한국축산식품학회지
Korean J. Food Sci. Ani. Resour.
Vol. 27, No. 4, pp. 496~500(2007)

Effect of Milk Containing *Streptococcus thermophilus* KACC 91147 on Blood Glucose Levels

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Streptococcus thermophilus KACC 91147 첨가우유 섭취가 혈당치에 미치는 영향

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ABSTRACT

The lactase activities of nine species of lactic acid bacteria were compared using the chromogenic substrate, *o*-nitrophenyl-β-D-galactopyranoside. *Streptococcus thermophilus* KACC 91147 had the highest lactase activity among a total of thirty strains of *Lactobacillus* and *S. thermophilus* tested, including commercial strains. *S. thermophilus* KACC 91147 released 0.30±0.12 mg/mL of galactose in treated milk A (10^7 CFU/mL) and 6.49±0.38 mg/mL in treated milk B (10^9 CFU/mL milk) over 2 hours. In milk tolerance tests, the blood glucose level (BGL) of 6 volunteers (2 males and 4 females) clinically diagnosed as lactose intolerant increased 3.0 mg/dl after drinking milk A, but a significant ($p<0.05$) additional increase of 11.2±4.18 mg/dl was found after drinking milk B. This result suggests that the addition of *S. thermophilus* KACC 91147 cells into milk aids the digestion of lactose in milk and ameliorates the symptoms of lactose-intolerant individuals due to the activity of lactase from the lactic streptococci.

Key words : Lactose intolerance, lactase, *Streptococcus thermophilus*, blood glucose level (BGL)

INTRODUCTION

Lactose is the primary carbohydrate of mammalian milk, and must be hydrolyzed to a monosaccharide in order to be absorbed by the small intestinal mucosa. A deficiency of intestinal lactase prevents hydrolysis of ingested lactose. Seventy-five percent of the world's population is estimated to be lactase-deficient, and lactose intolerance is very common among Asian, South American, and African persons. Lactose intolerance can be clinically diagnosed by milk tolerance test (Roy, 2003).

Culture-containing fluid milk products such as acidophilus milk and bifidus milk are thought to aid the digestion of the lactose in those products by lactose-intolerant individuals, but strain selection and optimization of culture preparation by the manufacturers are important to provide appropriate strains for the purpose of such application (Sanders *et al.*, 1996). Levri *et al.* (2005) reviewed 90 articles to assess the efficacy of oral probiotics in adults with lactose intolerance, and concluded that the supplement of probiotics in general did not alleviate the symptoms and signs of lactose intolerance in adults, but some evidence suggested that specific strains, concentrations, and preparations of the probiotics are effective (Kolars *et al.*, 1984; Lin *et al.*, 1991; Martini *et al.*, 1991; Savaiano *et al.*, 1984). Sanders *et al.* (1996) reported lactase activity of *S. thermophilus* was much greater than that of bifidobacteria or lactobacilli, and the activity was dependent on strain. Lin *et al.* (1990) showed non-fermented milk containing yogurt cultures at the concentration of 10^8 CFU/mL milk was 3-folds more effective in reducing breath hydrogen concentrations than that of lower concentration

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(10^7 CFU/mL milk). However, there was no evidence on the effect of the milk with culture on BGL of lactose intolerance. The purpose of this study was to obtain the candidate lactic acid bacteria with high lactase activity and to show the effect of the addition of the culture on BGL of those who are suffering from lactose intolerance.

MATERIALS AND METHODS

Sources and maintenance of bacterial strains

Eleven strains of ATCC (American Type Culture Collection), one strain of KCTC (Korean Collection for Type Cultures), one strain of KACC (Korean Agricultural Culture Collection) and eight commercial strains of lactobacilli and streptococci were used. Both the strain of actobacilli and streptococci was maintained by transferring biweekly in new MRS broth (Difco Laboratories, Detroit, MI, USA) containing 1% lactose. *S. thermophilus* commercial strains and KACC 91147 were maintained by transferring biweekly in new M17 broth (Difco) containing 1% lactose.

