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Original article

Identification of lactic acid bacteria isolated from Tarkhineh, a traditional Iranian fermented food

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ABSTRACT

Tarkhineh is a traditional Iranian fermented product produced from a mixture of doogh and wheat grout. The purposes of the present study were identifying of lactic acid bacteria (LAB) isolated and Changes of lactic acid bacteria flora throughout spontaneous fermentation of Tarkhineh. Results have shown a total of ten strains of LAB were isolated from Tarkhineh on the 3th day of fermentation using MRS agar plates and identified on the basis of morphological, biochemical, and physiological characteristics. The isolates were identified as *L.nagelii(67%), L.bifermentans(21.3%), Leu.cermoris(6%), L.fructosus(1.45%), L.fermentum(1%), L.intestinalis(0.9%), L.agilis(0.9%) L.acidipiscis(0.9%)* was reported, and approximately %1 of isolated samples remained unknown. The naturally occurring lactic acid bacteria load was found to vary between 1.97×10^5 and 4.3×10^5 cfu/gr. The main source of lactic acid bacteria was found to be the doogh.

1. Introduction

Fermented milk-cereal mixtures play an important role in the diets of many people in the Middle East (Ibanoglu etal., 1996). Tarkhineh is one of them. It is a traditional Iranian food, generally prepared with wheat grout, doogh(churned sour milk) and turnip in the ratio of 1:2.8:0.3 The mixture is kneaded with vegetables, salt, leaven and spices, then fermented with doogh bacteria and yeast. it fermented for 3-6 days. After fermentation the mixture is sun dried, Tarkhineh could be stored for 1-2 years under dry and cold storage conditions. It consumed as soup by cooking in water. Tarkhineh-like food consumed in other countries such as Iraq, Turkey and Egypt.

Fermented milk products have always been considered a highly nutritious food containing significant concentrations of protein, fat, minerals and micronutrients. The doogh makes up for the amino acids limited in cereal (lysine and methionine) and wheat grout (par-boiled cracked wheat) is a good source of certain minerals (Fe, Cu and Mn) that are deficient in milk (Tamime et al., 1999).

Tarhana is a good source of minerals, organic acids and free amino acids which make it healthy for children, the elderly and medical patients. In addition, it is a good source of vitamins such as thiamine, riboflavin and vitamin B12 (Ibanoglu *et al*. 1995). Ascorbic acid, niacin, pantothenic and folic acid are also present (Ekinci, 2005, Ekinci and Kadakal 2005). Lacticacid bacteria (LAB) from Doogh also aid in absorption of nutrients, which would otherwise, be indigestible or poorly digestible. LABs are a group of gram-positive bacteria linked to a constellation of morphological, metabolic and physiological characteristics. They are included in the group of non-spore forming, non-respiring cocci or rods, cataase-negative, devoid of cytochromes; non-aerobic but aero-tolerant fastidious, acid tolerant and strictly fermentative with lactic acid as the major end products during the fermentation of carbohydrates. LABs are widely distributed in nature. They have been isolated from grains, green plants, dairy and meat products, fermenting vegetables (Salminen etal,. 2011) and traditional Iranian foods such as Tarkhineh.

The purpose of this study was to determine the amount of micro flora of Tarkhineh and Isolation and Identification Lactic Acid Bacteria (LAB) from it. Another purpose is studying Changes in microbiological composition of Tarkhineh during fermentation. The identity of the culture was based on characteristics of the Lactic Acid Bacteria as presented in Bergey's Manual of Determinative Bacteriology, carrying out microscopy (morphology), Gram straining and fermentation of different carbon sources.

2. Materials and methods

2.1. Materials

In this study, the ingredients (wheat, doogh, yeast, turnip, salt and leaven) used in Tarkhineh preparation were purchased from local markets in Mashhad, Iran. The protein content of wheat, based on total weight, was 12.2%. The doogh used was (pH 3.7) made from cow's milk and had a fat content of 3.0% (wet basis). Yeast was baker's yeast in active dry form.

