ELSEVIER

International Dairy Journal 12 (2002) 579–589

INTERNATIONAL DAIRY JOURNAL

www.elsevier.com/locate/idairyj

Influence of compounds associated with fermented dairy products on the growth of lactic acid starter and probiotic bacteria

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Received 15 September 2001; accepted 28 January 2002

Abstract

The growth of 24 strains of lactic acid starter bacteria (*Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Lactococcus lactis*) and 24 strains of probiotic bacteria (*Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus paracasei*, *Lactobacillus rhamnosus* and bifidobacteria) in liquid media containing different substances was assessed. The substances used were salts (NaCl and KCl); sugars (sucrose and lactose); sweeteners (acesulfame and aspartame); aroma compounds (diacetyl, acetaldehyde and acetoin); natural colorings for fermented milk (red, yellow and orange colorings); flavoring agents (strawberry, vanilla, peach and banana essences); flavoring–coloring agents (strawberry, vanilla and peach); nisin, natamycin and lysozyme. Bacterial growth in the presence of natural fruit juices (green apple, kiwi, pineapple, peach and strawberry) with or without neutralization and cell viability in lactic acid acidified (pH 4 and 5) milk for 4 weeks at 5°C were also studied.

Some compounds (KCl, sweeteners, aroma compounds, natamycin, flavoring agents and the peach flavoring–coloring agent) did not influence the growth of the strains in the concentrations commonly used in the dairy industry. The effect of other substances (especially flavoring–coloring agents) on the growth of lactic acid starters and probiotic bacteria was strain-dependent. Natural fruit juices weakly inhibited mainly *S. thermophilus* strains. Cell viability during cold storage in acidified milk was satisfactory for *L. delbrueckii* subsp. *bulgaricus* and *L. casei* group strains. For *L. acidophilus* and *Bifidobacterium*, the decreases in cell counts at pH 5 were negligible. Nevertheless, decreases from 1.6 to 6.2 and from 0.1 to 7.6 log orders, respectively were observed at pH 4.

Keywords: Lactic acid starters; Probiotic bacteria; Additives; Dairy products; Growth inhibition

1. Introduction

Food additives are indispensable for (and sometimes even characteristic of) milk drinks and other dairy products (Spreer, 1998). These additives become part of the food, in order to provide some very specific and precisely defined sensory characteristics such as taste, appearance, consistency, or shelf life (Nakazawa & Hosono, 1992; Gilliland, 1998; Spreer, 1998). Combinations of different bacterial strains belonging to the genera *Lactobacillus, Streptococcus* and *Bifidobacterium*, have been used traditionally in fermented dairy products to promote human health (Prasad, Gill, Smart, & Gopal, 1998; Dunne et al., 1999). These microorganisms are selected on the basis of medical, scientific and technological criteria (Collins, Thornton, & Sullivan, 1998). The cultures used must tolerate the manufacturing process which they are to undergo so as to prepare a bioproduct (Charteris, Kelly, Morelli, & Collins, 1998) and maintain cell viability during storage. Strain survival in the product will depend on many factors such as pH, presence of preservatives (Charteris et al., 1998) and even the occurrence of potential microbial growth inhibitors (Collins et al., 1998). Some common additives used in the dairy industry are salts, sugars, fruits, sweeteners, colorings, flavorings, nisin, natamicyn and lysozyme. The effects of additives on the growth of lactic acid starter and probiotic bacteria have not been extensively studied (Samona & Robinson, 1993; Gomes, Teixeira, & Malcata, 1998; Rada & Dlabal, 1998; Vachon & Ustunol, 1998) and further information is still needed. Beyond the additives used in the dairy industry, some products of the lactic acid starter metabolism (diacetyl, acetaldehyde, lactic acid) could

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0958-6946/02/$ - see front matter
PII: S0958-6946(02)00046-8

be associated with the loss of viability of added probiotic bacteria (Post, 1996). The aims of this work were to determine the influence of additives commonly used in the dairy industry on the growth of lactic acid starter bacteria (*Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus* and *Lactococcus lactis*) and probiotic bacteria (*Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus paracasei*, *Lactobacillus rhamnosus* and bifidobacteria); as and to assess the influence of compounds produced by the lactic acid starter bacteria during their growth in milk (aroma compounds and lactic acid).

2. Materials and methods

2.1. Strains

The strains used in this study are shown in Table 1. For simplicity, "*L. casei* group" will refer to the three species used (*L. casei*, *L. paracasei* and *L. rhamnosus*) identified earlier as belonging to this group (Klein, Pack, Bonaparte, & Reuter, 1998).

2.2. Culture media and incubation conditions

Lactobacilli (aerobiosis) and bifidobacteria (anaerobiosis, GasPak System-Oxoid, Basingstoke, Hampshire, England) were cultured in MRS broth (Biokar, Beauvais, France) at 37°C. Lactococci and streptococci were grown in Elliker broth (Biokar, Beauvais, France) at 25°C and 37°C, respectively, aerobic incubation. For lactobacilli and bifidobacteria, cell enumerations were carried out on MRS agar (Biokar, Beauvais, France, 48 h at 37°C, aerobiosis and anaerobiosis, respectively). For lactococci and streptococci, viable counts (48 h at 25°C and 37°C—respectively, aerobiosis) were performed on Elliker agar (Biokar, Beauvais, France).

