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Viability of *Bifidobacterium infantis* and *Lactobacillus casei* subsp. *rhamnosus* in Starter Milk

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Abstract: The aim of this study is to evaluate the viability of *Lactobacillus casei* subsp. *rhamnosus* and *Bifidobacterium infantis* in starter milk, using time and temperature as variables, as well as to evaluate the viability of these micro-organisms over other bacteria normally found in milk, such as *Enterococcus faecalis*, *Escherichia coli*, *Bacillus subtilis*.

Key words: *Bifidobacterium infantis*, *Lactobacillus casei* subsp. *rhamnosus*, viability

Introduction

Foods that contain carbohydrates, as with any organic material, are an ideal substrate for the development of all types of micro-organisms. Under certain conditions (reduction of oxygen levels, variations in temperature, acidity, salinity, etc.), micro-organisms develop and metabolize these carbohydrates, turning them into lactic acid or acetic acid, sugars, alcohols or lipids. For this reason such foods are much more easy for the higher organism to make use of, since it finds that part of the product has already been bio-transformed. In animal models it has been possible to demonstrate the beneficial effects of bacteria administered in a controlled manner and this opens up a line of research for achieving their application in human foods in order to benefit health (Calvo, 2001). New food products are currently being proposed with a high *Bifidobacterium* sp. and *Lactobacillus acidophilus* content, representing, by way of example, 4% of all milk sold in France and around 25% of fermented milk products in Sweden. It has been estimated that around 80% of products on the world market contain strains of *Bifidobacterium* and around 45% of European dairy industries produce derivatives containing probiotic bacteria (Diplock *et al.*, 1998).

The beneficial effects of the presence of *Bifidobacterium* and *Lactobacillus* species in the gastro-intestinal tract depend on their viability and metabolic activity, encouraged by complex hydrocarbons and other bifidogenic factors found in the product itself (Ducluzeau, 1993; Gournier-Chateau, 1994; Marquina, 2001; Mazza, 1998; Salminen *et al.*, 1998; Sanders, 1999) meaning that the aim of this study is to evaluate the viability of *Lactobacillus casei* subsp. *rhamnosus* and *Bifidobacterium infantis* in starter milk, using time and temperature as variables, as well as to evaluate the viability of these micro-organisms over other bacteria normally found in milk, such as *Enterococcus faecalis*, *Escherichia coli*, *Bacillus subtilis*.

This study worked with two probiotic strains:

Bifidobacterium infantis and *Lactobacillus casei* subsp. *rhamnosus*, which had been previously identified. Strains of *Enterococcus faecalis*, *Escherichia coli* and *Bacillus subtilis* were also analyzed.

Bacteria were listed using MRS Agar (De MAN J. C., ROGOSA M. and SHARPE M.E.) as a culture medium for probiotic strains and TSA Agar (Tryptic Soy Agar) for the three remaining strains. The method used for counting viable micro-organisms was the surface method. In order to verify the viability of *E. coli* in milk, McConkey's Agar plates were seeded.

The original inocula prepared with each of the strains were of the order of 10⁸ UFC/ml. Once the probiotic strains had been counted (initial count) they were inoculated into a starter milk (commercial product) rich in carbohydrates (lactose), vitamins and mineral salts, at a temperature of 26 °C. A count was taken (time zero = t) from this preparation, and then a second (t+1) after leaving the inoculated milk for one hour in the fridge (Temp. 8 °C).

The same procedure was followed after mixing the probiotic strains with each of the three remaining strains. All tests were carried out in triplicate. All the strains except *E. coli*, being tested in milk, were seeded in MRS Agar and incubated at 37 °C for 48 hours, in anaerobiosis (5% CO₂), while the milk sample inoculated with *E. coli* was also seeded at the same time in TSA and McConkey's and incubated at a temperature of 35 °C for 24 hours in anaerobiosis.

The initial probiotic strain count was in the order of 10⁷ UFC/ml. With the starter milk inoculated at room temperature (26 °C), it was observed that the viability of the probiotic strains when they were on their own was in the order of 10⁸ UFC/ml.

In the samples inoculated with the probiotic strains in combination with *E. coli* and *E. faecalis*, it was observed that the count remained at 10⁷ UFC/ml. Combined with *Bacillus subtilis* the count was in the order of 10⁶ UFC/ml.

The figures obtained after the inoculated milk had

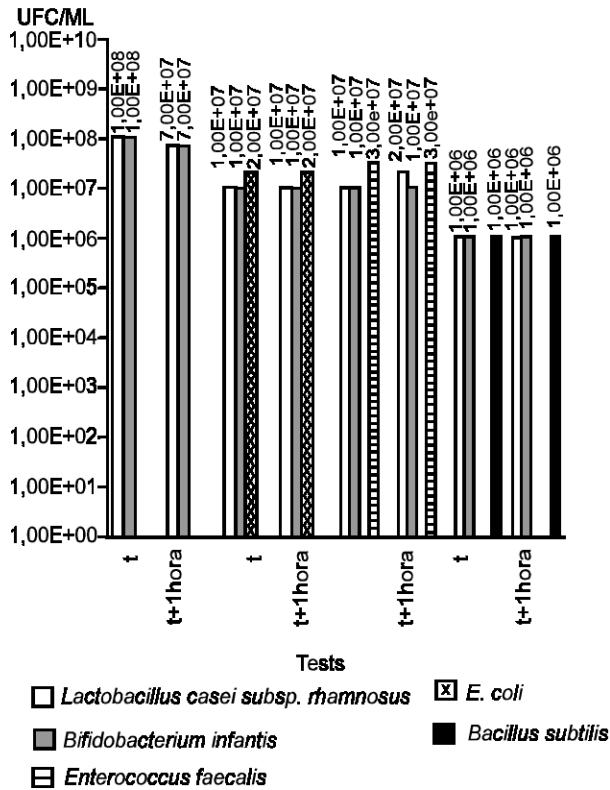


Fig. 1: Evaluation of probiotic micro-organisms combined with other micro-organisms in starter milk in relation to time

remained in the fridge (8 °C) for a period of one hour were 10⁷ UFC/ml., in all cases except for the combination of probiotics and *Bacillus subtilis*, where they remained at 10⁶ UFC/ml. The results can be seen in Fig. 1.

This study allows us to conclude that the viability of the probiotic strains *L. casei subsp. rhamnosus* and *B. infantis*, at the moment at which they are ingested, whether this is at the time that they are prepared or after they have remained in the fridge for one hour are suitable for achieving positive health results, given that previous studies advise that in order for the consumer actually to benefit from a physiological effect it should be ensured that the probiotic bacteria survive in products to be ingested in concentrations of at least 10⁶ UFC/ml.

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