2.2. Production of Tarkhineh

Production method of Tarkhineh with ingredients ratio is presented in Figure (1) and table (1). Turnip was grated and blended with doogh, salt and leaven. Then wheat grout added to this mixture. They were kneaded in a bowl for formed Tarkhineh dough. At the end of kneading, it was separated in to tree parts. first part (a) of dough was fermented at 10^0C, the second part (b) was fermented at 20^0C and the third part was fermented at 37^0C for 3 days in an incubator. It was kneaded daily to facilitate fermentation. Samples were taken at 0th, 1st, 2nd, and 3rd days of fermentation to investigate the chemical and microbiological changes. Before fermentation, all samples were filled in small jars and closed. Samples were analyzed at 0th, 1st, 2nd, 3rd days of the storage period (Erbaş etal., 2005).

2.3. Samples

In the present study, 6 samples of laboratory fermented Tarkhineh were prepared. After collection, samples were transported to the laboratory and analyzed. 25g from samples were homogenized with 225mL sterile sodium citrate solution 2% (w/v), in a Stomacher 400 (Seward Medical, London, UK). Serial decimal dilutions (10^{-2} to 10^{-5})

were made in 0.1% (w/v) peptone solution.(Abdi etal.,2006) Decimal dilution of these samples were mixed with MRS medium (AEB, France) and incubated at 37ºC for 48-72 h under anaerobic conditions.(Lengkey etal., 2009) The numbers of LAB were measured by the plate count on MRS agar (Difco Laboratories, Detroit, USA) mold and yeast were counted on Potato Dextrose agar (Nissui) incubated for 72 h at 30°C. Each LAB colony was purified twice by streaking on MRS agar. Colonies were counted as viable numbers of Microorganisms (colony forming unit (CFU) g^{-1} of Tarkhineh) (Duan etal., 2008)0.1 mL of the diluents were streaked on Nutrient agar for total bacteria counts.

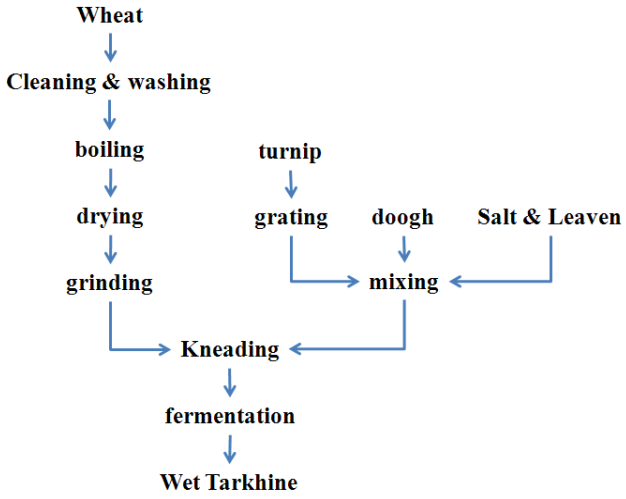

Table 1
Ingredients ratio of Tarkhineh

Ingredient	%	
	Type 1	Type 2
wheat grout	23.7	23.66
Doogh	66	65.7
Turnip	8	7.89
Leaven	0.5	0.47
Salt	1.8	2.28

Fig. 1. Flow diagram for traditional Tarkhineh production.

2.4. Determination of ph values and acidity of the test samples

The pH values of each sampels were determined at 25C using a pH meter (WTW-Inolab Level 3 Terminal, Weilheim, Germany) (Yilmaz etal., 2010). Acid content was determined by titrating with 0.1N NaOH with the indicator of phenoluphetalin until it's colure changes to pink.

2.5. Morphological, physiological, and biochemical tests

Gram stain of LAB and morphological characteristics were determined after 48 h of incubation on MRS agar. Catalase activity and gas production from glucose were determined (Duan etal., 2008). Growth at different temperatures was detected in MRS agar after incubation at 15 and 45^0c. Fermentation of Carbohydrates was determined. The carbohydrates tested were, D(+) galactose, lactose, fructose, maltose, D mannitol, melibiose, D(-) raffinose, sorbitol, D(+) (Merck, Darmstadt, Germany), and glucose (Erdogrul etal., 2006). Gas production from glucose were tested in phenol red broth with 2% glucose (Abdi etal.,2006).

