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Effect of temperature on growth and metabolism of probiotic bacteria in milk

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Abstract

The growth and metabolism of six probiotic strains with documented health effects were studied in ultra-high temperature (UHT) treated milk supplemented with 0.5% (w/v) tryptone or 0.75% (w/v) fructose at different temperatures. The probiotic strains were *Lactobacillus acidophilus* La5, *Lb. acidophilus* 1748, *Lb. johnsonii* LA1, *Lb. rhamnosus* GG, *Lb. reuteri* SD 2112 and *Bifidobacterium animalis* BB12. Fermentation was followed for 48 h at 20, 30, 37 and 45 °C and the samples were analysed for pH, log cfu mL^{-1}, volatile compounds, organic acids and carbon dioxide. All six probiotic strains showed very different profiles of metabolites during fermentation, however, the two *Lb. acidophilus* strains were the most alike. All strains, except *Lb. reuteri* SD 2112, showed viable cell numbers above 6.5 log cfu mL^{-1} after 48 h fermentation at 30, 37 and 45 °C. The probiotic strains produced different amounts of metabolic products according to temperature and fermentation time illustrating the importance of controlling these parameters.

Keywords: Fermented milk; Probiotic bacteria; Organic acids; Volatile compounds; CO_2

1. Introduction

During the past twenty years there has been a tremendous increase in the worldwide sales of cultured products containing probiotic bacteria. Today, most probiotic strains are used in yoghurts, fermented milks, ice creams and pharmaceutical products for their anecdotal health effect (Mattila–Sandholm, 1999). Increasing knowledge underlines the important role of the intestinal flora for maintaining health and in the prevention of disease. Probiotics offer dietary means to support the balance of intestinal flora (Holzapfel, Haberer, Snel, Schillinger, & Huis in't Veld, 1998). The microorganisms primarily associated with this balance are lactobacilli and bifidobacteria.

Several aspects have to be taken into consideration in the selection process of probiotic organisms. Safety aspects reviewed lately by Salminen et al. (1998) and Saarela, Mogensen, Fondén, Mättö, and Mattila–Sandholm (2000) include specifications as to human origin, non-pathogenicity and antibiotic resistance characteristics. Factors related to the technological and sensory aspects of the probiotic food products are of utmost importance since only by satisfying the demands of consumers can the food industry succeed in promoting the consumption of functional products in the future (Mattila–Sandholm, Myllärinen, Crittenden, Mogensen, Fondén, & Saarela, 2002). To maintain confidence in probiotic products it is important to demonstrate good survival of the bacteria in food products during their specified shelf life. In order for any beneficial effect in humans to develop, the viable cell count should be above 6 log cfu g^{-1} in order to supply a sufficient "daily dose" of 10^6–10^9 viable bacteria (Samona & Robinson, 1991; Lee & Salminen, 1995; Vinderola, Bailo, & Reinheimer, 2000). In addition, a pleasant taste and an attractive texture are essential for all food products, regardless of the "health message" of the product (Saxelin, Grenov, Svensson, Fondén, Reniero, &

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Mattila-Sandholm, 1999). The final concentration of lactate should be about 8000 mg kg^{-1} and the pH between 4.2 and 4.4 in order for the sensoric qualities of sourness and firm coagulum to be satisfactory (Narvhus, 1996).

There is limited published information concerning the technological production of fermented probiotic milk products and of the metabolic pathways followed by specific probiotic organisms during the fermentation of milk. The shift in metabolic pathways in response to environmental conditions is well documented in the literature in the case of homofermentative and heterofermentative lactobacilli (Axelsson, 1998). However, no information is available about the shift in metabolism of probiotic bacteria grown in milk in response to environmental changes. Metabolic changes are very important from a technological standpoint, since the amount of organic acids and volatile compounds is important in the development of flavour and texture of the fermented product. However, the metabolic changes may also be important for the microorganisms to obtain energy and to maintain the $NAD^+/NADH+H^+$ balance (Axelsson, 1998; Lopez de Felipe & Hugenholtz, 1999).

