Effect of Using Propionic Acid Bacteria as an Adjunct Culture in Yogurt Production

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ABSTRACT

Propionibacteria are able to produce a wide variety of food components beneficial to human health. In this study, yogurt was produced by using the adjunct starter cultures *Propionibacterium jensenii* B1264 and *Propionibacterium thoenii* (jensenii) P126. Although the total solids and protein contents of the yogurts did not show any significant differences, titratable acidity of the control sample (YC-380) remained lower than that of *Propionibacterium* spp.-supplemented yogurts during 15 d of storage. The yogurts produced by YC-380 + P126 cultures had the firmest structure (0.26 N). The highest acetaldehyde (29.35 mg/kg) content was obtained with yogurt made with YC-380 + P126 + B1264 on d 1. The addition of propionibacteria to yogurt did not have any negative effect on the counts of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* in yogurt. During the first week of storage, propionibacteria counts remained high, suggesting that yogurt provided a good environment for these organisms. This new product would provide not only beneficial health effects, but also a new alternative product to plain set-type yogurt.

Key words: set-type yogurt, propionic acid bacterium, adjunct starter culture

INTRODUCTION

Yogurt, which potentially contains beneficial microflora from lactic acid fermentation, offers the consumer more than just the conventional nutrients of a dairy product. In recent decades, there has been a trend to produce yogurt by using different starter cultures to provide more functional properties with different flavor alternatives for consumers. *Propionibacterium* spp. are used as starter cultures in dairy fermentations, where they play an important role in the development of characteristic flavors and eye production in Swiss-type

cheeses because of the production of propionate, acetate, and CO₂ (Langsrud and Reinbold, 1973; Biede and Hammond, 1979). Propionibacteria are used in several fermented dairy products and have potential as probiotics (Thiel et al., 2004). The potential benefit of propionibacteria in healing colonic mucosa has been reported (Michel et al., 2005). Huang and Adams (2003) observed that *Propionibacterium jensenii* 702 was able to survive the in vivo gastrointestinal tract transit in rats with no adverse effects on the animals. The probiotic influence of propionibacteria is based on the production of propionic acid, bacteriocins, and vitamin B₁₂; better exploitation of fodder; growth stimulation of other beneficial bacteria; and the ability to survive during gastric digestion (Kaneko et al., 1994; Perez-Chaia et al., 1999; Holo et al., 2002; Hugenholtz et al., 2002; Warminska-Radyko et al., 2002; Ekinici and Barefoot, 2006).

Metabolic products of probiotic bacteria, such as bacteriocins, organic acids, diacetyl, and other low molecular weight metabolites, may contribute to the control of undesired microorganisms and thus to a prolonged shelf life of a food (Glatz, 1992). Selected strains of these bacterial genera can be applied as “protective” cultures with an in situ production of antimicrobials. Such an example on the market is Bio Profit (Danisco Deutschland GmbH, Niebüll, Germany), a coculture of *Lactobacillus rhamnosus* LC705 (DSM 7061) and *Propionibacterium freudenreichii* ssp. *shermanii* JS (DSM 7067), which is suggested for the growth suppression of yeasts and molds (Suomalainen and Mäyrä-Mäkinen, 1999). The aim of this study was to produce set-type yogurt by using selected strains of propionibacteria as adjunct cultures and to investigate their microbial, physical, and chemical properties.

