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Evaluation of microbial diversity during the manufacture of Fior di Latte di Agerola, a traditional raw milk pasta-filata cheese of the Naples area

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Microbial diversity of the raw milk for the production of Fior di Latte di Agerola and its changes during cheesemaking were studied. Viable counts showed that at the end of curd ripening, loads of lactic acid bacteria, both mesophilic and thermophilic rods and cocci, higher than those commonly evidenced in similar cheeses produced by using natural or commercial starters, were detected. Identification of 272 isolates, supported by molecular diagnostic aids, evidenced representative cultures of a high number of bacterial taxa of interest as participating in the process, although most of the isolates belonged to *Lactococcus lactis* and *Lactobacillus helveticus* species. RAPD-PCR and REA-PFGE biotyping were performed for the isolates of the above species and it was shown that most of the strains isolated from the raw milk occurred during the whole cheesemaking process, and an active role of these strains in the fermentation was supposed. The results offer further proof of the importance of the raw milk as source of technologically interesting strains of lactic acid bacteria capable of driving the fermentation of traditional cheeses.

Keywords: Microbial diversity, raw milk cheese, *Lactobacillus helveticus*, *Lactococcus lactis*, Fior di Latte di Agerola.

Fior di Latte is a high-moisture Mozzarella cheese produced from cows' milk throughout the Southern regions of Italy and largely consumed as fresh cheese within one or two days after manufacture. A production of 220 400 tonnes was estimated for the year 2004 by Databank (www.databank.it). This type of cheese belongs to the historical heritage of the dairy tradition of Southern Italy; nevertheless, its increasing consumption during recent decades promoted the differentiation of many manufacturing processes, mainly depending on the size of the cheese factories. In fact large amounts are industrially produced from pasteurized cows' milk, often also imported, and commercial starters, usually containing *Streptococcus thermophilus* or mixtures of this microorganism with thermobacteria. More traditionally, natural whey or milk cultures are used to inoculate raw milk in the cheese vat. Finally, in many other cases, strictly local milk arising from the evening milking is refrigerated and stored for 8–10 h, then used for cheesemaking without starter addition in mixture with the milk from the morning milking. Differences occur also as far as curd ripening before the stretching in hot water and moulding: in the fastest industrial processes, the curd ripens in the presence of its whey in the cheese vat, at 35–37 °C; in traditional processing, the lactic fermentation is slower, as the curd, after cutting and whey draining, ripens at environmental temperature for 8–12 h according to the season, to reach a pH value of about 5·0.

Fior di Latte di Agerola represents the most celebrated product traditionally obtained through the activity of only the native milk microflora. It is produced by some hundreds of small dairy factories situated around the small town of Agerola as well as throughout the mountains of Sorrento Peninsula, called *Lactares Montes* by the ancient Romans. Its fame is surely due also to the fact that for a long time its production arose from the high-quality and fat-rich milk of a local breed (ecotype) named Agerolese, descended from the mating of autochthonous cattle with imported Jersey cattle and no longer existing because of its low yield.

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Table 1. Flow sheet of the cheesemaking process for Fior di Latte di Agerola, and the sampling points

Processing phases	Samples
Milk delivery to the cheese factory (evening)	
Cleaning by centrifugation	
Storage tank	Raw milk at arrival (M_0)
Refrigeration at 4–7 °C	
Storage in insulated tank for 8–10 h	
Transfer into the cheese vat together with the milk delivered in the morning	Raw milk in the cheese vat (M_F)
Heating at 35–37 °C	
Rennet addition	
Clotting within 20–30 min	
Curd cutting to hazelnut size within 30–40 min	
Whey draining	Curd before ripening (C_0)
Transfer of the curd on draining table	
Curd ripening at room temperature for 10–12 h	Curd after ripening (C_F)
Curd milling	
Stretching in water at 80–90 °C	
Moulding in pieces of about 400 g	
Hardening in cold water	
Salting in brine at 18°Bé for 5 min	Final product† (P_F)
Packaging in plastic or paper bag	
Immediate dispatching in refrigerated car at 4 °C	

† The curd is immediately stretched after the ripening and the final product is collected after 15 min from the collection of C_F

Moreover, in spite of recent studies on microbiota occurring in natural starters and during cheesemaking of other types of Mozzarella cheese (Coppola et al. 1985; Coppola et al. 1988; Parente et al. 1997; Morea et al. 1998; Morea et al. 1999) the microflora responsible for manufacturing processes of unripened pasta filata cheeses carried out without any starter addition is still unknown. The typical quality of such a cheese, surely depending also on the activities of a complex and fortuitous microflora, may be threatened by more and more stringent hygienic practices, since specific knowledge about microbial species and strains necessary to the achievement of the best results is lacking. This work was therefore carried out with the aim of evaluating and describing, by both conventional and molecular methods, microbial diversity and its variation during the whole process of making Fior di Latte di Agerola at one of the oldest cheese factories of the above area, producing this product since 1840.

