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Original article

A multiple strain starter for water-buffalo Mozzarella cheese manufacture

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Summary — The effect of different incubation conditions for a multiple strain starter developed for Mozzarella cheese manufacture from water-buffalo milk was studied. The original starter was prepared by mixing intermediate cultures of the different strains grown separately and was composed by mesophilic (*Streptococcus lactis* subsp. *lactis* 25%, *Str. lactis* subsp. *diacetylactis* 25%, *Str. faecalis* subsp. *faecalis* 7%, *Leuconostoc mesenteroides* 8%, *Kluyveromyces marxianus* 0.02%) and thermophilic (*Str. salivarius* subsp. *thermophilus* 20% and *Lactobacillus delbrueckii* subsp. *lactis* 15%) strains. To facilitate the production of the starter preparation for cheesemaking the mesophilic strains were grown together at 22°C, 30°C or 37°C and the thermophilic strains were grown together at 37°C, 40°C or 45°C. The best results in terms of acid production and species composition were obtained when the thermophilic species were incubated at 37°C and the mesophilic species at 22°C or 30°C. The effect of the inoculation level (2, 5 or 10%) on acid production was also tested for some combinations.

Mozzarella cheese — water-buffalo milk — multiple strain starter

Résumé — Un levain mixte pour la production du fromage «Mozzarella». *On a étudié l'effet de différentes conditions d'incubation sur un levain à souches multiples mis au point pour la production du fromage «Mozzarella» à partir du lait de bufflonne. Le levain original avait été préparé en mélangeant différentes souches microbiennes cultivées séparément, dans les proportions suivantes :* Streptococcus lactis *subsp.* lactis *25%,* Str. lactis *subsp.* diacetylactis *25%,* Str. faecalis *subsp.* faecalis *7%,* Leuconostoc mesenteroides *8%,* Kluyveromyces marxianus *0.02%,* Str. salivarius *subsp.* thermophilus *20%, et* Lactobacillus delbrueckii *subsp.* lactis *15%. Pour rendre plus simple la préparation du levain, en vue de son emploi en fromagerie, on a cultivé ensemble les espèces mésophiles à 22°C, 30°C ou 37°C et les espèces thermophiles à 37°C, 40°C ou 45°C. Les meilleurs résultats en termes de production d'acide et de composition en espèces ont été obtenus en incubant les souches thermophiles à 37°C et les mésophiles à 22°C ou à 30°C. On a aussi testé, pour quelques combinaisons de levains, l'effet de la quantité d'inoculum (2, 5 ou 10%) sur la production d'acide.*

fromage «Mozzarella» — lait de bufflonne — levain mixte

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INTRODUCTION

Mixed strain and natural whey starters have been used for centuries in cheese manufacture but, because of their unpredictable performance, they have generally been replaced by single and multiple strain starters in modern cheese making practice (Thunell and Sandine, 1985).

In an earlier work (Coppola *et al.*, 1988) we described the microbiology of the natural whey cultures utilized as starters in the manufacture of Mozzarella cheese from raw water-buffalo milk in Southern Italy. They are dominated by a complex association of thermophilic *(Streptococcus salivarius* subsp. *thermophilus)* and mesophilic streptococci *(Str. lactis* subsp. *lactis, Str. Lactis* subsp. *diacetylactis)* and thermophilic lactobacilli (mainly *Lactobacillus delbrueckii* subsp. *lactis* and *Lb. helveticus).* Enterococci *(Str. faecalis, Str. faecium),* leuconostocs *(Leuconostoc dextranicum, Leuc. lactis, Leuc. cremoris)* and yeasts can also reach high numbers. They are often heavily contaminated by coliforms (Coppola *et al.*, 1988; Addeo and Coppola, 1983). Although rational cheesemaking practice and the current Italian regulations for soft unripened cheese manufacture (Ministero della sanità della Repubblica Italiana, 1978) require the pasteurization of milk and the replacement of natural whey cultures with a defined starter culture system, whey starters are still in use in the manufacture of Mozzarella cheese from water-buffalo milk in Southern Italy. This is because, according to the cheesemakers, the use of the commercially available starter cultures (associations of *Str. salivarius* subsp. *thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* and/or *Lb. helveticus)* causes an impoverishment of the organoleptic characteristics of water-buffalo Mozzarella cheese, which is one of the most highly valued unripened «pasta filata» cheeses produced in Italy and is recognized by Italian law as a «typical» cheese (Repubblica Italiana, 1980).

