





Research Article

Assessment of Growth and Technological Performances of Yogurt Bacteria Isolated From Local Turkish Yogurts

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Abstract: Growth patterns and some technological performances of five *Streptococcus thermophilus* and seven *Lactobacillus delbrueckii* subsp. *bulgaricus* strains isolated from yogurt samples which were collected from remote rural areas of Anatolia, Turkey were assessed. The single isolates were screened for milk acidification performances, production of volatile organic compounds and texture developing properties, and selected isolates were combined to be used in yogurt-making. Yogurt samples were subjected to sensory evaluations as well as fermentation profiles. Finally, the combinations of one *Streptococcus thermophilus* isolate with all *Lactobacillus delbrueckii* subsp. *bulgaricus* strains were found to have potential for further evaluations regarding their suitability for commercial applications.

Keywords: yogurt, starter, flavor, texture

1. Introduction

Yogurt is perhaps one of the oldest fermented foods and is believed to be spread from Central Asia to the rest of the World via migration routes of nomadic people [1]. Although the health benefits of yogurt dates back to 6000 BC in Ayurvedic Indian scripts, its scientific demonstration was rather new. In the early 20th century, *Lactobacillus delbrueckii* subsp. *bulgaricus* (formerly named *Bacillus bulgaricus*) was isolated from yogurt and associated with longevity in the Bulgarian peasant people [2]. Current standard procedures and regulations call for the addition of *Streptococcus thermophilus* (*Str. thermophilus*) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (*Lb. bulgaricus*) in the manufacture of yogurt [3, 4]. The textural and organoleptic properties of yogurt are largely determined by the strains of these two species. The harmony between two or more strains of these species is essential for development of a well-balanced aroma/flavor and texture in yogurt. For this reason, many studies have been dedicated to screen the strains of these two yogurt bacteria for their suitability for commercial applications [5, 6]. Very few number of screened strains have reached a commercial success since these strains have to meet basic selection criteria for being used as starter culture in yogurt-making. These criteria include safety for human consumption, acidification rate of milk, aroma producing and

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texture promoting capacities, growth kinetics, bacteriophage resistance, sustainable production at commercial level, *i.e.*, reproducibility and suitability for freeze-drying and so on [5, 7]. Today, many of these traits of yogurt bacteria may well be improved using modern molecular techniques [8, 9].

In yogurt production, back-slopping fermentation model has been largely replaced by commercial culture-based fermentation model in many countries. This shift has eventually caused a progressive loss of microbial diversity and characteristic sensory properties of traditional yogurt. The consumers' expectations from yogurt have changed in recent years. Standard consumers demand more natural yogurt with taste and textural properties that they are used to. In order to meet consumers' demand towards local tastes and textures in yogurt, a re-designing of yogurt production has become a necessity for yogurt industry. Therefore, efforts have been accelerated to find out the novel yogurt starter strains from the local diversity of lactic acid bacteria [6, 10-12]. The common screening protocol for wild type *Str. thermophilus* and *Lb. bulgaricus* strains follow isolation of colonies using a suitable culturing medium (M17 agar for the former and MRS agar for the latter species), and phenotypic (*i.e.*, morphological examination, growing at different temperatures, Gram-staining, sugar fermentation, salt and acid resistance) and genotypic (Real Time-PCR, MALDI-TOF, DGGE, PFGE, ribotyping, fluorescent *in situ* hybridization, 16S rRNA sequencing, metagenomics analysis *etc.*) identification of the colonies [13]. Today, genotypic identification of lactic acid bacteria is achieved with high sensitivity by using one or more of the techniques mentioned above. However, most difficult part of culture development studies is to find the best combination of *Str. thermophilus* and *Lb. bulgaricus* strains to obtain yogurt with a balanced taste/aroma and texture. To do so, all isolated and identified strains should be subjected to technological performance tests and culture combinations should be prepared based on the results of these pre-selection tests. These combinations are further used in the manufacture of yogurt with desired characteristics.

Present study aimed at screening the isolates of *Str. thermophilus* and *Lb. bulgaricus* which were previously isolated from local fermented dairy products in Anatolia, Turkey for their growth patterns, technological performances and suitability for yogurt-making.

2. Materials and methods

2.1 Bacterial strains and chemicals

Table 1. Characteristics of the isolates from traditional yogurt samples in Anatolia, Turkey

	Isolate no	Strains	16S rRNA Gene Bank	Dairy product	Isolation location
<i>Str. thermophilus</i>	ST1	MGB80-7	HM058270.1	Yogurt	Bolu
	ST2	PON458	KC545934.1	Yogurt	Ermenek
	ST3	IMAU11370	KP764074.1	Yogurt	Bolu
	ST4	S9	CP013939.1	Yogurt	Ermenek
	ST5	KLDS SM	CP016026.1	Yogurt	Ermenek
<i>Lb. bulgaricus</i>	LB1	SB25	KJ868760	Yogurt	Ermenek
	LB2	MGB27-2	HM058081.1	Yogurt	Sertavul
	LB3	IMAU11365	KP764119.1	Yogurt	Mut
	LB4	NWAFU1436	MG551099.1	Yogurt	Mut
	LB5	SKB1083	MK564723.1	Yogurt	Merzifon
	LB6	MN-BM-F01	CP013610.1	Yogurt	Merzifon
	LB7	JCM 1002	LC063162.1	Yogurt	Merzifon

Bacterial strains were obtained from culture collection formed within the scope of a project entitled “*Development*

of *Industrial Yogurt Starter Combinations Using Local Sources*” (Project No: 112D052, financially supported by The Scientific and Technological Research Council of Turkey-TUBITAK). The characteristics of the strains are given in Table 1. The strains used in this study were obtained from traditional yogurt samples collected from different rural areas of Anatolia, Turkey. In total, 5 *Str. thermophilus* and 7 *Lb. bulgaricus* strains were screened for their growth properties and technological performances. All chemicals were supplied from Sigma-Aldrich Inc. (Sigma-Aldrich Inc., St. Louis, MO, USA) unless otherwise stated. Skimmed milk powder and cow’s milk were supplied from Ankara University Dairy Farm and yogurt productions were carried out at Ankara University Department of Dairy Technology Pilot Dairy Plant.

