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Microflora and acidification properties of yogurt and yogurt-related products fermented with commercially available starter cultures

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Yogurts and yogurt-related milk products were produced using 44 commercially available starter cultures from 8 suppliers. The yogurt starters consisted of the classical yogurt microflora and the yogurt-related cultures containing *Lactobacillus acidophilus* and/or *Bifidobacterium* spp. instead of or in addition to the yogurt bacteria. The counts of lactobacilli in the fresh yogurts varied between 5.5×10^7 and 6.5×10^8 CFU/ml, and the counts of streptococci varied from 3.5×10^7 to 1.2×10^9 CFU/ml. About 80% of the yogurts had higher counts of cocci than rods. During storage of the products for 2 weeks at 6°C the stability of the microflora differed markedly among the cultures. In the fresh yogurt-related products the *L. acidophilus* counts ranged from 4.0×10^5 to 2.6×10^8 CFU/ml; bifidobacteria were found at levels between 4.0×10^6 and 2.6×10^8 CFU/ml. In most products reduced viable counts of these bacteria were observed after 2 weeks. Titratable acidity increased on average by 22.3% in the yogurts, and by 14.9% in the yogurt-related products during storage. In most products a higher amount of L(+)- than D(−)-lactic acid was found.

Key words: Yogurt; Yogurt-related products; Microflora; Acidification properties

Introduction

According to international statistics, the consumption of fermented milk products is still increasing (International Dairy Federation, 1990; Klupsch, 1989a). This effect is not only due to the expanding variety, but also to sensory aspects as well as to proposed therapeutic properties of these products (Conway et al., 1987; Deeth and Tamime, 1981; Fernandes et al., 1987; Gurr, 1987; Kim, 1989). Particularly in recent years, specific benefits have been attributed to *Lactobacillus acidophilus* and to different species of *Bifidobacterium* (Driessen and De Boer, 1989; Gilliland, 1989; Hunger, 1989; Johnson et al., 1987; Klaver and Kingma, 1989; Tamime and Robinson, 1988). These microorganisms are used for ferment-

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ing the so-called yogurt-related milk products, in addition to or instead of the classical thermophilic yogurt microflora. In order that live microorganisms from the fermented milk products yield specific effects in the human intestine, high initial viable counts as well as high survival rates during stomach passage are necessary. This has given rise to discussions about the establishment of limits or guidelines for viable bacterial count in these products (Cogitore, 1984; Driessen and De Boer, 1989; Kurmann, 1982; Romero et al., 1989; Rohm et al., 1990). Furthermore, changes in the bacterial population during the storage period in retail stores must be taken into consideration (Sinha et al., 1989). Thus, nutritive aspects are one reason having led to the creation of 'new' fermented milk products. Besides this, starter cultures not showing 'over-acidification' (acidification during storage) and with an increased ratio of L(+)-lactate/D(−)-lactate content have been developed and are offered by many culture manufacturers. This has also contributed to the growing outlet and the diversification of fermented milk products.

In the present study we describe the results of screening experiments with a representative number of commercially available yogurt and yogurt-related starter cultures, regarding their bacterial population and their acidification behaviour. For this purpose fermented milk products were produced under laboratory conditions, followed by chilled storage comparable with practical conditions.

Experiments

Starter cultures

A description of the yogurt and yogurt-related starter cultures is given in Table I. Cultures were provided by the following manufacturers (in alphabethical order): Centro Sperimentale del Latte (CSL), Milano, Italy; Chr. Hansen's Laboratorium A/S, Horsholm, Denmark; Lacto-Labo, Dangé St. Romain, France; Laboratoires Miles-Marschall, Épernon, France; National Institute for Dairy Research (NIZO), Ede, The Netherlands; Laboratoire Roger, La Ferté sous Jouarre, France; Sanofi Bio-Industries, Hamm, Germany; Laboratorium Wiesby, Niebüll, Germany. An arbitrary designation code A–H was chosen for the culture manufacturers. Cultures were obtained either as lyophilized or deep-frozen concentrates and maintained according to the guidelines of the producers.

