Note

Fermentation of Jerusalem Artichoke with or without Lactic Acid Bacteria Starter Cultures

Ken-ji YOKOI^{1*}, Ken-Ichi KAWASAKI^{1,4}, Go NISHITANI³, Akira TAKETO² and Ken-Ichi KODAIRA³

¹ Toyama Prefectural Food Research Institute, 360 Yoshioka, Toyama, 939-8153, Japan

² Department of Applied Physics and Chemistry, Fukui University of Technology, Gakuen, Fukui, 910-8505, Japan

³ Molecular Biology Group, Faculty of Engineering, Toyama University, 3190 Gofuku, Toyama, 930-8555, Japan

⁴ Present address: Laboratory of Aquatic Food Science, Department of Fisheries, Faculty of Agriculture, Kinki University, Nakamachi, Nara, 631-8505, Japan.

Received May 2, 2005; Accepted June 16, 2006

The present study investigated the fermentation of Jerusalem artichoke with or without the addition of *Leuconostoc mesenteroides*, *Lactobacillus casei*, or *Lactococcus lactis* as a starter. Seasoning brine, containing 2.5% NaCl and about 1.0×10^7 cfu/ml starter bacteria when added, was mixed with sliced Jerusalem artichoke and fermented at 20°C. Whether the starters had been added or not, lactic acid bacteria became predominant in the brine, and consequently acidity was increased. Fermentation was completed in 6–7 days. Bacterial flora in the brines of fermented products was investigated by amplified ribosomal DNA restriction analysis (ARDRA) and sequencing of the 16S rRNA gene. When *L. mesenteroides* or *L. lactis* was used as a starter, the added strain was predominant in the product, suggesting attainment of controlled fermentation. In the case of *L. casei*, the starter strain did not prevail, and the final microbial flora was different from that of natural fermentation. These results indicate that *L. mesenteroides* and *L. lactis* have the potential to control Jerusalem artichoke fermentation.

Keywords: *Leuconostoc mesenteroides*, *Lactobacillus casei*, lactic acid bacteria, Jerusalem artichoke, ARDRA, 16S rDNA sequence

Introduction

The Jerusalem artichoke is a plant rich in inulin, which is known as a prebiotic (Van Loo *et al.*, 1995; Roberfroid and Delzenne, 1998), and is attractive as a material of functional foods. In several prefectures of Japan, the tuber of this plant has been used primarily in pickling (Sakamoto, 2002). The unsavory flavor of the fermented product, however, has limited their spread; however, scientific attempts to improve the manufacturing process and the taste of the product have not yet been performed.

Lactic acid bacteria (LAB) play an important role in food fermentation, cause the characteristic flavor changes associated with fermentation, and exercise a preservative effect on the fermented product (Holzapfel, 1997; Gardner *et al.*, 2001). In most cases, vegetables can be fermented by the bacteria that naturally exist on the raw material (Vaughn, 1985). However, this type of fermentation often leads to variations in the properties of products. Traditionally, by trial and error, skills have developed to control fermentation by defining such conditions as temperature and salt concentrations.

This report deals with fermentation of Jerusalem artichoke, using LAB as the starter for stabilization of microbial flora.

Materials and Methods

Microorganisms and media *L. mesenteroides* LM 057 (Rhodia, USA), *L. casei* Hakkoushinuchi (Taiyo Kouryou Co., Osaka, Japan) and *L. lactis* TF1 isolated from a naturally fermented Jerusalem artichoke preparation were used in this study. Each strain was inoculated in MRS broth (Difco) and grown for 24 hours at 30°C. Before use, the culture was washed twice in 0.85% NaCl solution.

Fermentation procedures Jerusalem artichoke (*Helianthus tuberosus*, cultivated in Toyama and Ishikawa Prefectures) was purchased from a local market in Toyama, Japan. Jerusalem artichoke tuber was sliced at an average thickness of 2 mm. Sliced Jerusalem artichoke (approx. 600 g) was packed into 2l plastic jars, then brine containing 3% glucose, 1% mirin (Japanese fermented seasoning), 2.5% NaCl and approximately 10^7 /mL specified bacteria was added. For “natural fermentation”, plain brine without bacterial cells was added. Final pack-out ratio of Jerusalem artichoke vs. brine was about 1: 1 (w/w). To prevent material from rising, a 2 mm thick plastic sheet and approximately 300 g of stones were placed over the material. All jars were stored at 20°C.

