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REVIEW

Research advances of deciphering Shalgam microbiota profile and dynamics

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Abstract

The relationship between the microbiota and their functions in the quality and characteristic flavors of the fermented foods that provide them autochthonous attributes has been remained elusive, so far. With the demand in elucidating the microbiota of the autochthonous fermented foods, the characterization of the shalgam microbiota via culture-dependent and culture-independent methods has been carried out. To shed light on shalgam microbiota harboring Lactic acid bacteria (LAB) and yeasts, microorganisms isolated from shalgam have been identified by culture-dependent methods including 16S rRNA and ITS (Internal Transcribed Spacer) gene regions sequencing, RAPD-PCR, Rep-PCR, and API CHL50. Culture-independent characterization methods such as 16S rRNA and ITS meta-barcoding sequencing were performed to pinpoint the microbial diversity within shalgam. More recently, bioinformatics and *in vitro* analysis of bacteria and yeast isolated from shalgam to find prospective probiotics and elucidate shalgam microbiota dynamics due to the types of salts used in shalgam production have been reported. In this review, we intend to collate the data on microorganisms identified via culture-dependent and culture-independent methods. Taken together, we presented a broad perspective on the shalgam microbiota and how future endeavors in shalgam microbiota research can move forward.

Introduction

Obtained through the fermentation of black carrot and/or turnip, a member of radish family vegetables, shalgam has been an autochthonous and unique fermented beverage for Turkey. According to the regulatory definition of the shalgam, it contains fermented black carrot, turnip, salt, sourdough extract, bulgur flour, and water. Even though shalgam resembles an Indian fermented drink kanji, the production methods and microbiota of both beverages differ from each other (Coşkun, 2017; Lamba et al., 2019; Özdemir & Güldemir, 2021; Tangüler et al., 2020).

With black carrot fermentation of LAB and yeast, shalgam has been manufactured through two different methods that are classified as traditional and direct production methods (Altay et al., 2013; Coşkun, 2017; Tangüler et al., 2022). Traditional shalgam production has been carried out within two consecutive fermentation steps, in which both steps simply have been connected via the use of the water extract from the first dough fermentation, as a starter culture for the second fermentation. At the first step of the traditional fermentation; yeast, bulgur flour, water, and salt are kneaded to form a dough, and subsequently the dough fermentation continued for three days. Having completed the dough fermentation, the extraction to obtain a water-based liquid that is enriched with yeast

and LAB is performed by washing the dough three to five times with water. Chopped black carrot, tap water, and salt are treated with the water extract from the first fermentation, and then the second fermentation commences. The second fermentation lasts for three-ten days till the final fermented shalgam reaches to its characteristic flavor and structure (Tangüler et al., 2017, 2022). Considering the long fermentation time and uncontrolled nature of traditional shalgam production, the direct production of shalgam was applied. Two different direct production methods have been utilized so far: adding 15% of previous batch production to a new shalgam fermentation batch and the inoculating baker`s yeast as a starter culture into a mix containing black carrot, turnip, salt, bulgur flour, and water (Canbaş & Fenercioğlu, 1984; Tangüler & Erten, 2012a). Shalgam production methods were shown in **Figure 1**. Shalgam has been deemed a functional food since it contains anthocyanin and phenolic compounds that provide health benefits to humans (Dereli et al., 2015; Ekinci et al., 2016; Kelebek et al., 2018; Kırca et al., 2007; Tazehkand & Valipour, 2019; Türker et al., 2004; Türkyılmaz et al., 2012). It was reported that the type of shalgam production methods affected anthocyanin quantity and profile in shalgam (Tangüler et al., 2021). Tanrıseven et al. (2020) tested the effects of production methods on shalgam, resulting in the determination of seven different anthocyanins in shalgam. 235.76 mg/L anthocyanin in shalgam was produced by the direct production methods, and 185.26 mg/L was produced by the traditional production methods (Tanrıseven et al., 2020). Phenolic compounds and anthocyanins were affected by newly proposed shalgam process steps such as pasteurization and depectinization for the clarification of fermented black carrot juice to obtain a clear visual of the final product of shalgam fermentation (Dereli et al., 2015). In traditionally produced shalgam samples that contained 16 different phenolic compounds, the amount of phenolic compounds was higher at the end of the second fermentation as compared to the first stage of shalgam fermentation (Toktaş et al., 2018). It was reported that the phenolic compounds and anthocyanins of the shalgam fermentation was affected not only by production methods, but also the materials used in shalgam altered the content of bioactive compounds in shalgam (Bayram et al., 2014). The source of phenolic compounds could be black carrots used in the shalgam production (Bayram et al., 2014). That was statistically confirmed by the correlation between increasing phenolic compounds with adding more black carrot into shalgam fermentation (Bayram et al., 2014). In parallel with that, the size of chopped black carrot affected quantities of anthocyanin and phenolic composition that were produced during shalgam fermentation (Tangüler et al., 2014). Grape pomace was added to shalgam fermentation to increase polyphenolic content and reveal to what extent grape pomace increased the ethanol content in shalgam. Up to 50% of grape pomace-added shalgam`s ethanol did abide by the legislatively authorized limit of ethanol content in shalgam, which is 0.5% ethanol (Akbulut & Çoklar, 2020).