Material and Methods

Sampling

Investigations were carried out in three production batches in springtime in order to avoid extreme weather. Milk delivered at the dairy factory (M_0) and after storage, immediately before cheesemaking (M_F), curd before (C_0) and after ripening (C_F), and the Fior di Latte (P_F) were sampled in sterile plastic containers and stored on ice in an isothermal bag prior to analysis. Sampling points and the cheesemaking process are summarized in Table 1.

Microbial counts, isolation and maintenance of bacterial isolates

Samples (10 g) were homogenized with 90 ml of sterile quarter-strength Ringer's solution in a Stomacher 400 (Lab Blender, Seward Medical, London, UK) serially diluted in the same diluent, and inoculated onto plates of M17 Agar (Oxoid, Garbagnate Milanese, Italy) supplemented with lactose at 10 g/l (LM17) for cocci, Rogosa Agar (Oxoid) for lactobacilli, Slanetz & Bartley Agar (Oxoid) for enterococci, LUSM Agar (Benkorroum et al. 1993) for leuconostocs, DRBC Agar with chloramphenicol selective supplement (Oxoid) for yeasts, Violet Red Bile Glucose (VRBG) Agar (Oxoid) for *Enterobacteriaceae*, and Baird Parker RPF (BP+RPF) Agar (Oxoid) for coagulase-positive staphylococci. Incubations were carried out according to manufacturers' recommendations. Randomly selected isolates were purified by streaking on the same medium and maintained at 4 °C as stab work cultures in agar containing $CaCO_3$ as neutralizing agent: by using Yeast Lactose Agar (Harrigan & McConce, 1976) for streptococci, lactococci and enterococci, and MRS Agar (Oxoid) for leuconostocs and lactobacilli.

Phenotypic identification

Phenotypic identification of lactococci, enterococci and thermophilic streptococci was carried out through physiological and biochemical techniques according to Teuber et al. (1991), Devriese & Pot (1995) and Schleifer et al. (1991), respectively. Lactobacilli and leuconostocs were identified according to Hammes et al. (1991) and Villani et al. (1997), respectively. Molecular validation of

Table 2. Reference strains used in the study

Species	Strain†
Enterococcus faecalis	ATCC 19433^T
Enterococcus faecium	ATCC 19434^T
Lactococcus lactis subsp. *lactis*	DSM 20481^T
Lactococcus garvieae	DSM 20684^T
Lactococcus raffinolactis	DSM 20443^T
Leuconostoc mesenteroides subsp. *mesenteroides*	DSM 20343^T
Streptococcus thermophilus	CNRZ 302
Streptococcus suis	SAP77 (Blaiotta et al. 2002)
Streptococcus parauberis	SAP99 (Blaiotta et al. 2002)
Lactobacillus amylovorus	DSM 20531^T
Lactobacillus gallinarum	DSM 10532^T
Lactobacillus crispatus	DSM 20584^T
Lactobacillus acidophilus	DSM 20079^T
Lactobacillus helveticus	DSM 20075^T

†DSM: Deutsche Sammlung von Mikroorganismen und Zelkulturen, Braunschweig, Germany; CNRZ: Centre National de Recherches Zootechniques, Jouy-en-Josas, France; ATCC: American Type Culture Collection, Rockville, Maryland, USA
^{T}Type strain

identification and strain typing were performed as described below. The reference strains listed in Table 2 were used as markers when required.

Molecular analyses

DNA extraction from bacterial cultures. Preparation of crude cell extracts was carried out as previously reported (Moschetti et al. 1998). One µl (about 25 ng) of the mixture was used directly as template for PCR amplification.

