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Characterization of probiotic bacteria involved in fermented milk processing enriched with folic acid

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ABSTRACT

Yogurt products fermented with probiotic bacteria are a consumer trend and a challenge for functional food development. So far, limited research has focused on the behavior of the various probiotic strains used in milk fermentation. In the present study, we characterized folic acid production and the sensory and textural characteristics of yogurt products fermented with probiotic bacteria. Yogurt fermented with *Lactobacillus plantarum* had improved nutrient content and sensory and textural characteristics, but the presence of *L. plantarum* significantly impaired the growth and survival of *Lactobacillus delbrueckii* ssp. *bulgaricus* during refrigerated storage. Overall, *L. plantarum* was a good candidate for probiotic yogurt fermentation; further studies are needed to understand the major metabolite path of lactic acid bacteria in complex fermentation.
Key words: yogurt, probiotic strain, folic acid, complex fermentation

INTRODUCTION

Probiotics are defined by the United Nations Food and Agriculture Organization/World Health Organization as "live microorganisms (bacteria or yeasts) that when ingested or locally applied in sufficient numbers confer one or more specified demonstrated health benefits for the host" (FAO/WHO, 2001). Lactic acid bacteria (**LAB**) have potential probiotic use as food-grade fermentation starters (Sanders et al., 2013). As well, certain LAB strains can synthesize folates, a family of B-complex vitamins that function in 1-carbon metabolism to allow the de novo synthesis of amino acids and nucleosides (Rossi et al., 2011). Because of the perceived health benefits of probiotic strains, their use has expanded rapidly in health-based products. Consumers nowadays have become more demanding of natural foods, and products created using environmentally friendly procedures are more attractive.

Probiotic-fermented milks possess many useful properties when they interact with host cells in the gastrointestinal tract, including anti-inflammatory activity (de Assis et al., 2016), antioxidant activity (Srivastava et al., 2015), antibacterial activity, and angiotensin I-converting-enzyme inhibitory activity (Abd El-Gawad, 2014; Rai et al., 2015). Folate deficiency is associated with many important diseases such as chronic heart failure, oxidative stress, and neural tube defects (Czeizel et al., 2013), but folic acid supplementation before and during early pregnancy (up to 12 wk of gestation) can prevent neural tube defects in offspring (Czeizel et al., 2011). Lactic acid bacteria strains with folate acid production are able to improve folate status and prevent folate deficiency in some deficient rodent models (Laiño et al., 2015). With probiotic supplementation, the interaction between adhesive probiotics and the host cells may trigger a signal cascade that leads to reductions in serum cholesterol and gastrointestinal infections, and improvements in lactose metabolism, inflammatory bowel disease, and immune system stimulation (Whelan and Quigley, 2013; Settanni and Moschetti, 2014).

However, no bacterial strain provides all of these potential benefits, not even probiotic strains. Folic acid concentrations in yogurts vary widely and are normally considered low because of inadequate selection of starter cultures and fermentation conditions. Traditionally, yogurt is fermented using *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* in the milk medium. These 2 strains establish a symbiotic relationship in milk and perform biochemical reactions that lead to a decrease in pH and the formation of a semi-solid texture and a distinctive yogurt flavor (Irigoyen et al., 2012). Promising probiotic strains, including *Bacillus, Enterococcus, Escherichia,* and *Propionibacterium* genera, and the yeast genus *Saccharomyces,* have been adopted in new yogurt products (Ceapa et al., 2013;

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Kanmani et al., 2013). However, from a technological point of view, the addition of different LAB strains to milk fermentation is a challenge. Texture, protein gel strength, and aroma can be affected by different kinds of probiotics in the milk fermentation process (Bouteille et al., 2013).

Yogurt is the most popular fermented dairy product, but limited research has focused on the behavior of the different probiotic strains during milk fermentation. The objectives of this study were to characterize the potential folic acid production of several species of lactobacilli (*Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus acidophilus*). We also evaluated texture parameters, post-acidification, and microorganism counts in probiotic yogurts during cold storage to determine the yogurt products' nutritional, textural, and sensory properties.

