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Original article

Microbiological characteristics of Parmigiano Reggiano cheese during the cheesemaking and the first months of the ripening

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Abstract — The aim of this work was to gain a comprehensive view of the microbiological characteristics of milk, natural whey starter, and cheese during the first months of ageing of Parmigiano Reggiano. A significant presence of different microbial groups in raw milk from the initial moments of production was rapidly substituted by various lactic acid bacteria. Natural whey starter contained a large number of thermophilic lactic acid bacteria (*Lactobacillus helveticus*, *L. delbrueckii* ssp. *lactis*, *L. delbrueckii* ssp. *bulgaricus*) and some facultatively heterofermentative lactic acid bacteria belonging to *L. rhamnosus*. Thermophilic lactic acid bacteria disappeared within 30 d. Rod-shaped mesophilic facultatively heterofermentative lactic acid microflora, consisting of *L. casei*, *L. paracasei* ssp. *paracasei*, *L. paracasei* ssp. *tolerans*, *L. rhamnosus* and pediococci, progressively increased up to the fifth month of ageing. Results showed that thermophilic lactobacilli were derived from natural whey starter whereas *Streptococcus thermophilus* originated from raw milk. Further, natural whey starter was the source of *L. rhamnosus* which was present throughout the entire period of the cheese ageing. The other components of non-starter lactic acid microflora derived from raw milk.

Parmigiano Reggiano / raw milk cheese / microbiology / ripening

Résumé — **Caractéristiques microbiologiques du fromage Parmigiano Reggiano dans la phase de fabrication et durant les premiers mois d'affinage.** Cet article a pour objectif de fournir une

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description complète des caractéristiques microbiologiques du lait, du lactosérum et du fromage Parmigiano Reggiano dans les premiers mois d'affinage. La présence significative de groupes microbiologiques différents dans le lait dès les premiers instants du processus de fabrication était rapidement remplacée par la microflore lactique thermophile. Les échantillons de lactosérum naturel contenaient un grand nombre de bactéries lactiques thermophiles (*Lactobacillus helveticus*, *L. delbrueckii* ssp. *lactis*, *L. delbrueckii* ssp. *bulgaricus*) et quelques bactéries lactiques hétérofermentaires facultatives appartenant à l'espèce *L. rhamnosus*. Les bactéries lactiques thermophiles ont disparu en l'espace de presque 30 jours pendant que la microflore lactique mésophile hétérofermentaire facultative en bâtonnets, composée de *L. casei*, *L. paracasei* ssp. *paracasei*, *L. paracasei* ssp. *tolerans*, *L. rhamnosus* et de pédiocoques, a augmenté progressivement jusqu'au 5^{e} mois d'affinage. *Streptococcus thermophilus*, la microflore lactique mésophile hétérofermentaire facultative, excepté *L. rhamnosus*, et les autres bactéries que nous avons isolées dans les fromages pendant la fabrication et les premiers mois d'affinage provenaient du lait cru.

fromage / Parmigiano Reggiano / lait cru / microbiologie / affinage

1. INTRODUCTION

Parmigiano Reggiano is a hard, cooked Italian cheese. It is produced with partly skimmed raw cow milk to which natural whey starter is added and which is then ripened for 12–24 months. The ripening period involves a sequential breakdown of milk components, such as fat, protein and lactose, by the enzymes of bacteria [5]. Consequently, knowledge of bacterial flora involved in cheese ripening is of prime importance in predicting and determining final cheese quality. In a previous work conducted during the advanced stages of Parmigiano Reggiano cheese ripening (from 5 to 24 months) we were able to ascertain the presence of lactic acid bacteria throughout the entire period of ageing, with a predominance of facultatively heterofermentative lactobacilli [4].

The availability of information on the microbiological characteristics of natural whey starters used in Parmigiano Reggiano cheesemaking is limited to a few reports [19, 20, 24]. Recent studies dealing with cheesemaking and the early stages of ripening have been performed relative to Grana Padano, a cheese very similar to Parmigiano Reggiano [1, 2, 18, 23]. There is, however, a clear distinction in the manufacturing technology of Parmigiano Reggiano and Grana Padano defined as follows: in Grana Padano production, milk from a single milking and lysozyme are used. Lysozyme does not only significantly influence the growth of butyric acid bacteria but also of lactic acid bacteria, consequently affecting curd acidification during the cheesemaking [9]. Further, significant differences in microbiological composition of the two cheeses during the advanced stages of ripening have been ascertained [4]. Indeed, what is lacking is a study that specifically focuses on the microflora (not only of the lactic acid group) during the entire period of ageing in Parmigiano Reggiano. Preliminary results obtained from a study of some samples drawn throughout the entire period of production and ageing of the cheese have demonstrated the existence of microbial species related to several diverse groups, which are mostly present in the early phases of cheesemaking [22]. Thus, we studied numerous samples of milk, natural whey starter and cheese during cheese-making up to the fifth month of ripening in order to arrive at an even more comprehensive view of the microbiology of Parmigiano Reggiano by confirming and ultimately broadening the knowledge of its microbial characteristics.

