

The microbiology of Kasseri cheese during the maturation

Thomas Bintsis *

Hellenic Open University, School of Science & Technology, Patra, Greece.

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Abstract

The microbiology of Kasseri cheese, a Protected Designation of Origin (PDO) cheese of pasta-filata type was studied in order to identify the dominant species and strains that may contribute to the maturation process. Chemical composition and microbiological quality of Kasseri cheese samples from two different dairies during the maturation was studied at 0, 7, 25, 60 and 90 days of the maturation. Lactic acid bacteria and *Enterococcus* spp. were found to be the dominant microflora in fresh cheese. *P. pentosaceus* and *P. acidilactici*, *E. hirae*, *E. faecium*, *E. durans* and *E. gallinarium*, together with facultatively heterofermentative lactobacilli were found to be the dominant microflora. Since these strains are dominating the microflora of Kasseri cheese during maturation, the enzymic system need to be further studied in order to select the proper strains for adjunct culture in Kasseri cheese.

Keywords: Kasseri cheese; Pasta-filata cheese; Enterococci; Pediococci

1. Introduction

Kasseri is a traditional Greek cheese of pasta-filata type. Other cheese categorized as pasta-filata are Provolone, Caciocavallo, Mozzarella and Kashkaval [1]. The PDO status for Kasseri was recognized by the EC in 1996 and amended in 2000 [1]. Traditionally, it was produced in the mountains of Pindos and Olympos. Because milk collection was difficult, shepherds carried out the coagulation of milk and the drainage of cheese curd in the mountains, in order to preserve the milk and diminish its volume, reducing, thus, the cost of transportation [1]. Several drained cheesecurds (called “baskies”), were gathered together and carried for processing to cheese plants. In the meantime, the pH of the baski dropped (to about pH 5.20) by the activity of the native microflora of the milk. Mature baski was sliced and kneaded in hot water in order to get a pasta-filata texture in nearby factories. According to traditional practice, Kasseri cheese was made from raw milk, because it was considered that kneading eliminated the pathogenic bacteria and controlled the native microflora [2]. The chemical composition of Kasseri cheese is presented in Table 1.

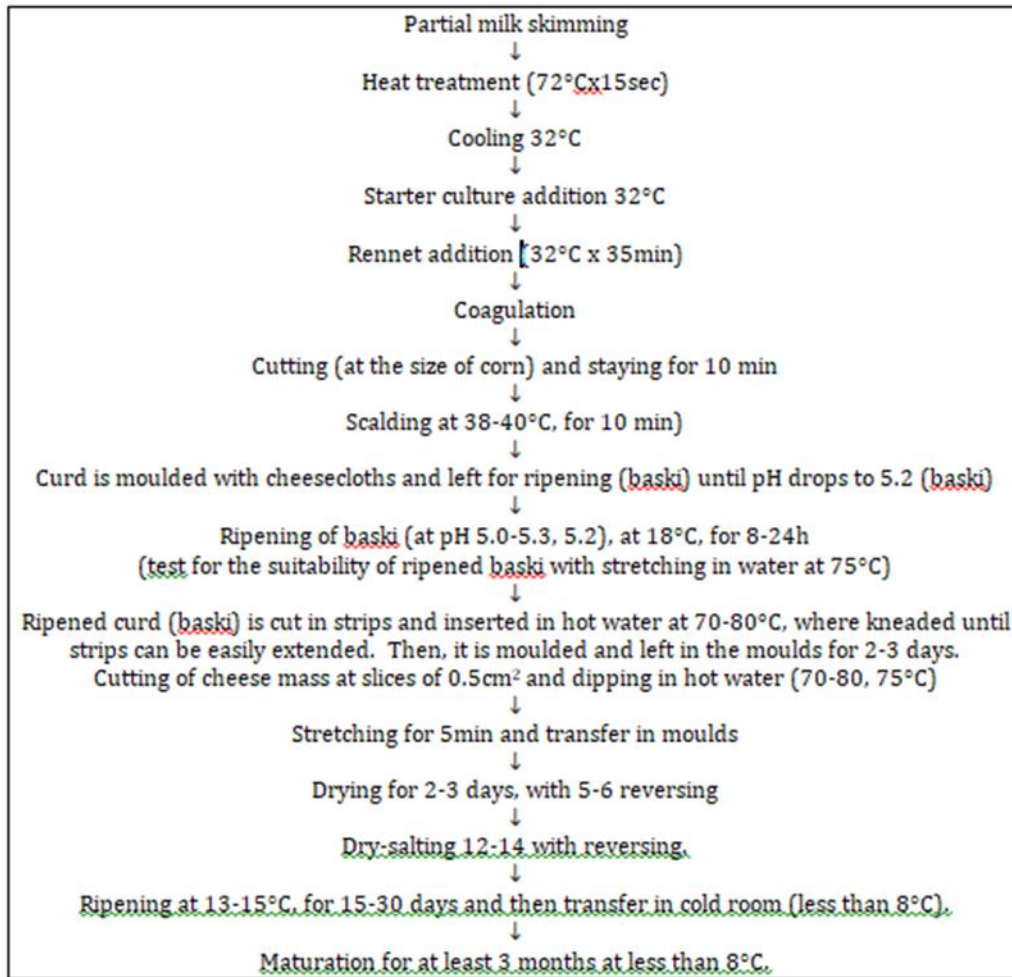
Table 1 Average composition of Kasseri cheese

