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Evaluation of the Antimicrobial Efficacy of some Fermented Traditional Turkish Beverages with Probiotic Potentials

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<ABSTRACT>

Turkey is a home country for a good number of fermented beverages derived from milk, cereals, fruits and vegetables, and several studies have reported the probiotic potentiality of these beverages. Probiotics, otherwise known as beneficial microorganisms possess the ability to exert antimicrobial effects, which is one of the most important selection criteria for their use in commercial products. In the current study, the antimicrobial activities of potential probiotic bacteria isolated from five fermented traditional Turkish beverages (boza, kefir, ayran, shalgam juice and hardaliye) were evaluated. The bacterial isolates were morphologically characterized and genotypically identified by 16S rRNA gene sequence analysis. The antimicrobial effects of the isolates against selected human pathogens were assessed using spot-on-the-lawn and agar well diffusion assays. Eighteen of the twenty-two strains displayed varying degrees of antagonism against the tested pathogens. Amongst the isolates, the strongest antimicrobial effects were exhibited by strains from boza, kefir and shalgam which can be attributed to their greater microbiota

diversity. Strain specificity in the activities of the obtained isolates and specificity with the different indicator pathogens tested was observed. The impressive antimicrobial effects exhibited by boza, kefir and shalgam isolates offer a promising health benefit to consumers of these fermented probiotic products.

Keywords: Probiotics, Lactic acid bacteria, Antimicrobial activity, Fermented foods and beverages.

1. Introduction

Fermented foods are defined as foods and beverages produced via controlled microbial growth, and by the conversion of food components through enzymatic actions (1). Fermentation enhances the preservation of foods as well as enables the transformation of raw materials into a new product with unique sensory properties (taste, aroma, texture etc.), improved nutritional values and functional properties (2, 3, 4). Foods and beverages that are prepared via fermentation processes constitute an important part of human nutrition in virtually every food culture around the globe (3). Fermented/pickled fruits and vegetables, fruit juices, tea leaves, cereals, roots and tubers are very popular and widely consumed in many regions of Europe, Asia, the Americas, Africa and Middle East (5). Generally, several genera of lactic acid bacteria (LAB) including *Lactobacillus, Streptococcus,* and *Leuconostoc* are predominant in fermented foods, but other bacteria as well as yeast and fungi also contribute to food fermentations (6). There are two main methods by which foods are fermented. First, foods can be fermented naturally, also known as "spontaneous ferments" or "wild ferments", a process whereby microorganisms are naturally present in the raw food ingredient or processing environment, e.g. sauerkraut, kimchi and certain fermented soy products (7). On the other hand, foods can also be fermented by the addition of starter cultures, often referred to as "culture-dependent ferments", e.g. kefir, kombucha (6). One method of preparing a culture-dependent ferment is "back-slopping", a process in which a little amount of a previously fermented batch is inoculated into the raw food, e.g. sourdough bread (1).

With a surging interest in gastrointestinal health in recent years, the consumption of fermented foods containing live microorganisms has gained popularity as an important dietary strategy for improving human health (1, 7). This popularity may not be unconnected with the rising consumer awareness about the concept of "probiotics". Probiotics (from Greek: meaning "for life") are "Live microorganisms which, when administered in adequate amounts, confer a health benefit on the host." (8). Human probiotic microorganisms mostly belong to the *Lactobacillus, Bifidobacterium, Streptococcus, Lactococus,* and *Enterococcus* genera (9). Furthermore, some strains of Gram-positive bacteria in the genus *Bacillus* as well as some yeast strains in the genus *Saccharomyces* are also commonly used in probiotic

products (10). The FAO/WHO (11), the European Food Safety Authority (EFSA) (12) and the FDA (Food and Drug Administration) have stipulated different criteria such as safety, functionality, technological usefulness and the absence of the risk of acquired resistance to antibiotics for probiotic strain selection that would exert beneficial effects on human health and could be used in the probiotics industry (9, 10, 14). Generally, one of the most important properties among the selection criteria is that a probiotic strain must produce antimicrobial substances and be antagonistic to pathogens. Antimicrobial activity of probiotics may be manifested by one or a combination of the following mechanisms including: (i) competition for limited nutrients, (ii) competition for adhesion sites, (iii) synthesis of various antimicrobial metabolites such as organic acids, H_2O_2, bacteriocins, etc. and (iv) inhibition of toxin production in pathogenic microorganisms (15, 16). For instance, lactic acid bacteria (LAB) produce lactic acid as well as other organic acids; thus, lower the environmental pH and consequently inhibit the growth of bacterial pathogens (17, 18).

Although, commercially-produced fermented foods usually serve as carriers for probiotic bacteria (6), that a particular food or beverage is produced by fermentation does not necessarily imply that it contains live microorganisms. For example, bread, wine, beer, and distilled alcoholic beverages are produced by yeast fermentation, but the producing organisms are either inactivated by heat (in the case of bread and some beers) or are physically removed by filtration or other means (in the case of wine and beer). Moreover, many fermented foods are heat-treated after fermentation to extend shelf-life or enhance food safety. Hence, soy sauce, sauerkraut and other fermented vegetables are made shelf-stable by thermal processing. Even non-thermally processed fermented foods may notwithstanding contain low levels of live and viable microorganisms simply due to unfavorable environmental conditions which decrease microbial populations over time (6). Furthermore, it is also pertinent to note that the absence of live microorganisms or probiotics in the final fermented product does not preclude a positive functional role. Several studies have reported some mechanisms through which fermented foods may exert beneficial effects on health and disease whether they contain live microorganisms or not. For instance, food-fermenting microbes may produce vitamins or other bioactive molecules in situ or inactivate anti-nutritional factors and yet be absent at the time of consumption (6). Prebiotics and other components found in fermented foods may also exert health benefits (1, 19). In addition, fermentation-derived metabolites may exert health benefits such as in the case of LAB which generate polyamines and bioactive peptides in both dairy and non-dairy fermented foods with potential effects on immune, metabolic and cardiovascular health (20).

There are sufficient historical and scientific records that have shown Turkey to be an origin country for a decent number of fermented probiotic beverages (21). Moreover, these

several fermented traditional foods are produced in many regions of Turkey from products that are indigenous to particular regions. Kefir, ayran, boza, hardaliye and shalgam juice seem to be the most widely known fermented traditional Turkish non-alcoholic beverages. The first two products are produced from milk while the last three are obtained from cereals, fruits and vegetables respectively, and their microbiota is composed mainly of LAB (22).

Kefir is a smooth, slightly foamy, whitish, viscous, slightly acidic, slightly carbonated mildly alcoholic fermented beverage that is widely consumed in Turkey (23, 24). The name kefir is derived from the Turkish word "Keyif" meaning "Joy/Pleasure/Good feeling" to express the feelings experienced after drinking it (21, 23, 24, 25, 26). Kefir can be produced by fermentation of different kinds of milk including cow, ewe, goat or plant-derived milk (26). It is traditionally produced by adding a starter culture known as "Kefir grains" to pasteurized milk. Kefir grains is a symbiotic consortium of lactic and acetic acids-producing bacteria as well as lactose-fermenting yeasts (e.g. *Kluyveromyces marxianus*) and non-lactose fermenting yeasts (e.g. *Saccharomyces cerevisiae, S. unisporus*) housed within a polysaccharide and protein matrix called kefiran (25, 27). Kefir has been reported to be tolerated well by people with lactose intolerance/maldigestion as it contains β-galactosidase enzyme expressing organism (e.g. *K. marxianus*) which hydrolyses lactose, and thus reduces lactose concentrations in the beverage (7).