2.2 Activation of the strains and preparation of working cultures for yogurt production

Activation of the strains was achieved by adding 20 µl of *Lb. bulgaricus* and *Str. thermophilus* strains into 5 ml of MRS and M17 broths, respectively. While the tubes containing *Lb. bulgaricus* strains were incubated at 43°C for 72h under anaerobic conditions, tubes containing the strains of *Str. thermophilus* were incubated at 37°C for 24h aerobically. 0.5 ml of pre-activated strains were inoculated into 50 ml of sterile reconstituted milk (10%, w/v) to obtain a working culture for yogurt-making. Reconstituted milk was incubated at 43°C until gelation was complete and the working cultures were kept at 4°C for 24h before use. Yogurt samples were prepared by inoculating 100 ml of heat-treated (at 90°C for 10 min) fresh cow’s milk [3.2% (w/v) milk fat and 3.0% (w/v) protein] with working culture (2%, w/v). Combined cultures were prepared by mixing single isolates of *Str. thermophilus* and *Lb. bulgaricus* at 1 to 1 ratio. Samples were incubated at 43°C until pH 4.6 was attained. Then the fermented milks were kept at 4°C for 14 days. The preparation of working cultures and yogurt samples is summarized in Figure 1.

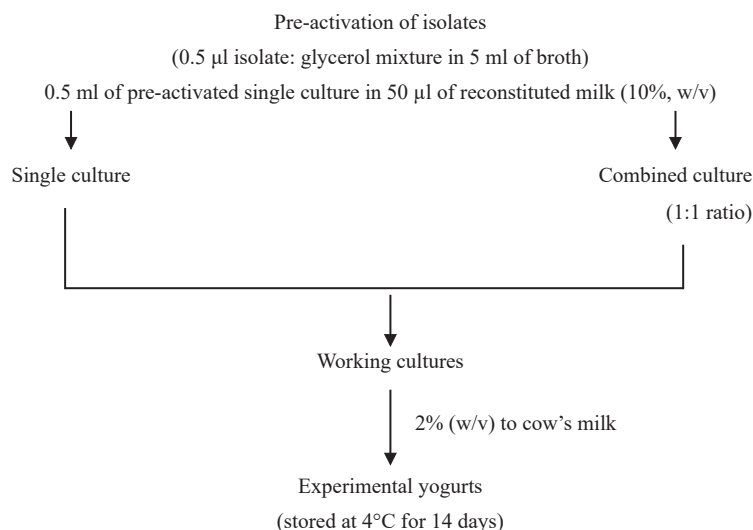


Figure 1. Schematic illustration of preparation of working culture and experimental yogurts. For incubation conditions refer to text

2.3 Determination of specific growth rates and growth patterns of the strains

Maximal growth rates of the strains were determined by measuring optical density of the growth mediums at 600 nm (OD_{600}) at the beginning and end of 24h growth period [14]. Twenty microliters of each activated strains were transferred into a suitable broth medium and incubated at 43°C anaerobically for *Lb. bulgaricus* and at 37°C aerobically for *Str. thermophilus*. OD_{600} values were measured using a UV/VIS spectrophotometer (Perkin Elmer Inc., Massachusetts, USA). When necessary, broths containing single strains were diluted with sterile pure water and in the calculation of maximal growth rate, the dilution rate was considered. Maximal growth rate (μ_{max}) of each strain was calculated using the following formula:

$$\text{Maximal growth rate } (\mu_{max}) = 2.303 \times (\text{OD}_{600\ 124} - \text{OD}_{600\ t0}) / 24 \quad (1)$$

Growth patterns of the isolates were determined by measuring OD₆₀₀ values throughout 24h incubation with 3h intervals. In order to avoid the erroneous results, culture conditions (batch and volume of growth medium, size of culture vessel and incubation conditions etc.) were kept same.

2.4 Textural properties of yogurt samples made by single strains

Large deformation textural characteristics of yogurt samples produced by single strains were determined by means of a Texture Profile Analyzer (model TX.2TA, Stable Micro Systems, Godalming, UK) using back extrusion mode. The measuring conditions were as follows: cell load, 5 kg; cylindrical probe with 20 mm diameter; penetration depth of probe 15 mm and penetration speed, 1 mm/s. Measurements were achieved at 4°C. Three measurements were done for each sample.

