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Short communication

Growth of *Lactobacillus paracasei* A13 in Argentinian probiotic cheese and its impact on the characteristics of the product

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ABSTRACT

The growth capacity of probiotic *Lactobacillus paracasei* A13, *Bifidobacterium bifidum* A1 and *L. acidophilus* A3 in a probiotic fresh cheese commercialized in Argentina since 1999 was studied during its manufacture and refrigerated storage at 5 °C and 12 °C for 60 days. Additionally, viable cell counts for probiotic bacteria in the commercial product are reported for batch productions over the last 9 years. *L. paracasei* A13 grew a half log order at 43 °C during the manufacturing process of probiotic cheese and another half log order during the first 15 days of storage at 5 °C, without negative effects on sensorial properties of the product. However, a negative impact on sensorial characteristics was observed when cheeses were stored at 12 °C for 60 days. Colony counts in the commercial product showed variations from batch to batch over the last 9 years. However, colony counts for each probiotic bacterium were always above the minimum suggested. Growth capacity of *L. paracasei* A13 in cheese during manufacturing and storage, mainly at temperatures commonly found in retail display cabinets in supermarkets (12 °C or more), would make it necessary to re-evaluate its role as possible probiotic starter and the consequences on food sensorial characteristics if storage temperature during commercial shelf life is not tightly controlled.

1. Introduction

According to the FAO/WHO, probiotics are "*live microorganisms which when administered in adequate amounts confer a health benefit on the host*" (FAO/WHO, 2002). After a massive incorporation of probiotic bacteria in fermented milks since early in the 90's, cheeses have also been a food targeted for carrying viable probiotic microorganisms. Incorporation of probiotics into cheese appears to be an encouraging alternative to the problem of survival in acid products because of the higher pH values of this kind of product, the closed matrix, and the high fat content, which might act as a protective factor for these organisms during both the food storage and the passage through the gastrointestinal tract (Stanton et al., 2003). A great variety of cheeses carrying probiotic bacteria as adjunct cultures has been reported, such as Cheddar, Cottage, Gouda, Crescenza, Egyptian Kariesh, semi-hard goat, Argentinian fresh, Canestrato Pugliese hard, Egyptian Tallaga, Estonian "Pikantne", white brined, white, Minas fresh, ewe's, semi-hard and petit-suisse cheese (Vinderola et al., in press). After a screening of a combination of probiotic strains suitable for fresh cheese, a commercial product of this type containing *Lactobacillus paracasei*, *L. acidophilus* and *Bifidobacterium bifidum* was launched in Argentina in May 1999 (Vinderola et al., 2000). In vivo studies were also carried out in mice in order to study the immunomodulating capacity of the product (Medici et al., 2004), showing that it is able to enhance the number of IgA producing cells in the small and large intestine lamina propria, which accomplishes an unquestionable role in the protection of mucosal surfaces (Tsuji et al., 2008). Nonstarter lactic acid bacteria (NSLAB) are adventitious lactic acid bacteria that contaminate cheeses. NSLAB grow poorly in milk, but they are able to grow in young cheeses (Stanley, 1998). Briggiler-Marcó et al. (2007) isolated, characterized and assessed the performance of NSLAB, mainly from the *L. casei* group, in soft and semi-hard cheeses, showing that they possess appropriate technological characteristics for their use as adjunct cultures in this kind of cheeses. The aim of this work was to report the viability of probiotic bacteria in industrial productions of Argentinian probiotic fresh cheese for the last 10 years and to evaluate their possible growth during the manufacture and shelf life of the product.

2. Materials and methods

2.1. Strains

Frozen cultures of commercial probiotic (*B. bifidum* A1, *L. acidophilus* A3 and *L. paracasei* A13) and starter (*Lactococcus lactis* A6 and *Streptococcus thermophilus* A4) bacteria were obtained from local providers. Cultures A4 and A6 were used as lactic starters for fresh cheese productions. The identity of probiotic and starters manufacturers has been kept undisclosed for confidentiality reasons.