chemical composition	percentage
Moisture	35.1-43.0
Fat in Dry Matter	32.0-52.3
Fat	26.2-29.6
Salt in Moisture	2.7-3.3
pH	5.5-5.7

Source: Anifantakis (1991) [3]

* Corresponding author: Thomas Bintsis
Hellenic Open University, School of Science & Technology, Patra, Greece.

The technology of Kasseri cheese is described in Figure 1.



Source: Zerfiridis (2001) [4]

Figure 1 Technology of manufacture of Kasseri cheese

2. Material and methods

Cheese samples were taken from two dairies (Dairy A and Dairy B) in Thessaly, Central Greece. Two samples from each dairy were taken from two cheese making days at 0 days (fresh cheese) and at 7, 25, 60 and 90 days. Analysis and methods used are shown in Table 2.

In addition, at critical for the maturation points, that is at 0.7 and at the end of the maturation (90 days) sixty (60) colonies were selected from MRS plates with 25-50 colonies. The colonies were purified on MRS agar and were classified and identified based on phenotypical characteristics as described by Bintsis et al. and Psoni et al. [11, 12].

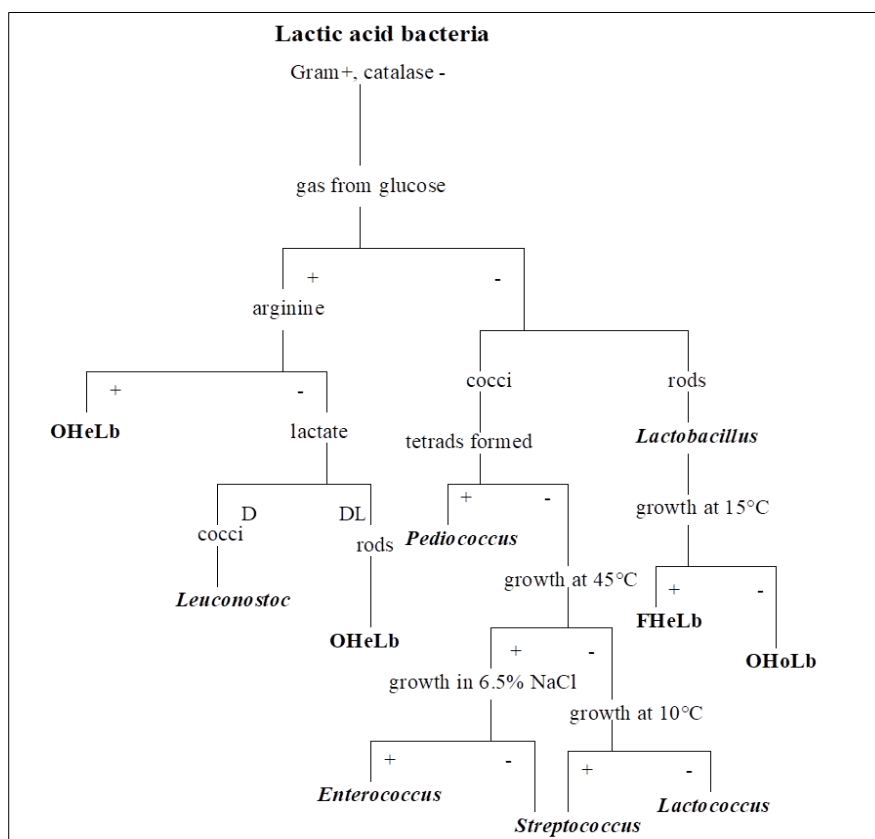
All cultures were stored in MRS Broth + glycerol at -70°C.