2.5 Determination of volatile aroma compounds of single strain yogurts

Aroma profiles of the yogurt samples produced by single isolates were determined using a Gas Chromatography/Mass (GC/MS) system (Agilent 7890A GC-5975 MSD, Agilent Technologies, CA, USA). The volatiles were extracted using divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber [15]. Five grams of yogurt sample were mixed with 10 µl of internal standards (2-methyl-3-heptanon and 2-methyl pentanoic acid in 81 mg/kg methanol) and the mixture was stirred at 50°C for 30 min. The volatile compounds were separated using a DB-Wax column (30 m, 0.25 mm, 0.25 µm; J&W Scientific, Folsom, CA, USA). Helium was used as the carrier gas (1 ml/min). The oven was held at 40°C for 10 min (desorption period), then increased 5°C *per* min to 110°C. The temperature was then raised by 10°C *per* min to 240°C to give a run time of 15 min. The mass spectrometer was set to record at 35-500 m/z (threshold 150) at a sampling rate of 5.19 scan/s. The quantification of the volatiles was achieved by comparing the peak area of each volatile compound with peak area of internal standard using the following formula and the results were expressed as proportional amount (mg/kg):

$$\text{Volatile compounds} = 81 \times \text{peak area of volatile compound} / \text{peak area of internal standard} \quad (2)$$

2.6 Descriptive sensory analysis of yogurt samples

Yogurt samples produced by combined yogurt cultures were subjected to descriptive sensory analysis using Spectrum™ Descriptive Analysis Method as described in Meilgaard et al. [16]. Panel group consisted of five persons who were trained previously on sensory evaluation of fermented dairy products including basic taste, texture and flavor identification. Each sensory trait was converted to metric values using Spectrum Universal intensity scale. In this spectrum, “0” represented the unidentified sensory trait and “15” represented the strongest sensory trait. Samples (100 ml) were served to the panelists in cups bearing a random 3-digit number at 10°C and maximum six yogurt samples were evaluated by the panel group at one session. Panelists were provided with bread stick and a glass of water between each sample.

2.7 Statistical analysis

Data were statistically processed with one-way analysis of variance (ANOVA) using the SPSS Software (SPSS ver. 17, SPSS Inc., Chicago, IL, USA). Differences between groups were determined by Least Significant Difference (LSD) test ($p < 0.05$). The experiment was repeated three times ($n = 3$).

3. Results and discussion

3.1 Maximal growth rates and growth patterns of single strains

Maximal growth rates (μ_{max}) and growth profiles of single strains are given in Table 2, and Figures 2 and 3,

respectively. Overall, the maximal growth rates of *Str. thermophilus* strains were much higher than *Lb. bulgaricus* strains. The highest maximal growth rates were found in the isolates ST3, ST4 and ST2, in decreasing order. While the maximal growth rates of *Str. thermophilus* strains ranged between 0.0736 and 0.2204 1/h, these figures were between 0.0032 and 0.0379 1/h in the *Lb. bulgaricus* strains. As seen in Figures 2 and 3, all *Str. thermophilus* strains reached the stationary phase of growth within 9-12 hours of incubation. Isolate ST1 showed rather an atypical growth pattern for *Str. thermophilus* and had a shorter lag phase than the rest of the *Str. thermophilus* strains. All *Lb. bulgaricus* strains had far longer lag phases of growth than *Str. thermophilus* isolates. *Lb. bulgaricus* strains had similar growth patterns with varying OD₆₀₀ values. Proto-cooperation between yogurt bacteria has long been known. Both yogurt bacteria stimulates the growth of each other by providing essential growth promoters which are needed by its partner organism for the growth [17]. The proto-cooperation between yogurt bacteria is not only on nutritional exchanges, but also dramatic physiological changes in these two bacteria are evident [18]. In mixed cultures, *Str. thermophilus* shows rather lower resistance to acidity than *Lb. bulgaricus* and hence, reaches to stationary phase earlier than its counterpart. The growth of *Str. thermophilus* slows down, to a great extent, at pH 4.2-4.4. On the contrary, *Lb. bulgaricus* is able to tolerate lower pH values, i.e. pH 3.5-3.8 [19].

Table 2. Maximal growth rates (μ_{max} , 1/h) of the isolates (n = 3)

<i>Str. thermophilus</i>	
ST1	0.0736 ± 0.0038 ^a
ST2	0.1405 ± 0.0195 ^b
ST3	0.2204 ± 0.0760 ^c
ST4	0.1669 ± 0.0176 ^d
ST5	0.1000 ± 0.0080 ^e
<i>Lb. bulgaricus</i>	
LB1	0.0032 ± 0.0002 ^f
LB2	0.0323 ± 0.0005 ^g
LB3	0.0379 ± 0.0010 ^h
LB4	0.0256 ± 0.0010 ⁱ
LB5	0.0137 ± 0.0010 ^j
LB6	0.0228 ± 0.0001 ^k
LB7	0.0148 ± 0.0002 ^l

*Different superscript letters indicate statistically different groups (p > 0.05)

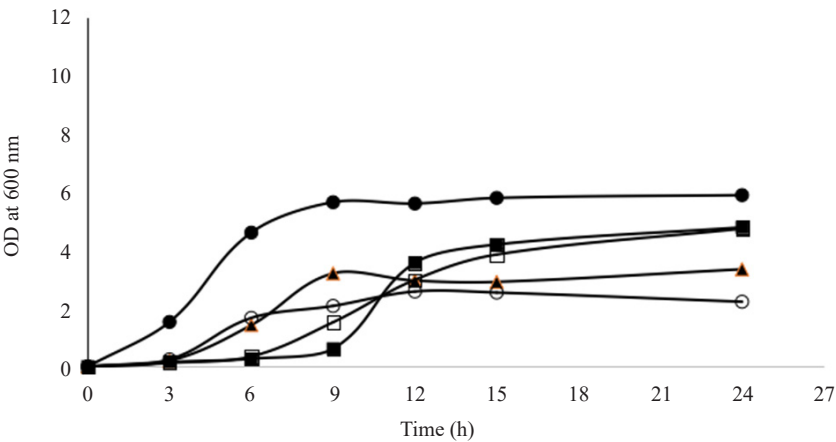


Figure 2. OD₆₀₀ values of *Str. thermophilus* isolates (n = 3). Isolate codes: (●) ST1, (▲) ST2, (◻) ST3, (■) ST4, (○) ST5. Error bars are smaller than symbol dimension