Table 1
Lactic acid starter and probiotic strains used in this study

Organism/strain	Origin[a]
S. thermophilus	
43, 175, 176	Natural milk cultures
A4, A5, A10, DC1, CC1	Comercial cultures
L. delbrueckii subsp. *bulgaricus*	
Ab1, Bb1, Cb1, Db1, Eb3, Eb4, Gb1, Hb2	Commercial cultures
L. lactis	
13-3, 15-1, 15-4, C12, SL3, SD5, Mo12, A6	Commercial cultures
B. bifidum	
A12, BBI, Bb12	Commercial cultures
35914	ATCC collection
B. longum	
A1, A7, BL	Commercial cultures
Bifidobacterium sp.	
A2	Commercial culture
L. acidophilus	
A3, A9, 08, 53, 5, CSL	Commercial cultures
1881, 1923	CNRZ collection
L. paracasei	
A13, A14, BRA, LB	Commercial cultures
L. rhamnosus	
A15, A16, LS	Commercial cultures
L. casei	
1874	CNRZ collection

[a] "Commercial cultures" indicates material provided by various commercial suppliers. Sources available on request.

2.3. Test materials and methodologies

2.3.1. Additives

The food additives and other compounds used in this study are shown in Table 2. The effect of them on strain growth was assessed by the growth-in-liquid-medium assay (GLM assay) as follows: overnight broth cultures were inoculated (2%) in test tubes containing 3 mL of culture medium (MRS or Elliker broth, for lactobacilli/bifidobacteria or cocci, respectively) plus the additives in the concentrations mentioned above. The relative growth (after 24 h) of the strains in the presence of each chemical assessed was expressed as the percentage of optical density at 600 nm ($O.D._{600\,nm}$), of the culture with respect to a control culture (without the addition of the chemical assessed). Values of a relative growth lower than 30% or higher than 70% were considered negative or positive results, respectively. Values of a relative growth ranging within these limits were regarded as weak.

2.3.2. Natural fruit juices

Fresh fruits (green apple, kiwi, pineapple, peach and strawberry) were washed, peeled, trimmed and centrifuged (6000 rpm, 30 min). Aliquots of each juice were neutralized (pH 7.0) with NaOH pellets (Mallinckrodt, Buenos Aires, Argentina). The supernatants (with or without neutralization) were sterilized (100°C, 15 min) and kept frozen (−80°C) until used. The effect of fruit juices on the growth was determined by the well-diffusion-agar assay (WDA assay) as follows: 20 mL of MRS or Elliker agar (Biokar, Beauvais, France), melted and tempered at 45°C, were vigorously mixed with 200 μL of an overnight culture of lactobacilli/bifidobacteria or cocci, respectively, and poured into Petri dishes. Wells of 10 mm in diameter were made in the agar layer and 180 μL of each fruit juice (natural or neutralized) were placed into each well. The plates were incubated aerobically (except for bifidobacteria that were incubated in anaerobiosis) overnight at 37°C; lactococci plates were incubated at 25°C. The diameters (mm) of the inhibition halos were recorded.

2.3.3. Lactic acid bacteria metabolites

The influence of aroma compounds (diacetyl, acetaldehyde and acetoin, Sigma, St. Louis, USA) was

Table 2
Food additives used in this study

Additive	Concentration[a] (% w/v)	Origin[b]
Salts		
NaCl	**1**, **2**	Sigma, St. Louis, USA
KCl	**1**, **2**	Sigma, St. Louis, USA
Sugars		
Sucrose	**5**, **15**, **20**	Cicarelli, Santa Fe, Arg.
Lactose	**5**, **15**, **20**	Cicarelli, Santa Fe, Arg.
Sweeteners		
Acesulfame	**0.03**, 0.12	Commercial supplier S1
Aspartame	**0.03**, 0.12	Commercial supplier S1
Natural colorings		
Carmine (red)	**0.027**, 0.054 (v/v)	Commercial supplier S2
Curcuma/Bixin (yellow)	**0.044**, 0.088 (v/v)	Commercial supplier S2
Bixin (orange)	**0.022**, 0.044 (v/v)	Commercial supplier S2
Flavorings		
Peach	**0.08**, 0.16 (w/w)	Commercial supplier S3
Strawberry 1	**0.08**, 0.16 (w/w)	Commercial supplier S3
Strawberry 2	**0.08**, 0.16 (w/w)	Commercial supplier S3
Vanilla	**0.07**, 0.14 (w/w)	Commercial supplier S3
Strawberry 3	**0.1**, 0.2	Commercial supplier S4
Banana	**0.1**, 0.2	Commercial supplier S4
Flavoring–colorings		
Strawberry 1	**0.4**, 0.8	Commercial supplier S5
Strawberry 2	**0.4**, 0.8	Commercial supplier S5
Peach	**0.4**, 0.8	Commercial supplier S5
Vanilla	**0.12**, 0.24	Commercial supplier S5
Others		
Nisin	**1**, 2 ppm	Commercial supplier S6
Natamycin	**5**, 10 ppm	Commercial supplier S7
Lysozyme	**25**, **50** ppm	Sigma, St. Louis, USA

[a] The concentrations chosen for this study correspond to those normally used in the Argentinean dairy industry (in bold type) and higher.