Ayran is an indigenous Turkish fermented milk beverage which is more or less a "national drink" widely consumed across the length and breadth of Turkey (21). It is produced in either of two ways, i.e. by the addition of water to yoghurt (homemade) or by the addition of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp*. bulgaricus* as starter cultures to standardized milk for fermentation (industrial production) (28). Basically, ayran is generated by blending yoghurt with 30–50 % of water and 0.5–1 % of salt (29).

Boza is a cereal-based fermented beverage produced by a combination of yeast and lactic acid fermentation of millet, maize, wheat, rye, rice semolina or flour and mixed with sugar or saccharine (30). It is a viscous liquid with a pale yellow colour and sweet slightly sharp to slightly sour taste which is widely consumed in Turkey due to its pleasant taste, flavour and nutritional properties (31). It is also popular and widely consumed in several Balkan countries on a daily basis (26, 32). In Turkey, boza is usually served alongside cinnamon powder and roasted chickpeas, and is generally considered to be beer's ancestor (30). Boza is produced at both an artisanal and industrial scale (33). Its preparation typically involves six stages viz. milling of raw materials (cereals), boiling, straining, cooling, sugar addition, and fermentation. A previously fermented boza is used as inoculum for the fermentation stage (21, 30, 31). Lactic acid fermentation by LAB produces antimicrobial metabolites such as lactic acid and increases acidity which supplies preservative effect

whereas the metabolites from alcoholic fermentation by yeasts bring about the mouthfeel and odor of boza (26, 30, 31). Boza is a good source of fibre, carbohydrate, protein and vitamins including riboflavin, thiamine, niacin and pyridoxine (26, 30).

Hardaliye is a kind of fruit-based fermented non-alcoholic traditional beverage which originates from Thrace, the European part of Turkey, and is produced from red grape juice and 0.2% crushed black mustard seeds (34, 35). Hardaliye is mostly homemade by the traditional method. The ingredients are pressed and allowed to ferment at room temperature for 5–10 days in wooden or plastic barrels. (22, 26, 33). Benzoic acid is sometimes added as a preservative especially at the industrial scale (26). Owing to its rich LAB flora, hardaliye has been described as a non-dairy probiotic beverage which aids digestion and also helps in the prevention of coronary heart disease (36). The nutritional value, functional properties and health benefits of hardaliye are derived from its ingredients and fermentation process. For example, grapes are rich in phenolic contents and provide strong antioxidant effects for the human body and thus, inhibit cancer cells formation, whereas the oils from mustard seeds exert medicinal effects on circulatory disorders, common cold and bronchitis as well as possess antimicrobial properties (34).

Shalgam juice (Şalgam) is a vegetable-based red colored, cloudy and sour beverage produced by LAB and yeast fermentation of a mixture of black or purple carrots (*Daucus carota*), turnips (*Brassica rapa*), bulgur (broken wheat) flour, salt, sourdough and water (37). It is widely consumed in many cities across the Mediterranean region of Turkey but has also in recent years become popular in metropolises such as Istanbul, Ankara and Izmir (38). Production comprises two stages with the first one termed "1st fermentation" which involves mixing bulgur flour (3%), salt (0.2%) and sourdough (0.2%) together with water and allowing it to ferment at room temperature for 3–5 days. In the second stage, cleaned and chopped black carrots (10–20%), sliced turnips (1–2%), salt (1–2%) and water are then added to the extract obtained from stage one and left to undergo a "2nd fermentation" for 3–10 days in a wooden barrel (37). The juice is then filtered and packaged in suitable containers, and chilli powder may also be added depending on consumer preference (26). Shalgam is typically made on a home-scale and consumed within 3 months from production time, although it is also produced commercially with an extended shelf life of 1-2 years using preservatives (26). Shalgam juice is a highly nutritional beverage due to its high mineral, amino acid, polyphenol and vitamin contents (37, 39).

There are quite a few studies and review papers that have reported the microbiological, chemical, nutritional and some probiotic properties of fermented traditional Turkish foods and beverages (22, 26). However, no study reporting the antimicrobial efficacies of the potential probiotic microbiota of these five major beverages (boza, kefir,

hardaliye, ayran and shalgam) was encountered in the literature. The current study, therefore, aimed to consolidate past works on the probiotic properties of the selected beverages specifically by investigating the antimicrobial activities of their microbiota against selected standard pathogens, thereby helping to validate their widely acclaimed beneficial effects on human health. Ultimately, the present study aims to contribute to public knowledge of fermented functional foods and probiotics, increase awareness of the Turkish populace about the health benefits derivable from their own indigenous fermented dairy and non-dairy beverages. It is hoped that in the long run this knowledge and awareness will result in increased consumption of these functional food products and, hence, result in an improved general public health.

2. Materials and Methods

2.1.   Isolation, Identification and Characterization of Target Microorganisms from Beverage Samples

Sampling was done by purchasing commercially produced and prepackaged fermented Turkish beverages from retail locations. Three different brands from each of the five beverages (boza, kefir, hardaliye, ayran and shalgam) were analyzed.

Tenfold serial dilutions of the beverage samples were prepared up to 10^{-6} with sterile 0.5 % peptone water accordingly. To isolate the target microorganisms 100 μL aliquot was taken from each dilution and spread-plated on four selective culture media namely de Man Rogosa and Sharpe (MRS) agar (Sigma-Aldrich®), *Bifidobacterium* Selective Medium (BSM), M17 Agar and Sabouraud Dextrose Agar (SDA- with the addition of 0.05 g/1 chloramphenicol). MRS and BSM plates were incubated for 72 hours at 37 °C in anaerobic jars (Oxoid, UK) containing Gaspack (AnaeroGen, Oxoid, UK) (oxygen level <1 %, CO_2 level 9-13 %). M17 Agar and SDA plates were incubated aerobically at 28-30 °C for 72 hours. After the incubation periods, colonies were randomly picked from the plates and sub-cultured on fresh plates of the same media. Morphologically characterized pure isolates were stored in the appropriate broths (MRS, BSM, M17 and YPEG broth media) containing 20 % glycerol at -20 and -80 °C until antimicrobial assays. For all subsequent assays, isolates were propagated twice and activated in the corresponding media at the appropriate incubation conditions.

Preliminary phenotypic and morphological characterization of the isolates was performed with catalase test and Gram-staining reaction to select the target groups of bacteria. Gram-stained smears were examined microscopically. Subsequently, molecular identification by 16S rRNA gene sequencing was performed on phenotypically characterized isolates. Bacterial genomic DNA was extracted from overnight cultures, using a Hibrigen

Bacterial Genomic DNA Isolation Kit according to the manufacturer's guidelines. PCR amplifications of the genomic DNA fragments of approximately 1500 bp of the 16S rRNA gene were performed with a Hibrigen 2X PCR Master Mix as described by (40), using the universal primers 16S rRNA (forward: 5'-TGGAGAGTTTGATCCTGGCTCAG-3'; reverse: 5'-TACCGCGGCTGCTGGCAC-3').