Laboratory production of fermented milk products

UHT milk ('Schärdinger Formil', 2.5% fat content) was enriched with 3% (w/v) full-cream milk powder and used as a base milk. For the manufacture of some products with certain cultures from Wiesby and Sanofi, their commercial bacterial growth-promoting substrates ('Bios 2000' and 'Nährboden', respectively) were added to the milk. After heat pretreatment of the base milk for 20 min at 90°C in 5-1 stainless steel beakers using a Löblich (Vienna, Austria) laboratory steam vessel, the milk was cooled to incubation temperature and then inoculated with the starter cultures at different strengths as suggested by the culture suppliers. Fer-

TABLE I

Starter cultures used in this study

Internal code no.	Producer [a]	Product type [b]	Culture type [c]	Typical property/use as described by the producers
Yogurt cultures				
1	A	N	FD	Rapid acidification; set-style, stirred, drinking yogurt
2		N	FD	Rapid acidification, rich flavour; increased viscosity; set-style, stirred yogurt
3	B	N	DF	Medium in flavour, low viscosity; drinking yogurt
4		N	DF	Medium in flavour, high viscosity;
5		N	DF	Rich in flavour, medium viscosity;
6		N	DF	Low in flavour, very high viscosity; yogurt with a high end-pH value
7		N	FD	Classical yogurt culture
8	C	N	FD	Set-style yogurt
9		S	FD	Stirred yogurt
10		S	FD	Stirred yogurt
11		S	FD	Stirred yogurt
12		S	FD	Mild; stirred yogurt
13	D	N	FD	Classical set-style yogurt
14		N	FD	Classical set-style yogurt
15		N	FD	Increased viscosity; set-style yogurt
16		N	FD	Increased viscosity; set-style yogurt
17		N	FD	High viscosity; set-style yogurt
18	E	S	FD	Stirred yogurt
19		S	FD	Set-style yogurt
20		S	FD	Stirred yogurt
21	F	N	FD	Mild, low viscosity; set-style and stirred yogurt
22 [d]		N	FD	Mild, low viscosity; set-style and stirred yogurt
23		N	FD	Set-style and stirred yogurt
24		N	FD	Set-style and stirred yogurt
25		N	FD	Increased viscosity; set-style and stirred yogurt
26 [d]		N	FD	Increased viscosity; set-style and stirred yogurt
27		N	FD	Increased viscosity; set-style and stirred yogurt
28		N	FD	Increased viscosity; set-style and stirred yogurt

TABLE I (continued)

Internal code no.	Producer [a]	Product type [b]	Culture type [c]	Typical property/use as described by the producers
29	G	N	FD	Set-style yogurt
30		N	FD	Set-style yogurt
Yogurt-related cultures				
31	A	N	DF	Mild, set-style product with *Lactobacillus acidophilus;* increased viscosity
32		N	DF	Mild, set-style product with *L. acidophilus*; increased viscosity;
33		N	DF	Mild, set-style product with *L. acidophilus*; increased viscosity
34		N	DF	Set-style product with *L. acidophilus* and *Bifidobacterium infantis*
35	C	N	FD	Mild, set-style product with *L. acidophilus* and *B. longum*
36		S	FD	Mild, stirred product with *L. acidophilus* and *B. longum*
37	H	N	FD	Mild, set-style product with *L. acidophilus* and *B. bifidum*; low over-acidification
38		S	FD	Mild; stirred product with *L. acidophilus* and *B. bifidum*;
39		N	FD	Mild product with *L. acidophilus* *B. bifidum*; increased viscosity, low over-acidification
40		N	FD	Mild, set-style product with *L. acidophilus* and *B. bifidum*; high viscosity, low over-acidification
41		N	FD	Mild, set-style product with *L. acidophilus* and *B. bifidum*; low over-acidification
42	H	S	DF	Acidophilus milk
43		S	DF	Bifighurt drinks with *B. bifidum*
44	C	S	FD	Acidophilus milk

[a] Arbitrary producer codes do not follow the listing in the text.
[b] N, Natural set product; S, stirred product.
[c] FD, Freeze-dried culture; DF, deep-frozen culture.
[d] Identical to previous culture, except that fermentation was performed until a lower pH value was obtained.

