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Original article

The microbial community and free amino acid composition in salted and fermented squid products

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Introduction

Salted and fermented product of aquatic organisms and their organs, called "shiokara" in Japanese, are traditional seafoods in Japan. It is typically made from squid mantle muscle together with its visceral part, liver, by treatment with NaCl. This product is called "ika-shiokara", where "ika" means squid in Japanese. These products are usually provided with taste-active compounds such as free amino acids and peptides during the fermentation process. While various factors apparently affect the fermentation process, the microbial community is considered to play important roles in this process, because taste-active compounds are usually produced by degradation of proteins with the action of not only endogenous and but also exogenous proteases. Fujii et al. [1] previously determined bacteria that dominated the ika-shiokara products by using a conventional culture method. According to their study, predominant bacteria in ika-shiokara were found to be *Staphylococcus* and *Micrococcus*. However, most bacteria are now known to be unculturable and metagenomic analysis has overcome such problems encountered in conventional microbial experiments.

The present study was carried out to determine the microbial community in the ika-shiokara products by the 16S rRNA metagenomic analysis. Furthermore, the contents of free amino acids were determined to correlate them with the above-mentioned microbial community.

Materials and methods

Shiokara production
Two types of ika-shiokara products were prepared in this study with the Japanese common squid *Todarodes pacificus*. For both types squid muscle and liver were separately washed, chopped, and mixed with 5 or 10% NaCl (w/w) at final concentrations in the products. Then salt-treated muscle and liver were mixed and subjected to the production of two types. One type named "akazukuri", now commonly produced in the ika-shiokara factories, was prepared from the 5% NaCl-treated mixture by adding seasonings and allowing to stand at 5°C for several days. The other named "namakouji-jukusei" is a traditional type, which was prepared from the 10% NaCl-treated mixture by adding malted rice and allowing to stand at 25°C for a few days.

Samples were collected from two types of the shiokara products to extract DNA and to analyze free amino acids.

DNA extraction and metagenomic analysis
Two methods were carried out for DNA extraction and compared for their purity. One method employed GNOME DNA Isolation Kit (MP Biomedicals, Santa Ana, CA, USA). The other employed CTAB buffer [2% cetyltrimethylammonium bromide, 2% polyvinylpyrrolidone, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris-HCl (pH 8.0)] and AMpure XP beads (Beckman Coulter, Pasadena, CA, USA). Both methods were carried out using the liquid part of the shiokara products. Then the V1-V3 region in the 16S rRNA gene was amplified with a bacteria-specific PCR primer set using the purified DNA as a template. The 16S rRNA amplicon products were sequenced with a MiSeq next generation sequencer (Illumina, San Diego, CA, USA). Acquired Illumina reads were joined by overlapping forward and reverse reads using the FLASH command [2] and analyzed by the SILVAngs server [3].

Furthermore, starting materials such squid mantle and foot muscles, malted rice, and liver were subjected to the screening of coexisting bacteria and subsequent DNA extraction. The mantle and foot muscles were soaked together to CTAB buffer, and the obtained solution was subjected to extraction and purification of

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DNA as described above. Malted rice powder and chopped salted liver were directly subjected to the extraction of DNA.

Free amino acid analysis

Free amino acids were analyzed by HPLC essentially according to Teerlink et al. [4]. Samples subjected to the determination of the free amino acid content were collected from the liquid part of the shiokara products.

Results and discussion

Microbial community

The extraction of DNA was more efficient and its purity was higher with CTAB buffer than with GNOME DNA Isolation Kit. Then we employed the former method for further experiments. As a result, we found that the most abundant bacteria belonged to *Vibrio* followed by *Psychrobacter* in the starting materials of squid muscle and akazukuri on the day when the production was started (day 0) (Fig. 1). This bacterial community was not changed dramatically during the fermentation process for 7 days. These results suggest that the microbial community in the starting material plays the major roles during the akazukuri preparation process. Meanwhile, namakouji-jukusei contained *Paracoccus* and *Cronobacter* as the most abundant bacteria on day 0, and then various bacteria including *Psychrobacter*, *Acinetobacter* and *Staphylococcus* on day 4. Thus, the bacterial community of namakouji-jukusei changed markedly.