PCR amplification of the 16S rRNA genes and subsequent purification of the amplicons were performed as previously described by (40). The PCR Amplification reactions contained 50 ng of DNA, 12.5 µL 2X Master mix, 0.3 mM of each primer in a final volume of 25 µL. Amplification reactions were performed as follows: an initial denaturation step at 94 °C for 2 minute which was followed by 35 cycles at 94 °C for 1 minute, then at 55 °C for 1 minute, 72 °C for 1.5 minute, and one last cycle at 72 °C for 7 minute for the final elongation step. The PCR products were then analyzed by electrophoresis in 1% agarose gels in Tris-Borate-EDTA (TBE) buffer with ethidium bromide and visualized by UV light. The PCR products were sequenced in an Applied Biosystems 3130 Genetic Analyzer. The results of DNA sequencing runs were assembled using the Chromas software. In order to identify the strains, the sequences of each lactobacilli strain were compared with those available in the databases of the National Center for Biotechnology Information (NCBI) using the BLAST search program (www.ncbi.nlm.nih.gov/BLAST/).

2.2. Evaluation of the Antimicrobial Activities of the Isolates

The antimicrobial activities of the obtained isolates were evaluated against selected common human microbial pathogens using spot-on-the-lawn and agar well diffusion methods. All strains used were sourced from the American Type Culture Collection (ATCC), and all pathogens were activated for 24 hours on Trypticase Soy Agar (TSA; Oxoid) prior to every assay. The standard indicator pathogenic strains used in this study are as follows: *Acinetobacter baumanii ATCC 19606, Bacillus cereus* ATCC 14579*, Escherichia coli* ATCC 25922*, Klebsiella pneumoniae* ATCC 4352*,* Methicillin-Resistant *Staphylococcus aureus* (MRSA) ATCC 33591, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella enterica* serovar Typhimurium ATCC 14028*, Staphylococcus aureus* ATCC 6538*, Staphylococcus epidermidis* ATCC 1228, Vancomycin-Resistant *Enterococcus faecalis* (VRE) ATCC 51299*,* and *Candida albicans* ATCC 10231.

2.2.1. Spot-on-the-Lawn Technique

The antimicrobial activity of the isolates was evaluated by the spot-on-the-lawn assay described by (41) with slight modification. A 2 µL inoculum taken from overnight broth culture was spotted in triplicates on the surfaces of their corresponding isolation media, i.e. MRS, BSM, M17 and SDA plates. The spots were allowed to dry for 1 hour at room temperature after which the plates were incubated appropriately. Incubation of M17 and

SDA plates was made at 30 °C, MRS and BSM at 37 °C in anaerobic jars (Oxoid, UK) containing Gaspack (AnaeroGen, Oxoid, UK) for 24 hours. After colony development, the spots were overlaid with 10 ml of soft (0.75 %) Trypticase Soy Agar (TSA) seeded with 1 % (v/v) of an active overnight culture of the target pathogenic strain which had been adjusted to McFarland 0.5 turbidity standard (1-1.5 x 10^8 CFU/mL). The overlaid plates were then incubated under appropriate conditions for 24 to 48 hours. After 24 to 48 hours of incubation, measurements of inhibition zones around the isolate colonies were taken from the outer edge of those colonies to the outer edge of the clear zones. Inhibition zones of > 20 mm, 10 - 20 mm, and < 10 mm were interpreted as strong, intermediate, and weak inhibitions respectively.

2.2.2. Agar Well Diffusion Assay

In order to obtain the cell-free supernatants (CFS) needed for the agar well diffusion assay, 24-hour activated isolates were grown in MRS, BSM (anaerobically at 37 °C), M17 and YPEG (aerobically at 30 °C) broths respectively for 24 hours. The cells were harvested by centrifugation of cultures (8000 rpm/min at 4 °C for 15 minutes). The obtained supernatants were sterilized by filtration (0.22 μm pore size, Millipore, Bedford, MA).

The antimicrobial efficacy of the potential probiotic isolates was then further examined using the agar well diffusion technique described by (42). Briefly, 20 ml of Mueller Hinton Agar (MHA) medium cooled to 45 °C was vigorously mixed with 200 μL of an overnight culture of each indicator pathogen (adjusted to 1– 1.5 x 10^8 CFU/ml by McFarland 0.5 Standard), poured into Petri dishes and allowed to solidify. Then 50 μl aliquots of the prepared supernatant (CFS) was placed in a 6 mm well excavated in the agar. Plates were maintained at 4 °C for 1 hour prior to incubation in order to allow for diffusion of the supernatant into the agar. Inhibition of the indicator pathogens was evaluated after incubation at 37 °C for 24 hours. The antimicrobial activity was expressed as the diameter of inhibition zones around the wells. Inhibition zones ≥ 10 mm were regarded as positive.

3. Results

A total of twenty-two bacterial strains comprising lactic and acetic acid bacteria were isolated from four (boza, kefir, shalgam and ayran) of the five fermented traditional Turkish beverages analyzed. No microbial strain was isolated from hardaliye. Boza and kefir contained the largest microbiota loads with eight isolates each followed by shalgam having five, which is due to their various raw materials and fermentation processes. The names of the 22 isolates as identified by molecular sequence analysis of their 16S rRNA and their distribution in the studied fermented traditional Turkish beverages are presented in Table 1.

In the spot-on-lawn assay, the bacterial strains isolated from boza, kefir and shalgam exhibited impressive antimicrobial activity against nearly all tested indicator pathogens at varying degrees. Moreover, the results presented in Table 2 clearly show that the obtained probiotic isolates displayed tremendous antagonistic activity against both Gram-positive (*B. cereus,* MRSA*, S. aureus, S. epidermidis,* VR *E. faecalis*) and Gram-negative bacteria (*A. baumanii, E. coli, K. pneumoniae, P. aeruginosa, S. enterica* serovar Typhimurium). *Lactobacillus nagelii* (B24), *Lactobacillus parabuchneri* (B25) and *Acetobacter peroxydans* (B27) isolates obtained from boza displayed the strongest antagonistic effects with ≥ 20 mm diameter inhibition zones against all tested bacterial pathogens. However, they exhibited no antifungal activity against *C*. *albicans*. MRSA was highly sensitive to *Lb nagelii* (B24) and *Lb parabuchneri* (B25) isolates from boza as well as to *Lb fermentum* (K13) and *Gluconobacter frateurii* (K26) isolates from kefir with 26.00, 22.10, 26.40 and 25.90 mm inhibition zones, respectively, but minimally sensitive to *Lactococcus lactis* (S2) obtained from shalgam (12.00 mm zone). *L. lactis* strains B7 and B10 isolated from boza showed medium antimicrobial activity against the bacterial pathogens and *Candida albicans* with ≥ 10 ≤ 20 mm diameter inhibition zones*. L. lactis* (B7) strain exhibited the strongest inhibitory effect (28.60 mm zone) while *L. lactis* (B10) and *Lb. fermentum* (K13) strains displayed medium antagonism producing 16.80 and 11.90 mm inhibition zones respectively against *C. albicans. Gluconobacter frateurii* (K26) and *Lb. fermentum* (K13) strains isolated from kefir also showed a very strong antibacterial activity creating 20.80 – 30.10 mm diameter inhibition zones against five bacterial pathogens comprising both Gram-positive (VRE, MRSA) and Gram-negative (*P. aeruginosa, K. pneumoniae* & *E. coli*). In contrast, another *Lb. fermentum* strain K12 also obtained from kefir displayed a weak antagonistic effect (< 10 mm zones) against the same pathogens. The strain *L. lactis* (S2) isolated from shalgam exhibited a medium inhibitory effect (9.60 – 15.70 mm zones) on all indicator bacterial pathogens except *A. baumanii*.

