The Microfloras of Traditional Greek Cheeses

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ABSTRACT Many traditional cheeses are made in Greece. Some of them are, in fact, types of the same cheese variety, whether or not they have different cheesemaking technologies, but are known by different local names. Twenty of them have been granted protected designation of origin status. In the 8th century BCE, Homer described a cheese thought to be the ancestor of feta, the main cheese manufactured in Greece from the ancient times until today. Meanwhile, various cheese types evolved through the centuries, and almost every area in Greece has its own cheesemaking tradition. Some cheese varieties are local, handcrafted products whose production has been handed down from generation to generation, and without interest in their continued production, these varieties will disappear. Other local varieties are made at small factories from pasteurized milk and commercial rennet and starter and are very different from the traditional versions. However, some milk producers still make their cheeses at home or at small dairies from raw milk, without any starter, or sometimes from thermized milk, with traditional yogurt as the starter. Their cheeses are the basis for the information presented in this review.

HISTORICAL BACKGROUND
It is commonly believed that cheesemaking started accidentally in a region of the Middle East known as the Fertile Crescent in the region of the Tigris and Euphrates rivers, some 8,000 years ago. Travelers from Asia seem to have transferred the art of cheesemaking to Europe.

Cheesemaking in Greece has a millennia-old tradition. In the 8th century BCE, in his Odyssey (IX, lyrics 218–223, 244–249), Homer described the shepherd and cheesemaker Polyphemus of the 12th century BCE, as well as his cheeses. Homer wrote (translated by Samuel Butler):

We soon reached his cave, but he was out shepherd, so we went inside and took stock of all that we could see. His cheesracks were loaded with cheeses, and he had more lambs and kids that his pens could hold. They were kept in separate flocks; first there were the hoggets, then the oldest of the younger lambs and lastly the very young ones all kept apart from one another; as for his dairy, all the vessels, bowls, and milk pails into which he milked, were swimming with whey. He curdled half the milk and set it aside in wicker strainers, but the other half he poured into bowls that he might drink it for his supper.

Diodorus (Diodorus Siculus; 1st century BCE), the Greek historian from Sicily, wrote that Aristeus, son of Apollo and grandson of Zeus, who had learned the art of cheesemaking from his nannies, the nymphs, was sent by the gods at Mount Olympus to teach the Greeks how to make cheese (Diodorus, Library of History, IV 81.1–81.2). Given the value of cheese as a food, it is not surprising that ancient Greeks considered cheese to be a divine invention and gift.

The cheese that Homer described may be the ancestor of feta, and it has been the main cheese manufactured in Greece since ancient times. Meanwhile, various cheese types evolved through the centuries so that now, each area and almost every island has its own unique tradition of cheese manufacture. However, some of them are very local and, in fact, are homemade. The new generation is not interested in learning how to make them, and their manufacture, unfortunately, tends to disappear. Some, even though local, are made at small factories from

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pasteurized milk and commercial rennet and starter and are still found in the market, even though they are very different from the traditional versions. There are some milk producers, however, who are fond of their tradition, who learned how to make cheese from their fathers and grandfathers, and who continue to manufacture their cheeses at home or at very small dairies from raw milk without any starter or, in some cases, from thermized milk with the addition of some quantity of traditional yogurt as a starter. Their valuable products are the basis for the information presented in this review.

Numerous traditional cheeses are still made throughout Greece. Some are types of the same variety of cheese but are known by different local names; they may have somewhat different steps in manufacture or possibly the same manufacturing process. Twenty of them were recognized as protected designation of origin (PDO) cheeses (Table 1), and others are awaiting that recognition. Greek traditional cheeses can be grouped into five categories according to their manufacturing technology: cheeses in brine, soft cheeses, semihard cheeses, hard cheeses, and whey cheeses.

**CHEESES IN BRINE (PICKLED CHEESES)**

**Batzos PDO**

**Traditional technology**

Batzos is a low-fat, semihard cheese with a sourish and lightly piquant taste and a large number of holes in the body, manufactured mainly in western Macedonia, northwestern Greece. Its technology involves steps which lead to enrichment of the whey by fat. The whey is then used to make butter, when sheep’s milk is used for cheese manufacture, or fat-rich manouri cheese, when goat’s milk or a mixture of goat’s milk and sheep’s milk (up to 30%) is used to make cheese. Batzos cheese is, therefore, a byproduct of either manouri cheese or butter production from the whey.

The milk is not heat treated, and coagulation (by traditional rennet) takes place within 50 min at 28 to 30°C. When the milk just begins to thicken, the manufacturer “hits” the milk with a thick wooden stick about 150 to 200 times; the milk is then left to rest for 35 to 40 min before being hit again about 300 to 350 times. During these hitting stages, a large proportion of fat is transferred to the whey. The curd is then left to settle, and it is later placed in cheesecloth, which is fastened, hung for draining, and ripened for 24 h. Some cheesemakers apply mild heat before transferring the curd to the cheesecloth. The next day, the ripened curd is cut into slices and salted with coarse salt; cheese pieces are then placed in tins and covered with brine (1, 2). Cheese is either consumed fresh (fried, plain, or with eggs) or stored in cool rooms.

**Changes in the microflora during storage**

Batzos cheese from raw ewe’s milk was made at a village in western Macedonia, northwestern Greece, by a shepherd whose family had produced this kind of cheese for generations. We monitored and quantified the traditional steps of manufacture, as follows. Morning ovine milk (40 liters) was initially strained through cheesecloth into an open kettle, and the milk was warmed slightly (32°C) using a gas burner. Coagulation took place within approximately 50 min. When the milk began to thicken, it was stirred with a thick wooden stick many times. On completion of the gelation stage, the curd was cut into small pieces, and the whey-curd mixture was stirred vigorously for about 5 min. The curd was then heated under continuous stirring to 41°C (within 11 min) and left (for ∼7 min) to settle to the bottom of the vat. The curd was then covered with cheesecloth, and the whey was removed. At this stage, the curd was wrapped in the cheesecloth and pressed by hand to help the pieces fuse together. The ends of the cheesecloth were then tied together, and after further pressing by hand to remove as much whey as possible, the curd was hung to drain. After 24 h at room temperature, gas holes appeared in the mass of the cheese; at this point, the cheese was cut into slices and salted on the surface with a

**TABLE 1** Traditional Greek PDO cheeses

<table>
<thead>
<tr>
<th>Cheeses in brine</th>
<th>Soft cheeses</th>
<th>Semihard cheese</th>
<th>Hard cheeses</th>
<th>Whey cheeses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batzos</td>
<td>Anevato</td>
<td>Kasseri</td>
<td>Formaella</td>
<td>Manouri</td>
</tr>
<tr>
<td>Feta</td>
<td>Galotyri</td>
<td></td>
<td>Graviera Agrafon</td>
<td>Xinomizithra Kritis</td>
</tr>
<tr>
<td>Kalathaki Limnou</td>
<td>Katiki</td>
<td></td>
<td>Graviera Kritis</td>
<td></td>
</tr>
<tr>
<td>Sfela</td>
<td>Kopanisti</td>
<td></td>
<td>Graviera Naxou</td>
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<tr>
<td></td>
<td>Pichtogalo Chanion</td>
<td></td>
<td>Kefalograviera</td>
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<td></td>
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<td></td>
<td>Ladotyri Mutilinis</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Metsovone</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>San Michali</td>
<td></td>
</tr>
</tbody>
</table>

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coarse-grained salt. On the next day, the cheese was put into tins, covered with brine (120 g/liter), and transferred to a cold room (4°C).

The cheese made from raw ewe’s milk in the spring contained lower microbial counts than the product made in the summer for all microbial groups except staphylococci (3). Higher levels of environmental contamination and faster growth of microorganisms due to the higher temperatures during the summer may have contributed to these higher levels.

Lactic acid bacteria (LAB), Enterobacteriaceae, and coliforms were the major components of the microflora during storage of the cheese (Fig. 1). Lactococci were the most abundant microbial group in the curd of cheese made in the spring, being between 0.77 (coliforms) and 5.67 (yeasts) log counts higher than the other groups in the curd. The counts of total nonstarter LAB (NSLAB) and lactococci in curd made in the spring increased during ripening for 24 h and then declined gradually during storage for 6 months, by almost 1.5 and 3.2 log<sub>10</sub> CFU/g, respectively (Fig. 1b). The number of enterococci rose by ∼2 log<sub>10</sub> at day 15 but decreased gradually thereafter to levels similar to the initial populations. LAB in cheese made in the summer underwent small changes during storage; their counts ranged between 7.24 and 7.99 log<sub>10</sub> CFU/g. Populations of enterococci, on the other hand, evolved similarly to those in the spring cheese.

The number of microorganisms indicative of the hygiene level, Enterobacteriaceae and coliforms, decreased gradually in both spring and summer cheeses during storage (Fig. 1a). However, their numbers, even after storage of the product for 6 months, were still high (10<sup>4</sup> to 10<sup>5</sup> CFU/g). These large populations represent a serious obstacle for marketing the product, even though no standards exist in Greece for these organisms. Enterobacteriaceae and coliforms declined faster in cheeses made in the summer (by over 3 log<sub>10</sub> CFU/g), and this is possibly related to the higher numbers of LAB in the curd of summer cheese and its lower pH (6.07 and 5.65 in the curd and ripened curd, respectively, of the spring cheese; 5.60 and 5.25 in the curd and ripened curd, respectively, of the summer cheese).

The number of staphylococci decreased in the cheeses from both seasons during storage by ∼1.5 and ∼2.0 log<sub>10</sub> CFU/g in spring and summer cheeses, respectively. Yeast counts tended to undergo various changes during storage and were counted at levels ≤10<sup>3</sup> CFU/g in spring cheeses, while in the products made in summer, they reached populations as high as 10<sup>5</sup> to 10<sup>6</sup> CFU/g and were higher than either Enterobacteriaceae and coliforms or enterococci in cheeses after 60 days of storage.

The cheese from raw goat’s milk was manufactured at a small creamery in northwestern Greece with milk obtained from local breeds by the same local method in winter, spring, and summer. The microbial counts of fresh cheese made in both spring and summer were more or less similar (Fig. 1). The environmental temperature in June the year when the cheeses were made was even lower than in March, so no obvious effect of season on the levels of microbial counts was recorded for the fresh product (ripened curd).

LAB, Enterobacteriaceae, and coliforms seem to predominate over the other microbial groups initially in the cheese made from raw goat’s milk. LAB appear to be the predominant microbial group during manufacture and storage (4). In addition, even though the numbers of Enterobacteriaceae and coliforms were high (over 6.5 log<sub>10</sub> CFU/g) in the fresh cheeses made in both seasons, they tended to decline considerably faster than in the cheeses from ewe’s milk, and they were either not detected in spring cheeses or found at very low levels (<200 CFU/g) in the product made in the summer after 6 months (Fig. 1a). However, the pH values of 5.30 to 6.06 and 5.20 to 5.70 for cheeses made in spring and summer, respectively, do not seem to be effective for their rapid reduction. Therefore, other factors, such as low storage temperature (4°C), brine concentration (7.54 to 13.50%), and antibacterial activity by LAB, may be the main agents regulating their survival. In addition, other microbial groups, such as staphylococci and enterococci, were enumerated at levels similar to Enterobacteriaceae and coliforms in the fresh (2-day-old) product. Both microbial groups tended to decline. Staphylococci decreased similar to Enterobacteriaceae populations during storage for 30 to 60 days. However, enterococci remained at high levels throughout storage and were counted at lower populations (by 10<sup>3</sup> to 10<sup>6</sup> CFU/g) during storage than those in the product from ewe’s milk; their evolution seemed to be similar for both products from ewe’s milk and/or goat’s milk.

Maximum levels of total LAB and lactococci were attained at 2 days (before salting) for the product from goat’s milk, and the same was observed for the product from ewe’s milk made in spring. Lactobacilli in spring and summer goat’s milk cheeses increased their numbers significantly until about half a month of storage and then decreased by 1 to 1.5 log<sub>10</sub> CFU/g in the 6-month-old cheese. A high brine concentration of 7.5 to 13.5% in the cheese obviously contributed to their decline.

Yeasts were counted at levels considerably lower than those in the products from ewe’s milk, possibly due to lower environmental contamination.
FIGURE 1 Numbers (log_{10} CFU/g; mean ± standard deviation [bars]) of *Enterobacteriaceae* and coliforms (a) and NSLAB (b) during manufacture and storage of batzos cheese from raw ewe’s and/or goat’s milk made in spring and summer. d, days. doi:10.1128/microbiolspec.CM-0009-2012.f1
Lactococcal counts in cheese made in the winter from goat’s milk were the highest of all microbial groups in cheese at day 1, being 0.87 to 5.70 log_{10} CFU/g higher than those of the other microbial groups. Total LAB, lactococci, staphylococci, enterococci, and yeasts increased their numbers by 0.25 to 1.16 log_{10} CFU/g after ripening for 1 day at room temperature, but the population of Enterobacteriaceae, coliforms, and lactobacilli remained almost unchanged. Enterobacteriaceae, coliforms, staphylococci, and LAB significantly decreased their numbers during storage. At the end of storage, lactobacilli were counted at significantly higher numbers than the initial count, and enterococci and yeasts underwent small changes.

**Evolution of NSLAB throughout ripening**

The evolution of lactic microflora in cheeses with PDO status is of particular interest because the biochemical activities of these organisms participate in cheesemaking and may play an acknowledged role in the development of organoleptic characteristics during ripening.

The lactation season had a clear effect on the predominance of certain NSLAB in batzos cheese made from ewe’s milk. While lactobacilli predominated (63.8% of the isolates) among LAB isolated from the ripened curd of cheeses made in the summer, enterococci were the most abundant bacteria (51.9% of the isolates found in the ripened curd) in the spring cheeses. Lactococci (mainly *Lactococcus lactis* subsp. *lactis*) were found only in the curds of spring cheeses (22.2% of the isolates). In cheese made in the winter from goat’s milk, lactococci and enterococci predominated throughout storage and lactobacilli were rarely found.