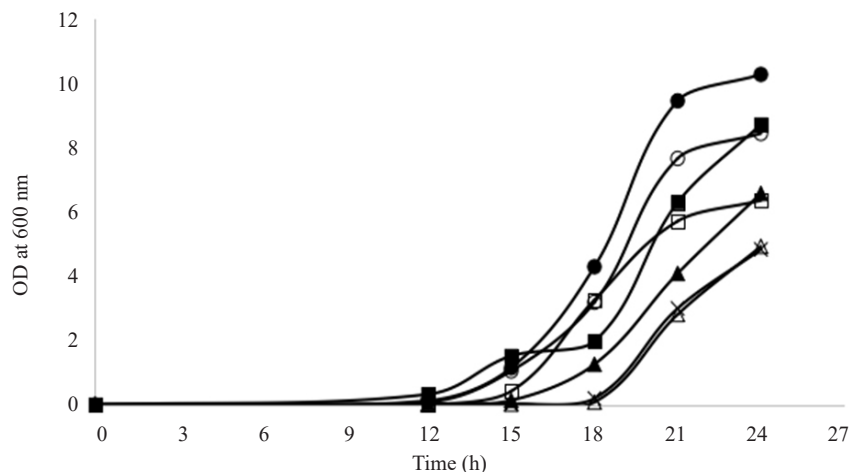


Figure 3. OD₆₀₀ values of *Lb. bulgaricus* isolates (n = 3). Isolate codes: (●) LB1, (○) LB2, (□) LB3, (■) LB4, (×) LB5, (△) LB6, (▲) LB7. Error bars are smaller than symbol dimension

3.2 Acidification profiles of yogurts

Acidification profiles of working cultures and yogurts made by single strains are given in Table 3. Among *Str. thermophilus* strains, the fastest gel formation of working cultures was observed in the ones inoculated with isolates ST3 and ST4 (gel formation time of working culture between 400-490 min). ST2 was the slowest acid-producing isolate with 960 min of gelation time. *Lb. bulgaricus* strains were rather slower acid-producers compared to *Str. thermophilus* strains. The average gelation time of the working cultures inoculated with *Lb. bulgaricus* strains were between 690 min and 1580 min. Working cultures were used in the manufacture of experimental yogurt samples at a level of 2% (w/v). Inoculated milks were incubated at 43°C until pH 4.7 was attained. The isolates ST4 and ST5 acidified the milk faster (within 235 min and 280 min, respectively) than the rest of the *Str. thermophilus* isolates. Among *Lb. bulgaricus* isolates, LB2 and LB4 were the fastest acid-producers (reaching pH 4.6-4.7 within 270-290 min). The decrease in pH of yogurt samples continued during 24 h-refrigeration, being more pronounced in the samples inoculated with isolates ST1 and ST5 in the *Str. thermophilus* group and LB1, LB2 and LB4 in the *Lb. bulgaricus* group.

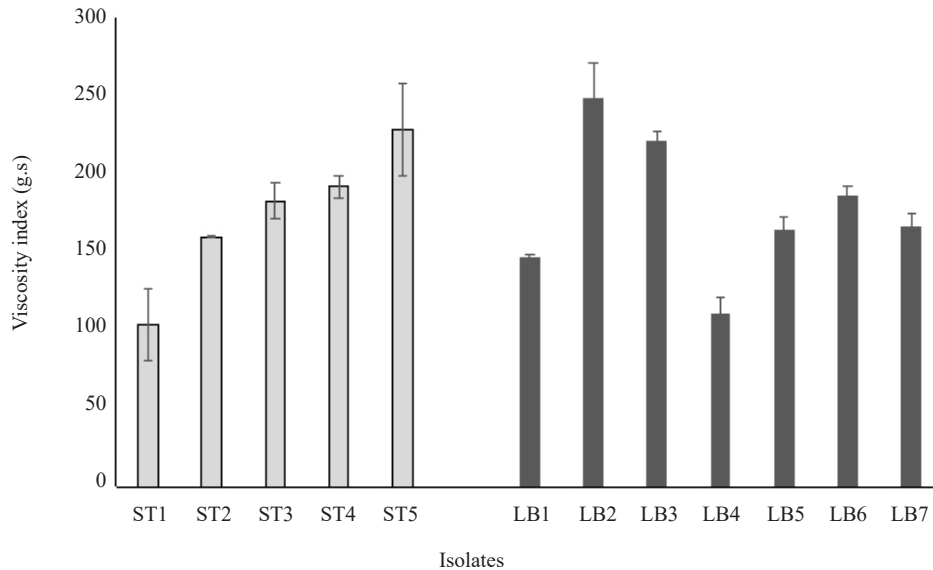
3.3 Textural properties of yogurt samples

Viscosity index and firmness values of 1-day old yogurt samples are presented in Figures 4 and 5, respectively. Among *Str. thermophilus* isolates, ST5 yielded the firmest yogurt with 484.98 g. Overall, except for the isolates LB2 and LB4, *Lb. bulgaricus* strains tested led to yogurt samples with lower firmness values than that made by *Str. thermophilus* strains. Firmness is defined as the maximum peak force during the first compression cycle and is required to create a certain degree of deformation in the product. Firmness is an important textural parameter for assessing the physical quality of yogurt [20]. The strength of the internal bonds making up yogurt body was the highest in the samples made by single isolates of ST5 and LB2 as evidenced by high cohesiveness values in these samples (not shown). The lowest consistency values were obtained in yogurts made by single isolates of LB5, LB6 and LB7. Consistency and viscosity index are related textural parameters; consistency indicates the thickness of the body of yogurt and viscosity index gauges the resistance of the sample to flow off the disc during back extrusion [21]. Yogurt samples made by isolate ST5 in the *Str. thermophilus* group and LB2 and LB3 in the *Lb. bulgaricus* group had the highest viscosity index values. The isolates ST5 and LB2 positively differ from the rest of the isolates in terms of their contributions to the textural properties of yogurt matrix.