Screening of the culture by lactase activity

Lactase activity was determined as described by Miller (1972). The cells of lactic acid bacteria were grown overnight in the MRS for lactobacilli or M17 for *S. thermophilus*. Cultures were then sub-cultured and grown to an absorbance of 0.7 to 1.0 at 600 nm wavelength, and chilled on ice for 20 min. An aliquot (adjusted so that lactase concentrations could be read on the standard curve) of this mixture was added to Z buffer (60 mM Na_2HPO_4, 40 mM $NaH_2PO_4 \cdot H_2O$, 10 mM KCl, 1 mM $MgSO_4 \cdot 7H_2O$, and 50 mM β-mercaptoethanol) to make 1 mL. Cells were permeabilized by adding 100 μL chloroform and 50 μL 0.1% SDS. This solution was mixed by vortex mixer for 10 sec. and then added 0.2 mL of o-nitrophenyl-β-D-galactopyranoside (4 mg/mL solution), which functioned as a lactose analogue and chromogenic substrate. After the mixture was incubated at 4, 37, and 55°C for 20 min, the reaction mixture was neutralized by the addition of 0.5 mL of 1 M Na_2CO_3 and centrifuged by a centrifue (VS-15CFN, VISION SCIENTIFIC, Korea) for 10 min at 14,000 rpm. The supernatant fluid was transferred into a cuvette, and the absorbance at 420 nm was measured. Because the absorbance at 420 nm o-nitrophenol is being disturbed by the scattering light from the cell debris, the absorbance at 550 nm was also measured for the corrections of the absorbance. The Miller unit of lactase activity was calculated by using following equation.

Miller Units

$$= 1{,}000 \times [(OD_{420} - 1.75 \times OD_{550})] / (T \times V \times OD_{600})$$

T = time of the reaction in minute, V = volume of culture used in the assay in mL

Activity staining of Lactase

Thirty milliliter of culture was harvested by centrifugation at 4°C for 30 min (5,000×g) after overnight incubation at 37°C and the cell pellet was washed twice with 20 mM sodium phosphate buffer (SPB, pH 7.4) and then re-suspended in 3 ml of SPB. The washed cell suspension was disrupted with an ultrasonic cell disrupter (VC750, SONICS & MATERIALS, USA) on an ice bucket. The cell debris was removed by centrifugation (10,000×g for 10 min at 4°C) and micro-filtration (0.45 μm, Millipore, USA). Lactase in the filtrate was examined by 12% native PAGE with activity staining (Hung *et al.*, 2001).

Determination of galactose in the cultured milk

Galactose concentration in the cultured milk was assayed to estimate the hydrolysis rate of lactose by the cells of *S. thermophilus* KACC 91147 which cannot utilize galactose. Galactose concentration in the cultured milk with *S. thermophilus* KACC 91147 was measured by using a total galactose assay kit (Interscientific Co., USA). Fifty microliters of milk sample was mixed with 500 μL 3% TCA solution. After centrifugation at 5,000×g for 10 min, 200 μL of supernatant was transferred into a test tube. Five hundred microliters of working enzyme (Reagent A) containing bacterial galactose dehydrogenase was added to the test tube and incubated the enzyme reaction mixture at room temperature for 30 min. Five hundred microliters of color solution (Reagent B) was then added and incubated for 2 min in order to the development of color. Absorbance was measured with a spectrophotometer (V-530, JASCO, Japan) at 570 nm. The concentration of galactose released by the hydrolysis of lactose in milk was calculated by a standard curve plotted with a series of galactose concentration (0, 0.2, 0.4, 0.6, 0.8, and 1 mg/mL).

Milk intolerance test

Six volunteers (2 males, 4 females) who are suffering from the lactose intolerance were asked to fast overnight. Blood glucose level of the volunteers was checked before and after drinking 500 mL of the milk. Three different milk samples were control milk, milk sample A containing low concentration (10^7 CFU/mL) of the cells, and milk sample B containing high concentration (10^9 CFU/mL) of the cells of

S. thermophilus KACC 91147. Milk was obtained from Korea National Liverstock Research Institute, and pasteurized by heating at 63°C for 30 min. The cells were grown in M17 broth containing 0.5% lactose and harvested by centrifugation at 3,000×g for 30 min at 4°C, and washed the cells pellets twice with 0.85% sterile saline. Three milk samples were fed in the morning with one week interval. BGL was measured using AccuCheck Compact (Roche, Diagnostics GmbH, Germany).

Statistical analysis

BGL data of six volunteers was analyzed by paired T-test using a Statistical Analysis System (SAS Institute, 1990).