PCR Assays. Oligonucleotide primers designed by Jensen et al. (1993) were used to amplify the 16S-23S rRNA intergenic spacer region from the conserved regions at the 3′ end of the 16S rRNA and the 5′ end of the 23S rRNA genes. PCR reactions were carried out as previously reported (Blaiotta et al. 2002). Specific detection of *Leuconostoc mesenteroides* subsp. *mesenteroides* strains was performed as previously described by using the specific primers LMMf and LMMr (Moschetti et al. 2000). A multiplex PCR assay described by Fortina et al. (2001) was employed for the specific identification of *Lactobacillus helveticus* isolates. RAPD-PCR typing was performed by using the primer Primm 239 (CTGAAGCGGA) and PCR conditions previously described by Moschetti et al. (1998). PCR products were separated by agarose (2% w/v) gel electrophoresis at 7 V cm^{-1} for 2 h. RAPD-PCR products were run for 3·5 h.

Restriction endonucleases analysis – Pulsed field gel electrophoresis (REA-PFGE). Intact genomic DNA was isolated as described by Moschetti et al. (1997). DNA inserts were digested in 200 µl of appropriate buffer supplemented with 40 U of *Sma*I (Promega). Electrophoresis of the restriction digests was performed by using the CHEF system (Bio-Rad Laboratories) with 1% (w/v) agarose gels and 0·5 × TBE as running buffer, at 10 °C. Restriction fragments were resolved in a single run, at constant voltage of 6 V cm^2 and an orientation angle of 120° between electric fields, by a single-phase procedure for 28 h with a pulse ramping between 1 and 30 s.

Electrophoretic pattern analysis. Bands were automatically detected by using the software Phoretic 1 advanced version 3.01 (Phoretix International Limited, Newcastle upon Tyne, UK). Cluster analysis was performed by the above software after band matching; the method described by Saitou & Nei (1987) was used to obtain the correlation matrix.

Direct isolation of DNA from dairy samples. DNA extraction from dairy samples was carried out as described by Coppola et al. (2001). In brief, 1 ml or 1 g of sample was frozen at −30 °C for 2 h. Then 600 µl of freshly prepared 0·4 M-NaOH plus 300 µl of trisodium citrate dihydrate (40% w/v; Wash Buffer A) were added to the defrozen sample. After shaking, the sample was incubated for 15 min at room temperature and then centrifuged at 25 400 ***g*** for 4 min. The pellet was resuspended twice in 1 ml of 5 × SSC and 150 µl of trisodium citrate dihydrate (40% w/v; Wash Buffer B) and then incubated for 10 min at room temperature. After centrifugation (25 400 ***g*** for 4 min) the pellet was purified with a synthetic resin (Instagene Bio-Rad Matrix, Bio-Rad Laboratories, Richmond CA, USA), according to the supplier's instructions. The resulting purified DNA was stored at −30 °C until use. One µl (about 25 ng) of the mixture was used directly as template for PCR amplification.

Polymerase chain reaction – Denaturing gradient gel electrophoresis (PCR-DGGE). The variable V3 region of the 16S rDNA was amplified by primers and conditions described by Ercolini et al. (2002). PCR products were analysed by DGGE by using a Dcode apparatus (Bio-Rad Labs, Hercules CA, USA). Samples were applied to 8% (w/v) polyacrylamide gels in 1 × TAE buffer. Parallel electrophoresis experiments were performed at 60 °C as previously described (Ercolini et al. 2002). Gels were electrophoresed for 10 min at 50 V and for 3·5 h at 200 V, stained with ethidium bromide for 5 min and rinsed for 20 min in distilled water.

Results

The microbial contents of the various samples analysed are reported in Table 3. The highest viable counts were detected at the end of the ripening for most of the microbial groups. The stretching procedure affected the

Table 3. Microbial counts (log CFU/ml or /g) on various media during cheesemaking of Fior di Latte di Agerola