2. MATERIALS AND METHODS

2.1. Cheesemaking

Raw milk, obtained by mixing the partly skimmed evening milk and the whole morning milk, was heated to about 22 °C. Natural whey starter was then added at 28–30 g·L^{-1} bringing the pH of the mixture to 6.2–6.3. Coagulation occurred at 32–33 °C due to the addition of calf rennet powder, then the curd was broken up for 2–4 min, cut into fragments and cooked at a temperature raised gradually to 42–44 °C, and then more quickly to 55–56 °C in 10–15 min. The curd was left covered by the whey for 40–60 min, then removed and placed inside a circular wooden mould. The cheese was held at about 20 °C for 3 d while it was turned at frequent intervals to facilitate complete whey drainage. The cheese round was then salted by immersion in brine (260–280 g NaCl·L^{-1} at 16–17 °C) for 20–24 d. After salting, the cheese was ripened at 16–18 °C and 85% relative humidity in store rooms, where it was frequently turned.

2.2. Samples

Samples were taken during cheesemaking and ripening (until the fifth month) of Parmigiano Reggiano cheese. Samples of natural whey starter, milk, whey after cooking and cheese were obtained from 15 different dairy plants located in various areas of production.

2.3. Physico-chemical, chemical and microbiological analyses

Water activity (a_w) was determined with an AquaLab Instrument (Decagon, Washington, USA).

Citrate content was determined enzymatically with Boehringer kits (Boehringer-Mannheim, Germany).

Natural whey starter, milk and whey after cooking (10 mL) or 10 g from the central portion of each cheese were withdrawn aseptically and homogenized with 90 mL of sterile quarter-strength Ringer's solution in a blender (Stomacher 400, Seward Medical, London, SE1 1PP, UK). Ten-fold dilutions were made in the same diluent. All the plates were seeded in duplicate.

2.4. Media and growth conditions

Thermophilic streptococci were counted in M17 agar (Unipath, Basingstoke, RG24 OPW, UK) after incubation for 72 h at 45 °C. Lactococci were counted in M17 agar (Unipath) after incubation for 72 h at 22 °C. Enterococci were counted in Slanetz & Bartley medium (Unipath) after incubation for 48 h at 37 °C. *Enterobacteriaceae* were counted in Violet Red Bile Glucose Agar (Unipath) after incubation for 48 h at 37 °C. Faecal coliform bacteria were counted in Violet Red Bile Lactose Agar (Unipath) after incubation for 48 h at 44 °C. *Micrococcaceae* were counted in Mannitol Salt Agar (Unipath) after incubation for 48 h at 30 °C. Lactic acid bacteria were counted in MRS agar (pH 6.3 ± 0.1) (Unipath) after anaerobic incubation (Anaerogen, Unipath) for 72 h at 22 °C and 45 °C.

Five colonies belonging to different types were randomly picked from each 25–50 colony count plate of MRS agar, M17 agar incubated at 22 °C, Slanetz & Bartley medium and Mannitol Salt Agar. Purified strains isolated from MRS agar were classified according to references [10, 33]. Purified strains isolated from M17 agar were identified according to references [11, 29]. Purified strains isolated from Slanetz & Bartley medium were identified according to reference [6]. Purified strains isolated from Mannitol Salt Agar were identified according to references [14–16, 27].

2.5. Physiological and biochemical examination

All isolates were characterized for morphology, Gram-stain and catalase test.