For these reasons, we decided to develop a new starter culture which would mimic the composition of the natural whey cultures as closely as possible. In some preliminary cheesemaking trials (to be published) a starter composed by about 25% *Str. lactis* subsp. *lactis,* 25% *Str. lactis* subsp. *diacetylactis,* 7% *Str. faecalis* subsp. *faecalis,* 8% *Leuconostoc mesenteroides,* 20% *Str. salivarius* subsp. *thermophilus,* 15% *Lb. delbrueckii* subsp. *lactis* (2 different strains) and 0.02% *Kluyveromyces marxianus* (as determined by place counts on the bulk starters; the actual volumetric ratio of the cultures was 1:2:0.5:2:1:2:0.5) gave satisfactory results (good acid production, excellent behaviour of the curd at stretching and excellent quality of the finished product). In these trials the single strains were grown separately and mixed just before the inoculation of the vat milk. This would obviously be impractical in actual cheesemaking practice. The number of starter tanks necessary would be reduced if the mesophilic strains *(Str. lactis* subsp. *lactis, Str. lactis* subsp. *diacetylactis, Str. faecalis* subsp. *faecalis, Leuconostoc mesenteroides and Kluyveromyces marxianus)* and the thermophilic strains *(Str. salivarius* subsp. *thermophilus* and the two strains of *Lb. delbrueckii* subsp. *lactis)* were grown in 2 different starter tanks only. A major problem with this approach would be to maintain the desired numerical ratio among the strains and the activity of the starter. The objective of this work was to select the most appropriate time/ temperature combination for the incubation of the bulk starters composed by the strains indicated above.

MATERIALS AND METHODS

Microbial strains

Str. lactis subsp. *lactis* 136, *Str. lactis* subsp. *diacetylactis RD, Str. faecalis* subsp. *faecalis* 221, *Str. salivarius* subsp. *thermophilus* 317, *Leuc. mesenteroides* L1, *Lb. delbrueckii* subsp. *lactis* 22 and 35, and a yeast strain tentatively identified as *Kluyveromyces marxianus* (25Y) from the culture collection of the Istituto di Microbiologia Agraria, Università degli Studi di Napoli, were used in this experiment. All the strains, except RD, were isolated from natural whey cultures utilized in the manufacture of Mozzarella cheese from water-buffalo milk. The strains were stored freeze-dried. Working cultures were grown in skim milk (Oxoid) and stored in the refrigerator.

Incubation conditions

All the mother cultures were grown separately (317, 22 and 35 at 37°C for 18 h; 136, RD, 221, 25Y, L1 at 30°C for 18 h) in skim milk (skim milk + 0.5% yeast extract for 25Y and L1). At the end of the incubation, mother cultures of 317, 22 and 35 were mixed at a 1:1:1 ratio (vol/vol) and the mixture was used to inoculate skim milk at the 2% level; mother cultures of 136, RD, 221, L1, 25Y were mixed at 1:2:0.5:2:0.5 ratio (vol/vol) and the mixture was used to inoculate skim milk at the 2% level. The thermophilic starters were incubated at 37°C (A), 40°C (B), 45°C (C) for 18 h. Further experiments showed that early stationary phase was reached by the thermophilic cultures after 4.5 h at 37°C, 4 h at 40°C and 3.5 h at 45°C : these incubation conditions were labeled H, I and L respectively. At the end of the incubation the cultures were promptly removed from the incubator and stored at 4°C until used. The mesophilic starter were incubated at 22°C (D), 30°C (E), 37°C (F) for 18 h. An attempt to grow all the strains together at 37°C was also made (G). Some incubation conditions (for example C, F and G) were discarded after a preliminary experiment while others (A, B, D, E, H, I and L) were replicated 2 to 6 times.