RESULTS AND DISCUSSION

Screening of lactic acid bacteria by lactase activity

Lactase acitivity of the nine species of LAB expressed by Miller unit was summarized in Table 1. Cells from four strains of *S. thermophilus* strains were shown to contain more lactase than those of twenty six strains of *Lactobacilli.* The ranges of lactase activity for *S. thermophilus* were from 885 to 51,464 units/mL, for lactobacilli from ND (less than 100) to 7,126 units/mL. Lactase activity of *S. thermophilus* was increased with the increment of the reaction temperature, and that of KACC 91147 was the highest among the streptococci. Sanders *et al.* (1996) reported a broad range of lactase activity from 11.8 to 189 units/cfu for streptococci and from 0.01 to 4.73 units/cfu for lactobacilli. As a consequence, amelioration effects of lactose intolerance could accomplish by the consumption of ordinary yogurt or thermophilus milk, the both is fermented by *S. thermophil*us (Sanders *et al.*, 1996).

The lactase activity of *S. themophilus* was visualized in a native PAGE followed by an activity staining (Fig. 1). The lactase activity of *S. themophilus* KACC 91147 was highest near 132 kDa. Molecular weight of the lactase of *Streptococcus thermophilus* can be calculated as 117 kDa from amino acid sequence (Bolotin *et al.*, 2004), the lactase activity was found above 132 kDa (Kim *et al.*, 2003). Lactase activity of *E.coli* from Sigma was found to be near 216 kDa, and the association state of subunits of microbial β-galactosidase is mostly known to be either dimeric or tetrameric (Kim *et al.*, 2003).

Release of galactose by *S. thermophilus* KACC 91147 in milk

Although lactose is efficiently transported into the cell and subsequently hydrolyzed by an intracellular β-galactosidase, many strains of *S. thermophilus* used in the dairy industry

Table 1. Lactase activity of the lactic acid bacteria by Miller Unit

Genus and Species	Strain	Lactase activity (Miller Unit)		
		10°C	37°C	55°C
Streptococcus thermophilus	KACC 91147	7771	32811	51464
	St-37	885	17492	20817
	Th-3	5784	23141	48329
	402	7621	15318	19944
Lactobacillus acidophilus	LA100	166	336	253
	ATCC 2181	1120	507	6135
Lactobacillus bulgaricus	ATCC 11977	ND	160	273
	ATCC 7995	594	349	191
	LB 207	2581	2516	1345
	ATCC 55163	168	ND	ND
Lactobacillus casei	912LC	ND	ND	ND
	ATCC 3109	107	ND	ND
Lactobacillus gasseri	ATCC 4962	ND	ND	ND
Lactobacillus helveticus	Lh-BO2	702	6236	6490
Lactobacillus rhamnosus	Wisbey 744	ND	ND	ND
Lactobacillus paracasei	KCTC 3169	ND	ND	ND
Lactobacillus plantarum	ATCC 10012	ND	ND	ND
	ATCC 14917	ND	ND	ND
	ATCC 49445	ND	ND	ND
	ATCC 8014	911	5310	7216

ND : less than 100.

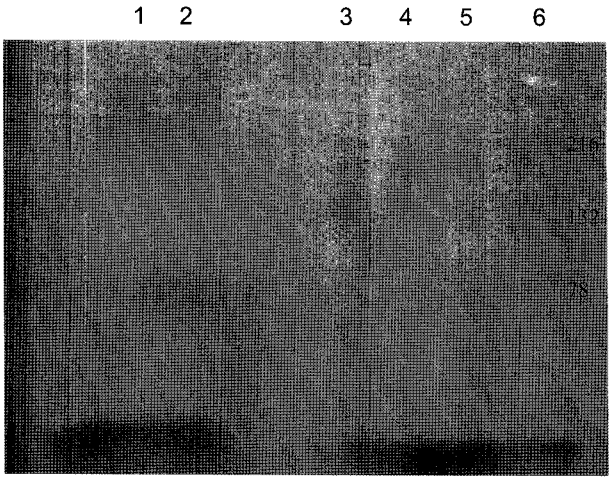

Fig. 1. Activity staining of *Streptococcus thermophilus* KACC 91147. 1: 5U of standard (Sigma G4155). 2: 1U of standard (Sigma G4155). 3: 10 μL of cell free extract of *S. thermophilus* KACC 91147. 4: 5 μL of cell free extract of *S. thermophilus* KACC 91147. 5: 1 μL of cell free extract of *S. thermophilus* KACC 91147. 6: Molecular weight standard (BIO-RAD 161-0324).

