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Production of tofu by lactic acid bacteria isolated from naturally fermented soy whey and evaluation of its quality

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ABSTRACT

Fermented soy whey (FSW) is a traditional tofu coagulant used extensively in China. In this study, 112 *Lactobacillus* spp., 18 *Streptococcus* spp. and 33 yeasts were isolated and identified from naturally fermented soy whey (NFSW). Lactic acid bacteria were found as the major effective strains, and 7 lactic acid bacterial strains could produce enough acid to coagulate soy milk. The pH values and acid production were also monitored during fermentation. The JMC-1 strain showing Gram-positive and rod-shaped was determined to have a high acid-producing activity among the 7 strains. API 50 CHL strip and phylogenetic analysis of 16S rDNA gene sequence demonstrated that the JMC-1 strain was identified as *Lactobacillus plantarum*. Lactic acid and acetic acid were the major organic acids in fermented soy whey, which increased during the fermentation. High-performance liquid chromatography revealed that the concentrations of these acids increased significantly as fermentation time was prolonged. Compared with $CaSO_4$ tofu and $MgCl_2$ tofu, the tofu prepared by *L. plantarum* JMC-1 was of better quality in terms of sensory evaluation, texture, yield, and retention capacity.

1. Introduction

Tofu is a well-known traditional food in many East Asian countries (Rossi, Felis, Martinelli, Calcavecchia, & Torriani, 2016). Tofu has been distributed worldwide because of high nutrient amounts and several potential health benefits to humans (Li, Qiao, & Lu, 2012; Yang et al., 2012). Tofu, also known as soybean curd, is a soft, cheese-like food prepared by curdling fresh hot soymilk with a coagulant.

Fermented soy whey (FSW) has been used as a traditional tofu (Mengkebilige, Chen, Wang, Li, & Bao, 2000; Qiao et al., 2010) coagulant for more than 600 years in China, especially in rural areas. In naturally fermented soy whey (NFSW) preparation, soy whey is used as a raw material fermented with various mixtures of microbes in natural environment. Soy whey is a byproduct of the preparation of soybean products, obtained during the process of pressing tofu, such as tofu and soy protein isolate, which contain high amounts of useful compounds (Xiao et al., 2015; Li, Wu, Wang, & Liu, 2014). Large amounts of soy whey are produced yearly; however, this production aggravates environmental pollution. FSW was used for coagulating tofu. Thus, use of FSW for coagulating tofu could help to reduce environmental pollution. Bittern tofu prepared with magnesium chloride ($MgCl_2$) (Molamma, Conrad, & Suresh, 2006) is too hard, and $MgCl_2$ is not good for our health. Tofu coagulated by calcium sulfate ($CaSO_4$) has an unpleasant taste, specifically a beany flavor and bitter taste. Compared with these two kinds of tofu, FSW tofu exhibits moderate hardness and better flavor.

Soymilk is coagulated with salts, acids, and enzymes during tofu production (Kohyama, Sano, & Doi, 1995). Acid- and FSW-coagulated tofu have been rarely investigated. Fermented soy whey contains a microbial community dominated by lactic acid bacteria (LAB) that play a major fermentative role and thus affect the acidity and flavor of tofu products (Qiao et al., 2010). The main organic acids in FSW are lactic acid and acetic acid (Qiao et al., 2010; Özcelik, Kuley, & Özogul, 2016). However, FSW is produced with different processing technologies dependent on geographical location and climate. Consequently, inconsistent flora is produced as a result of different environmental conditions, which makes it difficult to consistently obtain high quality tofu. The quality of FSW tofu varies in terms of predominant microorganisms. Moreover, the fermentation time of NFSW is almost 3 days. Thus, predominant microorganisms must be isolated and identified from NFSW for extensive industrial processing. To the best of our knowledge,

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proximate NFSW composition, bacterial phases, and different bacterial activities on FSW tofu have been seldom analyzed. Changes in organic acids in FSW in different fermentation stages and differences among FSW tofu, bittern tofu, and gypsum tofu have been rarely evaluated.

This work aimed to screen and isolate a high-acid-producing bacteria from NFSW for the industrial production of FSW tofu or other soy products. Changes in pH, acid production, and organic acid of FSW during fermentation were evaluated. The solidification characteristics and good flavor of FSW tofu were also investigated.

2. Materials and methods

2.1. Sample collection

A sample of naturally fermented soy whey was collected from Hunan Province, China. The pH value of the sample was 3.95. The fermented soy whey (FSW) was made by fermenting soy whey at 28 °C for 3 days.

2.2. Proximate composition analyses

Proximate composition analyses were conducted using standard methods (AOVO, 2000). The total protein was determined by micro-Kjeldahl method using a protein conversion factor of 6.25, and crude fat was determined by the Soxhlet extraction method. Ash was assayed using the AOAC method (Marmouzi, EI Madani, Charrouf, Cherrah, & EI Abbbes Faouzi, 2015). The soluble solid content was assayed by refractometer after filtration. All the results were reported on a wet basis.