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2.2. Cheese making

Argentinian probiotic fresh cheese was manufactured at a cheese factory in Bella Italia, Santa Fe, Argentina. Lots of 9000 L of raw milk were standardized at the dairy plant to a fat content of 1.6–1.8% and stored at 5 °C until being processed. Then, it was pasteurized following a high temperature–short time process (18 s at 74 °C) and cooled. Frozen cultures of lactic and probiotic bacteria were inoculated (1% wt/wt) at the beginning of batch fermentations. The inoculated milk was ultrafiltered to a concentration of 40–42% of total solids and the retentate was maturated for 1 h 45 min at 43 °C. Milk clotting took place along the line production. The molded curd was cut into 300 g portions, salted, and vacuum-packed. Cheese samples were ripened at 5 °C or 12 °C for 60 days. The mean values of some physicochemical cheese parameters are: pH 5.29 ± 0.11, moisture 58 ± 1.15% wt/wt, fat 12 ± 0.43% wt/wt, proteins 23 ± 0.84% wt/wt, salt 0.9 ± 0.03% wt/wt, ash 3.4 ± 0.05% wt/wt, DM 40.8 ± 1.03% wt/wt, and calcium 0.6 ± 0.04% wt/wt.

2.3. Microbiological analysis

Bacterial counts from cheeses were performed for each batch of cheese manufactured at the dairy plant since its launch in the year 1999. Five samples were randomly taken from each batch manufactured for the determination of probiotic viability. For assessing the growth capacity during the manufacturing process and refrigerated storage, samples were taken during the manufacturing process at times of 0, 15, 45 min, 1 h 45 min (beginning of clotting), 2 h 15 min, 2 h 45 min (end of clotting), 3 h 45 min (beginning of salting), 4 h 45 min and 5 h 45 min, after the addition of probiotic cultures, and at times of 0, 5, 10, 15, 20, 30, 45 and 60 days of storage at 5 °C and 12 °C. These experiments were carried out in duplicate, in two independent trials. Cheese portions (20 g) were aseptically sampled and suspended in 180 mL of 2% sodium citrate solution and homogenized (3 min) in a Stomacher Lab-Blender 400. Serial dilutions were made in sterile peptone water consisting of 0.1% (wt/vol) casein peptone (Microquin, Santa Fe, Argentina) and plated following the surface plate technique on different media for viable counts. Lactic Bacteria Differential agar (Hi-Media, Bombay, India) was used to enumerate *Streptococcus thermophilus* (37 °C, 3 days, aerobiosis) and *Lactococcus lactis* were counted on Elliker agar (Biokar, Beauvais, France) (25 °C, 3 days, aerobiosis). *B. bifidum* and *L. paracasei* were enumerated on LP-MRS agar (37 °C, 3 days, anaerobiosis GasPak system, Oxoid) whereas Bile-MRS agar was used to enumerate *Lactobacillus acidophilus* (37 °C, 3 days), according to Vinderola and Reinheimer (2000).

2.4. Sensorial analysis

Cheeses maintained for 60 days at 5 °C or 12 °C were analysed for sensorial properties according to the standard FIL-IDF 99C:1997 (sensory evaluation of dairy products by scoring-reference methodology), by a trained-panel of sensorial evaluation of the dairy plant.

2.5. Statistical analysis

Results are the mean ± standard deviation. Data were analysed using the one-way ANOVA procedure of SPSS software. The differences among means were detected by the Duncan's Multiple Range Test (SPSS, 1996). Data were considered significantly different when $P<0.05$.

