原 報

Microbiological Properties of Maziwa lala, A Kenyan Traditional Fermented Milk of Masai Community in Kenya

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1. Introduction

Fermented milks such as yogurt, ymer, filmjolk, langfil, villi, kefir and kumiss are manufactured in modern factories using identified strains of dairy lactic acid bacteria such as *Lactobacillus delbrueckii* subsp. *bulgaricus, Streptococcus salvarius* subsp. *thermophilus, Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* as starters. In addition, therapic fermented milk, which are expected for the consumers to improve lactose tolerance in lactose-intolerant people, reduce the number of the pathogenic microorganisms in the intestinal tract, reduce serum cholesterol levels and activate the immune systems, are manufactured using *Lactobacillus acidophillus, Lactobacillus casei* subsp. *casei* and *Bifidobacterium* at present[1~4].

On the other hand, the traditional fermented milks, such as Dahi in India, Dadih in Indonesia, Labanh in the Middle East and Maziwa lala in Kenya, have still been produced by spontaneous fermentation of bovine, ovine or buffalo milk with natural microorganisms all over the world. The microflora in such fermented milks are different in each products probably due to differences between the manufacturing locations and conditions of fermentation. For example, the floras in Dadih are different in each data reported by Imai et al.[5], Hosono et al.[6] and Zakaria et al.[7].

In east Africa, several natural fermented milks, such as Maziwa lala, Busaa, Uji, Muratina and Mnazi, are manufactured[8,9]. Maziwa lala, one of the natural fermented milks of east Africa, is prepared as follows; a gourd is washed with hot water and rubbed with the burnt end of some chopped sticks, letting the charcoal break inside. After the charcoal pieces are removed, raw milk or boiled milk is put into the gourd and kept for 2~7 days at room temperature.

In this study, we investigated the microflora of Maziwa lala to compare with previous data by Miyamoto et al.[8] and determined the viscosity of curds prepared with the strains of lactic acid bacteria isolated from Maziwa lala to clarify the role of each strain in the property of Maziwa lala.

2. Materials and Methods

2.1 Maziwa lala samples

Two Maziwa lala samples were collected from two families in a village near Narok, 200 km west of Nirobi in October, 1996. Sample (A) was prepared by pouring fresh milk into a gourd followed by spontaneous fermentation by natural microorganism at room temperature (around 25℃) for 2 days. Sample (B) was prepared by pouring pasteurized milk into a gourd followed by spontaneous fermentation by natural microorganisms at room temperature (around 25℃) for 7 days. The samples were immediately brought to Japan at 4℃ and subjected to the experimental procedures within 48 hours.

2.2 Counting bacterial numbers and isolation of microorganisms

Fresh Maziwa lala was homogenized and diluted with several folds of distilled water. Each dilution of Maziwa lala was poured into petridishes containing MRS, M17, BCP and Standard Plate Count agar medium (SPC)[10] followed by incubation at 25℃ for 48 or 72 hr under aerobic conditions, but that in MRS broth was incubated at 25℃ for 72 hr under anaerobiotic conditions. After incubation, single colonies were counted and each strain of bacteria was repeatedly isolated from the colonies. Taxonomic properties of the isolates were examined and identified according to Bergey's Manual of Systematic Bacteriology[11] and Manual for Identification of Medical Bacteria[12]. Identification of genera and species was done using several tests as shown in Table 1, 2 and 3, respectively.

2.3 Viscosity measurement for the curd

Lact. lactis subsp. *lactis* MZW 4 (LL) and *Leuc. mesenteroides* subsp. *mesenteroides dextranicum* MZW 36 (LD) isolated from Maziwa lala were selected as starters for the preparation of the curd. Reconstituted commercial skim milk (10%w/v) was boiled for 10 min, followed by cooling to room temperature, and then inoculated with either *Lact. lactis* subsp. *lactis* MZW 4 or *Leuc. mesenteroides* subsp. *mesenteroides dextranicum* MZW 36. Each overnight culture inoculum in skim milk (LL 2%, LD 2%,

Table 1 Classification of strains isolated from Maziwa lala (A).

