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Selection of probiotic bacteria for the fermentation of a soy beverage in combination with *Streptococcus thermophilus*

C.P. Champagne [a,*], J. Green-Johnson [b], Y. Raymond [a], J. Barrette [a], N. Buckley [c]

[a] Food R&D Centre, Agriculture and Agri-food Canada, 3600 Casavant, St. Hyacinthe QC, Canada J2S 8E3
[b] Faculty of Science, University of Ontario Institute of Technology, 2000 Simcoe Street North, Oshawa ON, Canada L1H 7K4
[c] Canadian Space Agency, 6767 Route de l'Aéroport, Longueuil QC, Canada J3Y 8Y9

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ABSTRACT

Lactobacillus delbrueckii subsp. *lactis* R0187, *Lactobacillus helveticus* R0052, *Lactobacillus rhamnosus* R0011 and *Bifidobacterium longum* R0175 were examined for their ability to grow in combination with *Streptococcus thermophilus* cultures in milk and a laboratory soy beverage (LSB; both standardized to 4.5% protein and 2.3% fat). Strains R0011 and R0187 did not rapidly acidify the soy beverage despite good growth rates on soy carbohydrates. The *S. thermophilus* populations in the LSB were similar to that of milk even though milk had 30% more buffering capacity. In milk but not in soy, symbiosis with respect to acidification rate was observed between *S. thermophilus* and *L. helveticus* or *B. longum*. The populations of *L. helveticus* in the fermented products were similar in pure cultures or in the presence of the streptococci. However *B. longum* did not compete well in the mixed culture. Fermentation conditions varied as a function of the ability of *S. thermophilus* strains to acidify media to a pH of 4.65 (between 8 and 24 h). The probiotic populations in the mixed culture were influenced by the *S. thermophilus* strain and by the time of fermentation. Variations in growth rates of the bacteria did not appear to be linked to differences in initial redox or α-amino nitrogen levels. Strain selection enabled the preparation of a mixed starter, probiotic-fermented soy beverage containing 1.1×10^8 CFU/mL of *L. helveticus* R0052, which represented approximately 13% of the total final population.

1. Introduction

Up to 93% of consumers believe certain foods have health benefits that may reduce the risk of disease (Clydesdale, 2004). This has created a strong demand for functional foods which are foods containing specific ingredients that can reduce the risk of having certain diseases (for example, diarrhoea, cancer, cardiovascular conditions). Worldwide, the dairy sector, which is strongly linked to probiotics, is the largest functional food market, accounting for nearly 33% of the broad market, while cereal products have just over 22% (Leatherhead Food International, 2006). Probiotic bacteria are the main "bioactive ingredients" added to the dairy matrix in order to generate this health benefit. Probiotics are defined as "live microorganisms which, when administered in adequate amounts, provide a health benefit the host" (Araya et al., 2002). Probiotic-containing drinks are the fastest-growing dairy product in Europe and data show that the global market for probiotic functional foods has grown by 19% in recent years and is expected to grow by 5% annually between 2006 and 2011 (Nutraingredients-USA, 2007). Picking up on the trend in dairy products, new probiotic-containing products have been launched, particularly in fruit-based drinks and cereals. Soy is an excellent candidate for such products (De Valdez & Giori, 1993). A first benefit of soy beverage fermentation is the reduction of its "beany" flavour (Blagden & Gilliland, 2005; Desai, Small, McGill, & Shah, 2002; Stern, Hesseltine, Wang, & Konishi, 1977). Soy is also considered a good substrate for functional foods since fermentation by probiotics has the potential to (1) reduce the levels of some carbohydrates which can be responsible for gas production in the intestinal system, (2) increase free isoflavone levels (Chien, Huang, & Chou, 2006; Wei, Chen, & Chen, 2007) and (3) favour desirable changes in bacterial populations in the gastrointestinal tract (Benno, Endo, Shiragami, Samaya, & Mitsuoka, 1987; Bouhnik et al., 2004). Soy also benefits bone health (Messina, Gugger, & Alekel, 2001, chap. 5), which is a concern for ageing people. It is noteworthy that bone health is also of concern to astronauts. Indeed, it was observed that astronauts exposed to a microgravity environment on extended space flights demonstrated significant bone mass loss (Lane, LeBlanc, Putcha, & Whitson, 1993). Therefore, a food which would benefit bone metabolism would be helpful to the elderly as well as the astronauts in the space or outpost (Moon, Mars) environments. Furthermore, reduced activity of the immune system is also a common result of ageing and extended space flight (Stein, 2001). Since some

* Corresponding author. Tel.: +1 450 768 3238.
E-mail address: ChampagneC@agr.gc.ca (C.P. Champagne).

