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Research Article

# Isolation and Identification of Lactic Acid Bacteria from Homemade Natural Pickles

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## Abstract

Pickles are both a traditional and commercial product type formed as a result of fermentation of fruits or vegetables. Many types of pickles prepared by traditional methods can be produced in different ways in terms of raw materials and chemical properties such as salt ratio and acidity. The aim of this study was to determine the existing natural microflora in home pickles and to identify the dominant species. 15 traditional home pickles were used as material for microbiological and some chemical analyses. Isolation of LABs (Lactic acid bacteria) from pickle samples and identification of the isolates by classical methods and API test kits were carried out. In the study, a total of 130 pickle-derived strains were isolated and 88 of them were grouped as Lactobacillus and 42 as Pedicoccus. In addition, total aerobic mesophilic bacteria, yeast and mould, lactobacillus lactococcus counts and pH, acidity and salt content were determined from microbiological parameters and chemical analyses. As a result, it was determined that the dominant flora present in the home pickle samples belonged to *L. plantarum*.

## Introduction

Fermentation is an effective preservation method for controlling pathogenic bacteria and microorganisms that cause food spoilage, which cause significant changes in the taste, aroma and nutritional properties of foods [1]. The basic principle of food preservation by fermentation is the breakdown of carbohydrates and the production of alcohol, $CO_2$ derivatives and various organic acids as end products [2,3]. Fermented products are foods obtained as a result of natural fermentation of plant and animal foods spontaneously or with the help of starter culture [4]. As a result of fermentation, products with high nutritional value, versatile, different taste and aroma, economically valuable, long-term preservation and unique products are obtained [5]. This method has been applied for many years in the preparation of some foods or in making them durable, and with this method, besides the absence of significant losses in the nutritional values of the raw materials, the desired flavour can be achieved by improving the sensory properties. In the production of pickles, which is one of the foods produced by fermentation, a single vegetable species is used as well as mixed vegetable species. The processing of these vegetables into pickles is completed with classification, washing processes, brining, fermentation, storage and packaging processes after harvesting and transport processes. The most effective stage on the final product is especially the fermentation and storage stage. In the fermentation stage, it is the lactic acid bacteria that are the main fermentation agent and give the product its properties by forming lactic acid in the product [6]. Lactic Acid Bacteria (LAB), which carry out fermentation, consist of strains of species found in the raw material and spontaneously developed in the product. The related Lactic Acid Bacteria suppress other microorganisms in the environment and manage the process that results in the decrease of pH and also the formation of organoleptic properties of fermented products [7].

Lactic acid fermentation is used in many traditionally produced fermented foods and beverages. Lactic acid fermentation is a method that has been preferred and applied for thousands of years in the preservation of fruits and vegetables, as well as a process that allows the production of highly consumable foods as a result of significant changes in the taste, aroma and structure of foods. Pickles have an important place among the foods where lactic acid fermentation is applied [5]. Pickle is a product which is formed by fermentation of vegetables and fruits with lactic acid bacteria in brine with a certain salt concentration or in their own water and has a long shelf life with the protective effect of lactic acid and salt in the environment. Various pickles are produced by subjecting many fruits and vegetables to lactic acid fermentation [8]. Pickles are generally consumed directly as an appetiser with meals and are frequently used in the preparation of various salads and canapés. Lactic acid fermentation not only increases the durability of the product, but also gives it its unique characteristics in terms of taste and colour [9]. The aim of this study was to identify Lactic Acid Bacteria (LAB) isolates from home pickles made by traditional method by using classical methods and API test kits and to determine the dominant flora species. It is also aimed to determine some microbiological and chemical properties of pickle samples.

## Materials and Methods

### Material

In the study, 15 different homemade pickle samples were used as material. The pickles were taken from different houses and brought to the laboratory in cold chain and analysed.

### Methods

In this study, isolation of lactic acid bacteria from pickle samples and physiological and biochemical tests were performed and the isolates were identified using API 50 CHL test kits. Lactic acid bacteria, yeast-mould, and total aerob mesophyll bacteria counts and chemical analyses of pH, titration acidity and salt were also performed.

### Preparation of dilutions from pickle samples

Sterile physiological water containing 0.85% NaCl and 0.1% peptone was used as dilution liquid. Ten ml each of the brine of the samples were transferred to Stomacher bags and 90 ml of sterile physiological water was added. This mixture was omogenized in a Stomacher (BagMixer 400, Intersience International, France) for 5 min and dilutions were obtained.