For the phenotypical characterization of LAB, the scheme shown in Figure 2 was used.

The identification procedure was integrated with sodium dodecyl sulphate polyacrylamide gel electrophoresis SDS-PAGE method for the 40 colonies. SDS-PAGE electrophoresis of cell proteins is described by Pot et al. and Piraino et al. [13, 14].

Table 2 Methods used for the analyses of Kasseri cheese

Parameter	Method
Chemical	
Moisture	Drying at 105°C [5]
Fat	Gerber method [6]
Salt	Determination of Chloride Content [7]
pH	Hanna pH meter, Hanna Instruments, Padova, Italy
Microbiological	
Total Microbial Count (TMC)	PCA, 30°C x 48 h [8]
Lactic Acid Bacteria	MRS, 30°C x 48 h [9]
Lactic Acid Bacteria	M17, 30°C x 48 h [9]
Lactic Acid Bacteria	Acetate Agar, 30°C x 48 h [9]
<i>Staphylococcus</i> spp.	Baird Parker Agar + Egg Yolk Tellurite Emulsion, 37°C x 48 h [9]
<i>Enterococcus</i> spp.	Citrate Azide Agar, 37°C x 72 h [10]
Coliforms	Violet Red Bile Lactose Agar, 30°C x 24 h [11]
Yeasts & Moulds	Acidified Potato Dextrose Agar, 25°C x 72 h [10]



FHeLb: facultative heterofermentative lactobacilli, **OHoLb:** obligatory homofermentative lactobacilli,
OHLeLb: obligatory heterofermentative lactobacilli.

Figure 2 Phenotypic characteristics used to classify lactic acid bacteria [Source: Piraino et al. (2006) [15]]

3. Results

The chemical composition of Kasserri cheese produced in the two Dairies studied is shown in Table 3. It can be seen that the pH of fresh cheese for both samples was close to 5, 3 and at this pH the procedure of stretching was carried out. Throughout maturation, only small differences were observed for both samples and for the same sample.

The results from the physicochemical analyses agree with the studies for the chemical composition during the maturation of Kasserri cheese [2, 16, 17].

The results for the microbiological analyses throughout the maturation are shown in Table 4. From Table 4 it can be seen that high numbers for the TMC were found for fresh cheese and for both samples (after the stretching at 70-80°C), while higher numbers were found in sample B. Differences in the quality of milk and the hygienic condition in the dairy plant are possible reasons for these differences. Lactic acid bacteria, which were counted on three nutrient agars, were found to be the dominant microflora in fresh cheese, while high numbers of enterococci were also counted. Enterococci were at higher numbers in sample B and in combination with the higher numbers for staphylococci and coliforms it can be said that the hygiene conditions in dairy B need to be improved. However, the numbers of both staphylococci and coliforms are declining during maturation.