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probiotic bacteria have been shown to enhance immune functions (Perdigon, Vintini, Alvarez, Medina, & Medici, 1999), fermentation with probiotics also has the potential of benefiting the elderly or the astronauts on extended microgravity duty. One of the aims of this study is to develop a fermented soy product containing probiotics with potential as a functional food on Earth and in space.

Numerous studies have been done on the growth of lactic cultures in soy beverages (Mital & Steinkraus, 1979). A feature of soy fermentation by probiotics is the strain-linked variability of the acidification rate. The study by Stern et al. (1977), which included eight *Lactobacillus acidophilus* cultures, and similar studies using bifidobacteria (Scalabrini, Rossi, Spettoli, & Matteuzzi, 1998; Tsangalis, Ashton, McGill, & Shah, 2002), revealed sharp differences between strains in the rate of acid production on carbohydrates found in soy. This literature shows that for pure cultures, strain selection is essential to obtain adequate acidification rates. Even then, with pure probiotic cultures, fermentation times required to attain a pH below 4.5 are typically 10 h or more at 37 °C (Angeles & Marth, 1971; Blagden & Gilliland, 2005; Chien et al., 2006; Garro, De Valdez, & De Giori, 2004; Murti, Lamberet, Bouillanne, Desmazeaud, & Landon, 1993;Kamaly, 1997; LeBlanc et al., 2004). Industrially, short fermentation times are preferable in order to increase plant output as well as reduce unwanted contaminating microorganisms. Furthermore, probiotic cultures alone can generate products with unpleasant flavours (Macedo, Soccol, & Freitas, 1998). A potential solution to these two problems is the use of mixed cultures with a yoghurt strain. However, little is known of how typical yoghurt starters mixed with probiotics will behave in soy beverages. Pairing of probiotic cultures can be very disadvantageous to some strains (Macedo et al., 1998), and data show that combinations with *Streptococcus thermophilus* can be detrimental to *L. acidophilus* (De Valdez & Giori, 1993) and bifidobacteria (Murti, Bouillanne, Landon, & Desmazeaud, 1993). Thus, the development of a fermented soy product containing probiotics will require strain selection for ability to grow in the substrate as well as ability to compete or even establish a synergy between strains. The main probiotic bacteria studied in the past for growth in soy beverages are *L. acidophilus, Lactobacillus fermentum* and the bifidobacteria. Little is known of probiotic *Lactobacillus rhamnosus, Lactobacillus helveticus, Lactobacillus delbrueckii* ssp. *lactis*. This study looked at the growth in soy of various bacteria included these lesser-used but promising probiotics and describes the development of a starter culture that can ferment soy rapidly while maintaining significant probiotic yields.

Pure cultures are rarely used in milk fermentations. To produce cheese, blends of *Lactococcus lactis* strains are used, while in yoghurt, combinations of streptococci and lactobacilli serve the purpose. In cheese making, blends of *L. lactis* strains must be carefully selected because of bacteriophage issues as well as potential antagonism (Hugenholtz, 1986; Lawrence, Thomas, & Terzaghi, 1976). Similarly, pairing of yoghurt cultures must be carried out keeping in mind that not all *S. thermophilus* and *Lactobacillus bulgaricus* blends are successful (Moon & Reinbold, 1976). While data are available on mixed cultures of *S. thermophilus* and *L. bulgaricus* in soy beverages (De Valdez & Giori, 1993), this study detailed growth and acidification by probiotic bacteria when combined with *S. thermophilus* strains.