With respect to the shalgam production process, anthocyanin and phenolic compound-rich shalgam might have problems during and after production (Erten et al., 2008; Karaoğlan, 2013; Tangüler et al., 2015); (iii) the lack of widely-used starter culture for traditional shalgam production; (iv) the absence of optimum process conditions aiming quality improvement and/or shelf life extension (Demir et al., 2006; Erten et al., 2008; Özdemir-Alper & Acar, 1996). Apart from those problems, manufacturing standard fermented food products is an issue to overcome for transitioning from the traditional production of autochthonous fermented foods to their industrial production (Materia et al., 2021). Although the standardization problem for shalgam production has not been reported in previous studies, traditional and direct production pose risks for industrial shalgam production as they are not optimized in terms of process inputs and conditions. Process conditions, microbiota, and shalgam ingredients have been considered to impact the end product of shalgam fermentation (Erten et al., 2008). Shalgam microbiota contributes to shalgam quality and flavors by assimilating and metabolizing available carbon sources such as sucrose, glucose, and fructose during the fermentation. In exchange of carbon source by the resident microbes in shalgam, the microbiota generates not only aroma compounds but also organic acids

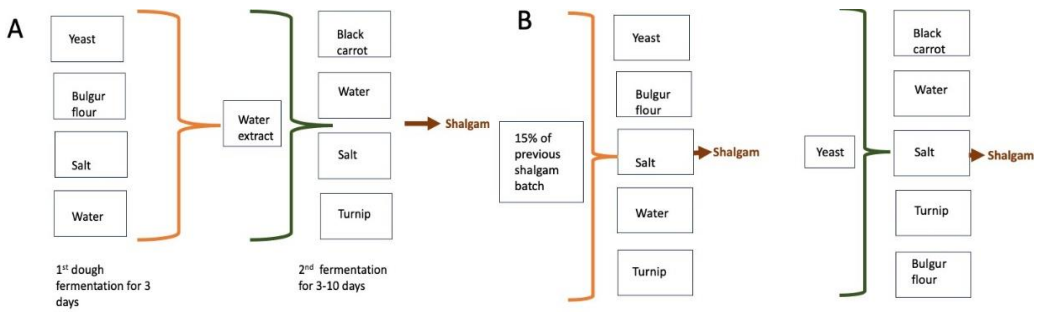

Figure 1. Process methods of the shalgam production methods. A) traditional shalgam production and B) direct shalgam production.

including lactic acid, acetic acid, citric acid, propionic acid, and succinic acid. The overall microbial community in shalgam entails ethanologenic yeast and LAB, resulting in main fermentation products as lactic acid and the trace amount of ethanol (Ekinci et al., 2016; Ulucan, 2019). Despite the presence of yeasts in shalgam, LAB-dominance in the shalgam microbiota has been reported (Ağırman & Erten, 2018; Demir et al., 2006; Erginkaya & Turhan, 2016; Özer & Çoksöyler, 2015; Tangüler & Erten, 2012a). It was indicated that the microbial load of LAB in shalgam microbiota has been influenced by pH, fermentation temperature, salt type and quantity, and black carrot size and quantity (Okçu et al., 2016: Tangüler & Erten, 2013). It was also reported that altering shalgam content by adding ayran, which is a water-added yogurt drink, increased the number of *Streptococcus* colonies after 7 days of storage at 4 ˚C as compared to the non-added shalgams (Uzay et al., 2021).