A common predominant microorganism for cheeses made from ewe’s or goat’s milk manufactured in both spring and summer is *Lactobacillus paraplastic* (Table 2). Moreover, the cheese from goat’s milk had a more complex predominant microflora of NSLAB species, possibly due to higher contamination from the environment of the small dairy. *Enterococcus faecium* predominated in batzos cheese from raw ewe’s milk made in the spring (40.8% of the isolates), followed by *L. lactis* subsp. *lactis* (22.2% of the isolates). In cheese made in the summer, *Lactobacillus paracasei* subsp. *paracasei* predominated (33%) and *L. plantarum* followed (30.8%). In cheeses made from raw goat’s milk, *Lactobacillus plantarum* was the most frequently isolated NSLAB in cheese made in the spring (37.8%), followed by *L. paraplastic* (28.3%) and *L. lactis* subsp. *lactis*. In the summer cheese, *L. lactis* subsp. *lactis* predominated (29.8%), *L. plantarum* then followed (29.1%), and *L. paraplastic* was also frequent (25.6%). In winter cheeses, however (4), lactococci (*L. lactis* subsp. *lactis*, mainly) predominated, enterococci were found abundantly (mainly *Enterococcus durans*), and lactobacilli were rarely isolated.

**Effect of NSLAB on cheese quality and flavor**

Lactococci are an important group of NSLAB of batzos cheese, and their predominance in the fresh product may suggest that this microbial group plays a principal role in

### Table 2

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>% of isolates from cheese made in:</th>
<th>Spring</th>
<th>Summer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sheep’s milk cheese</td>
<td>Goat’s milk cheese</td>
<td>Sheep’s milk cheese</td>
</tr>
<tr>
<td><em>Lactococcus lactis</em> subsp. <em>lactis</em></td>
<td>22.2</td>
<td>11.9</td>
<td>29.8</td>
</tr>
<tr>
<td><em>Lactococcus raffinolactis</em></td>
<td>2.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Lactobacillus plantarum</em></td>
<td>37.8</td>
<td></td>
<td>29.1</td>
</tr>
<tr>
<td><em>Lactobacillus paraplastic</em></td>
<td>14.8</td>
<td>28.3</td>
<td>30.8</td>
</tr>
<tr>
<td><em>Lactobacillus pentosus</em></td>
<td>7.4</td>
<td>2.2</td>
<td>2.8</td>
</tr>
<tr>
<td><em>Lactobacillus paracasei</em> subsp. <em>paracasei</em></td>
<td></td>
<td>33.0</td>
<td></td>
</tr>
<tr>
<td><em>Lactobacillus sake</em></td>
<td>5.9</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td><em>Lactobacillus curvatus</em></td>
<td>5.9</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td><em>Lactobacillus brevis</em></td>
<td>4.4</td>
<td>4.4</td>
<td>0.7</td>
</tr>
<tr>
<td><em>Lactobacillus buchneri</em></td>
<td>0.7</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td><em>Carnobacterium piscicola</em></td>
<td>0.7</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td><em>Carnobacterium diversin</em></td>
<td>0.7</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>7.4</td>
<td>13.8</td>
<td></td>
</tr>
<tr>
<td><em>Enterococcus faecium</em></td>
<td>40.8</td>
<td>21.3</td>
<td></td>
</tr>
<tr>
<td><em>Enterococcus durans</em></td>
<td>3.7</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td><em>Enterococcus hirae</em></td>
<td>0.7</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td><em>Enterococcus pseudoavium</em></td>
<td>0.7</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td><em>Leuconostoc spp.</em></td>
<td>1.1</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td><em>Pediococcus spp.</em></td>
<td>3.7</td>
<td>3.7</td>
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the initial acidification of milk and curd. However, enterococci also occur frequently, especially in cheeses made in the winter and spring, and may contribute in the development of organoleptic characteristics during ripening.

The rate and the intensity of acidification in milk determine the acidifying ability of the test strains. Regarding the rate of acidification, fast isolates lower the milk pH by more than 1.25 pH units after 6 h, and they are seldom found in batzos cheese made from goat’s milk (5). Concerning the intensity of acidification, i.e., the ability to reduce the pH of the milk at 24 h, three main groups of isolates were distinguished: high acidifying isolates, showing a pH decrease of more than 2 pH units (group I); the group of medium acidifying activity, showing a pH drop ranging between 1.5 and 2.0 pH units (group II); low acidifying isolates, causing a pH decrease of less than 1.5 pH units (group III). Groups I and III contained isolates obtained throughout the whole lactation season. In group II, isolates from winter and summer cheeses were allocated. It is also noteworthy that the majority of the high acidifying isolates were derived from fresh cheese. It is, therefore, tempting to speculate that these isolates act as starters to drive the initial acidification of the curd.

Concerning the acidifying ability of enterococcal isolates from goat’s milk cheese, these organisms could be considered slow acid producers (6). An interesting tendency of increased activity of the isolates with the cheese age was noticed, which suggests that enterococci may form acid mainly at the late stages of cheese storage.

With respect to proteolytic and peptidase activity, biotypes of lactococci characterized by a very different activity are found (5). The majority of the lactococcal isolates from goat’s milk cheese made in the winter exhibited Lys→Leu→Pro aminopeptidase activity, while more than 50% of the isolates from summer cheeses showed higher Leu-aminopeptidase activity than Lys-aminopeptidase activity and stronger Pro-aminopeptidase activity than the isolates from winter cheese. This property might be important for the degradation of β-casein (β-CN), which is rich in proline. It is also clear from the above that cheeses in each season may undergo differential proteolysis during storage.

Enterococci exhibited low aminopeptidase activity and higher dipeptidase activity and preferentially degraded Lys- and/or Leu- rather than Ala-p-nitroanilides (6). The isolates from 30- and 60-day-old cheeses exhibited low aminopeptidase activity, in general. Their contribution to batzos cheese flavor may, therefore, be considered not significant in this respect.

αs-CN was degraded preferentially by both lactococci and enterococci. In comparison, lactococci were observed to have a stronger degradation ability on both caseins than did enterococci. Therefore, lactococci may be considered more important for cheese proteolysis.

Both lactococci and enterococci from goat’s milk batzos cheese inhibited the growth of undesirable and other target microorganisms. Lactococci inhibited, preferentially, Escherichia coli O157:H7. Associative growth studies showed that when E. coli O157:H7 was grown in the presence of L. lactis subsp. lactis strain M7D2, its growth was lower by 1 and 2 log10 CFU/g at 8 h (milk pH, 5.21) and 24 h (pH, 4.38), respectively. Most lactococci exhibited antagonistic activity against Yersinia enterocolitica, and some of them inhibited the growth of Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes, and Enterococcus faecalis. Enterococci, on the other hand, were found to exert an interesting antagonistic activity, and together, the isolates inhibited the growth of B. cereus, S. aureus, E. coli, and L. monocytogenes. In addition, most of them inhibited E. faecalis, many (∼30%) inhibited Streptococcus thermophilus, and a great number of them affected, even though slightly, the growth of Listeria innocua. It is possible, therefore, that the NSLAB grown in batzos cheese may be responsible for the regulation of growth of food-borne pathogens and cheese safety.

Therefore, due to the different predominant lactic acid microflora in batzos cheese made in each season, the high level of heterogeneity of the technological properties of lactococci and enterococci, and their different modes of action according to the season, it appears that the cheeses produced in winter, those produced in spring, and those produced in summer undergo differential ripening changes during storage and develop unique flavor compounds.

Feta Cheese PDO
Traditional technology
Feta cheese is a traditional Greek cheese manufactured from ewe’s milk or mixtures of it with goat’s milk, with the latter not exceeding 30%. Feta is a soft, white, brined cheese made in Greece at least since Homeric times. It is produced between January and May, using raw milk without starters at farms in isolated mountainous areas or at home. The cheese has a white color, its texture is smooth and firm, and it is sliceable (the name feta means “slice” in Greek).

The traditional steps of cheese manufacture are as follows. Directly after collection, warm milk is strained...
through cheesecloth, and it is then curdled in a galvanized copper vat by a quantity of traditional rennet made from the abomasum of unweaned kids. For this purpose, pieces from the dried abomasum are put into slightly salted water for 1 to 3 days, and the extract constitutes the traditional rennet. This rennet type seems to contribute to the development of a very pleasant aroma and pepperish taste. After curdling, the curd is cut crossways and left for some time for partial exclusion of the whey. The curd is transferred onto cheesecloth with either a wooden ladle or a plate. The cheesecloth is fastened and left for several hours until drainage is completed. In the past, the cheesecloth was made from ewe’s wool woven by the women of the family, and the free threads of the cloth were burnt before use. The curd is either cut in four pieces, the shape of a quoin, or in rectangular pieces, for storage in barrels or tins, respectively. It is then dry-salted with coarse salt at room temperature and put into wooden barrels or tins. During salting, a surface microflora is developed (slime) which is thought to contribute greatly to cheese ripening and flavor. The barrels or tins are filled with brine (7% salt content) and left to ripen in cool cellars of the house for at least 2 months (2).

Changes of the microflora during ripening and storage

Traditional feta was made by farmers in different areas and altitudes, from raw ewe’s milk from local herds. In the drained curd (fresh cheese at day 1) of cheeses, the microbial populations were counted and found to be at high levels (Fig. 2).

The cheeses made at a small traditional creamery in southwestern Greece from ewes fed at low altitude (<200 m) had counts ranging from 6.37 (yeasts) to 9.94 (total aerobic counts) in the drained curd. For those

![FIGURE 2](MicrobiolSpectrum/Fig2.png)
cheeses made in the same area at high altitude (∼1,500 m) in a separate room of the house, the microbiota of the fresh cheese was counted at levels of 4.30 log_{10} CFU/g (yeasts) to 7.04 log_{10} CFU/g (gram-negative organisms). Cheeses made at a farm in western Macedonia, northwestern Greece, situated at an altitude of ∼850 m, had counts as high as 5.57 log_{10} CFU/g (yeasts) to 9.37 log_{10} CFU/g (LAB on MR5 agar) in the drained curd, while cheeses made at a farm in the northern Peloponnesse, southern Greece, at an altitude of ∼800 m contained microbial populations ranging from 4.30 log_{10} CFU/g (yeasts) to 8.42 log_{10} CFU/g (LAB, lactococci) at day 1. All cheeses were manufactured in the late spring.

It is obvious, therefore, that fresh feta cheese made in different areas and/or at different altitudes may differ with respect to microbial counts and the predominant microflora. Additionally, yeasts constitute the microbial group found at the lowest level in feta cheese. Factors such as milking conditions, milk storage, animal and personal hygiene, and cleaning practices might have influenced the levels and the composition of the milk and cheese microfloras.

The general trend for the various microbial groups was a decline in numbers throughout ripening and storage. For the cheese made at a traditional dairy in southwestern Greece (low altitude), the following changes were recorded: total viable counts, Enterobacteriaceae, and coliforms declined by ≥1 log_{10} CFU/g after ripening at room temperature for 4 days, while the cheese was accepting some surface salting (brine concentration, 1.5 to 2.0%) and had reached a pH lower than 5.0. After that, both Enterobacteriaceae and coliforms declined significantly, by 4.76 and 5.24 log_{10} CFU/g at 60 days. At this time, the cheese pH was ~5.0 and the brine concentration was around 5%. It seems possible that these two factors regulate the survival of the various microbial groups throughout feta cheese ripening. In the mature cheeses (∼2-month-old) manufactured in the other Greek areas and altitudes, both factors were found at different levels. The cheese pH, in particular, was measured at considerably lower levels (southwest, high altitude, 3.20; Peloponnesse, 4.60; northwest, 3.96), and at the same time, Enterobacteriaceae and coliforms were not detected in the mature cheeses.

NSLAB predominated over the other microbial groups throughout ripening and storage of cheese made in southwestern Greece at low altitude. Total LAB decreased slightly, while lactococci at the end of storage had populations significantly lower than in the drained curd. Enterococci, on the other hand, tended to increase their counts initially and underwent small changes throughout storage. Their populations ranged between 10^6 to 10^9 and 10^5 to 10^6 CFU/g for lactococci and enterococci, respectively. In cheese manufactured at a high altitude in southwestern Greece, NSLAB populations were found at considerably lower levels than those above. Very similar populations of total LAB, lactococci, and enterococci, as high as 10^5 CFU/g, were counted in the fresh cheese, while after ~2 months of ripening and storage, their counts were less than 10^2 CFU/g. NSLAB populations of cheeses made in northwestern Greece were high and similar to those counted in cheese made in the northern Peloponnesse, both areas of similar altitudes. Total LAB decreased with storage, and lactococcus counts of mature cheese were lower than in the drained curd by 1.6 (Peloponnesse) and more than 5 (northwestern Greece) log_{10} CFU/g. Enterococcus counts, on the other hand, decreased in the mature cheese by 0.6 (northwestern Greece) to 2.2 (Peloponnesse) log_{10} CFU/g.

The levels of gram-negative bacteria were reduced by 4 to 8 log_{10} CFU/g. Yeast counts were either decreased for cheeses of northwestern Greece (by 4.1 log_{10} CFU/g) and southwestern Greece (low altitude, 1.6 log_{10} CFU/g; high altitude, more than 5 log_{10} CFU/g), or increased (by 1.2 log_{10} CFU/g) for the cheese made in the Peloponnesse. Staphylococci were eliminated in the cheeses from all areas except northwestern (low altitude) Greece, where they were counted at levels as high as 10^5 CFU/g in the mature cheese.

