS medium for *Lactobacilli* isolation, and purified through streak plate method. Purified strains were stored at -20ºC and registered in Faculty of Agriculture Saba Basha, Alexandria University Culture Collection (FABA).

Thirty seven *Lactobacilli,* Gram-positive and catalase-negative isolates were identified to the genus level using phenotypic ($CO_2$ production, growth at 10 and 45ºC) and biochemical (carbohydrate fermentation) characterization [10]. The strains then were technologically characterized for flavor formation, Exopolysaccharide (EPS) and acid production, autolytic and proteolytic activity and antimicrobial effect as described by [11].

Selected strains characterization was confirmed using genotypic 16S rRNA approach. The full length 16S rRNA gene of two bacterial isolates; KP623 (*Lb. plantarum*) and KP654 (*Lb. delbrueckii* subsp *lactis*), was amplified via PCR, partially sequenced by using an AB 373 DNA sequence (Applied Biosystem, Mubarak city for scientific research) [12]. The nucleotide sequences were subjected to BLAST (basic local alignment search tool) program to align sequences with homologous sequences in the Genbank. Software Bioedit was used to align the query with other sequences in the GenBank then phylogenetic tree of the bacterial isolates was drawn. The sequences of 16S rRNA was deposited to the GenBank database under the accession numbers; KX378140 (KP623) and KX378141 (KP654).

## 2.3 Starter Cultures and Karish Cheese Making

Depending on technological characteristics; two *Lactobacilli* strains with antimicrobial activity were selected; KP623 (*Lb. plantarum*) and KP654 (*Lb. delbrueckii* subsp *lactis*) for application. Three treatments of Karish cheese were made of defatted, pasteurized cow's milk; control (T1), inoculated with commercial mesophilic starter culture of Karish cheese MA011 (*Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*) obtained from (Rhodia Food/ Danisco France), (T2) and (T3) inoculated individually via two selected LAB isolates; KP623 (*Lb. plantarum*) and KP654 (*Lb. delbrueckii* subsp *lactis*) respectively. Based on a preliminary experiment; MA011 starter culture and both Lactobacilli isolates were enriched in sterilized skimmed milk to reach $10^6$-$10^7$ CFU $mL^{-1}$ viable count prior to inoculation in milk for cheese making [13]. Cheeses were analyzed in fresh, after 3 and 10 days of storage at 4ºC for microbiological characteristics, chemical composition, sensory evaluation and texture properties.

## 2.4 Chemical Analysis of Karish Cheese

Total protein in cheese samples was determined by semi macro Kjeldahel method according to [14]. The conventional Gerber's method as described in [14] was used for determination of cheese fat content. Total solid determined according to [15]. pH was determined using a glass electrode (HANNA pH 211 instrument microprocessor pH meter, Romania). Titratable acidity (TA) was determined as Lactic acid % of cheese weight using NaOH (N/9).

## 2.5 Texture Analysis

Texture properties of fresh Karish cheese samples were evaluated using Texture Analyzer (CNF/Faranell, England). Refrigerated Karish cheese samples were prepared in cubes with dimensions (50 x 50 x 50 mm) and centrally positioned beneath the probe. Speed was 1mm $s^{-1}$ and 10 mm was the distance of penetration at ambient temperature (approximately 25℃). Data were collected on computer and the parameters texture profile parameters; hardness, consistency and adhesiveness were calculated from LFRA texture analyzer and computer interface as described by [16]. The analyses were carried out in duplicates; the means and standard deviation of data was calculated.

## 2.6 Sensory Evaluation

Karish cheese samples of the three treatments were cut into pieces and placed on white plates. Samples were tempered at ambient temperature (20 ±2℃) and then presented to the panelists in a random order. Water was provided between samples. The two Karish cheese treatments; T2 and T3 prepared using isolated *Lactobacilli* strains were evaluated organolepticaly after zero, 3, and 10 days of ripening, at Faculty of Agriculture, Saba Basha Alexandria University by 8-10 graders, and compared with control treatment (T1) of Karish cheese made using the mesophilic starter culture. Each panel assessed cheese separately for flavor (smell and taste), texture and appearance. A list containing the mast widely accepted description of flavor, off-flavor and texture of soft cheese was present to aid the panelists in carrying out the organolyptic evaluation. The scale for flavor (smell and taste) was 1, bad; 2, sufficient; 3, good; 4, very good. The scale for texture or appearance: 1, soft; 2, normal; 3, firm /hard. Intensity remarks were on scale from (1-4) 1, slightly; 2, moderate; 3, strong; 4, very strong. The graders gave also the cheese overall grade out of (100) for total

acceptability. The sensory evaluation procedure was modified from the method described previously [17,18]. The average of 5 replicates of sensory evaluation data with standard deviation was determined.

