

## Abstract of Thesis/Dissertation

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Title : Study on the characteristic properties of Gambian traditional fermented milk  
(ガンビアの伝統的な発酵乳の特性に関する研究)

## Abstract

Milk and fermented milk are popular with people of all walks of lives and ages in The Gambia, as it is well known to contain useful nutrients. There is little information about the microbiota of Gambian traditional FM, although there are reports of the existence of pathogens such as genus *Bacillus*, *Clostridia*, *Listeria*, *Escherichia*, *Salmonella*, and *Staphylococci*, in Gambian fermented milk (FM). Recently, diarrheal disorders in children under the age of 5 have been frequently reported in The Gambia (Saha *et al.* 2013), suggesting that pathogenic microorganisms present in unpasteurized milk and fermented milk are one of the causes. However, consumers and producer have little knowledge about not only such problems but also the characteristics of milk and dairy products produced in The Gambia. Therefore, this research first aimed to elucidate the microbial profile of Gambian traditional FM “Kosam” and evaluate its safety.

FM samples were collected at two regions (Central River Region: CRR, Lower River Region: LRR) in The Gambia in 2019. The microbiota of these samples was analyzed by culture-dependent methods and Illumina sequencing. The number of lactic acid bacteria (LAB) in FM from CRR and LRR was  $8.27 \pm 0.08$  log CFU/mL and  $7.21 \pm 0.09$  log CFU/mL, respectively. Whilst no coliforms and *Escherichia coli* were detected in FM from CRR (CRR-FM), that from LRR (LRR-FM) contained  $5.73 \pm 0.17$  log CFU/mL of coliforms and  $4.82 \pm 0.13$  log CFU/mL of *E. coli*. The dominant viable

LAB in CRR-FM was *Lactobacillus delbrueckii*, followed by *S. lutetiensis*, while that from LRR-FM was *Lc. lactis*. The metagenomic analysis using Illumina sequencing also revealed that these species were dominant in Gambian traditional FM. Furthermore, it also revealed the possibility of the presence of pathogens such as *Klebsiella* spp. These results reveal that some traditional Gambian fermented milk is not microbiologically safe.

To produce safe FM in The Gambia in the future, the LAB isolates from Gambian traditional FM were characterized on the basis of their acid production of reconstituted skimmed milk (RSM) and the bacteriocin-like inhibitory activity among isolates. Among LAB isolates from CRR-FM and LRR-FM, the proportions of the strains which lowered the pH of RSM to 6.0 or less after the incubation at 35°C for 24 h were 79% and 16%, respectively. The majority of *Lc. lactis* strains isolated from both Gambian FM could not decrease the pH value of RSM to less than 6.0. On the other hand, among LAB isolated from CRR-FM, some of *Lb. delbrueckii*, *Leu. mesenteroides*, *Limosilactobacillus fermentum*, and *Streptococcus lutetiensis* strains could reduce the pH to less than 5.0. The pH of CRR-FM and LRR-FM were 4.7 and 5.4, respectively, suggesting that the proportion of strains to produce acid in milk affects the pH of Gambian FM. Furthermore, the decrease in pH was also suggested to affect the number of coliforms and *E. coli* in Gambian FM.

A bacteriocin-like inhibitory activity assay among isolates was performed by the agar well diffusion assay. Twenty strains (9 species) isolated from Gambia FM were used as indicators. *S. lutetiensis* strains showed bacteriocin-like inhibitory activity against 15 indicator strains (5 species), suggesting that they have a relatively wide range of antibacterial activity. Similarly, *Lc. lactis* strains and *Lb. delbrueckii* strains showed the activity against 7 strains (3 species) and 6 strains of indicators (2 species), respectively. All of *E. faecalis* strains showed the bacteriocin-like inhibitory activity against three *Lb. delbrueckii* strains as indicators. In contrast, *E. faecalis*, *Lb. plantarum*, *Lb. fermentum*, *Lc. lactis*, and *Leu. mesenteroides* used as indicators were not inhibited by all other LAB strains isolated from Gambian FM. These results suggest that the bacteriocin-like active substances produced by LAB strains are involved in the formation of the microbiota of Gambian FM.

From the *S. lutetiensis* strains having a relatively wide range of antibacterial activity, *S. lutetiensis* G3067 was selected as a strain with higher bacteriocin-like activity. In addition, a bacteriocin produced by this strain was purified and characterized. The cell-free supernatant (CFS) prepared from MRS broth culture of *S. lutetiensis* G3067 was subjected to three preparation steps: chloroform extraction, ammonium sulfate precipitation, anion exchange chromatography.

Bacteriocin-like inhibitory activity assay was performed by an agar well diffusion method using *Lb. delbrueckii* subsp. *delbrueckii* NBRC13953<sup>T</sup> as an indicator strain. By these purification steps, bacteriocin activity increased from 20 AU/mL in CFS to 800 AU/mL in Peak 1 on anion exchange chromatography, with a yield of 16.7%. Peak 1 fraction was inactivated by proteinase K and eluted as a single peak on HPLC. In addition, it was detected as a broad band at the position between 1.0 and 3.5 kDa on Tricine SDS-PAGE, and its estimated molecular weight was calculated as 3.0 kDa from its migration. These results revealed that this component was a bacteriocin. This bacteriocin, named bacteriocin G3067, had thermal stability against the heat treatment at 40°C, 50°C and 60°C, for 30 min. Furthermore, after treatment at 70°C, 100°C and 121°C for 30 min, its activity increased significantly compared with the unheated sample. In addition, an activity of bacteriocin G3067 was not changed by the addition of divalent metal ions. These properties suggest that bacteriocin G3067 may be available for food processing.

This study enhanced the knowledge concerning Gambian FM and contributed to the elucidation of microbial communities. It also provided an opportunity to preserve LAB strains isolated from Gambian FM as microbial resources. These findings will become important not only for Gambian politicians and bureaucrats to consider laws and regulations, but also to promote eating safe dairy products for the general public in The Gambia.