2.5.1. Strains isolated from MRS

Gas production from glucose was observed in MRS broth with citrate omitted and containing inverted vials (Durham). Amygdalin, arabinose, arbutin, cellobiose, fructose, galactose, glucose, gluconate, inulin, lactose, maltose, mannitol, mannose, melezitose, melibiose, ribose, rhamnose, 2-ketogluconate, n-acetylglucosamine, salicin, sucrose and xylose fermentation were determined by adding the test substances (filter-sterilized) after autoclaving to the basal medium (MRS broth without glucose, citrate and meat extract but with 40 mg·L^{-1} bromocresol purple). Tubes were incubated at 22 °C or 45 °C for 10 d and observed 3, 6 and 10 d after inoculation. Arginine hydrolysis was tested in MRS broth without glucose and meat extract but containing 3 g·L^{-1} arginine and 2 g·L^{-1} sodium citrate replacing ammonium citrate. Ammonia was detected using Nessler's reagent. Aesculin hydrolysis was determined with the method of Sharpe et al. [26]. The lactic acid optical isomer was determined in MRS broth using the reaction kits provided by Boehringer-Mannheim (kit 139.084 and D-lactate dehydrogenase). Growth at 15 °C and 45 °C was examined in MRS broth. Acetoin production was determined in MRS broth using the Voges-Proskauer test. Pediococci growth was also determined in MRS broth containing 100 and 150 g·L^{-1} of NaCl and at 50 °C in MRS broth.

2.5.2. Strains isolated from M17 agar

Growth at 40 °C, in the presence of 40 and 65 g·L^{-1} of NaCl was observed. Galactose, lactose, maltose, melezitose, melibiose, raffinose and ribose fermentation was determined by adding the test substances (filter-sterilized) after autoclaving to MRS broth without glucose, citrate and meat extract but with 40 mg·L^{-1} of bromocresol purple. Tubes were incubated at 22 °C for 10 d and observed 3, 6 and 10 d after inoculation. Arginine hydrolysis was tested in MRS broth without glucose and meat extract but containing 3 g·L^{-1} arginine and 2 g·L^{-1} sodium citrate replacing ammonium citrate. Ammonia was detected using Nessler's reagent.

2.5.3. Strains isolated from Slanetz & Bartley medium

Growth at 50 °C, in the presence of 1 g·L^{-1} methylen blue and 65 g·L^{-1} NaCl was observed. Arabinose and galactose, lactose, maltose, melezitose, melibiose, raffinose and ribose fermentation was determined by adding the test substances (filter-sterilized) after autoclaving to MRS broth without glucose and meat extract but with 40 mg·L^{-1} bromocresol purple. Tubes were incubated at 22 °C or 45 °C for 10 d and observed 3, 6 and 10 d after inoculation. Arginine hydrolysis was tested in MRS broth without glucose and meat extract but containing 3 g·L^{-1} arginine and 2 g·L^{-1} sodium citrate replacing ammonium citrate. Ammonia was detected using Nessler's reagent.

2.5.4. Strains isolated from Mannitol Salt Agar

Sensitivity to furazolidone [32] and lysostaphin [25] was used to separate the staphylococci from the micrococci. Production of pigment [30] was observed. Staphylococci were assayed for coagulase activity, detection of DNAse [28], urease (Unipath), novobiocin resistance [13] and acid production from cellobiose, maltose, mannitol, mannose, raffinose, sucrose and xylose in MSA without mannitol and agar. The following assays were performed on micrococci cultures: arginine and esculin hydrolysis, acid production from glucose, glycerol and mannose, growth at 37 °C, growth on Nutrient Agar supplemented with 75 g·L^{-1} NaCl (w/v), growth on Simmons Citrate Agar [13] and susceptibility to lysozyme [16].

Table I. Microbial counts carried out during the cheesemaking and the ripening of Parmigiano Reggiano.
Tableau I. Dénombrements microbiologiques effectués pendant la fabrication et l'affinage du Parmigiano Reggiano.