[b] Sources of materials indicated as "Commercial suppliers" available upon request.

studied by the GLM assay, as described above. The concentrations chosen were those commonly produced by dairy starters in fermented dairy products (Benito de Cardenas, Portillo, Rivadeneira, Medina, & Oliver, 1992; Tridjoko, Bouillanne, Landon, & Desmazeaud, 1992; Imhof, Glattli, & Bosset, 1995) and higher (500 and 1000 ppm). To determine the effect of lactic acid on cell viability, broth cultures of the strains were harvested in the stationary phase by centrifugation at 12,000*g* for 5 min at 5°C, washed twice in 50 mM K_2HPO_4 (pH 6.5) and resuspended in the same buffer. The cell suspensions were inoculated (1.5% v/v) in test tubes containing reconstituted (10% w/v) skim milk (Merck, Darmstadt, Germany) acidified with lactic acid (90%, Anedra, Buenos Aires, Argentina) at pH 6.5 (control), 5 and 4, without a head-space. The samples were cold stored (5°C) for 4 weeks. Before and after the incubation period, cell enumerations were performed using the culture media and the incubation conditions described above.

2.4. Growth kinetics

According to the results obtained with the techniques described above, growth kinetics of some strains were determined. The strains were grown in broth in the presence (test curve) or absence (control curve) of some of the food additives studied. Bacterial growth was measured by $O.D._{600\,nm}$.

2.5. Statistical analysis

Experiments were carried out in duplicate. The results of the survival assayed during cold storage in acidified milk were analyzed using the one-way ANOVA procedure of SPSS. The differences among means were evaluated by the Duncan's multiple range test (Lizasoain & Joaristi, 1995).

3. Results and discussion

The worldwide production and consumption of fermented milk products increased dramatically during the last quarter of the past century due to the introduction of fruit-flavored and aromatic yogurts. Sensory characteristics play an important role in the product acceptance by consumers. In this sense, dairy products are almost always consumed first and foremost due to their flavor. When designing a dairy product the desired sensory properties must be taken into account together with the tolerance of the specific dairy microorganisms to the chemicals used to attain those properties. Nowadays, probiotic bacteria are widely used as adjunct cultures for fermented milk and cheeses. These products contain a great variety of added

compounds and their influence on lactic acid starter and probiotic bacteria is not very well known. The list of dairy additives includes salts, sugars, sweeteners, colorings, flavorings, fruits and preservatives as nisin, natamicin and lysozyme (Çon, Çakmakçi, Çaglar, & Gokalp, 1996; Charteris et al., 1998; Gardini, Lanciotti, Guerzoni, & Torriani, 1999; Lee, Nomoto, Salminen, & Gorbach, 1999).

Among the substances tested, only acesulfame, natural red and orange colorings (carmine and bixin, respectively), acetoin, flavoring of peach and strawberry 3, and natamycin did not interfere with the growth of the lactic acid starter and probiotic bacteria strains used in this study, at both concentrations tested (data not shown). Some other additives (KCl, sucrose, aspartame, flavoring of strawberry 1 and 2, flavoring of vanilla and banana and flavoring–coloring commercial mixture of peach) were inhibitory but only at the highest concentrations tested. For the other compounds, growth inhibitions were observed even at the concentrations that are commonly used in the dairy industry (Tables 3–5).

Sweeteners (acesulfame and aspartame), at the concentration normally used in fermented dairy drinks (0.03%) were not inhibitory for lactic acid starter and probiotic bacteria. The highest concentration tested (0.12%) of aspartame was only inhibitory for three lactic acid bacteria strains and one probiotic strain. Likewise, Samona et al. (1993), working with aspartame (0.04 and 0.08%) had not detected any influence during growth of bifidobacteria in milk.

There are a wide variety of yogurts produced in the world. Depending on the type of product, the total sugar content (mainly sucrose and lactose) ranges from 5.2% to 21% (Birollo, Reinheimer, & Vinderola, 2000). In this work, probiotic bacteria proved to be less sensitive to the presence of sugars than lactic acid starter bacteria. Among the former, only some strains of bifidobacteria were inhibited by 15% or 20% of sucrose and/or lactose. On the other hand, 15% of both sugars was inhibitory for some strains of all lactic acid starter species studied. Furthermore, three *Lactococcus lactis* strains did not grow even in the presence of 5% of lactose, but no strain was inhibited by 5% sucrose. A great variability in the viability of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* was previously observed by Birollo et al. (2000) in yogurts with a different content of total sugars.

Diacetyl is a flavor compound commonly found in fermented dairy products that possesses antimicrobial activity. At concentrations of 50 ppm, it strongly inhibited the growth of *Escherichia coli* (Kang & Fung, 1999). According to our study, aroma compounds (diacetyl, acetaldehyde and acetoin) were not inhibitory for the growth of lactic acid starter or probiotic microflora at the concentrations commonly present in fermented milk and cheeses (data not shown). At higher levels (500 and 1000 ppm), acetoin proved to be innocuous for the species tested while acetaldehyde and diacetyl showed inhibitory effects. Among probiotic bacteria, most *Bifidobacterium* strains were inhibited by both compounds at 1000 ppm while the majority of *L. acidophilus* strains were inhibited by 1000 ppm of diacetyl. On the other hand, a higher number of lactic acid starter strains (including all *S. thermophilus* strains) were sensitive to these compounds.

