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Microbial Ecological Studies on Traditional Food Fermentation

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Research aims

Many kinds of fermented foods have been manufactured by traditional procedure all over the world. In many cases, appropriate microorganisms grow selectively during the fermentation process without strict microbiological regulation. However, the microbial composition and their association are difficult to clarify by cultivation-isolation methods. In addition to the culture-based methods, in this study, molecular microbial ecological approaches were applied to obtain further insight into a traditional brewing technology.

Methods

Pot vinegar has been manufactured in Kagoshima prefecture since 1805. After steamed rice, water and rice-koji are added into a ceramic pot, additional rice-koji is spread onto the liquid surface (floating koji). The pot is placed outdoors for around 3 months. The fermentation broth was collected from three fermentation pots several times during the fermentation period. Total DNA was extracted from the harvested cells and used for PCR-denaturing gradient gel electrophoresis (DGGE). The procedures of DNA extraction and PCR-DGGE were described previously[1].

Results

Chemical analyses of the fermentation broth indicated that saccharification and alcohol production simultaneously proceeded for around 20 days after starting the fermentation. And then, acetic acid concentration increased gradually with the consumption of ethanol and reached 5% at the end of fermentation (day 90). Koizumi *et al.* reported the similar results in 1989[2]. Lactic acid concentration increased in a few days after starting the fermentation and was kept at 100–150 mM during the process. The production of lactic acid correlated with the decrease in pH below 4 at the initial stage.

PCR-DGGE profile indicated the succession of the bacterial community around day 15–30 when acetate concentration started to increase (Fig. 1). The similar banding profile was observed in three fermentation pots. Although the banding profiles of the starting materials (day 0) were different from one another, the three pots showed the nearly same bands on day 1. This indicates the rapid growth of favorable bacteria in a day to achieve the stable fermentation.

The saccharificaion/alcohol fermentation process was characterized by the bands which were related to *Lactobacillus fermentum* (bands 1–4), *Lactococcus lactis* (band 6), or *Pediococcus acidilactici* (band 8). And then, the bands related to *Acetobacter pasteurianus* (band 9) and *Lactobacillus acetotolerans* (band 7) were detected in the acetate fermentation process. In contrast, *Lactobacillus casei* and *Lactobacillus plantarum* were isolated from the saccharification/alcohol fermentation process. The previous studies also reported the isolation of *Lactobacillus casei* subsp. *casei* and *Lactobacillus plantarum* with high frequency[3,4]. However, these lactic acid bacteria were not detected in the major bands of the PCR-DGGE profile. Furthermore, the low similarity of band 1, 2, or 3 to the closest relative may indicate that these bacteria are novel *Lactobacillus* species.

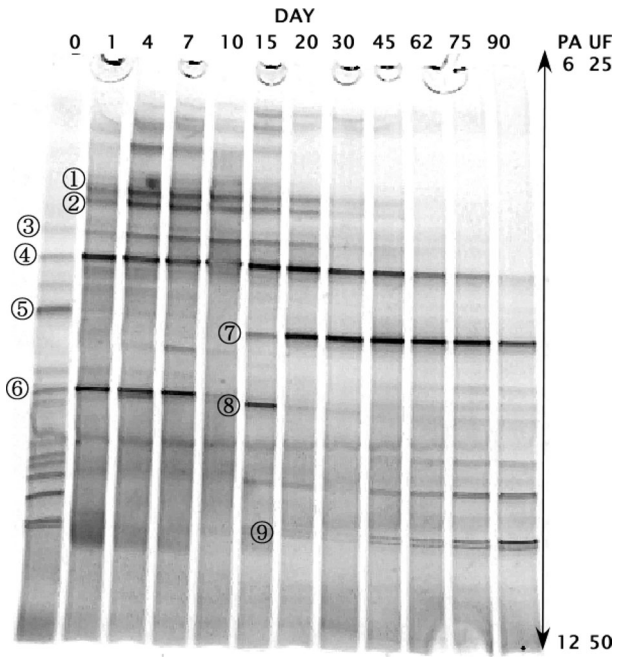

Fig. 1. PCR-DGGE profile during the manufacturing process
The arrow represents the linear gradient of polyacrylamide concentration (PA 6–12%) and DNA denaturants (UF 25–50%). The bands indicated by the circled number were excised to determine the DNA sequences. The closest relative in DNA databases to each band is as follows. 1, *Lactobacillus fermentum* (97%); 2, *L. fermentum* (97%); 3, *L. fermentum* (97%); 4, *L. fermentum* (98%); 5, *Aspergillus oryzae* (98%); 6, *Lactococcus lactis* (100%); 7, *Lactobacillus acetotolerans* (100%); 8, *Pediococcus acidilactici* (100%); 9, *Acetobacter pasteurianus* (100%).

Conclusion

PCR-DGGE analysis represented the succession of bacterial community during the pot vinegar fermentation process. The change in the fermentation pattern was clearly characterized by the change in the banding profile. The same succession was observed in the three pots examined. The bacteria detected as major bands were different from the isolated microorganisms.

References

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