The predominant microflora of feta cheese and its possible role in cheese ripening

The predominant surface microflora of fresh cheese (at 4 days, when the microbial slime starts appearing) is composed of LAB, yeasts, and salt-tolerant microbes. Leuconostoc, Lactococcus lactis, and Lactobacillus plantarum constitute the predominant LAB microflora developed at day 4, while at the end of ripening at room temperature (~20 days), the LAB microflora is composed of lactobacilli, with L. plantarum being the predominant species (7). The predominant yeast species at 4 days were Saccharomyces cerevisiae and Debaryomyces hansenii (8), while at the end of ripening at room temperature, Saccharomyces cerevisiae predominated. The halotolerant surface microflora was composed of staphylococci, micrococci, enterococci, and coryneform bacteria. Staphylococcus saprophyticus predominated the halotolerant bacteria content on the surface of cheese at day 4, while coryneforms were commonly found in the 20-day-old cheese (9). As indicated by their enzyme systems, lactobacilli of the cheese
surface may affect proteolysis, yeasts participate in both proteolysis and lipolysis, and staphylococci contribute to cheese lipolysis.

Lactococci were the most abundant group among the NSLAB found in the fresh cheese (43.6% of the isolates) of southwestern Greece (low altitude), with \textit{L. lactis} subsp. \textit{lactis} predominating. Lactobacilli were also isolated more frequently (23.1% of the isolates) than enterococci, and leuconostocs were equal to lactobacilli in abundance. The NSLAB microflora of cheese at 60 days was composed of lactobacilli only, with \textit{L. paraplanatarum} and \textit{L. paracasei} subsp. \textit{paracasei} being isolated at the same frequency (38.3% of the isolates) and predominating among lactobacilli. The NSLAB predominating in the fresh cheese made at high altitude in the same area were enterococci (33.3%) and lactobacilli, with \textit{E. faecalis} and \textit{L. paracasei} subsp. \textit{paracasei} predominating. In cheese made in the northern Peloponnese, only enterococci were found in the drained curd, with \textit{E. faecalis} predominating (54% of the isolates), while the NSLAB isolated from the mature cheese were lactobacilli, mainly heterofermentative (75% of the isolates). From the drained curd of cheese made in northwestern Greece, only lactococci (almost exclusively \textit{L. lactis} subsp. \textit{lactis}) were isolated. In cheese at ~2 months, only lactobacilli were present and \textit{Lactobacillus pentosus} was the predominant species (81.8% of the isolates). As shown in Table 3, each cheese type had its own NSLAB profile, with respect to species composition, with only two common species, \textit{Lactobacillus buchneri} and \textit{E. faecium} (unpublished data). It is expected, therefore, that the types of traditional feta manufactured in each geographic area, even in each creamery of Greece, mature differently due to the biochemical activity of different predominant NSLAB and may each develop a unique flavor.

Air has been recognized as a source of microbial contamination in dairy plants since the early 1900s. Dairy plant operations pay great attention to the effect of air on product quality. Contamination of milk during curdling in an open tank or of cheese during draining and dry salting provides microorganisms with a variety of sites for colonization. The numbers of airborne microorganisms may differ between plants, and the

### TABLE 3 NSLAB species’ in feta cheese manufactured in various parts of Greece

<table>
<thead>
<tr>
<th>Species</th>
<th>Presence in feta cheese from area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Northwestern Greece, (Livadero village ~850-m altitude)</td>
</tr>
<tr>
<td></td>
<td>Palaiomanina village (&lt;200-m altitude)</td>
</tr>
<tr>
<td>\textit{Lactococcus lactis} subsp. \textit{lactis}</td>
<td>+</td>
</tr>
<tr>
<td>\textit{Lactococcus lactis} subsp. \textit{cremonis}</td>
<td>-</td>
</tr>
<tr>
<td>\textit{Lactococcus garvieae}</td>
<td>+</td>
</tr>
<tr>
<td>\textit{Lactococcus raffinolactis}</td>
<td>-</td>
</tr>
<tr>
<td>\textit{Lactobacillus paracasei} subsp. \textit{paracasei}</td>
<td>+</td>
</tr>
<tr>
<td>\textit{Lactobacillus paracasei} subsp. \textit{tolerans}</td>
<td>-</td>
</tr>
<tr>
<td>\textit{Lactobacillus planatarum}</td>
<td>+</td>
</tr>
<tr>
<td>\textit{Lactobacillus paraplanatarum}</td>
<td>+</td>
</tr>
<tr>
<td>\textit{Lactobacillus pentosus}</td>
<td>+</td>
</tr>
<tr>
<td>\textit{Lactobacillus curvatus}</td>
<td>-</td>
</tr>
<tr>
<td>\textit{Lactobacillus brevis}</td>
<td>-</td>
</tr>
<tr>
<td>\textit{Lactobacillus bucheri}</td>
<td>+</td>
</tr>
<tr>
<td>\textit{Enterococcus faecalis}</td>
<td>-</td>
</tr>
<tr>
<td>\textit{Enterococcus faecium}</td>
<td>+</td>
</tr>
<tr>
<td>\textit{Enterococcus durans}</td>
<td>-</td>
</tr>
<tr>
<td>\textit{Enterococcus avium}</td>
<td>-</td>
</tr>
<tr>
<td>\textit{Enterococcus hirae}</td>
<td>-</td>
</tr>
<tr>
<td>\textit{Leuconostoc pseudomesenteroides}</td>
<td>+</td>
</tr>
<tr>
<td>\textit{Leuconostoc mesenteroides} subsp. \textit{mesenteroides}</td>
<td>-</td>
</tr>
<tr>
<td>\textit{Leuconostoc mesenteroides} subsp. \textit{dextranicum}</td>
<td>-</td>
</tr>
</tbody>
</table>

*From fresh and 2-month-old cheese.*
same is observed with the predominant types (10). Thus, lactococci were found to predominate in the air of the cheesemaking room of plant A, while the predominant airborne microfloras of plant B were micrococi and bacilli. On the other hand, lactococci and mesophilic lactobacilli predominated over the other LAB in the air of cheesemaking rooms; however, the species profile differed between creameries A and B, with just two common species (Lactococcus lactis subsp. cremoris and Lactobacillus bifermentans) found in the air of both (Table 4). A significant number of aerobic bacteria derived from the air could grow at 4°C and in 6.5% NaCl, and both aerobic bacteria and LAB exhibited acidifying and proteolytic activity. Therefore, there is a possibility that airborne bacteria contaminating cheese milk in the open vats of traditional cheesemaking rooms or dairies may multiply and contribute to the ripening changes in the cheese during manufacture, ripening, and storage.

“Wild” isolates of lactococci predominating in fresh feta cheese made in northwestern Greece were found to produce acid in milk and may drive the initial acidification of milk during cheesemaking. A high percentage (94.6%) of lactococci exhibited high acidifying capacity with respect to their ability to decrease the milk pH at 24 h ($\Delta$ pH$_{24h}$) [change in pH at 24 h] > 2.0 pH units; unpublished data). There was also a tendency of the isolates to either not degrade at all (35.14% of the isolates) or partially decrease (5.90 to 43.85%) $\beta$-CN of

**TABLE 4** Species of LAB isolated from MRS agar plates exposed to the air of cheesemaking and draining-salting rooms at two creameries (A and B) making traditional Greek cheeses (mainly feta)

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Strains (% of the isolates) isolated from air in:</th>
<th>Cheesemaking room</th>
<th>Draining-salting room</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Lactococci</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. lactis subsp. lactis</td>
<td></td>
<td>39.5</td>
<td>21.4</td>
</tr>
<tr>
<td>L. lactis subsp. cremoris</td>
<td></td>
<td>5.3</td>
<td>6.2</td>
</tr>
<tr>
<td>L. garvieae</td>
<td></td>
<td>18.8</td>
<td></td>
</tr>
<tr>
<td>Leuconostocs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. lactis</td>
<td></td>
<td>2.6</td>
<td>7.2</td>
</tr>
<tr>
<td>L. cremoris</td>
<td></td>
<td>2.6</td>
<td>11.1</td>
</tr>
<tr>
<td>L. mesenteroides</td>
<td></td>
<td>6.3</td>
<td>14.3</td>
</tr>
<tr>
<td>L. pseudomesenteroides</td>
<td></td>
<td>6.3</td>
<td>14.3</td>
</tr>
<tr>
<td>Weissella</td>
<td></td>
<td>10.5</td>
<td>28.6</td>
</tr>
<tr>
<td>W. paramesenteroides</td>
<td></td>
<td>2.7</td>
<td>14.3</td>
</tr>
<tr>
<td>W. congusa</td>
<td></td>
<td>2.7</td>
<td>14.3</td>
</tr>
<tr>
<td>Enterococci</td>
<td></td>
<td>2.6</td>
<td>6.2</td>
</tr>
<tr>
<td>E. faecium</td>
<td></td>
<td>2.6</td>
<td>6.2</td>
</tr>
<tr>
<td>Enterococcus sp.</td>
<td></td>
<td>2.6</td>
<td>6.2</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td></td>
<td>5.3</td>
<td>6.2</td>
</tr>
<tr>
<td>L. bifermentans</td>
<td></td>
<td>5.3</td>
<td>6.2</td>
</tr>
<tr>
<td>L. buchneri</td>
<td></td>
<td>2.6</td>
<td>5.6</td>
</tr>
<tr>
<td>L. coryneformis subsp. torquens</td>
<td></td>
<td>2.6</td>
<td>5.6</td>
</tr>
<tr>
<td>L. curvatus</td>
<td></td>
<td>7.9</td>
<td>7.1</td>
</tr>
<tr>
<td>L. casei</td>
<td></td>
<td>7.9</td>
<td>7.1</td>
</tr>
<tr>
<td>L. paracasei subsp. paracasei</td>
<td></td>
<td>12.5</td>
<td>22.2</td>
</tr>
<tr>
<td>L. paracasei subsp. tolerans</td>
<td></td>
<td>12.5</td>
<td>22.2</td>
</tr>
<tr>
<td>L. pentosus</td>
<td></td>
<td>18.4</td>
<td>5.5</td>
</tr>
<tr>
<td>L. plantarum</td>
<td></td>
<td>18.4</td>
<td>5.5</td>
</tr>
<tr>
<td>L. paraplantarum</td>
<td></td>
<td>5.6</td>
<td></td>
</tr>
<tr>
<td>L. rhamnosus</td>
<td></td>
<td>18.8</td>
<td>5.6</td>
</tr>
<tr>
<td>L. sake</td>
<td></td>
<td>5.6</td>
<td></td>
</tr>
<tr>
<td>Yeasts</td>
<td></td>
<td>18.8</td>
<td>7.2</td>
</tr>
</tbody>
</table>
Lactobacilli obtained from the cheese were found to ex-
feta cheese as well as obligately heterofermentative
S. aureus, and some exhibited an antibacterial activity towards
S. aureus, Y. enterocolitica, Listeria innocua, Clostridium
tyrobutyricum, and Clostridium sporogenes. Faculta-
tively heterofermentative lactobacilli from the surface of
feta cheese as well as obligately heterofermentative
lactobacilli obtained from the cheese were found to ex-
habit antifungal activity (12). The former group also
showed a broad spectrum of antibacterial activity, with
preference toward Listeria monocytogenes and other
food-borne pathogens. Considering the above activities,
the unfriendly cheese environment, with respect to
pH and NaCl, and the rapid decline of undesirable
microorganisms in the mature cheese contribute to the
microbiological safety of mature traditional feta cheese.
Lactobacilli, leuconostocs, and pediococci grown in feta
may also contribute to the ripening changes and flavor
formation through their biochemical activity.

Lactobacillus paracasei subsp. paracasei strains pro-
duce acid in milk and lower the milk pH to ~4.0 to 4.5
after 24 h. They also exhibit Leu-, Ala-, and Lys-
aminopeptidase activities (13) and accumulate amino
acids in the milk at various quantities, depending on
the strain (14). Besides aminopeptidase, strains of these
species also have intracellular dipeptidyl aminopepti-
dase, dipeptidase, endopeptidase, and carboxypepti-
dase activities and preferentially degrade either β-
or α-CN. L. paracasei subsp. paracasei strains also produce
esterases, and their activity is lower with higher-molec-
ular-weight fatty acids (13).

Lactobacillus plantarum, on the other hand, may
grow to high cell densities in feta cheese and exhibit
biotechnologically important characteristics, such as acid-
ifying and proteolytic activity (15). Their acidifying ca-
acity is low and strain dependent, with a ΔpH24h of <1 pH
unit. Amino acid amounts accumulated in the milk may
diff, depending on the strain. L. plantarum exhibits Leu-
and Val-aminopeptidase, β-galactosidase, α-glucosidase,
β-glucosidase, and N-acetyl-β-glucosaminidase activities,
and through these activities, it degrades milk constitu-
ents, such as peptides, glycolipids, and glycoproteins.
L. plantarum may also contribute to ripening changes of
feta cheese by degrading triglycerides through their weak
esterase-lipase activities.

Pediococcus pentosaceus, whenever found in feta
(16), may form acid at low levels (ΔpH24h < 0.5 pH
units) as well as diacetyl and acetaldehyde (40) and
may contribute to proteolysis through Leu-, Val-, and Cy
aminopeptidase activities as well as phosphoamidase
activity (17). Milk triglycerides may be affected through
a very weak esterase-lipase activity and glucosides by
enzymes such as β-galactosidase, β-glucosidase, and N-
acetyl-β-glucosaminidase exhibited by the majority of
the isolates. Crude cell-free extracts from P. pentosaceus
preferentially hydrolyzed β-CN (18) and exhibited high
protease, aminopeptidase, and dipeptidyl aminopepti-
dase activities.

Survival of pathogens in feta cheese
Escherichia coli O157:H7 inoculated into cheese milk
increases its population during the first 10 h of cheese-
making and then decreases after 24 h, coincidentally with
the drop of pH to ≤5.1 (19). Its load is decreased rapidly
during ripening (population of 106 CFU/g), and it is not
detected after about 1 1/2 months of ripening. In addi-
tion, when the acidity of the cheese is developed quickly
(pH 4.6 within 48 h), initial populations of 103 to 108
CFU of Yersinia enterocolitica decrease rapidly, and
no viable cells are detected after 72 to 120 h (20).