With the exception of Murti, Lamberet, et al. (1993), there has been no standardization of protein or total solids when milk and soy beverages are compared as growth substrates (Angeles & Marth, 1971; Macedo et al., 1998; Mital, Steinkraus, & Naylor, 1974). Protein (Bury, Jelen, & Kimura, 1998) or carbohydrate concentration (Chang & Stone, 1990) both affect growth of lactic cultures. Therefore comparisons in un-standardized media raise the concern that observed differences could be due to the chemical nature of the ingredients in the matrices and their concentration. In this study, evaluations of mixed cultures in milk and soy substrates were made under standardized conditions. Redox level has also been shown to influence the growth of probiotic bacteria in milk, particularly oxygen-sensitive species (Dave & Shah, 1997b), however, it is not known if milk or soy beverages have different redox levels. Another objective of this study was to compare growth of cultures in soy and milk substrates on a standardized protein and fat basis, and to determine the impact of differences in redox level, buffering capacity, total solids level and carbohydrate level.

2. Materials and methods

2.1. Cultures

The following probiotic strains were graciously supplied by Institute Rosell-Lallemand (Montréal, Canada): *S. thermophilus* R0083, *Lactobacillus delbrueckii* subsp. *lactis* R0187 (*L. lactis throughout the text*), *L. helveticus* R0052, *L. rhamnosus* R0011 and *Bifidobacterium longum* R0175. *S. thermophilus* R0083 can also be obtained from Abiasa (St-Hyacinthe, QC Canada). Strains ST5, Y12S and Y24S of *S. thermophilus*, were taken from the Food Research and Development Center (FRDC) of Agriculture and Agri-food Canada (St. Hyacinthe, QC, Canada) culture collection. Stock cultures were prepared by mixing MRS-grown (lactobacilli and *B. longum)* or M17-grown (streptococci) cultures with sterile rehydrated skim milk 20% (w/w) and glycerol 20% (w/v) in a 2:5:5 ratio, placing 1 mL in Cryovials (Wheaton, Milleville NJ, USA) and freezing at −80 °C. The glycerol and milk solutions were previously sterilized, respectively at 121 °C for 10 min and 110 °C for 5 min (real temperature and holding time). Strains R0011, R0052, R0175 and R0187 were selected because of their potential to improve health by modulating the immune system (Easo, Measham, Munroe, & Green-Johnson, 2002; Wallace, Bradley, Buckley, & Green-Johnson, 2003; Wood, Keeling, Bradley, Johnson-Green, & Green-Johnson, 2007), preventing infection (Johnson-Henry et al., 2004; Johnson-Henry et al., 2005) reducing the symptoms of stress (Gareau, Jury, MacQueen, Sherman, & Perdue, 2007; Zareie et al., 2006) and producing bioactive compounds (Fiander, Bradley, Johnson-Green, & Green-Johnson, 2005). In clinical trials, these microbes have been used to treat acute gastrointestinal infections (Kocian, 1994), reduce pain and bloating associated with irritable bowel syndrome (Benes, Kretk, & Tompkins, 2006) and maintain remission in patients with ulcerative colitis (Haskey & Dahl, 2006). The data support the definition of these strains as probiotics.

For the preparation of the inocula, 100 mL of sterile MRS (Difco, Sparks, USA) and M17 (EMD, Darmstadt, Germany) broths were inoculated with 1 mL of a thawed stock culture and incubated at 37 °C until a pH of 4.5 was reached. The incubation time varied between 11 and 16 h as a function of the strain. The MRS medium was prepared as proposed by the manufacturer, but lactose content of M17 was adjusted to 1% (w/v) instead of the customary 0.5%. For the bifidobacteria, 0.1% of ascorbic acid (BioShop, Burlington ON, Canada) was added to the MRS broth by adding 1 mL of a sterile solution of 10% ascorbic acid (as an antioxidant) to 100 mL of pre-sterilized MRS broth.

2.2. Milk and soy beverages

All commercial soy beverages (CSB) contain supplements. For our studies, the commercial product with the fewest supplements was used: natur-a™ (Nutrisoya, St-Hyacinthe QC, Canada). The label of natur-a™ reported the following ingredients: filtered water, soybeans (certified biological OCPP/PRO-CERT Canada), calcium carbonate, natural flavours, palmitate of vitamin A, vitamins B2, D2 and B12. It contained 3.2% protein and 1.6% fat.