Shalgam's characteristic flavors and aroma compounds could be attributed to the contingency of shalgam microbiota, urging researchers to elucidate the microbial diversity of shalgam through microbial characterization methods. Hitherto, culture-dependent (RAPD-PCR, Rep-PCR, API CHL50, ITS gene sequencing, and 16S rRNA gene sequencing) and culture-independent (16S and ITS metabarcoding) microbial characterization methods have been deployed in **Figure 2**. This review was intended to provide insights gained from the shalgam microbiota research for deeper understanding of the microbial diversity in shalgam.

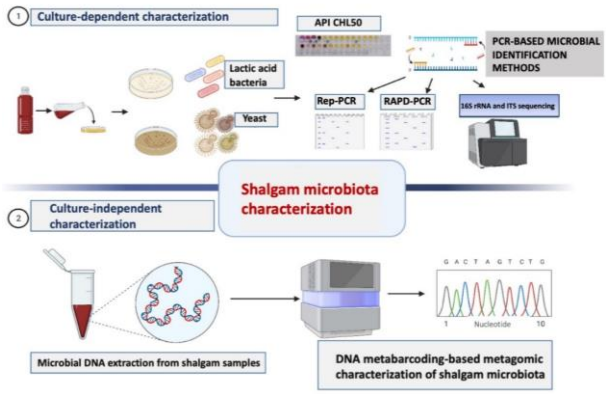

Figure 2. Schematic representation of shalgam microbiota characterizations.

Culture-dependent microbial characterization of shalgam microbiota

The studies on elucidating the microbial community of sourdough revealed that LAB has more diversity than yeasts in sourdough. As a result of that, LAB outperformed yeast in terms of the contribution to generating aroma compounds and improving shelf life stability (Gobbetti et al., 2016; Minervini et al., 2019). Thus, LAB diversity is of great importance for shalgam due to the use of the LAB-rich water extract that is obtained from the dough fermentation at the beginning of traditional shalgam production (Erten et al., 2008). In the culture-dependent characterization of shalgam microbiota, resident, and intact microorganisms in shalgam can be reproduced under the metabolic and physiological requirements provided in the culture medium. Up to now, for the microbial identification of LAB isolated from shalgam microbiota, the culture-dependent characterization methods such as API 50 CHL test, 16S rRNA gene sequencing, Randomly Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR), and Repetitive element sequence-based Polymerase Chain Reaction (Rep-PCR) were utilized (Ağırman et al., 2021; Arıcı, 2004; Baser et al., 2012; Erginkaya & Hammes, 1992; Kafkaskıray, 2020; Mete et al., 2017; Tangüler & Erten, 2012a, b ; Tangüler et al., 2014).

API 50 CHL was used to identify LAB and related genera. Incubated in Man Rogosa Sharpe (MRS) medium, isolated microorganisms were put into API 50 CH test kits that harbor 49 carbohydrates. In microbial fermentation of carbohydrates located in API 50 CH test kits, acids produced after microbial fermentation would change the pH of strips so that the color in strips shifts to indicate the occurrence of microbial fermentation. The color pattern created by microbial fermentation helps identify isolated microorganisms at the species level (Schilinger & Lücke, 1987). The first microbial isolation from shalgam was carried out in traditionally produced shalgam samples, identifying *Lactiplantibacillus plantarum*, *Levilactobacillus brevis*, and *Limosilactobacillus fermentum* as isolated LAB. The identification was initially performed by inspection of the color of colonies, cellular morphologies, and finally by performing the API 50 CH test (Erginkaya & Hammes 1992). In another microbial isolation effort, Arıcı (2004) found that *Lacticaseibacillus paracasei* subsp. *paracasei*, and *L. plantarum* were dominant microorganisms in shalgam.