Aeromonas hydrophila inoculated into cheese milk is
entrapped in the curd during cheesemaking and increases
its numbers during the first 8 to 16 h of cheesemaking (21).
The decrease in pH to 4.4 to 4.8 within 2 days is accom-
panied by a concomitant significant (P < 0.001) decrease
in A. hydrophila and, finally, to undetectable numbers of
A. hydrophila within 72 to 120 h after curdling.

Touloumissio Cheese (Touloumotsi)
Touloumissio is a cheese that is preserved in skin bags
(touloumia). It is, in fact, feta cheese with some modi-
fications in technology. The cheese is manufactured from
raw ovine or caprine milk or mixtures of them.

The milk is curdled as for feta. After salting on tables and
ripening in barrels, the cheese is washed thoroughly, cut
into small pieces, and put in skin bags. The bags, preferably
from goat skin, have previously been shaved, treated with
salt, cleaned, and then reversed. The cheese pieces are put
next to each other. After filling the bags with either brine
or cold boiled and salted (5%) milk (for a better quality
product), the opening is closed and the skins are kept in
cool stores, with moderate humidity, to ripen. The bags are
put on a board, and then they are frequently turned to
L. brevis and/or esterase-lipase activities exhibited by the NSLAB of touloumissio may contribute to fat hydrolysis through weak esterase activity. It is also possible that the NSLAB of touloumissio changes of the cheese. E. faecalis, E. durans, Enterococcus casseliinaus, and Enterococcus malodoratus were found less frequently. Lactobacilli constituted 35.3% of the NSLAB isolates. Lactobacillus plantarum was the most frequent species (50.7% of the lactobacilli) and was detected in all cheese samples examined. Lactobacillus casei and L. buchneri were also found quite frequently, and Lactobacillus coryniformis and Lactobacillus brevis were rarely present. Lactococci (2.9% of the isolates), leuconostocs (1.4% of the isolates), and pediococci (2.9% of the isolates) were also found in some cheese samples.

The NSLAB of touloumissio may contribute to the cheese ripening and flavor through their strong hydrolytic activities detected in wild isolates from the cheese. The L. casei strains were found to display strong Leu-aminopeptidase and α-glucosidase activities, whereas L. plantarum strains displayed strong β-galactosidase activity. The β-galactosidase and α-glucosidase activities of L. brevis were strong, as also was observed for their Val-aminopeptidase activity. L. coryniformis also exhibited strong Leu-aminopeptidase and α-glucosidase activities. In addition, strong β-galactosidase and α-glucosidase activities were displayed by Leuconostoc mesenteroides subsp. mesenteroides, while the isolates of Weissella paramesenteroides were characterized by strong β-galactosidase and α-glucosidase activities. It also seems possible that the NSLAB of touloumissio may contribute to fat hydrolysis through weak esterase and/or esterase-lipase activities exhibited by L. casei and L. brevis. Therefore, NSLAB of touloumissio may degrade milk constituents and contribute to ripening changes of the cheese.

Kalathaki Limnou PDO
Kalathaki Limnou PDO is made on the island of Limnos by a technique similar to that used for feta, with the exception that the curd is drained into round baskets where it is also surface salted. In spite of a very similar technology, feta cheese was found to contain significantly higher quantities of total C_{2,0} to C_{8,0} free fatty acids and total free fatty acids than kalathaki of Limnos.

Sfela (Feta of Fire) PDO
Sfela is a semihard cheese produced in the southern Peloponnese, particularly in Messinia province. The cheese is often called “feta of fire” because the curd is cooked at 36 to 38°C. The cheese is made from raw ovine milk or mixtures of ovine and caprine milk. Sfela ripens in barrels, like feta, and the milk is curdled like feta. After coagulation, the manufacturer puts heat under the vat to briefly cook the coagulum. The cooked curd is drained in cheesecloth, and it is then put on tables, where it is inverted regularly to obtain a round shape. The cheese is then cut in slices and dry salted. While the cheese is draining, the manufacturer makes myzithra from the whey. Ground myzithra is used to cover the slices of sfela, which are then put into skin bags or barrels. The gaps between the pieces are filled with ground myzithra. Brine is then added, the opening of the bag is closed, and the cheese is transferred to cold storage to ripen, like touloumissio. Slices in barrels are put in layers, taking care to avoid gaps. The gaps, whenever formed, are filled with myzithra. After 1 to 2 weeks at room temperature, the brine is replaced, and some days later, the barrels are transferred to cold storage (4 to 6°C).

Teleme
Teleme is a soft and pleasant-tasting white-brined cheese made from all milk types or mixtures thereof. Teleme seems to have originated from northern Romania. The cheese is made year-round at home or in creameries. It is manufactured all over the country, primarily on the mainland. The technology of manufacture of teleme cheese differs from that used for feta in the procedure of draining and salting. The molded curd of teleme is subjected to pressure to expel the whey. After the blocks of teleme are drained, they are immediately immersed in brine (usually 18% NaCl) to allow penetration of salt.

In cheese made at home by a producer from raw goat milk, high counts of aerobic bacteria, LAB, psychrophils, and proteolytic and lipolytic bacteria were counted throughout ripening of the cheese. Proper hygiene practices in the cheesemaking room resulted in low coliform and yeast counts in the curd (mean log counts of 3.04 and 1.99, respectively).

Levels of most microbial groups increased during storage for 15 days, when coliforms, staphylococci, and lipolytic bacteria reached their peak growth (5.95, 5.36, and 7.46 log_{10} CFU/g, respectively). At the same time, the cheese pH was significantly decreased.
FIGURE 3 Populations (log_{10} CFU/g) of different microbial groups during manufacture and ripening of teleme cheese made from raw goat’s milk. (a) Total aerobic count and other microbial groups; (b) NSLAB. doi:10.1128/microbiolspec.CM-0009-2012.f3
creased to ~5.5. A mean pH value in the range of 4.15 to 4.50 from 15 to 90 days and an increasing brine concentration up to 6.21% in the 90-day-old cheese may be considered unfavorable for most microbial groups, and their combined effect results in a significant decrease in numbers of coliforms and staphylococci. Total aerobic counts reached their maxima at 75 days, and the same was observed with NSLAB (mean log counts [CFU/g]: total, 8.55; lactobacilli, 7.82; enterococci, 6.92).

NSLAB predominated over the other microbial groups during storage, and their population (6.12 to 8.55 log10 CFU/g) (Fig. 3b) was unaffected by the salt concentration until 75 days. Yeasts were favored by the cheese environment, possibly due to their salt resistance, and increased significantly to ~104 CFU/g after 90 days of storage. Psychrotrophic organisms were not severely affected by the pH and salt content of the cheeses, although, from 15 days onward, a slight decrease in their numbers was observed. However, high counts were not accompanied by any defect in the cheese.

The lactic microflora of the cheese was complex, and 20 different species were found throughout storage. Leuconostocs predominated in curd, and lactococci (L. lactis) were found more frequently (31.1% of the isolates) than lactobacilli, enterococci, and/or leuconostocs in the 15-day-old cheese. At the end of storage (90 days), 61.5% of the isolates were lactobacilli, 1.6% were lactococci, 28.7% were enterococci, and 8.2% were leuconostocs. Lactococci, predominating even in the 75-day-old cheese, may play an important role in the ripening changes of this cheese. Lactobacillus plantarum, which was the dominant Lactobacillus species throughout storage of the cheese, is salt resistant and represented 14.8%, 21%, 33%, and 48% of the isolates in fresh curd and 15-, 75-, and 90-day-old cheese, respectively. L. paracasei subsp. paracasei was also isolated with increased frequency as ripening progressed, and enterococci appeared to be an important microbial group. Enterococcus faecium was the most frequently isolated species in the curd (20% of the isolates) and dominated over E. faecalis and E. durans from the curd to the 90-day-old cheese.

**SOFT CHEESES**

**Anevato PDO**

Anevato is a spreadable cheese made from raw ovine or caprine milk or mixtures of both. The traditional cheese has a pleasant taste, especially when made from goat’s milk. The cheese is produced at home as well as at creameries in the mountain region of western Macedonia, in northwestern Greece, and nearby Thessaly.

Traditionally, anevato cheese was produced in western Macedonia by shepherds with large flocks of goats and sheep. They added rennet to milk obtained in the morning, just before taking the cattle out for feeding. During the day, the curd was “raised” and was ready to be drained upon their return late in the afternoon. The shepherds used to visit their villages once a week to see their families and sell their cheese.

Studies were conducted on anevato cheese made at a small creamery in northwestern Greece from raw goat milk throughout the whole lactation season (25). Whole milk from local herds was left at 18 to 20°C to sour by the activity of the natural flora. Liquid rennet was added to the milk at a pH of about 6.2, and coagulation took place in 12 h. The curd was cut (2- by 2-cm pieces) and left to rest (usually 4 to 5 h) until the pieces came to the surface of the whey (anevato means a cheese that is raised). The curd was put in cheesecloth and drained for 24 h. After draining, salt was added and the curd was thoroughly mixed and put in plastic containers (about 5 kg). The cheese was then refrigerated (4°C) until sold. The microbial counts of the various microbial groups were high (Fig. 4).

The total counts and NSLAB found in the raised curd increased significantly after draining the curd for cheese manufactured in January. Populations of gram-negative bacteria, halotolerant organisms, enterococci, yeasts, psychrotrophs, and proteolytic bacteria increased by 0.27 to 1.12 log. Multiplication during curd draining for ~8 h at room temperature, indicated by a pH decrease from 5.13 to 4.47, and physical entrapment of the microorganisms in the curd may account for these increases. Enterobacteriaceae and coliforms, on the other hand, decreased during draining, probably due to unfavorable conditions created by the increasing population of NSLAB and the lower pH.

Similar trends for microbial counts were observed in curds made in March and May. However, the initial contamination by psychrotrophs, yeasts, and NSLAB was higher in the raised curd from March and May than in curd made in January. The lower pH (March, 4.43 to 4.28; May, 4.25 to 4.20) of the curd and the warmer environment of cheese made in March and May can be considered the main factors regulating the growth of the microflora, along with the inhibitory activity exhibited by the higher numbers of NSLAB of the raised curd in March and May. It seems, therefore, that the higher acidity favored the growth of yeasts, the warmer environment may have contributed, to some extent, to a faster growth of psychrotrophs, and the lower pH along
FIGURE 4 Counts (log_{10} CFU/g; mean ± standard deviation [bars]) during manufacture and storage of anevato cheese made throughout the whole lactation season. (a) Enterobacteriaceae and coliforms; (b) NSLAB; (c) other microbial groups. doi:10.1128/microbiolspec.CM-0009-2012.f4

Figure 4 continues on next page
with the inhibitory effect of higher numbers of NSLAB are, mainly, responsible for the reduced numbers of Enterobacteriaceae and coliforms, especially in the raised curd of cheese made in May.

LAB and gram-negative organisms were the major components of the cheese microflora throughout the entire lactation season. The general trend for gram-negative organisms was a gradual decrease during storage at 4°C. Their counts were still high after 60 days. In spite of their high numbers, neither off-flavor nor any other defects caused by certain psychrotrophic gram-negative bacteria were detected in the cheese, due possibly to the low pH and the absence of active strains.

NSLAB decreased in number upon storage for 15 days. Total LAB decreased their numbers by 0.89, 1.02, and 0.99 log_{10} CFU/g for cheese made in the winter, spring, and summer, respectively. The respective declines of lactococci were 0.76, 0.98, and 1.82 log_{10} CFU/g. After that, various tendencies were recorded for cheese made at the various seasons, but at the end of storage, similar populations were enumerated in cheese made throughout the whole lactation season. Enterococci were also present at relatively high levels in all cheeses (10^4 to 10^7 CFU/g). They decreased gradually after salting, but there were no seasonal effects on their numbers.

Enterobacteriaceae and coliforms decreased gradually during storage and disappeared rapidly from cheese made in May (they were not detected in cheese after salting). A significantly faster decline of these organisms in cheese made in March in comparison to the cheese made in January was also recorded. These differences in behavior in cheese made at different seasons may be related, mainly, to lower pH and higher numbers of NSLAB, but the combined effect of pH (January, 5.13 to 4.21; March, 4.43 to 4.25; May, 4.25 to 3.49) and salt concentration (January, 1.47 to 1.78; March, 1.84 to 1.71; May, 2.2 to 2.8) might have had some influence.

Halotolerant organisms tended to decrease during storage. Growth rates of yeasts were lower in cheese made in January (3.49 to 5.43 log_{10} CFU/g) than those in cheese made in March (4.35 to 6.32 log_{10} CFU/g) and even lower than those made in May (5.38 to 7.05 log_{10} CFU/g). Their ability to multiply at these high levels in the cheese kept at 4°C suggests that the yeasts were psychrotrophic and that they, in fact, determine the quality and shelf life of anevato cheese.

Lactococci dominated in the cheese up to 15 days (51.2 to 70.7% of the isolates). Leuconostoc spp. were present at low levels initially but occurred more fre-
Lactobacilli became predominant at 30 and 60 days (Fig. 5). Lactococci, mainly *L. lactis* subsp. *lactis* bv. *diacetylactis*, could be considered responsible for cheese manufacture, and the floating of the curd after coagulation is attributed to CO₂ production from citrate by these microorganisms.

Enzyme activities exhibited by lactococci from anevato were found to be weak (25), suggesting that milk constituents may be slightly hydrolyzed by lactococci during manufacture and storage. However, an extremely high number of them (31% of the isolates), compared to findings with other cheeses (5, 26), were fast acid producers and lowered the milk pH to levels of ≤5.2 after 6 h. Considering this property, as well as their predominance in the curd, one could speculate that, in fact, the autochthonous lactococcal microfloras of anevato cheese act mainly as starter cultures during production of the cheese.

**Galotyri PDO**

Galotyri is a soft cheese with a sour taste made from ovine milk at the end of the lactation season. The cheese is made in all regions of the country by processes which differ from region to region. The main traditional methods of cheese manufacture are described below (2).

