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Evaluation of lactic acid bacterial strains of boza for their exopolysaccharide and enzyme production as a potential adjunct culture

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ABSTRACT

Boza is a non-alcoholic beverage obtained from fermented cereals. Thirteen lactic acid bacteria (LAB), previously isolated from boza were identified and evaluated to determine the various technological properties for selecting appropriate strains as adjunct culture in boza. Each isolate was checked for purity, Gram-stained and tested for the catalase and oxidase activity and then subjected to identification by polymerase chain reaction (PCR) with partial 16S rRNA gene sequencing. The tests for carbohydrate fermentation and enzyme profiles were carried out with the API 50 CHL and API ZYM galleries, respectively. Exopolysaccharide (EPS) production of strains was determined by Fourier transform infrared spectroscopy (FT-IR) and quantified by the phenol sulphuric acid method. To our best knowledge, this is the first study reporting on *Lactococcus garvieae* (E32), *Pediococcus parvulus* (E42) and *Streptococcus macedonicus* (A15) in boza. All strains, except *S. macedonicus* (A15) produced EPS. *Leuconostoc citreum* (E55) and *Lactococcus lactis* (A47) were the highest EPS producing strains, yielding 2.39 ± 0.49 and 1.98 ± 0.23 g/L of EPS, respectively. *Lactobacillus paracasei* (D41), *Lactobacillus plantarum* (B2), *Lactococcus lactis* (F39) and among low-EPS producing strains *Lactobacillus coryniformis* (C55), *L. paracasei* (E8), and *P. parvulus* (E42) were evaluated to be promising candidates as potential adjunct culture in boza. The variety of enzyme production was also concern. *Lc. garvieae* (E32) was found to produce the largest variety of enzymes among the strains. FT-IR spectroscopy can be used for the assessment of EPS production by microorganisms reliably and accurately.

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1. Introduction

Boza is a cereal-based fermented beverage that can be produced by boiling coarsely ground cereals such as millet, rice, wheat, maize and cracked wheat in water. The ratio of cereal and water is approximately 1–5. The boiling process is completed until the raw material becomes thick soup-like consistency. The slurry is then cooled, distributed to the containers, and finally subjected to fermentation process. The fermentation process takes one to two days at 20–22 °C. Boza is consumed without any treatment by all age-groups including children and elderly particulary in autumn and winter. Cinnamon powders are sprinkled over boza for better flavor.

Several members of the lactic acid bacteria (LAB) participate in boza fermentation itself as actively growing bacteria including *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Enterococcus* and *Weissella* [1–4]. Since these microorganisms have complex nutritional requirements [5], cereals and cereal-based products possess the nutrients necessary for LAB requirements, and they are accepted as suitable environments to maintain the growth and vitality of the bacteria [6].

Exopolysaccharides (EPS) are polymers with a high molecular weight that are produced in situ by microorganisms in fermented milk products and are evaluated as an alternative food additives [7,8]. EPS, synthesized by certain lactic acid bacteria, *Streptococcus*, *Lactobacillus*, *Lactococcus* and *Leuconostoc* are GRAS status [7]. They are soluble in water and form a gel as the viscosity of food increases [8].

There are limited products produced by fermented cereals for human consumption. Boza is one of the well-known fermented cereal-based beverage in Turkey, Balkan countries and South Russia [9]. In boza fermentation, the use of starter cultures is not common. The manufacturers use a back slopping technique with indigenous strains. A portion of a prior fermentation is used as an inoculum for the following batch. However, boza spoils quickly, and it is

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Table 1
Physico-chemical properties of the boza samples.

Physico-chemical properties	Boza samples		
	1[a]	2[b]	3[a]
Dry matter (%)	22.74 ± 3.22	14.39 ± 1.31	24.07 ± 2.45
Total sugar (%)	22.62 ± 2.11	6.63 ± 1.00	16.00 ± 1.98
Protein (%)	0.46 ± 0.01	0.65 ± 0.07	0.56 ± 0.05
Ash (%)	0.44 ± 0.05	0.17 ± 0.01	0.24 ± 0.03
Total acidity (%)	0.25 ± 0.03	0.30 ± 0.05	0.27 ± 0.02
pH	3.95 ± 0.45	3.68 ± 0.25	3.47 ± 0.58
Water activity	0.94 ± 0.04	0.92 ± 0.08	0.91 ± 0.13

[a] Sample 1 and 3 produced by wheat and maize.
[b] Sample 2 produced by millet.

very susceptible temperature differences between day and night during transporting. Because of short shelf-life (only 2–3 weeks) and unsuitable for refrigeration, boza may contain pathogenic bacteria, produce undesired fermentation products such as alcohol Thus the quality and sensory characteristics of boza is not stable and sometimes producing abdominal discomfort and flatulence.