Media (Incubation conditions)	Samples†				
	M_0	M_F	C_0	C_F	P_F
LM 17 Agar (30 °C; 48 h)	6·398±043	7·149±0·18	7·704±0·33	9·146±0·70	8·02±0·2
LM 17 Agar (42 °C; 48 h)	6·125±0·33	7·316±0·17	8·001±0·19	7·716±0·20	8·14±0·2
Rogosa Agar (30 °C; 48 h)	5·079±0·45	5·279±0·16	5·658±0·22	7·267±0·14	6·45±0·22
Rogosa Agar (42 °C; 48 h)	4·544±0·26	4·756±0·16	4·903±0·12	6·695±0·26	6·49±0·18
Slanetz & Bartley Agar (37 °C; 48 h)	6·863±0·16	6·097±0·33	7·234±0·20	7·699±0·11	7·28±0·02
LUSM Agar (30 °C; 72 h)	4·571±0·33	5·092±0·12	4·949±0·14	6·681±0·98	ND
DRBC Agar (30 °C; 5 d)	4·217±0·61	4·279±0·45	4·881±0·17	5·568±0·09	ND
VRBG Agar (30 °C; 48 h)	3·69±0·15	3·43±0·11	5·10±0·04	6·61±0·16	1·698±0
BP+RPF Agar (37 °C; 24 h)	2·954±0	4·30±0	5·30±0	5·00±0	ND

† Samples were collected during cheesemaking of Fior di Latte: M_0, raw whole cows' milk; M_f milk before cheesemaking; C_0, curd before ripening; C_F, curd after ripening; P_F, Fior di Latte cheese
ND not detectable

lactic acid bacteria (LAB) loads causing a decrease of about 1–2 log depending on the cases and could eliminate the undesired microorganisms (Table 3). Loads of cocci as determined on LM17 were higher than the loads of rods determined on Rogosa both in the milk at the beginning and throughout the whole cheesemaking process. Leuconostocs counted on LUSM were shown to be particularly sensitive to the thermal treatment occurring during the stretching step.

Colonies from countable plates of LM17 Agar, Rogosa Agar, LUSM and Slanetz & Bartley agar were randomly isolated, purified and identified. A total of 165 cocci and 107 rods were isolated. By means of phenotypic identification based on biochemical assays, three species of *Lactococcus*, four of *Enterococcus*, three of *Streptococcus*, two of *Leuconostoc*, one of *Weissella* and twelve of *Lactobacillus* were identified (Table 4). *Lactococcus lactis* among cocci and *Lactobacillus acidophilus* (group) among rods were the dominant species according to the biochemical identification.

For a number of isolates (42 enterococci, 37 streptococci, 8 leuconostocs, 69 lactococci and 53 lactobacilli) molecular methods were applied in order to achieve unequivocal identification. As summarized in Table 4, 24 presumptive *Enterococcus faecalis* were confirmed by 16S-23S spacer analysis as well two *Enterococcus faecium*. Four isolates identified as *Enterococcus solitarius* and 12 unidentified *Enterococcus* spp. were shown to be *Enterococcus faecalis* by the same method.

The 16S-23S spacer analysis was performed to identify 37 streptococci of which 34 were shown to be *Streptococcus thermophilus*, two *Streptococcus suis* and one *Streptococcus parauberis* (Table 4). Eight *Leuconostoc mesenteroides* subsp. *mesenteroides* were analysed by a specific amplification as described in Materials and Methods; all the strains were shown to give positive response after the amplification thus confirming the phenotypic identification.

Spacer analysis was further applied and gave confirmation of the phenotypic identification of 50 *Lactococcus lactis* and 11 *Lactococcus raffinolactis* strains. By contrast, three isolates identified as *Lactococcus plantarum* and five isolates identified as *Lactococcus lactis* were shown to be *Lactococcus lactis* and *Lactococcus garvieae*, respectively (Table 4). The 53 isolates identified as belonging to the *Lactobacillus acidophilus* group were shown to be *Lactobacillus helveticus* by means of species-specific PCR (Fortina et al. 2001).

To monitor the occurrence of dominant biotypes during the cheese production, PCR-RAPD assays were performed on the 53 *Lactococcus lactis* and the 55 *Lactobacillus helveticus* isolates. We chose one representative species among rods and one among cocci for the biotyping. As shown in the dendrogram in Fig. 1, 21 RAPD profiles were distinguished among *Lactococcus* isolates displaying a high degree of DNA polymorphism. Notably RAPD profile C grouped 7 isolates (probably 7 isolates of the same strain) that occurred during the whole cheesemaking process. Besides, strains characterized by profiles H and E were isolated from milk to the ripened curd. RAPD PCR of *Lactobacillus* isolates yielded only four clusters (results not shown). For a more appropriate biotyping of these last strains *Sma* I-REA-PFGE analysis was performed. In Fig. 2, the dendrogram of similarity retrieved from the REA-PFGE analysis of *Lactobacillus helveticus* strains (55) is reported. Thirteen profiles could be distinguished at 95% similarity; most of the strains showed patterns C, H and I with 12, 12 and 9 strains respectively. Moreover, a lower but significant number of strains were characterized by patterns M and G. All the above groups of strains occurred from the beginning up to the end of the ripening of the curd of Fior di Latte cheese.