Table 3. Acidification capacities of the strains (n = 3)

Isolates	Time to set for working cultures (min)	Incubation time of yogurt (min)	pH of yogurt at the end of incubation period	pH of yogurt after overnight refrigeration
<i>Str. thermophilus</i>				
ST1	680 ± 14.0 ^a	470 ± 45.0 ^a	4.67 ± 0.018 ^{aA}	4.52 ± 0.011 ^{acB}
ST2	960 ± 11.0 ^b	660 ± 9.00 ^b	4.68 ± 0.029 ^{aA}	4.67 ± 0.044 ^{bA}
ST3	490 ± 14.0 ^c	435 ± 30.0 ^a	4.66 ± 0.033 ^{aA}	4.61 ± 0.060 ^{bB}
ST4	400 ± 31.0 ^d	235 ± 4.00 ^c	4.53 ± 0.011 ^{bA}	4.52 ± 0.023 ^{acA}
ST5	660 ± 16.0 ^a	280 ± 7.00 ^d	4.69 ± 0.013 ^{aA}	4.56 ± 0.019 ^{aB}
<i>Lb. bulgaricus</i>				
LB1	960 ± 16.0 ^b	805 ± 22.0 ^c	4.70 ± 0.055 ^{adA}	4.56 ± 0.011 ^{aB}
LB2	690 ± 45.0 ^a	290 ± 10.0 ^f	4.64 ± 0.064 ^{aA}	4.51 ± 0.018 ^{cB}
LB3	1.440 ± 35.0 ^c	715 ± 33.0 ^g	4.65 ± 0.055 ^{aA}	4.64 ± 0.029 ^{bA}
LB4	810 ± 44.0 ^f	270 ± 55.0 ^f	4.43 ± 0.028 ^{caA}	4.33 ± 0.008 ^{dB}
LB5	1.580 ± 29.0 ^g	672 ± 14.0 ^b	4.68 ± 0.038 ^{aA}	4.67 ± 0.010 ^{bA}
LB6	741 ± 10.0 ^h	827 ± 15.0 ^c	4.70 ± 0.024 ^{adA}	4.70 ± 0.019 ^{bA}
LB7	1.578 ± 8.00 ^g	677 ± 38.0 ^{bg}	4.72 ± 0.005 ^{dA}	4.69 ± 0.066 ^{bA}

*Different lower case superscript letters in the same columns and upper case superscript letters in the same line indicate statistically different groups (p < 0.05)

**Figure 4.** Viscosity index values of the yogurt samples made by single cultures of *Str. thermophilus* or *Lb. bulgaricus* (n = 3)

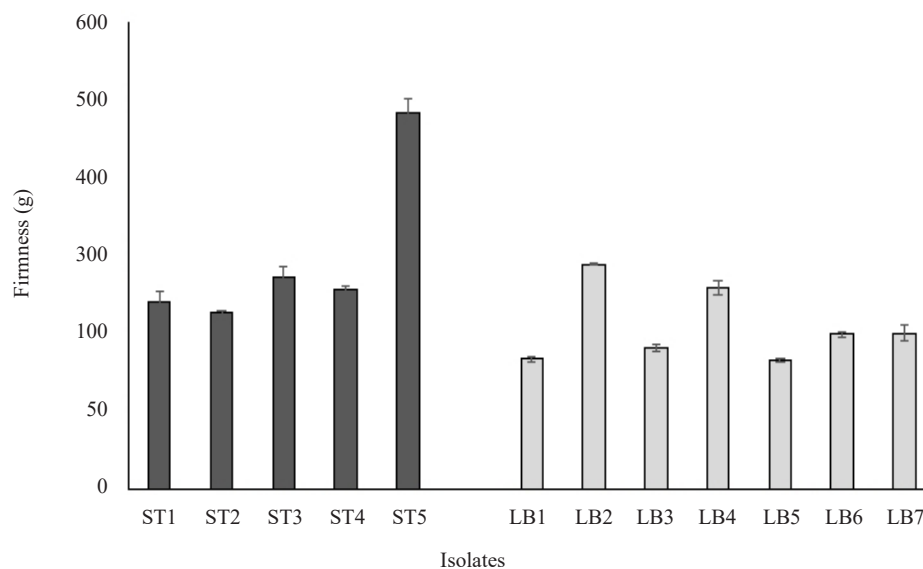


Figure 5. Firmness values of the yogurt samples made by single cultures of *Str. thermophilus* or *Lb. bulgaricus* (n = 3)

Table 4. Major aroma compounds of yogurts made by single strains of *Str. thermophilus* or *Lb. bulgaricus* (mg/kg) (n = 3)