On the other hand, in the agar well diffusion assay, the cell-free supernatants (CFS) of ten out of twenty-two isolates exhibited medium antimicrobial effects (≥ 10 ≤ 20 mm zones) against bacterial and *Candida albicans* pathogens (Table 3). However, *Pseudomonas aeruginosa* was strongly inhibited by *Lb. fermentum* (B4), *Lb. pentosus* (S5) and *Lb. plantarum* (S8) strains with 25.10, 23.40, and 19.10 mm diameter zones respectively. The CFS of *Lb. fermentum* (B6) with a 25. 40 mm zone of inhibition displayed the strongest antagonistic activity against the yeast pathogen *C. albicans*. Furthermore, *L. lactis* strains (K18 and A22), *Lb. fermentum* strains (B4 and K13), *Lactobacillus plantarum* strains (K14 and K15) had medium inhibitory effects with 10.20 – 15.80 mm zones on the pathogenic yeast *C. albicans.* The weakest antibacterial effects were displayed by the supernatants of *L.*

lactis (K18) and *Lb. plantarum* (S8) both with an 8.30 mm inhibition zone against *P*. *aeruginosa* and *K*. *pneumoniae* indicator strains respectively.

4. Discussion

Among the known mechanisms of action by which probiotics exert their beneficial effects on human health is antagonism against microbial pathogens via the production of antimicrobial metabolites. In the present study, the antimicrobial efficacy of some potential probiotic bacteria isolated from fermented traditional Turkish beverages against 11 common human pathogens has been evaluated. Thus, with respect to antimicrobial effects, the potentials of boza, kefir and shalgam to bestow some probiotic health benefits to the consumers have been validated through the present study. Other probiotic properties of Turkish boza and kefir were reported in a previous study by (43). Kefir was also reported in a previous study to exhibit antimicrobial activity which is attributable to the lactic acid, acetic acid, bacteriocins, hydrogen peroxide, acetaldehyde, volatile acids, diacetyl, and/or carbon dioxide produced by the bifidobacteria and LAB strains present in the drink (44). Although ayran is the most widely consumed amongst the fermented Turkish beverages examined, its inherent LAB strains exhibited no significant antimicrobial effects. No lactic acid bacterial or other potential probiotic strain was isolated from commercially available hardaliye in this study. This reason is thought to be due to the presence of the preservative benzoic acid which is usually added at the start of the fermentation process. It has, therefore, been determined that commercial hardaliye lacks probiotic properties considering the fact that probiotics must be found alive and in sufficient quantities in final products. In order to retain the potentially probiotic natural microflora of commercial hardaliye, production without the use of benzoic acid or other chemical preservatives is recommended. In the same vein, no live LAB strain was found in some brands of commercial shalgam that were examined. Similar to hardaliye, the reason is also thought to be due to the presence of preservatives and/or additives such as sodium benzoate which is declared in the ingredients list on label.

Pathogenic bacterial multidrug resistance as well as biofilm formation have led to the ineffectiveness of the antibiotics available in the treatment of infections whereas the application of probiotics has been considered functional in preventing and/or counteracting biofilm-related infections (45). Antagonism by antimicrobial metabolites has been considered as an important property in the selection of potential probiotics for the maintenance of a healthy microbial balance in the gut. LAB, mostly the lactobacilli due to their capacity to alienate bacterial pathogens via the production of some antimicrobials such as organic acids (mainly lactic acid), bacteriocins, H_2O_2 etc., acquire this enviable property for probiotic potentiality and thus are a maintainable substitute to the synthetic antibiotics (46). Lactic

acid typically diffuses into pathogenic bacterial cell which disrupts the cell membrane integrity thereby causing damage to it as well as retarding their metabolic processes and preventing growth (47). Within the scope of this study, the antimicrobial activities of LAB and acetic acid bacteria strains isolated from fermented Turkish beverages were assessed by two methods namely spot-on-the-lawn and agar well diffusion methods. Through this study, it has been possible to evaluate the potential beneficial effects of fermented traditional Turkish beverages on human health as regards antimicrobial efficacy. Eighteen strains namely *Lb. fermentum* (B4), *Lactococcus lactis* (B7), *Lb. fermentum* (B6), *L. lactis* (B10), *Lb. fermentum* (K13), *Lb. fermentum* (K12)*, Lb. nagelii* (B24), *Lb. parabuchneri* (B25), *L. lactis* (S2), *L. lactis* (K18)*, L. lactis* (A22), *Lb. plantarum* (K14), *Lb. plantarum* (K15), *Lb. fermentum* (B3), *Lb. pentosus* (S5)*, Lb. plantarum* (S8), *Gluconobacter frateurii (*K26) and *Acetobacter peroxydans* (B27) exhibited varying degrees of antimicrobial activities against the tested pathogens. Furthermore, differences in the antibacterial effect of different strains of the same species of isolates were observed. This result has confirmed that the antibacterial activity and health benefits imparted by probiotic bacteria are strain specific rather than being species- or genus-specific. It is therefore crucial to note that no single strain will provide all the proposed benefits, not even strains of the same species, and also not all strains of the same species will be effective against defined health conditions (48, 49). This specificity has also been confirmed by previous studies in terms of the microorganism strain, the metabolites it produces, and even the susceptibility pattern of the test organism to antimicrobials (50). It therefore goes without saying that in order to obtain maximum health benefits, the addition of a mixture of various strains of probiotic organisms to diets is imperative.

Many bacteriocins isolated from LAB are usually active against Gram-positive bacteria while Gram-negative bacteria generally exhibit little sensitivity to bacteriocins. The difference in resistance between Gram-positive and Gram-negative bacteria may be due to differences in the cell envelopes (51). However, production of lactic acid in high concentrations in combination with bile salts has an inhibitory effect on the growth of pathogenic Gram-negative bacteria in the intestinal tract (52). The present study has reported antagonistic activity of LAB strains against both Gram-positive and -negative bacteria alike. This finding is in accordance with a similar study conducted by (53) which reported that the CFS of a *Lb. plantarum* strain displayed broad spectrum antimicrobial activities against Gram-positive and -negative bacteria as well as against yeast *C. albicans*. *P. aeruginosa*, *K. pneumoniae* and *E. coli* were reported to be sensitive at medium levels to *Lb. plantarum* and *L. lactis* strains isolated from Turkish boza and *B. cereus* (a Gram-positive bacterium) was also strongly inhibited by the same LAB strains (54). Also, the inhibitory

effect of *Lb. plantarum* and *Lb. fermentum* strains used as starter organisms in the production of bread and bakery products, against rope-forming *Bacillus cereus* was previously reported (55, 56). Many *Lb. plantarum* strains have been reported to produce bacteriocins known as plantaricins (57) which are compounds that are highly diverse in their activities and structures and have been reported to be particularly active against gastrointestinal as well as food-borne pathogens (58). In the present study, *Lactococcus lactis* strains (B7 & B10), *Lactobacillus fermentum* strains (K12 & K13), *Gluconobacter frateurii* (K26), *Lactobacillus nagelii* (B24), *Lactobacillus parabuchneri* (B25), *Acetobacter* peroxydans (B27), and *Lactococcus lactis* (S2) isolates showed better antibacterial activity by spot-on-lawn method. The excellent antibacterial activity reported of *Lactobacillus parabuchneri* against all indicator pathogens in this study is in contrast to the results obtained by (59) who had reported that *Lb. parabuchneri* strain isolated from a Brazilian ovine cheese had little inhibitory effect against all tested pathogens. *Lb. parabuchneri* is an obligatory heterofermentative bacterium occasionally isolated from cheeses (60). Considering that heterofermentative LAB typically ferment glucose to yield ethanol, acetic acid and carbon dioxide in addition to lactic acid as by-products as against homofermentative LAB which produce only lactic acid, they possess the ability to antagonize highly pathogenic bacteria. It is, therefore, noteworthy that this is the first study to have reported the presence of *L. parabuchneri* in a Turkish boza drink. LAB strains that produced larger inhibition zones against *E. coli, S. aureus* and *S.* Enteritidis in a previous study were reported to be heterofermentative (61).