Genus*	A	B	C	D
Gram staining	+	+	+	+
Cell form	S	S	R	S
Growth at 10℃	±	±	+	+
Growth at 25℃	+	+	+	+
Growth at 30℃	+	+	+	+
Growth at 37℃	+	+	+	+
Growth at 45℃	−	−	−	+
Growth at pH 9.2	+	+	−	+
Growth at pH 9.6	−	−	−	+
Gas from glucose	−	+	+	−
Dextran formation	±	+		±
Growth in Anaerobic condition	+	+	+	+
Catalase	−	−	−	−
NH_3 from Arginine	+	−	−	

* A; *Lactococcus*, B; *Leuconostoc*, C; *Lactobacillus*, D; *Enterococcus*

Table 2 Classification of strains isolated from Maziwa lala (B).

Genus*	A	B	C
Gram staining	+	+	+
Cell form	S	S	R
Growth at 10℃	±	±	+
Growth at 25℃	+	+	+
Growth at 30℃	+	+	+
Growth at 37℃	+	+	+
Growth at 45℃	−	−	−
Growth at pH 9.2	+	+	−
Growth at pH 9.6	−	−	−
Gas from glucose	−	+	+
Dextran formation	±	+	
Growth in Anaerobic condition	+	+	+
Catalase	−	−	−
NH_3 from Arginine	+	−	−

* A; *Lactococcus*, B; *Leuconostoc*, C; *Lactobacillus*

Table 3 Physiological characteristics of strains of lactic acid bacteria isolated from Maziwa lala.

Test	Reaction				
L–Arabinose	±	+	−	+	−
Cellobiose	+	+	+	+	−
Esculin	+	+	+	+	±
Fructose	+	+	+	+	+
Galactose	+	+	+	+	+
Glucose	+	+	+	+	+
Lactose	+	+	−	+	+
Maltose	+	+	+	+	+
Mannitol	−	−	−	−	−
Mannose	+	+	+	+	+
Melezitose	−	−	−	−	−
Melibiose	−	+	+	+	−
Raffinose	−	+	+	+	−
Rhamnose	−	−	−	−	−
Ribose	+	±	+	+	+
Salicin	+	+	−	+	+
Sorbitol	−	−	−	+	−
Trehalose	+	+	+	+	−
D–Xylose	−	+	+	−	−
L–Xylose	−	−	−	−	−
Identification*	A	B	C	D	E

* A; *Lactococcus lactis* ssp. *lactis*
B; *Leuconostoc mesenteroides* ssp. *mesenteroides dextranicum*
C; *Leuconostoc paramesnteroides*
D; *Lactobacillus plantarum*
E; *Lactobacillus curvatus*

LL+LD 1+1%) was added to the skim milk and incubated at 30°C for 48 hours under the aerobic conditons. The curds were kept at 5°C for 16 hours before being tested.

A rotational viscometer B8L–HM (Tokyo Keiki Co. Ltd., Tokyo) with an HM–1 spindle was used to measure viscosity. The shear rates were 60 rpm and conducted at 5°C for the viscosity test. Viscosity are indicated as means and standard deviations in pa.s. The significance of differences among treatment groups was determined by analysis of variance with Duncan's multiple-range test (SAS Institute, Cary, NC). Results were considered significant at $p < 0.05$.

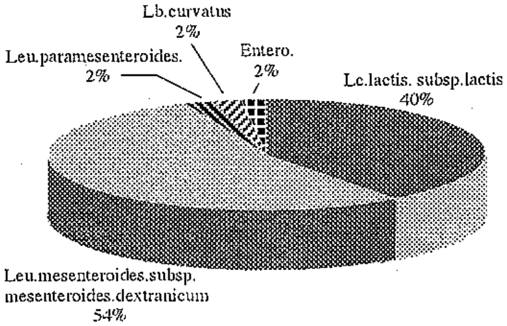
3. Results and Discussion

The bacterial counts in Maziwa lala (A) and Maziwa lala (B) were found to be 5.2×10^7 and 1.3×10^9 c.f.u./ml, respectively. The bacterial count in Maziwa lala (B) was 25 times as large as that in Maziwa lala (A). It was considered to be due to the differences of the conditions of fermentation, pasteurization of milk and fermented time.