MATERIALS AND METHODS

Bacterial Cultures

Propionibacterium thoenii (jensenii) P126 and *Propionibacterium jensenii* B1264 were obtained from the Clemson University Food Microbiology culture collection, Clemson, South Carolina (Grinstead and Barefoot, 1992; Ratnam et al., 1999). They were cultivated in

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Table 1. Combination of starter cultures used in yogurt preparation

Starter culture used for yogurt production	Bacterial species	Inoculation amount, %	Incubation time, ¹ min
YC-380	<i>Streptococcus thermophilus</i> and <i>Lactobacillus delbrueckii</i> ssp. <i>bulgaricus</i>	2% YC-380	150 ± 5 ^a
P126	<i>Propionibacterium thoenii</i> (jensenii) P126	2% P126	530 ± 25 ^b
B1264	<i>Propionibacterium jensenii</i> B1264	2% B1264	542 ± 32 ^b
YC-380 + P126	<i>S. thermophilus</i> , <i>L. delbrueckii</i> ssp. <i>bulgaricus</i> , and <i>P. thoenii</i> (jensenii) P126	1% YC-380 + 1% P126	158 ± 6 ^a
YC-380 + B1264	<i>S. thermophilus</i> , <i>L. delbrueckii</i> ssp. <i>bulgaricus</i> , and <i>P. jensenii</i> B1264	1% YC-380 + 1% B1264	164 ± 5 ^a
YC-380 + P126 + B1264	<i>S. thermophilus</i> , <i>L. delbrueckii</i> ssp. <i>bulgaricus</i> , <i>P. thoenii</i> (jensenii) P126, and <i>P. jensenii</i> B1264	1% YC-380 + 0.5% <i>P. thoenii</i> (jensenii) P126 + 0.5% <i>P. jensenii</i> B1264	152 ± 9 ^a

^{a,b}Means with different superscripts differ ($P < 0.05$).

¹Means ± SEM.

sodium lactate broth that consisted of 1% trypticase soy broth without dextrose (BBL; Baltimore Biological Laboratories Inc., Cockeysville, MD), 1% yeast extract (Difco Laboratories, Detroit, MI), and 1% sodium lactate syrup (60% wt/wt; Fisher Scientific Co., Pittsburgh, PA) in distilled water (Grinstead and Barefoot, 1992). Sodium lactate agar was prepared from sodium lactate broth by adding 1.8% agar (BBL). *Propionibacteria* were grown at 32°C under flowing CO₂ (0.4 L/h) for 48 h (Ratnam et al., 1999; Ekinici and Barefoot, 2006). All cultures were maintained in the appropriate growth medium containing 20% glycerol and stored at -80°C.

A commercial YC-380 starter culture, which is a combination of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* (Chr. Hansen-Peyma, Istanbul, Turkey), was used in the production of control yogurt samples.

Yogurt Preparation

Commercial UHT milk containing 3.4% fat was heated to 30°C, and commercial NDM was added to increase milk TS to 15%. Total solids fortification was carried out based on TS analysis. The milk was homogenized stepwise with an UltraTurrax homogenizer (Dix 900, Heidolph, Kelheim, Germany) at 60°C for 2 min. After homogenization, milk was heated to 85°C for 15 min and then cooled to 43°C. Different combinations of bacterial cultures were added (Table 1). The inoculation rate was 2% for all samples. Inoculated milk was distributed in 200-mL plastic cups and incubated at 42°C until it reached pH 4.6. After fermentation, yogurt samples were removed and stored at 4°C for 15 d and analyzed at d 1, 7, and 15.

Proximate Analysis

The samples were analyzed in triplicate for acidity, fat, protein, ash, and TS content. The pH values of the

yogurt samples were measured by using a pH meter (Inolab, WTW Measurement Systems Inc., Fort Myers, FL). Acidity was expressed as a percentage of lactic acid (AOAC, 1990). Fat content was determined by the Gerber method (British Standards Institution, 1955); for yogurt, this was done by using an 11-g sample of yogurt in the butyrometer. Total solids were calculated after evaporation of the water present in the samples placed in an oven (105°C) for 48 h (until constant mass was obtained) according to the Turkish Standards Institution method (TS 1330; Turkish Standards Institution, 1999). The micro-Kjeldahl method was used to determine total protein contents of yogurt (AOAC, 1990) and ash by heating a 5-g sample in a muffle furnace at 100°C for 1 h, 200°C for 2 h, and 550°C overnight (Marth, 1978).

