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Occurrence and growth of lactic acid bacteria species during the fermentation of shalgam (salgam), a traditional Turkish fermented beverage

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ABSTRACT

Shalgam (salgam) is a traditional lactic acid beverage produced from the lactic acid fermentation of black carrot, turnip, rock-salt, sourdough, bulgur flour and drinkable water. The aim of this study was to examine quantitatively the occurrence and growth of lactic acid bacteria during the course of fermentation using the traditional production method consisting of two stages: First fermentation and second fermentation. Isolated strains from experiments made at university laboratory, and large and small scale production plants in industry were identified by morphological, physiological and biochemical characterisations and using the commercially available system API 50 CH for the characterisation of carbohydrate fermentation patterns. The number of LAB increased during the fermentations. The most dominant LAB during the first and second fermentations was *Lactobacillus plantarum*. Next to *L. plantarum, Lactobacillus paracasei* subsp. *paracasei* was quantitatively the most important LAB recovered from all fermentations. *Lactobacillus brevis* and *Lactobacillus fermentum* were also subsequently determined in some fermentations. Low populations of *Leuconostoc mesenteroides* subsp. *mesenteroides*, *Pediococcus pentosaceaceus*, and *Lactobacillus delbrueckii* subsp. *delbrueckii* were present at the beginning but died off during the fermentation.

1. Introduction

Alcoholic and non-alcoholic fermented beverages are produced throughout the world. Some beverages produced through alcoholic fermentation such as wine and beer are of great commercial importance worldwide (Waites, Morgan, Rockey, & Higton, 2001, 288 pp.). Some fermented beverages obtained as a result of lactic acid or lactic acid and alcohol fermentations are of minor economic importance in global terms. They are, however, produced commercially in some countries. These beverages may be exemplified as ayran (Ozer, 2006, 488 pp.), boza (Zorba, Hancioglu, Genc, Karapinar, & Ova, 2003), kefir, koumiss (Tamime & Marshall, 1997), shalgam (salgam) (Erten, Tanguler, & Canbas, 2008).

Shalgam, a traditional lactic acid fermented beverage, is a red coloured, cloudy and sour soft drink; it is mainly produced in some provinces of Southern Turkey, especially in Adana, Mersin, Hatay and Kahramanmaras in homes, villages and industries. Shalgam is widely consumed with food and as a refreshing drink in mainly these provinces. It has also become a popular beverage in such metropolitan cities as Istanbul, Ankara and Izmir Metropolies (Canbas & Deryaoglu, 1993; Canbas & Fenercioglu, 1984).

The raw materials used for shalgam production are black carrot (*Daucus carota* ssp. *sativus* var. *atrorubens* Alef.) which is the main raw material, turnip (*Brassica rapa* L.), rock-salt, sourdough, bulgur flour and drinkable water (Erten et al., 2008).

Although no standard manufacturing method is available for shalgam production and processing is different from one plant to another, there are two main processing methods for commercial production: the traditional method and the direct method (Erten et al., 2008).

The traditional method consists of two stages: First (Dough) fermentation and second (Carrot or main) fermentation. The first fermentation is performed for the enrichment of lactic acid bacteria (LAB) and yeasts. The mixture of bulgur flour, salt, sourdough and adequate water is left for fermentation at room temperature for 3–5 days. The fermented mixture of bulgur flour and sourdough is extracted with adequate water for three to five times. The extracts obtained from first fermentation are combined with sorted and chopped black carrot, salt, if available sliced turnip and adequate water in a tank for second fermentation. Second fermentation is naturally carried out for 3–10 days at ambient temperature. After

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the completion of fermentation, fermented juice is removed from the vessel and marketed. In direct production method, first fermentation (Dough fermentation) is not carried out. The chopped black carrots, salt, if available sliced turnip, bakers' yeast (*Saccharomyces cerevisiae*) or sourdough and adequate water are transferred to a tank and allowed to ferment at ambient temperature (Erten et al., 2008).

It is generally accepted that the main fermentation agents of shalgam are LAB which are responsible for the acidification process by converting sugars into mainly lactic acid and other end compounds, giving shalgam its typical taste and flavour (Erten et al., 2008). LAB can be divided into two groups: homofermentative and heterofermentative. Homofermentative LAB such as some *Lactobacillus* (*L.*), *Pediococcus* (*P.*), *Lactococcus* (*Lac.*) and *Streptococcus* produce lactic acid as the major or sole metabolic end product of glucose fermentation using Embden-Meyerhof-Parnas pathway. Heterofermentatif LAB such as *Leuconostocs* (*Leu.*), *Oenococcus*, *Weissella* and some *Lactobacillus* form equimolar amounts of lactate, CO_2 and ethanol or acetate depending on air via hexose monophosphate or pentose pathway (Blandio, Al-Aseeri, Pandiella, Cantero, & Webb, 2003; Caplice & Fitzgerald, 1999; Holzapfel & Wood, 1995). Lactobacilli play an important role in several food fermentations such as vegetables, meats and dairy products (Bernerdau, Vernoux, Henri-Dubernet, & Gueguen, 2008; Caplice & Fitzgerald, 1999). They are classified within the three groups: obligately homofermentative, facultatively heterofermentative and obligately heterofermentative lactobacilli. They are generally most-acid tolerant of LAB (Axelsson, 1998; Hammes & Vogel, 1995; Stiles & Holzapfel, 1997). Lactic acid fermented cucumber and olive fermentations are dominated and terminated primarily by *Lactobacillus plantarum* (Harris, 1998). *L. plantarum* is a facultatively heterofermentative lactobacilli (Axelsson, 1998; Hammes & Vogel, 1995; Stiles & Holzapfel, 1997).