Microbial Analysis Periodically during the fermentation process, a brine sample was taken from each batch and diluted with 0.1% peptone water. The pour plate technique was used for the enumeration of microorganisms. The number of total viable bacteria was deter-

* To whom correspondence should be addressed.
E-mail: yokoi@agri.pref.toyama.jp

Fig. 1. Microbiological changes and acid production in the brine of fermented Jerusalem artichoke: natural fermentation (A), *L. mesenteroides* starter (B), *L. casei* starter (C), *L. lactis* starter (D). ●, Total viable bacterial cells; ○, Lactic acid bacteria; ▲, Yeast; ▽, Titrative acidity; ■, pH.

mined using plate count agar (Nissui Seiyaku Co., Tokyo, Japan) after incubation at 37°C for 2 days. For LAB, plate count agar containing Brom Cresol Purple (Nissui Seiyaku Co.) was used. Yeast was counted on a Potato Dextrose agar plate (Nissui Seiyaku Co.) containing chloramphenicol (100 µg/mL) after incubation at 28°C for 5 days.

Chemical analysis During fermentation, pH was measured periodically using a pH meter (Hitachi, Japan). Titrative acidity (calculated as % lactic acid) was determined using 50 mM NaOH according to the method of Etchells *et al.* (1964).

Extraction of total DNA from microorganism The bacterial colony formed on PLC agar was picked up and proliferated in Tryptone glucose yeast broth (0.5% Tryptone, 0.1% glucose, 0.25% Yeast extract, pH 7.1) at 37°C for 24 h. A portion (1.5 ml) of culture was harvested and the cells were washed once and suspended in 50 µl of TE-buffer (10 mM Tris-Cl, 1 mM EDTA; pH 8.0). After adding 0.05 g of glass beads (diameter 0.1 mm), cells were disrupted by vortexing for 1 min. Glass beads and cell debris were then removed by centrifugation at 10,000 g for 5 min and the supernatant was used as total DNA solution.

Amplified Ribosomal DNA Restriction Analysis (ARDRA) ARDRA analysis was performed according to the method of Fernandez *et al.* (1999). PCR amplification of 16S rDNA genes was carried out in a 50-µL reaction volume containing 5 µL of DNA solution, 0.3 µM (each) primer, 0.2 mM (each) deoxyribonucleotide triphosphate, 1 mM MgSO₄, and 1 U KOD-plus-DNA polymerase (Toyobo, Osaka, Japan). Primers used for PCR were 8F (5'-AGAGTTTGATCAT-

GGCTCAG-3') corresponded to positions 8-27 of *E. coli* 16S rDNA, and 15R (5'-AAGGAGGTGATCCAGCCGCA-3') corresponded to the complement of positions 1540-1520. PCR reaction was performed with a DNA Thermal Cycler (Takara, Ohtsu, Japan) using one denaturation step at 94°C for 2 min followed by 30 cycles of reaction at 94°C for 15 s, 50°C for 30 s, and 68°C for 90 s. The amplification products were checked by agarose gel electrophoresis in 1.0% gels. Gels were stained with ethidium bromide and visualized with UV light. The 16S rDNA amplification products (0.5 µg) were digested with Hae III (Takara, Japan) and analyzed by agarose gel electrophoresis in 1.5% gels.

16S rDNA sequencing and identification Amplified 16S rDNA was ligated into the Hinc II site of the pUC 118. Positive clones with 16S rDNA inserts were partially sequenced using a Long Read Tower DNA sequencer (Amersham, USA). Universal M13 sequencing primer (5'-GTTTCCAGTCACGACGTTGTA-3') and M13-RV sequencing primer (5'-GAGCGGATAACAATTTCACACAGG-3') were used in sequencing reactions. The obtained sequences (around 700 bp) were compared to Genbank data, using the BLAST analysis program, in order to identify the bacterial species.