Comparing the results of the two methods adopted in this study, *L. lactis* strains (B7 & B10) as well as *Lb. fermentum* (K13) strain showed antifungal activity against *Candida albicans* by spot-on-the-lawn method, but the same effect was not observed with agar well method. The lack of positive results by agar well diffusion method may be due to the indiffusibility of the secreted molecules since this is the basis of agar well assay. In contrast, *Lb. plantarum* (K14, K15), *Lb. fermentum* (B3, B4, B6), *L. lactis* (K18), *Lb. pentosus* (S5) and *Lb. plantarum* (S8) strains showed antimicrobial activity by agar well diffusion but no antagonism was recorded with spot-on-the-lawn method. Since they showed negative results by spot-on-the-lawn method, it is thought that the isolates may not secrete any primary or secondary metabolites other than bacteriocins. Therefore, spot-on-the-lawn method has been found in this study to be more effective than agar well diffusion method in the determination of antimicrobial activity. This finding was consistent with the results reported by (62) who investigated the antagonistic effects of LAB strains against Gram-negative bacteria using these two methods and found spot-on-the-lawn to be more effective. However, utilizing the probiotic strain *Bifidobacterium bifidum* against *Salmonella enterica*

serovar Enteritidis, agar well diffusion method was demonstrated to be better in determining antagonism than the other two methods (disk diffusion and spot-on-the-lawn) employed (63). The inhibitory activity on tested bacteria by the spot-on-the-lawn method is seen as better, but it could be as a result of the synergy of all metabolites viz. lactic acid, acetic acid, diacetyl, bacteriocin etc., as they are being produced during the assay period (64). The variation in antibacterial activities as depicted by different authors might be due to the number of the colony forming units (CFU) of the LAB used (in spot-on-the-lawn) and/or the quantity of culture supernatant used (in agar well diffusion) as well as the activity and diffusibility of the bacteriocins possessed in it (46, 65).

Conclusions

Probiotics are increasingly gaining overwhelming attention from both the food industry and the academia. Probiotic foods constitute a significant part of the functional foods market worldwide. The presence of these good microbes in fermented food products all over the world, and their ability to combat pathogenic and spoilage microbes using different mechanisms of action, has been validated with several scientific studies. From the perspective of antimicrobial health benefits, the present study consolidates past studies demonstrating the probiotic potentials of fermented traditional Turkish beverages. The study has substantiated the antimicrobial efficacy of the potential probiotic strains isolated from kefir, boza and shalgam against an array of human pathogens comprising both Gram-positive and -negative bacteria as well as yeast (*Candida albicans*). In total, 16 lactic acid bacteria and 2 acetic acid bacteria isolates were found to be antagonistic at varying degrees against the tested bacterial and yeast pathogens. In the current study, boza and kefir followed by shalgam were found to be more effective in terms of antimicrobial activity against human pathogens. The outstanding antimicrobial efficacy of boza and kefir in particular is believed to be connected with the greater diversity of their microflora as well as the absence of chemical preservatives. It has, therefore, been determined that boza, kefir and shalgam compared to other fermented traditional Turkish beverages analyzed, are the most promising probiotic candidates. From this perspective, regular consumption of boza, kefir and shalgam in adequate quantities is recommended for the treatment and/or prevention of various diseases. In addition, going by the results obtained in this study as well as previous studies on other probiotic properties, boza, kefir and shalgam may also be applicable as bio-therapeutics/nutraceuticals against bacterial and yeast infections in humans, although *in vivo* studies would be useful in validating their efficacy. Due to the presence of beneficial nutrients and substances such as probiotics and/or antioxidants, boza, kefir, Ayran, shalgam and hardaliye can also be generally regarded as functional foods.

Other probiotic properties of the microbiota of these fermented Turkish beverages have been reported individually in previous studies. However to the best of our knowledge, this is the first study that evaluated the antimicrobial properties of the isolates obtained from all five beverages against a broad spectrum of human microbial pathogens. The study has to a large extent achieved its objective. Future research efforts should be directed toward animal testing and/or clinical trials to better evaluate the antimicrobial as well as overall probiotic effects of Turkish boza, kefir and shalgam on human. Furthermore, suitable biopreservatives for commercial hardaliye and shalgam drinks should be investigated in order to retain their beneficial microflora whilst extending their shelf lives. Alternatively, probiotic strains and starter cultures resistant to benzoic acid may be investigated in further studies for fortification of commercial hardaliye since no LAB strain was isolated from any hardaliye sample which is thought to be due to the presence of chemical preservative. Ayran which is a "national drink" is also recommended for fortification with carefully selected and promising probiotic strains from the *Bifidobacterium* and *Lactobacillus* genera in order to enhance its probiotic functional qualities.

Acknowledgements

References

1. M. L. Marco, D. Heeney, S. Binda, C. J. Cifelli, P. D. Cotter, B. Foligné, M. Gänzle, R. Kort, G. Pasin, A. Pihlanto, et al., *Curr. Opin. Biotechnol*., 2017, **44**, 94 LINK doi:10.1016/j.copbio.2016.11.010

2. R. W. Hutkins, Microbiology and Technology of Fermented Foods, 2nd Edn., Wiley, Hoboken NJ, 2018

3. J. P. Tamang and D. Samuel, Fermented Foods and Beverages of the World, 1st ed., eds. J. P. Tamang and K. Kailasapathy, CRC Press, Taylor and Francis Group, New York, 2010

4. K. H. Steinkraus, *Comp Rev Food Sci Food Saf*., 2002, 1, 23

5. J. Josephsen and L. Jespersen, Handbook of Food and Beverage Fermentation Technology, eds. O. R. Fennema, Y. H. Hui, M. Kerel, P. Walstra and J. R. Whitaker, Marcel Dekker, New York, 2004

6. S. Rezac, C. R. Kok, M. Heermann and R. Hutkins, *Front. Microbiol.*, 2018, 9, 1785 LINK doi:10.3389/fmicb.2018.01785

7. E. Dimidi, S. R. Cox, M. Rossi and K. Whelan, *Nutrients,* 2019, 11, 1806 LINK https://doi.org/10.3390/nu11081806

8. FAO/WHO. Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria Cordoba, Argentina. 1-4 October, 2001

9. P. Markowiak and K. Śliżewska, *Nutrients*, 2017, 9, (9), 1021 LINK https://doi.org/10.3390/nu9091021

10. O. Simon, *Adv. Pork Prod.*, 2005, 16, 161

11. Food and Agriculture Organization (FAO), Guidelines for the Evaluation of Probiotics in Food. FAO; London, ON, Canada: Report of a Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food. 30 April–1 May, 2002

12. European Food Safety Authority (EFSA), Opinion of the Scientific Committee on a request from EFSA related to a generic approach to the safety assessment by EFSA of microorganisms used in food/feed and the production of food/feed additives. *EFSA J.*, 2005, 226, 1