Production of fermented milk products using probiotic lactic acid bacteria is a major challenge to dairies as milk is not, on the whole, a good growth medium for these organisms. Growth and metabolism of five probiotic strains in ultra-high temperature (UHT) milk supplemented with tryptone and fructose at 37 °C have recently been studied by Østlie, Helland, and Narvhus (2003). In this study, the effect of different incubation temperatures on the growth and metabolism of six probiotic strains with documented health effects were studied in ultra-high temperature (UHT) semi-skimmed milk supplemented with 0.5% tryptone (w/v) or 0.75% fructose (w/v). Their ability to produce organic acids, volatile compounds and carbon dioxide at different temperatures was focused on.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Lactobacillus johnsonii LA1 was kindly supplied from Nestec Ltd, Lausanne, Switzerland; *Lb. rhamnosus* GG (ATCC 53103) from Valio Ltd, Helsinki, Finland; *Lb. reuteri* SD 2112 from Biogaia Biologics, Stockholm, Sweden; and *Lb. acidophilus* LA-5 and *Bifidobacterium animalis* BB12 from Christian Hansen, Oslo, Norway. *Lb. acidophilus* NCFB 1748 was obtained from the National Collection of Food Bacteria, Reading, England. The strains were subcultured three times in de Man Rogosa Sharpe (MRS) broth (Difco Labs., Detroit, MI, USA) at 37 °C overnight, before a final inoculation for making concentrated stock cultures. Cysteine hydrochloride (0.05%, w/v, Sigma, St. Louis, MO, USA) was added to MRS broth for culturing *B. animalis* BB12 and *Lb. johnsonii* LA1. Frozen concentrated stock cultures were made as described by Østlie et al. (2003). The concentrated cultures (10 ×) were stored in 3 mL lots at −80 °C.

2.2. Production of fermented milk

One bottle containing 300 mL UHT milk (1.5% (v/v) fat, TINE, Oslo, Norway) was inoculated with 1% (v/v) of the frozen culture. One bottle was inoculated for each incubation temperature. The milk to be inoculated with *Lb. rhamnosus* GG was supplemented in advance with 0.75% (w/v) filter-sterilized fructose (Merck, Darmstadt, Germany) and the milk for the other strains was supplemented with 0.5% (w/v) filter sterilized tryptone (Oxoid Ltd., Hampshire, England). Forty millilitre lots of the inoculated milk were aseptically distributed in 50 mL sterile bottles. One bottle was prepared for each sampling time and was used for all analyses except the CO_2 measurement. For CO_2 measurement, 10 mL lots of the freshly inoculated milk were aseptically distributed into sterile headspace vials (20-CV, Chromacol Ltd, Trumbell, USA) and sealed with sterile septa (20-CB3, Chromacol Ltd) and aluminium crimp caps (20-ACB3, Chromacol Ltd). One vial was prepared for each sampling time. Both bottles and vials were incubated at 20, 30, 37 and 45 °C for 0–48 h. Viable microbial counts, pH, volatile compounds, organic acids and carbon dioxide were determined in the incubated milk after 0, 4, 8, 12, 18, 24 and 48 h incubation.

2.2.1. Viable microorganisms

Samples were diluted in peptone–saline water (0.9%, w/v saline; 0.1%, w/v peptone) and viable counts of the probiotic strains were determined on MRS agar (Difco) after anaerobic incubation at 37 °C for 3 days (BBL GasPakPlus System, Becton Dickinson Microbiology Systems, Sparks, MD, USA).

2.2.2. Chemical analysis

All pH measurements were made during fermentation using a Radiometer (pHM 92) pH meter with a combined glass electrode and temperature probe (Radiometer, Copenhagen, Denmark). The pH meter was calibrated using standard buffer solutions (Merck) at pH 4.0 and 7.0.

Volatile compounds were analysed by headspace gas chromatography according to the method of Narvhus, Østeraas, Mutukumira, and Abrahamsen (1998) as described by Østlie et al. (2003).

Organic acids were analysed by high pressure liquid chromatography (HPLC) using a modification of the