At 7 days of ripening, an increase in the numbers of the most important microbial groups is observed. This part of ripening is carried out at room temperature (Figure 1) and is critical for the development of the dominant microflora. These microfloras contribute through their enzymic activities to the biochemical changes taken place during maturation [1]. Lactic acid bacteria were found to be present at high numbers at both cheese samples, and *Enterococcus* spp. were at high numbers as well.

From 25 days and then, during the maturation, which take place in cold rooms, a gradual reduction in the numbers of main microbial groups is observed for both cheese samples.

Similar results for the development of the microfloras during the maturation of Kasserri cheese [17], Kafalotyri cheese [18,19], Tourkish Kasha cheese [20], Italian Caciocavallo cheese [21] and Spanish Manchego cheese [22] have reported.

It is interesting to note the absence of yeasts and moulds in Kasserri cheese, throughout the maturation. Yeasts constitute an important part of the microfloras in certain cheeses [23], where, with the high enzymic activities contribute to the development of the cheese flavour [24].

Ten colonies from the counts of LAB were selected from each sample from the fresh, at 7 days and at 90 days (a total of 60 colonies). The colonies were purified and phenotypically characterized and classified as shown in Figure 2. The percentage of the main microbial groups at 0, 7 and 90 days are shown in Table 5.

In addition, the method of SDS-PAGE electrophoresis of cell proteins was used as suggested from Pot et al. and Piraino et al. [13, 14]. The results are presented, in the form of dendrogram (Figure 3). The selected colonies were divided in groups using the statistical program Gel-Compar. Thus, the first group was identified as *Enterococcus durans*, with a low percentage (50%). The next group was identified as *Enterococcus hirae* with a higher percentage (70%), whereas, with the same percentage was identified the next group as *Pediococcus acidilactici*. The fourth group was identified as *Pediococcus pentosaceus* with 75%, while the grouping percentages for *Enterococcus gallinarum* and *Enterococcus faecium* were 40 and 60% respectively.

Table 3 Chemical characterization of Kasserri cheese samples from two different dairies during the maturation

	Fresh		7 days		25 days		60 days		90 days	
	A	B	A	B	A	B	A	B	A	B
pH	5.32 ± 0.25	5.31 ± 0.25	5.33 ± 0.25	5.32 ± 0.29	5.43 ± 0.25	5.41 ± 0.25	5.48 ± 0.25	5.42 ± 0.29	5.47 ± 0.05	5.43 ± 0.04
Moisture	45.22 ± 0.63	46.27 ± 0.52	43.87 ± 1.31	45.70 ± 1.45	43.10 ± 1.63	44.52 ± 1.73	42.52 ± 0.81	43.75 ± 1.00	42.35 ± 1.35	43.37 ± 1.31
Salt	1.63 ± 0.34	1.83 ± 0.21	1.20 ± 0.13	1.64 ± 0.58	1.49 ± 0.48	1.62 ± 0.43	1.52 ± 0.42	1.68 ± 0.38	1.32 ± 0.26	1.29 ± 0.39
Salt in moisture	3.48 ± 0.72	3.80 ± 0.44	2.67 ± 0.35	3.45 ± 1.20	3.33 ± 0.97	3.50 ± 0.81	3.43 ± 0.89	3.70 ± 0.74	3.02 ± 0.52	2.89 ± 0.84
Fat in Dry Matter	46.87 ± 0.61	48.39 ± 0.42	45.62 ± 0.73	48.00 ± 1.63	45.30 ± 2.17	47.97 ± 1.20	44.68 ± 1.45	47.11 ± 0.59	45.14 ± 2.31	47.43 ± 1.33

A, B: Dairies A and B Figures are mean values in duplicate analyses for two samples from each dairy

Table 4 Microbiological analyses during the maturation of Kasserri cheese from samples from two dairies