Physical Analysis

Syneresis. One hundred grams of yogurt sample was weighed on a filter paper (Whatman 4, Clifton, NJ) resting on top of a funnel connected to a volumetric flask. After 2 h of drainage at 7°C, the quantity of whey collected in a 50-mL graduated cylinder was used as an index of syneresis (Farooq and Haque, 1992).

Texture. The firmness of the yogurt was measured with a Lloyd Instruments TAPlus texture analyzer (Ametek Inc., Hants., UK) with an 11-mm cylinder probe and is expressed in newtons. The probe (0.100 N) was inserted into each product to a depth of 15 mm and at a speed of 24 mm/min. The instrument was connected to a computer fitted with a Nexygen MT program (Lloyd Instruments, Hants., UK) to measure gel strength. Firmness was evaluated immediately after removal from cold storage at 4°C. Measurements were taken at 2 points far away from each other for each sample.

Microbiological Analyses

One gram of yogurt sample was 10-fold serially diluted with 0.15% sterile peptone water (Acumedia, Baltimore, MD), and viable numbers were enumerated by using the pour plate technique. Coliform counts were estimated by using violet red bile agar (Merck, Darmstadt, Germany) incubated at 37°C for 24 to 48 h. Yeast and mold enumeration was carried out on potato dextrose agar (International Dairy Federation, 1992) incubated at 25°C for 5 d.

The counts of *Streptococcus* spp. and *Lactobacillus* spp. were enumerated on M17 agar and de Man, Rogosa, Sharpe agar (Merck), respectively, by incubating the plates at 37°C for 48 h. *Propionibacterium* spp. were cultivated on sodium lactate agar as described in Ekinci and Barefoot (2006) and grown at 32°C under flowing CO₂ (0.4 L/h) for 4 to 5 d.

Determination of Flavor Compounds

Determination of volatile flavor substances (acetaldehyde, diacetyl, acetoin, ethanol, and propionic acids) in yogurt samples was done by using the headspace method, slightly modified as described in Guzel-Seydim et al. (2000). Four milliliters of yogurt sample was transferred into headspace vials on d 1, 7, and 15 during yogurt storage. Gas chromatography analysis was performed by using a Perkin-Elmer Auto System XL (Perkin-Elmer, Milan, Italy) equipped with a flame-ionization detector. The following conditions were used for gas chromatography analysis: capillary column, PE-Wax 52 CB (30 m × 0.32 mm; film thickness of 0.25 µm; Perkin-Elmer); column temperature programmed for 5 min at 35°C, 1 min at 50°C, and 1 min at 150°C; injector and detector temperatures, 180 and 200°C, respectively; carrier gas, helium at a flow rate of 40 mL/min; split ratio, 1:20 mL/min. Peak identification of volatile substances was based on the retention times of the external individual reference standards. Standard solutions of acetaldehyde, acetoin, diacetyl, ethanol, and propionic acid were prepared with distilled deionized water. Standard solutions were analyzed as described previously for the samples.

Determination of Organic Acids

One gram of each sample was diluted into 3 mL of 0.01 M KH₂PO₄ (pH 8.0) and centrifuged at 1,760 × *g* for 2 min. After centrifugation, 1 mL of upper phase was filtered through 0.45-µm Gelman Acrodisc filters (Pall GmbH, Dreieich, Germany) and injected into a Shimadzu class LC-VP HPLC system (Shimadzu Scientific Instruments, Columbia, MD) equipped with a UV-visible detector (SPD-10AV vp) and pump (LC-6AD)

and with class LC-VP software; the mobile phase was 0.05 M H₃PO₄ prepared in water and adjusted to pH 2.2 with NaOH. The elution was conducted at room temperature by using a YMC Pack-ODS-AM column (250 × 4.6 mm i.d., 5 mm; Perkin-Elmer) at a flow rate of 0.5 mL/min. The UV detector was set at 210 nm. Initial identity assignment of organic acids was based on comparison of retention data obtained with the UV detector for standard compounds and sample components. Quantification was achieved by using peak areas from external calibration with standard solutions (formic, lactic, orotic, citric, and uric acids, Sigma Aldrich Co., Steinheim, Germany).