Enumeration

Decimal dilutions (in Ringer's solution, quarter strength) and enumerations were carried out on the mother cultures and on the bulk starters using the media described below.

Thallous acetate Tetrazolium Glucose Agar (TITGA) (Barnes, 1956) was surface inoculated for the differential enumeration of *Str. faecalis* subsp. *faecalis*. The plates were incubated at 37°C for 48 h. *Str. lactis* subsp. *lactis* and *Str. lactis* subsp. *diacetylactis* also grew on this medium but their colonies were clearly distinguishable from those of the enterococcus. The medium described by Reddy *et al.* (1972) (RA) was used for the differential enumeration of the citrate utilizing/ fermenting strains. The plates were surface inoculated and incubated at 30°C for 5 d in a Gas-pack jar with a CO_2-generating kit (BBL). The colonies of *Str. lactis* subsp. *diacetylactis* RD were undistinguishable from those of *Str. faecalis* subsp. *faecalis* 221 : both were white, round, 2—4 mm with a 5 mm clear halo. *Str. lactis* subsp. *lactis* 136 colonies were white, round, 2—3 mm without halo. Elliker Lactic Agar (ELA) (Elliker *et al.*, 1956) was used for the total count of the mesophilic strains and for the differential enumeration of *Leuc. mesenteroides* L1. Plates were surface inoculated and incubated at 30°C for 48 h in a Gas-pack jar with a CO_2-generating kit (BBL). L1 colonies were mucoid, translucent, 4—5 mm in diameter, clearly distinguishable from those of the other strains, which were white, round, 2—3 mm in diameter. In a preliminary experiment Mayeux Agar (Mayeux *et al.*, 1962) was also used for the differential enumeration of L1 but its use was discontinued because L1 was easily counted on ELA. Yeast Dextrose Chloramphenicol Agar (ISO/DIS, 1985) was used for the differential enumeration of 25Y. Plates were incubated at 30°C for 5 d. Tryptose-Proteose-Peptone-Yeast-Eriochrome Black Agar (TPPYA) (Bracquart, 1981) was used for the differential enumeration of *Str. salivarius* subsp *thermophilus* 317 and *Lb. delbrueckii* subsp. *lactis* 22 and 35. The plates were surface inoculated and incubated for 48 h at 37°C in a Gas-pack jar with a CO_2-generating kit (BBL). The coccus colonies were round, dark brown/violet, slightly convex, 1—1.5 mm while the rods' colonies were translucent, irregular, rough, 1—1.5 mm in diameter. In a preliminary experiment

differential counts of these strains were also performed on Yoghurt Lactic Agar (YLA) (Matalon and Sandine, 1986). Although this medium allowed good growth and differentiation of the 2 species, its use was discontinued because the presence of large opaque haloes around the coccus colonies made the differential count on crowded plates complicated.

Acidity and acid production ability of the bulk starters

At the end of the incubation period, pH and titratable acidity (expressed as °SH/50) were measured on the bulk starters. The thermophilic and mesophilic starters were mixed in a 2:3 ratio in all the possible combinations (A:D, A:E, A:F, B:D, B:E, B:F, C:D, C:E, H:D, I:D, L:D, G) and the mixtures were inoculated in skim milk at the 2% level. A, B, C, D, E, F, G taken alone were also used to inoculate skim milk at the 2% level. Moreover, to test the effect of inoculum level on acid production ability, A, D, E, A:D, A:E were inoculated in skim milk at the 5% and 10% levels while H, I, L, H:D, I:D, L:D were inoculated at the 5% level. The inoculated milk was incubated at 37°C (the cheese vat temperature) and pH was measured hourly with a Beckman PHI43 pH-meter. Although this might not be the better model from a biological point of view, a linear relationship apparently existed between the pH and the time (in h). The parameters of the equation $pH = a + bt$ were estimated with the least square method and the rate constants b were compared using a Student's *t*-test described in Steel and Torrie (1980).