11 LAB species from 135 microorganisms isolated from commercial shalgam samples were identified via API CHL 50 test. Except for one shalgam sample, among isolated LAB species *L. plantarum*, *L. brevis,* and *Leuconostoc mesenteroides* subsp. *mesenteroides/dextranicum* were always present (Tangüler & Erten, 2012c). Accordingly, the same group showed that *L. plantarum* was the dominant LAB species in shalgam (Tangüler & Erten, 2012b, 2012c). To ascertain the shalgam microbiota alterations during traditional production, microbial isolation was performed for two stages of fermentation. It was found that although *Lactobacillus delbrueckii* subsp. *delbrueckii*, *Leuconostoc mesenteroides* subsp. *cremoris*, *Leuconostoc mesenteroides* subsp. *mesenteroides,* and *Pediococcus pentosaceus* were isolated at the first stage of the traditional production, they vanished at the end of the second fermentation (Tangüler & Erten, 2012b). Aiming to choose the appropriate starter cultures for shalgam production, commercial and lab-made shalgam samples were subjected to culturing and 447 candidate strains for starter cultures were obtained. Having evaluated the strains in the face of hurdles that might

occur during shalgam production, 18 strains identified via API 50 CHL test were potential starter cultures. *L. plantarum, L. paracasei* subsp. *paracasei*, *P. pentosaceus, L. delbrueckii* subsp. *delbrueckii, L. mesenteroides* subsp. *mesenteroides/dextranicum, Limosilactobacillus fermentum, Lactococcus lactis, L. penthosus,* and *Lentilactobacillus buchneri* were identified species among 18 strains (Tangüler & Erten, 2013). Previously, Tangüler & Erten (2013) isolated three LAB species (*L. plantarum, L. paracasei* subsp. *paracasei,* and *L. fermentum*). They were separately utilized as a starter culture for shalgam production. When *L. plantarum* was used for the shalgam production as a starter culture, the highest amount of volatile organic compounds was generated in shalgam samples (Tangüler et al., 2017). From 42 commercial shalgam samples, 21 LAB with phenolic acid decarboxylase activity were identified via API 50 CHL test. *L. mesenteroides* subsp. *mesenteroides/dextranicum*, *L. plantarum*, *P. pentasaceus*, *L. acidophilus,* and *L. helveticus* were identified LAB species, *L. plantarum* was the most isolated and identified LAB at about 28.57% of all isolates among isolated strains (Okçu et al., 2016). Traditionally produced shalgams were kept for five days at 25 ˚C after the second fermentation to trace the source of biogenic amines found in shalgams. In the study, LAB isolation was performed during the second fermentation. API 50 CHL test-based characterization of traditionally produced shalgam samples demonstrated *Lactobacillus subsp*. (51 strains), *Lactococcus* subsp. (three strains), *Streptococcus* subsp. (one strain), and *Leuconostoc* subsp. (one strain) that can generate biogenic amine putrescine during the fermentation (Mete et al., 2017). Erginkaya and Turhan (2016) isolated ten bacteria and ten yeasts from two stages of traditional shalgam fermentation. The result of the identification of isolated bacteria that was applied onto API 50 CHL test strips indicated *L. plantarum* and *L. pentosus* as isolated LAB; *Saccharomyces cerevisiae* and *Candida krusei* as isolated yeasts in shalgam samples.