Statistical Analysis

Statistical analyses were conducted by using the GLM procedure (PROC GLM) of SAS (SAS Institute Inc., 1999). Separation of the means (*P* < 0.05) was accomplished by Duncan's multiple range test. All experiments and analyses were replicated 3 times. All analyses within experimental replications were performed in duplicate.

RESULTS AND DISCUSSION

The use of propionibacteria as a sole starter culture significantly affected the incubation length of yogurt samples in our study. Average values of incubation length were shown in Table 1. Because of the slow growth of propionibacteria cultures, longer fermentation times were observed when the bacteriocin-producing cultures *P. jensenii* B1264 (2%) and *P. thoenii* (*jensenii*) P126 (2%) were used alone as starter cultures for production of a new fermented dairy product (approximately 9 h). Incubation time was similar to that of yogurt bacteria when propionic acid bacteria were used with YC-380 in yogurt production; therefore, a combination of a commercial YC-380 starter culture and *Propionibacterium* spp. was used. It has been reported that probiotic bacteria grow slowly in milk because of the lack of proteolytic activity, and the usual practice is to add yogurt bacteria (*S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*) to probiotic products to reduce the fermentation time (Shihata and Shah, 2000; Antunes et al., 2004).

The results of titratable acidity, pH, TS, fat, and protein analyses are shown in Table 2. Decreases in pH of YC-380 + B1264 and YC-380 + P126 samples were similar to the control sample on d 1 (*P* > 0.05). The pH values of all yogurt samples gradually dropped during cold storage, similar to the results reported by Guzel Seydim et al. (2005). Titratable acidity of the control sample (YC-380) remained lower (*P* < 0.05) than that

Table 2. The effect of different starter cultures on the pH, TS, fat, titratable acidity, and protein contents of yogurt during storage at d 1, 7, and 15¹

Item	Storage time, d	YC-380	YC-380 + P126	YC-380 + B1264	YC-380 + P126 + B1264
pH	1	4.11 ± 0.04	4.06 ± 0.04	4.03 ± 0.01	4.07 ± 0.04
	7	3.97 ± 0.02	3.93 ± 0.03	3.94 ± 0.02	3.99 ± 0.02
	15	3.92 ± 0.02	3.84 ± 0.01	3.84 ± 0.02	3.91 ± 0.03
TS, %	1	14.08 ± 0.02	14.28 ± 0.04	14.28 ± 0.05	14.20 ± 0.09
	7	14.13 ± 0.06	14.33 ± 0.07	14.29 ± 0.05	14.08 ± 0.06
	15	14.09 ± 0.07	14.29 ± 0.03	14.29 ± 0.04	14.04 ± 0.03
Fat, %	1	3.15 ± 0.06	3.15 ± 0.08	2.98 ± 0.08	2.98 ± 0.10
	7	3.12 ± 0.08	3.12 ± 0.08	3.03 ± 0.08	3.17 ± 0.08
	15	3.12 ± 0.08	3.13 ± 0.08	3.03 ± 0.05	3.00 ± 0.13
Titratable acidity, % lactic acid	1	1.31 ± 0.01 ^x	1.34 ± 0.01 ^x	1.30 ± 0.01 ^x	1.30 ± 0.01 ^x
	7	1.49 ± 0.08 ^y	1.65 ± 0.02 ^y	1.67 ± 0.02 ^y	1.62 ± 0.05 ^y
	15	1.70 ± 0.07 ^{a,z}	1.96 ± 0.01 ^{b,z}	1.92 ± 0.05 ^{b,z}	1.89 ± 0.09 ^{b,z}
Protein, %	1	3.77 ± 0.05	3.63 ± 0.05	3.55 ± 0.06	3.63 ± 0.03
	7	3.54 ± 0.04	3.44 ± 0.07	3.31 ± 0.04	3.45 ± 0.05
	15	3.16 ± 0.05	3.13 ± 0.05	3.04 ± 0.03	3.23 ± 0.04

^{a,b}Means in the same row with different superscripts differ ($P < 0.05$).