NaCl is widely used in the food industry as a preservative agent, to impart sensory characteristics and to satisfy the human daily requirements. Additionally, NaCl is important for controlling cheese ripening (Reinheimer, Renzulli, Rubiolo, Bailo, & Binetti, 1997). The salt content of fermented dairy products might jeopardize the cell viability of the probiotic cultures (Gomes, Teixeira, & Malcata, 1998). In our work, lactic acid bacteria appeared to be more sensitive to salts, particularly NaCl, than probiotic bacteria. Contrary to the findings of Gomes et al. (1998), *Bifidobacterium* strains were more sensitive to salts than *L. acidophilus* strains since only some strains of *Bifidobacterium* were inhibited by 2% of NaCl among the probiotic strains used in this study. *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* were the more sensitive lactic acid species; strains of the former were affected by 2% salts and strains of the latter were affected by 1% and 2% of NaCl and 1% of KCl. In general, KCl was less inhibitory than NaCl. According to these results, the NaCl concentration used (0.9%) in Argentinean probiotic cheese (Vinderola, Prosello, Ghiberto, & Reinheimer, 2000) seems to jeopardize neither probiotic nor lactic acid starter bacteria cell viability.

Natural colorings for fermented milk (carmine, curcuma/bixin and bixin) did not affect the growth of probiotic bacteria. No inhibitions were observed for *L. delbrueckii* subsp. *bulgaricus* either. Only the cocci strains assessed (mainly *S. thermophilus*) were inhibited at curcuma/bixin (yellow colorant for yogurts) concentrations commonly used in the dairy industry.

Flavorings of strawberry (1 and 2), vanilla or banana affected only a few strains of yogurt bacteria (*S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*). On the other hand, flavoring–colorant commercial mixtures proved to have an important inhibition potential since growth inhibitions were observed even at the concentrations recommended by suppliers, except for the flavoring–colorant commercial mixture of peach, for all the strains assessed with the exception of those belonging to the *L. casei* group or *L. lactis*.

Lysozyme is an attractive preservative because it inhibits food spoilage and bacteria which are harmful to human health (Spahr & Url, 1994) but does not significantly affect the growth of thermophilic lactobacilli and streptococci, at levels up to 25 ppm (Lodi, Oggion, Vezzoni, & Carini, 1983). In some countries,

Table 3
Growth of probiotic bacteria in liquid medium (MRS broth, 37°C) in the presence of aroma compounds and additives used in the dairy industry after 24 h incubation

Organism[a]	Diacetyl	FC strawberry 1[b]		FC strawberry 2[b]		FC vanilla[b]		Organism[a]	Aspartame	Nisin (ppm)	
	1000 ppm	0.4%	0.8%	0.4%	0.8%	0.12%	0.24%		0.12%	1	2
L. acidophilus								*L. casei* group			
A3	−	−	−	−	−	−	−	A13	+	+	+
A9	−	−	−	−	−	w	−	A14	+	+	+
08	−	+	w	+	w	+	w	A15	w	−	−
53	−	−	−	−	−	−	−	A16	+	+	+
CSL	+	−	−	−	−	−	−	LB	+	w	−
La5	−	−	−	−	−	−	−	BRA	+	+	+
CNRZ 1881	−	−	−	−	−	−	−	LS	+	−	−
CNRZ 1923	−	+	+	+	w	+	w	CNRZ 1874	+	−	−

Organism[a]	NaCl	Sacarose (%)		Lactose (%)		Acetaldehyde (ppm)		Diacetyl (ppm)		FC strawberry 1[b]	FC strawberry 2[b]	Nisin
	2%	15	20	15	20	500	1000	500	1000	0.8%	0.8%	2 ppm
Bifidobacterium												
A1	−	−	−	−	−	+	−	−	−	w	w	−
A2	+	+	+	+	−	+	−	+	−	+	+	−
A7	−	−	−	−	−	+	+	−	−	w	+	+
A12	+	+	+	+	−	+	−	+	+	+	w	+
BBI	+	+	+	+	−	−	−	+	−	+	+	−
Bb12	+	+	+	+	+	+	+	+	−	w	w	−
BL	+	+	+	+	−	+	−	+	+	w	w	−
ATCC 35914	nd	nd	nd	nd	nd	nd	nd	nd	nd	w	−	−

[a] For these strains, a normal growth (+) was recorded in the presence of the aroma compounds and food additives from Table 2 not included here.
[b] Flavoring–coloring commercial mixture.
−: No growth ($A_{600\,nm}$ <30% of the control culture).
+: Normal growth ($A_{600\,nm}$ >70% of the control culture).
w: Weak growth (30% < $A_{600\,nm}$ <70% of the control culture).
nd: Not determined.

