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Characteristics of the Alcoholic Milk Product Fermented by *Lactococcus lactis* subsp. *lactis* TA29 and *Saccharomyces exiguus* SK2

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A cultured milk product was made by fermenting 10% reconstituted skim milk with *Lactococcus lactis* subsp. *lactis* TA29 and *Saccharomyces exiguus* SK2. *L. lactis* TA29 and *S. exiguus* SK2 grew up to 1.0×10^9 and 2.0×10^6 cfu/ml, respectively. After the fermentation 21% of lactose was hydrolyzed, pH was lowered to 4.2, and titratable acidity and alcohol concentration were increased to 0.96 and 0.023%, respectively. When the fermented milk was stored at 4°C for 9 days, the viable cell counts for *L. lactis* TA29 and *S. exiguus* SK2 were 6.5×10^5 and 1.6×10^6 cfu/ml, respectively. The alcoholic fermented milk prepared in this experiment was more inhibitory against some pathogenic bacteria including *C. perfringens* than commercial yoghurt products tested.

Kefir is a cultured milk product obtained by mixed acidic and alcoholic fermentation. It is produced by the addition of kefir grains to the milk, which are strained at the end of fermentation for reuse. These grains are particles of milk solids containing lactose fermenting yeasts, streptococci and lactobacilli (12). The yeasts impart to kefir its characteristic flavor, a slight fizziness and a low alcohol content (6). Kefir grains vary in size (0.5-3 cm diameter) and resemble cauliflower florets. Its microflora is held together by a matrix of fibrillar material composed largely of insoluble carbohydrate (11).

Production of kefir as a health food is gaining interest in many countries (10). Most commonly kefir is made by a single-stage fermentation of skimmed milk. Alternatively, another fermentation method involves an initial bacterial lactic fermentation followed by a yeast alcoholic fermentation (6). In both methods, the fermented kefir after straining of the grains is kept refrigerated for flavor, alcohol, diacetyl and CO_2 development. In order to facilitate large scale production, efforts were aimed towards the production of kefir from a fast single-use starter culture.

In this work we studied the characteristics of milk product fermented with *Lactococcus lactis* subsp. *lactis* TA 29 and *Saccharomyces exiguus* SK2.

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Key words: fermented milk, *Lactococcus lactis*, *Saccharomyces exiguus*, pathogenic bacteria, kefir

MATERIALS AND METHODS

Strains and Culture Conditions

Lactococcus lactis subsp. *lactis* TA29 isolated from raw milk maintained in M 17 broth (14). *Saccharomyces exiguus* SK2 was isolated from kefir grain and maintained on YM agar (3).

Clostridium perfringens ATCC 13124 was grown in reinforced clostridial broth (Difco) in anaerobic pressure tubes (Bellco Glass). *Staphylococcus aureus* ATCC 12600, *Salmonella typhimurium* KFRI 191 and *Escherichia coli* ATCC 11775 were grown in nutrient broth (Difco).

Preparation of Fermented Milk

The mixed culture of *L. lactis* subsp. *lactis* TA29 and *S. exiguus* SK2 was made in autoclaved reconstituted skim milk (10% w/v) at 23°C for 24 h and transferred twice for making inoculum. For the preparation of fermented milk, 2% of inoculum was added to the 10% reconstituted skim milk pasteurized at 85°C for 30 min. The inoculated milk was incubated at 23°C for 24 h and ripened at 10°C for 3 days.

Analytical Methods

Cell growth was measured by colony counts using layered plates (7). M 17 agar and Potato Dextrose Agar (Difco) were used for colony counts of *L. lactis* subsp. *lactis* TA29 and *S. exiguus* SK2, respectively.

The concentrations of lactose, glucose, galactose, and

ethanol were determined using the enzymatic method (1). The acidity was measured by the titration method (2).

The inhibitory effect of fermented milks on pathogenic bacteria was determined by agar diffusion assay (5). Autoclaved EG agar (Eiken, Tokyo) without horse blood was cooled to 45°C, inoculated with a test pathogen grown for 24 h, and poured into petri dishes. The fermented milk (0.2 ml) was added to stainless steel cylinders (6 mm, inner diameter; 8 mm, outer diameter; 10 mm, length) placed on the surface of the agar plate. After incubation at 37°C for 24 h, the diameters of inhibition zones were measured.

RESULTS AND DISCUSSION

S. exiguus SK2 isolated from kefir grain was not grown by single culture in autoclaved homogenized milk (Fig. 1), but the strain was grown to 4.0×10^6 cfu/ml with *L. lactis* subsp. *lactis* TA29. These results indicate that *S. exiguus* SK2 can not utilize lactose in milk for itself (3), but the strain utilizes glucose or galactose hydrolyzed from lactose by *L. lactis* TA29 in a mixed culture. There was no difference in the growth of *L. lactis* TA29 between single and mixed culture.