	Log colony forming units·mL^{-1}				Log colony forming units·g^{-1}								
	Natural whey starter	milk	Whey after cooking	Curd	Cheese								
					24 h	5 d	8 d	15 d	30 d	60 d	90 d	120 d	150 d
Lactic Acid Bacteria at 45 °C	8.34 (0.85)	3.26 (0.24)	6.61 (0.95)	7.11 (0.46)	7.01 (0.22)	7.45 (0.78)	7.28 (0.79)	6.92 (0.74)	6.88 (0.78)	7.23 (1.17)	7.46 (0.56)	7.23 (0.81)	6.84 (0.55)
Lactic Acid Bacteria at 22 °C	3.42 (0.33)	4.78 (0.45)	4.44 (1.03)	4.3 (0.67)	5.26 (0.87)	5.35 (0.56)	5.86 (0.88)	6.48 (1.01)	7.32 (0.76)	7.46 (0.66)	7.5 (0.89)	7.74 (0.69)	7.52 (0.74)
Thermophilic Streptococci	< 2	2.9 (0.98)	4.4 (0.67)	2.3 (0.78)	< 2	< 2	< 2	< 2	< 2	ND	ND	ND	ND
Lactococci	ND	4.5 (0.67)	3.49 (1.04)	3.6 (0.77)	4.4 (1.22)	3.2 (0.99)	3.1 (0.65)	2.14 (1.23)	< 2	< 2	ND	ND	ND
Enterococci	ND	2.82 (0.87)	2.46 (0.67)	3.2 (1.3)	3.51 (0.78)	3.45 (0.88)	3.92 (0.55)	4.02 (0.61)	4.17 (0.66)	3.49 (0.88)	2.99 (0.76)	2.18 (0.45)	2.00 (1.0)
Enterobacteriaceae	ND	2.65 (0.59)	2.56 (0.68)	1.3 (0.4)	A	A	A	A	A	ND	ND	ND	ND
Coliforms	ND	2.06 (0.45)	1.95 (0.55)	1.2 (0.23)	A	A	A	A	A	ND	ND	ND	ND
Micrococcaceae	ND	4.28 (0.87)	3.56 (0.61)	3.1 (0.89)	3.26 (1.01)	3.15 (0.67)	3.1 (0.76)	3.3 (1.11)	3.26 (0.99)	2.70 (0.77)	2.4 (0.65)	2.1 (1.02)	1.8 (0.88)

ND: not determined; A: absent in 1 g or mL; mean ± standard deviation.

ND : non déterminé ; A : absent dans 1 g ou mL ; moyenne ± écart type.

3. RESULTS

Table I shows the results of microbial counts from samples throughout the entire observation period.

Lactic acid bacteria counts at 45 °C were maximum in natural whey starter and minimum in the vat milk. The counts showed a nearly constant growth trend in the cheese until the fifth month of ageing. At 22 °C, the lactic acid bacteria counts were minimum in natural whey starter and progressively increased until the fifth month of ageing.

Thermophilic streptococci showed counts less than log 2 in natural whey starter but were present in vat milk, they presented an insignificant count in the cheese after 24 h. Lactococci were detectable until the 15th day but were then no longer recovered. Enterococci were also found throughout the period of observation although present in low amounts. *Enterobacteriaceae* and coliforms were absent after 24 h. *Micrococcaeae* demonstrated a progressively decreasing trend starting from vat milk.

The lactic acid microflora isolated from milk (Tab. II) were essentially facultatively heterofermentative mesophilic strains with a predominance of *L. paracasei* ssp. *paracasei* and of some strains ascribable to *L. paracasei* ssp. *tolerans* and to other species; at 45 °C few thermophilic species were isolated. At 45 °C, the microflora in the starter was composed of *L. helveticus* and of some strains of *L. delbrueckii* ssp. *bulgaricus* and of *L. delbrueckii* ssp. *lactis*, whereas at 22 °C *L. rhamnosus* and *L. brevis* were principally isolated. In both curd and whey after cooking, mainly *L. helveticus* was found at 45 °C and *L. paracasei* ssp. *paracasei* at 22 °C.

From this point on, it was apparent that the thermophilic strains progressively decreased and a sequential development of facultatively heterofermentative mesophilic lactic acid bacteria gradually became more evident. In parallel, a reduction of citrate concentration was observed while the values of a_w were in agreement with the possibility of growth of lactic acid bacteria during the period of this study (Fig. 1).

Among lactococci (Tab. III) *Lactococcus lactis* ssp. *lactis* was predominant together with *L. raffinolactis, L. plantarum* and *L. lactis* ssp. *cremoris*. Among enterococci (Tab. IV) *Enterococcus faecalis* was the predominant species accompanied by *E. faecium*. Among *Micrococcaceae* (Tab. V), *Kocuria kristinae, K. rosea, Kytococcus sedentarius, Arthrobacter agilis* and certain strains ascribable to *Staphylococcus* spp. have been isolated. However, all *Micrococcaeae* turned out to be coagulase and thermonuclease negative.