After milking, the milk is strained, boiled, and then put in a pot made of either clay or galvanized copper. After 24 h, it is salted (4% NaCl) and left for another 2 days. During this time, the milk is mixed every 3 h. It is then transferred to a skin bag. This is repeated several times until the bag is filled. The opening of the bag is closed, and the cheese is put on a board on which ash is scattered to absorb the moisture. The bags are turned from time to time to prevent mold growth. The cheese ripens for 3 months. While some traditional producers hang the skin bag instead of placing it on the board, nowadays, the cheese usually ripens in barrels instead of

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**FIGURE 5** Distribution of NSLAB (percentage of isolates) during manufacture and storage of anevato cheese. doi:10.1128/microbiolspec.CM-0009-2012.f5
skin bags. In this case, to avoid the growth of molds, the cheese surface is covered with melted fat. Manufacturers in some areas may use a small quantity of rennet to curdle the milk. In many areas, the cheese producers make yogurt with the milk, put it in the skin bag, and then add to it pieces of fresh feta (proportion, 5/1) and salt (4%). In other regions, the producers make another type of galotyri; they make strained yogurt, transfer it into the skin, and add salt. Retail cheeses have a high moisture content (76.4%).

Artisanal cheese is manufactured by heating the milk to 85 to 90°C and then adding “natural” starter, which contained microbial populations ranging from 1.66 (enterobacteria) to 8.0 (lactococci) log10 CFU/g (27). The NSLAB microflora of the cheese samples were composed primarily of heterofermentative arginine-negative mesophilic lactobacilli and secondarily of lactococci. Gas-producing NSLAB of the Leuconostoc group made up a minor part of the NSLAB microflora, and yeasts were present at high levels (10^4 CFU/g).

Although the population of NSLAB did not undergo major changes during storage, yeast counts grew significantly to levels exceeding 7 log10 CFU/g after 14 days of storage at 4°C, due to conditions favorable for their growth (pH 4.1 at 14 days). Yeast growth resulted in slimy yellowish colonization spots on the cheese surface after 14 days at 4°C, which made the cheese inedible after 21 days.

The hygienic quality of the cheese was in compliance with the microbiological criteria of European Union regulation 2073/2005 with respect to enterobacterial counts (≤100 CFU/g in the majority of the samples), coagulase-positive staphylococci in soft nonripened cheeses (≤100 CFU/g), and absence of salmonella. The only safety concern was the detection of L. monocytogenes in 3 samples (2 artisanal, 1 industrial). This obviously was a postprocessing contamination, which may indicate poor hygienic conditions in the plants.

As a postprocess contaminant, E. coli O157:H7 survived better in the artisanal cheese than in commercial cheese kept at 4 and 12°C. In addition, its survival was better at 12°C than 4°C, probably due to yeasts which grew on the cheese surface (28). Under the same conditions, L. monocytogenes survived similarly in both cheese types and storage temperatures (29).

Katiki PDO
Katiki is a soft cheese made in the Domokos area and other parts of mountainous Greece, from goat’s and ewe’s milk from local breeds. In the Aitolia-Akarnania region, the cheese is also commonly known as tsalafouti. The milk is left at room temperature (20 to 22°C) to sour and curdle with or without rennet. It is then drained in cheesecloth, salt is added (1%), and the cheese is kept refrigerated until it is consumed.

In samples obtained from six different productions from a small dairy in the Domokos area, the cheese pH was low (range, 3.8 to 4.2); therefore, fecal coliforms were not detected in any of the samples and coliforms were also found at low levels (10^3 CFU/g) (30). The predominant microfloras, the NSLAB and yeasts, were counted at mean levels of 10^7 and 10^6 CFU/g, respectively, while entercoccal populations ranged between 10^4 and 10^5 CFU/g. Enterococci were found abundantly (60.4%) among the predominant NSLAB population. Lactobacilli were present quite often (23.5%), and weisellae and leuconostocs were less frequently (9.8% and 5.9%, respectively) isolated. E. durans was the most frequently found species (27.5% of the isolates), followed by L. plantarum (17.6%) and Enterococcus faecium (13.7%). Other species, such as Enterococcus fæciæm, Enterococcus hiræ, E. faecælis, L. mesenteroides subsp. cremoris, Leuconostoc lactis, W. paramesenteroides, Weisella viridescens, L. paracæsi subsp. paracæsi, and L. paracæsi subsp. tolerans, were also found less frequently.

Kopanisti PDO
Kopanisti is a cheese variety with a characteristic peppery taste and is made from cow’s milk in the Cyclades islands of the Aegean Sea. Its manufacture is not standardized, but there are some common steps followed by producers. The milk is clotted with a small quantity of rennet over a period of a few hours. The curd is drained in cheesecloth, after which a quantity of older cheese (mana) is added, and the curd is kneaded by hand and formed into balls the size of a small orange. The cheese is then left in clay pots for several days until a green or blue-green mold appears on the surface. The balls are then sprinkled with fine salt and kneaded again so that the mold spreads evenly throughout the mass of the cheese. The cheese is put in pots and pressed by hand so that no air gaps remain in the cheese mass. Its surface is covered with a dry piece of cheesecloth, upon which a weight is put to press the cheese. The cheesecloth is changed every day. The cheese ripens for 1 to 2 months, until a peppery taste develops and it is ready for consumption (2, 31).

In cheese samples obtained from retail shops, there was a wide range in pH, NaCl percentage, and moisture percentage, due to the fact that the cheeses had been manufactured on five different islands and the producers did not follow the same process. The combined effect of the low pH values (<5.2) and high brine concentration...
(>6.5% for the 44% of the samples) might have influenced the numbers and types of microorganisms that finally occur in the cheese. Thus, coliforms were not found in 60% of the samples and were detected in small numbers (10^3 CFU/g) in the rest of the cheeses. Micrococci and staphylococci were virtually absent (31).

The cheese environment did not adversely affect the growth of NSLAB, and these organisms were present in high numbers (>10^3 CFU/g) in 86% of the samples. Salt-resistant lactobacilli (L. plantarum and L. casei) were the most frequently isolated species (40% and 12.2% of the isolates, respectively) from a large number (84%) of cheeses. Other lactobacilli found in some cheeses were L. curvatus, L. xylosus, L. brevis, L. buchneri, L. cellobiosus, and L. viridescens. E. faecium, E. faecalis, E. casseliflavus, E. durans, and pediococci (P. pentosaceus) were also present in cheese samples.

Yeasts were probably favored by the cheese environment, and their population exceeded 10^3 CFU/g in about 70% of the positive samples. Pichia membranifaciens and Pichia fermentans were the predominant species, accounting for 69% and 17% of the yeast isolates, respectively. The molds developed in kopanisti cheese were characterized as Penicillium commune (31).

Pichtogalo Chanion PDO

Pichtogalo Chanion PDO is a soft, spreadable cheese manufactured from ewe’s milk or mixtures of ewe’s and goat’s milk, which is left to sour for 24 h. The cheese is drained, salt is added at 1%, and the product is ready for consumption. The product is made at Chania on the island of Crete.

The gross composition of this cheese was found to be as follows (32): moisture content, 61.63% ± 0.67%; fat in dry matter, 54.03% ± 7.73%; protein content, 14.23% ± 1.72%; salt content, 1.02% ± 0.38%; water activity (a_w), 0.99 ± 0.003; pH, 4.36 ± 0.25.

The plate counts of the various microbial groups were high in retail cheese samples. In five samples, coliforms and Enterobacteriaceae ranged between 1.0 × 10^1 to 2.0 × 10^3 and <10 to 2.7 × 10^5 CFU/g, respectively. LAB, lactococci, and lactobacilli predominated over the other microbial groups and were counted at mean levels of 1.1 × 10^8 and 1.4 × 10^8 CFU/g, respectively. Mean plate counts of enterococci were also high (2.8 × 10^7 CFU/g; range, 1.8 × 10^4 to 1.3 × 10^8 CFU/g). Counts of staphylococci were <10 to 3.8 × 10^2 CFU/g. Yeasts were present at levels as high as 10^4 to 10^6 CFU/g and constituted the spoilage microflora of this cheese (unpublished data). Salmonella spp. and L. monocytogenes were not detected, coagulase-positive staphylococci were present in 6.45% of the samples, and B. cereus and sulfite-reducing clostridia were isolated from 14.51% and 40.32% of the samples, respectively. The cheeses contained high populations of coliforms, enterococci, yeasts, molds, and psychrotrophic bacteria, while LAB constituted the predominant cheese microflora (32).

SEMIHARD CHEESES

Kasseri PDO

Kasseri is a traditional Greek cheese of the pasta filata type with a pleasant flavor. The cheese was introduced to Greece from the neighboring Balkan countries at the end of the 19th century, initially to Thessaly, central Greece, and its manufacture eventually spread to other areas of the country. Plain ovine or mixtures of it with caprine or bovine (5 to 10%) milk are used for the cheese manufacture (1, 2).

The milk is curdled at 32°C within 30 min. The coagulum is cut (the size of corn), and for a better ripening, it is usually cooked at 38°C. After removal of the majority of the whey, the curd is cut with a knife into large pieces, which are then transferred on a table and rubbed by hand on wet cheesecloth to loosen the particles of curd. Then the ends of the cheesecloth are fastened together tightly, and the resulting product, called baski, is left to drain by a weight equal to that of the curd and ripened over a period of 8 to 24 h at 18 to 20°C. The ripened baski is cut into small, thin, uniform slices, which are put into a basket (kafino). The basket is dipped in water (at ~70°C), where it is turned, in a way, to facilitate the penetration of the water between the slices. Then the cheese is kneaded for uniformity. It is cut into pieces and introduced into the molds, where the cheese remains for 2 to 4 days to harden. Twenty-four hours after production, the molds are transferred to storage (18°C) and are surface salted. The cheese, after the end of salting, is thoroughly washed with warm water (40 to 50°C) and then with cold water and is left to dry for 5 to 10 days. The ripening continues in cold storage (0 to 2°C).

Baski contains NSLAB (10^6 to 10^8 CFU/g), lactobacilli (10^6 to 10^8 CFU/g), lactococci (10^4 to 10^8 CFU/g), and thermophilic (growth at 42°C) cocci (10^5 to 10^8 CFU/g), as well as enterococci (10^2 to 10^7 CFU/g) (unpublished data). The heat treatment of the ripened baski results in a considerable decrease in numbers of total NSLAB (by 1 to 2 log_{10} CFU/g), lactobacilli (by 1 to 4 log_{10} CFU/g), and mesophilic cocci (by 3 log_{10} CFU/g), but a smaller reduction in numbers of thermophilic cocci is recorded in the fresh kasseri cheese. Therefore, ther-
mophilic streptococci of the *Streptococcus thermophilus* group are found quite frequently. Lactococci (mainly *L. lactis* subsp. *lactis*) and leuconostocs (mainly *L. mesenteroides*) are isolated at lower frequencies. However, the predominant NSLAB microflora of ripened *baski* and fresh kasser is composed, mainly, of enterococci and lactobacilli (38.9% and 39.8% of the isolates, respectively). Facultatively and obligately heterofermentative lactobacilli are found, among which *L. paracasei*, *L. paraplantarum*, and *L. rhamnosus* were the most frequently isolated.

The pH of both *baski* and fresh cheese was over 5.0. The moisture content of kasser ranged between 42 and 46%, and the brine concentration of the mature cheese was around 3%. These factors regulate the growth and survival of the microflora during ripening.

The majority of lactococci of kasser exhibited a satisfactory acidifying capacity and lowered the milk pH to levels of ≤4.0 after 24 h of growth in milk. At the same time, proteolysis of milk by lactococci resulted in accumulation of amino acids in the milk at levels exceeding 100 ppm (in l-glycine equivalent) for the majority of them. Lactococci also produced antibacterial substances, possibly bacteriocins, which inhibited the growth of similar or related species. With regard to their antagonistic activities against food-borne pathogens, lactococci preferentially inhibited the growth of *E. coli* O157:H7, *Yersinia enterocolitica*, and *Staphylococcus aureus*, as well as *B. cereus* (inhibition zone, >1 cm).

Thermophilic streptococci formed less acid in milk than lactococci after 24 h and lowered the milk pH by 1.4 to 2.20 pH units for the *S. thermophilus* group and by 1.50 to 2.20 units for *Streptococcus macedonicus*. *S. macedonicus* is frequently found in traditional Greek kasseri (33). There was a high heterogeneity between the isolates regarding their proteolytic activity, with *S. macedonicus* being more active than *S. thermophilus*. Thermophilic streptococci exhibited less antagonistic activity than the lactococci. However, there were some (20%) which strongly inhibited the growth of *S. aureus*, *Y. enterocolitica*, and *E. coli* O157:H7, while more than 40% of isolates exhibited inhibitory effects against *B. cereus*.

It seems, therefore, that the natural NSLAB microflora developed during kasseri cheese production may contribute to cheese production and ripening changes by their biochemical activities and possibly regulate the microbial growth and survival of undesirable microorganisms.

**Krassotyri (Possias)**

Krassotyri, or possias, has a slightly sour taste and organoleptic properties very much affected by the properties of wine sediment, into which the cheese is placed after ripening (1). The wine sediment is called *possia*, from which the cheese was named. The cheese is made from ovine or caprine milk or mixtures of both, mainly on the island of Kos in the Dodecanese.

The milk from the afternoon milking is heated until boiling, and it is then transferred into shallow clay pots. The next morning, the milk fat from the milk surface is removed, and the milk is thoroughly mixed with fresh milk from the morning milking. The milk is coagulated at 32 to 34°C within 1 to 1.5 h with rennet (usually traditional). The curd is cut and is then transferred into baskets (*tyrovolia*) to drain for 2 to 4 h. After that, the baskets are removed, the surface of the cheese is salted, and then the cheese is put into vessels. The cheese is transferred to a cool place to ripen for 20 to 30 days, and after that, it is removed from the vessels and put on shelves to dry. The dry cheese is again put in the vessels, and wine sediment is added to cover the cheese. In this environment, the cheese ripens for one more week. At the end, the cheeses obtain a reddish color from the sediment (1).