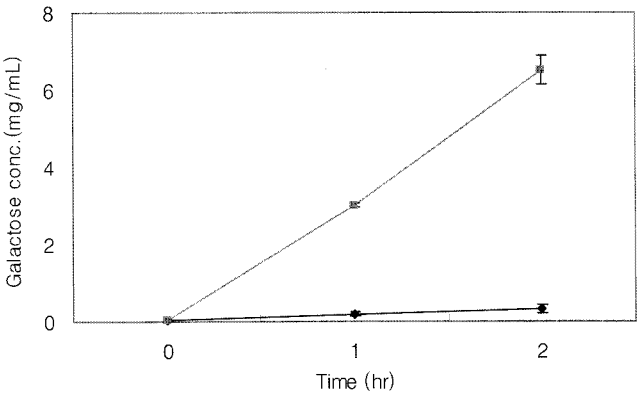

Fig. 2. Galactose concentration in milk at 37°C with the milk A (10^7 CFU/mL; ◆) and the milk B (10^9 CFU/mL; ■) containing *Streptococcus thermophilus* KACC 91147.

ferment only the glucose moiety of lactose, while the galactose moiety is excreted into the medium in equimolar amounts with the lactose uptake (Hutkins *et al.*, 1985; Hutkins and Morris, 1987; Thomas and Crow, 1984). Since *S. themophilus* KACC 91147 is deficient in galactose utilizing mechanism, lactose hydrolysis in milk could be estimated by the concentration of galactose released from the lactose hydrolyzed. As shown in Fig. 2, at low concentration of *S. thermophilus* KACC 91147 (10^7 CFU/mL milk), only 0.30± 0.12 mg/mL of galactose was determined after 2 hr at 37°C, while 6.49±0.38 mg/mL was at higher concentration of that (10^9 CFU/mL milk).

Amelioration effect on BGL

Table 2 shows the changes in the mean BGL of six volunteers following the administration of 500 mL control milk, culture-containing milk A and B. The mean BGL administered control milk gradually increased from 85.7 mg/dl to 90.8 mg/dl after 60 min. However, the mean BGL with both the culture-containing milk A and B was increased up to Δ 4.2 and Δ 11.2 mg/dl until 30 min. but decreased prominently to Δ -1.8 and Δ -9.5 mg/dl respectively. The changes in the mean BGL was dependent on the concentration of the cells of *S. thermophilus* KACC 91147. We inferred that the *S. thermophilus* KACC 91147 in milk increase BGL by lactose hydrolysis. Owing to the limit of the commercial blood glucose test kit, we could not discriminate between glucose and galactose. It can be assumed that the decrease of BGL in 60 min is caused by the activation of galactose utilizing enzyme system. It is well known that galactose is metabolized in species ranging from *E. coli* to mammals predominantly via a series of sequential reactions, the Leloir pathway. Deficiency of any one of these enzymes such as galactokinase, uridylyltransferase, or epimerase in humans results in a form of the inherited metabolic disorder, galactosemia (Fridovich-Keil, 2006). In most people except galactosemia, galactose is converted to UDP galactose by Leloir pathway, and UDP galactose can be converted to UDP glucose by UDP galactose 4-epimerase. UDP glucose is the glucosyl donor for the synthesis of glycogen. Therefore, it was thought that lactic acid bacteria in dairy products could be applicable for the control of blood glucose level of diabetes by consuming glucose and by providing glucosyl donor, as well. More con-

Table 2. The changes of the mean blood glucose level after administration of cultured milk (N=6) (Unit : mg/dl)

Treat	Time				
	0[a]	15	30	45	60
Control	85.7±2.40[b]	88.3±2.65	88.7±1.61	90.3±3.19	90.8±3.11
Cultured milk A	Δ 0±2.68	Δ 0.7±2.3	Δ 4.2±3.09	Δ 1.2±2.18	Δ -1.8±1.72
Cultured milk B	Δ -0.8±3.62	Δ 6.2±4.06	Δ 11.2±4.18*	Δ -4.2±4.17	Δ -9.5±3.08*

a : 0 means just before drink milk.
b : Mean ± S.E.
* : Statistically significant (p<0.05).

trolled study with animal models should be done to see whether lactic acid bacteria in dairy products are effective in lowering BGL *in vivo* conditions.

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(2007. 8. 7. 접수/2007. 12. 5. 채택)