3- Results and Discussion

Results of Microbial analysis and physicochemical tests are given in Table (2). After series of purification on MRS agar, 400 isolates were found to be Gram-positive and catalase negative. The isolates were identified as *L.nagelii(67%), L.bifermentans(21.3%), Leu.cermoris(6%), L.fructosus(1.45%), L.fermentum(1%), L.intestinalis(0.9%), L.agilis(0.9%)* , *L.acidipiscis(0.9%),* and approximately %1 of isolated samples remained unknown. Frequency of Lactobacillus has been reported in various food components (Torres etal, 2006). Isolates (1, 8), (L.fructosus), Isolates (2), (L.fermentum), Isolates (4), (Leu.cermoris) , Isolates (5), (L.intestinalis) ,. Isolates (6), (L.agilis), Isolates (7), (L.acidipiscis) Isolates (9), (L.nagelii) and Isolates (10), (L.bifermentans) was diagnose Isolates (3), was not identified. (Figure 4 to 12). The microbial changes in during laboratory fermentation of Tarkhineh are shown in Figure2. The LAB counts on MRS increased from 1.97×10^5 to 4.3×10^5 cfu/gr. The largest increase in the numbers of LAB was noted during the first 24 h of fermentation and further incubation led to decrease. The yeast counts

increased from 1.04×10^5 to 3×10^4 cfu/gr. The pH changes of Tarkhineh are shown in Figure3. In the present investigation, the pH of tharkhineh ranged between 4.19 and 4.43. Using phenotypic methods for identifying LAB isolated during production of Tarkhineh under laboratory conditions Yilmazer (1994) isolated S. thermophilus, P. pentosaceus, L. mesenteroides, L. casei spp. casei, L. curvatus, L. delbrueckii spp. bulgaricus, L. helveticus, L. fermentum, L. cellobiosus, L. brevis while 10.5% of the isolates could not be identified with the phenotypic methods used. In a similar study, Lazos et al.(1993), using phenotypic methods, found S. lactis, S. diacetylactis, L. cremoris, L. lactis, L. casei, L. delbrueckii spp. bulgaricus and L. acidophilus to be the predominant LAB. Earlier phenotypically based studies investigating the LAB present in Kishk (final product), a product similar to Tarkhineh, revealed that the product mainly contained L. brevis,L. plantarum and L. casei (Morcos et al., 1973; El-Gendy, 1983) The results are consistent with previous studies, but there are differences between strains of Lactobacillus, due to differences in the microbial load of used doogh as and the type and amount of material in the areas (Sengun etal., 2009).

Gobbetti et al., (1994) proposed that lactic acid bacteria create an acidic environment conducive to yeast proliferation while the yeasts provide vitamins and other growth factors such as amino acids for the lactic acid bacteria. The simultaneous increase in numbers of both LAB and yeasts may therefore be attributed to their symbiotic association the results are in agreement with those reported in study. The decrease in pH and increase in lactic acid followed the same trend as reported for other natural fermented foods (Mohammed et al., 1991; Choi et al., 1994).

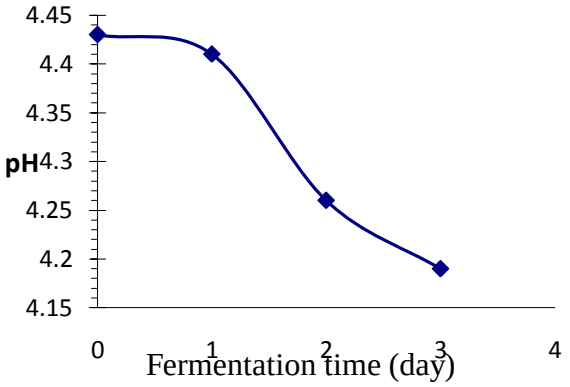

Fig. 3. Changes in pH during fermentation

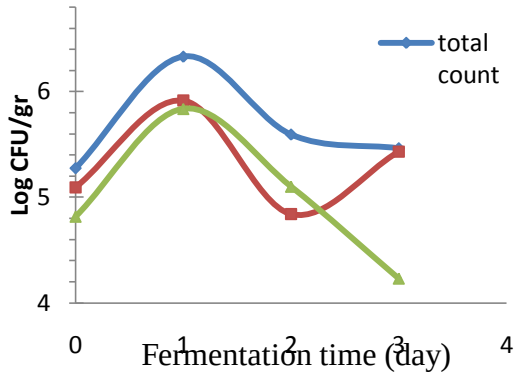

Fig.2. Changes in M.O during fermentation

Lactobacillus highest frequency among the identified material was to be allocated. Greater presence of Lactobacillus in fermented dairy products has been reported in various. Another reason for the difference of microflora appeared among various fermented products in different regions was probably related to chemical and physical factors, including substrates, NaCl concentration and fermentation temperature (Cho etal., 2006; Wood, 1998). Due to the high content of lactic acid bacteria in the Tarkhineh , a traditional Iranian fermented food, industrial production and the optimization of the Tarkhineh's production process is proposed and we can use this food as a dietary supplement and a functional food.

