African Journal of Biotechnology Vol. 9(35), pp. 5735-5738, 30 August, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.579
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Fermentation characteristics of Korean pear (*Pyrus pyrifolia* Nakai) puree by the *Leuconostoc mesenteroides* 51-3 strain isolated from *Kimchi*

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Accepted 8 July, 2010

A lactic acid bacterial strain showing fast growth and high acid production when cultured in Korean pear puree was isolated from *Kimchi*. This strain was analyzed by using the API 50 CHL kit and 16S rRNA sequencing and was thus identified as *Leuconostoc mesenteroides* 51-3. Korean pear puree was fermented with the *L. mesenteroides* 51-3 strain at 30°C for 12 h. The changes in pH, titratable acidity and viable cell count during fermentation were investigated. The pH and titratable acidity of the pear puree were 4.06 and 0.66%, respectively, after 12 h of fermentation. The viable cell count of *L. mesenteroides* 51-3 rapidly increased to 3.7 × 10^9 CFU/g after 12 h of cultivation. The content of lactic acid and acetic acid was determined to be 0.138 and 0.162%, respectively, after 12 h of fermentation. When the fermented pear puree was stored at 4°C, the pH, titratable acidity and viable cell count remained fairly constant for 14 days.

Key words: Fermentation, Korean pear puree, *Leuconostoc mesenteroides*.

INTRODUCTION

The Korean pear (*Pyrus pyrifolia* Nakai) is one of the most abundantly produced fruits in South Korea and is mainly composed of 85-88% water, 10-13% carbohydrates, 0.3% proteins and 0.2% lipids (Hwang and Park, 2006). It is primarily consumed as a fresh fruit, and therefore, most investigators have concentrated on optimizing the storage conditions so as to minimize the physicochemical changes occurring during the long-term storage of freshly harvested pears. Storage methods such as controlled-atmosphere (CA) storage and electron-beam irradiation have been studied (Chung et al., 2009; Kim et al., 2008), and some of these have been adopted.

Although a study has been conducted to isolate functional foodstuffs such as dietary fibers and pectins from pears (Zhang et al., 2005), the use of pear-derived products in the food and beverage industry is currently poor. Methods of fermenting pear puree, which can improve the flavor and functionality of pears, have neither been extensively studied nor adopted. As mentioned earlier, the Korean pear has a sugar content of 10 – 13%; this suggests that the fruit can be fermented.

Lactic acid bacteria (LAB) play an important role in the production of a large number of fermented foods such as yoghurt, cheese and *Kimchi* (Kalui et al., 2010). In recent times, there has been considerable interest in the use of LAB as probiotics, that is, as live microbial food supplements that benefit humans by improving the intestinal microbial balance. Probiotic microorganisms exhibit properties that are beneficial to human or animal health. They can protect humans against infections caused by harmful bacteria in the intestines, thus preventing diarrhea; they also prevent constipation by acidifying the intestine, enhance immunity and reduce allergic reactions

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Abbreviations: CA, Controlled-atmosphere; **LAB,** lactic acid bacteria; **HPLC,** high performance liquid chromatography; **CFUs,** colony-forming units; **MRS,** Man, Rogosa and Sharpe.

and the risk of developing colonic cancer (Mercenier et al., 2002). In recent times, the continuous increase in the demand for healthful and safe foods and in health consciousness among consumers has lent the probiotic food industry a central role in moderating consumer health (Khan and Ansari, 2007).

In this study, LAB was screened to determine their suitability for use as a fermentation starter in the fermentation of the Korean pear for the production of a yogurt-like fruit-based probiotic food.

MATERIALS AND METHODS

Isolation and identification of LAB from *Kimchi*

Kimchi, purchased from the local market in Hongseong, was used as a screening source for LAB. LAB were isolated through a 2-step screening procedure. First, diluted *Kimchi* juice was spread-plated on Lactobacilli Man, Rogosa and Sharpe (MRS) agar plates containing 0.3% $CaCO_3$ and incubated at 30°C for 36 h. Colonies forming a clear zone on the plates were selected (Choi et al., 2009) and cultured in pear puree at 30°C for 15 h on a rotary shaker at 150 rpm. Finally, microorganisms showing high titratable acidity and viable cell counts were selected. The strains were identified on the basis of their carbohydrate-fermentation pattern, using the API 50 CHL kit (bioMerieux Inc., Marcy l'Etoile, France) and 16S rRNA sequencing analysis (Yoon et al., 1998).

Pear puree media preparation and fermentation

Peeled and cored pear pieces were crushed to a puree by using a mechanical blender. This puree, without any added components, was used as the fermentation medium. The LAB preculture was grown in MRS broth at 30°C for 15 h on a rotary shaker at 150 rpm. LAB were harvested from the preculture broth by centrifugation (3,000 × *g* for 15 min) and washed twice with physiological saline. The washed pellet was suspended in the same volume of physiological saline as that of the preculture broth. Further, 50 g of fresh pear puree was poured into a sterilized 500-ml baffled flask and inoculated with 5% (v/v) of the washed LAB suspension. The final culture was performed under aerobic conditions at 30°C for up to 12 h.