^{x-z}Means within the same item and in the same column with different superscripts differ ($P < 0.05$).

¹Means ± SEM. YC-380 = *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*; YC-380 + P126 = *S. thermophilus*, *L. delbrueckii* ssp. *bulgaricus*, and *Propionibacterium thoenii* (jensenii) P126; YC-380 + B1264 = *S. thermophilus*, *L. delbrueckii* ssp. *bulgaricus*, and *Propionibacterium jensenii* B1264; YC-380 + P126 + B1264 = *S. thermophilus*, *L. delbrueckii* ssp. *bulgaricus*, *P. thoenii* (jensenii) P126, and *P. jensenii* B1264.

of *Propionibacterium* spp.-supplemented samples after 15 d of storage because of the production of propionic acid by *Propionibacterium* spp. and lactic acid by lactic acid bacteria. Total solids and protein contents of the yogurts did not show any significant ($P > 0.05$) differences among different treatments.

Rheological Properties

As shown in Figure 1, YC-380 + P126 + B1264 samples had the highest serum separation. Means were 37.25, 36.05, and 33.50% on d 1, 7, and 15, respectively. Serum separation amounts in control samples and YC-380 + P126 samples were similar. Syneresis of the all samples decreased during storage. Serum separation of YC-380 + P126 + B1264 was higher than control yogurt samples during storage ($P > 0.05$). Higher acidity stimulates syneresis in yogurt (Tamime and Robinson 1999). Differences in formation of the protein matrix caused by the different metabolic activities of lactic acid bacteria and propionic acid bacteria might have caused the significant changes in syneresis among treatments (Tamime and Deeth, 1980). La Torre et al. (2003) obtained similar results in set-type yogurt and bioyogurt.

The firmness of yogurt depends on the TS content of the product and also on protein-protein interactions (Tamime and Deeth, 1980; Gastaldi et al., 1997; Penna et al., 2001; Kristo et al., 2003). The use of different starter cultures had a significant effect on the firmness of the samples during storage (Figure 2). Parallel to the syneresis results, the yogurts produced by the com-

bination of cultures YC-380 + P126 had the firmest structure (0.26 N), whereas the samples produced by the combination of cultures YC-380 + P126 + B1264 showed the lowest firmness, with the value of 0.22 N ($P < 0.05$). The reason for the better structure of YC-380 + P126 might be related to exopolysaccharide production by propionibacteria. Dairy propionibacteria have been reported to produce extracellular slime or polysaccharide that increases the viscosity of liquid cultures (Skogen et al., 1974). *Propionibacterium thoenii* (jensenii) P126 produces extracellular slime in liquid cultures (Ekinci and Barefoot, 2006).

Microbiological Analyses

No coliforms, yeasts, or molds were found during the storage of yogurt. The changes in viable counts of different combinations of bacterial cultures are shown in Table 3. The viable counts of *S. thermophilus* in control samples during storage changed from 8.33 log cfu/g on d 1 to 6.33 log cfu/g on d 15 ($P < 0.05$). The addition of propionibacteria to yogurt did not have a negative effect on the counts of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* in yogurt ($P > 0.05$), suggesting that the activities of propionibacteria did not interfere with those of the lactic starters. There were significant decreases in the populations of *L. delbrueckii* ssp. *bulgaricus* during storage in all samples; lower storage temperatures and overacidification have been reported to limit the growth of *L. delbrueckii* ssp. *bulgaricus* (Kneifel et al., 1992). The counts of propionibacteria decreased by

Figure 1. Changes in serum separation (syneresis) of yogurt samples during 15 d of storage at 4°C. Data points represent averages of 3 experiments.