4. DISCUSSION

In the early stages of Parmigiano Reggiano cheese production, it was possible to verify the presence of a variegated microflora constituted essentially by thermophilic and mesophilic lactobacilli, thermophilic streptococci, lactococci, enterococci, enterobacteria and *Micrococcaceae*. According to the literature, natural whey starter has proven to be free of any undesirable microorganisms but instead to be populated mainly by thermophilic lactic acid bacteria [8, 18–21, 24]. Moreover it was possible to ascertain a relatively more complex microbiological composition of natural whey starter due to the reoccurring isolation of facultatively heterofermentative strains ascribable essentially to *L. rhamnosus*.

The microbiological quality of the milk used for cheesemaking proved to be quite satisfactory. In cooked whey enterobacteria and *Micrococcaceae* showed counts analogous to those ascertained in raw milk because of their resistance to the cooking process. On the contrary, after 24 h the enterobacteria were uncountable probably thanks to antagonistic activities or to competition

Table II. Identification of lactic acid bacteria isolated from MRS agar plates incubated at 22 °C and 45 °C during the cheesemaking and the ripening of Parmigiano Reggiano cheese (number of strains).

Tableau II. Identification des bactéries lactiques isolées à partir de milieu MRS gélosé incubé à 22 °C et 45 °C pendant la fabrication et l'affinage du fromage Parmigiano Reggiano (nombre de souches).

	Incubation temperature	Samples												
						Ripening time (days)								
	(°C)	Milk	Starter	Whey	Curd	1	5	8	15	30	60	90	120	150
Number of strains identified	22	38	25	26	35	62	68	58	73	87	71	61	79	72
	45	27	47	37	51	92	80	58	99	63	67	70	72	65
Lactobacillus brevis	22	0	6	3	0	16	23	14	8	18	12	0	0	0
	45	0	0	0	0	0	0	0	0	0	0	0	0	0
L. casei	22	0	0	0	0	0	0	0	0	0	0	0	8	12
	45	0	0	0	0	0	0	0	0	0	0	0	0	0
L. paracasei ssp. *paracasei*	22	32	0	21	35	34	30	38	24	26	21	36	31	29
	45	20	0	0	0	0	0	0	0	9	11	14	12	10
L. paracasei ssp. *tolerans*	22	2	0	1	0	12	15	6	9	12	5	8	10	7
	45	0	0	0	0	0	0	0	0	0	0	0	0	0
L. rhamnosus	22	0	19	0	0	0	0	0	32	31	33	17	30	24
	45	0	0	0	0	0	0	0	30	28	33	36	41	39
L. curvatus	22	4	0	1	0	0	0	0	0	0	0	0	0	0
	45	0	0	0	0	0	0	0	0	0	0	0	0	0
L. helveticus	22	0	0	0	0	0	0	0	0	0	0	0	0	0
	45	4	42	29	31	37	26	28	19	21	4	0	1	3
L. delbrueckii ssp. *bulgaricus*	22	0	0	0	0	0	0	0	0	0	0	0	0	0
	45	1	5	8	0	35	23	8	20	5	4	5	0	0
L. delbrueckii ssp. *lactis*	22	0	0	0	0	0	0	0	0	0	0	0	0	0
	45	2	0	0	20	20	31	22	30	0	4	0	6	4
Pediococcus acidilactici	22	0	0	0	0	0	0	0	0	0	0	0	0	0
	45	0	0	0	0	0	0	0	0	0	11	15	12	9

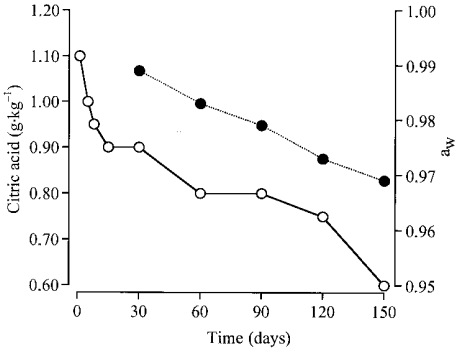

Figure 1. Changes in citric acid content (●) and water activity (○) during the ripening of Parmigiano Reggiano cheese.

Figure 1. Évolution du contenu de l'acide citrique (●) et de l'activité de l'eau (○) pendant l'affinage du fromage Parmigiano Reggiano.

for fermentable sugars rather than to the cooking steps in cheesemaking.

During the various stages of cheesemaking rod-shaped thermophilic microflora could be ascribed to *L. helveticus*, the predominant species, *L. delbrueckii* ssp. *bulgaricus* and *L. delbrueckii* ssp. *lactis* according to various literature relative to grana cheeses [8, 18, 23].