13. A. Anadón, M. R. Martínez-Larrańaga and M. A. Martínez, *Regul. Toxicol. Pharmacol.*, 2006, 45, 91 LINK doi:10.1016/j.yrtph.2006.02.004

14. F. Gaggia, P. Mattarelli and B. Biavati, *Int. J. Food Microbiol.*, 2010, 141, 15 LINK doi:10.1016/j.ijfoodmicro.2010.02.031

15. M. B. Romond, Z. Haddon, Mialcareck, et al., *Anaerobe*, 1997, 3, 131

16. S. Pithva, P. Ambalam, J. M. Dave, et al., Science against microbial pathogens: communicating current research and technological advances, ed. A. Méndez-Vilas, 2011

17. M. Saarela, G. Mogensen, R. Fonden, et al., *Journal of Biotechnology,* 2000, 84, 197

18. D. Sharma and B. S. Saharan, *Int J Microbiol.*, 2014, 1, (7), 698

19. N. Salazar, M. Gueimonde, C. G. de Los Reyes-Gavilan and P. Ruas-Madiedo, *Crit. Rev. Food Sci. Nutr.*, 2016, 56, 1440

20. E. Pessione and S. Cirrincione, *Front. Microbiol.*, 2016, 7, 74

21. O. T. Aladeboyeje and N. O. Sanli, *International Journal of Life Sciences and Biotechnology*, 2021, 4, (3), 546 LINK DOI:10.38001/ijlsb.936982

22. F. Altay, F. Karbancıoglu-Güler, C. Daskaya-Dikmen, et al., *International Journal of Food Microbiology*, 2013, 167, 44

23. A. Y. Tamime, Fermented Milk, ed. A.Y. Tamime, Blackwell Science Ltd, Oxford, UK, 2006

24. A. M. O. Leite, M. A. Miguel, R. S. Peixoto, A. S. Rosado, J. T. Silva and V. M. Paschoalin, *Braz. J. Microbiol.*, 44, 341, 2013

25. F. Lopitz-Otsoa, A. Rementeria, N. Elguezabal and J. Garaizar, *Rev. Iberoam. Micol.*, 2006, 23, 67

26. B. Kabak and A. D. W. Dobson, *Critical Reviews in Food Science and Nutrition*, 2011, 51, 248

27. E. Simova, D. Beshkova, A. Angelov, T. Hristozova, G. Frengova and Z. Spasov, *J. Ind. Microbiol. Biotechnol.*, 2002, 28, 1

28. Turkish Food Codex (TFC) Regulations, Fermented Milk products, Regulation No: 2009/25, Republic of Turkey Ministry of Food, Agriculture and Livestock, Ankara, Turkey, 2009

29. A. Köksoy and M. Kilic, *International Dairy Journal*, 2003, 13, (10), 835

30. M. Arici and O. Daglioglu, *Food Reviews International*, 2002, 18, (1), 39

31. S. Yegin and M. Fernandez-Lahore, Handbook of Plant-based Fermented Food and Beverage Technology, eds. Y. H. Hui and O. E. Evranuz, CRC Press, Boca Raton, US, 2012

32. S. Todorov, M. Botes, C. Guigas, et al., *J Appl Microbiol.*, 2008, 104, 465

33. A. J. Marsh, C. Hill, R. P. Ross, et al., *Trends Food Sci Technol*, 2014, 38, 113 LINK https://doi.org/10.1016/j.tifs.2014.05.002

34. M. Arici and F. Coskun, *Food Microbiology*, 2001, 18, 417

35. H. Aydogdu, S. Yildirim, A. K. Halkman and T. Durgun, *Gida*, 2014, 39, 139 (In Turkish)

36. F. C. Prado, J. L. Parada, A. Pandey and C. R. Soccol, *Food Research International*, 2008, 41, 111

37. H. Erten, H. Tangüler and A. Canbas, *Food Reviews International*, 2008, 24, 352

38. H. Tangüler and H. Erten, *LWT — Food Science and Technology*, 2012, 46, 36

39. B. Incedayi, V. Uylaser and U. Copur, *J. of Food, Agriculture & Environment*, 2008, 6, 31

40. S. S. Grosu-Tudor, M. M. Stancu, D. Pelinescu, et al., *World J Microbiol Biotechnol*, 2014, 30, 2459 LINK https://doi.org/10.1007/s11274-014-1671-7

41. R. Tour´e, E. Kheadr, C. Lacroix, et al., *Journal of Applied Microbiology*, 2003, 95, (5), 1058

42. B. Ripamonti, A. Agazzi, C. Bersani, et al., *Anaerobe*, 2011, 17, 97

43. M. Dogan and H. Ozpinar, *Kafkas Univ Vet Fak Derg*, 2017, 23, (4), 555

44. F. Cetinkaya and T. Elal-Mus, *Ankara Üniversitesi Veteriner Fakültesi Dergisi (Veterinary Journal of Ankara University)*, 2012, 59, 217

45. C. Vuotto, F. Longo and G. Donelli, *Int. J. Oral Sci.*, 2014, 6, 189 LINK doi:10.1038/ijos.2014.52

46. D. Halder, M. Mandal, S. Chatterjee, et al., *Biomedicines*, 2017, 5, (2), 31 LINK doi: 10.3390/biomedicines5020031

47. R. C. Reuben, P. C. Roy, S. R. Sarlkar, A. S. M. Alam and I. K. Jahid, *J. Dairy Sci.* 2019, 103, (2), 1223

48. N.P. Shah, *International Dairy Journal*, 2007, 17, 1262

49. C. Hill, F. Guarner, G. Reid, et al., *Nat. Rev. Gastroenterol. Hepatol.*, 2014, 11, 506 LINK doi:10.1038/nrgastro.2014.66

50. R. Eid, J. E. Jakee, A. Rashidy, et al., *Probiot. Health*, 2016, 4, 138

51. F. B. Elgado, M. A. R. V. Guerra, R. A. Macayan, et al., *Int J Food Microbiol*, 2004, 95, 11

52. A. A. A. Argyri, G. B. Zoumpopoulou, K.-A. G. C. Karatzas, E. B. Tsakalidou, G.-J. E. D. Nychas, E. Z. D. Nychas and C. C. A. Tassou, *Food Microbiol,* 2013, 33, 282

53. T. H. Lin and T. M. Pan, *Journal of Microbiology, Immunology and Infection*, 2019, 52, (3), 409 LINK http://dx.doi.org/10.1016/j.jmii.2017.08.003

54. M. Kivanc, M. Yilmaz and E. Cakir, *Turkish Journal of Biology*, 2011, 35, 313

55. O. Mentes, R. Ercan and M. Akcelik, *Food Control*, 2007, 18, (4), 359

56. R. Denkova, S. Ilieva, Z. Denkova, et al., *Biotechnol Biotechnol Equipment*, 2014, 28, (3), 487

57. J. Nissen-Meyer, A. G. Larsen, K. Sletten, et al., *J Gen Microbiol*, 1993, 139, 1973

58. C. A. West and P. J. Warner, *FEMS Microbiol*, 2001, 49, 163

59. S. Meira, V. Helfer, R. Velho, et al., *Journal of Dairy Research*, 2012, 79, (1), 119 LINK doi:10.1017/S0022029911000884

60. M. Coton, F. Berthier and E. Coton, *FEMS Microbiology Letters*, 2008, 284, 150

61. H. Harnentis, Y. Marlida, Y. S. Nur, W. Wizna, M. A. Santi, N. Septiani, F. Adzitey and N. Huda, *Veterinary world*, 2020, 13, (9), 1922 LINK https://doi.org/10.14202/vetworld.2020.1922-1927