Compounds	Isolates											
	ST1	ST2	ST3	ST4	ST5	LB1	LB2	LB3	LB4	LB5	LB6	LB7
Acetic acid	0.445	0.698	0.554	0.815	0.376	0.436	0.593	0.251	1.776	0.169	0.417	0.113
Butyric acid	0.219	0.312	0.128	0.481	0.358	0.215	0.301	1.510	2.905	0.059	0.148	0.311
Hexanoic acid	0.924	1.229	0.889	0.866	1.011	0.393	0.967	0.619	2.247	0.212	0.426	0.121
Octanoic acid	1.814	2.897	0.998	0.405	0.515	0.258	0.341	nd	2.634	0.152	2.059	0.182
Decanoic acid	0.889	1.577	1.028	0.231	0.161	0.105	0.192	0.242	0.712	0.068	0.078	0.128
Nonanoic acid	0.292	0.839	0.334	nd	0.635	0.358	0.776	0.708	0.065	0.574	0.374	0.061
Acetaldehyde	1.105	2.484	2.229	3.506	2.220	0.341	1.833	2.870	0.347	1.935	0.686	1.920
Diacetyl	nd	nd	0.419	0.682	0.072	0.486	1.314	0.707	0.175	0.068	0.107	nd
Acetoin	0.732	0.872	0.122	0.540	2.821	0.222	0.584	0.503	0.757	0.604	0.247	0.765
4-Octanone	0.210	0.193	0.187	0.670	0.685	0.329	1.205	0.160	nd	nd	nd	0.251
3-Pentanol	0.852	0.535	0.087	nd	0.098	0.789	0.146	0.463	0.405	0.475	0.087	nd
2-Butanone-3-methyl	0.410	0.297	0.455	0.620	0.653	0.107	0.119	nd	nd	nd	0.133	nd

nd: not determined

3.4 Volatile compounds of yogurt samples made by single strains

The major volatile compounds of yogurt samples made by single strains of *Str. thermophilus* and *Lb. bulgaricus* are presented in Table 4. Carboxylic acids and ketones showed high relative abundance in yogurt samples. Among the carboxylic acid compounds, octanoic, hexanoic and decanoic acids were the most abundant acids in both yogurt groups. Especially, octanoic acid which is responsible for sweat cheesy flavor was produced at high quantities by some strains (*i.e.*, ST1, ST2, ST3, LB4 and LB6). Yogurts made by isolates ST2 and ST3 contained higher amount of decanoic acid which is responsible for rancid flavor. Acetaldehyde is the major aroma compound of yogurt and is produced during

the fermentation process of milk at far greater quantities than any other volatile compounds [22]. With the exception of the isolates LB1, LB4 and LB6, the rest of the strains produced acetaldehyde at higher quantities (between 1.105 and 3.506 mg/kg). Major ketones in the yogurt samples were acetoin, diacetyl, 4-octanone and 2-butanone-3-methyl. Acetoin (2-butanone-3-hydroxy) contributes to the characteristic flavor of yogurt together with acetaldehyde, acetone and diacetyl, and the isolate ST5 was found to be potent acetoin producers. Diacetyl (2,3-butanedione), which is mainly responsible for the buttery flavor in foods, was more abundant in yogurts made by isolate LB2. The relatively higher abundance of carboxylic acids and ketones in single strain yogurts were reported by Dan et al. [23] who screened 17 *Lb. bulgaricus* strains isolated from local fermented cow or yak milk products for volatile compounds profiles.

3.5 Properties of yogurts made by combined cultures

It has long been known that yogurt bacteria have a weak ability to grow and produce lactic acid when used alone in yogurt than their ability to grow and develop acidity when used in combination [24]. Combined cultures also contribute to the aroma and texture of yogurt in a positive manner. Selection of strains for a culture combination is of critical importance for commercial applications. Among the selection criteria of single strains for a culture combination are fast acidification during fermentation and slow acidification during cold-storage, development of a texture and balanced flavor, resistance against bacteriophages and more importantly reproducibility. In some cases, when a single strain finds a proper partner strain, their combined action in yogurt is far better than what they do as a single strain. From this fact, in the present work, all the single isolates of *Str. thermophilus* and *Lb. bulgaricus* were combined with each other and used in the manufacture of yogurt. The combinations that were able to reduce milk pH to 4.6 in ≤ 500 min were taken into further analyses and the rest of the combinations were eliminated.

Table 5. Acidity profiles of experimental yogurts made by combined isolates (n = 3)