3. Results and discussion

Probiotic bacteria are added to fermented dairy products as adjunct cultures and those products are thought to serve as mere vehicles for them until reaching the consumer. This is due to the slower growth capacity in cow's milk of probiotic bacteria as compared to traditional starter lactic acid bacteria (*Streptococcus thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, *Lactococcus lactis*) (Champagne and Gardner, 2005) and maybe also to the little knowledge about the outcome (rheologic and sensorial properties) of a mixed fermentation involving starter lactic acid and probiotic bacteria. During the manufacturing process of Argentinian probiotic fresh cheese, starter lactic acid bacteria are able to grow at least two log orders and remain without further changes during the shelf life of the product stored at 5 °C. However, at 12 °C, counts of *S. thermophilus* increased almost one log order until reaching 9.2 ± 0.1 log orders by the end of the refrigerated storage (60 days). On the contrary, counts of *L. lactis* in cheeses stored at 12 °C were lower than six log orders. We hypothesize that the counts of the strain used might have diminished in the cheese matrix due to autolysis (O'Donovan et al., 1996). Although considered a mesophilic (Mannu et al., 2000) lactic acid bacterium, in this study *L. paracasei* A13 was able to grow approximately a half log order at 43 °C during the manufacturing process of probiotic cheese (Fig. 1). This finding is in accordance with that of Farnworth et al. (2007), who reported that, under certain conditions, probiotic bacteria may participate together with starter lactic acid bacteria, in the fermentation process. The growth of probiotic bacteria in a food matrix is a scarcely reported phenomenon, since, for example in fermented milks, the most widely used vehicle for probiotic bacteria, no growth is expected to occur mainly due to the acidic conditions of the product. No evident growth was observed for *B. bifidum* A1 or for *L. acidophilus* A3 during the manufacture of Argentinian fresh probiotic cheese. The capacity of a probiotic bacterium to, at least partially, grow during the manufacturing process of the product used as vehicle, might allow a reduction in the initial content of the strain, reducing the costs of its addition to the food product. When the viability of probiotic bacteria in cheese samples was monitored during the refrigerated storage at 5 °C, a significant increase (approximately half log order) in the number of viable cells of *L. paracasei* A13 was observed from day 15 until the end of the shelf life (Fig. 2). For *L. acidophilus* A3 there was also a significant increase from 6.5 ± 0.2 log orders cfu/g (0 d) to 7.2 ± 0.1 log orders cfu/g at day 30, this cell count remained without significant changes until day 60. On the contrary, *B. bifidum* A1 in cheese held at 5 °C diminished from 7.3 ± 0.1 log orders cfu/g (0 d) to 6.4 ± 0.2 log orders cfu/g at day 30 or 6.7 ± 0.3 log orders cfu/g at day 60. More evident changes in the counts of probiotic bacteria were observed in samples kept at 12 °C for 60 days, a temperature that is maybe closer to those found in retail display cabinets in supermarkets according to Willocx et al. (1994) and Evans et al. (2007). For *L. paracasei* A13, an increase of more than 1.5 log orders in cell counts were monitored by day 15 of storage, this being the increment of almost 2 log orders from day 30 until day 60 (Fig. 2). No statistical significant changes were observed for *B. bifidum* A1, whereas

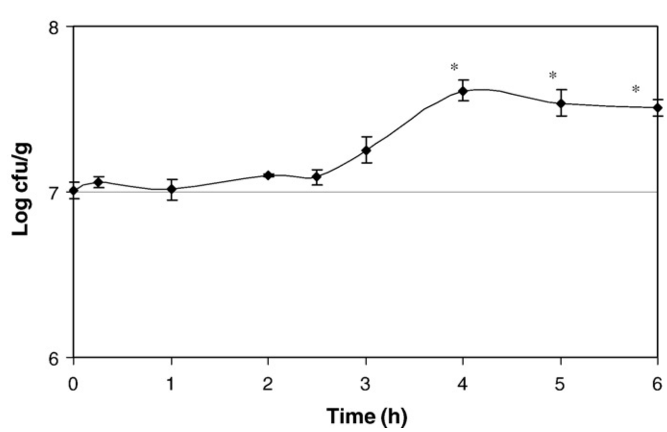

Fig. 1. Growth of *L. paracasei* A13 during the manufacturing process of Argentinian probiotic fresh cheese. * Significantly different from the colony count at time 0 ($P<0.05$).

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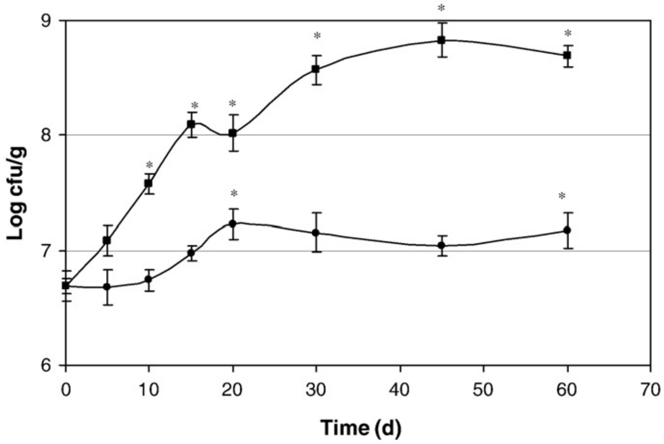

Fig. 2. Growth of *L. paracasei* A13 during the refrigerated storage of Argentinian probiotic fresh cheese at 5 °C (●) or 12 °C (■). * Significantly different from the colony count at time 0 ($P<0.05$).