Shalgam production and consumption are increasing in Turkey. Its quality is closely related to the microbial ecology, mainly LAB, of fermentation. As we are aware, there are no available commercial LAB starter cultures isolated from their natural environments to do shalgam fermentations. Shalgam is produced with uncontrolled fermentation which leads to a variation in terms of quality and stability of the product. Therefore, it is important to select the starter cultures for the controlled fermentations to produce a better product.

Despite this fundamental contribution of microorganisms, shalgam microbiology is complex and not known in detail (Erten et al., 2008). This is the first study about identification of LAB that develops during dough fermentation. On the other hand, very limited studies (Arıcı, 2004; Erginkaya & Hammes, 1992) have identified some species of LAB that develop during carrot fermentation. However, these studies are qualitative in contribution and quantitative knowledge describing the development of individual LAB species during fermentation is lacking. The objective of this research was to examine the quantitative development of individual LAB species during fermentation of shalgam produced in laboratory, by small scale and large scale producers in industry. Those species isolated in present study will be the source to select the starter cultures for future studies and then for industrial usage.

2. Materials and methods

2.1. Materials

For shalgam production, black carrot and turnip were kindly provided by Icenbilir Shalgam Company (Adana, Turkey). Other raw materials were purchased from market.

2.2. Methods

2.2.1. Shalgam production

Shalgam A was produced at the Biotechnology Laboratory of Department of Food Engineering, Cukurova University. Shalgams B and C were produced by commercial large and small scale producers in industry, respectively.

Shalgam A production by traditional method was carried out according to Erten et al. (2008). Traditional method consisted of two stages. At stage one (first or dough fermentation), bulgur flour (30 g L^{-1}), rock-salt (2 g L^{-1}), sourdough (2 g L^{-1}) made with the incubation of baker's yeast at 30 °C for 24 h and adequate drinkable water were mixed and kneaded for the formation of dough. The dough obtained was fermented in a tank at 25 °C for 3 days. After this time, about 20 L of water was added into fermented mixture, blended well and extracted for 15 min. The extraction was done four times. The extracts obtained from the first fermentation were combined to perform the second (carrot or main) fermentation with sorted and chopped black carrots (150 g L^{-1}), rock-salt (10 g L^{-1}), sliced turnip (10 g L^{-1}) in a 100 L of closed stainless steel tank. If necessary, adequate water was added to fill the tank. Fermentation was carried out at 25 °C and followed daily by measuring pH and total acidity as lactic acid. Shalgam A was produced in triplicate at the laboratory.

Commercial samples, shalgam B from large scale producer and shalgam C from small scale producer were also made by traditional method according to the producers in industry. Samples were only taken during the second fermentations which were carried out at room temperature.

2.2.2. Enumeration and isolation of LAB

During the first (dough) fermentation, daily samples of 25 g of dough were dispersed aseptically into 225 mL of sterile physiological saline (8.5 g L^{-1}) and mixed thoroughly. During the second (carrot) fermentation, shalgam samples (200 mL) were taken from the centre of 100 L of fermentation vessel. Samples were serially diluted (10^{-1} to 10^{-8}) in sterile physiological saline and spread-inoculated (0.1 mL) onto plates of de Man, Ragosa, Sharpe (MRS) Agar (Merck AG, Darmstadt, Germany), supplemented with 50 mg L^{-1} cycloheximide to prevent the growth of yeasts and moulds. Plates were incubated at 30 °C for 3–5 days in jars made anaerobic with GasPaks (Anaerocult® A, Merck AG, Darmstadt, Germany) for colony development. The LAB colonies were counted on their morphology from the plates and various colony types were selected and purified by restreaking three times on MRS agar (without cycloheximide) to obtain pure cultures. Gram-positive and catalase-negative isolates were maintained as liquid cultures in MRS broth with 200 g L^{-1} sterilized glycerol at −20 °C for subsequent identification (Bae, Fleet, & Heard, 2006; Coulin, Farah, Assavo, Spillmann, & Puhan, 2006; Ozcangaz, 2000, p. 101; Vieira-Dalodé et al., 2007).

2.2.3. Characterisation of isolates of LAB

A total of 312 isolates were identified by morphological, physiological and biochemical characterisation. Isolates were tested for Gram reaction, catalase formation, cell morphology, CO_2 production from glucose, ability to grow at 10 °C and 45 °C and at pH 4.4 and 9.6, tolerance to 65 g L^{-1} and 180 g L^{-1} salt, hydrolysis of arginine, nitrate reduction, acetoin formation and MR-VP test. Subsequently, API 50CH galleries and API CHL medium (BioMérieux, France) were used according to the manufacturer's instructions for determining the utilization of 49 carbohydrates of isolated strains. APILAB PLUS database identification software (BioMérieux, France) was used to interpret the results (Harrigan & McCance, 1990, 452 pp.; Randazzo, Heilig, Restuccia, Giudici, &