Free amino acids

The total free amino acid content in akazukuri was about 88 µmol/g, which was much lower than that in namakouji-jukusei (228 µmol/g). However, both akazukuri and namakouji-jukusei shiokara products contained taurine almost at the same level as the most dominant amino acid (about 30 µmol/g) (Fig. 2). It was noted that namakouji-jukusei contained glutamic acid and leucine at high concentrations. These results indicate that the metabolite composition of namakouji-jukusei was markedly affected by the fermentation process probably in association with changes in the microbial community.

We are currently analyzing samples collected periodically from both types of ika-shiokara products and results to be obtained will be reported elsewhere.

References

1. Fujii T et al. (1994) Nippon Suisan Gakkaishi 60: 265–270
2. Magoc T et al. (2011) Bioinformatics 27: 2957–2963
3. SILVAngs: http://www.arb-silva.de/ngs/
4. Teerlink T et al. (1994) Clin Chem 40: 245–249

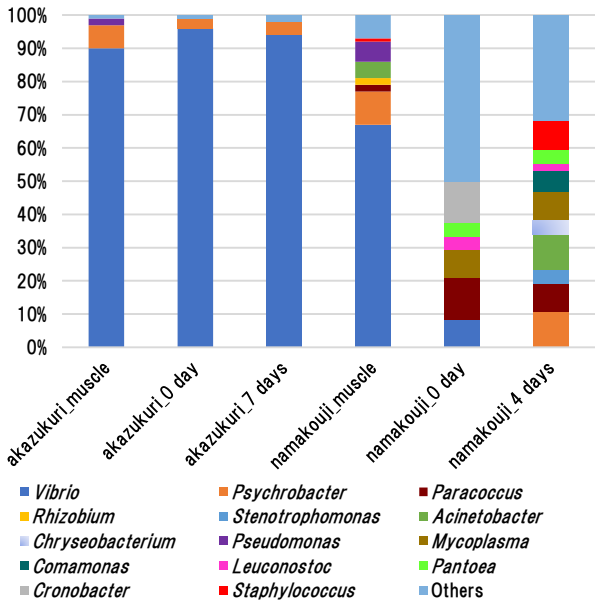

Fig. 1. Microbial communities of two types of ika-shiokara products, akazukuri and namakouji-jukusei. 16S rRNA metagenomic datasets were analyzed by the SILVAngs sever.

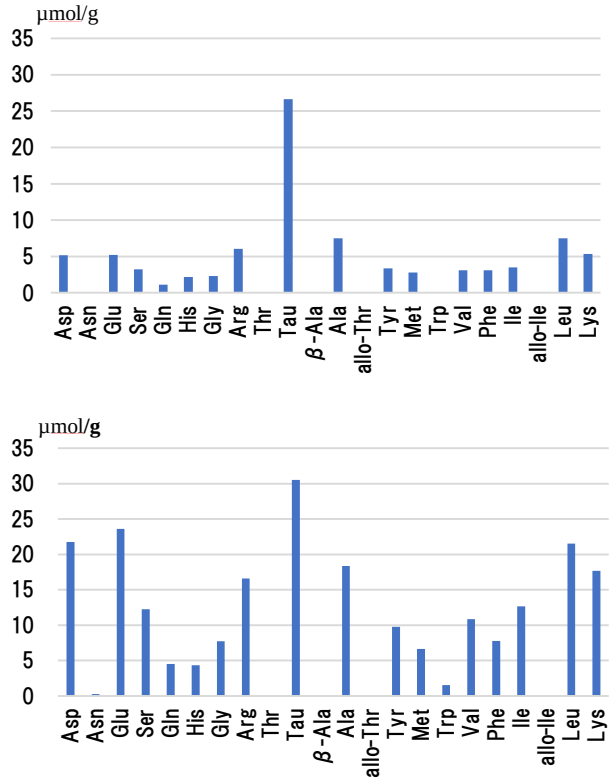

Fig. 2. The contents of free amino acids in akazukuri on day 7 (upper panel) and namakouji-jukusei on day 4 (lower panel) after preparation of the ika-shiokara products.