Figure 2. Changes in the firmness of yogurt samples during 15 d of storage at 4°C. Data points represent averages of 3 experiments.

Table 3. Viability of starter cultures in yogurts during 2 wk of storage at 4°C¹

Culture	Storage time, d	YC-380, log cfu/g	YC-380 + P126, log cfu/g	YC-380 + B1264, log cfu/g	YC-380 + P126 + B1264, log cfu/g
<i>Streptococcus thermophilus</i>	1	8.33 ± 0.01	8.15 ± 0.02	8.19 ± 0.06	8.22 ± 0.04
	7	7.96 ± 0.02	7.06 ± 0.04	7.17 ± 0.05	7.30 ± 0.03
	15	6.33 ± 0.02	6.17 ± 0.03	6.27 ± 0.01	6.19 ± 0.03
<i>Lactobacillus delbrueckii</i> ssp. <i>bulgaricus</i>	1	8.74 ± 0.10	8.15 ± 0.06	8.31 ± 0.01	8.54 ± 0.10
	7	7.85 ± 0.02	7.15 ± 0.06	7.70 ± 0.11	7.50 ± 0.09
	15	4.91 ± 0.06	4.89 ± 0.04	4.28 ± 0.05	4.20 ± 0.08
<i>Propionibacterium thoenii</i> P126	1	—	7.20 ± 0.04	7.45 ± 0.06	7.21 ± 0.02
<i>Propionibacterium jensenii</i> B1264	7	—	6.31 ± 0.02	6.33 ± 0.01	6.34 ± 0.04
	15	—	5.28 ± 0.14	5.31 ± 0.04	5.31 ± 0.03

¹Means ± SEM. YC-380 = *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*; YC-380 + P126 = *S. thermophilus*, *L. delbrueckii* ssp. *bulgaricus*, and *Propionibacterium thoenii* (jensenii) P126; YC-380 + B1264 = *S. thermophilus*, *L. delbrueckii* ssp. *bulgaricus*, and *Propionibacterium jensenii* B1264; YC-380 + P126 + B1264 = *S. thermophilus*, *L. delbrueckii* ssp. *bulgaricus*, *P. thoenii* (jensenii) P126, and *P. jensenii* B1264.

2 log cycles at the end of storage ($P < 0.05$). During the first week of storage, propionibacteria culture counts for YC-380 + P126, YC-380 + B1264, and YC-380 + P126 + B1264 decreased from 7.20 to 6.31 log cfu/g, 7.45 to 6.33 log cfu/g, and 7.21 to 6.34 log cfu/g, respectively. The results showed that this product provided a good carrier for these organisms. The pH of propionibacteria-supplemented yogurts also decreased to 3.9 during this period, resulting in a decrease in viable propionibacteria counts.

Flavor Compounds

Although a long list of volatile organic compounds has been identified in yogurt (Law, 1981), in many studies only a few of them have proved to have a decisive influence on aroma because of their comparatively high concentrations. Acetaldehyde is recognized as a major flavor component in yogurt, and the evaluation of its flavor, according to some authors, is based mainly on acetaldehyde production by the starter culture (Tamime and Deeth, 1980).

The flavor compounds produced by the starter bacteria were mainly synthesized during fermentation and the first 24 h of storage (Table 4). In control samples as well as in propionibacteria-supplemented samples, the prevailing constituents were acetaldehyde and acetoin. The highest acetaldehyde (29.35 mg/kg) content of yogurt samples was obtained with yogurt made with propionibacteria-supplemented starter cultures (YC-380 + P126 + B1264) on d 1. These results could be attributed to variations in the metabolic activities that existed in microbial species or to differences within strains of the same species (Beshkova et al., 1988; Georgala et al., 1995). In general, the level of carbonyl compounds decreased during cold storage; this could be associated with further metabolic activity of the starter