	Fresh		Day 7		Day 25		Day 60		Day 90	
	A	B	A	B	A	B	A	B	A	B
TMC	2.2 x10 ⁷	2.5 x10 ⁷	6.8 x10 ⁷	5.5 x10 ⁷	1.6 x10 ⁷	2.7 x10 ⁷	8.3 x10 ⁶	1.1 x10 ⁷	6.9 x10 ⁶	9.2 x10 ⁶
LAB-MRS	1.4 x10 ⁷	2.3 x10 ⁷	2.7 x10 ⁷	5.8 x10 ⁷	7.8 x10 ⁶	2.2 x10 ⁷	4.3 x10 ⁶	2.4 x10 ⁷	4.1 x10 ⁶	1.3 x10 ⁷
LAB-M17	1.8 x10 ⁷	1.9 x10 ⁷	3.7 x10 ⁷	4.3 x10 ⁷	1.5 x10 ⁷	1.8 x10 ⁷	5.1 x10 ⁶	2.1 x10 ⁷	3.9 x10 ⁶	1.8 x10 ⁷
LAB-Ac.	1.3 x10 ⁷	1.1 x10 ⁷	4.3 x10 ⁷	3.7 x10 ⁷	1.8 x10 ⁷	1.1 x10 ⁷	3.6 x10 ⁶	1.2 x10 ⁷	4.1 x10 ⁶	8.1 x10 ⁶
Staphylococci	<100	2.5x10 ³	2 x10 ²	1.4 x10 ³	1.1 x10 ²	6.5 x10 ²	<100	<100	<100	<100
Enterococci	4.5x10 ⁴	4.2x10 ⁶	1.3 x10 ⁵	2.2 x10 ⁶	1.5 x10 ⁵	8.8 x10 ⁵	6.5x10 ⁴	5.5 x10 ⁵	7.3 x10 ⁴	3.3 x10 ⁵
Coliforms	<10	2.6x10 ⁴	<10	4.4 x10 ⁴	<10	2.7 x10 ³	<10	2.2x10 ³	<10	6.5 x10 ²
Yeasts	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100

A, B: Dairy A and B Figures are mean values from duplicate analyses for two samples for each dairy

Table 5 Classification of colonies from MRS as identified using phenotypical characteristics

Microbial group	Fresh	Day 7	Day 90
<i>Pediococcus</i> spp.	5 (25%)	7 (35%)	10 (50%)
<i>Enterococcus</i> spp.	10 (50%)	9 (45%)	6 (30%)
<i>Lactobacillus</i> spp.	5 (25%)	4 (20%)	4 (20%)
Σύνολο	20 (100%)	20 (100%)	20 (100%)