16S rRNA gene sequence-based identification of bacteria has been widely used as the gold standard. 16S rRNA gene of bacteria contains different vector regions that has different gene sequence. The variation within these vector regions that can be different from bacteria to bacteria makes 16S rRNA gene sequencing a nascent identification method for stratification (Weinroth et al., 2022). The very first culture-dependent characterization of shalgam based on 16S rRNA gene sequencing was reported *L. casei*, *L. plantarum*, *L. plantarum* subsp. *argentoratensis*, *L. acidophilus*, *L. brevis*, *L. helveticus*, *L. paracasei* subsp. *paracasei*, *L. paracasei* subsp. *tolerans*, *L. parabrevis*, *L. reuteri*, *L. delbrueckii* subsp. *lactis*, *L. delbrueckii* subsp. *delbrueckii*, *L. delbrueckii* subsp. *indicus*, *L. gasseri,* and *L. sharpeae* as LAB from shalgam (Baser et al., 2012). The effects of production methods and using starter cultures (*L. plantarum*, *L. fermentum,* and *L. paracasei* subsp. *paracasei*) on shalgam microbiota were sought in another study (Tangüler et al., 2015). The microbial isolation was carried out for laboratory-made shalgams, and samples were collected at the beginning and the end of the fermentation. of 38 isolated LAB, nine species including *L. mesenteroides* subsp. *mesenteroides*, *L. plantarum*, *P. pentosaceus*, *L. casei*, *Lactobacillus sp*., *L. buchneri*, *Ln. parabuchneri*, *L. brevis*, and *L. pentosus* were found in the study. Regardless of the production methods used in the study, it was interesting to note that even though *P. pentosaceus* and *L. mesenteroides* subsp. *mesenteroides* were present at the beginning of the fermentation, those strains were not found at the end of the fermentation (Tangüler et al., 2015). 21 LAB strains isolated from commercial shalgams were characterized through species-specific PCR, and then their stratifications were further confirmed by 16S rRNA gene sequencing. *L. plantarum*, *L. plantarum* subsp. *argentoratensis*, *L. casei*, *L. paracasei* subsp. *paracasei*, *L. sharpeae*, *L. brevis*, *L. parabrevis*, *L. reuteri*, *L. delbrueckii* subsp. *lactis*, *L. delbrueckii* subsp. *delbrueckii*, *L. delbrueckii* subsp. *indicus*, *L. helveticus*, *L. gasseri*, and *L. acidophilus* were reported in commercial shalgam samples (Ekinci et al., 2016).

60 isolated bacteria from shalgam and gilaburu were initially characterized by Fourier Transform Infrared (FTIR) to determine whether the isolates did belong to LAB or not. Having determined that 41 out of 60 isolated bacteria did belong to the *Lactobacillus* genera, 16S rRNA gene sequencing was performed for LAB isolated from shalgams and gilaburu. *L. plantarum, L. fermentum*, and *L. pentosus* were the three main strains identified in shalgams (Akman et al., 2021).

Yeasts, a part of the shalgam microbiota, have been isolated from shalgam samples as well. A study was conducted to identify yeasts and bacteria during the shalgam fermentation. Two-stage identification process was geared towards identifying 110 bacteria and 36 yeast isolated from traditionally produced shalgams. Initially, Rep-PCR with $(GTG)_5$ primers facilitated the identification of isolated microorganisms. Then, 16S rRNA and 26S gene sequencing were carried out for the identification of bacteria and yeast species, respectively. As indicated in the study, *L. plantarum* and *S. cerevisiae* were the only isolated strains at the end of the dough fermentation of traditionally produced shalgams. *L. plantarum*, *L. brevis, L. lactis*, *Bacillus circulans*, *Pantoea agglomerans*, *Staphylacoccus pasteuri*, *L. mesenteroides*, *Paenibacillus cucumis*, *Micrococcus yunnanensis*, and *Staphylococcus hominis* as bacteria; *S. cerevisiae* and *Pichia kudriavzevii* as yeasts were identified in shalgams (Kafkaskıray, 2020).

Kahve et al. (2022) performed yeast isolation and identified yeasts through Inter-priming binding sites (iPBS) retrotransposon marker system and ITS gene sequencing. iPBS retrotransposon marker system has been proposed to characterize yeasts, previously (Aydın et al., 2020;İbrahim et al., 2022). In this system, transposons, which are mobile and short DNA

fragments, are in different regions of the organisms` genome for adaptation to the environment and resistance to stressors. Transposons cause alterations in the genome, supporting organisms` phenotypical adaptation to any stressors in the living environment. Retrotransposons, a class of transposons, has been inserted a DNA fragment into the genome, in which the insertion is mediated by RNA. Therefore, the way of genomic DNA insertions (retrotransposons) would help organisms uphold a higher copy number of insertion DNA in the genome, enabling genetic diversity within yeasts (Boeke & Devine, 1998). By PCR-based amplification of inter-priming binding sites (iPBS) retrotransposons in the yeast genome, the identification of isolated yeasts has been carried out by Aydın et al. (2020). Similar to the 16S gene region, ITS (Internal Transcribed Spacer) gene sequencing has been carried out to identify isolated yeasts. With the help of iPBS system and confirmation by ITS gene sequencing, 172 yeasts isolated from shalgam samples were stratified. The sampling for shalgam characterization was performed at four different fermentation time periods (0,7,14, and 21 days) of four different commercial shalgams produced using traditional and direct methods. *Pichia kudriavzevii*, *Saccharomyces cerevisiae*, *Pichia fermentans*, *Candida oleophila*, *Kazachstania bulderi*, and *Geotrichum candidum* as yeasts were identified via iPBS. In the study, *Pichia* yeast was reported to be the most isolated yeasts at 77.9% of 172 isolated yeasts (Kahve et al., 2022).