Decimal dilutions were then prepared for each sample using the same dilution liquid [10].

### Isolation of lactic acid bacteria from pickle samples

For the isolation of lactic acid bacteria, MRS (de Man Rogosa Sharpe) agar (Oxoid) and M17 agar (Oxoid) were inoculated using the spreading method. The plates cultured on MRS agar were incubated under anaerobic conditions and the plates cultured on M17 agar were incubated under aerobic conditions at 30 °C for 24 hours 30 and at the end of incubation, colonies with different characteristics were selected and inoculated on MRS agar by drawing method and the plates were incubated at 30 °C for 48 hours. In order to obtain pure cultures from single fallen colonies, triple drawings were made on the relevant medium and the microscopic appearance of the pure cultures obtained were examined and Gram staining, catalase and oxidase tests were performed. Gram (+), catalase and oxidase (-), cocci or rod-shaped bacteria were preserved as frozen cultures in liquid media containing 20% glycerol at -80 °C for identification purposes [11].

API (Analytical Profile Index) test kits are widely used today to identify microorganisms. Studies have shown that lactic acid bacteria can be easily identified at species/subspecies level with API 50 CHL test kit [12,13]. The results obtained from the test kit are identified with the bacterial species in the database of the test computer programme. API 50 CHL kit (bioMérieux®SA, France) was used for the identification of the isolates and the evaluation was performed by APIWEBTM.

### Other microbiological analyses of pickle samples

For total aerobic mesophilic bacteria (TAMB) count, Plate Count Agar (PCA) (Oxoid) was inoculated by smear method and incubated at 30-32 °C for 48 hours, for yeast-mould count, Potato Dectrose Agar (PDA) (Oxoid) was inoculated and incubated at room temperature (25 °C) for 5 days, For the enumeration of lactic acid bacteria, MRS (de Man, Rogosa, Sharpe Agar) and M17 (Oxoid) agars were inoculated and the plates inoculated on MRS agar were incubated under anaerobic conditions and the plates inoculated on M17 agar were incubated under aerobic conditions at 30 °C for 24 hours [10].

### Chemical analyses

The brine of pickle samples were analysed for pH, total acidity (% lactic acid) and % salt content. Thermo Orion Star A211 type pH meter was used for pH analysis. pH meter was calibrated with standard buffer solutions and then the measurement process was carried out. The salt content of pickle samples was determined according to (Anonymous. [14] and the % acidity of the samples was determined by titrating the samples with 0.1 N NaOH [8].

### Statistical analysis

Data were statistically compared using SPSS for Windows Release ver. 20 package programme was used for statistical comparison. Results are given as mean ± standard deviation (SD).

## Results

The distribution of lactic acid bacteria isolated and identified from pickle samples by traditional method according to the samples is given in Table 1. microbiological analysis results are given in Table 2 and chemical analysis results are given in Table 3. A total of 130 strains were isolated from the pickle samples used in the study. Among these, 88 strains (67.69%) belonged to Lactobacillus genus (*L. brevis*, *L. pentosus*, *L. plantarum*, *L. delbrueckii spp. lactis*, *L. Buchneri*, *L. acidophilus* and *L. paracasei spp. paracasei*). 42 strains (32.31%) belonged to the genus Pediococcus (*P.pentosaceus*, *P. damnosus* and *Pediococcus spp.*). Of the 88 Lactobacillus isolates, 33 were *L. plantarum* (25.38%), 14 were *L. brevis* (10.76%), 5 were *L. delbrueckii spp. lactis* (3.85%), 11 were *L. pentosus* (8.46%), 6 *L. buchneri* (4.62%), 13 *L. acidophilus* (10%) and 6 *L. paracasei spp. paracasei* (4.62%). Of the 42 Pediococcus isolates, 23 were identified as *P. pentosaceus* (17.69%), 11 as *P. damnosus* (8.46%) and 8 as *Pediococcus spp* (6.15%).