Analysis

The pH of the fermented pear puree was measured using a pH meter (720P, Istek, Seoul, Korea). The titratable acidity was determined by titrating 5 g of the sample with 0.01 N NaOH, and the result was expressed as the percentage of lactic acid. The organic acid content was analyzed with high performance liquid chromatography (HPLC) on a Dionex system (Sunnyvale, CA, USA) (Oh and In, 2008). LAB growth was assessed by counting the viable cells after plating the culture on MRS agar plates. The plates were incubated at 30°C for 36 h, and the culture density in terms of colony-forming units (CFUs) was estimated.

RESULTS AND DISCUSSION

Isolation and identification of LAB

Among the 10 strains isolated from *Kimchi*, the selected strain (51-3 strain) showed the highest titratable acidity (1.02%) and viable cell count (2.9×10^9 CFU/g) after culture in pear puree at 30°C for 15 h. Using the API 50 CHL kit, we tentatively identified and classified strain 51-3 as *Leuconostoc mesenteroides* ssp. *mesenteroides/dextranicum* (Table 1). Because the partial 16S rRNA sequence of the 51-3 strain shared 99% similarity with that of *L. mesenteroides*, this strain was eventually identified as *L. mesenteroides* (Figure 1). *L. mesenteroides* KACC 91945P was recently isolated from *Kimchi* and found to be suitable for the fermentation of Korean pear puree (In et al., 2010). Despite the use of the same screening tools for selecting the *L. mesenteroides* 51-3 strain and the *L. mesenteroides* KACC 91945P strain, the 2 strains exhibited slightly different carbohydrate-utilization patterns in the case of mannitol, arbutine, gluconate and 5-keto-gluconate; the utilization of these sugars was positive in the *L. mesenteroides* KACC 91945P strain (In et al., 2010) but negative in the *L. mesenteroides* 51-3 strain. This shows that the *L. mesenteroides* 51-3 strain is considerably different from the *L. mesenteroides* KACC 91945P strain.

Microbial changes during Korean pear puree fermentation

The changes in cell growth, pH and titratable acidity of Korean pear puree fermented with the *L. mesenteroides* 51-3 strain were examined (Figure 2). While the pH of the fermented broth decreased from 5.65 to 4.17 after 6 h of fermentation, its titratable acidity increased from 0.221 to 0.617% and was maintained between 0.6 and 0.7% until 12 h of cultivation. The content of lactic and acetic acid in the fermented pear puree was determined to be 0.138 and 0.162%, respectively, after 12 h of fermentation. These results are similar to those obtained in previous studies involving LAB cultivation in pear puree (In et al., 2010) and in a solution of milk and fruit juice (Ko and Kang, 1997). When a solution of milk and apple juice was fermented with the *Lactobacillus acidophilus* KCTC 2189 strain, the pH of the mixture drastically decreased from 6.2 to 4.08 after 24 h of fermentation (Ko and Kang, 1997). In the present study, the pear puree was initially inoculated with approximately 10^8 CFU/g of the *L. mesenteroides* 51-3 strain. After fermentation in pear puree for 12 h, the viable cell count rapidly increased to 3.7×10^9 CFU/g. These data implied that the Korean pear puree itself contained sufficient nutrients for the growth of bacteria in general and can be utilized for the manufacture of yogurt-like fermented fruit-based products, without the addition of nutritional supplements.

The pear puree that had been fermented by the *L. mesenteroides* 51-3 strain was stored at 4°C for 2 weeks, and the changes in viable cell count, pH and titratable acidity were examined (Table 2). While the pH of the fermented pear puree slightly decreased from 3.61 to 3.26 after 14 days of storage, its titratable acidity increased

Table 1. Utilization of various carbohydrates by *Leuconostoc mesenteroides* 51-3 strain isolated from *Kimchi*

Carbohydrate	Reaction	Carbohydrate	Reaction
Control	-	Esculine	+
Glycerol	-	Salicine	+
Erythritol	-	Cellobiose	-
D-Arabinose	-	Maltose	+
L-Arabinose	+	Lactose	-
Ribose	+	Melibiose	+
D-Xylose	+	Saccharose	+
L-Xylose	-	Trehalose	+
Adonitol	-	Inuline	-
Methyl-β-xyloside	-	Melezitose	-
D-Galactose	+	D-Raffinose	+
D-Glucose	+	Amidon	-
D-Fructose	+	Glycogene	-
D-Mannose	+	Xylitol	-
L-Sorbose	-	β-Gentiobiose	-
Rhamnose	-	D-Turanose	+
Dulcitol	-	D-Lyxose	-
Inositol	-	D-Tagatose	-
Mannitol	-	D-Fucose	-
Sorbitol	-	L-Fucose	-
Ethyl-α-D-mannoside	-	D-Arabitol	-
Methyl-α-D-glucoside	+	L-Arabitol	-
N-Acetylglucosamine	+	Gluconate	-
Amygdaline	-	2-Keto-gluconate	-
Arbutine	-	5-Keto-gluconate	-

+: Positive reaction, -: negative reaction.

