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α-Galactosidase and proteolytic activities of selected probiotic and dairy cultures in fermented soymilk

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Abstract

The metabolic activities of *Lactobacillus acidophilus* (LAFTI® L10 and La4962) *Bifidobacterium* (*lactis* LAFTI® B94 and *longum* Bl536), *Lactobacillus casei* (LAFTI® L26 and Lc279), *Lactobacillus delbrueckii* ssp. *bulgaricus* Lb1466 and *Streptococuss thermophilus* St1342 were assessed in soymilk. Strains were initially analyzed for α-galactosidase activity and organic acid production in MRS broth at 37 °C. Consequently, soymilk was fermented with each strain and cell growth, production of organic acid, metabolism of oligosaccharides and proteolytic and ACE-inhibitory activities were assessed during 48 h of incubation at 42 °C. All strains exhibited variable α-galactosidase activity, with *Bifidobacterium lactis* B94 showing the highest activity. The oligosaccharide metabolism depended on α-galactosidase activity. *B. lactis* B94, *S. thermophilus* St1342 and *L. acidophilus* La4962 reduced raffinose substantially by 77.4%, 64.5% and 55.9%, respectively. All strains reached the desired therapeutic level of 10^8 cfu/ml in soymilk after 48 h at 42 °C. The hydrolysis of protein in soymilk likely depended on strain ($P < 0.0001$) and time ($P < 0.0001$). The strains also released bioactive peptides with ACE-inhibitory activities between 17% and 43%.

Keywords: Fermented soymilk; Raffinose; Stachyose; α-Galactosidase activity; ACE-inhibitory activity

1. Introduction

Soy-based foods may provide a range of health benefits to consumers due to their hypolipidemic, anticholesterolemic and antiatherogenic properties, and reduced allergenicity (Favaro Trindade, Terzi, Turgo, Della Modesta, & Couri, 2001). They also contain isoflavones, which have been linked to reduced risk of most hormone-associated health disorders (Kurzer, 2000). However, consumption of soymilk is hindered, due to the presence of unpleasant off-flavours carried over from soy beans. These characteristic flavours are caused by *n*-hexanal and -pentanal, which occur in beans as a product of breakdown of unsaturated fatty acids (Arai, Suzuky, Fujimake, & Sakurai, 1996; Scalabrini, Rossi, Spettoli, & Matteuzzi, 1998). In addition to these aldehydes, soymilk contains various oligosaccharides, including raffinose and stachyose, that may cause a gastrointestinal discomfort to consumers (Tsangalis & Shah, 2004).

Raffinose and stachyose are α-galactosides of sucrose, comprising three and four monomeric units, respectively, and are non-digestible in the gut due to the absence of α-galactosidase in the human intestinal mucosa. Consequently, intact oligosaccharides pass directly into the lower intestine, where they are metabolized by bacteria that possess this enzyme, resulting in the production of gases (Tsangalis & Shah, 2004). This problem could be alleviated by using a specific enzyme, α-galactosidase, or an organism that possesses high α-galactosidase activity, to minimize the content of flatulence-causing oligosaccharides in the product (Scalabrini et al., 1998). Several

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Bifidobacterium strains have been reported to produce varying levels of α-galactosidase, which metabolize α-galactosyl oligosaccharides in soymilk (Scalabrini et al., 1998). Soymilk is a good medium for growing *Bifidobacterium* because it contains oligosaccharides that are fermented by most of the strains belonging to this genus (Liu, 1997; Scalabrini et al., 1998).

Bifidobacterium spp., *Lactobacillus acidophilus*, and *L. casei*, have been associated with health-promoting effects and are classified as probiotic organisms since they are thought to improve the microbial balance in the human gastrointestinal tract (GIT) (Schrezenmeir & de Vrese, 2001). Health benefits attributed to probiotics include antimicrobial, antimutagenic, anticarcinogenic and antihypertensive properties (Lourens-Hattingh & Viljeon, 2001). Antihypertension has been reported to be mediated through inhibition of angiotensin-converting enzyme (ACE) (Nakamura et al., 1995). This enzyme plays a major role in the regulation of blood pressure. ACE converts angiotensin-I to a vasoconstrictor, angiotensin-II, and the inactivation of the vasodilator bradykinin. ACE inhibition results in an antihypertensive effect (Fuglsang, Rattray, Nilsson, & Nyborg, 2003; Saito, Nakamura, Kitazawa, Kawai, & Itoh, 2000). Many ACE-inhibitory peptides have been derived from food proteins (Donkor, Henriksson, Vasiljevic, & Shah, 2005; Saito et al., 2000; Wu & Ding, 2001).

