



Research Article

Volume 2 - Issue 2: 79-85 / April 2019

ISOLATION AND IDENTIFICATION OF LACTIC ACID BACTERIA FROM FERMENTED CHICKPEAS

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Received: November 11, 2018; **Accepted:** January 31, 2019; **Published:** April 01, 2019


Abstract


In this study, lactic acid bacteria were isolated from the fermented chickpeas, which is a leavening agent and phenotypically identified. Chickpea seeds were traditionally fermented and sampling was performed at the end of the fermentation (at 40°C for 16h). A total of 60 isolates of lactic acid bacteria were phenotypically identified in chickpea fermentation liquid. *Lactococcus lactis* was the dominant species (90%) in the samples, followed by *L. plantarum* (6.7%) and *L. brevis* (3.3%). According to the results, fermented chickpea is a good source as a leavening agent for chickpea-bread production.

Keywords: Chickpea, Leavening agent, *Lactococcus*, *Lactobacillus*, Identification

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Cite as: Aydın F, Çebi K. 2019. Isolation and identification of lactic acid bacteria from fermented chickpeas. *BSJ Agri*, 2(2): 79-85.

1. Introduction

Chickpea bread, a kind of traditional bread, is made by using fermented chickpea seed as a leavening agent. Being one of the main materials of natural leaveners, chickpea is also one of the most important foods in cuisine and chickpea (*Cicer arietinum* L.) is one of the most important legumes crops in the world (Singh and Basu, 2012; Silva et al., 2014; Adebo et al., 2017). In addition, it is one of the oldest and widely consumed pulses in the world; it is a staple food crop particularly in tropical and subtropical areas (Alajaji and El-Adawy, 2006; Dida Bulbula and Urga, 2018). Chickpeas are also part of the food pyramid, recommended for a healthy diet. Furthermore, chickpeas provide a significant resource for diet fiber in addition to

its rich protein, mineral (Ca, Fe, K, Mg, and P), some vitamins (thiamine, niacin, and ascorbic acid) (Meng et al., 2010; Aguilar et al., 2015) and unsaturated fatty acids (linoleic, oleic) and the essential amino acids which are insufficient in wheat flour (Zafar et al., 2015; Shrivastava and Chakraborty, 2018). Depending on its species and injection conditions, the content of the chickpea generally consists of 38–73% carbohydrates, 16–31% protein, 1.5–6% lipid, 2–10% cinder and 2–9% cellulose (Chavan et al., 1987; Akçin, 1988; Miao et al., 2009).

Chickpeas can be soaked and then cooked for eating, or used to prepare bread leaven and turnip juice. Moreover, in some regions of Turkey (especially Southern and Eastern regions) it is roasted, grinded and consumed as a coffee-like drink. In addition, various fermented products

are made of chickpea in Middle Asia, the Middle East and Africa countries. Especially in India, chickpea is found in common fried foods and appetizers such as *dhokla*, *dosa* and *idli* (Hancioğlu, 2003).

As a traditional product in Turkey, chickpea bread is produced in small bakeries or in homes. In the production, chickpea seed is used as a leavening agent. To prepare traditional chickpea leaven, chickpeas are cut into small pieces and then placed in boiled and cooled water at 45-50°C and a small amount of salt is added into the mixture. It is then left to ferment at approximately 37-40°C for 15-16 hours and fermentation can spontaneously occur. At the end of fermentation, chickpea fermentation liquid is used to prepare the chickpea dough (Tamerler, 1975; Hancioğlu, 2003). LAB is known as the most important industrial microorganisms in the production of various fermented products (Batish et al., 1997; Rees, 1997; Liu et al., 2011). LAB is generally used as a starter culture in the food industry. Due to their bacteriocin production features, they offer potential biologic protection to extend the shelf life of foods. In addition, they are an ingredient of probiotic products and some of them lead to food deterioration. (Kiran and Osmanoğlu, 2011).

Lactic acid bacteria have a key role in the fermentation of sour dough. They help the development of acidity and leave various amino acids and small peptides in the dough environment, increase the development of other microorganisms and metabolic activities, and enhance flavour, aroma, and rheological features of the dough. In addition, they retard the staling of the bread and mould and bacteria-sourced deterioration (Martinez-Anaya, 1996; Schieberle, 1996; Gobbetti, 1998; Angioloni et al., 2005).

In bread fermentation, bacterial fermentation has a significant role in the formation of flavour and aroma. In different bread types, lactic acid bacteria such as *Lactobacillus sanfrancisco*, *Leuconostoc mesenteroides*, *L. delbrueckii*, *L. fermenti*, *Pediococcus cerevisia*, *Enterococcus faecalis* are isolated (Aydn, 2000).

