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Influence of *Lactobacillus plantarum* on yogurt fermentation properties and subsequent changes during postfermentation storage

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ABSTRACT

This study aimed to evaluate the influence of 9 *Lactobacillus plantarum* with broad-spectrum antibacterial activity on fermented milk, including changes to the fermentation characteristics (pH, titration acidity, and viable counts), texture profile, relative content of volatile compounds, and sensory evaluation during 28-d storage at 4°C. First, *L. plantarum* IMAU80106, IMAU10216, and IMAU70095 were selected as candidates for further study because of their excellent coagulation and proteolytic activities. Subsequently, these *L. plantarum* were supplemented to fermented milk produced by commercial yogurt starters (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*) and a panel of parameters reflecting product quality was subsequently monitored along 28-d postfermentation storage. The pH value and titration acidity of the fermented milk mildly fluctuated, whereas the *L. plantarum* viable counts remained stable along the storage period. Fourteen key volatile compounds were detected in the fermented milk by gas chromatography-mass spectrometry, and some flavor compounds were uniquely present in the *L. plantarum*-supplemented fermented milk (including 2,3-pentanedione, acetaldehyde, and acetate). No significant difference was shown in the sensory evaluation scores between samples with or without *L. plantarum* supplementation, but a gradual decrease was observed over storage in all samples. However, when *L. plantarum* was added, apparent shifts were observed in the overall quality of the fermented milk based on principal component analysis and multivariate ANOVA, particularly in the texture (adhesiveness) and volatile flavor compound profiles (acetaldehyde). Compared with *L. plantarum* IMAU80106 and IMAU10216, both the texture and volatile flavor profiles of IMAU70095 were closest to those of the control without adding the adjunct bacteria, suggesting that IMAU70095 might be the most suitable strain for further application in functional dairy product development. The current work has explored the potential of applying *L. plantarum* in fermented milk by performing thorough physical and chemical characterization. Our work is of intense interest to the dairy industry.
Key words: *Lactobacillus plantarum*, fermented milk, adjunct culture, postfermentation storage

INTRODUCTION

Fermented milk is produced by the acidification action of lactic acid bacteria (**LAB**) during metabolism, which results in important physicochemical, sensory, and microbiological changes in the milk (Casarotti et al., 2014). The primary LAB involved in food fermentation include the starter cultures, adjunct cultures, and probiotic cultures. In industrial production, commercial yogurt starters, namely *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*, are often used to ferment pasteurized milk (Fisberg and Machado, 2015); thus, dairy products are considered as the most suitable carriers for probiotics (Lourens-Hattingh and Viljoen, 2001). Probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2001). Therefore, the development of novel functional products by incorporating probiotic bacteria in fermented milk is a current focus in the dairy industry (Georgieva et al., 2009). Adjunct cultures may also confer additional properties to the fermented products. For example, they may enhance the flavor, texture, or visual appearance of the products. The application of probiotic adjunct culture has also become a popular practice.

To provide health benefits, the probiotic adjunct culture must overcome the physical and chemical barriers such as acid and bile stress in the gastrointestinal tract (Del Piano et al., 2006) and maintain a high viability

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(at least 10^6 cfu/g) throughout the specified product shelf life (Tripathi and Giri, 2014). The most commonly used probiotic bacteria are LAB; in particular, lactobacilli and bifidobacteria are considered to have the highest potential. Among the lactobacilli, *L. plantarum* is a versatile and widely distributed species. Its dual role as an indigenous human gut inhabitant and a safe starter culture in food fermentation (De Vries et al., 2006) has made it a preferred choice to be used in novel functional product development. Many previous reports found that consuming *L. plantarum*-containing fermented milk brings in vivo functionality to the host (Chiu et al., 2006; Nasrabadi et al., 2011). In our previous study, 9 *L. plantarum* were identified to suppress the growth of 5 common foodborne pathogens (namely *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Shigella flexneri*) that could be present in fermented milk (Li et al., 2015); thus, they have great potential to serve also as natural probiotic biopreservatives. Nevertheless, this continues to be a difficult task due to the stressful environment created for the probiotic bacteria during fermentation and storage.

On the other hand, a good adjunct bacterium should neither adversely affect product quality and sensory properties such as texture, taste, chemical content, and flavor nor enhance acidification during product storage. However, during the processes of milk fermentation and product storage, the viable bacteria including any adjunct culture would still continue to convert milk lactose to lactic acid and other flavor compounds (Kök-Tas et al., 2013), causing a series of physical and chemical changes. Although some previous studies have successfully selected adjunct cultures that do not alter the sensory profile and texture of prebiotic-containing yogurts (Hekmat and Reid, 2006; Kailasapathy, 2006), more comprehensive and objective evaluation of the changes occurred during fermented milk storage will be necessary to set up guidelines for product quality assurance.

The objective of this study was to test the effect of 9 *L. plantarum* strains on milk fermentation and post-fermentation storage of the products over 28 d. These *L. plantarum* strains were chosen based on their high in vitro tolerance to low pH, artificial digestive juices, and bile salts, together with their strong anti-bacterial capacity in fermented milk (Li et al., 2015). To quantify the changes, we measured a wide spectrum of parameters, including the pH, titratable acid (**TA**), viable counts, texture profile, sensory quality, and volatile flavor compounds, along the monitored time course. Data were then analyzed with principal component analysis (**PCA**) and multivariate ANOVA (**MANOVA**). Our long-term goal is to identify probiotic adjunct cultures that are suitable for developing novel functional fermented milk products.

MATERIALS AND METHODS

Bacterial Isolates and Reagents

Nine isolates of *L. plantarum* with broad-inhibition activity against 5 enteric pathogens (Li et al., 2015) were obtained from the Lactic Acid Bacteria Culture Collection of the Key Laboratory of Dairy Biotechnology and Engineering, Inner Mongolia Agricultural University, Inner Mongolia, China. These bacteria were originally isolated from food and were identified as *L. plantarum* using a combination of traditional physiological and biochemical identification methods and 16S rRNA gene sequence analysis; their 16S rRNA gene sequences were submitted to the GenBank database (Table 1; Zhang et al., 2012). All isolates were stored long term in a skim milk medium (SMM, NZMP Co. Ltd., Wellington, New Zealand) at −80°C and recovered in de Man, Rogosa, and Sharpe broth (Oxoid Ltd., Basingstoke, Hampshire, UK) at 37°C for 24 h following standard methods (Vinderola et al., 2000) before experiments. Commercial yogurt starter cultures (YF–L904), which contained *S. thermophilus* and *L. delbrueckii* ssp. *bul-*

Table 1. Origins of the 9 *Lactobacillus plantarum* strains

Strain	GenBank accession no.	Sample type	Sampling region	Reference
IMAU80106	GU125529	Pickle	Sichuan	Zhang et al., 2012
IMAU10996	HM218698	Yogurt	Inner Mongolia	Zhang et al., 2012
IMAU10216	GU138544	Sour dough	Inner Mongolia	Zhang et al., 2012
IMAU70095	GQ131211	Sour porridge	Inner Mongolia	Bao et al., 2012
IMAU70023	GQ131139	Sour porridge	Inner Mongolia	Yu et al., 2011
IMAU30043	FJ749637	Koumiss	Xinjiang	Zhang et al., 2012
IMAU50045	FJ749445	Dairy fan acid whey	Yunnan	Yu et al., 2012
IMAU30162	FJ749719	Koumiss	Xinjiang	Zhang et al., 2012
IMAU40082	FJ749357	Fermented yak milk	Qinghai	Bao et al., 2012

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