Table 4
Growth of lactic acid starter bacteria in liquid medium (MRS broth, 37°C for lactobacilli; Elliker broth, 37°C for streptococci and 25°C for lactococci) in the presence of aroma compounds, flavorings and flavoring–colorings commercial mixtures after 24 h incubation

Organism[a]	Acetaldehyde[b]		Diacetyl (ppm)		Curcuma/bixin		F1[c]	F2[d]	FV[e]	FB[f]	FC1[g] (%)		FC2[g](%)		FCV[g](%)		FCP[g]
	500	1000	500	1000	0.044%	0.088%	0.16%	0.16%	0.14%	0.2%	0.4	0.8	0.4	0.8	0.12	0.24	0.8%
S. thermophilus																	
A5	–	–	–	–	+	–	+	–	–	+	–	–	–	–	–	–	–
A5	–	–	–	–	–	–	+	+	+	+	+	–	–	–	–	–	+
A10	–	–	–	–	–	–	–	+	+	+	+	w	–	–	–	–	+
DC1	–	–	–	–	–	–	+	+	+	+	–	–	–	–	–	–	+
CC1	–	–	–	–	–	–	+	+	+	+	–	–	–	–	–	–	+
43	–	–	–	–	–	–	+	+	+	+	–	–	–	–	–	–	+
175	–	–	–	–	–	–	+	+	+	+	–	–	–	–	–	–	+
176	–	–	–	–	–	–	+	+	+	+	–	–	–	–	–	–	+
L. bulgaricus																	
Ab1	+	+	+	–	+	+	+	+	+	+	w	–	–	–	w	–	+
Bb1	+	–	+	–	+	+	+	+	+	–	–	–	–	–	–	–	+
Cb1	+	+	+	–	+	+	+	+	+	w	–	–	–	–	+	+	+
Db1	+	–	+	–	+	+	+	+	+	w	–	–	–	–	–	–	+
Eb3	–	–	+	–	+	+	+	+	+	w	–	–	–	–	–	–	+
Eb4	+	–	+	–	+	+	+	+	+	w	–	–	–	–	–	–	+
Gb1	+	+	+	+	+	+	+	+	+	w	–	–	–	–	+	–	+
Hb2	+	+	+	–	+	+	+	+	+	w	–	–	–	–	–	–	+
L. lactis																	
A6	+	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+
15-1	+	+	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+
15-4	–	–	–	–	–	–	+	+	+	+	+	+	+	–	+	+	+
C12	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+
Mo12	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+
13-3	+	+	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+
SD5	–	–	+	–	+	–	+	+	+	+	+	+	+	+	+	+	+
SL3	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+

[a] For these strains, a normal growth (+) was recorded in the presence of the aroma compounds and food additives from Table 2 not included here.
[b] In ppm.
[c] Flavoring of strawberry 1.
[d] Flavoring of strawberry 2.
[e] Flavoring of vanilla.
[f] Flavoring of banana.
[g] Flavoring–coloring commercial mixtures of strawberry 1 and 2, vanilla and peach, respectively.
–: No growth ($A_{600\,nm}$ < 30% of the control culture). +: Normal growth ($A_{600\,nm}$ > 70% of the control culture). w: Weak growth (30% < $A_{600\,nm}$ < 70% of the control culture).

lysozyme is used in cheese technology for the prevention of late blowing of cheeses (Spahr et al., 1994), although its relative high cost currently limits its widespread use (Russel, Hugo, & Ayliffe, 1991). Lysozyme could be used in probiotic semihard and hard cheeses but it would be important to know the resistance of probiotic cultures to it. In the present study, no effect was observed for probiotic bacteria while for lactic acid starter bacteria, a strain selection would appear mandatory if lysozyme is used at levels of 50 ppm, or higher.

Natamycin is an antifungal antibiotic that is permitted in some countries for surface preservation of cheese but is not active against bacteria (Russel et al., 1991). The results obtained in this study showed that it did not affect the growth of lactic acid starter and probiotic bacteria.

Nisin, a polypeptide-type antibiotic produced by *L. lactis*, is active against spore-forming bacteria. It is permitted in many countries for the preservation of processed cheeses and other dairy products (Russel et al., 1991). The antimicrobial spectrum of nisin is narrow and limited to Gram-positive bacteria, including *Bacillus, Clostridium* and lactic acid bacteria (Spahr et al., 1994, Wirjantoro, Lewis, Grandison, Williams, & Delves-Broughton, 2001). In this study only *L. acidophilus* and *S. thermophilus* strains proved to be resistant to nisin (2 ppm). In accordance with Rada et al. (1998) and Kot, Murad, and Bezkorovainy (2001), the sensitivity of bifidobacteria was variable, but resistant strains could be selected. Several strains of the other species tested in this study were inhibited by 1 ppm of this additive.

Table 5
Growth of lactic acid starter bacteria in liquid medium (MRS broth, 37°C for lactobacilli; Elliker broth, 37°C for streptococci and 25°C for lactococci) in the presence of additives used in the dairy industry after 24 h incubation