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Table 2
Biochemical characteristics and suger test of LAB isolated from Tarkhineh and comparison with reference LAB strains.

code	Catalaze test	Geram test	Gas from glucose	raffinose	gluconate	melibiose	lactose	sorbitol	sucrose	fructose	galactose	mannitol	maltose	glucose
1	-	+	-	-	-	-	-	-	-	-[1]	-	-	-	+
2	-	+	-	+	+	+	+	-	+	+	+	-	+	+
3	-	+	-	+	-	+	-	-	-	-	-	d	-	+
4	-	+	-	-[2]	-[2]	-	+[1]	-[2]	+[1]	-	d	-	d	+
5	-	+	-	+	-[2]	-[1]	-[2]	-	+	-[2]	+[2]	+	-[2]	+
6	-	+	-	+	-	-[1]	+	-	+	-[1]	+	+	+	+
7	-	+	-	-	-	-	-[2]	-	+	+[2]	+[2]	+	-[2]	-[1]
8	-	+	-	-	-	-	-	-	-	-[1]	-	-	-	+
9	-	+	-	-	-[2]	-	-	-[2]	+	+[2]	+	+	+[2]	+
10	-	+	-	+[1]	-	-	-	-	-	+	-[1]	+	+	+[1]

Was not corresponding with results reported in the Bergey's Manual.
This sugar was not examined in Bergey's Manual.
d: delay.

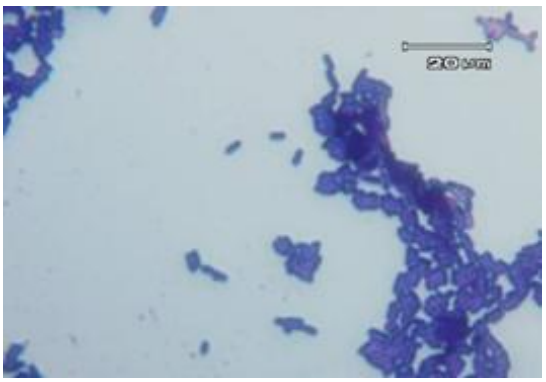

Fig. 4. Isolates (1, 8), (L.fructosus).

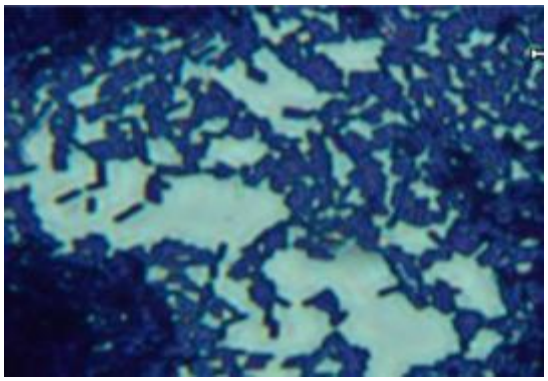

Fig. 5. Isolates (2), (L.fermentum).

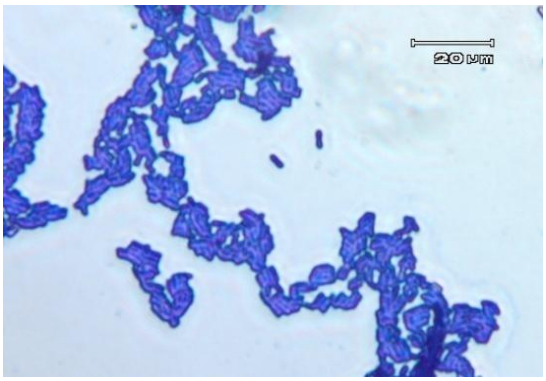

Fig. 6. Isolates (3), was not identified.