The dynamics of the microbial population was monitored during the manufacture by analysing the DNA directly extracted from dairy samples by PCR-DGGE. DGGE profiles of the amplified V3 region of the different

Table 4. Occurrence of different species of lactic acid bacteria during cheesemaking of Fior di Latte di Agerola

Bacterial species (phenotype)	Bacterial species (genotype) Taxon	Method†	Samples‡ M_0	M_F	C_0	C_F	P_F	Total
Lactococcus lactis	*Lactococcus garvieae*	A	1 (−5)§	1 (−5)	3 (−6)			5
Lactococcus lactis subsp. *lactis*	*Lactococcus lactis subsp. lactis*	A	9 (−5)	12 (−7)	11 (−7)	13 (−8)	5 (−7)	50
Lactococcus plantarum	*Lactococcus lactis subsp. lactis*	A	1 (−5)		1 (−6)	1 (−7)		3
Lactococcus raffinolactis	*Lactococcus raffinolactis*	A		8 (−6)	2 (−6)	1 (−8)		11
Enterococcus faecalis	*Enterococcus faecalis*	A	4 (−4)	3 (−5)	8 (−5)	9 (−5)		24
Enterococcus faecium	*Enterococcus faecium*	A	2 (−4)					2
Enterococcus solitarius	*Enterococcus faecalis*	A	1 (−4)	3 (−4)				4
Enterococcus spp.	*Enterococcus faecalis*	A		1 (−6)	4 (−6)	5 (−7)	2 (−7)	12
Streptococcus thermophilus	*Streptococcus thermophilus*	A		4 (−7)	5 (−7)	8 (−7)	17 (−6)	34
Streptococcus spp.	*Streptococcus parauberis*	A	1 (−5)					1
Streptococcus spp.	*Streptococcus suis*	A		1 (−6)	1 (−5)			2
Leuconostoc gelidum						1 (−4)		1
Leuconostoc mesenteroides subsp. *mesenteroides*	*Leuconostoc mesenteroides* subsp. *mesenteroides.*	B		4 (−5)	2 (−4)	2 (−5)		8
Weissella paramesenteroides				1 (−3)	2 (−4)	5 (−5)		8
Lactobacillus acidophilus (group)	*Lactobacillus helveticus*	C	6 (−3)	21 (−4)	13 (−5)	13 (−5)		53
Lactobacillus helveticus	*Lactobacillus helveticus*	C	1 (−4)		1 (−5)			2
Lactobacillus casei subsp. *casei*			1 (−3)		2 (−5)			3
Lactobacillus paracasei			1 (−4)					1
Lactobacillus paracasei subsp. *paracasei*			1 (−4)	2 (−4)				3
Lactobacillus curvatus			1 (−3)		1 (−5)			2
Lactobacillus sakei			8 (−3)	6 (−3)	1 (−5)			15
Lactobacillus delbrueckii subsp. *bulgaricus*			1 (−4)		1 (−5)			2
Lactobacillus graminis			3 (−3)		1 (−5)			4
Lactobacillus homohiochii			1 (−3)	1 (−4)	1 (−4)			3
Lactobacillus maltaromicus			1 (−4)	11 (−4)				12
Lactobacillus pentosus			1 (−4)					1
Lactobacillus plantarum			1 (−3)		1 (−4)	4 (−6)		6

† A: PCR 16S-23S rDNA Spacer analysis; B: Specific amplification for *Leuconostoc mesenteroides* subsp. *mesenteroides* (Moschetti et al. 2000) C: Specific PCR amplification of *Lactobacillus helveticus* (Fortina et al. 2001). When genotype and molecular methods are not indicated the phenotypic identification had given results consistent with the cited diagnostic procedures

‡ Samples were collected during cheesemaking of Fior di Latte: M_0, raw whole cows' milk; M_f milk before cheesemaking; C_0, curd before ripening; C_F, curd after ripening; P_F, Fior di Latte cheese;

§ Number of isolates from each sample with isolation dilution in parenthesis

samples are depicted in Fig. 3. Seven bands were detected in M_0, nine bands were recovered in C_0, while six and two bands were distinguished in C_F and P_F, respectively.

