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Development of fermented instant Chinese noodle using *Lactobacillus plantarum*

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Abstract

A new-type of instant Chinese noodle was developed with the application of lactic acid fermentation by lactobacilli. Since the pH value of the noodle sheets is alkaline with *kansui* (around 8.5), alkaline tolerance is required for the lactobacilli to ferment noodle sheets. The screening of the lactobacilli strains suitable for the fermentation was conducted using 46 strains from 12 species (including subspecies) of lactobacilli. Several strains of *Lactobacillus pentosus* and *Lactobacillus plantarum* were found to be fermenters. Among these, *L. plantarum* NRIC 0380, that showed the highest fermentation rate and favorable modification of noodle, was selected as the best strain, and was employed for the pilot scale manufacture of instant Chinese noodle. During fermentation, *L. plantarum* NRIC 0380 produced lactic acid to about 11 g/kg noodle sheet after 24 h with a concomitant pH decrease from an initial of about 7.9 down to 3.9. Sensory test after rehydration with boiled water revealed that the fermented instant Chinese noodle sheets at pH 7.5 had increased hardness, elasticity and light sour taste.

Keywords: Lactobacilli; *Lactobacillus plantarum*; Instant Chinese noodle

1. Introduction

Among lactic acid bacteria (LAB), lactobacilli are widely used for the preparation of fermented food products such as yoghurt, fermented milk, fermented sausage, bread and pickles (Caplice and Fitzgerald, 1999). The effects of lactic acid fermentation ranges from improvement of nutritional quality (Shahani and Chandan, 1979), formation of sour taste and flavor, increased preservation period due to the production of antimicrobial substances (Caplice and Fitzgerald, 1999), to the maintenance of health as probiotics (Ouwehand et al., 2002). Therefore, the application of lactobacilli for the fermented food production seems quite important.

Strong wheat is widely used as the raw material for bread, Chinese noodle and spaghetti. Sourdough seems to be an only example of the wheat fermentation by lactobacilli. Sourdough is the ecosystem where lactobacilli are in association with *Saccharomyces* yeasts (Gobbetti, 1998). A few studies have been reported on the production of antifungal organic acids (Corsetti et al., 1998) or bacteriocins (Messens and de Vuyst, 2002) by the lactobacilli isolated from sourdough.

In this study, we intend to develop a new-type of instant Chinese noodle with improved texture and taste over traditional noodles by the application of lactic acid

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fermentation using lactobacilli to boost domestic production of strong wheat and create new-bio industry in Japan. Strong wheat and *kansui* are the typical materials for the manufacture of instant Chinese noodle (Kubomura, 1998). *Kansui* is the mixture of alkaline salts such as potassium carbonate and sodium carbonate. The major role of *kansui* in noodle preparation is to increase the noodle elasticity and the development of yellow color by reacting with flour flavones. Thus, *kansui* is an important ingredient for the manufacture of instant Chinese noodle (Miskelly, 1996). The presence of *kansui* increases the pH value of the dough to about 8.5. There are no reports on alkaline noodle fermentation, except for the application of alkaline resistant bacterial fermentation in Chinese noodles in order to increase its preservation period (Saito et al., 2003). Among LAB, aerococci, enterococci and tetragenococci have been reported to grow even at pH 9.6, whereas, lactobacilli were inhibited under such alkaline conditions (Axelsson, 1993). Thus, it is a challenge to ferment alkaline noodle sheets by lactobacilli. Therefore, the aim of this study is to screen many lactobacilli of food origin for noodle sheet fermentation, and to manufacture novel fermented instant Chinese noodle with improved texture and taste.