RESULTS

The results of the counts on the mother cultures and bulk starters are shown in Table I.

The number of *Str. lactis* subsp. *diacetylactis* RD was estimated subtracting the counts of *Str. faecalis* subsp. *faecalis* 221 on TITG from those of the citrate utilizing colonies on Reddy's Agar. Since replicates were available for treatments D (22°C) and E (30°C), an analysis of variance was carried out on the results. Total counts, counts of 136, RD, 221 and 25Y were not significantly different between the treatments, while the count of L1 at 30°C was lower ($P = 0.05$) than that at 22°C. The results of the counts of 136 and RD on Reddy's agar and on ELA for treatment F (mesophilic starters grown at 37°C) were contradictory and no sensible estimate could be made of their number. Similar problems were experienced with treatment G (mesophilic and thermophilic strains grown together at 37°C). The counts of the different strains for this treatment (when available) were as follows (Log(cfu/ml)) : total count : 9.46; 136 : 8.45; L1 : 7.08; 221 : 6.6; 25Y : 4.4.; 317 : 8.53; 22 + 35 : 8.37. No estimate could be made for RD, while the result for 317 is probably overestimated (136, 221 and RD would probably grow on TPPYA at 37°C, giving the same colony morphology as 317). Although every care was used in standardizing the preparation and use of Reddy's agar, this medium often gave results which were inconsistent or whose interpretation was difficult. An analysis of variance was carried out on the results of the counts for starter A, B, H, I and L (thermophilic strains grown respectively at 37°C for 18 h, 40°C for 18 h, 37°C for 4.5 h, 40°C for 4 h, 45°C for 3.5 h). Differences between the counts of *Str. salivarius* subsp. *thermophilus* 317, *Lb. delbrueckii* subsp. *lactis* 22 + 35 and total counts among the treatments were never significant. Moreover, counts of 317 were not significantly different from those of 22 + 35 within treatments.

The pH and titratable acidity of the bulk starters are shown in Table II. When more than 1 replicate was available the mean

Table I. Starter counts in the mother cultures and bulk starters (mean with standard error in parentheses).
Dénombrement des ferments dans les cultures-mères et les levains (moyenne avec erreur standard entre parenthèses).

Strains		*Count (Log (cfu/ml))*		
A. Mesophiles	*m.c.*	*D*	*E*	*F*
		(22°C 18 h)	*(30°C 18 h)*	*(37°C 18 h)*
Str. lactis subsp. *lactis* 136	9.10 (0.01)	8.35 (0.38)	8.92 (0.21)	n.d.
Str. lactis subsp. *diacetylactis* RD	8.81 (0.12)	8.76 (0.32)	9.18 (0.43)	n.d.
Str. faecalis subsp. *faecalis* 221	8.60 (0.36)	7.54 (0.72)	7.24 (0.16)	7.48
Leuc. mesenteroides L1	8.09 (0.29)	7.46 (0.30)	6.67 (0.08)	7.60
Kluyveromyces marxianus 25Y	6.10 (0.33)	4.71 (0.29)	4.64 (0.34)	4.98
Total count on the bulk starter		9.11 (0.04)	9.15 (0.11)	9.15
B. Thermophiles	m.c. (37°C 18 h)	A (40°C 18 h)	B (45°C 18 h)	C
Str. salivarius subsp. *thermophilus* 317	8.95 (0.29)	8.57 (0.23)	7.03(0.32)	7.48
Lb. delbrueckii subsp. *lactis* 22	8.32 (0.30)			
Lb. delbrueckii subsp. *lactis* 35	8.07 (0.68)			
Lb. delbrueckii subsp. *lactis* 22 + 35		8.69 (0.22)	8.62 (0.22)	8.92
Total count in the bulk starters		8.95 (0.18)	8.68 (0.25)	8.93
	H (37°C 4.5 h)	I (40°C 4 h)	L (45°C 3.5 h)	
Str. salivarius subsp. *thermophilus* 317	7.97 (0.32)	8.62 (0.31)	8.66 (0.32)	
Lb. delbrueckii subsp. *lactis* 22 + 35	7.53 (0.22)	7.69 (0.22)	7.99 (0.23)	
Total count in the bulk starters	8.70 (0.24)	8.70 (0.23)	8.86 (0.25)	