Culture-independent microbial characterization of shalgam microbiota

Culturing the microbiota of fermented food might not be enough to reproduce the full spectrum of microbial diversity. The microbiota consists of a variety of cell types and states, such as intact, viable, non-viable, autolyzed cells, and cell lysates. In culture-independent characterization, it is likely to identify those cells within the microbial community of a fermented food (Carraro et al., 2011). Ekici et al. (2022) conducted the very first and the only culture-independent characterization of traditionally fermented shalgam microbiota. Towards that end, the microbial diversity in shalgam that is traditionally produced was investigated by taking samples from six time points (3, 7, 10, 13, 17, and 20 days) during 20 days of the second fermentation. The bacterial identification without microbial isolation was performed via DNA extraction and PCR amplification of 16S rRNA V4-V5 vectors. For yeast identification in shalgam samples, the ITS2 gene from extracted DNA extracts was amplified via PCR. After PCR-amplified DNA samples were sequenced, *Candida boidinii,* and *Saccharomyces cerevisiae*, *L. mesenteroides* and *L. lactis* were reported as dominant yeast and bacteria species, respectively (Ekici et al., 2022). On the contrary to the culture-independent characterization of shalgam samples, in the culture-dependent characterization of shalgam, Arici (2004) pointed out *L. plantarum* and *L. paracasei* subsp. *paracasei* as dominant LAB species. To the best of our knowledge, never have 13 LAB species and 15 yeasts found in this study been reported in shalgam, previously. It is also interesting to note that even though *Weisella* species have never been reported in culture-dependent characterization of shalgam, the culture-independent characterization of shalgam demonstrated *Weisella* presence in shalgam microbiota for the first time. As compared to the culture-dependent characterization of shalgam microbiota, *Pichia* and *Lacticasei* group bacteria including *L. paracasei, L.casei* were not found in the metabarcoding analysis (Ekici et al., 2022). Through the metagenomic analysis of shalgam samples, 35 bacteria and seven yeasts were found at the end of shalgam fermentation. With the help of culture-independent characterization of shalgam samples, it was found that the bacterial diversity in shalgam fluctuated dynamically during the fermentation while yeast diversity in the microbiota remained less fluctuated as compared to bacterial species (Ekici et al., 2022). At **Table 1**, the identified LAB and yeast species were collated and classified as culture-dependent and culture-independent characterizations of shalgam samples microbiota so that it can be inferred that characterization methods might lead to the identification of different microorganisms in shalgam.

Bioinformatic analysis of whole genome sequenced LAB isolated from shalgam

Having resilience in dealing with harsh conditions of gastrointestinal transit and the capability to bind intestinal mucosa, probiotics confer health benefits (Gumustop & Ortakci, 2022). Isolated microorganisms from shalgam might carry probiotic traits and be robust under the gastrointestinal conditions. *In vitro* assays of isolated *Pichia kudriavzevii* (Gumustop & Ortakci, 2022), *L. plantarum* subsp. *plantarum* W2, *L. fermentum* Akhavan E3, and *L. Pentosus* XL963 (Akman et al., 2021) were carried out to vet the probiotic traits of isolated microorganisms. Recently, three isolated LAB strains` (*L. plantarum* DY46, *Liquorilactobacillus nagelii* AGA58, and *L. fermentum* AGA52) genomes were sequenced and genomic data were evaluated *in silico* (Yetiman et al., 2022, 2023; Yetiman & Ortakçı, 2023). For *L. fermentum* AGA52 isolated from shalgam, *in silico* analysis of the probiotic traits, carbohydrate utilization capacity, bacteriophage resistance, antioxidant capacity, and *in vitro* analysis of the ability of cholesterol degradation, and gamma amino butyric acid (GABA) producing capability were appraised.