mentation was carried out either using the direct-inoculation of step-wise propagation technique, depending on the type of culture. In the case of deep-frozen culture concentrates it was necessary to prepare dilutions of the freshly-thawed

cultures in a 0.15 M phosphate buffer solution (pH 6.8), prior to milk inoculation. For the production of natural set products, the inoculated milk was filled into 180 ml polystyrene beakers, followed by sealing with aluminium covers. For the production of stirred products, the inoculated milk was filled into 2-1 glass bottles. In order to simulate the production of stirred yogurt the content of the glass bottle was shaken vigorously for 1 min before filling the fermented product into polystyrene beakers. Fermentation was performed under thermophilic conditions, as advised by the culture producers and using thermostated water-baths (Grant W38, Cambridge, U.K.). Only with cultures no. 18, 19, 20 and 38 fermentation was carried out at 32°C as specified. The process was stopped by rapidly cooling the products to 6°C using a circulating water bath (Haake T41, Berlin, Germany). During the fermentation pH was monitored by using pH meters (WTW pH 537, Weilheim, Germany) equipped with Ingold (Steinbach, Germany) 405-60-TT-S7 glass electrodes, connected with a Yokogawa μR-100 6-channel recorder (Tokyo, Japan).

Viable counts

Appropriate decimal dilutions of the milk products were prepared in 0.1% sterile peptone water. The enumeration of characteristic yogurt bacteria was performed according to the provisional standard method of the International Dairy Federation (1983) using the M17 medium for the examination of *Streptococcus salivarius* subsp. *thermophilus* at 37°C for 48 h under aerobic conditions, and the acidified MRS medium for the examination of *Lactobacillus delbrueckii* subsp. *bulgaricus* at 37°C for 72 h under an anaerobic atmosphere containing 10% CO_2, 10% H_2 and 80% N_2 using an anaerobic incubator (Scholzen, Kriens, Switzerland).

Populations of *Lactobacillus acidophilus* were enumerated on TGV (Trypton Glucose Meat Extract)-agar (Galesloot et al., 1961) containing 2% (w/v) sodium chloride. Plates were incubated anaerobically (see above) at 37°C for 48 h.

Viable counts of *Bifidobacterium* spp. were determined according to Klaver (1989) using an MRS medium supplemented with 5% (v/v) mutton blood, 0.05% (w/v) cysteine and 0.1% (w/v) bile salt, and incubated anaerobically (see above) at 37°C for 72 h.

From each product two samples were examined in duplicates. Based on preceding trials, the accuracy of determinations of colony forming units (CFU) was $\pm 25\%$. Selective growth of the bacteria on the different agar media was confirmed by microscopical examination of typical colonies and by checking pure isolates with the API 50 CH system (API bioMérieux, Vercieu, France).

Acidification properties and chemical analyses

Changes in pH were monitored as described above. Potential acidity of the products was estimated based on Soxhlet Henkel degrees (°SH) and titrated with 0.1 N sodium hydroxide.

L(+)- and D(−)-lactic acid content was examined by means of an HPLC method described by Olieman and De Vries (1988); a Shimadzu (Tokyo, Japan) HPLC system was used. Lactose content was determined enzymatically using an UV test combination no. 176303 from Boehringer (Mannheim, Germany).

Results

Viable counts

Table II summarizes the viable count ranges found in the fresh products 24 h after laboratory production and after a storage period of 2 weeks at 6°C. Ratios of typical yogurt bacteria (lactobacilli vs. streptococci) varied from 1 : 0.1 for no. 12 to 1 : 21.8 for no. 13 (results not shown). In general, most of the fresh yogurts (about 80%) contained higher numbers of cocci than rods. With two selected cultures, nos. 21 and 25, a second series of production (nos. 22 and 26) to a lower pH value (4.30 instead 4.60; see Table III) was performed. In these yogurts the ratios between rods and cocci were distinctly higher than in the corresponding 'low acid' products (1 : 0.4 in no. 22 vs. 1 : 2.3 in no. 21, and 1 : 0.3 in no. 26 vs. 1 : 1.5 in no. 25).