62. B. H. Cadirci and S. A. Citak, *Pak. J. Nutr.*, 2005, 4, 237

63. N. Rahimifard and M. Naseri, *J. Bacteriol. Mycol.*, 2016, 2, (3), 61 LINK DOI:10.15406/jbmoa.2016.02.00024

64. A. H. Con and H. Y. Gokalp, *Meat Sci*, 2000, 55, 89

65. S. Choyam, D. Lokesh, B. B. Kempaiah, et al., *Frontiers in Microbiology,* 2015, 6, 1034.

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<Tables>

Table 1

Nomenclature of lactic acid bacteria isolated from fermented Turkish beverages based on molecular typing by 16S rRNA sequence analysis

Beverage Source	Isolate ID code	16S rRNA Percent similarity (%)	Closely related species
Boza	B3	100	*Lactobacillus fermentum*
	B4	98.81	*Lactobacillus fermentum*
	B6	99.37	*Lactobacillus fermentum*
	B7	98.97	*Lactococcus lactis*
	B10	97.14	*Lactococcus lactis*
	B24	87.81	*Lactobacillus nagelii*
	B25	99.05	*Lactobacillus parabuchneri*
	B27	94.93	*Acetobacter peroxydans*
Kefir	K11	99.62	*Lactobacillus fermentum*
	K12	98.66	*Lactobacillus fermentum*
	K13	99.25	*Lactobacillus fermentum*
	K14	98.09	*Lactobacillus plantarum*
	K15	99.80	*Lactobacillus plantarum*
	K18	98.24	*Lactococcus lactis*
	K19	98.24	*Lactococcus lactis*
	K26	99.32	*Gluconobacter frateurii*
Shalgam	S2	98.38	*Lactococcus lactis*
	S5	98.86	*Lactobacillus pentosus*
	S8	99.04	*Lactobacillus plantarum*
	S16	89.75	*Acetobacter pasterianus*
	S17	87.09	*Lactobacillus plantarum*
Ayran	A22	99.59	*Lactococcus lactis*

Table 2

Antimicrobial Activity of Isolates against standard pathogens (Spot-on-the-Lawn Method)

ISOLATE	INHIBITION ZONES (mm)										
	Acinetobacter baumanii	*Bacillus cereus*	*E. coli*	*Klebsiella pneumoniae*	MRSA	*Pseudomonas aeruginosa*	*S. enterica serovar Typhimurium*	*Staph. aureus*	*S. epidermidis*	VRE *faecalis*	*Candida albicans*
Lactococcus lactis (B7)	-	27,70	17,20	16,80	-	10,80	16,30	20,50	13,5	6,40	28,60
L. lactis (B10)	-	-	18,60	-	-	-	-	-	12,20	-	14,20
Acetobacter peroxydans (B27)	26,70	24,60	24,00	20,40	-	26,80	25,9	23,40	28,60	20,80	-
Gluconobacter frateurii (K26)	27,80	24,20	-	25,80	26,00	-	-	-	-	22,40	-
Lb. nagelii (B24)	20,10	23,80	22,30	21,60	22,10	17,80	22,00	22,20	20,00	21,90	-
Lb. fermentum (K13)	-	-	24,30	24,90	26,40	30,10	-	-	-	20,80	11,90
Lb. fermentum (K12)	-	11,30	-	7,00	-	-	10,80	10,50	8,80	-	-
Lb. parabuchneri (B25)	24,90	15,50	24,00	23,80	25,90	22,50	20,50	22,30	17,80	21,20	-
L. lactis (S2)	-	11,80	11,30	12,50	12,00	12,80	15,70	9,80	9,60	11,30	-

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Table 3

Antimicrobial Activity of Isolates against standard pathogens (Agar Well Diffusion Method)

| ISOLATES	INHIBITION ZONES DIAMETER (mm)										
	Acinetobacter baumanii	*Bacillus cereus*	*E. coli*	*Klebsiella pneumoniae*	MRSA	*Pseudomonas aeruginosa*	*S. enterica serovar Typhimurium*	*Staph. Aureus*	*S. epidermidis*	VRE *faecalis*	*Candida albicans*
Lb. fermentum (B4)	11,60	-	-	9,70	8,50	25,10	8,40	9,30	10,00	-	11,00
Lb. fermentum (B6)	9,10	-	-	-	-	9,80	9,90	-	-	11,70	25,40
L. lactis (K18)	-	-	-	-	-	8,30	-	-	-	-	10,20
L. lactis (A22)	10,50	-	-	-	-	-	-	-	-	-	10,90
Lb.fermentum (K13)	11,50	-	-	-	-	-	12,70	-	-	-	14,30
Lb.plantarum (K14)	12,30	15,90	9,50	-	13,60	-	-	13,20	11,20	11,40	15,80
Lb.plantarum (K15)	11,80	-	9,20	9,90	9,30	-	-	11,60	11,00	10,90	14,80
Lb. fermentum (B3)	10,50	-	-	10,80	-	-	11,10	7,40	-	-	-
Lb. pentosus (S5)	12,40	-	-	-	15,60	23,40	10,40	-	-	-	-
Lb. plantarum (S8)	12,80	-	9,50	8,30	-	19,10	-	-	-	-	-

<Figure captions>

Fig. 1. Representative images showing the antibacterial effects of isolates obtained from fermented Turkish probiotic beverages against Gram-positive bacteria
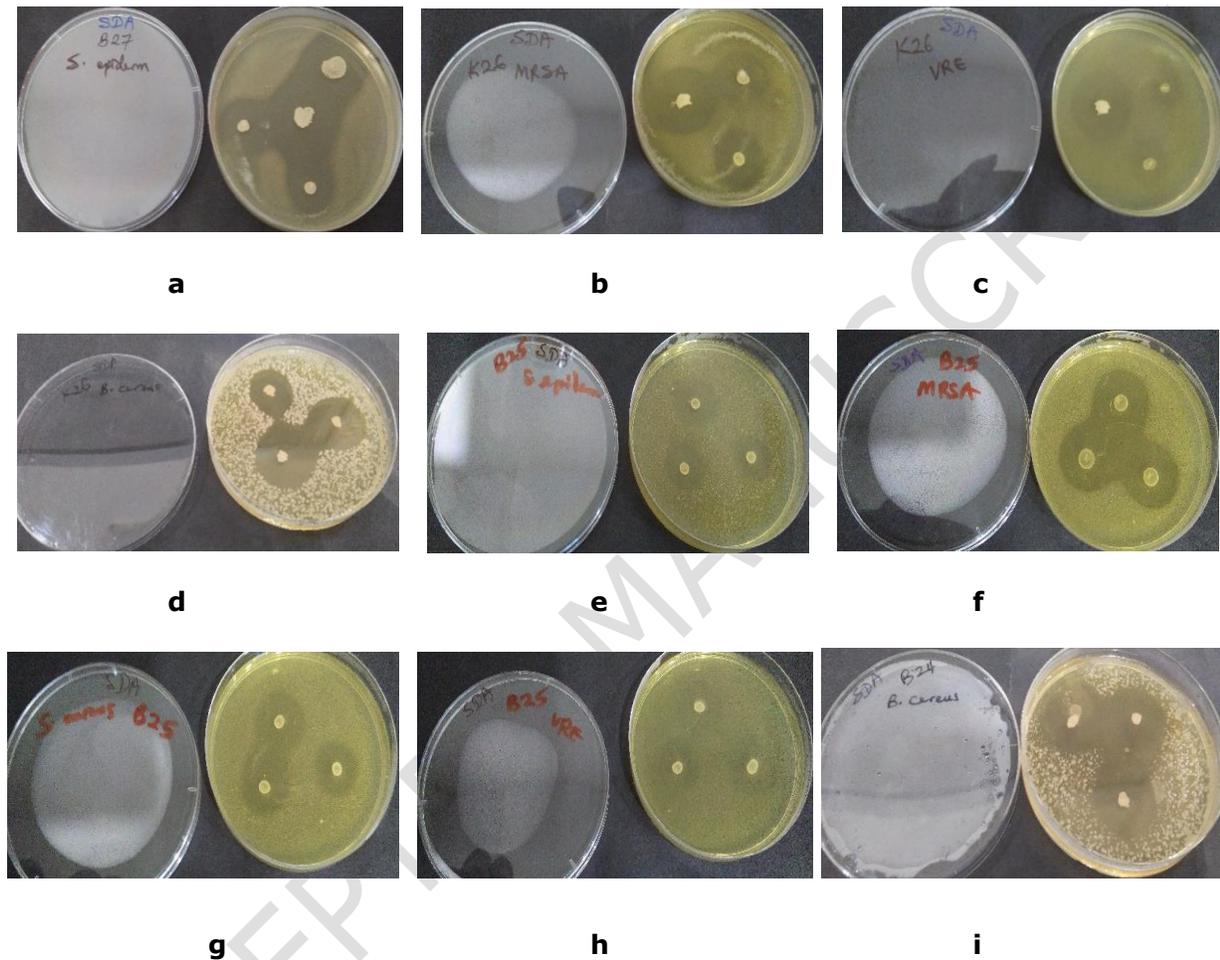
Fig. 1