On the basis of the results obtained from *in silico* and *in vitro* assays, the ability to utilize cholesterol and produce GABA corroborated that *L. fermentum* AGA52 had probiotic attributes (Yetiman et al., 2023). In line with that, as a result of bioinformatic analysis of *L. plantarum* DY46 genome isolated from shalgam, antibiotic resistance genes were present in its genome. Moreover, its genome consisted of a plantaricin-

Table 1. The list of LAB and yeasts identified based on culture-dependent and culture-independent microbial characterization methods

Culture-dependent microbial identification	
LAB	*Lactiplantibacillus plantarum, Limosilactobacillus fermentum*, Lacticaseibacillus paracasei subsp. paracasei*, Lacticaseibacillus casei*, Lacticaseibacillus paracasei subsp.tolerans*, Levilactobacillus brevis, Lactiplantibacillus plantarum subsp. agentoratensis*, Lentilactobacillus buchneri*, Lentilactobacillus parabuchneri*, Pediococcus pentosaceus*, Leuconostoc mesenteroides, Lactococcus lactis, Lactobacillus coryniformis*, Lactobacillus delbrueckii subsp. delbrueckii*, Leuconostoc mesenteroides subsp. jonggajibkimchii*, Lactiplantibacillus paraplantarum*, Liquorilactobacillus nagelii** and *Lactiplantibacillus pentosus**
Yeasts	*Saccharomyces cerevisiae, Candida krusei, Pichia kudriavzevii*, Pichia fermentans*, Candida oleophila*, Kazachstania bulderi** and *Geotrichum candidum**
Culture-independent microbial identification	
LAB based on Meta-barcoding metagenomics analysis	*Leuconostoc mesenteroides, Lactococcus lactis, Leuconostoc pseudomesenteroides*, Leuconostoc inhae*, Weissella confusa*, Lactococcus raffinolactis*, Leuconostoc kimchii*, Leuconostoc lactis*, Lactiplantibacillus plantarum, Lactococcus piscium*, Weissella soli*, Levilactobacillus brevis, Lactobacillus curvatus*, Lactococcus garvieae*, Leuconostoc fallax*, Liquorilactobacillus nagelii, Lactobacillus paracollinoides** and *Lactobacillus paracollinoides**
Yeasts based on Meta-barcoding metagenomics analysis	*Saccharomyces cerevisiae, Candida Bodinii*, Wickerhamomyces anomalus*, Rhodotorula mucilaginosa*, Barnettozyma californica*, Trichosporon coremiiforme*, Typhula ishikariensis*, Naganishia albida*, Rhodotorula glutinis*, Meyerozyma guilliermondii*, Trebouxia sp. *, Acremonium antarcticum*, Sporidiobolus salmonicolor*, Candida humilis*, Malassezia restricta** and *Rhodotorula diobovata**

Strains with asterisk **(*)** indicate that the isolated species were only found in the characterization methods used in the table.

producing gene cluster, except for the gene that can produce Pln J peptide (Yetiman et al., 2022). The same group reported that they managed to isolate *Liquorilactobacillus nagelii* from shalgam. The *Liquorilactobacillus nagelii* AGA58 genome and probiotic traits have been evaluated *in silico* and *in vitro*. As a result of bioinformatic analysis on the *Liquorilactobacillus nagelii* AGA58 genome, *Liquorilactobacillus nagelii* AGA58 carried an A2 lantipeptide producing gene (Yetiman & Ortakçı, 2023). In the light of *in vitro* and *in silico* analysis of shalgam-originated LAB that might confer probiotic traits, it can be purported that shalgam might be considered as a probiotic beverage.

The effect of different salts on the shalgam microbiota

The mineral content of shalgam determined by Inductively Cold Plasma (ICP) analysis has been reported in the published literature (Ağırman et al., 2021; Demir et al., 2004; Özdemir-Alper & Acar, 1996; Yılmaz-Ersan & Turan, 2012). Minor and major elements in shalgam have been found via ICP-OES (Inductively Cold Plasma-Optical Emission Spectrometry) analysis. In terms of abundance and detectability in shalgam samples, Na, K, Ca, Mg, and P were considered as major elements while minor elements were indicated as Fe, Cu, Zn, Ni, Mn, Pb, and Sn (Yılmaz-Ersan & Turan, 2012). When Aspergillus aculeatus Pectinex Ultra SP-L enzyme and citric acid were added into the shalgam fermentation, ICP analysis demonstrated shalgam samples treated with the enzyme and citric acid contained Na, K, Ca, Mg, and Fe elements. Also, the highest Mg levels in shalgam samples were archived when Pectinex Ultra SP-L enzyme and citric acid were present during the fermentation (Demir et al., 2004).