Discussion

Fior di Latte di Agerola cheese is an unripened pasta filata cheese made from raw cows' milk according to a very traditional process. Producers firmly maintain their product to be distinct from other similar cheeses that are produced with raw milk but with the addition of a milk culture of lactic acid bacteria as a starter. In the case of the cheese here considered, only the native microflora are involved. The present study was aimed at defining, by both conventional and molecular methods, the microbial populations occurring during the whole production process.

The raw milk used is strictly local, often produced by small farms and delivered to the cheese factory twice a day. Its microbiological quality resembles that of a typical milk for cheesemaking, with some millions of acidifying bacteria, and a minor occurrence of yeasts, *Enterobacteriaceae* and staphylococci. The storage of such a milk at low temperature for 8–10 h is a well-consolidated procedure, also practised in southern Italy within manufacture of hard and long-ripened pasta filata cheeses such as Caciocavallo or Provolone. The storage practice involves a further growth of lactic streptococci but without great variation of the whole structure of the microbial

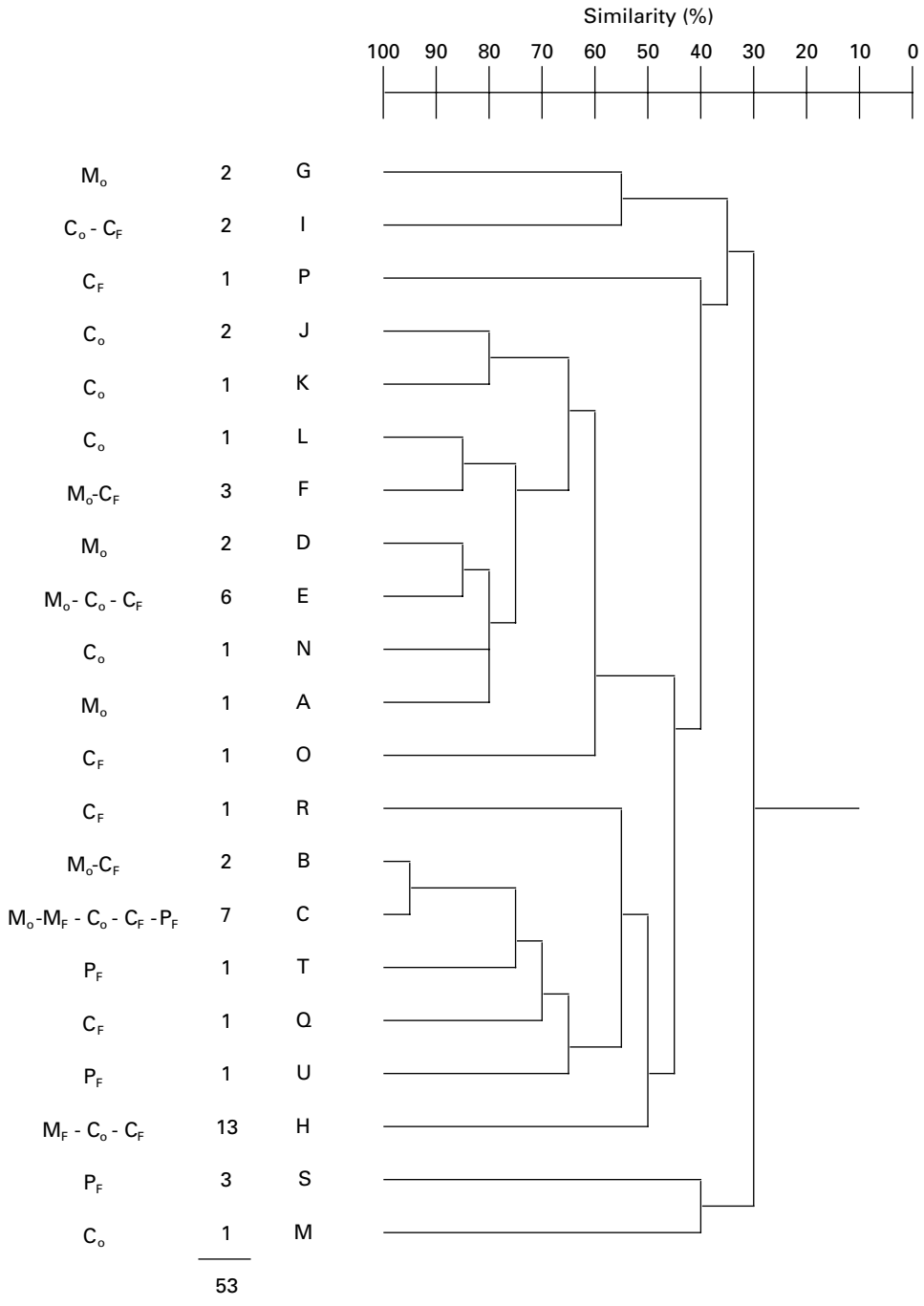

Fig. 1. Dendrogram of similarity showing the relationship between RAPD-PCR patterns of *Lactococcus lactis* strains.

population and with an insignificant decrease of milk pH from 6·7 to around 6·6 (results not shown). With the subsequent phases of the cheesemaking process, and after whey draining, a fresh curd with a higher microbial content is obtained. Curd ripening occurs on stainless steel draining tables at room temperature, but starting with a curd at almost the vat temperature (around 32–33 °C). The process takes 10–12 h, depending on the season. It is stopped when curd pH reaches values as low as 5·0–5·1, corresponding to the suitability of the curd to be stretched in hot water. At this point the curd microbial content is at its highest.

In the present work viable counts of both mesophilic and thermophilic bacteria, arising only from the native milk microflora, were higher than the loads reported by others who analysed samples during the manufacture of

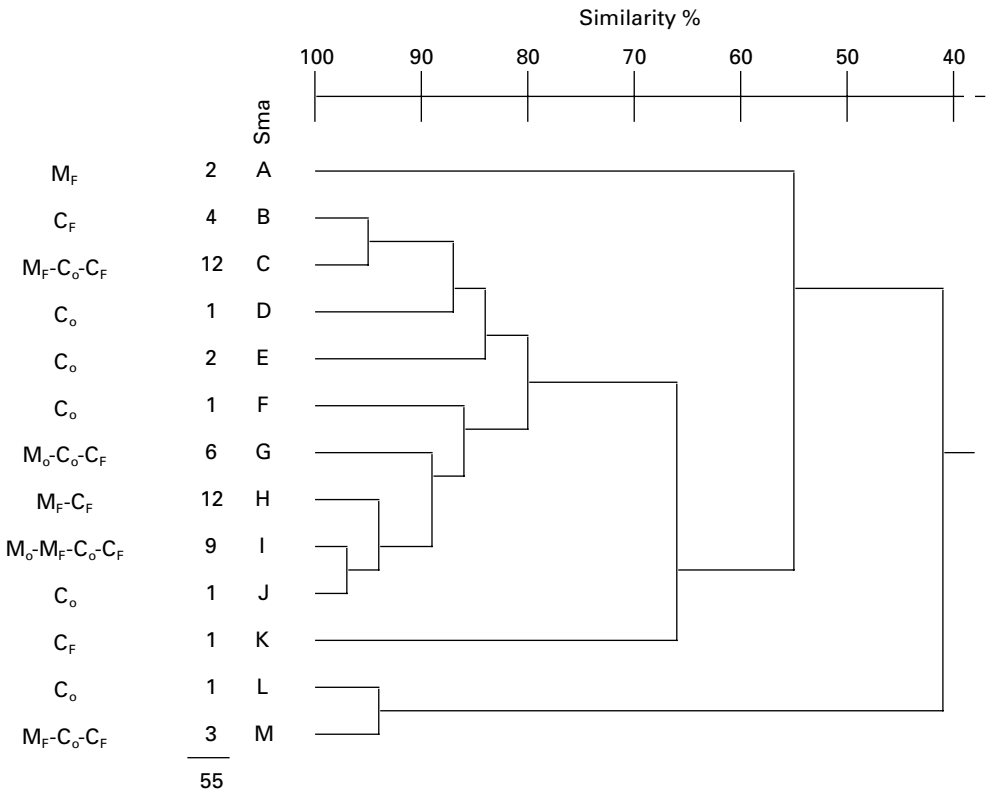

Fig. 2. Dendrogram of similarity showing the relationship between *SmaI* REA-PFGE patterns of *Lactobacillus helveticus* strains.