### 2.7 Statistical Analysis

Statistical analysis was performed using Analytical Software SPSS® 13.0 (Statistical Package for the Social Sciences) (2005).

## 3. RESULTS

### 3.1 Evaluation of Collected Karish Cheese Microbial Quality

Table 1 exhibits microbial analyses of collected 15 Karish cheese samples compared with The Egyptian standards for Kariesh cheese 1008 4/2005 [19]. As revealed from results, the collected samples harbor microbial contaminants exceed the acceptable level according to Egyptian standards 1008 4/2005. The coliform count as fecal indicator and gram negative foodborne bacteria varied between a minimum of 4.88 and maximum of 5.47 with average of 5.18 $Log_{10}CFU\ g^{-1}$. Gram positive foodborne bacteria; *Staphylococcus* spp. presence varied between 2.04 and 2.97 with an average of 2.51 $Log_{10}CFU\ g^{-1}$. Another unacceptable microbial parameter in collected samples was the yeasts and molds; the count was between 4.78 and 5.13 with average of 4.94 $Log_{10}CFU\ g^{-1}$ failed to conform to the Egyptian standards. The TBC is considered high since it reached 10.65 $Log_{10}CFU\ g^{-1}$ while the LAB count was 8.72 $Log_{10}CFU\ g^{-1}$ in average.

### 3.2 Isolation, Characterization and 16S rRNA Identification of *Lactobacilli* Strains

LAB *Lactobacilli* groups isolated from Karish cheese samples are illustrated in Table 2. Group B; Facultative heterofermentative showed its dominance since it occupied more than 50% of total isolated *Lactobacilli*, followed by Group C; obligatory heterofermentative (producing lactic acid, carbon dioxide, ethanol, and/or acetic acid in equimolar amounts) with 37.84% and finally comes Group A; obligatory homofermentative (producing more than 85% lactic acid) with only 10.81%.

Technological characterization of isolated *Lactobacilli* strains nominated selection of two strains; KP623 (*Lb. plantarum*) and KP654 (*Lb. delbrueckii* subsp *lactis*) for their significant properties that showed in Table 3. The most important property was that the two strains showed antimicrobial activity against *E. coli* with diameter 1.7 and 1.2 cm respectively. The strain *Lb. delbrueckii* subsp. *lactis* KP654 showed intermediate autolytic activity (69 to 40%), while the strain *Lb. plantarum* KP623 was classified as a poor autolysis culture (39 to 4%), according to [20] classification. On the other hand, the strain *Lb. delbrueckii* subsp. *lactis* KP654 also showed proteolytic effect. Both strains showed slow acid producing ability and none of them produced EPS.

Phenotypically pre-identified *Lactobacilli* strains; KP623 and KP654 were selected for confirming identification via 16S rRNA approach, depending on possessing antimicrobial activity and other preferable technological characteristics. Phylogeny trees (Figs. 1 and 2) were drawn using the results of BLAST (basic local alignment search tool) analysis. Sequences were screened for chimeras using BioEdit sequence alignment editor and analysis program. The sequence data of the two isolated strains were deposited to the GenBank Data Library under the accession numbers; KX378140 (KP623) and KX378141 (KP654). Their partial sequences were as follows;

**KP623** [*Lactobacillus plantarum*] (accession number KX378140, bases 1 to 242)
GGGAAACCTGCCCAGAAGCGGGGGATAACACCTGGAAACAGATGCTAATACCGCATAACAACTT
GGACCGCATGGTCCGAGCTTGAAAGATGGCTTCGGCTATCACTTTTGGATGGTCCCGCGGCGTA
TTAGCTAGATGGTGGGGTAACGGCTCACCATGGCAATGATACGTAGCCGACCTGAGAGGGTAAT
CGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAG

**KP654** [*Lactobacillus delbrueckii* subsp. *lactis*] (accession number KX378141, bases 1 to 685)
GACTACCAGGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCCTCAGCGTCAGTTGCAGACC
AGAGAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTCCACCGCTACACATGGAGT
TCCACTCTCCTCTTCTGCACTCAAGAATGACAGTTTCCGATGCAGTTCCACGGTTGAGCCGTGGG
CTTTCACATCAGACTTATCATTCCGCCTGCGCTCGCTTTACGCCCAATAAATCCGGACAACGCTT
GCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGACTTTCTGGTTGATTACCGT
CAAATAAAGACCAGTTACTGCCTCTATCCTTCTTCACCAACAACAGAGCTTTACGATCCGAAGAC

CTTCTTCACTCACGCGGCGTTGCTCCATCAGACTTGCGTCCATTGTGGAAGATTCCCTACTGCTG
CCTCCCGTAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCCGATCAGTCTCTCAACTCGGCT
ACGCATCATTGCCTTGGTAGGCCTTTACCCCACCAACTAGCTAATGCGCCGCGGGCTCATCCTA
AAGTGACAGCTTGCGCCGCCTTTCAAACTTGAATCATGCGATTCATGTTGTTATCCGGTATTAGC
ACCTGTTTCCAAGTGGTATCCCAGTCTTTAGGGCAGAT

### 3.3 Microbial Quality of Produced Karish Cheese

Table 4 illustrates microbiological properties of Karish cheese treatments. It was observed that; the LAB counts were comparable to total counts, and none of the undesired microorganisms; coliform or *Staphylococcus*. spp., had appeared in all products until the last tested cheese age (10 days). Furthermore, it is noticeable that, although the total counts showed almost the same range in collected samples and the three treatments, but LAB counts in the treatments were elevated significantly with time progress. On the $10^{th}$ day of storage; yeasts & molds started to appear in T1 (control) treatment (2 and 1 $CFUg^{-1}$/ averaged 0.15 $Log_{10}CFU\ g^{-1}$), but these results were within the acceptable limit of Egyptian standards 1008 4/2005. The bright side of these results was that the yeasts and molds did not show up in treatments T2 and T3 that used the isolated antagonistic *Lactobacilli* as individual starter cultures; KP623 (*Lb. plantarum*) and KP654 (*Lb. delbrueckii* subsp *lactis*) respectively, until the $10^{th}$ day of storage.

### 3.4 Chemical Analysis of Karish Cheese Treatments

Data presented in Table 5 showed chemical composition of Karish cheese treatments. Comparing to T1 (control), results of the selected *Lactobacilli* strains; KP623 (*Lb. plantarum*) and KP654 (*Lb. delbrueckii* subsp *lactis*) used in cheese making did not show significant changes on chemical composition except for slight significant decrease in protein/ dry matter % coincided with relative significant increase in moisture content. Other chemical properties were comparable to control.

### 3.5 Sensory Evaluation of Karish Cheese

Sensory evaluation of Karish cheese treatments is exhibited in Table 6. The highlighted result was that, up to the end of storage period; the graders gave all the cheese treatments high overall grade ranged between 90 and 93 that indicated high acceptability. On comparing to T1 (control), the results showed that organoleptic properties were significantly affected when using selected *Lactobacilli* strains as individual starter cultures. Cheese made with *Lb. plantarum* (KP623) was relatively special when fresh where it described as creamy and good flavor, soft and smooth texture. However, the flavor of cheese produced by the two isolated strains has been described as Karish cheese-like and their appearance described as normal.

### 3.6 Texture Profile Analysis of Karish Cheese Treatments

Texture profile analyses parameters; Hardness, Adhesiveness, Springiness, Cohesiveness, Gumminess and Chewiness of fresh Karish cheese treatments are represented in Fig. 3. Both treatments, T2 and T3 that used isolated *Lactobacilli*; KP623 (*Lb. plantarum*) and KP654 (*Lb. delbrueckii* subsp. *lactis*) respectively, showed less values in all texture profile parameters comparing to control treatment (T1) especially in chewiness that was about 50% of the control.