Based on practical experiences, a relative change of ±50% of the original viable count was used as a measure for a significant change in bacterial populations during storage. In about 45% of the yogurts the lactobacilli counts increased during 2 weeks. On the other hand, a relative increase of the streptococci was observed only with 25% of the yogurts, whereas 30% (nos. 3, 5, 6, 12, 16, 17, 19, 26, 28) were constant in their bacterial population (results not shown).

In the yogurt-related products, the viable counts of the bacteria other than the classical yogurt flora showed markedly reduced counts after storage for 2 weeks (Table II), with the exceptions that the product microflora of nos. 34 and 39 was stable (results not shown).

Acidification properties

As can be seen from Table III, the duration of time needed for fermentation differed among the cultures. Most fermentations were stopped at pH 4.6, in order

TABLE II

Bacterial counts of the products fermented with the cultures described in Table I

Bacterial composition	Viable count range (CFU/ml)	
	Fresh products [a]	Stored products [b]
Yogurts (n = 30)		
Lactobacillus delbrueckii subsp. *bulgaricus*	5.5×10^7–6.5×10^8	3.4×10^7–5.3×10^8
Streptococcus salivarius subsp. *thermophilus*	3.5×10^7–1.2×10^9	1.0×10^6–1.0×10^9
Yogurt-related products (n = 14)		
L. delbrueckii subsp. *bulgaricus*	6.0×10^6–4.6×10^8	1.4×10^7–5.4×10^8
S. salivarius subsp. *thermophilus*	1.4×10^7–1.1×10^9	1.1×10^7–1.0×10^9
L. acidophilus	4.0×10^5–7.7×10^8	4.0×10^4–2.5×10^8
Bifidobacterium sp.	4.0×10^6–2.6×10^8	4.3×10^4–9.2×10^7

[a] Results obtained with fresh products stored for 24 h at 6°C.
[b] Results obtained with products stored for 14 days at 6°C.

to obtain relatively mild products. In general, the acidity of the fresh yogurts ranged from 34 °SH (nos. 1, 2) to 52.2 °SH (no. 12). Considering the whole period, relative increases in titratable acidity of 12.0% (no. 18) up to 35.3% (no. 24) were measured. The mean value of 'over-acidification' of all yogurts was 22.3%. In contrast to this, the yogurt-related products showed an average increase of 14.9% in titratable acidity, ranging from 1.6% (no. 41) to 27.3% (no. 32). In most products a main portion of the L(+)-lactate enantiomer was found. Only products obtained with cultures no. 9, 11, 12, 22, 26, 35 and 36 has a relatively higher D(−)-lactate content. As mentioned above, with two selected cultures (nos. 20, 21 and 25, 26) two kinds of yogurts, 'mild' and 'sour', were produced. Corresponding results demonstrate, that the 'sour' products had differing lactate proportions compared with those of the 'mild' yogurts. Residual lactose contents of the yogurts showed a mean of 4.40, those of the yogurt-related products of 4.59 g/100g.

Discussion

Both the microbiological composition as well as the stability of the viable count during storage of the products are important properties of yogurt and yogurt-related starter cultures.

The present study has shown that the fresh and the stored yogurts contain total bacterial counts within the range of 10^7–10^9 CFU/ml. These results are in general accordance with earlier findings (Hamann and Marth, 1984; Rohm et al., 1990; Sinha et al., 1989). It has been proposed by Hamann and Marth (1984) and by Kurmann (1982) that yogurt should contain at least 10^6 CFU/ml viable microorganisms at the time of sale. In Italy viable counts between 1 and 5 million bacteria per g are recommended for yogurt (Spolaor et al., 1989). Rohm and Lechner (1988) found that most retail products contained more than 10^7 CFU/ml, and this value could be accepted as a possible limit.

Changes in the bacterial counts during storage of the products were culture-dependent. Similar effects have been described by Hamann and Marth (1984) and by Sinha et al. (1989). It was observed by the former authors that the viable population of yogurt microorganisms increased initially after the yogurt manufacture, and then decreased during prolonged refrigerated storage of the product. It is evident from several reports (e.g., Hamann and Marth, 1984; Hunger, 1985; Rohm et al., 1990; Romero et al., 1989) that the population can be affected by the storage temperature.