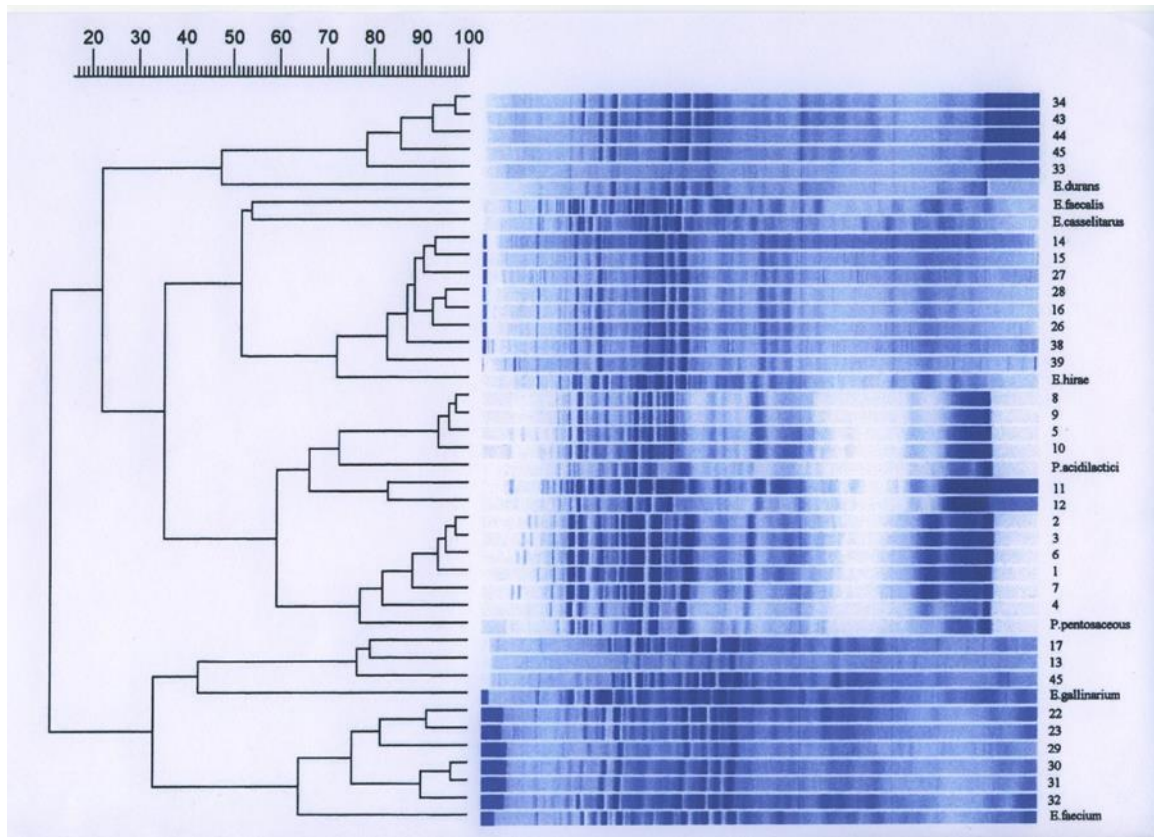


Figure 3 Dendrogram of protein fractions of isolated LAB extracts from Kasseri cheese and reference strains of LAB, using the method of SDS-PAGE electrophoresis

Table 6 Phenotypical characteristics of lactic acid bacteria isolates from Kasseri cheese

		Cell morphology	Catalase	10	15	45	Gas from glucose	NH ₃	6.5% NaCl	pH 9.6	After SDC-PAGE
7A1	1	C	W	+	ND	-	-	+	-	-	<i>P. pentosaceus</i>
7A2	2	C	-	+	ND	-	-	+	-	-	<i>P. pentosaceus</i>
7A3	3	C	W	+	ND	-	-	+	-	-	<i>P. pentosaceus</i>
7A4	4	C	W	+	ND	-	-	+	-	-	<i>P. pentosaceus</i>
7A6	5	C	-	+	ND	-	-	w	+	+	<i>P. acidilactici</i>
7A10	6	C	-	+	ND	-	-	+	-	-	<i>P. pentosaceus</i>
1A1E6	7	C	-	+	ND	-	-	+	-	-	<i>P. pentosaceus</i>