In the food industry, acidification and enzymatic processes which result from the development of lactic acid is used to enhance flavour, scent and texture features of various fermented foods. The interest in this bacteria group has recently increased and the researches performed in this field have deepened due to the following reasons: These bacteria are dominant in the natural flora of fermented food materials commonly consumed by people, they produce exopolysaccharide and used as starter culture, found in probiotic products and also produce a special protein called bacteriocin as well as extra shelf-life to foods. However, some lactic acid bacteria lead to the deterioration of foods (Rebecchi et al., 1998; Nout, 1994; Caplice and Fitzgerald, 1999; Diop et al., 2010; Soro-Yao et al., 2014). For these reasons, the definition of lactic acid bacteria is important both in industrial and scientific terms. The aim of the study was

to isolate and identify lactic acid bacteria in the liquid phase of a fermentation chickpea.

2. Material and Method

2.1. Preparation of Chickpea Fermentation Liquid

A total of 100 g of dried chickpeas were cut into pieces similar to wheat and put in a sterilized bottle. 350 ml boiled and cooled water at 50°C and 1 g salt were added to the mixture. This mixture was fermented at 40°C for 16 hours. At the end of the fermentation, chickpea fragments were filtered and separated and the chickpea fermentation liquid was obtained.

2.2. Physicochemical Analysis

After parallel samples were taken from the perfusate, pH values were determined by using a pH-meter (ATI ORION 420, MA 02129, USA).

2.3. Isolation and Identification of Lactic Acid Bacteria

Lactic acid bacteria were enumerated on MRS Agar (de Man Rogosa Sharpe, Oxoid) and M17 Agar (Oxoid) through the surface spread method used to isolate the lactic acid bacteria and were incubated at 30°C for 48 hours. At the end of the incubation period, 15 colonies were randomly selected and purified by streaking on on the MRS Agar and M17 Agar medium. After incubation at 30°C for 48 hours, isolates were tested to Gram strains, microscopic imaging and catalase (Bactident catalase, Merck) tests (Harrigan, 1998).

Gas production from glucose and arginine hydrolysis tests were applied on the isolates. API 50 CHL kit (BioMerieux, SA, France) was used to identify the isolates and an assessment was conducted using APIWEB™ (Apiweb™ standalone V 1.2.1 Ref 40012, Biomérieux®) and was determined according to the methods described by Schillinger and Lücke (1987). Sugar fermentation pattern was determined using API 50 CHL (BioMerieux, SA, France) and the identification was performed by APIWEB™.

3. Results and Discussion

The pH values of chickpea fragments, water and salt mixture was found to be 6.34. The pH value of chickpea leaven obtained after the chickpea fragments were fermented in pure water and salt environment for 16 hours was found to be 4.88. This situation could result from the substances produced by microorganisms during fermentation.

These results on pH value are parallel with the values found by other researchers. In studies conducted on different periods, Bayfield and Young (1967), Dıđrak and Özçelik (1991) and Özkaya (1992) reported that the pH value of the dough decreases due to the substances (lactic acid, acetic acid, CO₂, etc.) developing in the environment and after a while, this decrease in pH slows down and becomes stable in the end. Other studies showed that the produced substances in fermentation such as lactic acid, acetic acid and CO₂, affect the environment's pH value and

the most important substance to increase acidity is lactic acid (Hancioğlu, 2003; Dikbaş, 2003).

Another study on chickpea leaven was found that the pH level in the environment decrease with especially the activities of *Lactobacillus plantarum*, *Lactobacillus pentosus*, *Lactobacillus sanfrancisco*, *Enterococcus mundtii*, *Enterococcus gallinarum*, *Pediococcus urinae-equi*, *Lactobacillus viridencens* and *Streptococcus thermophilus* species and *Saccharomyces cerevisiae* (Hancioğlu 2003).

The fermentation-sourced increase in environmental acidity brings along a lot of advantages. It helps the microflora's pH levels developing in chickpea leaven and the new microflora developing during the fermentation to

decrease. In other words, it increases the environment's acidity (Työppönen et al., 2003; Devlieghere et al., 2004) and prevents the growth of undesired microorganisms, thus giving the dough a clearer flavour and aroma (Baykara, 2006).

As shown at Table 1 and 2, arginine hydrolysis and CO₂ formation tests were applied on isolates, which had feature of showing Gram positive and catalase negative reaction. The out of 60 lactic acid bacteria isolated from chickpea leaven, 54 isolates were found to be *Lactococcus lactis* ssp *lactis*, four isolates were *Lactobacillus plantarum* and two isolates were *Lactobacillus brevis*.