and the standard error are shown. Treatments can be grouped as follows on the basis of multiple mean comparison on pH : A = B = C > E > D = I = L > H. The figure is more complex for titratable acidity where there is overlapping among mean groups. A, B, and C apparently have the highest and H, I, and L the lowest titratable acidity. The pH of the natural whey cultures is around 4.2 and their titratable acidity is around 27°SH/50.

In this experiment the acid production of the starters in skim milk at 37°C (the vat temperature in Mozzarella cheese manufacture from water-buffalo milk) was chosen as an index of starter performance. A linear relationship apparently existed between pH and time in h and the parameters of the regression equations are shown in Table III. Analysis of the variance showed that all the regressions were highly significant ($P < 0.005$). The estimated time to reach pH 4.9, which is considered the optimal pH for curd stretching (Altiero *et al.*, 1984), is also shown. The acid production rates (b) were compared using a Student's *t*-test : when the values in Table III are followed by the same letter they are not significantly different from one another at the 95% confidence level. The estimated equation for acid production of the natural whey starter culture in water-buffalo milk during the manufacture of the cheese is pH =

Table II. Acidity of the bulk starters (mean with standard deviation in parentheses).
Acidité des levains (moyenne avec erreur standard entre parenthèses).

Starter	*pH*	*°SH/50*
A (Therm.37°C 18 h)	3.95 (0.07) d	25.45 (0.49) de
B (Therm.40°C 18 h)	3.87 (0.10) d	31.71 (0.70) f
C (Therm.45°C 18 h)	3.90 (0.02) d	30.57 (0.49) ef
D (Mes.22°C 18 h)	5.17 (0.38) b	12.54 (1.68) c
E (Mes.30°C 18 h)	4.44 (0.05) c	20.43 (1.20) d
F (Mes.37°C 18 h)	4.41	19.11
G (Mes. + Therm.37°C 18 h)	3.98	24
H (Therm.37°C 4.5 h)	6.17 (0.12) a	5.67 (0.64) a
I (Therm.40°C 4 h)	5.20 (0.15) b	6.30 (0.77) ab
L (Therm.45°C 3.5 h)	5.23 (0.15) b	11.47 (1.07) bc

Means followed by the same letter are not significantly different from each other ($P < 0.05$).
Les valeurs suivies de la même lettre ne diffèrent pas significativement entre elles ($P < 0.05$).

6.47–0.43t with a $t_{4.9} = 3.39$. It is evident that this situation is quite different from acid production in skim milk; previously we showed that acid production in skim milk can be much lower than that in water-buffalo milk (Coppola *et al.*, 1988). However, the estimated equation for acid production rate for starter A + D used at the 2% level in 2 cheese-making trials was pH = 6.61–0.32t with $t_{4.9} = 5.34$. This shows that the acid production rate of the multiple strain starter in water-buffalo milk is approximately equal to that in skim milk but still much lower than that of the natural whey culture.