The textural characteristics of boza are very important for the quality of final product. If necessary, hot water after cooking process can be added to modify boza viscosity. Boza is a highly viscous drink, but gel formation is not desired. Thus, the strain used for starter culture in boza should not be an EPS producer. Microbial enzyme activities during fermentation process are largely responsible for the formation of flavor compounds, because cereals are cooked for boza production. The aim of this study was to determine the lactic acid bacteria in boza for selecting appropriate indigenous strains as adjunct/starter cultures. EPS production of selected strains was studied to establish one of selection criteria. Therefore, the enzymatic properties of selected strains were also determined.

2. Materials and methods

2.1. Materials

Thirteen strains of lactic acid bacteria (LAB) previously isolated from boza obtained from three major boza manufacturers in Istanbul were used in this study. The physico-chemical characteristics of boza samples were shown in Table 1. Strains were identified by PCR using partial 16S rRNA gene sequencing as explained below. *Lactobacillus* (*L.*) *paracasei* (D41, E8), *Lactobacillus plantarum* (B2), *Lactococcus* (*Lc.*) *lactis* (A47, F39), *Leuconostoc* (*Ln.*) *citreum* (E55, A31, B56) and *Streptococcus macedonicus* (A15) (recent proposed name by Schlegel et al. [10] *S. gallolyticus* subsp. *macedonicus*) were isolated from boza made of wheat and maize. Whereas, *L. coryniformis* (C55), *Lc. garvieae* (E32), *Pediococcus* (*P.*) *parvulus* (E42) and *Weissella* (*W.*) *confusa* (C19) were isolated from boza made of millet only. The culture media and the temperatures used for isolation of each strain and the raw materials used for boza production were shown in Table 2.

2.2. The physico-chemical characteristics of boza samples

The physico-chemical analyses conducted for the samples are as follows. Dry matter was determined with a forced draft oven. Protein, ash, pH was analyzed with a Kjeldhal 1030 protein apparatus, combustion at 550 °C, and a Jenway 3010 type pH meter, respectively. Total sugar contents along with Turkish Standard [11], water activity (a_w) was determined by a Decagon PawKit (AquaLab, Washington, USA). Also, total titritable acidity was analyzed and expressed as % lactic acid by the method from Thyyagaraja et al. [12]. All analyses were carried out with duplicate samples.

2.3. Isolation and enumeration of LAB

Culture-dependent methods were used for the determination of lactic biodiversity in boza. Lactic acid bacteria were isolated and enumerated from boza by using three different culture media namely de Man, Rogosa and Sharpe (MRS) agar (Merck, Darmstadt, Germany), Rogosa agar (Merck, Darmstadt, Germany) and M17 agar (Merck, Darmstadt, Germany) and two different incubation temperatures such as at 30 °C and 37 °C for 48 h. 25 g boza sample was added to 225 ml sterile phosphate buffer solution in aseptic conditions and suitable dilutions were made. 0.1 ml of the sample was inoculated onto three different culture media.

2.4. Identification of lactic acid bacteria by PCR

Cultures were subcultured, to check purity. Also, cultures were Gram-stained and tested for catalase and oxidase activity, and then all sample identification were confirmed by PCR. The reactions of carbohydrate fermentation were determined by API50CHL with Apilab Plus software (BioMerieux, Istanbul, Turkey). In this study, partial 16S rRNA gene sequencing was used with the universal primers: forward 5′ CCG TCA ATT CCT TTG AGT TT 3′ and reverse 3′ AGA GTT TGA TCC TGG CTC AG 5′ [13]. The resulting PCR product was sequenced; and sequence similarity search was carried out with BLAST (Basic Local Alignment Search Tool). Comparisons of reference strain and isolates sequences were performed using Clustal W alignment algorithm. The dendogram constructed (Neighbor-joining) from the partial 16S sequences of isolates and matching sequences from the reference strains found in databases (NCBI gene bank) is presented in Fig. 1. Their accession numbers are as follows: *L. coryniformis* (NR029018.1), *L. paracasei* (JQ680426), *L. plantarum* (KC454277.1), *Lc. garvieae* (AB598960), *Lc. lactis* (HE805077.1), *Ln. citreum* (DQ489736.1), *P. parvulus* (NR029136), *S. macedonicus* (NR074404.1) and *W. confusa*

Table 2
The raw materials used in boza production, the media and the temperatures used for the isolation of each strain.

LAB species	Raw materials	Isolation medium	Temperature (°C)
Lactobacillus coryniformis (C55)	Millet	Rogosa[a]	30
Lactobacillus paracasei (D41, E8)	Millet; maize and wheat	Rogosa	37
Lactobacillus plantarum (B2)	Millet; maize and wheat	MRS[b]	37
Lactococcus garvieae (E32)	Millet	MRS	37
Lactococcus lactis (A47, F39)	Millet; maize and wheat	M17[c]	37
Leuconostoc citreum (E55, A31, B56)	Millet; maize and wheat	MRS	37
Pediococcus parvulus (E42)	Millet	MRS	30
Streptococcus macedonicus (A15)	Millet; maize and wheat	MRS	37
Weissella confusa (C19)	Millet	Rogosa	37

[a] Rogosa agar (Merck).
[b] de Man, Rogosa and Sharp (MRS) agar (Merck).
[c] M17 agar (Merck).

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