Table 1. Gram reaction, catalase activity, arginine hydrolysis and CO₂ formation of isolates obtained from M17 agar.

Isolate Code	Gram Reac.	Catalase	Arginine	CO ₂	Name of isolates
M17 S1	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
M17 S3	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
M17 S4	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
M17 S7	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
M17 S8	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
M17 S9	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
M17 S10	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
M17 S12	+	-	+	-	<i>Lactobacillus plantarum</i>
M17 S13	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
M17 S14	+	-	+	+	<i>Lactobacillus brevis</i>
M17 S15	+	-	+	+	<i>Lactobacillus brevis</i>
M17 S16	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
M17 S18	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
M17 S21	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
M17 S22	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
M17 S23	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
M17 S25	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
M17 S27	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
M17 S29	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
M17 S30	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
M17 S31	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
M17 S33	+	-	+	-	<i>Lactobacillus plantarum</i>
M17 S35	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
M17 S36	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
M17 S37	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
M17 S38	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
M17 S39	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
M17 S42	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
M17 S43	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
M17 S44	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>

There are a very limited studies conducted on isolated and identified lactic acid bacteria from chickpea leaven. Sáez et al. (2018) isolated and identified LAB from kabuli chickpeas planted and consumed in northwestern Argentina and an important increase (P < 0.05) of total mesophiles and LAB counts were seen from the first day of fermentation raising from initial values of 4.57 ± 0.34 and 0.88 ± 0.35 log CFU/g of unfermented flours (doughs) to 10.40 ± 0.39 and 9.61 ± 0.21 log CFU/g after 5 back-slopping steps, respectively. In a study, cowpeas and chickpeas were allowed to undergo natural fermentation for 4 days at 25°C. The microorganisms isolated from cowpeas and chickpeas were *Lactobacillus casei*, *L.*

leichmanii, *L. plantarum*, *Pediococcus pentosaceus* and *P. acidilactici*. *Lactobacillus helveticus* was found in chickpeas only (Zamora and Fields, 1979). In the another study the major bacteria identified in the soaked fermented chickpea were *Clostridium* sp., *Bacillus* sp. and *Enterococcus* sp. but only *Clostridium* sp. produced gas from sugar and hydrolysis gluten during dough fermentation which gives Kaak it is special texture (Kyyaly et al., 2017). In similar researchers studying the microflora of fermented chickpea perfusate, it was found that *Lactobacillus* spp., *Pediococcus* spp. and *Clostridium* spp. are dominant species in the environment (Katsaboxakis and Mallidis, 1996). In a study conducted

on microflora of chickpea leaven, *Enterococcus mundtii*/*E. gallinarum*, *E. casseliflavus*, *Lactobacillus plantarum*/*pentosus*, *L. sanfrancisco*, *L. viridescens*, *L. bifermantans*, *Pediococcus urinea-equi*, *Streptococcus thermophilus*, *Lactococcus lactis* subsp. *cremoris* were found as lactic acid bacteria (Hancioğlu, 2003).

Table 2. Gram reaction, catalase activity, arginine hydrolysis and CO₂ formation of isolates obtained from MRS agar.

Isolate Code	Gram Reac.	Catalase	Arginine	CO ₂	Name of type
MRS S1	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
MRS S2	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
MRS S3	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
MRS S4	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
MRS S5	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
MRS S6	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
MRS S7	+	-	+	-	<i>Lactobacillus plantarum</i>
MRS S9	+	-	+	-	<i>Lactobacillus plantarum</i>
MRS S10	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
MRS S12	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
MRS S16	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
MRS S19	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
MRS S21	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
MRS S22	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
MRS S24	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
MRS S25	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
MRS S26	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
MRS S27	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
MRS S28	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
MRS S29	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
MRS S30	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
MRS S31	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
MRS S35	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
MRS S37	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
MRS S38	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
MRS S39	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
MRS S40	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
MRS S41	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
MRS S42	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>
MRS S44	+	-	+	-	<i>Lactococcus lactis</i> ssp <i>lactis</i>