7B3E7	8	C	-	+	ND	-	-	+	+	+	<i>P. acidilactici</i>
7B4E8	9	C	-	+	ND	-	-	+	+	+	<i>P. acidilactici</i>
7B7	10	C	-	+	ND	-	-	+	+	+	<i>P. acidilactici</i>
C1	11	C	-	+	ND	+	-	+	+	-	<i>P. pentosaceus</i>
C7	12	C	-	+	ND	+	-	+	+	-	<i>P. pentosaceus</i>
1A2E1	13	C	-	+	ND	+	-	+	+	-	<i>E. gallinarium</i>
1A9E2	14	C	-	+	ND	+	-	+	+	+	<i>E. hirae</i>
3A1E4	15	C	-	+	ND	+	-	+	+	+	<i>E. hirae</i>
3A2E5	16	C	-	+	ND	+	-	+	+	+	<i>E. hirae</i>
A28E3	17	C	-	+	ND	+	-	+	+	+	<i>E. gallinarium</i>
7A5	22	C	-	+	ND	-	-	+	+	+	<i>E. faecium</i>
7A7	23	C	-	+	ND	-	-	+	+	+	<i>E. faecium</i>
7A8	26	C	-	+	ND	-	-	+	+	+	<i>E. hirae</i>
7A9	27	C	-	+	ND	-	-	+	+	+	<i>E. hirae</i>
7B2	28	C	-	+	ND	-	-	+	+	+	<i>E. hirae</i>
7B5	29	C	-	+	ND	-	-	+	+	+	<i>E. faecium</i>
7B6	30	C	-	+	ND	+	-	+	+	+	<i>E. faecium</i>
7B10	31	C	-	-	ND	-	-	+	+	+	<i>E. faecium</i>
C4	32	C	-	+	ND	-	-	+	+	-	<i>E. faecium</i>
C8	33	C	-	+	ND	+	-	+	+	-	<i>E. durans</i>
C9	34	C	-	+	ND	+	-	-	+	-	<i>E. durans</i>
7C4	43	C	-	+	ND	+	-	+	+	-	<i>E. durans</i>
7C5	44	C	-	+	ND	+	-	-	+	-	<i>E. durans</i>
7C6	45	C	-	+	ND	+	-	+	+	-	<i>E. durans</i>
1A5		R	-	ND	+	ND	-	ND	ND	ND	<i>Lactobacillus</i> spp.
1A9		R	-	ND	+	ND	-	ND	ND	ND	<i>Lactobacillus</i> spp.
7A11		R	-	ND	+	ND	-	ND	ND	ND	<i>Lactobacillus</i> spp.
7B1		R	-	ND	+	ND	-	ND	ND	ND	<i>Lactobacillus</i> spp.
7B9		R	-	ND	+	ND	-	ND	ND	ND	<i>Lactobacillus</i> spp.
1B7		R	-	ND	+	ND	-	ND	ND	ND	<i>Lactobacillus</i> spp.
1B5		R	-	ND	+	ND	-	ND	ND	ND	<i>Lactobacillus</i> spp.
1B6		R	-	ND	+	ND	-	ND	ND	ND	<i>Lactobacillus</i> spp.
1B10		R	-	ND	+	ND	-	ND	ND	ND	<i>Lactobacillus</i> spp.

C: cocci, R: rods, ND: not carried out, +: positive reaction, -: negative reaction.

4. Discussion

Pediococci and enterococci constitute the main microbial groups that dominate the maturation of Kasseri cheese. From the 40 isolates at 0 and 7 days, 31 were found to be cocci (12 *Pediococcus* spp. – 6 *P. pentosaceus* and 4 *P. acidilactici* and 19 *Enterococcus* spp. – 6 *E. hirae*, 6 *E. faecium*, 5 *E. durans* and 2 *E. gallinarium*) and 9 rods (facultatively heterofermentative lactobacilli).

Tsakalidou et al. [25] identified strains from Kasseri cheese using the method of SDS-PAGE, and found *E. faecium*, *Staphylococcus* spp., *Lactococcus lactis*, *Lactobacillus paracasei* subsp. *tolerans*, *Lactobacillus delbrueckii*, *Lactobacillus fermentum*, *Streptococcus thermophilus* and a strain which was first classified as *Streptococcus* spp. and then as a new species with the name *Streptococcus macedonicus*.

Pediococci have been reported to be the dominant microflora of certain cheeses. Very early, Dacre [26] reported pediococci to be an important part of Cheddar cheese microflora and constituted the 25% of the microflora at 6 months. The presence of pediococci has been reported in Cheddar cheese [27, 28, 29, 30, 31], Caciocavallo Pugliese cheese [32] and in mature Kefalotyri [19], and is possible to contribute to the development of the flavor and aroma of each cheese.