Yogurt-related milk products containing *L. acidophilus* and/or bifidobacteria are a microbiologically sensitive group of fermented foods. In particular, bifidobacteria usually exhibit a weak growth in milk (Hunger, 1989). Nevertheless, some synergistic growth-promoting effects between *L. acidophilus* and *B. bifidum* are known, which can be utilized in the manufacture of these products (Klaver and Kingma, 1989). The performance of these bacteria in such products can also be improved by adding growth-promoting additives (e.g., vitamin-enriched protein

TABLE III

Acidification properties of the starter cultures and biochemical parameters of the corresponding milk products

Culture no.	Duration (h) of fermentation	Final pH value [a]	Acidity (°SH) at 6°C				%Ratio of L(+)-lactate vs. D(−)-lactate content [b]	Residual lactose content [b] (g/100 g)
			Fresh product [b]	7d	14d	21d [c]		
1	2.5	4.60	34.0	41.5	42.8	44.0 (29.4)	69/31	4.53
2	3.0	4.60	34.0	40.8	41.8	45.0 (32.4)	100/0	4.55
3	3.2	4.35	43.3	48.7	53.0	53.1 (22.6)	100/0	4.43
4	3.6	4.35	45.8	51.7	53.5	56.7 (23.8)	100/0	4.16
5	3.0	4.35	49.0	51.9	55.0	57.0 (16.3)	100/0	4.27
6	4.0	4.55	38.1	41.5	44.0	44.8 (17.6)	100/0	4.51
7	3.0	4.60	39.6	42.5	45.6	46.0 (16.2)	100/0	4.36
8	2.7	4.60	45.4	51.5	52.5	53.0 (16.7)	55/45	4.34
9	3.2	4.40	45.6	51.8	55.0	59.1 (29.6)	49/51	4.48
10	3.7	4.40	47.9	52.9	53.1	54.7 (14.2)	64/36	4.23
11	3.5	4.40	51.5	53.9	59.3	60.6 (17.7)	41/59	4.24
12	4.3	4.40	52.2	53.4	60.0	60.1 (15.1)	42/58	4.26
13	2.7	4.60	45.2	50.4	52.8	52.9 (17.0)	100/0	4.15
14	2.4	4.60	42.4	48.0	51.1	51.4 (21.2)	100/0	4.20
15	2.4	4.60	40.2	45.9	50.5	52.6 (30.8)	100/0	4.44
16	2.4	4.60	42.0	50.0	50.0	50.0 (19.0)	100/0	4.74
17	2.8	4.60	39.5	45.5	50.5	51.6 (30.6)	100/0	4.76
18	10.3 [d]	4.20	48.3	50.5	52.0	54.1 (12.0)	100/0	4.15
19	11.1 [d]	4.25	47.0	51.4	54.0	57.4 (22.1)	100/0	4.23
20	11.2 [d]	4.25	50.0	53.8	56.1	59.4 (18.8)	100/0	4.21
21	2.9	4.60	36.6	42.0	44.8	45.3 (23.8)	68/32	4.65
22 [e]	3.0	4.30	38.4	42.0	45.6	48.2 (25.5)	38/62	4.60
23	3.1	4.60	39.0	42.0	44.0	50.8 (30.3)	58/42	4.62
24	3.0	4.60	36.8	46.0	48.5	49.8 (35.3)	60/40	4.63
25	3.1	4.60	39.6	48.8	52.0	52.2 (31.8)	60/40	4.50
26 [e]	3.5	4.30	39.8	44.0	46.8	48.0 (22.4)	44/56	4.61
27	2.3	4.60	40.6	44.0	48.2	49.5 (21.9)	56/44	4.34
28	2.6	4.60	38.6	43.6	49.0	49.2 (27.5)	63/37	4.60
29	3.3	4.35	46.4	50.9	52.8	53.0 (14.2)	100/0	4.08
30	2.9	4.40	43.3	47.2	49.3	49.5 (14.3)	100/0	4.30
31	6.3	4.50	31.0	34.4	35.4	36.2 (16.8)	100/0	4.60
32	4.2	4.60	33.0	36.6	41.2	42.0 (27.3)	100/0	4.60
33	6.2	4.60	35.0	36.8	39.2	43.4 (24.0)	100/0	4.27
34	6.6	4.60	39.9	47.8	48.9	50.0 (25.3)	100/0	4.55
35	2.3	4.60	43.2	45.6	49.1	49.4 (14.4)	40/60	4.47
36	3.5	4.40	49.2	55.4	60.4	61.7 (25.4)	36/64	4.29
37	5.2	4.60	37.5	37.9	39.0	39.4 (5.0)	98/2	4.82
38	11.6 [d]	4.40	41.7	42.0	42.0	43.2 (3.6)	65/35	4.81
39	6.6	4.60	37.0	39.2	39.4	40.2 (8.6)	75/25	4.81
40	6.5	4.60	36.8	39.0	39.4	40.1 (9.0)	61/39	4.84
41	4.6	4.60	37.4	37.6	37.6	38.0 (1.6)	86/14	4.79
42	19.5	4.20	34.0	37.3	40.0	40.2 (18.2)	77/23	4.50
43	20.0	4.40	46.4	52.2	56.7	57.5 (23.9)	100/0	4.30
44	15.3	4.20	48.0	48.7	49.1	50.4 (5.0)	100/0	4.60