MATERIALS AND METHODS

Strains and Cultures

We purchased *L. bulgaricus* (No. 1.1855) and *S. thermophilus* (No. 1.1854) from China General Microbiological Culture Collection Center. We stored *L. plantarum* (ATCC 14917), *L. acidophilus* (ATCC 4356), and *L. casei* (ATCC 15008) in our laboratory at −80°C in culture plus glycerol (20%, vol/vol) for further use. Raw milk was produced by Ningbo Dairy Group Co., Ltd., and had a fat content of 3.2%. All bacterial strains were cultured at 37°C in de Man, Rogosa, and Sharpe (**MRS**) medium before milk fermentation.

Fermentation Process

To prepare the mother culture, we inoculated heat-treated milk (90 to 95°C for 5 min) with 3% (wt/wt) *Lactobacillus* strains anaerobically at 37°C for 18 h, and maintained it at 4°C until milk fermentation (within 48 h). Milk fermentation took place at 43°C for 6 h and post-ripening at 4°C for 18 h before sensory evaluation and texture analysis. During acidification, pH decreased below 4.6. Milk gelation occurs at pH 5.2 to 5.4, when lactose converts to lactic acid in milk medium. The pH value of the yogurt was determined by inserting the electrode (Mettler Toledo, Shanghai, China) directly into the yogurt samples.

Folic Acid Production of Different Strains

We purchased a folic acid reference standard (CAS No. 59–30–3) from Sigma-Aldrich (Shanghai, China). We performed analysis of folic acid production from the various *Lactobacillus* strains using HPLC on an Zorbax Eclipse XDB-C18 (4.6 × 250 mm, Agilent Technologies, Santa Clara, CA) at 280 nm. Mobile phase A consisted of 20% H_3PO_4 in water (vol/vol, pH 7.2), phase B consisted of 100% methanol (vol/vol), and the ratio of A to B was 90:10 under a flow rate of 1 mL/min.

Sensory Evaluation and Texture Analysis

Ten panelists were recruited for the sensory evaluation analysis. Panelists were asked to taste the samples in the order given and evaluate the appearance, taste, and aroma of the fermented milk. For each test, replicates were conducted to improve the power of analysis and detect true discriminators. All sensory attributes were evaluated using a scale of 1 to 9, where 1 was very low and 9 was very high.

Texture of the different kinds of yogurt were evaluated using a TAHD Plus Texture Analyzer (Stable Micro Systems, Godalming, UK) with a back extrusion rig and a 35-mm compression disk attached to an extension bar using 50-kg load cells (Ciron et al., 2010). Three replications were made at a pre-test 1.0 mm/s, test of 1.0 mm/s and post-test 10.0 mm/s at 30 mm above sample surface, penetrating to a depth of 30%. We used mean values to obtain a force-time curve.

Acidification Profile Determination

Production of acid was assessed by changes in pH of the yogurt during milk fermentation. Yogurt samples were weighed to 40.0 g and the pH measurements were performed using a Mettler Toledo M 220 pH meter (Mettler Toledo Instruments).

Microbial Counts and Stability

A 1-mL sample of yogurt was transferred to a screw-capped tube containing 9 mL of sterile saline water. Further dilutions were made from this original dilution, and the bacteria were counted after fermentation in duplicate by using MRS agar medium, which is selective for the *Lactobacillus* strains. The stability of the bacteria in the yogurt was assessed following analysis at 1, 7, 14, 21, and 28 d of refrigerated storage through microbial counts in selective medium.

Statistical Analysis

The experiment was repeated 3 times. Statistical analyses between groups were carried out using a 2-tailed Student's *t*-test with SPSS 13.0 software. Data are presented as means ± standard deviations. A *P*-