## 4. DISCUSSION

The obtained data from microbial quality assessment of collected Karish cheese samples indicated their inferior hygienic quality with a great chance of being a cause of foodborne illness. According to the Egyptian Standard ES 1008-2000; 50 % of cheese samples are not accepted due to the high counts of coliform group as fecal indicator contamination mostly in Karish variety [21]. *Staphylococci* is usually the dominant pathogens in traditional Karish cheese, this microbe was found in about 10% of examined Karish cheese samples [22]. The International Commission on Microbiological Specifications for Foods has classified cheese as a high risk potential hazard. A high yeast count often indicates neglected hygienic measures during production and handling, contamination of raw material, unsatisfactory sanitation, or unsuitable time and temperature during storage and/or production [3]. The high obtained TBC may be attributed to the high LAB content. Similar counts were reported by [22] for lactic acid bacteria (1.02 X $10^8$ CFU $g^{-1}$/

8.08 $Log_{10}$CFU $g^{-1}$) for Karish cheese in Egyptian markets. Although the Egyptian standards No.1008 4/2005 did not mention guidelines for acceptable levels of total bacterial count (TBC) for Karish cheese, but they obligate the pasteurization or any equivalent heat treatment of milk.

Lactic acid *Lactobacilli* bacteria may play the main role in the fermentation and organoleptic properties of Karish cheese because of their common presence where, *Lactobacilli* was reported to form a significant part of the final market cheese, but the origin of the *Lactobacilli* in this product remains unclear [23]. That may translate the diversity of *Lactobacilli* groups isolated from Karish cheese samples.

*Lactobacilli* have the longest history as bio-therapeutic agents which serve the commercial interest in using LAB as natural food preservative for the antimicrobial systems possessed by these bacteria [24]. This was the main basis of nominated two *Lactobacilli* isolates, KP623 and KP654 as they showed inhibitory zone diameter of (1.7 and 1.2 cm respectively) against *E. coli*. Enhanced autolysis and high amino-peptidase activities showed by the strain *Lb. delbrueckii* subsp. *lactis* KP654 gave it an advantage where this can be a limiting factor in the rapid formation of flavor constituents during ripening, while slow acid producing ability of the two strains did not affect their selection as this is not a cornerstone in Karish cheese manufacture. The obtained results were in agreement of those reported by [20].

The phylogeny trees applies not only to the organisms that house genes but also to the evolutionary history of the genes themselves [25]. It is obviously clear through phylogeny trees of the two selected isolates (Figs. 1 and 2), their match with the strains' sequence identification which confirm the obtained results.

The microbial analyses of Karish cheese treatments announced the high microbial quality of these products achieving the research main goal. The results highlighted that of the dominant microorganisms present in the products are LAB; the intended added starters. These results are in agreement with [26], who reported antagonistic activity of *Lactobacillus* against *Staphylococcus*. spp. and coliform. On comparing with Table 1, these results also reflect the success in obtaining optimum hygienic conditions throughout the production process unlike the traditional procedure. Increased LAB counts could be relied on the absence of nutrients competition between LAB and other microorganisms, especially in the individual culture treatments; T2 and T3. LAB are known to compete with other microbes by modifying the microenvironment by their metabolic end products [27]. Yeast and molds absence in T2 and T3 till the $10^{th}$ day of cold storage, may translate the antimicrobial effect of those two promising strains which achieve the main aim of this study in better microbial quality and extended shelf life of produced Karish cheese. These results agreed with [28] opinion who stated that; LAB isolated from various fermented foods produce organic acids and a high diversity of antimicrobial agents, which are responsible for the upkeep of quality and the palatability of fermented foods.

Decrease in protein/ dry matter % is considered an advantage since it reflected positively on sensory and texture analyses of the isolated strains' products, resulting soft and smooth product with decreased hardness, adhesiveness and chewiness. Other chemical properties were comparable to control and kept the characteristics familiar to average consumer. Similar results were reported by (Awad, 2016).

Sensory analysis translated the chemical and texture analyses (Table 5 and Fig. 3), where less protein/ dry matter % decreased hardness and compacted structure of the new products causing softness. Keeping organoleptic characteristics similar to conventional Karish cheese with higher hygienic quality is encouraging. These results agreed with what earlier reported that *Lactobacilli* are members of autochthonous non-starter lactic acid bacteria (NSLAB) microbiota of traditional raw milk cheeses and contribute to peculiar flavors of traditional cheeses [29].

Texture profile analyses results confirmed the relation with the chemical properties showed in Table 5; where compact structure was a result of relatively higher protein/ dry matter % of T1 (control). The higher moisture content the more smooth cheese that coats the mouth during mastication [16]. Such improvement in textural characteristics could increase consumer acceptability to these new products.