cultures during the storage period. Acetaldehyde, which is the main flavor substance in yogurt, metabolized to ethanol via alcohol dehydrogenase of *S. thermophilus* (Ozer, 2006). Concentration of acetaldehyde significantly decreased during cold storage at d 15 for all samples ($P < 0.05$). Relatively high activity of ethanol production was recorded in all yogurts on d 1. Ethanol concentration was higher in yogurts made with propionibacteria-supplemented cultures compared with control yogurt. Ethanol concentration decreased greatly by the end of storage. Although the diacetyl contents of all yogurts were considerably increased until d 7 ($P < 0.05$), they proceeded to decrease perceptibly for the control and YC-380 + P126-supplemented yogurts at the end of storage. The decreases in diacetyl and acetaldehyde contents in all yogurt samples by the end of the storage period could be due to hydrolysis by microbial enzymes to form other substances (Beshkova et al., 1988; Georgala et al., 1995; Tamime and Robinson, 1999). As expected, propionibacteria-supplemented yogurts had a higher propionic acid content than control yogurts ($P < 0.05$; Table 4). Propionibacteria converts lactate to propionic and acetic acids during heterolactic fermentation (Hettinga and Reinbold, 1972). The amounts of propionic acid for yogurts made with YC-380 + P126, YC-380 + B1264, and YC-380 + P126 + B1264 cultures increased from 41.99 to 60.72, 35.95 to 67.17, and 35.70 to 51.12 mg/kg, respectively, during cold storage.

Organic Acids

The average concentrations (mg/g) of organic acids in yogurts are shown in Table 5. It is evident that the added starter culture bacteria had accomplished the main fermentation and, as a consequence, there was a high concentration of lactic acid in all the products.

Table 4. Production of flavor compounds (mg/kg) by yogurt cultures during storage at d 1, 7, and 15¹

Flavor compound, mg/kg	Storage time, d	YC-380	YC-380 + P126	YC-380 + B1264	YC-380 + P126 + B1264
Acetaldehyde	1	28.44 ^{a,x}	8.97 ^{b,x}	25.21 ^{b,x}	29.35 ^{a,x}
	7	20.03 ^{a,x}	18.23 ^{a,x}	26.82 ^{b,x}	18.91 ^{a,y}
	15	14.29 ^{a,y}	12.40 ^{a,y}	16.33 ^{a,y}	12.36 ^{a,z}
Acetoin	1	31.95 ^{a,x}	43.43 ^{b,x}	32.86 ^{a,x}	32.82 ^{a,x}
	7	27.58 ^{a,x}	33.50 ^{b,y}	28.73 ^{a,x}	26.01 ^{a,y}
	15	7.35 ^{a,y}	21.28 ^{b,z}	19.05 ^{b,y}	18.02 ^{b,y}
Ethanol	1	6.63 ^{a,x}	7.58 ^{a,x}	6.50 ^{a,x}	3.61 ^{b,x}
	7	2.89 ^{a,y}	3.09 ^{a,x}	2.86 ^{a,y}	4.33 ^{b,x}
	15	0.94 ^{a,y}	3.86 ^{b,x}	1.42 ^{a,y}	3.01 ^{b,x}
Diacetyl	1	0.21	0.39	0.30	0.43
	7	0.59	0.53	0.49	0.90
	15	0.15	0.17	0.66	0.48
Propionic acid	1	14.52 ^{a,x}	41.99 ^{b,x}	35.95 ^{b,x}	35.70 ^{b,x}
	7	25.64 ^{a,x}	25.84 ^{a,y}	25.60 ^{a,y}	34.13 ^{b,x}
	15	37.30 ^{a,z}	60.72 ^{b,x}	67.17 ^{b,z}	51.12 ^{c,y}

^{a-c}Means within the same row with different superscripts differ ($P < 0.05$).

^{x-z}Means within the same flavor compound and in the same column with different superscripts differ ($P < 0.05$).