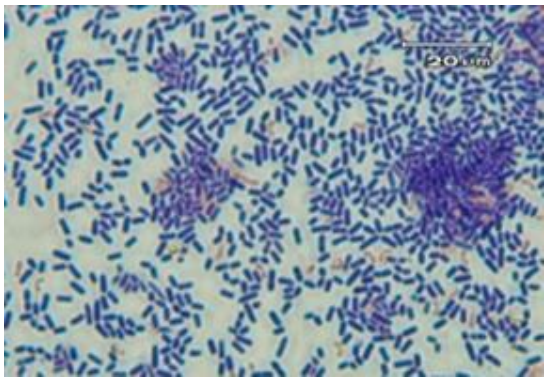

Fig. 7. Isolates (4), (Leu.cermoris).

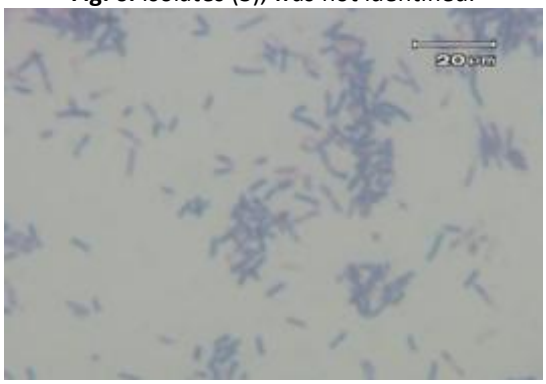

Fig. 8. Isolates (5), (L.intestinalis).

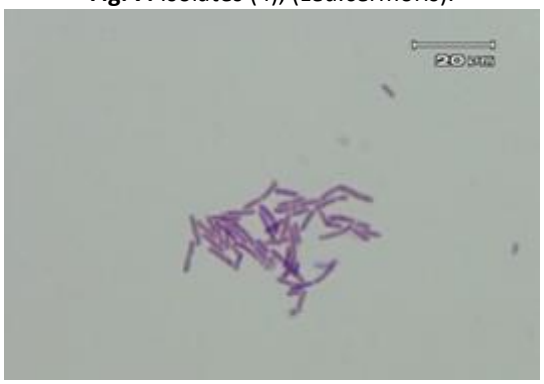

Fig. 9. Isolates (6), (L.agilis).

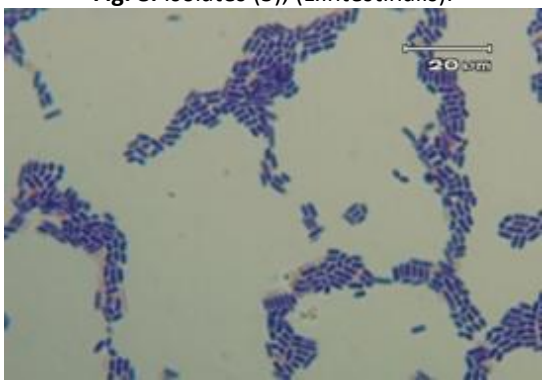

Fig. 10. Isolates (7), (L.acidipiscis).

Fig. 11. Isolates (9), (L.nagelii).

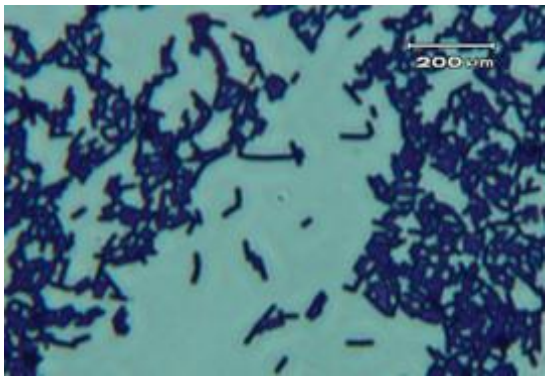

Fig. 12. Isolates (10), (L.bifermentans)

References

Abdi, R., Sheikh-Zeinoddin, M., Soleimanian-Zad, S. (2006). Identification of lactic acid bacteria from traditiona Iranian lighvan cheese, Pakestanjornal of biological science, 9, 99-103.

Cho, J., Lee, D., Yang, C., Jeon, J., Kim, J., Han, H. (2006).Microbial population dynamics of kimchi, a fermented cabbage product.*FEMS microbiology letters, 257*(2), 262-267.