Regarding lactic acid microflora present during ageing, it was possible to verify that raw milk was the source of facultatively heterofermentative mesophilic lactobacilli, lactococci and *S. thermophilus* while *L. rhamnosus* derived from natural whey starter. In fact, no strains belonging to *L. rhamnosus* were isolated from raw milk. This indeed depends on its good thermo-resistance and on its aciduric property [4] (natural whey starter is obtained by incubating the whey from the previous day's production at 54–55 °C with a temperature gradient lowered to 28–30 °C within a 24 h time period and final pH drops to 3.20–3.30 [24]). On the other hand, during cheese ripening, the same species was isolated at 45 °C because of its heat resistance.

Some thermophilic lactic acid bacteria were isolated up to the fifth month of ageing, thus demonstrating that they sometimes do not disappear within just a few hours of cheesemaking as reported in related literature [8, 23]. This highlighted the variability of the lactic acid microflora responsible for raw milk cheese ripening.

This study consequently demonstrated that a variegated lactic acid microflora participated in the ripening process. However, concurring with previous publications, the thermophilic bacteria generally disappeared in a brief time (1–3 months), thus releasing a wealth of enzymes [3, 7, 8, 17, 31]. An undoubtedly important contribution was made by the mesophilic lactic acid bacteria (essentially *L. casei*, *L. paracasei*, *L. rhamnosus*, pediococci) which presented meaningful counts in the fifth month of ageing, thereby showing a significantly higher level compared to what has been reported thus far in literature with specific reference to Grana Padano [1]. This type of microflora undoubtedly played an active role during the entire period of ageing as well as a passive and gradual one, which could be attributed to enzyme release following autolysis of mesophilic lactic acid bacteria cells that takes place in the advanced stages of ageing [4].

The growth of lactic acid bacteria during the first months of ageing paralleled a gradual decrease in citric acid concentration. This finding demonstrated, in agreement with those noted by Jimeno et al. [12] regarding hard Swiss-type cheese, that the growth of facultatively heterofermentative lactic acid bacteria was in all probability connected to their ability to utilize citrates as the sole source of energy.

Moreover, it is necessary to question whether the minor microflora, such as micrococci, enterobacteria, lactococci and thermophilic streptococci which vanished relatively quickly, play a role in the process of cheese ripening by releasing their enzyme complexes.

Table III. Biochemical characterization of lactococci isolated from Parmigiano Reggiano cheese.
Tableau III. Caractérisation biochimique des lactocoques isolés du fromage Parmigiano Reggiano.

Identification	Number of strains	Lactic acid isomer(s)	Growth at 40 °C	Growth in 4% NaCl	Growth in 6.5% NaCl	Arginine hydrolysis	Galactose	Lactose	Maltose	Melibiose	Melezitose	Raffinose	Ribose
L. lactis ssp. *lactis*	78	L(+)	+	+	–	+	+	+	+	–	–	–	+
L. raffinolactis	22	L(+)	–	–	–	+	+	+	+	+	+	+	+
L. plantarum	26	L(+)	+	+	–	–	–	–	+	–	+	–	–
L. lactis ssp. *cremoris*	11	L(+)	–	–	–	–	+	+	–	–	–	–	–

Table IV. Biochemical characterization of enterococci isolated from Parmigiano Reggiano cheese.
Tableau IV. Caractérisation biochimique des entérocoques isolés du fromage Parmigiano Reggiano.

Identification	Number of strains	Lactic acid isomer(s)	Growth at 50 °C	Growth in 0.1% methylene blue	Growth in 6.5% NaCl	Arginine hydrolysis	Arabinose	Galactose	Lactose	Maltose	Melibiose	Melezitose	Raffinose	Ribose
E. faecalis	52	L(+)	+	+	+	+	–	+	+	+	–	–	–	+
E. faecium	18	L(+)	+	+	+	+	+	+	+	+	–	–	–	+

Table V. Biochemical characterization of micrococci isolated from Parmigiano Reggiano cheese.
Tableau V. Caractérisation biochimique des microcoques isolés du fromage Parmigiano Reggiano.