Results and Discussion

Natural fermentation Fig. 1 (A) shows the change in the microbial flora, pH, and acidity of Jerusalem artichoke fermented without a starter. In this case, the number of total viable cells, 1.0×10^3 /mL at the beginning of fermentation, rapidly increased to 3.1×10^7 /mL, then decreased moderately, finally reaching 8.3×10^8 /mL. Change in the

Fig. 2. ARDRA patterns of bacteria in the brine of fermented Jerusalem artichoke by natural fermentation (A), using *L. mesenteroides* starter (B), *L. casei* starter (C), *L. lactis* starter (D). ARDRA patterns of pure starter culture (E) were also indicated. Ten random colonies on the agar plate were analyzed. Lane M indicated 100 bp marker DNA ladder.

Table 1. Identification by 16S rDNA partial sequence.

Strain	Identification	Identity	Source
N1	<i>W. soli</i>	97.8%	Natural fermentation
M1	<i>L. mesenteroides</i>	98.9%	<i>L. mesenteroides</i> starter fermentation
C1	<i>L. casei</i>	99.6%	<i>L. casei</i> starter fermentation
C2	<i>L. mesenteroides</i>	99.6%	<i>L. casei</i> starter fermentation
L1	<i>L. lactis</i>	99.3%	<i>L. lactis</i> starter fermentation

LAB number was nearly equal to the change in the number of total viable cells. Initially, the number of yeast cells was 1.6×10^4 /ml, then increased 35-fold, decreased transiently, and finally increased to 600-fold of the initial density. Titrativity acidity slowly increased, and after 1 week of fermentation, the acidity reached approximately 0.35%, while the pH decreased to 4.1. Amplified ribosomal DNA restriction analysis indicated that ten colonies, randomly isolated from a naturally fermented lot, belong to a single strain (Fig. 2A). The sequencing experiments demonstrated the presence of *Weissella soli* in the naturally fermented preparation (Table 1). Similarly, a single strain, *Lactococcus lactis*, dominated the other lot. These results indicated that LAB was predominant and grew remarkably well in natural fermentation, although the dominant strain varied, depending on the lot of Jerusalem artichoke. Brine added for seasoning contained glucose, salts, and mirin, thus it might promote the growth of the microbes associated with artichoke thereby increasing

the acidity. A preliminary sensory evaluation indicated that the products fermented for 6–7 days did not taste well (immature taste) and had an off-flavor. Among the ten independent experiments, six gave the same unfavorable results, whereas four samples showed increased acidity and tasted good. In the latter cases, *L. lactis* was predominant (data not shown). Although spontaneous lactic acid fermentation was successful in some cases, in general, fermentation without a starter was neither predictable nor controllable (Vaughn, 1985; Holzapfel, 1997).
Fermentation with Leuconostoc mesenteroides starter (LM-starter) Fig. 1 (B) shows the change in the microbial flora, pH, and acidity of the fermented Jerusalem artichoke with the addition of LM-starter. As a natural consequence of the starter addition, the LAB count was high (5.0×10^7 /ml) from the beginning of fermentation. Total cells and LAB followed almost the same proliferation pattern: plateaued at 3 days, followed by a decline. Initially, the viable cell count for yeast was approximate-

ly 3.0×10^3 /ml, and decreased to 1/1000 followed by a rapid increase 3 days later. Both the increase in acidity and the decrease in pH were comparatively rapid. In amplified ribosomal DNA restriction analysis, as might be expected, all isolates from the LM starter-added sample exhibited an identical restriction pattern (Fig. 2B). Subsequent DNA sequencing experiments demonstrated that the isolates were identical to *L. mesenteroides*. The products subjected to 6–7 days of fermentation had good taste and flavor. Regarding the use of *L. mesenteroides*, the results were reproducible (throughout the four repeated experiments). These results indicated that control fermentation of Jerusalem artichoke was achieved using *L. mesenteroides* as a starter.