Organism[a]	NaCl (%)		KCl (%)		Nisin (ppm)		Lysozyme (ppm)		Sacarose (%)		Lactose (%)			Aspartame
	1	2	1	2	1	2	25	50	15	20	5	15	20	0.12%
S. thermophilus														
A5	+	–	+	+	+	+	+	+	+	–	+	+	–	–
A5	+	–	+	+	+	+	+	+	+	+	+	–	+	+
A10	+	–	+	+	+	+	+	+	–	–	+	–	–	+
DC1	+	–	+	+	+	+	+	w	–	–	+	–	–	+
CC1	+	+	+	+	+	+	+	+	+	–	+	–	–	+
43	+	+	+	+	+	+	+	+	+	–	+	+	–	+
175	+	–	+	–	+	+	w	w	–	–	+	+	–	+
176	+	–	+	+	+	+	+	+	+	–	+	–	–	+
L. delbrueckii subsp. *bulgaricus*														
Ab1	+	+	+	+	+	+	+	+	+	–	+	+	–	+
Bb1	–	–	+	–	–	–	+	–	–	–	+	+	–	+
Cb1	+	+	+	+	+	+	+	+	+	+	+	+	–	+
Db1	–	–	+	–	–	–	+	+	+	–	+	+	–	+
Eb3	–	–	+	+	–	–	+	+	–	–	+	–	–	+
Eb4	+	–	+	+	–	–	+	–	+	+	+	–	–	+
Gb1	+	+	+	+	–	–	+	+	+	+	+	+	–	+
Hb2	+	–	+	+	–	–	+	w	–	–	+	–	–	+
L. lactis														
A6	+	+	+	+	+	+	+	+	+	–	–	–	–	+
15-1	+	+	+	+	+	+	+	+	–	–	+	–	–	–
15-4	+	–	+	+	–	–	+	+	–	–	–	–	–	–
C12	+	+	+	+	+	w	+	+	+	–	+	+	–	+
Mo12	+	+	+	+	+	+	+	w	+	–	+	+	+	+
13-3	+	+	+	+	+	w	+	+	+	–	–	–	–	+
SD5	+	+	+	+	+	w	–	–	–	–	+	–	–	+
SL3	+	+	+	+	+	w	+	+	+	+	+	–	–	+

[a] For these strains, a normal growth (+) was recorded in the presence of all the aroma compounds and the food additives from Table 2 not included here.
–: No growth ($A_{600\,nm}$ <30% of the control culture).
+: Normal growth ($A_{600\,nm}$ > 70% of the control culture).
w: Weak growth (30% < $A_{600\,nm}$ <70% of the control culture).

Since fermented dairy products have a high nutritional quality, the dairy industry is continually experimenting with the production of different types of yogurts, including fruit-added yogurts (Çon et al., 1996). However, there are insufficient data regarding the effect that fruits may have on the survival of microflora of these products (Venizelou, Kehagias, Samona, & Koulouris, 2000). The effect of natural fruit juices on the growth of lactic acid starter and probiotic bacteria used in this study is shown in Table 6. Among the juices assessed, strawberry juice showed the highest inhibition capacity, in accordance with Lee et al. (1999), since it inhibited strains of all species used except for *L. casei* group strains. For the remaining juices, pineapple and kiwi affected strains of three species, green apple strains of 2 species and peach strains of one species. Lactic acid starter bacteria, principally streptococci and lactococci, proved to be more sensitive to fruit juices than probiotic bacteria. *S. thermophilus* was inhibited by all fruit juices tested while *L. delbrueckii* subsp. *bulgaricus* was not affected by them except four strains inhibited by strawberry juice. For probiotic bacteria, the strains of the *L. casei* group were insensitive while only one strain of *L. acidophilus* (CNRZ 1881) and *Bifidobacterium* (*B. longum* A1) showed some degree of inhibition by three and one fruit juice, respectively. Neutralized juices inhibited neither lactic acid starter nor probiotic bacteria, indicating that acid injury was responsible for the inhibitory effect (Frank & Hassan, 1998). Venizelou et al. (2000) reported a suppression of growth for yogurt bacteria in the presence of high levels of fruit juices, this effect being more evident in strawberry yogurt and for *L. delbrueckii* subsp. *bulgaricus* more than for *S. thermophilus*. On the other hand, the addition of fruit flavors (cherries, oranges, strawberries and bananas) or sweeteners to yogurt did not show a significant effect on total bacteria (Çon et al., 1996).

Table 6
Inhibition of lactic acid starter and probiotic bacteria by natural fruit juices (well-diffusion agar assay, well diameter: 10 mm)

Species/strain	Inhibition halo (mm)				
	Strawberry	Pineapple	Peach	Kiwi	Green apple
S. thermophilus strains	22.3±1.9[a,b]	20.6±1.8[b]	18.3±2.2[b]	23.5±2.8[c]	17.7±2.1[a,b]
L. lactis strains	16.3±2.8[b]	16.0 ± 2.4[b]	—	18.2±3.0[b]	14.8±2.3[d,b]
L. delbrueckii subsp. *bulgaricus* strains	15.1±2.1[e,b]	—	—	—	—
L. acidophilus CNRZ 1881	16.7±1.1[b]	14.3±1.2[b]	—	14.9±0.8[b]	—
B. longum A1	11.7±0.7[b]	—	—	—	—
L. casei group	—	—	—	—	—

[a] For these means, *S. thermophilus* 176 was not included since it was not inhibited by strawberry or green apple juices.
[b] Partial growth inhibition (less turbidity in the halo than in the rest of the Petri plate).
[c] Some strains were partially inhibited while others were completely inhibited (complete absence of turbidity within the halo in the Petri plate).
[d] For this mean, *L. lactis* Mo12, SD5 and SL3 were not included since they were not inhibited by strawberry juice.
[e] For this mean, *L. delbrueckii* subsp. *bulgaricus* Ab1, Bb1, Cb1 and Gb1 were not included since they were not inhibited by strawberry juice.
−: Normal growth (absence of halo).