In cheese samples purchased from retail shops, the NSLAB constituted the predominant microflora of the cheese and were found at similar levels on the cheese surface and interior (34). The salt-tolerant organism and coliform populations were lower on the cheese surface than in the interior by ~1 log_{10} CFU/g. This was due to the effect of *possia* and not of the pH and/or the NaCl, which were similar for the cheese surface (4.38 and 2.38%, respectively) and interior (4.38 and 2.49%, respectively). *Possia* also affected the composition of the lactic microflora. Thus, the surface LAB microflora was composed of lactobacilli and enterococci (50% each), with *E. faecium* predominating (37.4% of the isolates). The LAB microflora of the cheese interior consisted of lactobacilli, enterococci, and lactococci, but lactobacilli predominated (65.5% of the isolates), *L. paraplantarum* and *E. faecium* were the most frequently isolated species (20.7% each). In addition, *L. rhamnosus*, *L. casei*, and *L. paracasei* were found in the cheese interior, but not on the surface, while *L. buchneri*, *Enterococcus gallinarum*, and *E. pseudoavium* isolated from the cheese surface were not found in the cheese interior. The mean moisture contents of the cheese surface and interior were 54.4 and 55.6%, respectively.
HARD CHEESES

Graviera Kritis PDO

Traditional technology

Graviera is the finest cheese among the Greek hard cheeses. It is distinguished by its pleasant aroma and fine taste. Sheep’s milk is mainly used for cheese manufacture, but cow’s milk or mixtures of it with sheep’s and goat’s milk are also used, the latter not exceeding 20%. The mature cheese has a smear rind and usually exhibits small or large irregular openings which could be characterized as slits or holes, rather than eyes. The product has a cylindrical shape, and its height is either 8 to 10 cm or 12 to 15 cm (1, 2).

Graviera was first manufactured in 1914 in the royal dairy of Lappa of Manolada by N. Zygouris, a dairy scientist. In 1917, a dairy school at Ioannina, Epirus, in northwestern Greece, was established, and its students learned how to make graviera. Later, technicians spread its technology to various dairies.

According to the narration of a shepherd from the Lasithi mountain on the island of Crete, the cheese is made as follows (35):

You collect the evening milk, you warm it and you leave it. The next morning you collect the cream and you put it in a clay pot and you salt it. You put the milk in the large vat, where you also add the morning milking and you stir the milk. You transfer the vat on a trivet and you put a small fire underneath to warm it. Then you add the rennet (from the stomach of a breast fed lamb) and within half an hour the milk is curdled (a wooden utensil) to cut it into very small pieces (the size of corn). This process lasts for about 15 min. Then the curd is heated to a temperature of 48 to 52°C and then removed from the heat. For about 1 h 15 min, the curd is stirred vigorously with a tarachtis (a wooden utensil used for stirring) to separate the whey (ouro) from the cheese curds (malaka). Salt (approximately 1%) is added. The cheese curd is left to settle on the bottom of the vat, from which it is collected using cheesecloth. The cheesecloth containing the curd is placed in a toupi (mold; a wicker or reed basket), which is put on the cheese press.

The cheesemaker kneads the cheese in the mold, so that it takes its form, and then covers it with the takos (wooden lid). The takos is pressed by a lever system, which permits control of the pressure applied to the cheese. The cheese in the mold is pressed for 24 h with low pressure (1 to 2 times the weight of the cheese) initially, which is gradually increased (to 12 times the weight of the cheese). For the same reason, the cheese is turned upside down three or four times during its drainage in the mold. The cheesecloth is also changed three times.

After the cheese has been drained sufficiently, it is put on shelves in the ripening cellar (14 to 16°C) for 14 to 15 days, and every morning, it is inverted and salted. The cheese ripens for 3 to 4 months.

Changes of the microflora during ripening and storage of Graviera Kritis cheese

Studies were conducted on Graviera Kritis manufactured at a small traditional factory on Crete from raw ewe’s milk. Curd before cooking contained high counts of the various microbial groups, ranging from 4.49 (yeasts) to 8.94 (total aerobic counts) log_{10} CFU/g. NSLAB, Enterobacteriaceae, and coliforms constituted the predominant microflora of the curd (Fig. 6).
FIGURE 6 Populations (log_{10} CFU/g; mean ± standard deviation [bars]) of microbial groups throughout manufacture and ripening of Graviera Kritis cheese. (a) Various microbial groups; (b) LAB. doi:10.1128/microbiolspec.CM-0009-2012.f6
Enterobacteriaceae and coliforms declined in the curd after cooking by 0.63 and 0.80 log10 CFU/g, respectively, and then reached their highest counts in cheese before salting, by increasing their populations by 0.49 and 0.40 log10 CFU/g (Fig. 6a). From this time onward, their population decreased by 4.13 and 4.63 log10 CFU/g at the end of the ripening. The pH values of the cheese (5.07 to 6.48) do not seem to be responsible for this significant reduction, since these organisms require pH values lower than 5.0 to 5.20 for inhibition. It seems likely that other factors, such as depletion of nutrients caused by the increase in the NSLAB population by 1.58 log10 CFU/g in the cheese before salting, as well as their antibacterial activities, may be the main agents regulating their survival.

NSLAB were the most abundant microbial group of cheese during ripening (Fig. 6b). Total LAB counts reached their maxima in the fresh (1 day) cheese before salting, being higher by 1.42 log10 CFU/g than the initial populations. Thereafter, they declined by 1.63 log10 CFU/g. A similar evolution of lactococci and lactobacilli during cheese aging was observed. Enterococci, on the other hand, increased by 2 log10 CFU/g to peak growth after salting and then decreased until the end of storage.

The levels of gram-negative bacteria were significantly reduced (by 5.15 log10 CFU/g) in the mature cheese. Staphylococci reached their maxima in cheese before salting and then declined by 4.02 log10 CFU/g, and yeasts populations were measured at levels of 3.04 (after salting) to 4.91 log10 CFU/g (90-day-old cheese) (Fig. 6a).

It seems possible, therefore, that the NSLAB population of traditional Graviera Kritis, surviving at high levels throughout cheese ripening, contributes significantly to ripening changes in the cheese.

The salt-in-moisture of the cheese ranged between 2.42 and 3.05%, and the moisture content of mature cheese was 31.52%. At this time, the 65.36% and 14.39% of αs- and β-CN, respectively, of the cheese was reduced by the native flora, mainly the lactic flora.

Evolution of the NSLAB microflora and its possible effect on cheese ripening and flavor

At the various steps of cheese manufacture and ripening, different NSLAB species were found (Table 5). Lactococci predominated in the curd (47.83 and 66.67% before and after cooking, respectively). The main Lactococcus species was L. lactis subsp. lactis. Its high frequency in the curd allows speculation that L. lactis acts to drive the initial acidification of the curd. It may also contribute to developing typical flavor in the traditional cheese through enzymatic activity. Leuconostocs in the curd constituted a significant part of lactic microflora (39.13% in the curd before cooking). Leuconostoc mesenteroides subsp. mesenteroides and Leuconostoc pseudomesenteroides found in the curd may be responsible for the formation of small holes in the cheese body. L. paracasei subsp. paracasei, L. plantarum, and L. brevis, found in the curd in low proportions, may play a minor role in open body formation by gas production from citrate or lactose, respectively.

The proportion of lactococci decreased with ripening, and these organisms were not present at 45 days. In contrast, the facultatively heterofermentative lactobacilli increased during ripening and constituted the 17.2, 89.3, and 84.1% of the isolates at 4, 45, and 90 days, respectively, suggesting a possible contribution to cheese ripening. L. paracasei subsp. paracasei predominated over the NSLAB in the mature cheese. The NSLAB microflora of the cheese was complex.

Enterococci constituted a low percentage of the predominant lactic microflora throughout cheese ripening. These organisms represented 4.3, 16.7, 13.8, 3.6, and 9.1% of the isolates in curd, before and after cooking, and in cheese after salting at 45 days and 90 days, respectively. E. faecalis was the most frequently found species. Leuconostocs constituted a minor portion of the cheese microflora throughout ripening, and weissellae and carnobacteria emerged during ripening of the cheese.

Lactococci from Graviera Kritis grown in milk formed low amounts of acid after 6 h, which increased significantly after 24 h. Lactococci of low and medium acidifying capacity were identified, but the majority (71.8%) exhibited strong acidifying activity and lowered the milk pH by more than 2 pH units after 24 h (Pavlidou S, Bozoudi D, Hatzikamari M, Tzanetakis N, Litopoulou-Tzanetaki E, unpublished data).

A significant variability among lactococcal isolates, with respect to casein degradation as well as a preference toward αs-CN degradation (by 89.9% of the isolates), was recorded after 4 days. β-CN was degraded by the majority of the isolates (94.9%) at a variable range (5.90 to 65.71%). The amino acids accumulated in the milk appeared after 24 h and increased significantly after 4 days, when 60% of lactococci formed amounts ranging from 2.09 to 157.46 ppm of L-glycine equivalents.

The isolates preferentially inhibited L. lactis subsp. lactis (71.8% of the isolates), S. thermophilus (20.8%), E. faecalis (56.4%), and L. helveticus (38.5%), suggesting possible bacteriocin formation. Isolates were found to preferentially (63.3%) inhibit Y. enterocolitica. Some isolates also adversely affected the growth of

Microfloras of Traditional Greek Cheeses

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E. coli (28.2%), Clostridium sporogenes (10.3%), and C. tyrobutyricum (15.4%).

It may therefore be concluded that lactococci predominating in Graviera Kritis cheese exhibit interesting technological characteristics. High numbers present in the curd may contribute to cheese manufacture by acidifying the milk, followed by casein degradation as ripening progresses. Lactococci of graviera may also protect the cheese from clostridial spoilage and contribute to cheese safety by inhibiting the growth of foodborne pathogens.

**Kefalograviera PDO**

Kefalograviera is a hard cheese with a firm body and many holes or eyes, a mild to moderately piquant flavor, and a color that ranges from nearly white to slightly yellow. A mixture of 60%/40% cow’s milk/sheep’s milk is usually used, but goat’s milk may also be incorporated in a proportion not higher than 20%. Kefalograviera is mainly manufactured in mountain regions of Greece (1).

Manufacture began in Greece in the mid-1960s. As the name of the cheese indicates, its technology has elements of both kefalotyri and graviera cheese. The fat content of the cheese milk is standardized to 3.3%. The milk is pasteurized; starter and rennet are added, as in the case of industrial kefalotyri; and coagulation takes place within 25 to 30 min. The coagulum is cut into particles, the size of corn kernels, and after 20 min of agitation, the temperature of the whey is raised to 47 to 48°C within 20 to 25 min. The curd is cooked at this temperature for 20 to 25 min under continuous agitation. The cheese is pressed like graviera.

**TABLE 5 NSLAB composition of Graviera Kritis cheese**

<table>
<thead>
<tr>
<th>Species</th>
<th>Presence in Graviera Kritis cheese</th>
<th>During ripening</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before cooking</td>
<td>After cooking</td>
</tr>
<tr>
<td><strong>Lactococci</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. lactis subsp. lactis</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>L. lactis subsp. cremoris</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>L. raffinolactis</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Leuconostocs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. mesenteroides subsp. dextranicum</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L. mesenteroides subsp. mesenteroides</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>L. pseudomesenteroides</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td><strong>Weissellae</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W. paramesenteroides</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>W. confusa</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td><strong>Enterococci</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. faecium</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>E. durans</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>E. avium</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>E. raffinosus</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>E. malodoratus</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td><strong>Lactobacilli</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. paracasei subsp. paracasei</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>L. paracasei subsp. tolerans</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>L. plantarum</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>L. paraplantarum</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>L. curvatus</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>L. pentosus</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>L. buchneri</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>L. parabuchneri</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>L. brevis</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>L. bifermentans</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>L. coryneformis</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td><strong>Carnobacteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. piscicola</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

Litopoulou-Tzanetaki and Tzanetakis
It is then placed in a room at 12 to 14°C for 24 h. The cheese is put in brine (18 to 20%) for 48 h, after which it undergoes dry salting. The cheese is finally washed with brine (12 to 14 Baumé [Be]), left to dry, placed in Cryovac packaging, and left to continue ripening for up to 3 months at 8 to 10°C (1).

**Kefalotyri**

Kefalotyri (kefalí, head; tyri, cheese) is characterized by high hardness, a salty taste, and strong flavor. It is made from sheep’s or goat’s milk or mixtures thereof. Cow’s milk may also be used. The cheese technology was introduced centuries ago from Italy to Greece. It is manufactured in various parts of Greece with technologies that differ from area to area, and it is traded under the name of the region where it is manufactured (Kriti, Naxos, Thessaly, Kefalonia, Epirus, etc.).

The milk is partially skimmed (5.8 to 6.0% fat content). After coagulation (within 30 to 35 min), the coagulum is cut into small particles (0.5 to 1.0 cm). The temperature of the coagulum is then raised (to 43 to 45°C) under continuous stirring, slowly initially and faster toward the end. The stirring is continued for 15 min. The curd is left to settle, the majority of whey is removed, and the coagulum is cut into pieces, approximately the size of a mold. The pieces are put into molds, lined with cheesecloth, and they are then put into a press. The initial pressure is small, and it is gradually raised to 10 to 12 times the weight of the cheese. The cheese is placed in the ripening room (14 to 16°C) for 24 h and then in brine (18 to 20 Be) for 1 to 2 days. It is transferred again in the ripening room, where it is given frequent dry saltings. The cheese is finally washed with brine and transferred to cold storage (2 to 4°C) for continued ripening (2).

Orinotyri (orinos, mountainous; tyri, cheese), a local name for kefalotyri, was made at a small traditional creamery located in a village of mountainous Epirus, northwestern Greece, from raw ewe’s milk. In five cheesemaking trials, the following observations were made (36).