2. Methods

2.1. Bacterial strains

Forty-six strains of the following 12 species (including subspecies) of *Lactobacillus* were obtained from the Japan Collection of Microorganisms (JCM, Wako, Japan), the Laboratory of Applied Microbiology, Graduate School of Agriculture, Hokkaido University (AHU, Sapporo, Japan) and the Culture Collection Center, Tokyo University of Agriculture (NRIC, Tokyo, Japan): *Lactobacillus delbrueckii* subsp. *lactis*, *L. helveticus*, *L. mali*, *L. casei* subsp. *casei*, *L. curvatus*, *L. paracasei* subsp. *paracasei*, *L. paracasei* subsp. *tolerans*, *L. pentosus*, *L. plantarum*, *L. sake*, *L. brevis* and *L. fermentum* (Table 1).

2.2. Screening of LAB strains for fermentation of instant Chinese noodle sheets

The strains of lactobacilli were cultured in half-strength MRS (de Man et al., 1960) broth under anaerobic conditions using mixed gases (N_2:H_2:CO_2 = 8:1:1) at 30 °C or 37 °C (depending on the optimal growth temperature of each type strain) until the late exponential phase of growth. Half-strength MRS broth contained (g/l): glucose, 10; Bacto Proteose Peptone No. 3 (Becton, Dickinson and Company), 5; 'Lab-Lemco' Powder (Oxoid), 5; Bacto Yeast Extract (Becton, Dickinson and Company), 2.5; Tween 80, 0.5; K_2HPO_4, 1; sodium acetate · $3H_2O$, 2.5; triammonium citrate, 1; $MgSO_4 \cdot 7H_2O$, 0.1; $MnSO_4 \cdot 4H_2O$, 0.025; and the pH was adjusted to 6.5 with HCl. The cells were harvested by centrifugation, washed with sterilized deionized water (SDW), and 0.3 g of wet cells were resuspended in 6 ml of SDW at a final concentration of around 5×10^9 cells/ml (bacterial cell suspension). The following components were mixed for 10 min using a KN-60W mixer (MK Seiko Co., Ltd., Nagano, Japan): 30 g of strong flour, 5 ml of SDW, 0.6 g of NaCl, 0.04 g of Na_2CO_3, 0.06 g of K_2CO_3 and 6 ml of bacterial cell suspension. The dough was sheeted using an IPM-500 noodle machine (Izumi Products Company, Nagano, Japan). At this stage, the pH value of the sheet was around 8.5. The sheets were put into plastic bags and incubated at 30 °C for fermentation. The pH value of the noodle sheets was measured after 0, 8 and 24 h of incubation as described in the analytical methods. The strains that decreased the pH value of the noodle sheets to less than 5.5 within 24 h incubation were selected as positive strains.

The noodle sheet fermentation of the selected strain, *L. plantarum* NRIC 0380, was analysed in details. The noodle sheets were analysed after 0, 6, 12 and 24 h of fermentation to measure pH, determine organic acids and count viable micro-organisms.

2.3. Pilot scale manufacture of fermented instant Chinese noodle and its evaluation

The following components were mixed for 18 min: 6 kg of strong flour, 1 L of SDW, 120 g of NaCl, 8 g of Na_2CO_3, 12 g of K_2CO_3 and 1 L of bacterial cell suspension prepared by culturing *L. plantarum* NRIC 0380 using DIFCO Lactobacilli MRS Broth (Becton, Dickinson and Company). The dough was sheeted, covered with plastic wrap and stored at 30 °C for fermentation. During fermentation, pieces of noodle sheets were collected after 0, 1.5, 4, 6, 8, 18 and 24 h, and organic acids, pH value and color were measured as described in analytical methods. Noodles were also cut from the sheets, steamed for 120 s, and fried using palm oil at 150 °C for 75 s to obtain instant Chinese noodle samples. Sensory evaluation of these samples was conducted as described in the analytical methods. Noodle sheets without LAB inoculation were used as the control.

2.4. Analytical methods

2.4.1. Sample preparation

Two grams of noodle sheets were suspended in 18 ml of SDW and homogenized using a homogenizer. The homogenates were used as samples for determination of viable count of micro-organisms. The homogenates were then centrifuged and the supernatants obtained were