Fermentation with *L. casei* starter (LC-starter) As shown in Fig. 1 (C), profiles of the viable cell counts were similar to those of the LM-starter. In the first three days, acidity increased slowly, as in natural fermentation. For 2 days, the number of yeast cells slowly increased, then decreased to 1/100 followed by a gradual increase. After 1 week of fermentation, acidity reached approximately 0.6%, which was lower than that of natural fermentation. ARDRA analysis indicated that the addition of the starter *L. casei* resulted in heterogeneous bacterial flora. Among the ten random isolates, six samples coincided well with *L. casei*, but the remaining four isolates were distinctly different from the starter (Fig. 2C). The DNA sequencing experiments demonstrated presence of *L. casei* and *L. mesenteroides* in the LC starter-added sample (Table 1). These results indicated that when *L. casei* was used as the starter, controlled fermentation was not achieved and the microbial flora was different from that of natural fermentation. Whether the *L. casei* starter was added or not, the taste of the final products was indistinguishable. Upon examination after fermentation, *L. casei* was comprised the majority (60–90%) of the microflora, differing from the starter-unadded control.

Fermentation with *L. lactis* starter (LL-starter) As mentioned above, spontaneous fermentation occasionally yielded tasty pickles, as a consequence of the propagation of a lactococcal strain. This strain was isolated and used as a starter in the fermentation of the artichoke tuber. As shown in Fig. 1D, the number of lactic acid bacteria increased 10-fold within 2 days, and thereafter remained nearly constant. Titration acidity augmented continuously during fermentation, and reached 0.5% at one week. Moreover, the fermented product had good flavor. The restriction fragment pattern of the ribosomal gene was identical to that of *L. lactis* TF1 (Fig. 2D, E). The identification was further confirmed by sequencing the gene (data not shown). Throughout 6 independent trials, the

starter strain dominated during and after fermentation. These results indicate that *L. lactis* TF1 is also suitable as a starter for the fermentation of Jerusalem artichoke.

As indicated above, natural fermentation resulted in an immature taste and off-flavor. The addition of LAB starter with seasoning brine improved both taste and flavor. By adding the starter, fermentation was completed within 6–7 days at 20°C. When pickled in miso, as in traditional fermentation, the completion of fermentation requires approximately two weeks or more. For industrial fermentation, it is important to stabilize the microbial flora and shorten the time duration in addition to improving the taste of the product. LAB-starter addition is considered to be effective for industrial fermentation. The taste and flavor did not particularly differ among the three starter strains (LM, LC and LL); however, microbial flora analysis indicated that LM-starter and LL-starter were more stable than LC-starter throughout the fermentation process. Thus, the addition of LM-starter or LL-starter is believed to considerably improve the fermentation of Jerusalem artichoke.

Acknowledgements We thank Mrs. S. Ikekawa for experimental support. This work was partially supported by a grant-in-aid for biotechnological research from the Ministry of Agriculture, Forestry and Fisheries of Japan.

References

- Etchells, J.L., Costilow, R.N., Anderson, T.E. and Bell, T.A. (1964). Pure culture fermentation of brined cucumbers. *Applied Microbiol.*, **12**, 523–535.
- Fernandez, A., Huang, S., Seston, S., Xing, J., Hickey, R., Criddle, C. and Tiedje, J. (1999). How stable is stable? Function versus community composition. *Appl. Environ. Microbiol.*, **65**, 3697–3704.
- Gardner, N.J., Savard, T., Obermeier, P., Caldwell, G. and Champagne, C.P. (2001). Selection and characterization of mixed starter cultures for lactic acid fermentation of carrot, cabbage, beet and onion vegetable mixtures. *Int. J. Food Microbiol.*, **64**, 261–275.
- Holzappel, W. (1997). Use of starter cultures in fermentation on a household scale. *Food control*, **8**, 241–258.
- Roberfroid, M.B. and Delzenne, N.M. (1998). Dietary fructans. *Annu. Rev. Nutr.*, **18**, 117–143.
- Sakamoto, A. (2002). Outogenous ground and cooking of Jerusalem Artichoke in Saga prefecture. *Nippon Chori Kagaku Kaishi*, **36**, 292–298 (in Japanese).
- Van Loo, J., Coussement, P., De Leenheer, L., Hoebregs, H. and Smits, G. (1995). On the presence of inulin and oligofructose as natural ingredients in the western diet. *Crit. Rev. Food Sci. Nutr.*, **35**, 525–552.
- Vaughn, R.H. (1985). The microbiology of vegetable fermentations. In *Microbiology of fermented foods*, Vol. 2, ed. by Wood, B.J.B., Elsevier, London, pp. 49–109.