Choi, S.Y., Beuchat, L.R., Perkins, L.M., Nakayama, T. (1994). Fermentation and sensory characteristics of kimchi containing potassium chloride as a partial replacement for sodium chloride. *International journal of food microbiology, 21*(4), 335-340.

Duan, Y., Tan, Z., Wang, Y., Li, Z., Qin, G., Huo, Y., et al. (2008). Identification and characterization of lactic acid bacteria isolated from Tibetan Qula cheese. *The Journal of general and applied microbiology, 54*(1), 51-60.

Ekinci, R. (2005). The effect of fermentation and drying on the water-soluble vitamin content of tarhana, a traditional Turkish cereal food.*Food chemistry, 90*(1), 127-132.

Ekinci, R., Kadakal, C. (2005).Determination of seven water-soluble vitamins in tarhana, a traditional Turkish cereal food, by high-performance liquid chromatography.*ACTA chromatographica, 15*, 289.

El-Gendy, S.M. (1983). Fermented foods of Egypt and the Middle East. Journal of Food Protection 46, 358–367.

Erbaş, M., Certel, M., Kemal Uslu, M. (2005).Microbiological and chemical properties of Tarhana during fermentation and storage as wet—sensorial properties of Tarhana soup.*LWT-Food Science and Technology, 38*(4), 409-416.

Erdogrul, Ö., Erbilir, F. (2006). Isolation and characterization of Lactobacillus bulgaricus and Lactobacillus casei from various foods.*Turkish Journal of Biology, 30*, 39-44.

Gobbetti, M., Corsetti, A., Rossi, J. (1994).The sourdough microflora. Interactions between lactic acid bacteria and yeasts: metabolism of carbohydrates. *Applied Microbiology and Biotechnology, 41*(4), 456-460.

Ibanoglu, S., Ainsworth, P., Hayes, G. D. (1996). Extrusion of tarhana: effect of operating variables on starch gelatinization. *Food chemistry, 57*(4), 541-544.

Ibanoglu, S., Ainsworth, P., Wilson, G., Hayes, G. D. (1995). The effect o fermentation conditions on the nutrients and acceptability of tarhana. *Food chemistry, 53*(2), 143-147.

Lazos, E., Aggelousis, G., Bratakos, M. (1993). The fermentation of Trahanas: a milk– wheat flour combination. Plant Foods for Human Nutrition 44, 45–62.

Lengkey, H., Balia, R., Togoe, I., Taşbac, B., Ludong, M. (2009).Isolation and identification of lactic acid bacteria from raw poultry meat.*Biotechnology in Animal Husbandry, 25*(5-6-2), 1071-1077.

Mohammed, S. I., Steenson, L. R., Kirleis, A. W. (1991). Isolation and characterization of microorganisms associated with the traditional sorghum fermentation for production of Sudanese kisra. *Applied and environmental microbiology, 57*(9), 2529-2533.

Morcos, S.R., Hegazi, S.M., El-Damhougy, S.T. (1973). Fermented foods in common use in Egypt, I. The nutritive value of kishk. Journal of the Science of Food and Agriculture 24, 1153–1156.

Salminen, S., Von Wright, A. (2011).*Lactic acid bacteria:microbiological and functional aspects*: CRC Press.

Tamime, A. Y., Barclay, M. N. I., McNulty, D., O'Connor, T. P. (1999).Kishk-a dried fermented milk/cereal mixture. 3. Nutritional composition. *Le Lait, 79*(4), 435-448.

Wood, B. J. B. (1998). Microbiology of fermented foods (2nd ed.). London: Springer.

Yilmaz, M.T., Sert, D., Demir, M.K. (2010). Rheological properties of Tarhana soup enriched with whey concentrate as a function of concentration and temperature. Journal of Texture Studies. 41(6):863-79.

Yılmazer, A.N. (1994). Tarhana üretiminde kullanılabilecek uygun bir laktik asit starter kombinasyonunun geliştirilmesi (The development of favorable lactic acid starter combination for Tarhana production), Ms. Thesis, Hacettepe University, Ankara, Turkey, 83 pp.

Sengun , I.Y., Nielsen, D. S., Karapinar, M., Jakobsen, M. (2009). Identification of lactic acid bacteria isolated from Tarhana, a traditional Turkish fermented food. International Journal of Food Microbiology. 135,105–111.