Fig. 2 shows the changes of viable cell number during fermentation at 23°C for 24 h, followed by ripening at 10°C for 3 days and storage at 4°C for 10 days. During the fermentation, *L. lactis* TA29 and *S. exiguus* SK2 were grown to 1.0×10^9 and 2.0×10^6 cfu/ml, respectively. During the ripening, the viable cell number of *L. lactis* TA29 decreased a little but that of *S. exiguus* SK2 increased a little. During the storage at 4°C, the viable cell number of *L. lactis* TA29 decreased greatly to 6.0×10^5 cfu/ml, but that of *S. exiguus* SK2 decreased only a little to 1.6×10^6 cfu/ml. This indicates that *S. exiguus* is more resistant to acid at low temperature than *L. lactis*. The cells of *L. lactis* TA29 might lose their viability during storage because the energy required to resist the lactic acid toxicity at low pH was not produced at 4°C.

It was reported that one ml of good-quality kefir showed viable counts of 10^9 lactococci and 10^4-10^5 yeast cells (8). This fermented milk contained more viable cells of yeast than kefir, but did not developed yeasty flavor. Wherever available in the world, kefir is considered an aid in the therapy of gastrointestinal disorders (8). The viable yeast cells in kefir may act some role in the therapy of gastrointestinal disorders because yeast culture is known to function beneficially in the animal gut (13).

Fig. 3 shows the change of sugar concentration during fermentation and ripening. About 21% lactose decreased during fermentation, but there was a little further reduction during ripening. Glucose levels decreased to zero during fermentation. About 96% galactose hydrolyzed from lactose was metabolized and only 0.41 g/l of galactose was remained after ripening.

The pH was lowered to 4.2 and titratable acidity increased to 0.96% lactic acid after ripening (Fig. 4). Ethanol concentration increased to 0.023% after ripening (Fig. 5). It is known that the final product of kefir has a pH of 4.0 and a titratable acidity of 1% lactic acid and contains 0.01-0.1% ethanol (4, 8).

The inhibitory effect of the alcoholic fermented milk

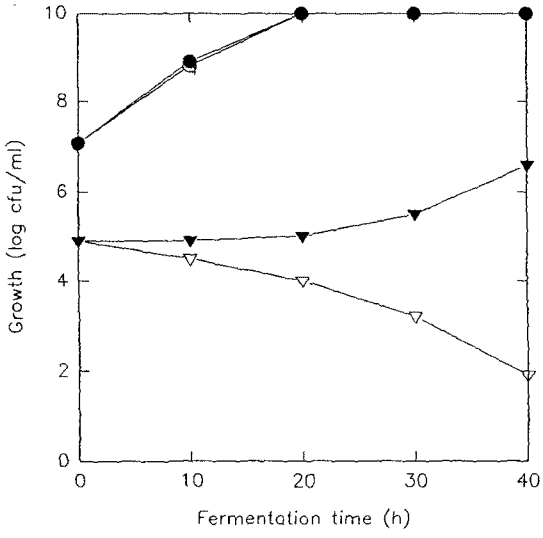

Fig. 1. Growth of *S. exiguus* SK2 and *L. lactis* TA29 in milk by single and mixed culture at 23°C.
▽, *S. exiguus* in single culture; ▼, *S. exiguus* in mixed culture; ○, *L. lactis* in single culture; ●, *L. lactis* in mixed culture.

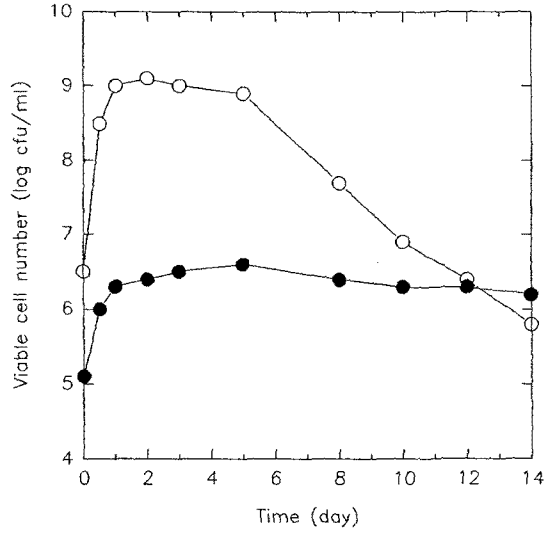

Fig. 2. Changes of viable cell number during fermentation at 23°C for 1 day, ripening at 10°C for 3 days, and storage at 4°C for 10 days.
○, *L. lactis* TA29; ●, *S. exiguus* SK2.