a: *Acetobacter peroxydans* (B27)- *S. epidermidis,*

b: *Gluconobacter frateurii* (K26)- MRSA,

c: *Gluconobacter frateurii* (K26)- VRE,

d: *Gluconobacter frateurii* (K26)- *B. cereus,*

e: *Lactobacillus parabuchneri* (B25)- *S. epidermidis,*

f: *Lactobacillus parabuchneri* (B25)- MRSA,

g: *Lactobacillus parabuchneri* (B25)- *S.aureus*,

h: *Lactobacillus parabuchneri* (B25)- VRE,

i: *Lactobacillus nagelii* (B24)- *B. cereus.*

Fig. 2. Representative images showing the antibacterial effects of isolates obtained from fermented Turkish probiotic beverages against Gram-negative bacteria
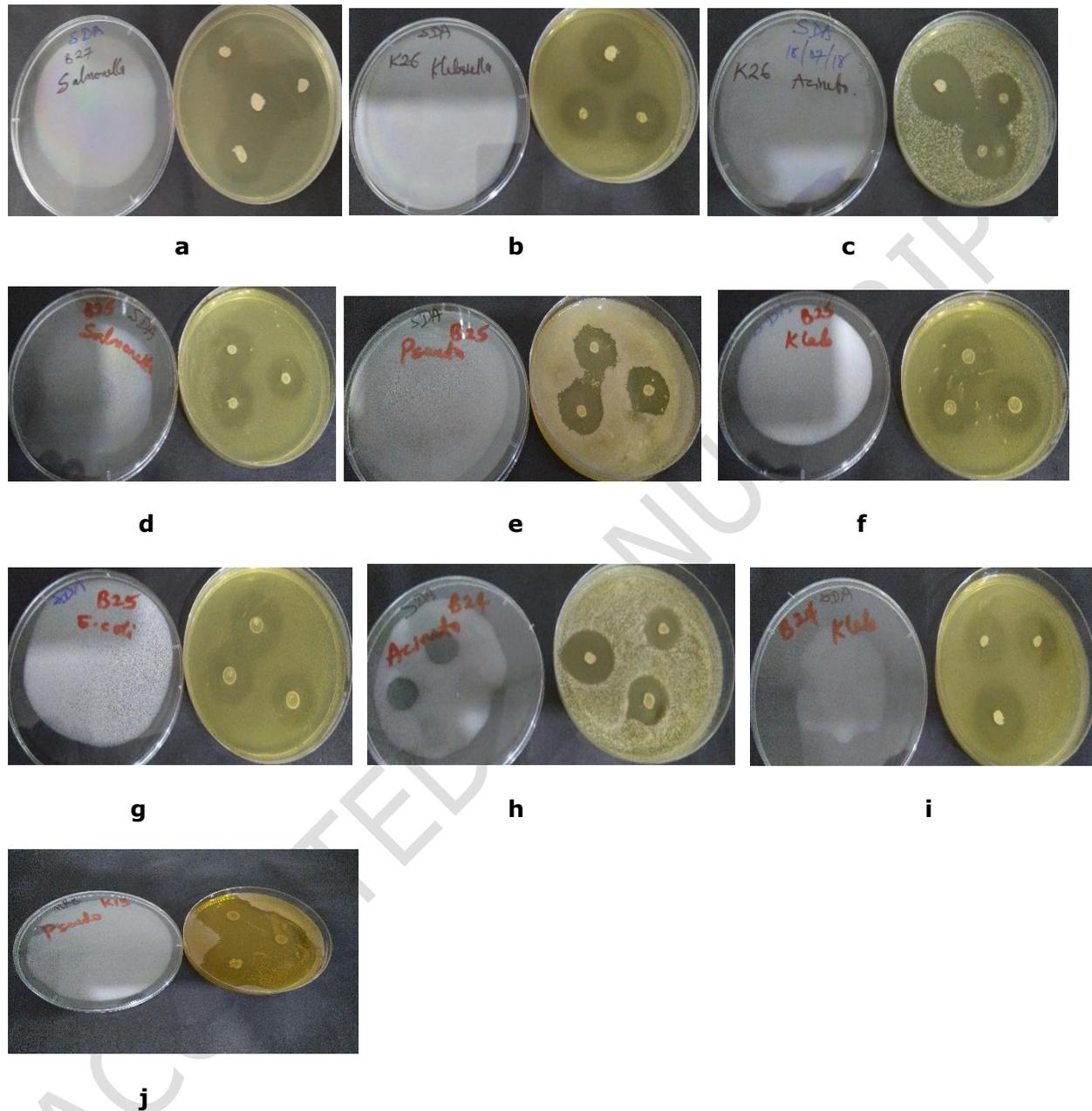
Fig. 2

a: *Acetobacter peroxydans* (B27)- *S. enterica* serovar Typhimurium,

b: *Gluconobacter frateurii (K26)- K. pneumoniae,*

c: *Gluconobacter frateurii (*K26)- *A. baumanii*,

d: *Lactobacillus parabuchneri* (B25)- *S. enterica* serovar Typhimurium*,*

e: *Lactobacillus parabuchneri* **(**B25)- *P. aeruginosa,*

f: *Lactobacillus parabuchneri* B25-*K. pneumoniae*

g: *Lactobacillus parabuchneri* **(**B25)-*E.coli ,*

h: *Lactobacillus nagelii* (B24)- *A. baumanii,*

i: *Lactobacillus nagelii* (B24)- *K. pneumoniae,*

j: *Lactobacillus fermentum* (K13)- *P. aeruginosa*

Fig. 3. Representative image showing the antifungal effect of *Lactococcus lactis* (B7) isolate obtained from boza against *C. albicans*

Fig. 3

Lactococcus lactis (B7) - *C. albicans*