Mozzarella cheese from cows' milk using natural whey cultures as starter (Morea et al. 1998; Morea et al. 1999). On the other hand, the results were similar to those detected in the traditional manufacture of water buffalo Mozzarella cheese by Ercolini et al. (2004) even though, in this last case, lower counts of thermophilic cocci were reported. Moreover, during the production of Fior di Latte di Agerola, a higher number of different LAB taxa could be evidenced as participating in the cheesemaking process in comparison with the above mentioned dairy manufactures. In the Fior di Latte di Agerola cheesemaking, as shown in this work, the dominant LAB species contributing to the processing are the mesophilic *Lactococcus lactis* and the thermophilic *Lactobacillus helveticus*. This last species was the dominant member of the genus *Lactobacillus* in natural starter cultures for other pasta filata cheeses (Parente et al. 1997) and as part of the microbiota of dairy samples during the manufacture of Mozzarella cheese (Morea et al. 1998). In the present study a significant number of isolates of *Lactobacillus helveticus* were molecularly characterized by REA-PFGE and it was shown that most of the strains belonged to only 3 (profiles C, H and I) out of 13 clusters. However, other biotypes (profiles G and M) occurred in the milk up to the ripened curd. These biotypes occurred in the raw milk and were shown actually to carry out the fermentation, as they were also isolated from the curd at the end of ripening. Other species of *Lactobacillus* (Table 4) were found but only at low concentration and only in the raw milk; therefore, they were not isolated from the ripened curd or Fior di Latte where, if present, they would occur at significant concentration.

The presence of *Lactococcus lactis* in cheese manufacture without starter addition is interesting as this species is recognized as the most important of the commercially used LAB (Stiles & Holzapfel, 1997). The technological properties of the isolates gathered in this study were not investigated but all the identified species can play a role in acidification and curd ripening. Biotyping of *Lactococcus lactis* by RAPD-PCR led to 21 profiles, indicating a very high degree of biodiversity within this species. Remarkably, strains characterized by profiles C, E and H constituted the majority of the isolates and were supposed to play a significant role in the fermentation, as they were isolated in all the samples from raw milk to curd after ripening.

Biotyping of both *Lactobacillus helveticus* and *Lactococcus lactis* underlined the importance of the raw milk as a source of important bacteria for the fermentation; in fact, the biotypes isolated from the curd after ripening arose, most of the times, from the raw milk.

PCR-DGGE analysis of the dairy samples was carried out in order to check the influence of the technological phases of the manufacture upon the overall microbiota.

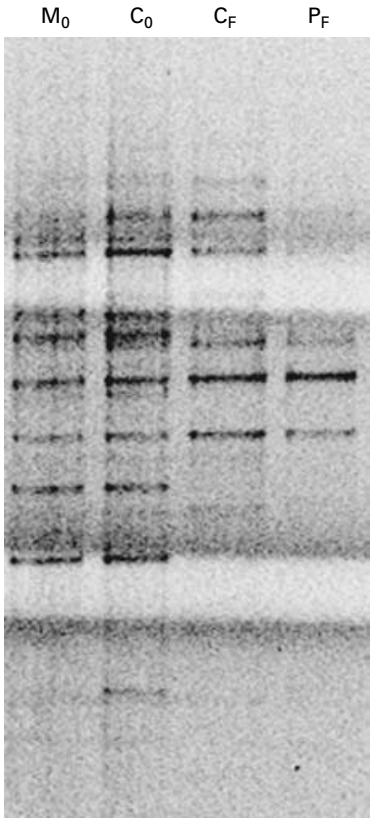

Fig. 3. PCR-DGGE profiles of (M_0) raw milk, (C_0) curd before and (C_F) after ripening, and (P_F) final ''Fior di Latte di Agerola'' cheese.

This is one of the simplest applications of this technique in food microbiology (Ercolini, 2004) and in this study it could show that the complex microbiota of the raw milk, composed of possibly seven different species was reduced by the curd ripening and stretching procedure leading to a Fior di Latte where only few species were detectable. However, as already observed in other studies (Ercolini et al. 2001, Ercolini, 2004) the level of microbial species diversity directly detectable by DGGE can underestimate the number of species (possibly cultivable on media) actually occurring in that particular sample.

This study of the manufacture of Fior di Latte di Agerola cheese highlighted a significant number of species and biotypes represented by more than ten different taxa that are related to different metabolic traits and which probably lead to the appreciated flavour and taste. Therefore, the raw milk has been once more shown as source of technologically interesting strains of LAB capable of driving the fermentation of traditional cheeses.

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