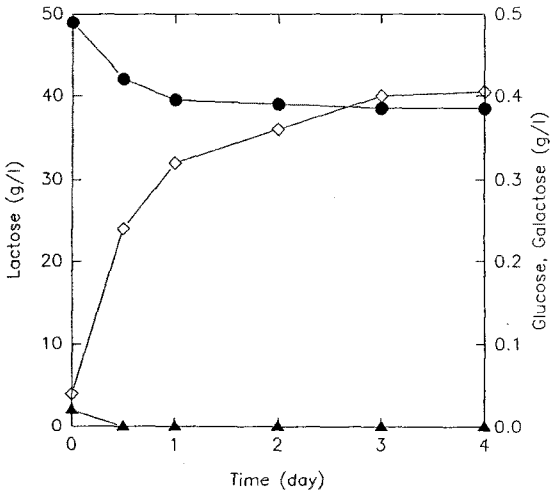

Fig. 3. Changes of the concentrations of lactose, glucose and galactose in alcoholic fermented milk during fermentation at 23°C for 1 day and ripening at 10°C for 3 days.
●, lactose; ▲, glucose; ◇, galactose.

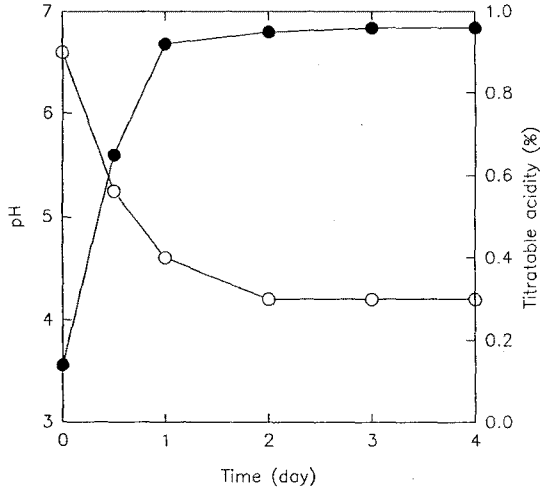

Fig. 4. Changes of pH and titratable acidity of the alcoholic fermented milk during fermentation at 23°C for 1 day and ripening at 10°C for 3 days.
○, pH; ●, titratable acidity.

on the growth of some pathogenic bacteria are given in Table 1. Fermented milk prepared in this work inhibited the growth of such pathogenic bacteria as *C. perfringens*, *S. aureus*, *S. typhimurium*, and *E. coli* more than the two commercial yoghurt products. Here, we can carefully speculate that this fermented milk may have more probiotic effects than yoghurt and ethanol in the alcoholic fermented milk may act some role in inhibiting the growth of the pathogenic bacteria.

Preliminary flavor acceptability test showed that the fermented milk had a weak flavor as compared with

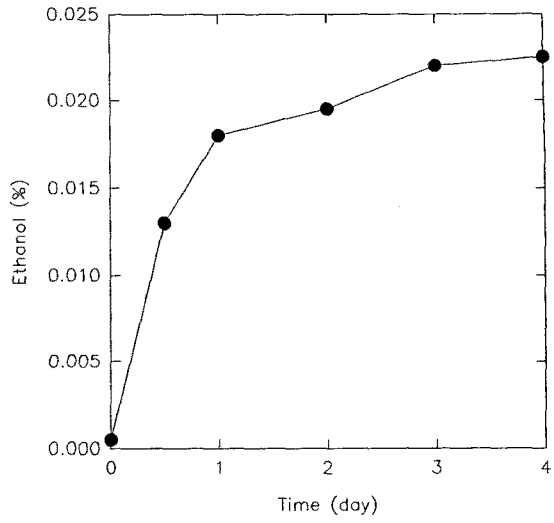

Fig. 5. Change of ethanol concentration of the alcoholic fermented milk during fermentation at 23°C for 1 day and ripening at 10°C for 3 days.

Table 1. Inhibitory effect of fermented milks on the growth of pathogenic bacteria.

	FM[a]	Yoghurt 1	Yoghurt 2
C. perfringens ATCC 13124	11.0[b]	9.0	8.5
S. aureus ATCC 12600	13.0	11.5	10.5
S. typhimurium KFRI 191	12.0	9.0	8.5
E. coli ATCC 11775	11.5	9.5	9.0

[a]FM, fermented milk made in this study; Yoghurt, commercial yoghurt.
[b]Diameter of inhibition zone (mm).

commercial yoghurt products and the fermented milk showed no fizziness due to the dissolved CO_2. In further study, heterofermentative lactobacilli should be added as starter for flavor development because they produce such flavor compounds as acetoin, diacetyl, CO_2, and ethanol in kefir (9).

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