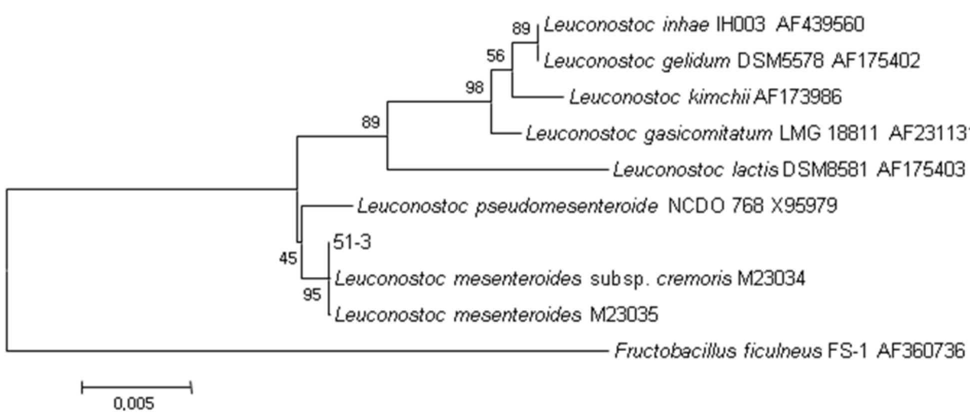

Figure 1. Phylogenetic tree based on 16S rRNA sequences showing the relationships between the *L. mesenteroides* 51-3 strain, various *Leuconostoc* species and some other related taxa.

from 0.67 to 0.97%. The *L. mesenteroides* 51-3 strain exhibited good survival throughout the storage period. The viable cell count remained above 10^9 CFU/g, thus maintaining the probiotic characteristics of the yogurt-like product. In conclusion, the present results indicate that the fermentation of Korean pear puree with the L. mesenteroides 51-3 strain may be useful in the development of yogurt-like fruit-based probiotic food.

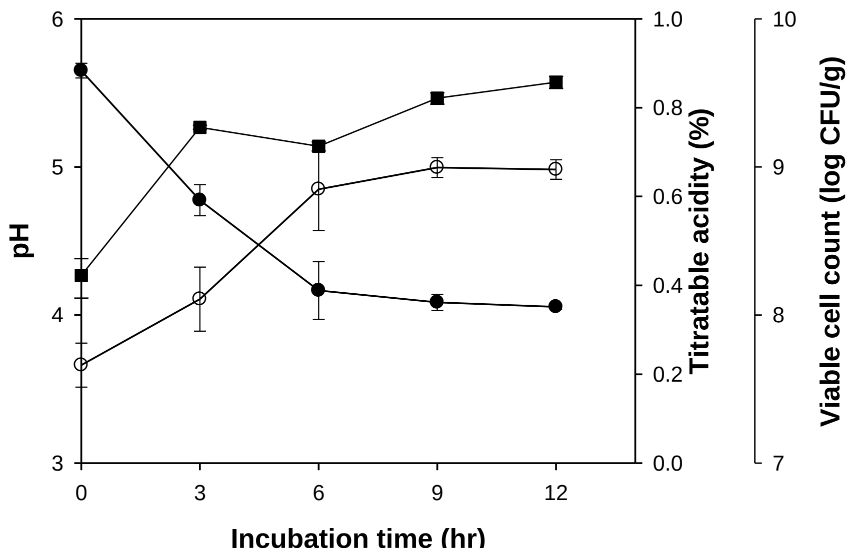

Figure 2. Changes in the growth of the *L. mesenteroides* 51-3 strain (■), the titratable acidity (●) and pH (○) of the fermented pear puree during lactic acid fermentation at 30°C.

Table 2. Changes in the characteristics of the fermented pear puree during storage at 4°C.

Parameters	Period of storage (days)						
	0	2	5	7	9	12	14
pH	3.61	3.50	3.24	3.20	3.16	3.26	3.26
Titratable acidity (%)	0.67	0.80	0.80	1.06	0.98	1.00	0.97
Viable cell count (CFU/g)	4.4×10^9	5.3×10^9	3.3×10^9	2.1×10^9	4.1×10^9	3.1×10^9	1.2×10^9

ACKNOWLEDGEMENT

This work was supported by the Regional Industrial Technology Development Program of the Ministry of Knowledge Economy, Republic of Korea.

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