In that study, only one species (*Lactobacillus plantarum*) was found to be the same as the species we isolated from chickpea leaven. *Lactococcus lactis* ssp *lactis* determined as dominant species in our study and *Lactobacillus brevis* were not found in this study. In another study ton sour dough, it was found *Carnobacterium divergens* (*L. divergens*), *L. brevis*, *L. amylophilus*, *L. sakei*, *L. acetotolerans*, *L. plantarum*, *Pediococcus pentosaceus*, *P. acidilactici* and *Tetragenococcus halophilus* (*Pediococcus halophilus*) (Gül, 2005). Gerçekaslan (2012) identified as *Lactobacillus plantarum* (61 isolate), 15 isolates *Lactococcus lactis* subsp *lactis*, 9 isolates *L. brevis*, 6 isolates *L. pentosus*, 3 isolates *Leuconostoc mesenteroides* subsp. *mesenteroides*, 3 isolates *L. fermentum*, 2 isolates *Leu. lactis*, 1 isolate *L. curvatus* subsp *curvatus*, 1 isolate *Leu. mesenteroides* subsp. *cremoris*, 11 isolates *Lactobacillus* spp. and 1 isolate *Leuconostoc* spp. from sourdough. Zhang et al. (2011) found *Lactobacillus plantarum* (37 isolates), *Leuconostoc citreum* (14 isolates), *Weissella cibaria* (10 izolot), *Weissella confusa* (7 isolate), *Lactobacillus paralimentarius* (8 isolate), *Lactobacillus helveticus* (7 isolate), *Lactobacillus mindensis* (4 isolate), *Lactococcus lactis* (3 isolate), *Lactobacillus curvatus* (3 isolate), *Lactobacillus rossiae* (3

isolate), *Leuconostoc mesenteroides* (2 isolate), *Lactobacillus brevis* (2 isolate), *Lactobacillus guizhouensis* (2 isolate), *Lactobacillus sanfranciscensis* (2 isolate), *Lactobacillus crustorum* (2 isolate), *Enterococcus durans* (1 isolate) and *Lactobacillus fermentum* (1 isolate). Reale et al. (2011) identified *Lactobacillus plantarum* (22 isolate), *Lb. brevis* (17 isolate), *Lb. casei* (2 isolate) and *Lb. paracasei* (2 isolate) in sourdough. *Lactococcus lactis* ssp. *lactis*, *Lactobacillus plantarum* and *Lactobacillus brevis* species that were identified from different sourdough was also found in our study. Also, Rizzello (2014) found *Lactobacillus plantarum*, *Lactobacillus sanfranciscensis*, *Leuconostoc mesenteroides*, *Lactobacillus fermentum*, *Weissella cibaria*, *Lactobacillus pentosus*, *Lactobacillus coryneformis*, *Lactobacillus rossiae*, *Lactobacillus brevis*, *Lactobacillus parabuchneri* and *Lactobacillus paraplanarum* in wheat-legume (chickpea, lentil and bean) sourdough. While *Lactobacillus plantarum* and *Lactobacillus brevis* determined in study, *Lactococcus lactis* ssp. *lactis* did not identified in the study. *Lactococcus lactis* ssp. *lactis* that was identified in our study is able to produce several bacteriocins including nisin, the best characterized and only bacteriocin

considered safe for consumption (GRAS, generally regarded as safe) (Teuber, 1995; Chen and Hoover, 2003; Cotter et al., 2005; Ortolani et al., 2010; Hyde et al., 2006; Soro-Yao, 2014). Additionally, it is known that many food-related lactic acid bacteria (*Enterococcus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, *Lactobacillus*, *Carnobacterium* and *Propionibacterium*) have antimicrobial features in food fermentation (Nout, 1994). These features ensure food safety and extend shelf life (Omemu and Faniran, 2011; Okerere et al., 2012; Ekwem, 2014; Soro-Yao, 2014).

It has been thought for centuries that metabolites of these bacteria present no danger for health in fermented foods and antimicrobial metabolites of LAB are regulatory agents. In addition, it is known that fermented foods and some LABs have natural, healthy and positive effects on consumer health (Hoover and Steenson, 1993; Vuyst and Vandamme, 1994). Because of all these features, these bacteria have become a very important to be need hard work on both industry and scientific perspective.

4. Conclusion

According to the results of this study, *Lactococcus* is the dominant flora in chickpea leaven with *Lactococcus* ssp. *lactis* being the dominant species, followed by *Lactobacillus plantarum* and *Lactobacillus brevis*. These bacteria, which were widely found in commercial starter culture preparates, were found in samples. Furthermore, in chickpea leaving production, lactic acid bacteria provides a low pH level for initial fermentation conditions such as 6.34. This value after 16 hours fermentation decreased to 4.88. Thus, isolating lactic acid bacteria strains can help to produce healthier and long life products.

Conflict of interest

The authors declare that there is no conflict of interest.

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