Table 7
Changes in viable cell counts (difference between counts at time 0 and after 4 weeks of cold storage (5°C), in log CFU mL^{-1}) of lactic acid starter and probiotic bacteria in milk acidified with lactic acid at different pH values

Organism	pH		
	6.5	5.0	4.0
S. thermophilus	0.19±0.30	0.16±0.32	6.04±0.46
L. delbrueckii subsp. *bulgaricus*	−0.14±0.35	−0.10±0.35	0.21±0.16
L. lactis	−0.45±0.18	−0.05±0.28	6.32±1.03
L. casei group	−0.01±0.42	−0.02±0.33	0.33±0.10
Bifidobacterium	0.24±0.47	0.15±0.41	0.37[a]±0.25
L. acidophilus	0.96±0.58	1.63±0.83	4.59±1.63

[a] For calculating this mean and standard deviation, losses in viable cell counts of *B. longum* A1 and A7 (6.3 and 7.6 log orders, respectively) were not included.
Negative value implies slightly higher cell counts at the end of the cold storage than at the beginning of it.

A satisfactory cell viability (count decrease <0.3 log) at pH 6.5 and 5 was observed for all the strains assessed in milk acidified with lactic acid (Table 7), except for *L. acidophilus* that suffered cell viability losses between 0.2 and 2.8 log (CFU mL^{-1}), depending on the strain, at pH 5. However, at pH 4, only *L. delbrueckii* subsp. *bulgaricus*, *Bifidobacterium* (except *B. longum* A1 and A7) and *L. casei* group strains did not experience a significant loss (count decrease <0.4 log) of cell viability during the 4 weeks of cold storage. For cocci, the cell viability losses were higher than 6 log (CFU mL^{-1}), while for *L. acidophilus* these values ranged from 1.7 to 6.2 log (CFU mL^{-1}). According to previous results (Gomes et al., 1998) a higher loss in cell viability was observed for *L. acidophilus* than for *B. lactis* in milk during cold storage. Hughes and Hoover (1995) also observed no decrease in cell counts for bifidobacteria cultures maintained in non fermented milk for 15 d and cell count lowering between 1 and 2 log (CFU mL^{-1}) in acidified milk (pH 5). As in our work, these authors concluded that the resistance to lactic acid observed for bifidobacteria was, in general, satisfactory.

Taking into account the preceding results from this study, the growth of some strains in the presence of some additives was evaluated by means of O.D. measurements. This allowed to show different behaviors: no effect, growth delay and complete growth inhibition. A clear bacteriostatic effect (complete growth inhibition) was observed for *L. acidophilus* A3 and A9 in broth cultures (24 h) with the addition of flavoring–coloring commercial mixtures of vanilla and strawberry 1 and 2 (data not shown). However, for *S. thermophilus* A5 (Fig. 1) a complete inhibition was observed during 24 h only for flavoring–coloring commercial mixtures of strawberry 2 while for the others two, the cultures experienced a delay of at least 10 h. Then, the strain grew up to reach O.D. values similar to the control. When *L. acidophilus* 53 and *L. paracasei* LB were grown in the presence of flavoring–coloring commercial mixtures of strawberry 1 (Fig. 2), only the former was delayed up to 10 h. Then it developed moderately. Fig. 3 shows that the growth of *L. lactis* A6 was delayed in different degrees depending on the flavoring–coloring commercial mixture considered. On the other hand, strains of *S. thermophilus* (A4 and A10) were completely inhibited in 24 h cultures by 0.044% (v/v) curcuma/bixin commercial mixture (data not shown).

Growth kinetics determined for lactobacilli showed that nisin (1 ppm) inhibited completely *L. thamnosus* LS but it did not affect the growth of *L. paracasei* A13 (Fig. 4). No effect was detected for *L. delbrueckii* subsp. *bulgaricus* Ab1 in the presence of lysozyme but this additive produced a complete growth inhibition on *L. delbrueckii* subsp. *bulgaricus* Bb1 up to 10 h (Fig. 5).

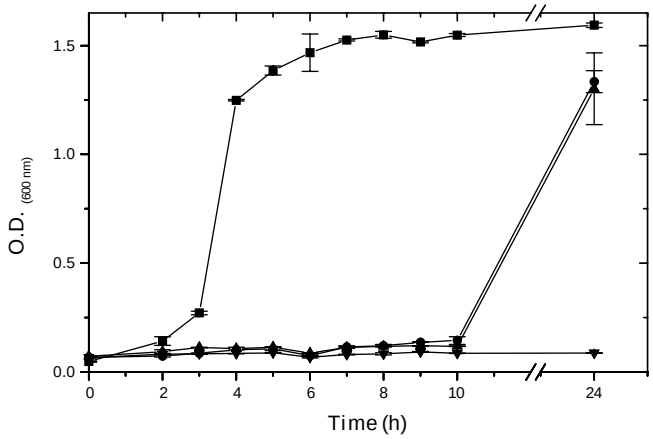

Fig. 1. Growth (O.D.$_{600\,nm}$, 37°C) of *S. thermophilus* A5 in the absence (■) and presence of flavoring–coloring commercial mixtures of 0.12% vanilla (●) and 0.4% strawberry 1 (▲) and 2 (▼).