Based on cell morphology, 45 isolates from Maziwa lala (A) were classified into 4 genera and identified as *Lactococcus, Leuconostoc, Lactobacillus* and *Enterococcus*. Using taxonomic characterization, 4 species were identified from genera as follows: *Lact. lactis* subsp. *lactis, Leuc. mesenteroides* subsp. *mesenteroides dextranicm, Leuc. paramesenteroides* and *Lb. curvatus*. The dominant species of lactic acid bacteria in Maziwa lala (A) were found to be *Leuc. mesenteroides* subsp. *mesenteroides dextranicm* (54%) followed by *Lact. lactis* subsp. *lactis* (40%), *Leuc. paramesenteroides* (2%) and *Lb. curvatus* (2%) as shown in Fig. 1.

On the other hand, 52 isolates from Maziwa lala (B) were classified into three genera and identified as *Lactococcus, Leuconostoc* and *Lactobacillus*. From taxonomic characterization, four species of lactic acid bacteria in Maziwa lala (B) were identified from genera as follows: *Lact. lactis* subsp. *lactis* (73%), *Leuc. mesenteroides* subsp. *mesenteroides dextranicm* (21%), *Lb. curvatus* (4%) and *Lb. plantarum* (2%) (Fig. 1). In this study, the dominant species of lactic acid bacteria in the two Maziwa lala samples were found to be *Lact. lactis* subsp. *lactis* and *Leuc. mesenteroides* subsp. *mesenteroides dextranicum* followed by *Lb. curvatus, Leuc. paramesenteroides* and *Lb. plantarum*, showing that in our Maziwa lala samples, the major lactic acid bacteri-

Sample (A)



Sample (B)

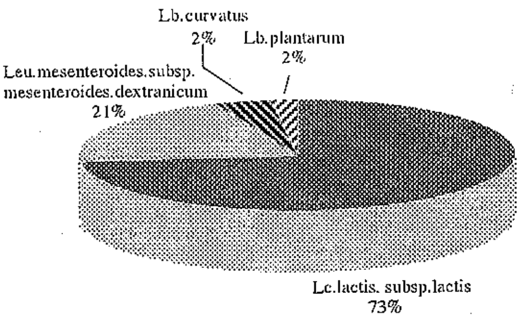


Fig. 1 The distribution of microorganisms isolated from Maziwa lala

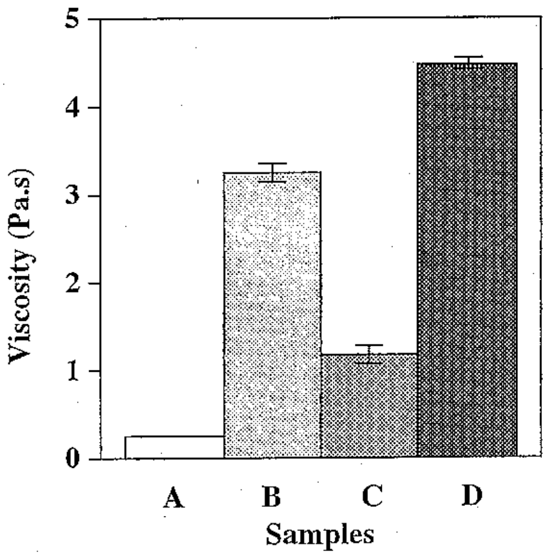


Fig. 2 The viscostiy of the curds produced by the strains isolated from Maziwa lala Values are means for three mesurements, with standard deviations indicated by bars. Means values were significantly different ($p<0.05$); A, control (skim milk) B, *Lact. lastis* subsp. *lactis* (2%) C, *Leuc. mesenteroides* subsp. *mesenteroides dextranicum* (2%) D, *Lact. lastis* subsp. *lactis* (1%) and *Luec, mesenteroides* subsp. *mesenteroides dextranicum* (1%).