¹YC-380 = *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*; YC-380 + P126 = *S. thermophilus*, *L. delbrueckii* ssp. *bulgaricus*, and *Propionibacterium thoenii* (jensenii) P126; YC-380 + B1264 = *S. thermophilus*, *L. delbrueckii* ssp. *bulgaricus*, and *Propionibacterium jensenii* B1264; YC-380 + P126 + B1264 = *S. thermophilus*, *L. delbrueckii* ssp. *bulgaricus*, *P. thoenii* (jensenii) P126, and *P. jensenii* B1264.

Lactic acid production increased slightly during storage, from 15.58 mg/g on d 1 to 21.85 mg/g on d 15 ($P < 0.05$), for the YC-380 + P126 + B1264 yogurts (Table 5). The lactic acid content of YC-380 + B1264 yogurts remained constant during cold storage. There was no significant difference in the concentrations of citric, for-

mic, and orotic acids in yogurt samples during 15 d of storage ($P > 0.05$).

CONCLUSIONS

Improved functional properties can be achieved by using adjunct probiotic starter cultures in the produc-

Table 5. Production of organic acids (mg/g) by yogurt cultures during storage at d 1, 7, and 15¹

Organic acids, mg/g	Storage time, d	YC-380	YC-380 + P126	YC-380 + B1264	YC-380 + P126 + B1264
Lactic acid	1	18.82 ^{z,ab}	17.42 ^{z,b}	19.89 ^a	15.58 ^{z,c}
	7	20.17 ^{y,a}	20.88 ^{x,a}	19.45 ^b	18.05 ^{y,b}
	15	22.47 ^{x,a}	19.97 ^{y,b}	19.34 ^b	21.85 ^{x,a}
Orotic acid	1	0.10	0.11	0.13	0.11
	7	0.10	0.13	0.14	0.12
	15	0.12	0.12	0.13	0.13
Citric acid	1	2.41 ^{x,a}	2.32 ^{y,a}	2.60 ^{y,a}	2.18 ^{x,b}
	7	2.25 ^{y,b}	2.52 ^{x,ab}	2.75 ^{x,a}	2.09 ^{x,b}
	15	2.56 ^{x,a}	2.31 ^{y,b}	2.44 ^{y,a}	2.41 ^{x,a}
Uric acid	1	0.07 ^y	0.07	0.09	0.07 ^y
	7	0.08 ^y	0.09	0.09	0.05 ^y
	15	0.12 ^x	0.08	0.10	0.09 ^x
Formic acid	1	3.76 ^{y,a}	3.45 ^{z,b}	4.08 ^{y,a}	3.34 ^{z,b}
	7	3.90 ^{y,b}	4.32 ^{x,a}	4.20 ^{x,ab}	4.33 ^{x,a}
	15	4.41 ^{x,a}	3.72 ^{y,c}	3.89 ^{z,bc}	4.11 ^{y,b}

^{a-c}Means within the same row with different superscripts differ ($P < 0.05$).

^{x-z}Means within the same organic acid and in the same column with different superscripts differ ($P < 0.05$).

¹YC-380 = *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*; YC-380 + P126 = *S. thermophilus*, *L. delbrueckii* ssp. *bulgaricus*, and *Propionibacterium thoenii* (jensenii) P126; YC-380 + B1264 = *S. thermophilus*, *L. delbrueckii* ssp. *bulgaricus*, and *Propionibacterium jensenii* B1264; YC-380 + P126 + B1264 = *S. thermophilus*, *L. delbrueckii* ssp. *bulgaricus*, *P. thoenii* (jensenii) P126, and *P. jensenii* B1264.

tion of fermented dairy products. In this research, propionibacteria were used as adjunct starter cultures with lactic acid bacteria in set-type yogurt production without adversely affecting the physical and chemical properties. Additionally, during the first week of storage, propionibacteria counts remained high, suggesting that yogurt provided a good environment for these organisms. This would provide not only beneficial health effects but also a new alternative product to plain set-type yogurt.

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