2.3. Enumeration and isolation of bacteria

2.3.1. Screening of major microorganism in fermented soy whey

Sample (1 ml) of naturally fermented soy whey (NFSW) was mixed with 9 ml of sterilized 0.85% NaCl (w/v) by vortex mixer. A series of appropriate dilution of the sample was inoculated into pour-plates and in triplicate. The de Man–Rogosa and Sharp (MRS) agar was used for the isolation of *Lactobacillus* spp., and M 17 was used for *Streptococcus* spp. and *Lactococcus* spp. They were incubated at 37 °C for 48–72 h. The Bengal medium for the isolation of yeast and molds were incubated for 3 days at 28 °C. The violet red bile agar (VRBA) for the isolation of coliforms was incubated for 24 h at 30 °C. After incubation, the plates with 30–300 colonies were enumerated and expressed as $\log_{10}$ of colony forming units per milliliter (cfu/mL) of samples.

All of the colonies were examined microscopically and checked for catalase reaction before the stocks were prepared. Gram-positive, catalase-negative colonies were identified initially as *Lactobacillus*. Colonies showing different appearance (size, shape, and color) (Li, Mutuvulla, Chen, Jiang, & Dong, 2012) were selected randomly and further purified by successive streaking using the same medium. The purified bacteria were resuspended and maintained in the same liquid medium containing 50% glycerol at −20 °C.

2.3.2. Soymilk coagulating capacity analysis

The strains were respectively inoculated into the MRS medium at inoculums concentration of 4% and incubated at 37 °C for 24 h for two successive transfers. The prepared inoculums was then inoculated into the sterile fresh soy whey at 4% (v/v) and incubated at 37 °C for 24 h for two successive transfers. The pH of each FSW was determined using a Schott pH Meter Lab 850 (model, Lab 850, Mainz, Germany). The bacteria and the corresponding FSW with a pH of less than 4.8 were marked.

Soybeans were washed, soaked overnight in water at room temperature, drained, rinsed, and blended with water (soybean to water ratio = 1:6 w/v) in a soymilk grinder (Model MJ-02, Hebei province, China) (Fig. 1). Subsequently, 40 ml of cooked soybean milk was placed in water bath at 85 °C. Then, 20 ml of the FSW was added into the soybean milk. FSW were capable of inducing soybean milk coagulation within 5–10 min at 85 °C, and then the corresponding bacteria were marked.

2.3.3. Determination of pH and the rate of acid production

The FSW of target bacteria were respectively inoculated into the fresh pasteurized soy whey medium at 4% (v/v) and incubated at 37 °C up to 24 h for two successive transfers. Then, 10 ml of fermentation broth was withdrawn after 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 h of incubation and stirred before measurement of pH and total acid. The pH of each fermentation broth of pure cultures was determined using a Schott pH Meter Lab 850 (model, Lab 850, Mainz, Germany). The acid production (g/100 ml, expressed as acid degree) of each fermentation broth was accomplished according to the method for total acid determination (Viljoen & Greyling, 1995). The rate (R) of acid production was determined in the exponential phase using the equation $R_M = \Delta M/\Delta t$ (ΔM = amount of acid produced [g/100 ml]; Δt = time [h]). Acidity in terms of lactic acid was determined by diluting an aliquot of the prepared sample with recently boiled distilled water. Then, 2–3 drops of 1% phenolphthalein solution was used as an indicator, and titration was conducted with 0.1 mol/L NaOH.

2.4. Identification of bacteria

2.4.1. Carbohydrate fermentation and assimilation test

API 50 CHL strip (Nigatu, Ahrné, & Molin, 2000) enabled identification of the isolates to species level. This system conducted 49 carbon assimilation or fermentation tests and included a database with 52 different species. All the procedures for LAB identification were conducted in accordance with the manufacturer's instructions. Bacterial suspension was distributed into each of the 50 couples and then overlaid with sterile paraffin oil. This set of strips was cultured at 37 °C for 48 h. During the culture time, the color of some couples changed from violet to black and was taken as a positive reaction.

2.4.2. 16S rDNA sequence analysis

2.4.2.1. DNA extraction and amplification. The JMC-1 strain was extracted for sequencing of 16S rDNA using the lysozyme method (Nurgul, Chen, Feng, & Dong, 2009; Liu, Zhang, Yi, Han, & Chi, 2016; Handa & Sharma, 2016). The primers pA 5′ AGAGTTTGATCCTGGCTCAG 3′ and pH 5′ TACGGCTACCTTGTTACGACTT 3′ were used for the amplification of the extracted DNA (1 μL). Then, 25 μL PCR system containing 18.25 μL deionized water, 0.5 μL of each 10 mM primer, 2.5 μL Easy Taq buffer, 2 μL of 2.5 mM dNTPs, 5 U of Taq polymerase, and 1 μL bacterial DNA was used. Amplification was implemented in PCR Thermocycler (Bio-Rad Mycycler™) programmed as follows: 94 °C for 3 min, 35 cycles at 94 °C for 30 s, 59 °C for 60 s, 72 °C for 2 min, and 72 °C for 10 min (final extension).

2.4.2.2. DNA sequencing and sequence analysis. The amplification products (5 μL) were extracted for DNA sequencing, and sequence analysis was conducted by electrophoresis in 0.8% agarose gels. DNA amplification of the products were performed by Sangon Company (Shanghai, China). The DNA sequences were submitted and deposited to The National Center for Biotechnology Information (NCBI) to find similar sequences using the basic local alignment search tool (BLAST) program. A set of the similar sequences was entered in the MEGA 3.1 to produce the phylogenetic tree.