Combinations		Fermentation time (min)	pH		Lactic acid (%)	
<i>Str. thermophilus</i>	<i>Lb. bulgaricus</i>		Day 1	Day 14	Day 1	Day 14
ST1	LB3	500 \pm 23.0 ^a	4.53 \pm 0.015 ^{aA}	4.34 \pm 0.005 ^{aB}	0.710 \pm 0.015 ^{aA}	0.760 \pm 0.033 ^{aB}
	LB5	255 \pm 15.0 ^b	4.24 \pm 0.013 ^{bA}	3.79 \pm 0.020 ^{bB}	0.830 \pm 0.005 ^{bA}	1.220 \pm 0.004 ^{bB}
ST2	LB5	500 \pm 2.5 ^a	4.34 \pm 0.015 ^{cA}	4.19 \pm 0.030 ^{cB}	0.668 \pm 0.001 ^{cA}	0.887 \pm 0.013 ^{cB}
ST3	LB5	300 \pm 14.0 ^{eg}	4.08 \pm 0.004 ^{dA}	3.93 \pm 0.003 ^{dB}	0.960 \pm 0.010 ^{dA}	1.030 \pm 0.020 ^{dB}
ST4	LB1	307 \pm 7.5 ^c	4.31 \pm 0.020 ^{cA}	4.20 \pm 0.020 ^{ceB}	0.730 \pm 0.030 ^{aA}	0.775 \pm 0.005 ^{aB}
	LB2	322 \pm 2.5 ^{de}	4.23 \pm 0.025 ^{bA}	4.17 \pm 0.035 ^{cB}	0.820 \pm 0.050 ^{bA}	0.920 \pm 0.030 ^{cB}
	LB3	327 \pm 7.5 ^e	4.29 \pm 0.025 ^{cA}	4.22 \pm 0.010 ^{cB}	0.820 \pm 0.030 ^{bA}	0.920 \pm 0.020 ^{cB}
	LB4	312 \pm 12.5 ^{de}	4.38 \pm 0.020 ^{fA}	4.34 \pm 0.015 ^{aA}	0.770 \pm 0.030 ^{ehA}	0.920 \pm 0.010 ^{eB}
	LB5	320 \pm 15.0 ^{de}	4.25 \pm 0.065 ^{bA}	4.09 \pm 0.015 ^{fB}	0.885 \pm 0.075 ^{fA}	0.965 \pm 0.065 ^{eA}
	LB6	375 \pm 15.0 ^f	4.44 \pm 0.065 ^{gA}	4.28 \pm 0.055 ^{gB}	0.710 \pm 0.070 ^{aA}	0.820 \pm 0.030 ^{fB}
	LB7	372 \pm 12.5 ^f	4.41 \pm 0.040 ^{gA}	4.24 \pm 0.015 ^{egB}	0.815 \pm 0.045 ^{bA}	0.915 \pm 0.045 ^{eB}
ST5	LB1	355 \pm 10.0 ^f	4.35 \pm 0.010 ^{efA}	4.16 \pm 0.006 ^{fB}	0.705 \pm 0.015 ^{aA}	0.805 \pm 0.025 ^{fB}
	LB2	315 \pm 15.0 ^{de}	4.37 \pm 0.015 ^{iA}	4.03 \pm 0.010 ^{iB}	0.750 \pm 0.020 ^{eA}	0.830 \pm 0.020 ^{fB}
	LB3	287 \pm 2.5 ^{gh}	4.44 \pm 0.020 ^{gA}	4.26 \pm 0.010 ^{gB}	0.770 \pm 0.030 ^{ehA}	0.820 \pm 0.000 ^{fB}
	LB4	305 \pm 5.0 ^c	4.29 \pm 0.005 ^{cA}	4.10 \pm 0.015 ^{fB}	0.780 \pm 0.010 ^{ehA}	0.915 \pm 0.045 ^{eB}
	LB5	270 \pm 25.0 ^{gh}	4.42 \pm 0.015 ^{gA}	4.28 \pm 0.015 ^{gB}	0.795 \pm 0.015 ^{hA}	0.810 \pm 0.010 ^{fA}
	LB6	265 \pm 20.0 ^h	4.52 \pm 0.015 ^{aA}	4.29 \pm 0.055 ^{gB}	0.790 \pm 0.010 ^{hA}	0.815 \pm 0.045 ^{fA}
	LB7	275 \pm 25.0 ^{gh}	4.51 \pm 0.000 ^{aA}	4.32 \pm 0.015 ^{aB}	0.765 \pm 0.005 ^{chA}	0.765 \pm 0.005 ^{aA}

*Different lower case superscript letters in the same columns and upper case superscript letters in the same line indicate statistically different groups (p < 0.05)

Table 6. Descriptive sensory evaluation of 14-day old experimental yogurts (n = 3)