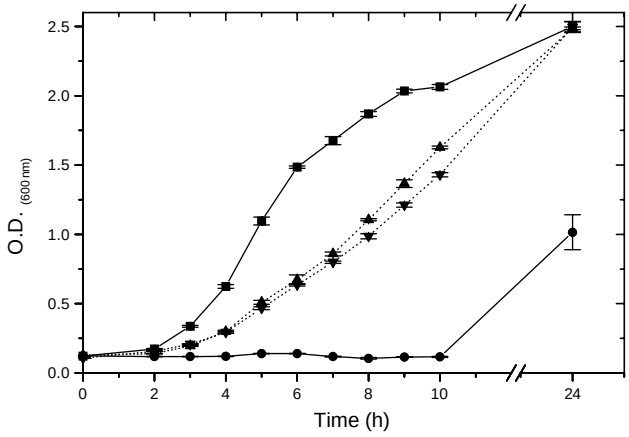

Fig. 2. Growth (O.D.$_{600\,nm}$, 37°C) of *L. acidophilus* 53 (full line) and *L. paracasei* LB (dot line) in the absence (■, ▲, respectively) and presence of flavoring–coloring commercial mixture of 0.4% strawberry 1 (●, ▼).

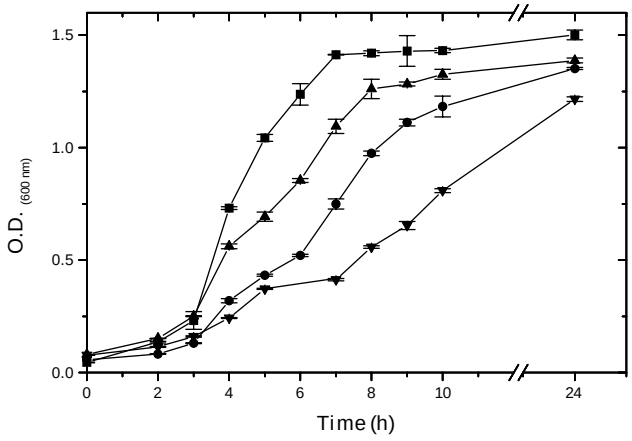

Fig. 3. Growth (O.D.$_{600\,nm}$, 37°C) of *L. lactis* A6 in the absence (■) and presence of flavoring–coloring commercial mixtures of 0.12% vanilla (●), 0.4% strawberry 1 (▲) and 2 (▼).

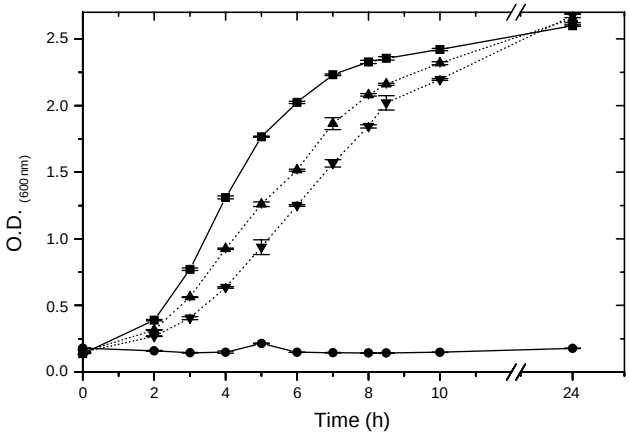

Fig. 4. Growth (O.D.$_{600\,nm}$, 37°C) of *L. rhamnosus* LS (full line) and *L. paracasei* A13 (dot line) in the absence (■, ▲, respectively) and presence of (1 ppm) of nisin (●, ▼).

Then, the strain was able to grow but not in the presence of 50 ppm of lysozyme.

4. Conclusions

Additives used in the dairy industry can significantly influence the growth and cell viability of lactic acid starter and probiotic cultures used for fermented products. It was demonstrated that, in general, probiotic bacteria were more resistant to dairy additives than lactic acid starter bacteria. Some of the compounds used were not inhibitory at the concentrations used for industrial manufacturing, while for others, strain-dependent effects were observed. Another group of additives influenced the bacterial growth only at concentrations higher than those used in practice.

The tolerance of starters and probiotic bacteria to dairy additives should be a selection criterion in order to

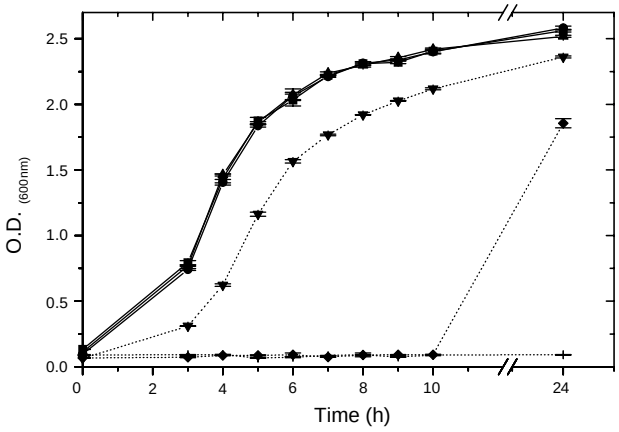

Fig. 5. Growth (O.D.$_{600\,nm}$, 37°C) of *L. delbrueckii* subsp. *bulgaricus* Ab1 (full line) and *L. delbrueckii* subsp. *bulgaricus* Bb1 (dot line) in the absence (■, ▼, respectively) and presence of 25 ppm (●, ◆, respectively) and 50 ppm (▲, +) of nisin.

achieve the best combination of strains for optimizing their growth and cell viability during the industrial process and the storage of the product.

Acknowledgements

This work was partially supported by the Agencia Nacional de Promoción Científica y Tecnológica from Argentina (Project PICT 97 No. 09-00000-00747). The authors are grateful for the assistance of the Lic. Nanci B. Bailo in this study.

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