hydrolysate powders) to the base milks and/or by chosing lower incubation temperatures than usually applied for the manufacture of classical yogurt (Klupsch, 1989b; Hunger, 1985). The present screening tests have shown that many of the fresh products contained bacterial populations of $> 10^8$ CFU/m^2. Similar viable counts were also reported by other researchers (Lee et al., 1988; Miller, 1981; Sharma and Prasad, 1986) for Acidophilus milks and Acidophilus yogurts. However, when comparing the classical yogurts with the yogurt-related products, the microbiological stability of the latter was less pronounced. Gilliland and Lara (1988) have also demonstrated that the *L. acidophilus* count in milk diminishes markedly during storage at 5°C, accompanied by losses of enzymatic activity.

'Over-acidification' is observed most frequently with 'classical' yogurts. This property contributes to other defects such as syneresis, increased reduction of viable counts and the accumulation of D(−)-lactic acid in the product. It is mainly due to the uncontrollable growth of strains of *L. delbrueckii* subsp. *bulgaricus* at low pH values and refrigerated temperatures (Kurmann, 1982). It can be influenced to a limited extent by applying 'good manufacturing practice' and by using cultures with reduced 'over-acidification' behaviour. Another possibility to circumvent this problem is to pasteurize the yogurt after its manufacture. Although this manipulation would aid the manufacturers considerably in handling and distribution, yogurt pasteurization is not commonly applied because many consumers wish to obtain fermented milk products containing live microorganisms providing an intact lactase activity (Driessen and De Boer, 1989; Deeth and Tamime, 1981). 'Mildly-acidifying' yogurt-related cultures containing *L. acidophilus* and bifidobacteria (Hunger, 1985; Klupsch, 1989b; Miller, 1981) offer the advantage of less 'over-acidification'. Furthermore, these cultures give reduced contents of the D(−)-lactate enantiomer in the products. The present study has shown that most of the yogurt and the yogurt-related cultures tested yielded products with increased L(+)-lactate proportions. It must, however, be taken into consideration that the ratio of lactate isomers in the products changes during storage (Kunath and Kandler, 1980).

Although modern biotechnology offers some possibilities to 'construct' starter cultures by means of genetic engineering, the traditional selection of microorganisms still plays an important role. The present screening study has demonstrated that many of the cultures are capable of fulfilling a series of current microbiological and biochemical requirements.

Notes to Table 3:

1–30, Yogurt cultures.

31–44, Yogurt-related cultures.

[a] Fermentation was stopped at this pH value.

[b] Data represent results obtained with fresh products stored for 24 h at 6°C.

[c] Relative percentage increase in acidity after storage for 21 days at 6°C indicated in parentheses.

[d] Fermentation was performed under mesophilic conditions (see text).

[e] Identical to previous culture, except that fermentation was performed until a pH value of 4.30 was obtained.

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