Combinations		Sensory characteristics					
<i>Str. thermophilus</i>	<i>Lb. bulgaricus</i>	Odor	Flavor	Texture	Aftertaste	Overall flavor effect	Remarks
ST1	LB3	Foreign (14) Acetaldehyde (2)	Sour (8)	Firm (10)	Yogurt (14)	Atypical (13)	Dominant sourness, bad odor development after 14 days of storage
	LB5	Foreign (8) Acetaldehyde (4)	Sour (14)	Firm (8),	Yogurt (14)	Atypical (14)	Dominant sourness, bad odor development after 14 days of storage
ST2	LB5	Cream-like (5), Acetaldehyde (4)	Sour (5), Creamy (4)	Firm (12)	Yogurt (14)	Balanced yogurt (12)	Suitable for commercial sour yogurt productions
ST3	LB5	Foreign (15) Acetaldehyde (4)	Foreign (15)	Weak body (6), Whey-off visible	Yogurt with dominant sourness (15)	Weak (14), Foreign (15)	Extremely acidic, not suitable for commercial productions
ST4	LB1	Acetaldehyde (14)	Sour (7) Yogurt-like (13)	Firm (11)	Yogurt with sourness (12)	Balanced yogurt (14)	Suitable for commercial sour yogurt productions
	LB2	Foreign (15) Acetaldehyde (3)	Sour (13), Yogurt-like (11)	Firm (10)	Yogurt with sourness (13)	Atypical (12)	Very weak commercial potential due to foreign odor
	LB3	Acetaldehyde (8)	Sour (12) Yogurt-like (8)	Firm (13)	Yogurt with sourness (14)	Weak yogurt (13)	Very weak commercial potential due to dominant sourness
	LB4	Acetaldehyde (12)	Sour (5) Yogurt-like (12) Bitter (7)	Firm (11)	Slight bitterness (12)	Balanced yogurt (12)	Slight quality loss during cold storage, weak commercial potential
	LB5	Acetaldehyde (10)	Sour (8) Yogurt-like (10) Bitter (5)	Firm (10)	Yogurt with sourness (8)	Balanced yogurt (12)	Weak commercial potential due to foreign odor
	LB6	Acetaldehyde (10)	Yogurt (10) Bitter (8)	Firm (12)	Yogurt (14)	Balanced yogurt (14)	Weak commercial potential due to bitterness
	LB7	Acetaldehyde (10)	Sour (10) Yogurt-like (12), Creamy (10)	Firm (11)	Creamy yogurt (10)	Balanced yogurt (13)	Weak commercial potential due to dominant creamy taste
ST5	LB1	Acetaldehyde (10)	Sour (4) Yogurt-like (12)	Firm (10)	Yogurt (12)	Balanced yogurt (14)	Very suitable for commercial yogurt production
	LB2	Acetaldehyde (13)	Sour (7) Yogurt-like (10) Creamy (13)	Firm (10)	Yogurt (12)	Balanced yogurt (14)	Very suitable for commercial yogurt production
	LB3	Acetaldehyde (12)	Sour (6) Yogurt-like (10) Nutty (5)	Firm (12)	Yogurt with slight sourness (14)	Balanced yogurt (15)	Very suitable for commercial yogurt production
	LB4	Acetaldehyde (12)	Sour (7) Yogurt-like (13)	Firm (11)	Yogurt with slight sourness (12)	Balanced yogurt (13)	Very suitable for commercial yogurt production
	LB5	Acetaldehyde (13)	Sour (4), Yogurt-like (13)	Firm (13)	Yogurt with slight sourness (13)	Balanced yogurt (14)	Very suitable for commercial yogurt production
	LB6	Acetaldehyde (10)	Sour (5) Yogurt-like (12)	Firm (12)	Yogurt with slight sourness (14)	Balanced yogurt (13)	Very suitable for commercial yogurt production
	LB7	Acetaldehyde (8)	Sour (8) Yogurt-like (5) Fruity (8)	Firm (11)	Yogurt with slight sourness (8)	Balanced yogurt (10)	Suitable for commercial yogurt production

*Numbers in brackets represent the average scores for each attribute (0: absent, 1: noticeable, 2.5: very light, 5: light, 7.5: light-medium; 10: medium, 12.5: medium-strong, 15: strong)

The fermentation time and variations in pH and percentage of lactic acid values of the combinations are given in Table 5. Fermentation end point was decided as 4.6 and the shortest fermentation time was noted in the combination of ST1/LB5 with 255 min. Except for the combinations of ST1/LB3 and ST2/LB5, all combinations reached to pH 4.6 within reasonable time period. Yogurt samples were stored at 4°C for 14 days and in all combinations, the pH values reduced during cold storage. In some combinations, the reduction in pH values was very sharp which is not desirable for commercial yogurt production. Overall, combinations of *Str. thermophilus* ST5 with all *Lb. bulgaricus* strains developed acidity at moderate levels and these combinations were evaluated as promising for commercial applications. The variation trend of lactic acid values of the yogurt samples were in line with the pH values.

Undoubtedly, fermentation kinetics of yogurt starter culture is the primary selection criteria of strain combinations. In industrial yogurt productions, yogurt cultures are expected to drop milk pH to *ca.* 4.6 within 4-5h, since yogurt production lines and daily shifts in yogurt factories are organized based on this figure. Therefore, any combinations created with the commercial production claim must meet this pre-requirement. Fermentation kinetics is important but not the sole criteria for starter culture selection. Yogurts produced by any combinations of yogurt bacteria should be organoleptically acceptable as well. The yogurt samples produced by using combined isolates were subjected to descriptive sensory analysis at day 14 (Table 6). A foreign odor in the combinations of ST1/LB3, ST1/LB5, ST2/LB5, ST3/LB5 and ST4/LB2 were noted by the panelists. In the rest of the samples, a characteristic acetaldehyde odor was dominant. Sourness was the dominant flavor characteristic in almost all combinations, being more pronounced in the combinations of ST1/LB5 and ST4/LB2. A dominant lemon-like sourness was noted in the combination of S19/ML4-1. In only three combinations (ST4 with LB4, LB5 and LB6) a bitterness was noted. No slimy body was reported for the samples. In one sample (ST3/LB5), a time-dependent wheying off was reported and the rest of the samples were found to be physically stable during cold storage.

4. Conclusion

In total, 5 *Str. thermophilus* and 7 *Lb. bulgaricus* strains were screened for their growth patterns and technological performances as single cultures, or for their suitability for yogurt-making as combined cultures. Some combinations showed very poor fermentation performances and these combinations were excluded from the study. Some combinations acidified the milk and reached the target pH (4.6 pH) within reasonable time period but these combinations were found to be unsuitable for yogurt production by the sensory test panel. Overall, the panel group stressed that combinations of ST5 with all *Lactobacillus bulgaricus* isolates may have potentials to commercialize. These combinations were also found to be suitable for yogurt-making based on their acidification performances. Future studies will focus on in-depth characterization of yogurts made by these selected combinations.

Author contribution

Dr. Irem Uzunsoy carried out growth pattern analysis, and microbiological studies. Dr. Sebnem Ozturkoglu Budak run volatile compounds analysis. Dr. Tuba Sanli coordinated sensory analysis. Dr. Birce Taban undertook microbiological studies. Prof. Dr. Aykut Aytaç, Prof. Dr. Nuray Yazihan and Prof. Dr. Barbaros Ozer were responsible for the conceptualization, management of the project, running microbiological and textural analysis, statistical evaluations and writing the paper.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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