Coppola et al. [33] isolated *Pediococcus* spp. from MRS agar during the maturation of Parmigiano Reggiano; interestingly, pediococci were found at the final stages of the maturation and *P. acidilactici* was, together with *Lactobacilli* spp. the dominant genus. In addition, Coppola et al. [34] reported that *P. acidilactici* was part of the dominant microflora of mature Parmigiano Reggiano (at 60, 90, 120 and 150 days) and the same authors concluded that pediococci play an important role at the maturation of such cheeses, contributing to the proteolysis and lipolysis with their enzymic activities. Bouton et al. [35] found pediococci in mature Comté cheese (1, 3 and 5 months but not in the fresh cheese, where enterococci and *Streptococcus thermophilus* were dominated. Tavaría & Malcata [36] reported the presence of *P. pentosaceus* in Serra de Estrela cheese but not in the milk used for the manufacture. *P. pentosaceus* was found in the dominant microflora of mature Manoura cheese, a hard cheese produced in Sifnos Island in Greece [37].

Pediococcus spp. is one of the most important lactic acid bacteria that are considered as probiotics [38]. Various strains of *Pediococcus* spp. produce a protein, also known as pediocin, which is considered an effective antimicrobial bacteriocin [38, 39]. Gandhi et al. [40] studied a peptidoglycan hydrolase, with a characterized antibacterial activity, from *P. acidilactici*, which may be interesting for antagonism with other bacteria in cheese and other fermented food products.

Attri et al. [41] reported that *P. acidilactici* had different proteolytic activities, β -galactosidase and antioxidant activities, and produced lactic acid; they concluded that *P. acidilactici* is a potential probiotic for humans with all the essential basic probiotic properties. In addition, Gandhi et al. [42] characterized a dipeptidyl peptidase-II from probiotic *P. acidilactici*. Recently, the whole genome of *P. acidilactici* was sequenced and analysed for its evolutionary relationship with other lactic acid bacteria [43]. The genome also encoded different enzyme activities required for utilization of various carbohydrates and encoded genes for probiotics properties.

Enterococci have reported to play a major role in ripening and aroma development in certain cheeses in southern Europe (Portugal, Spain, Italy and Greece) [44]. In Cebreiro, Kefalotyri, Manchego, Picante da Beira Baixa and Teleme cheeses, enterococci are the predominant microorganisms in the fully ripened cheese [44]. The beneficial role of enterococci in cheese-making has attributed to the lipolysis of milk by esterases [45] and enterococcal strains have been included in certain starter cultures.

However, some *Enterococcus* spp., especially *E. faecium* and *E. faecalis* may be considered as opportunistic pathogens, and, in case they carry antibiotic resistance genes and possible virulence factors, they would constitute a definite health risk [44].

It is interesting to note the absence of *Lactococcus* spp. from Kasseri cheese and this can be possibly explained by their thermosensitivity, [46] as they cannot survive the kneading stage. Similarly, Aran [20] reported the absence of lactococci from the Kashar cheese, a Turkish cheese from the category of pasta-filata. On the other hand, enterococci, which are thermoresistant microorganisms, were found in the dominant microflora of Kashar cheese [20]. *E. faecium* and *E. durans* were the dominant species. The presence of high numbers of *Enterococcus* spp. has reported for cheese such as Manchego [22], Mozzarella [47, 48] Serra [49], Kefalotyri [19], Feta and Telemes [50], Cebreiro [51] and Compté cheese [35].

5. Conclusion

The quality and the contribution of the microbiology during the maturation of Kasseri cheese were studied, together with the changes throughout. The main microbial groups were identified. It was found that the dominated strains belong to the genera of *Pediococcus*, *Enterococcus* and *Lactobacillus*. More specifically, *P. pentosaceus* and *P. acidilactici*, *E. hirae*, *E. faecium*, *E. durans* and *E. gallinarium*, together with facultatively heterofermentative lactobacilli. The biotechnological properties of these strains are needed to be further studies in order to select adjunct cultures for the standardized production of Kasseri and other pasta-filata cheese.

Compliance with ethical standards

Acknowledgments

The provision of cheese samples from the two dairies from Trikala, Greece is acknowledged.

Disclosure of conflict of interest

The author hereby declares that there is no conflict of interest that could arise.

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