Enterobacteriaceae and coliforms in 10-day-old cheese were high (mean log counts, 7.94 and 7.41 log₁₀ CFU/g, respectively), and their average counts in 3-month-old cheese were significantly reduced by 3.02 and 7.76 log₁₀ CFU/g, respectively, compared to counts in the fresh cheese. The pH values of fresh cheese were high (mean pH, 6.31), being reduced by 0.52 pH units in the mature cheese. Cooked curds have lower whey and lactose content than uncooked curds, and the resulting cheese has a higher pH, which permits survival of Enterobacteriaceae and coliforms. The significant reduction of pH in the mature cheese and the increase in salt-in-moisture content (from 4.13% to 6.64%) affect these organisms. The high cheese pH did not favor the growth of yeasts; these organisms were counted at low levels in the fresh cheese (<200 CFU/g), and they were not detected in the mature product. S. aureus was not detected in either fresh or mature cheese.

Levels of NSLAB decreased significantly with ripening (Fig. 7a). Total LAB, lactobacilli, lactococci, and enterococci were found at lower levels in the 3-month-old cheese by 1.13, 1.30, 1.10, and 2.05 log₁₀ CFU/g compared with cheese at 10 days. The high pH values in the fresh cheese suggest a weak lactose fermentation ability by the NSLAB. Moreover, high pH values do not seem to have influenced their decline. An increasing NaCl concentration up to 6.64% in the mature cheese was unfavorable for salt-sensitive NSLAB and likely caused a reduction in their levels.

Enterococci predominated in the fresh orinotyri. It is well known that these organisms are thermoduric and may be derived from contaminated raw milk due to poor cleaning practices at the farm. Lactococci, lactobacilli, and pediococci were also less frequently found in the fresh cheese (Fig. 7b).

The NSLAB microflora was complex in both 10-day- and 3-month-old cheese. Fifteen different species were characterized in the fresh cheese, with E. faecalis being predominant (24.7% of the isolates), followed by P. pentosaceus (23.0%), L. lactis subsp. lactis (16.5%), and L. curvatus (11.6%). In the 3-month-old cheese, 14 different species were found, and the predominant species were E. faecalis (25.0%), E. faecium (10.3%), L. paracasei subsp. paracasei (8.8%), and L. coryniformis subsp. torquens (8.8%). There were also species found in the fresh cheese (E. faecalis subsp. liquefaciens, E. malodoratus, Lactococcus garvieae, L. paracasei subsp. tolerans, L. curvatus) which were not detected in the mature cheese and others that emerged as ripening progressed and were found in the 3-month-old product (Lactococcus raffinolactis, L. coryniformis subsp. torquens, L. plantarum, L. paraplantarum, and Lactobacillus bombochii).

All of the L. lactis subsp. lactis isolates from orinotyri exhibited weak abilities to reduce milk pH after 6 h. Regarding intensity of acidification, there were two clusters of isolates, the slow and the medium capacity, which resulted in pH values after 24 h of ≥5.0 and <5.0, respectively. Lactococci that ferment lactose slowly contained both β-galactosidase and phospho-β-galactosidase, while those that ferment the sugar rapidly contained only phospho-β-galactosidase. Low
### Counts (log_{10} CFU/g; mean ± standard deviation [bars]) of NSLAB in fresh and mature orinotyri (a) and their distribution (b). (a) NSLAB; (b) distribution of genera or groups of NSLAB. doi:10.1128/microbiolspec.CM-0009-2012.f7

<table>
<thead>
<tr>
<th>Time of ripening</th>
<th>Total LAB</th>
<th>Lactobacilli</th>
<th>Lactococci</th>
<th>Enterococci</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh cheese</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>60</td>
<td>17.1</td>
<td>10.7</td>
<td>5.7</td>
</tr>
<tr>
<td>60</td>
<td>60</td>
<td>8.6</td>
<td>9.7</td>
<td>5.7</td>
</tr>
<tr>
<td>100</td>
<td></td>
<td>2.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Distribution of genera or groups of NSLAB

- **Lactococci**
- **Enterococci**
- **Leuconostocs**
- **Pediococci**
- **Facultatively heterofermentative lactobacilli**
- **Obligately heterofermentative lactobacilli**
acidifying ability of lactococci from this cheese was, therefore, related to β-galactosidase production. Other oxidases produced were α-galactosidase (70.3% of the isolates) and β-glucosidase (88.9%), α-mannosidase and β-galactosidase were found rarely, and N-acetyl-β-glucosaminidase was also detected quite often (25.9% of the isolates). The isolates exhibited a weak exopeptidase system consisting of Leu-, Val-, and Cys-aminopeptidase. Lipolytic esterase-lipase activities were also detected in more than 50% of L. lactis subsp. lactis isolates, which determines their ability to contribute to cheese lipolysis. With respect to proteolytic activity in skimmed milk, it was possible to find strains with very different activities. It is well known that L. lactis subsp. lactis exhibits higher proteolytic activity in milk than L. casei or L. plantarum by cell wall-associated proteinases, and this explains its faster growth rate in milk.

Considering that L. lactis subsp. lactis was among the predominant NSLAB of this kefalotyri-type cheese, this part of the cheese microflora may produce acid, proteolyze, and degrade milk fat, thus contributing to changes during cheese manufacture and ripening.

**Ladotyri PDO**

Ladotyri is a hard cheese, produced mainly on the island of Mytilini. It is considered a high-quality type of kefalotyri. The cheese is made from ewe’s or mixtures of ewe’s and goat’s milk, raw at home or pasteurized in dairies. Ladotyri is made by different methods, but the overall technology is very similar to that used for kefalotyri manufacture. Ladotyri is preserved in olive oil (ladi, olive oil; tyri, cheese).

In cheese made from raw ewe’s milk, high microbial counts of coliforms (1.8 × 10⁵ CFU/g), yeasts (5.8 × 10⁴ CFU/g), halotolerant bacteria (1.5 × 10⁵ CFU/g), psychrotrophs (1.3 × 10⁶ CFU/g), and LAB constituted the predominant microflora (mean counts, 3.2 × 10⁸ CFU/g and 4.1 × 10⁸ CFU/g, for lactococci and lactobacilli, respectively). Enterococci predominated (58%) over lactobacilli (36.2%), lactococci, S. thermophilus, and leuconostocs. The cheese pH was over 5, and its percent moisture and NaCl contents were ~31% and ~3%, respectively (37).

**Manura PDO**

Manura is a hard traditional Greek farmhouse cheese variety manufactured by producers on the island of Sifnos in the Cyclades complex in the Aegean Sea from raw ovine or a mixture (50:50) of raw ovine and caprine milk from local herds. The cheese is made as follows: the milk is neither heat-treated nor inoculated with a starter culture. Animal rennet is used to coagulate the milk. The stomach is salted heavily (4 teaspoons of salt) and kept in a clean vessel. The rennet solution is made as follows: the liquid secreted from the stomach in the vessel (about 1 teaspoon) is used to curdle one large cup of milk. The whey obtained from this curdling is used to fill the vessel containing the stomach, and adequate salt is added. In 2 or 3 days, the whey-rennet liquid becomes peppy and is ready for use. A quantity of the liquid is used to curdle freshly obtained warm milk within an hour of milking. The coagulum is cut into pieces, the size of a nut, and after a thorough stirring, it is left to settle at the bottom of the vat. After removal of the whey, the curd is transferred to clean baskets (tyrovolia) to drain. The cheeses are salted for 2 to 3 days with coarse salt, on a different side each day, after removal from the basket and inversion. Thereafter, the cheeses are removed from the baskets and put on beds of straw to dry for 3 to 4 months; during this period, the cheeses are frequently (initially, every day) inverted to avoid sticking. The cheeses are then put into barrels with red wine to “soften” for about a week (5 to 10 days). They are thereafter transferred into barrels and covered with wine sediment for 1 day. The cheeses are then moved to empty barrels, where they are kept until sold. The cheeses thus treated have a final reddish surface. The cheese dimensions are as follows: height, ~8 cm; diameter, ~12 cm; mean weight, ~650 g (38).

Studies were conducted on manura cheese manufactured at a farm on Sifnos by a shepherd who owned a flock of around 50 sheep and produced cheese throughout the whole lactation period for local consumption. Microorganisms developed better on the cheese surface than in the interior. The wine and wine sediment appeared to have an inhibitory effect on the growth of the cheese microflora, and the microbial counts decreased more rapidly on the cheese surface and were lower than populations in the interior following removal from the wine (Fig. 8). Total NSLAB, lactococci, and lactobacilli, as well as staphylococci, micrococci, and yeasts, were found in significant numbers on the surface of the fresh cheese and cheese after drying on the straw bed for ~3 months. In the cheese interior, NSLAB dominated throughout ripening.

The pHs of the cheese surfaces were higher than those of the interiors (pH range: surface, 4.97 to 5.41; interior, 4.39 to 4.88), and the same trend was observed for the salt-in-moisture percentage (surface, 4.26 to 9.79; inte-
The decrease in microbial counts during ripening was likely due to the increase in NaCl and the decrease in moisture (to 36.4% in the mature cheese). This effect was especially evident for the microflora of the cheese interior, probably because of the more salt-resistant microflora of the surface. The more rapid decline of the microorganisms on the cheese surface during ripening in red wine is also due to the inhibitory effect of phenolic compounds (acid phenols and tannins) and ethanol of red wine.

Enterobacteriaceae and coliforms, which are indicators of cheese quality, were not detected in the final product. Micrococcaceae were present in significant numbers in the fresh cheese and cheese after drying ($10^7$ and $10^3$ CFU/g for surface and interior, respectively) and decreased significantly to low levels ($\leq 10^2$ CFU/g) at the end of the ripening. Yeast populations declined in the final product by 4.28 and 2.77 log$_{10}$ CFU/g for the cheese surface and interior, respectively. Gram-negative bacteria growing on the cheese surface increased by 0.60 log$_{10}$ CFU/g during drying and then decreased by 3.85 log$_{10}$ CFU/g. Counts in the cheese interior decreased gradually, and in the final product, they were 2.03 log$_{10}$ CFU/g lower than the initial levels.

NSLAB increased during drying on the straw bed and thereafter decreased by 4.06 and 3.63 log$_{10}$ CFU/g on the cheese surface and interior, respectively. A substantial decrease in counts of lactococci (4.78 and 2.81 log$_{10}$ CFU/g, for cheese surface and interior, respectively) and lactobacilli (5.71 and 2.29 log$_{10}$ CFU/g) was recorded after peak growth at 3 months. Enterococcal counts also fell to very low levels on the cheese surface, and their numbers were reduced by 1.77 log$_{10}$ CFU/g in the cheese interior.

**FIGURE 8** Counts (log$_{10}$ CFU/g; mean ± standard deviation [bars]) of different microbial groups at three main points of ripening of manura cheese. Gram-ve, gram negative. doi:10.1128/microbiolspec.CM-0009-2012.f8
Leuconostoc mesenteroides subsp. cremoris was isolated in significant amounts from the fresh cheeses (44.4% and 48% of the isolates from the surface and the interior, respectively) and the surface of the cheese after drying on the straw bed (41.6% of the isolates) (Table 6). In addition, Weissella paramesenteroides comprised a high proportion of the isolates from the surface (37.1%) and interior (20.6%) and was also found frequently (20.8%) on the surface of the cheese after drying. In contrast, lactococci were found at low frequencies (3.7%; *L. lactis* subsp. *cremoris*) in manura. The facultatively heterofermentative lactic bacilli, *L. paracasei* subsp. *paracasei* and *L. curvatus*, were the principal species of lactobacilli isolated from the cheese interior at increased frequencies from fresh cheese (8% for both) versus cheese after drying on the straw bed (21.8 and 17.4%, respectively), and the same was observed for *Lactobacillus hilgardii*. However, once the cheese was put into the wine and wine sediment, its lactic microflora almost disappeared, probably because of the effect of the wine constituents. Thus, new LAB species appeared after about 10 days in the wine, such as *Lactobacillus bifermentans* and *L. brevis* (55% and 31.6% of the isolates from surface and interior, respectively) and *Pediococcus pentosaceus* (40% and 42.1% of the isolates from surface and interior, respectively). These organisms may be contaminants from the wine.

Consequently, leuconostocs predominating in fresh manura cheese could contribute to the development of an open texture inside the cheese, and lactobacilli would participate, mainly, in the development of cheese flavor. Lactococci, on the other hand, appear mainly responsible for the acidification of the milk.

**Metsovone PDO**

Metsovone is a hard smoked cheese of the pasta filata type, manufactured from raw whole cow’s milk or mixtures of cow’s, sheep’s, and goat’s milk (the last not exceeding 20%) in the region of Metsovo, Epirus, from which its name is derived (1).