Table 1
Sensorial properties of Argentinian probiotic fresh cheese maintained for 60 day at 5 °C and 12 °C.

Attribute	Temperature of storage	
	5 °C	12 °C
Color	Yellowish and bright white.	Pale and opaque white-beige.
Aroma	Soft, lactic, like ripened cream.	Strong, acid, like fermented cream, slightly rancid.
Taste	Lactic, creamy, slightly acid, somewhat bitter and spicy.	Very bitter, acid and rancid.
Texture	Creamy, slightly elastic and sticky, poorly-melting and soluble.	Easily splittable, grainy, rough, unstable, a bit plastic, melting and soluble.
pH	5.16 ± 0.12	4.61 ± 0.09

L. acidophilus A3 significantly increase its cell count from 6.5 ± 0.2 log orders cfu/g (0 d) to 7.4 ± 0.2 log orders by day 30, maintaining its counts until day 60. Fig. 3 shows the colony counts of probiotic bacteria in selected industrial productions of the commercial product. Cell counts ranged from 6 to almost 9 log orders cfu/g. Although fluctuations in cell counts were observed from batch to batch in the dairy plant during the last 9 years, the level of probiotic bacteria was always above 10^6 cfu/g, whereas in the majority of the points sampled, it was Q3 yet to be above 10^7 cfu/g, the minimum usually suggested for probiotic bacteria in foods (Ross et al., 2005). In general, viable cell counts of *L. paracasei* were above those of *L. acidophilus* and *B. bifidum* in the product. The capacity of *L. paracasei* A13 to grow during the manufacture and refrigerated storage of the product might justify the higher levels of this strain usually found compared to the two other probiotic strains included in the product. However, the microbiological changes observed in cheeses kept at 12 °C impacted negatively on the sensorial properties of the product (Table 1). The growth of starter and/or probiotic bacteria in the food matrix that serves as vehicle might seem an attractive issue for the functionality of the products. However, the impact of their metabolic activity on the food sensorial characteristics should be carefully evaluated before encouraging its growth in the food matrix. For example, the mesophilic and slow proteolytic capacity of *L. casei*, able to modify the reological and sensorial characteristics of the food, should be considered before its inclusion as probiotic in semi-hard cheeses ripened under certain conditions of mild temperature. Moreover, its capacity to grow in milky media might convert it into a target for bacteriophage attack, as recently reported for *L. paracasei* in fermented milks (Capra et al., 2006a, 2006b). In cheeses, most probiotic cultures partially lose their viability during storage (Champagne and Gardner, 2005), but there are examples of species that even grow in this environment. This is the case of propionibacteria in swiss-type