**Table 1. Microbiological analyses of Karish collected samples and Egyptian standards**

| Microbial item | Min $Log_{10}$ CFU $g^{-1}$ | Max $Log_{10}$ CFU $g^{-1}$ | Average $Log_{10}$ CFU $g^{-1}$ | ±SD | Egyptian Standard ES 1008 4/2005 ($Log_{10}$ CFU $g^{-1}$) |
|---|---|---|---|---|---|
| Total Bacterial Count (TBC) | 10.40 | 10.91 | 10.65 | 0.36 | No guidelines* |
| Lactic Acid Bacteria (LAB) | 8.53 | 8.91 | 8.72 | 0.27 | No guidelines* |
| Coliform | 4.88 | 5.47 | 5.18 | 0.42 | Not exceed 1 (10 CFU $g^{-1}$) |
| *Staphylococcus* spp. | 2.04 | 2.97 | 2.51 | 0.66 | Absent in 1 g |
| Yeasts & Molds | 4.78 | 5.13 | 4.95 | 0.25 | Yeast: Not exceed 2.6 (400 CFU $g^{-1}$)<br>Molds: Not exceed 1 (10 CFU $g^{-1}$) |

*Minimum, maximum and average values based on 15 collected Karish cheese samples; *No guidelines of acceptability according to total bacterial and LAB count in Egyptian standards 1008 4/2005 for Karish cheese*

**Table 2. Total LAB *lactobacilli* groups isolated from Karish cheese samples**

| Group | No. of isolate | % | Gram | Catalase | Growth at | | Co2 |
|---|---|---|---|---|---|---|---|
| | | | | | 10℃ | 45℃ | |
| Rod (*Lactobacilli*) | 37 | | | | | | |
| Group A | 4 | 10.81 | + | - | - | + | - |
| Group B | 19 | 51.35 | + | - | + | + or - | - |
| Group C | 14 | 37.84 | + | - | - or + | + or - | + |

*Group A: Obligatory homofermentative, Group B: Facultative heterofermentative, Group C: Obligatory heterofermentative*

**Table 3. Technological characteristics of selected isolated *lactobacilli* strains**

| Strain | Species | Antimicrobial activity | | Proteolysis | Acid producing ability | Autolytic activity% | EPS |
|---|---|---|---|---|---|---|---|
| | | (+ ; -) | Diameter (cm) | (+ ; -) | | | |
| KP623 | *Lb. plantarum* | + | 1.70 | - | Slow | 39.0 | - |
| KP654 | *Lb. delbrueckii* subsp. *lactis* | + | 1.20 | + | Slow | 40.3 | - |

**Table 4. Microbiological properties of Karish cheese treatments**

| Treatment | Age of cheese (days) | Total count $Log_{10}CFU\ g^{-1}$ | LAB $Log_{10}CFU\ g^{-1}$ | Coliform $Log_{10}CFU\ g^{-1}$ | *Staphylococcus* spp. $Log_{10}CFU\ g^{-1}$ | Yeasts & Molds $Log_{10}CFU\ g^{-1}$ |
|---|---|---|---|---|---|---|
| T1 | Fresh | $10.38\pm0.042^{Ca}$ | $10.32\pm0.134^{Ca}$ | ND | ND | ND |
| Control | 3 | $11.45\pm0.148^{Aa}$ | $11.43\pm0.113^{Ab}$ | ND | ND | ND |
| (MA011) | 10 | $10.78\pm0.071^{Bb}$ | $10.74\pm0.120^{Bb}$ | ND | ND | 0.15±0.212 |
| T2 | Fresh | $10.49\pm0.085^{Ca}$ | $10.45\pm0.064^{Ca}$ | ND | ND | ND |
| *Lb. plantarum* | 3 | $12.15\pm0.141^{Aa}$ | $12.04\pm0.126^{Aa}$ | ND | ND | ND |
| (KP623) | 10 | $11.20\pm0.113^{Ba}$ | $11.18\pm0.175^{Ba}$ | ND | ND | ND |
| T3 | Fresh | $10.40\pm0.127^{Ca}$ | $10.38\pm0.028^{Ba}$ | ND | ND | ND |
| *Lb. delbrueckii* subsp. *lactis* | 3 | $12.08\pm0.092^{Aa}$ | $12.00\pm0.057^{Aa}$ | ND | ND | ND |
| (KP654) | 10 | $11.23\pm0.177^{Ba}$ | $10.18\pm0.106^{Bc}$ | ND | ND | ND |