To lower the sodium chloride (NaCl) concentration in shalgam, when 1.5% NaCl was used, shalgam was prepared with black carrot juice concentrate and whey was the most appealing in the sensorial evaluation of shalgams (Güven et al., 2019). In the face of the health hazards of excessive salt consumption, it has been targeted to reduce salt content and/or substitute table salts with other salts used in shalgam production (Ağırman & Erten, 2018, Ağırman et al., 2021; Güven et al., 2019). Such actions to overcome health concerns related to NaCl reduction might lead to alterations in the aroma profile of fermented food as exemplified in cheese (Brandsma et al., 2022). The sodium salts used in the fermentation of shalgam were replaced with calcium and potassium salts and following that the effect of the salts on the microbiota of shalgam was investigated through culture-dependent characterization of shalgam samples. The highest amount of LAB was found when NaCl and potassium chloride (KCl) were used together in shalgam production. It has also been observed that different salts did not have any effect on the number of LAB colonies in the dough fermentation of shalgam. However, a decrease of 2 log CFU/mL in the total number of mesophilic aerobic bacteria was observed in all salts during shalgam fermentation (Ağırman & Erten, 2018). In the following study of the same group, shalgam samples totally containing 1.5% chloride salts prepared with KCl+$CaCl_2$ (50%+50%) and KCl+$CaCl_2$+NaCl (33.3%+33.3%+33.3%) salt mixes were culture-dependently characterized. The stratifications in the

study were first performed by RAPD-PCR to sort out LAB among isolates and followed by 16S rRNA gene sequencing. L. paracasei was found in the water extract from the first fermentation and eight-day fermented shalgams prepared with NaCl+KCl (50%+50%), $NaCl+CaCl_2$ (50%+50%), and $NaCl+KCl+CaCl_2$ (33.3%+33.3%+33.3%) salt mixtures, indicating that L. paracasei was the most persistent strain found through shalgam production. L. paracasei was identified throughout the fermentation in the presence of $CaCl_2$ and KCl while the shalgam fermentation with NaCl salt did not help L. paracasei remain in the second and the fourth day of shalgam fermentation. Throughout the shalgam fermentation prepared with $KCl+CaCl_2$ (50%+50%) and $NaCl+KCl+CaCl_2$ (33.3%+33.3%+33.3%) salt mixtures, L. lactis isolated from shalgams was the most persistent strain. It was interesting that L. pentosus and L. coryniformis were isolated when only NaCl (100%) salt was used in the shalgam fermentation (Ağırman et al., 2021).

Even though the types of salts` effects on shalgam microbiota were elucidated through culture-dependent characterization of shalgams made with different salts and salts mixtures, little did we know how other mineral contents of shalgam would alter shalgam microbiota throughout the fermentation.

Conclusion

In culture-based characterization of shalgam, the isolation of LAB has been the focal point. With the help of ever-growing state-of-art technologies such as next-generation sequencing Nanopore and PacBio, and MALDI-TOF-MS as a phenotype-based identification method, more comprehensive microbiota characterization of shalgam can be applied in the future. The structure-function relationship in a fermented food, which shows the contribution of microorganisms to the production of compounds generated during fermentation, has not yet been fully exploited in shalgam. Recently, research on unraveling which microorganisms can produce which aromatic compounds has been carried out through co-occurrence and network analysis in fermented foods (Wang et al., 2019; Wu et al., 2022). Also, finding the core microbiota of autochthonous fermented foods has accelerated the development of autochthonous starter cultures (Wu et al., 2022). Also, experimental studies intertwining the production of aroma components and the microbiota in shalgam have not been conducted yet. By conducting metagenomic analysis and *in silico* analysis of the metagenomic data, it will be possible to reveal which microorganisms in shalgam can produce anthocyanins, aroma components, phenolic compounds.

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