To raw milk, whey from the previous day (25 to 30 degrees Soxhlet-Henkel acidity) is added (2%) as a

### TABLE 6 NSLAB composition grown on cheese surface and interior of manura cheese throughout ripening

<table>
<thead>
<tr>
<th>Species</th>
<th>Fresh cheese (after salting)</th>
<th>After drying on straw bed (~3 mo)</th>
<th>After removal from wine sediment (~100 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Surface Interior</td>
<td>Surface Interior</td>
<td>Surface Interior</td>
</tr>
<tr>
<td>Lactococci</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. lactis</em> subsp. <em>cremoris</em></td>
<td>1 (3.7)</td>
<td>3 (12.5)</td>
<td></td>
</tr>
<tr>
<td>Leuconostocs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. mesenteroides</em> subsp. <em>cremoris</em></td>
<td>12 (44.4)</td>
<td>12 (54.5)</td>
<td>10 (41.6)</td>
</tr>
<tr>
<td>Weissellae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>W. confusa</em></td>
<td>1 (5.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>W. paramesenteroides</em></td>
<td>10 (37.1)</td>
<td>5 (22.7)</td>
<td>5 (20.8)</td>
</tr>
<tr>
<td><em>W. viridescens</em></td>
<td>1 (4.5)</td>
<td></td>
<td>2 (8.7)</td>
</tr>
<tr>
<td>Pediococci</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. pentosaceus</em></td>
<td>1 (3.7)</td>
<td></td>
<td>8 (40.0)</td>
</tr>
<tr>
<td><em>P. dextrinicus</em></td>
<td>2 (8.3)</td>
<td></td>
<td>8 (42.1)</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Facultatively heterofermentative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. paracasei</em> subsp. <em>paracasei</em></td>
<td>2 (7.4)</td>
<td>2 (9.1)</td>
<td>1 (4.2)</td>
</tr>
<tr>
<td><em>L. paraplanatum</em></td>
<td>1 (3.7)</td>
<td></td>
<td>5 (21.8)</td>
</tr>
<tr>
<td><em>L. coryneformis</em></td>
<td>1 (4.2)</td>
<td></td>
<td>1 (4.3)</td>
</tr>
<tr>
<td><em>L. curvatus</em></td>
<td>1 (4.2)</td>
<td></td>
<td>4 (17.4)</td>
</tr>
<tr>
<td><em>L. pentosus</em></td>
<td>1 (4.2)</td>
<td></td>
<td>1 (4.3)</td>
</tr>
<tr>
<td>Obligately heterofermentative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. bifermentans</em></td>
<td>2 (9.1)</td>
<td>1 (4.2)</td>
<td>5 (26.3)</td>
</tr>
<tr>
<td><em>L. brevis</em></td>
<td></td>
<td></td>
<td>11 (55.0)</td>
</tr>
<tr>
<td><em>L. hilgardii</em></td>
<td>2 (9.1)</td>
<td></td>
<td>6 (31.6)</td>
</tr>
<tr>
<td>Total</td>
<td>27 (100)</td>
<td>22 (100)</td>
<td>24 (100)</td>
</tr>
<tr>
<td></td>
<td>23 (100)</td>
<td></td>
<td>20 (100)</td>
</tr>
</tbody>
</table>

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Xinotyri

Xinotyri is a farmstead cheese manufactured from raw goat’s milk from indigenous breeds on the island of Naxos (Cyclades complex). Often, a small amount (~5%) of ewe’s milk is mixed with goat’s milk. The milk is coagulated in about 24 h at room temperature. Occasionally, a small amount of cheese whey from the previous day is used as a starter. The curd is transferred to cheesecloth to drain for 2 to 3 h, and then dry salt (1.5%) is added and the curd is kneaded for the uniform dispersion of salt. The curd is transferred into plastic truncated conical molds for 3 to 4 days and inverted daily. Afterward, the molds are removed and the cheese is left to ripen on wooden shelves for 30 to 45 days, depending on the season of the year. While ripening, the cheese is turned over every 1 to 2 days and, when necessary, brushed with brine to prevent the growth of molds. The cheese rind becomes wrinkled and yellowish, whereas its interior is whitish to yellowish and compact (39).

In studies of the traditional cheese processed (three cheesemaking trials) at a small creamery located in a village in the mountains of Naxos, the following microbiological changes were observed (39).

Early in ripening (6 days), the cheeses contained significantly higher populations of mesophilic LAB and lactococci than thermophilic LAB and streptococci. During the later stages of ripening, there were major declines in populations of NSLAB, significantly higher for the mesophilic versus thermophilic NSLAB populations. Enhanced survival of the thermophilic NSLAB may be interpreted by their higher tolerance to the harsh conditions of high acidity, low pH, and extensive dehydration (moisture, 17%) during cheese ripening.

Enterococci and yeasts were present at high levels (10^6 and 10^7 CFU/g, respectively) early in ripening and declined to levels as low as 10^3 CFU/g for enterococci and 10^5 CFU/g for yeasts. Enterobacteriaceae, which were 10^2 CFU/g by day 6, fell below 10 from day 22 onward, and Micrococcaceae fell by 2 log_{10} CFU/g from day 22 to 6 months from their counts (3.4 log_{10} CFU/g) at day 6. The cheese was free of Salmonella and Listeria and contained <2 log_{10} CFU/g of coagulase-positive staphylococci.

Melichloro (Melipasto)

Melichloro is the traditional cheese of the island of Limnos. The cheese is made from raw ewe’s milk at the end of the lactation season.

Fresh warm milk is coagulated within 1 h. The curd is cut into very small pieces and left to rest for 15 min. After removal of the whey, it is transferred into baskets for draining on wooden shelves. During draining, the cheese in the baskets is inverted 3 to 4 times within an hour. Ten hours after curdling, the cheese is dry salted several times on each side. Then the baskets are put in a wooden cage and hung from a tree under the sun to dry, and the baskets are inverted every day. After 4 to 5 days, the baskets are removed and the cheese wheels are left in the cage for further drying. When they are hard enough, the cheeses are washed with sea water and dried with a cloth.

The mean pH of five cheese samples was 4.49 ± 0.2, the moisture content was 31.4% ± 1.5%, and the mean brine concentration was measured at 5.8% ± 2.1%.

**WHEY CHEESES**

**Manouri PDO**

Manouri is a whey cheese that has many similarities to myzithra, but it is creamier and less salty, with a delicate texture and superior sensory quality. Manouri is a traditional product of western Macedonia in northwestern Greece, and it has long been made from the whey obtained during the production of batzos, a semihard cheese derived from caprine milk or mixtures of caprine and ovine milks. Today, manouri is also produced from the whey of cheeses made solely with ovine milk, and although it is a whey cheese, manouri is the main product of this cheesemaking process. The overall technology of manouri manufacture has much in common with the process for making myzithra, but the final moisture content (50 to 60%) is lower.

Due to the high moisture content and an initial pH above 6.0, whey cheeses are susceptible to microbial spoilage and therefore have a limited shelf life, even under refrigeration. The shelf life of commercial manouri is 30 days, a time span typical for this type of cheese.

Studies were conducted on manouri cheese made after making batzos by a shepherd at a village in western
Macedonia, northwestern Greece. Analyses of cheeses made in the spring and summer indicated the survival of heat-sensitive microorganisms, such as Enterobacteriaceae and yeasts, during the production of manouri cheese.

The initial mean log counts of yeasts in the interior of manouri cheeses were 4.65 and 2.41 in spring and summer, respectively (Fig. 9). The higher numbers in the spring are to be expected, as the airborne yeast flora tends to be highest in the spring, following the opening of leaf and flower buds.

The levels of Enterobacteriaceae, coliforms, lactococci, and enterococci found initially on the surfaces of cheeses made in the spring were 2.67, 2.30, 0.89, and 0.65 log cycles higher, respectively, than counts in the interiors of the same cheeses. The differences in numbers suggest possible contamination from the cheesecloth and growth on the cheese surfaces during draining. The surfaces of cheeses made in the summer had higher initial numbers of Enterobacteriaceae, coliforms, and yeasts than the cheese interiors by 1.57, 1.47, and 1.12 log cycles, respectively. It seems likely that the whey used to make cheeses in the summer was more heavily contaminated with Enterobacteriaceae, coliforms, and lactococci than that used earlier in the year, so that the resulting cheeses had higher counts than the spring cheeses. This whey could have resulted from milk with higher microbial numbers.

The high mean pH values of manouri cheese (6.78 to 7.33) were favorable for the growth of the different microbial groups, and their counts increased significantly during storage. In addition, the salt-in-moisture content (2.53 to 3.72%) of the cheese did not appear to be sufficient to influence their growth. Furthermore, the

![FIGURE 9 Numbers (log_{10} CFU/g; mean ± standard deviation [bars]) of the various microbial groups on the surface and the interior of manouri cheese made in spring and summer. doi:10.1128/microbiolspec.CM-0009-2012.f9](ASMscience.org/MicrobiolSpectrum)
combined effect of salt, pH, and low temperature was not effective in controlling the growth of enteric pathogens in manouri; these factors possibly acted selectively on microbiological survival and permitted the growth of salt-tolerant, psychrotrophic bacteria and yeasts. Moreover, a storage temperature of 4°C allowed longer survival of Enterobacteriaceae, and this observation is consistent with the findings for Enterobacteriaceae in manouri cheese made in spring and summer. In addition, the total aerobic counts in the interior of the cheeses made in the spring reached $10^5$ CFU/g after 5 days, and similar levels were detected initially in cheeses made in the summer. Regarding sanitary recommendations, this number is considered the cutoff point for similar food products; due to increases in acidity and alterations in sensory properties, such aerobic mesophilic counts can be responsible for failures of whey cheeses.

LAB were the major microflora in cheeses made in the spring. LAB showed little change in the cheese interior throughout storage or on the cheese surfaces until up to day 10; an increase of 0.78 log$_{10}$ CFU/g was then observed. The lactococci and enterococci increased significantly throughout storage and reached higher numbers on the cheese surfaces than in the interiors by 1.66 and 1.76 log$_{10}$ CFU/g, respectively. Even though LAB multiplied to high levels during the storage period, the pH did not seem to decline, possibly because of the presence of lactic acid-utilizing yeasts.

Higher counts of Enterobacteriaceae and coliforms were measured throughout storage at 4°C in cheeses made in the summer than in the spring cheeses. Moreover, microorganisms developed better on the surfaces of cheeses made in the summer than on cheeses made in the spring, and differences in log counts between final and initial numbers were greater by 0.57, 1.70, 1.72, and 0.38 log$_{10}$ CFU/g for Enterobacteriaceae, coliforms, yeasts, and staphylococci, respectively. The same pattern was observed for counts of LAB, lactococci, and enterococci. These observations point to a higher initial contamination of cheeses made in summer by psychrotrophic bacteria and yeasts.

At around day 20 for the spring cheeses and day 15 for the summer varieties, visible colonies of yeasts and bacteria started appearing on the surfaces of the cheeses. Enterobacteriaceae obtained from cheeses stored for 20 days at 4°C were characterized, and Hafnia was the dominant organism (68.75% of the total). This finding supports the observation that psychrotrophic microorganisms are among the main contaminants of the cheese and that they multiply during refrigerated storage. A range of yeasts, representing eight different species, were observed in manouri cheese at 20 days, with Debaryomyces hansenii, Pichia membranifaciens, and Pichia farinosa being the most frequently isolated species (25.0%, 32.1%, and 14.3%, of the isolates, respectively). Gram-positive, catalase-positive cocci were characterized mainly as staphylococci.

The isolates of Enterobacteriaceae, staphylococci, and, to a lesser extent, yeasts were proteolytic, but the free amino acid (N-NH$_2$) content of the cheese did not increase significantly during storage (136.4 to 225.2 mg/kg in glycine equivalent). The milk fat was not degraded to any great extent (acid degree value, 0.09 to 0.19) by the lipolytic activity of bacteria and yeasts. The main enzymes detected in selected isolates of Enterobacteriaceae were leucine, aminopeptidase, and phosphohydrolase.

It appears that the shelf life of the product is determined by the surface microbial growth and not by deteriorations caused by their enzyme activities.

**Xinomizithra PDO**

Xinomizithra is a type of myzithra cheese made from the whey obtained during the production of kefalotyri and graviera from sheep’s and goat’s milk. It is a traditional product of Crete. For the production of xinomizithra, myzithra is first manufactured, which is cooked and drained more severely, and it is then pressed for 1 week. During this time, it also turns sour (xino means sour). Salt is added, and the cheese is mixed. Then it is placed in barrels, which are transferred to ripen at 5 to 10°C for a period of 2 months.

The mean compositional characteristics of xinomizithra samples were as follows: pH, 4.75 (range, 4.40 to 5.50); moisture, 38.6% (range, 33 to 41.2%); NaCl, 1.86% (range, 1.45 to 2.35%); fat in dry matter, 30 to 34% (range, 26.86 to 34.88%). One sample was contaminated by Enterobacteriaceae and coliforms ($10^6$ CFU/g), which also contained staphylococci ($10^2$ CFU/g). Lactococci (mean, 2.9 × $10^8$ CFU/g) ranged from 2.2 × $10^6$ to 1.4 × $10^9$ CFU/g, lactobacilli (mean, 6.2 × $10^7$ CFU/g) were counted at levels ranging from 3.2 × $10^5$ to 1.0 × $10^8$ CFU/g, and enterococcal populations were high (mean, 4.3 × $10^6$ CFU/g; range, 1.1 × $10^6$ to 2.2 × $10^7$ CFU/g). Mean plate counts of yeasts were 2.5 × $10^7$ CFU/g (range, 37.4 × $10^2$ to 5.6 × $10^7$ CFU/g). These high counts indicate high contamination from the environment but also favorable conditions in the cheese for yeast growth (unpublished data).
**Xinotyri (Klotsotyri, Giza, Artymi, or Prentza)**

Xinotyri (xyno, sour; tyri, cheese) is the cheese type that is made from the whey obtained during the production of butter. It has a pleasantly sour and salty taste. During production, whey is placed in a copper vat, where it is heated until curdled. The curd is left to cool and then drained through cheesecloth for several hours. The drained cheese is cut in pieces or, more commonly, rubbed and salted, and then it is put in either barrels, skin bags, or tins for 2 or 3 months to ripen (2).

The cheese pH is <5.0 (mean, 4.57; range, 4.4 to 4.74), the mean moisture percentage is 40.4% (range, 37.1 to 43.7%), and the mean NaCl percentage is ~1.4%. The mean percentage of fat in dry matter is 14.8%. Coliforms and *Enterobacteriaceae* were not detected in the cheese, and LAB were found at quite low levels (mean counts: LAB, 1.1 × 10^4 CFU/g; lactococci, 4.0 × 10^2 CFU/g; lactobacilli, 5.5 × 10^3 CFU/g; enterococci, <10 CFU/g). Staphylococci were absent, and yeasts were detected in one cheese sample at 2.6 × 10^3 CFU/g (unpublished data).

**OTHER TRADITIONAL GREEK CHEESES**

Armigiotyri is produced throughout the Greek islands, except Crete. It is made from raw goat’s milk by shepherds at farms with the technology used to produce kefalotyri. Chlorotyri is a semisoft cooked cheese made throughout Greece from all milk types. It is a fresh cheese kept in brine until consumption. Kathoura is a cheese made mainly from goat’s milk by shepherds on farms on the island of Ikaria. The curd is gently cooked, and the cheese is either consumed after draining or cut into thick slices, salted, and kept in barrels or tins with brine.

**REFERENCES**