*ND; Not detected; Data are the mean of duplicates ±SD; $^{a,b,c,...}$ Means values in the same column between different treatments at same age marked with unlike letters are significantly different ($p<0.05$); $^{A,B,C,..}$ Means values in the same column between same treatment at different age marked with unlike letters are significantly different ($p<0.05$)*

**Table 5. Chemical composition of Karish cheese treatments**

| Treatment | Age of cheese (Days) | Moisture % | Protein/ dry matter % | Fat/ dry matter % | pH | Acidity % |
|---|---|---|---|---|---|---|
| T1 | Fresh | $71.5^{Ac}$ | $58.95^{Aa}$ | $3.02^{Aa}$ | $4.46^{Aa}$ | $1.63^{Aa}$ |
| Control | 3 | $71.01^{Bc}$ | $58.30^{Ba}$ | $3.04^{Aa}$ | $4.40^{Aa}$ | $1.74^{Aa}$ |
| (MA011) | 10 | $69.97^{Ca}$ | $59.27^{Aa}$ | $3.09^{Aa}$ | $4.41^{Aa}$ | $1.83^{Aa}$ |
| T2 | Fresh | $73.5^{Aa}$ | $56.23^{Bb}$ | $3.02^{Aa}$ | $4.57^{Aa}$ | $1.36^{Aa}$ |
| *Lb. plantarum* | 3 | $72.75^{Ba}$ | $56.88^{Ab}$ | $3.03^{Aa}$ | $4.55^{Aa}$ | $1.48^{Aa}$ |
| (KP623) | 10 | $70.92^{Ca}$ | $53.65^{Cc}$ | $3.03^{Aa}$ | $4.54^{Aa}$ | $1.65^{Aa}$ |
| T3 | Fresh | $72.15^{Ab}$ | $56.58^{Ab}$ | $3.01^{Aa}$ | $4.48^{Aa}$ | $1.42^{Aa}$ |
| *Lb. delbrueckii* subsp. *lactis* | 3 | $71.87^{Ab}$ | $55.10^{Bc}$ | $3.02^{Aa}$ | $4.38^{Aa}$ | $1.51^{Aa}$ |
| (KP654) | 10 | $70.6^{Bb}$ | $54.42^{Cb}$ | $3.03^{Aa}$ | $4.43^{Aa}$ | $1.70^{Aa}$ |

*Data are the mean for duplicates; $^{a,b,c,...}$ Means values in the same column between different treatments at same age marked with unlike letters are significantly different ($p<0.05$); $^{A,B,C,..}$ Means values in the same column between same treatment at different age marked with unlike letters are significantly different ($p<0.05$)*

**Table 6. Sensory evaluation of Karish cheese treatments**

| Treatments | Flavor (smell and taste) | | Appearance (Body and Texture) | | Overall grade (100) |
|---|---|---|---|---|---|
| | Grade[a] | Description (intensity)[c] | Grade[b] | Description (intensity)[c] | |
| **Fresh** | | | | | |
| T1 (control) | 4 ± 0.2 | Karish cheese- like(4) | 2 ± 0.5 | Normal (2), firm (2) | 93 ± 4.3[a] |
| T2 (KP623) | 3 ± 0.5 | Creamy (1), good flavor (4) | 2 ± 0.2 | Soft (1), smooth | 92 ± 2.4[b] |
| T3 (KP654) | 3 ± 0.6 | Karish cheese- like(1) | 2 ± 0.4 | Normal (2) | 90 ± 5.3[c] |
| **3 days** | | | | | |
| T1 (control) | 4 ± 0.1 | Karish cheese-like (3), good flavor (3) | 2 ± 0.3 | Normal (2) | 93 ± 3.5[a] |
| T2 (KP623) | 4 ± 0.1 | good flavor (3) | 2 ± 0.5 | Normal (1) | 91 ± 5.2[b] |
| T3 (KP654) | 3 ± 0.5 | Karish cheese - like(2) | 2 ± 0.5 | Normal (2) | 90 ± 2.7[c] |
| **10 days** | | | | | |
| T1 (control) | 4 ± 0.2 | Typical Karish cheese (4) | 2 ± 0.5 | Normal (2) | 93 ± 3.7[a] |
| T2 (KP623) | 4 ± 0.1 | Karish cheese- like(2) | 2 ± 0.7 | Normal (1) | 91 ± 4.1[b] |
| T3 (KP654) | 3 ± 0.5 | Karish cheese- like(1) | 2 ± 0.7 | Normal (2), good texture (2) | 90 ± 5.6[c] |