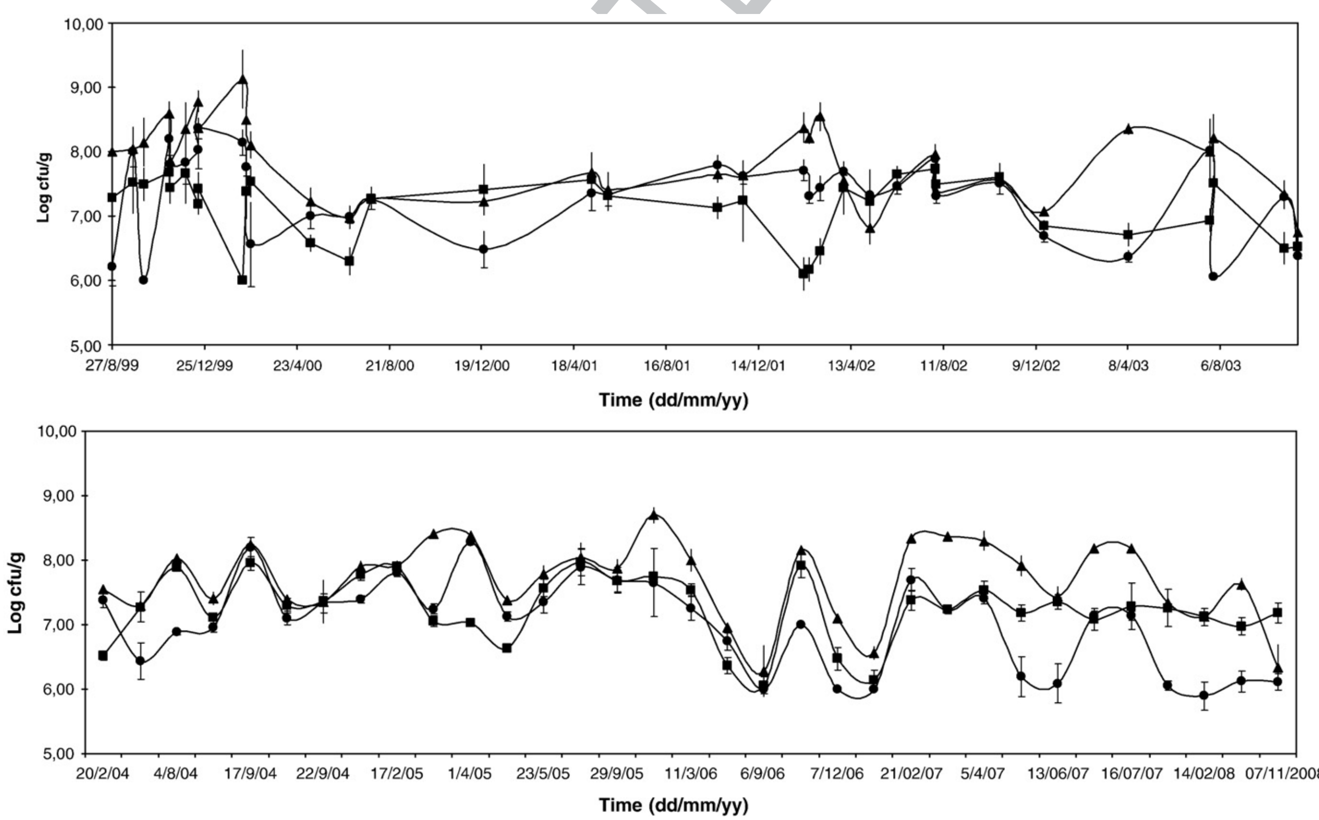

Fig. 3. Colony counts of *L. paracasei* A13 (▲), *B. bifidum* A1 (●) and *L. acidophilus* A3 (■) in different selected industrial productions of Argentinian fresh cheese in the period 1999–2008.

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cheese (Thierry et al., 1994) or *Lactobacillus casei* during the ripening of Cheddar (Stanton et al., 1998). Argentinian probiotic fresh cheese would be another example of a food matrix that allows the growth of certain probiotic bacteria during certain conditions of cold storage. This fact would warrant the arrival of high counts of probiotic bacteria to consumers, and it should be taken into account at the moment of inoculating them into milk for cheese manufacture. However, if temperature conditions are not tightly controlled and monitored during cold storage in retail cabinets, sensorial changes due to metabolic active cells might impact negatively in the consumer's acceptance of the product. The particular characteristics of the food used as vehicle for probiotic bacteria (chemical composition, manufacture and storage conditions) and the possibility of probiotic growth, should be taken into account before determining the minimum amount of probiotic bacteria required to name the food as a probiotic food. No generalizations or establishment of minimal quantities of probiotic bacteria in foods should be made without taking into account the capacity of specific strains to grow in the food matrix under specific conditions of manufacture and storage as individual adjuncts or in combination with other probiotics.

4. Conclusions

L. paracasei A13 included in Argentinian fresh cheese developed during the manufacturing process of the product at 43 °C and during the storage and shelf life of the product at 5 °C, increasing its count in approximately one log order, without negatively affecting the sensorial properties of the food matrix. A higher storage temperature (12 °C) allowed a higher growth of the strain but negatively affected the sensorial characteristics of the product.

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