Identification	Number of strains	Pigment	Growth at 37 °C	Arginine hydrolysis	Esculin hydrolysis	Growth with 7.5% NaCl	Acid from glucose	Acid from mannose	Acid from glycerol	Lysozyme susceptibility	Growth on Simmons citrate agar
Kocuria kristinae	46	orange	+	–	+	+	+	+	+	–	–
Kocuria rosea	31	pastel red	+	–	–	+	+	–	–	–	–
Kytococcus sedentarius	14	white	+	+	–	+	+	–	–	–	–
Arthrobacter agilis	5	red	–	–	–	+	+	–	–	–	–

These results ultimately showed a homogeneous microbiological composition in the Parmigiano Reggiano cheese ripening. On the other hand, the role of raw milk in the production of this cheese was manifested by the slight biodiversity found among the analysed samples.

REFERENCES

[1] Bosi F., Rebecchi A., Cappa F., Bottazzi V., Battistotti B., Lactic acid bacteria for grana cheese production. Part IV: Distribution and characterization of mesophilic lactic acid bacteria, Sci. Tecn. Latt. Casearia 44 (1993) 205–214.

[2] Bottazzi V., Sarra P.G., Vescovo M., Bersani C., Characteristics of lactic acid bacteria in natural whey cultures. 4. Cultures characterization tests, Sci. Tecn. Latt. Casearia 29 (1978) 367–381.

[3] Chapot-Chartier M.M., Les autolysines des bactéries lactiques, Lait 76 (1996) 91–109.

[4] Coppola R., Nanni M., Iorizzo M., Sorrentino A., Sorrentino E., Grazia L., Survey of lactic acid bacteria isolated during the advanced stages of the ripening of Parmigiano Reggiano cheese, J. Dairy Res. 64 (1997) 305–310.

[5] Davies F.L., Law B.A., Advances in the microbiology and biochemistry of cheese and fermented milk, Elsevier Appl. Sci. Publ., New York, USA, 1984.

[6] Devriese L.A., Collins M.D., Wirth R., The genus *Enterococcus*, in: Balows A., Trüper H.G., Dworkin M., Harder W., Schleifer K.H. (Eds.), The Prokaryotes, Springer-Verlag Inc., New York, USA, 1992, pp. 1465–1481.

[7] El-Kholy W., El-Soda M., Ezzat N., El Shafei H., Autolysis and intracellular enzymes release from cheese related dairy lactobacilli, Lait 78 (1998) 439–452.

[8] Giraffa G., Mucchetti G., Addeo F., Neviani E., Evolution of lactic acid microflora during Grana cheese-making and ripening, Microbiol. Aliment Nutr. 15 (1997) 115–122.

[9] Grazia L., Chiavari C., Castagnetti G.B., Losi G., Experimental manufacturing of Parmesan cheese from milk with added lysozyme: starter acidification and effect on thermophilic lactic acid bacteria, Sci. Tecn. Latt. Casearia 35 (1984) 384–393.

[10] Hammes W.P., Weiss N., Holzapfel W., The genera *Lactobacillus* and *Carnobacterium*, in: Balows A., Trüper H.G., Dworkin M., Harder W., Schleifer K.H. (Eds.), The Prokaryotes, Springer-Verlag Inc., New York, USA, 1992, pp. 1534–1594.

[11] Hardie J.M., Whiley R.A., The genus *Streptococcus-oral*, in: Balows A., Trüper H.G., Dworkin M., Harder W., Schleifer K.H. (Eds.), The Prokaryotes, Springer-Verlag Inc., New York, USA, 1992, pp. 1421–1449.

[12] Jimeno J., Lazaro M.J., Sollberger H., Antagonistic interactions between propionic acid bacteria and non-starter lactic acid bacteria, Lait 75 (1995) 401–413.

[13] Kloos W.E., Tornabene T.G., Schleifer K.H., Isolation and characterization of micrococci from human skin, including two new species *Micrococcus lylae* and *Micrococcus kristinae*, Int. J. Syst. Bacteriol. 24 (1974) 79–101.

[14] Kloos W.E., Schleifer K.H., Götz F., The genus *Staphylococcus*, in: Balows A., Trüper H.G., Dworkin M., Harder W., Schleifer K.H. (Eds.), The Prokaryotes, Springer-Verlag Inc., New York, USA, 1992, pp. 1369–1420.

[15] Koch C., Schumann P., Stackebrandt E., Reclassification of *Micrococcus agilis* (Ali-Cohen 1889) to the genus *Arthrobacter* as *Arthrobacter agilis* comb. nov. and emendation of the genus *Arthrobacter*, Int. J. Syst. Bacteriol. 45 (1995) 837–839.