*[a]Grade (1-4): 1: bad, 2: sufficient, 3: good, 4: very good; [b]Grade on scale from (1-3) : 1 : soft, 2 : normal, 3 : firm/hard; [c]Intensity remarks on scale from (1-4): 1: slightly, 2: moderate, 3: strong, 4: very strong; Data are the mean of 5 replicates ±SD; [a,b,c,..] Means values in the same column between different treatments at same age marked with unlike letters are significantly different (p<0.05)*

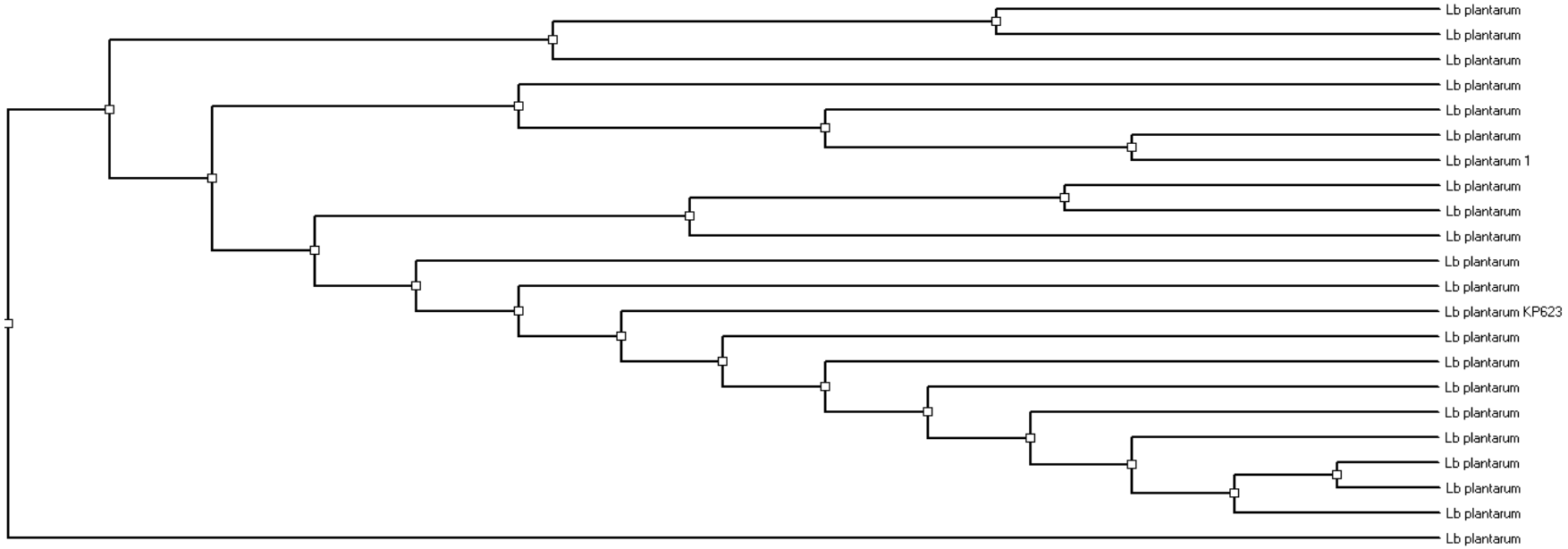


**Fig. 1. Phylogenetic tree of partial sequence of the selected isolate KP623 [*Lb. plantarum*]**

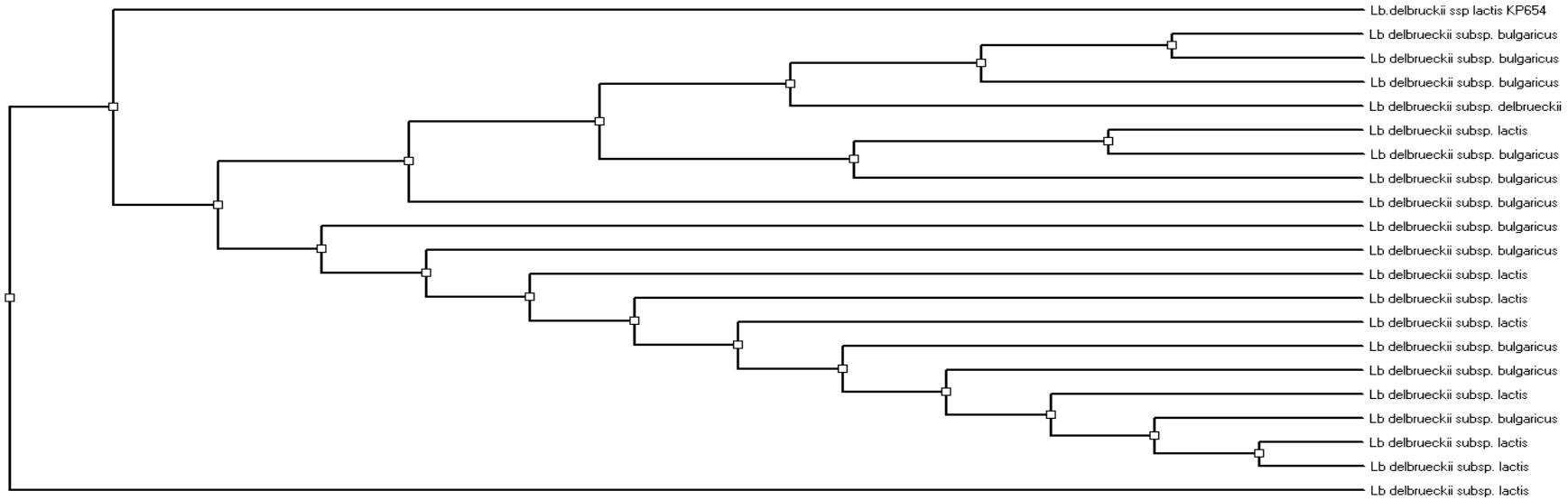


**Fig. 2. Phylogenetic tree of partial sequence of the selected isolate KP623 [*Lb. delbrueckii* subsp. *Lactis*]**

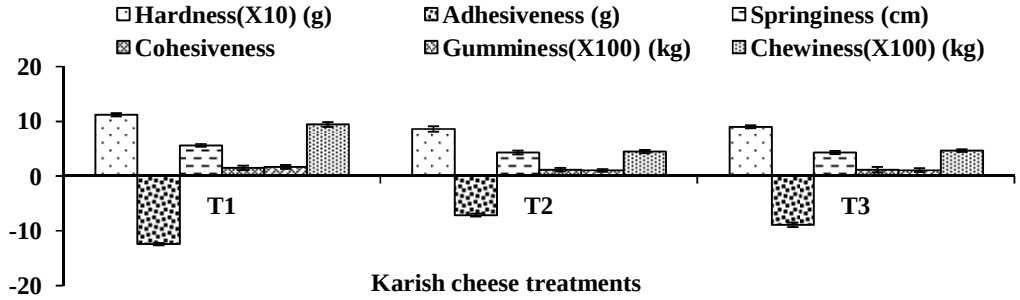


**Fig. 3. Texture profile analyses of fresh Karish cheese treatments**

*T1: Control (MA011); T2: Lb. plantarum (KP623); T3: Lb. delbrueckii subsp. lactis (KP654); Data represented are the mean for duplicates ±SD*

## 5. CONCLUSION

This study introduces two newly isolated, genetically identified antagonistic *lactobacilli* strains as bio-preservative starter cultures in Karish cheese which achieve more than one target; enhance initial microbial quality and prevent post contamination due to the antimicrobial activity they possess which extended products' shelf life. They also affected positively on chemical composition which translated into improved organoleptic and texture profile analyses and moreover they conserved the autochthonous flavor of Karish cheese that is well known to average consumer. Upraised results encouraged authors to recommend using these two new *lactobacilli* starter cultures KP623 (*Lb. plantarum*) and KP654 (*Lb. delbrueckii subsp. lactis*) to improve hygienic quality and extend shelf life which lead to a safe product avoiding foodborne illness, decreasing economic burden due to rapid spoilage and above all guaranteeing good health and well-being; one of the most important sustainable development goals in developed countries.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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