al starters were two species, *Lact. lactis* and *Leuc. mesenteroides*. The microflora in Maziwa lala had been previously reported by Miyamoto et al.[8]. According to their data, the microflora in those Maziwa lala is 24% *Lactobacillus curvatus* and *Lactobacillus plantarum*, 33% *Lactococcus cremoris, Lactococcus lactis* and *Enterococcus faecium*, and 43% *Leuconostoc mesenteroides*. The differences between our data and that of the previous study should be due to differences between the manufacturing locations and conditions of fermentation.

Based on the above data, the typical strains in Maziwa lala were selected for the test to measure viscosity of the curds prepared by fermentation of those. The viscosities of several curds were shown in Fig. 2. The control with skim milk was 0.234±0.003 Pa.s. The curd produced by the mixed starter of *Lact. lactis* subsp. *lactis* (1%v/v) and *Leuc. mesenteroides* subsp. *mesenteroides dextranicum* (1%v/v) showed the highest value of the viscosity, 4.473±0.062 Pa.s. Subsequently, the viscosity of the curd produced by *Lact. lactis* subsp. *lactis* (2%v/v) was 3.238±0.100 Pa.s. In addition, whey-off were clearly observed in these curds. On the other hand, the viscosity of the fermeted milk produced by single strain of *Leuc. mesenteroides* subsp. *mesenteroides dextranicum* (2%v/v) was 1.160±0.099 Pa.s, significantly higher than that of the control, but whey-off and the formation of the curd was not clearly observed. In general, *Lact. lactis* subsp. *lactis*, which is used as a starter for the manufacturing of cheese, is well known to contribute to acidogenesis, while *Leuc. mesenteroides* subsp. *mesenteroides dextranicum* must contribute to produce the favorite flavor of the cheese. According to our results of the test, the dominant strains in Maziwa lala, *Lact. lactis* subsp. *lactis* and *Leuc. mesenteroides* subsp. *mesenteroides dextranicum*, may be suggested to contribute to the formation of curds and flavour in Maziwa lala, respectively.

4. Conclusion

The major species of lactic acid bacteria in Maziwa lala were *Lact. lactis* subsp. *lactis* and *Leuc. mesenteroides* subsp. *mesenteroides dextranicum*, followed by *Lb. curvatus, Leuc. paramesenteroides* and *Lb. plantarum*. According to the viscosity test, *Lact. lactis* subsp. *lactis* was suggested to contribute to the formation of curd in Maziwa lala.

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ケニヤのマサイ族の生産する伝統的発酵乳：マジワララの微生物学的特性

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ケニアの一般家庭より伝統的発酵乳であるMaziwa lalaを採取し，その菌叢および流動特性について検討した。生菌数はMaziwa lala (A) で5.2×10^7 c.f.u/ml, (B) で1.3×10^9 c.f.u/mlであった．また，Maziwa lala (A), (B) からそれぞれ分離した45菌株，54菌株を同定試験に供した結果，主要菌種は*Lact. lactis* subsp. *lactis*と*Leuc. mesenteroides* subsp. *mesenteroides dextranicum*であり，これらが全菌株の90%以上を占めていた。

スキムミルク培地で，これら主要菌種である*Lact. lactis* subsp. *lactis, Leuc. mesenteroides* subsp. *mesenteroides dextranicum*およびこれら2種を混合（1:1）したものを培養し，粘性試験を行った。その結果，混合培養したものが最も粘性が高く，また，風味が良かったことから，これら2菌種がMaziwa lalaの製品特性を形成するものであることが示唆された。