DISCUSSION AND CONCLUSIONS

The high intrinsic variability of the plate count methods makes any comparison between the strain populations in the different starters very difficult. The only strain which seems to be significantly affected by the incubation temperature is *Leuc. mesenteroides* L1 whose counts are lower ($P < 0.05$) at 30°C than at 22°C. This is not surprising since the temperature optimum for leuconostocs is around 22°C and it is common practice to incubate mixed starters containing these strains at 18—22°C (Thunell and Sandine, 1985). The apparently large difference between the counts of *Str. salivarius* subsp. *thermophilus* 317 and the counts of *Lb. delbrueckii* subsp. *lactis* strains 22 and 35 was not significant. It is evident from the above that only rough approximations can be made on the actual strain ratios in the bulk starters. Since the mesophilic and thermophilic starters were mixed in a 3:2 (vol/vol) ratio to make the actual starter, it can be calculated from the data in Table I that the combinations that give ratios closer to the desired one were A + D and A + E and, to a lower extent, I + D and L + D (which are low in thermophilic lactobacilli and high in *Str.* lactis *subsp. diacetylactis* (see Table IV).

The data in Table III allow much more interesting comparisons among the

Table III. Parameters of the equation pH= a + bt, estimated for the acid production of the starters. $t_{4.9}$ is the estimated time in h and min to reach pH 4.9. Values of b followed by the same letter are not significantly different from each other ($P < 0.05$).
Paramètres de l'équation pH = a + bt, évalué pour la production d'acide par les levains. $t_{4,9}$ est le temps évalué en h et min pour arriver à pH = 4,9. Les valeurs de la colonne «b» suivies de la même lettre ne sont pas significativement différentes entre elles (P < *0,05).*

Starter	*a*	*b*	R^2	$t_{4.9}$
A (Therm.37°C 18 h) – 2%	6.51	– 0.24 bcde	0.82	6.42
A (Therm.37°C 18 h) – 5%	6.17	– 0.23 bc	0.97	5.31
A (Therm.37°C 18 h) – 10%	5.88	– 0.21 b	0.95	4.40
B (Therm.40°C 18 h) – 2%	6.71	– 0.30 ghi	0.99	6.02
C (Therm.45°C 18 h) – 2%	6.51	– 0.13 a	0.90	12.23
D (Mes.22°C 18 h) – 2%	6.54	– 0.28 bcdefg	0.93	5.51
D (Mes.22°C 18 h) – 5%	6.29	– 0.27 bcdefg	0.91	5.08
D (Mes.22°C 18 h) – 10%	6.10	– 0.26 bcdefg	0.90	4.36
E (Mes.30°C 18 h) – 2%	6.47	– 0.26 bcdefg	0.90	6.02
E (Mes.30°C 18 h) – 5%	6.18	– 0.25 bcdef	0.90	5.07
E (Mes.30°C 18 h) – 10%	5.88	– 0.22 b	0.85	4.27
F (Mes.37°C 18 h) – 2%	6.44	– 0.24 bcd	0.96	6.25
G (Mes. + Th.37°C 18 h) – 2%	6.49	– 0.22 b	0.92	7.13
H (Therm.37°C 4.5 h) – 2%	6.62	– 0.27 bcdefg	0.86	6.25
H (Therm.37°C 4.5 h) – 5%	6.46	– 0.31 ghi	0.86	6.25
I (Therm. 40°C 4 h) – 2%	6.50	– 0.34 hi	0.96	4.45
I (Therm.40°C 4 h) – 5%	6.32	– 0.34 hi	0.94	4.12
L (Therm.45°C 3.5 h) – 2%	6.48	– 0.36 hi	0.97	4.24
L (Therm.45°C 3.5 h) – 5%	6.43	– 0.33 ghi	0.92	4.34
A + D – 2%	6.60	– 0.30 ghi	0.95	5.40
A + D – 5%	6.35	– 0.30 fghi	0.96	4.50
A + D – 10%	6.12	– 0.29 cdefgh	0.97	4.12
A + E – 2%	6.56	– 0.30 defghi	0;94	5.32
A + E – 5%	6.33	– 0.29 defghi	0.93	4.55
A + E – 10%	6.04	– 0.27 cdefgh	0.96	4.13
A + F – 2%	6.52	– 0.25 bcdefg	0.96	6.28
B + D – 2%	6.74	– 0.32 ghi	0.98	5.45
B + E – 2%	6.62	– 0.30 efghi	0.98	5.44
B + F – 2%	6.60	– 0.27 bcdefg	0.94	6.17
C + D – 2%	6.58	– 0.24 bcdef	0.99	7.00
C + E – 2%	6.59	– 0.24 bcdef	0.98	7.02
H + D – 2%	6.54	– 0.29 cdefghi	0.81	5.39
H + D – 5%	6.46	– 0.31 ghi	0.88	5.03
I + D – 2%	6.50	– 0.37 hi	0.95	4.16
I + D – 5%	6.41	– 0.38 i	0.94	3.57
L + D – 2%	6.43	– 0.38 hi	0.97	4.04
L + D – 5%	6.43	– 0.38 hi	0.96	4.02