[16] Kocur M., Kloos W.E., Schleifer K.H., The genus *Micrococcus*, in: Balows A., Trüper H.G., Dworkin M., Harder W., Schleifer K.H. (Eds.), The Prokaryotes, Springer-Verlag Inc., New York, USA, 1992, pp. 1300–1311.

[17] Lortal S., Rousseau M., Boyaval P., van Heijenoort J.V., Cell wall and autolytic system of *Lactobacillus helveticus* ATCC 12046, J. Gen. Microbiol. 137 (1991) 549–559.

[18] Lucchini F., Bertuzzi S., Rebecchi A., Bottazzi V., Brambilla E., Lactic acid bacteria for grana cheese production. Part VI: Different features of whey natural culture, Sci. Tecn. Latt. Casearia 45 (1994) 95–104.

[19] Matteuzzi D., Studi sulla microflora dei sieroinnesti del formaggio Parmigiano-Reggiano. Nota I: Identificazione delle specie più rappresentative, Ann. Microbiol. 17 (1967) 201–210.

[20] Matteuzzi D., Studi sulla microflora dei sieroinnesti del formaggio Parmigiano-Reggiano. Nota II: Distribuzione biometrica dei caratteri "potere proteolitico" e "potere acidogeno" nella specie *Lactobacillus jugurti*, Ann. Microbiol. 17 (1967) 211–219.

[21] Mucchetti G., Addeo F., Neviani E., The history of cheese production with DOP characteristics. Production, use and composition of whey starter for Grana Padano and Parmigiano Reggiano cheese: considerations on the relationship between whey starter and DOP, Sci. Tecn. Latt. Casearia 49 (1998) 281–311.

[22] Nanni M., Coppola R., Iorizzo M., Sorrentino A., Sorrentino E., Grazia L., Lactic acid bacteria during the ripening of Parmigiano-Reggiano, Sci. Tecn. Latt. Casearia 48 (1997) 211–216.

[23] Neviani E., Carini S., Microbiology of Parmesan cheese, Microbiol. Aliment. Nutr. 12 (1994) 1–8.

[24] Sacchetti M., Zambonelli C., Acidità e carico microbico nei sieri-innesto della zona tipica del "Parmigiano-Reggiano", Latte 37 (1963) 351–360.

[25] Schleifer K.H., Kloos W.E., A simple test for separation of staphylococci from micrococci, J. Clin. Microbiol. 1 (1975) 337–338.

[26] Sharpe M.E., Fryer T.F., Smith D.G., Identification of the lactic acid bacteria, in: Gibbs B.M., Skinner F.A. (Eds.), Identification methods of Microbiologist, Academic Press, London, UK, 1966, p. 72.

[27] Stackebrandt E., Koch. C., Gvozdiak O., Schumann P., Taxonomic dissection of the genus *Micrococcus*: *Kocuria* gen. nov., *Nesterenkonia* gen. nov., *Kytococcus* gen. nov., *Dermacoccus* gen. nov., and *Micrococcus Cohn* 1872 gen. emend., Int. J. Syst. Bacteriol. 45 (1995) 682–692.

[28] Tatini S.R., Hoover D.G., Lachica R.V.F., Methods for the isolation and enumeration of *S. aureus*, in: Speck M.L. (Ed.), Compendium of Methods for the microbiological examination of food, American Public Health Association, Washington D.C., USA, 1984.

[29] Teuber M., Geis A., Neve H., The genus *Lactococcus*, in: Balows A., Trüper H.G., Dworkin M., Harder W., Schleifer K.H. (Eds.), The Prokaryotes, Springer-Verlag Inc., New York, USA, 1992, pp. 1482–1501.

[30] Tiecco G., Microbiologia degli alimenti di origine animale, Edagricole, Bologna, Italy, 1982.

[31] Valence F., Lortal S., Zymogram and preliminary characterization of *Lactobacillus helveticus* autolysins, Appl. Environ. Microbiol. 65 (1995) 3391–3399.

[32] von Rheinbaden K.E., Hadlok R.M., Rapid distinction between micrococci and staphylococci with furazolidone agar, Antonie van Leeuwenhoek 47 (1981) 41–51.

[33] Weiss N., The genera *Pediococcus* and *Aerococcus*, in: Balows A., Trüper H.G., Dworkin M., Harder W., Schleifer K.H. (Eds.), The Prokaryotes, Springer-Verlag Inc., New York, 1992, USA, pp. 1503–1507.