The ability of lactic acid bacteria (LAB) to ferment the available carbohydrates in a growing medium varies with strains. Matsuoka, Sasago, and Sekiguchi (1968) found that *Streptococuss thermophilus* produced a greater amount of acid in soymilk than did *Lactococcus lactis* or *L. delbrueckii* ssp. *bulgaricus*. Mital, Steinkraus, and Naylor (1974) also reported that certain organisms, such as *S. thermophilus*, *L. acidophilus*, *L. cellobiosis* and *L. plantarum*, which utilize sucrose, exhibited significant growth and produced substantial amounts of acid in soymilk. Others, such as *L. delbrueckii* ssp. *bulgaricus*, grew poorly in soymilk because of their inability to ferment sucrose and other carbohydrates in soymilk. A similar finding was also reported by Wang, Kraidej, and Hesseltine (1974). The use of LAB in preparing fermented soy products has received much attention (Cheng, Thompson, & Brittin, 1990; Karleskind, Laye, Halpin, & Morr, 1991; Lee, Morr, & Seo, 1990). Several studies on α-galactosidase activity and metabolism of α-galactosyl oligosaccharides by *Bifidobacterium* strains in soymilk have been reported but there is a lack of detailed information in the literature about the behaviour of probiotic organisms (*L. acidophilus* and *L. casei*) and their importance as part of the starter cultures for making fermented soy products. The aims of this study were (a) to assess the suitability of soymilk as a substrate for growth and acid development by selected probiotic organisms and by *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*, (b) to examine the metabolism of oligosaccharides by these selected organisms, and (c) to monitor their proteolytic and ACE-inhibitory activities in soymilk.

2. Materials and methods

2.1. Bacterial cultures

Pure strains of *L. acidophilus* LAFTI® L10, *B. lactis* LAFTI® B94 and *L. casei* LAFTI® L26 were kindly provided by DSM Food Specialties (Moorebank, NSW, Australia). *S. thermophilus* St1342, *L. delbrueckii* ssp. *bulgaricus* Lb1466, *L. acidophilus* La4962, *B. longum* Bl 536 and *L. casei* Lc279 were obtained from the culture collection of Victoria University (Werribee, Australia). The lyophilized organisms were propagated in deMann Rogosa Sharpe (MRS) broth (Oxoid, West Heidelberg, Australia) according to the manufacturer's instructions at 37 °C with the exception of *L. delbrueckii* ssp. *bulgaricus* Lb1466 which was grown at 42 °C. For propagation of *Bifidobacterium*, sterile MRS broth was supplemented with 0.05% (w/v) L-cysteine hydrochloride to provide anaerobic condition and to stimulate their growth (Ravula & Shah, 1998). After three successive transfers in MRS, the activated organisms were inoculated at 1% (v/v) level into 10 ml of sterilized commercial soymilk (Simply Soy, Sanitarium, NSW, Australia) supplemented with 2% (w/v) glucose and 1% (w/v) yeast extract for the manufacturing of the fermented soymilk.

2.2. Extraction of crude α-galactosidase

One of the requirements for good growth of cultures in soy-based media is the activity of α-galactosidase. All organisms (*L. acidophilus* L10, *B. lactis* B94, *L. casei* L26, *S. thermophilus* St1342, *L. delbrueckii* ssp. *bulgaricus* Lb1466, *L. acidophilus* La4962, *B. longum* Bl536 and *L. casei* Lc279) were assessed for α-galactosidase activity according to the methods of Scalabrini et al. (1998) and Tsangalis and Shah (2004). Briefly, the organisms were activated by two successive propagations in MRS broth at 37 °C for 20 h. Subsequently, 5% (v/v) of active culture was inoculated into 250 ml of MRS broth and incubated at 37 °C for 48 h. In order to ascertain the metabolic characteristics of probiotics and *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* in soymilk, α-galactosidase activity was examined in MRS basal broth supplemented with 2% (w/v) glucose, 2% (w/v) raffinose or a mixture of 1% (w/v) each of raffinose and glucose. During fermentation, 50 ml aliquots were withdrawn aseptically at 6, 12, 24, and 48 h and stored at 2 °C. Bacterial cells were harvested by centrifuging at 4000*g* for 10 min at 4 °C, using a Sorvall RT7 refrigerated centrifuge (Newtown, Conn., USA). The cell pellet was washed in 20 ml of cold 50 mM sodium citrate buffer (pH 5.5) and centrifuged at 4000*g* for 10 min and this was repeated twice. Finally cells were resuspended in 10 ml of the same buffer, placed in an ice bath and sonicated (Unisonics, Pty Ltd. Sydney, Australia) three times for 5 min. The cell debris was removed by centrifugation at 10,000*g* for 30 min at 4 °C. The supernatant was used as a crude enzyme extract.