starters. Starter C (thermophilic strains grown at 45°C) had the lowest acid production rate (b). This could be due to the low pH of the starter at the end of the incubation, which could have impaired the acid production ability of the micro-organisms. In fact the same starter grown at 45°C for 3.5 h and stored refrigerated

Table IV. Estimated percentages of the single starter strain in the bulk starters (data from Table I; mesophilic and thermophilic bulk starters mixed in a 3:2 vol/vol ratio).
Pourcentages estimés de chaque ferment dans les levains (données d'après le Tableau I; les levains mésophiles et thermophiles sont mélangés en rapport 3/2 vol/vol).

Strain	Starter												
	A+D	A+E	A+F	B+D	B+E	B+F	C+D	C+E	G	H+D	I+D	L+D	ideal
136	22	36	n.e.	29	42	n.e.	25	38	10	24	20	19	25
RD	37	39	n.e.	47	45	n.e.	41	41	n.e.	60	49	46	25
221	1	0.6	1	1.4	0.7	2	1	0.7	0.1	4	3	3	7
L1	2	0.1	1.8	2	0.2	2	2	0.2	0.4	3	2	2	8
25Y	0.004	0.002	0.004	0.005	0.003	0.005	0.004	0.002	0.001	0.01	0.0004	0.0004	0.02
317	18	11	16	2	1	2	1	0.7	11	6	23	24	20
22 + 35	20	12	18	17	10	16	30	2	8	2	3	5	15

n.e. : no estimation was possible

for 15 h had a high «b» value (– 0.36). This is true, too, for A (37°C for 18 h, b = – 0.21 — – 0.24) and H (37°C for 4.5 h, b = – 0.27 — – 0.31) while B (40°C for 18 h, b = – 0.30) and I (40°C for 4 h, b = – 0.34) were not significantly different. Both, however, had a much higher acid production rate than C. Also, no significant difference was found among the different inoculation levels for each starter. However, due to the lower initial pH of the inoculated milk, at higher inoculation levels, pH 4.9 was attained sooner. Among the associations, I + D and L + D had the highest acid production rate (b = – 0.38 pH unit/h). However, they were not significantly different ($P > 0.05$) from A + D, A + E, B + D and B + E.

From the above it can be tentatively concluded that the desired ratio among the strains in our starter for Mozzarella cheese can be best maintained growing the mesophilic strains (136, RD, 221, L1 and 25Y) at 22°C for 18 h and the thermophilic strains at 37°C for 18 h (combination A + D). An acceptable strain ratio can be also obtained using the same incubation conditions for mesophilic strains and growing the thermophilic strains at 40°C for 4 h or at 45°C for 3.5 h, followed by refrigeration until the moment of use (combinations I + D and L + D). It should be noted that the apparently higher acid production rate (b = – 0.37 — – 0.38) is not significantly different from that of A + D (b = – 0.30) when they are compared by a Student's *t*-test. The use of higher inoculation levels (5% or 10% instead of 2%) could allow a shorter preparation time for Mozzarella cheese but would increase the costs for starter preparation.

The lower acid production rate of the multiple strain starter in cheese-making trials when compared to the natural whey culture is probably the price for more constant performances and sanitary production of Mozzarella cheese for water-buffalo milk. Studies are in progress to improve the performances of the multiple strain starter.

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