**Table 1:** Distribution of Lactic acid bacteria isolated and identified from homemade pickle samples by samples.

| Species | KT1 | KT1 | KT1 | KT1 | KT1 | KT1 | KT1 | ST1 | ST1 | ST1 | ST1 | ST1 | BT1 | BT1 | BT1 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *L. plantarum* | 3 | 1 | 2 | - | 2 | 1 | - | 3 | 5 | 2 | 4 | 3 | 2 | 3 | 2 | 33 |
| *L. brevis* | 5 | - | - | 1 | 2 | - | - | 1 | - | 2 | 2 | - | 2 | - | 1 | 14 |
| *L. pentosus* | 2 | - | 3 | - | - | 1 | 1 | 2 | - | - | 1 | - | 1 | - | - | 11 |
| *L. buchneri* | 1 | - | - | - | 2 | - | - | - | - | 1 | 1 | - | - | 1 | - | 6 |
| *L. delbrueckii spp. lactis* | - | - | 2 | 1 | - | - | - | - | - | 1 | - | - | 1 | - | - | 5 |
| *L. acidophilus* | 3 | - | 2 | 1 | - | - | - | 2 | - | 1 | - | - | 2 | - | 2 | 13 |
| *L. paracasei spp. paracasei* | - | - | 1 | - | - | 2 | - | - | 1 | - | - | - | - | 2 | - | 6 |
| *P. damnosus* | 1 | - | 3 | 1 | - | - | - | 2 | - | 1 | 1 | - | 1 | 1 | - | 11 |
| *Pedicoccus spp.* | - | - | 2 | - | 1 | - | - | 2 | - | 1 | - | - | 1 | - | 1 | 8 |
| *P. pentosaceus* | - | 3 | 2 | 1 | - | - | 2 | 6 | 2 | 2 | - | - | 3 | 2 | - | 23 |
| *Total* | 15 | 4 | 17 | 5 | 7 | 4 | 3 | 18 | 6 | 11 | 9 | 3 | 13 | 9 | 6 | 130 |

KT: Mixed Pickle; ST: Cucumber Pickle; BT: Chilli Pickle.

**Table 2:** Microbiological analysis results of homemade pickle samples (LogCFU/ml).

| Pickles of types | TAMB Count | Lactobacillus Count (MRS) | Lactococci Count (M17) | Yeast-Mild Count |
|---|---|---|---|---|
| Mixed 1 | 5.63 | 6.64 | 5.63 | 4.73 |
| Mixed 2 | 5.94 | 7.01 | 5.61 | 3.34 |
| Mixed 3 | 4.84 | 6.6 | 5.25 | 2.85 |
| Mixed 4 | 5.25 | 7.02 | 2.73 | <1 |
| Mixed 5 | 4.87 | 7.05 | 5.76 | 3.82 |
| Mixed 6 | 5.13 | 6.96 | <1 | <1 |
| Mixed 7 | 5.02 | 7.55 | <1 | 3.38 |
| Cucumber 1 | 5.00 | 6.67 | 2.45 | 4.23 |
| Cucumber 2 | 4.54 | 6.15 | 2.13 | 4.38 |
| Cucumber 3 | 4.32 | 5.81 | 1.46 | 3.95 |
| Cucumber 4 | 5.01 | 6.03 | <1 | 4.01 |
| Cucumber 5 | 4.52 | 5.81 | <1 | 3.74 |
| Pepper 1 | 2.85 | 5.98 | 1.45 | 2.7 |
| Pepper 2 | 3.14 | 6.01 | 2.67 | 2.82 |
| Pepper 3 | 3.08 | 5.63 | <1 | 3.35 |
| Minimum | 2.85 | 5.63 | <1 | <1 |
| Maximum | 5.94 | 7.55 | 5.63 | 4.73 |
| Mean | 6.54 | 7.68 | 3.17 | 4.41 |

**Table 3:** Chemical analysis results of homemade pickle samples.

| Samples | pH | Total Acidity (%lactic acid) | Salt Concentration (%) |
|---|---|---|---|
| Mixed 1 | 3.24 | 0.98 | 2.63 |
| Mixed 2 | 3.44 | 1.20 | 2.87 |
| Mixed 3 | 3.54 | 1.00 | 3.25 |
| Mixed 4 | 3.55 | 1.12 | 3.73 |
| Mixed 5 | 3.87 | 1.02 | 4.16 |
| Mixed 6 | 3.63 | 0.96 | 4.05 |
| Mixed 7 | 3.82 | 1.25 | 4.32 |
| Cucumber 1 | 3.54 | 1.37 | 4.45 |
| Cucumber 2 | 3.92 | 1.68 | 3.95 |
| Cucumber 3 | 3.62 | 0.93 | 3.73 |
| Cucumber 4 | 3.41 | 1.80 | 3.65 |
| Cucumber 5 | 3.52 | 1.82 | 3.57 |
| Pepper 1 | 3.50 | 1.18 | 3.55 |
| Pepper 2 | 3.65 | 1.20 | 3.67 |
| Pepper 3 | 3.70 | 1.16 | 3.45 |
| Minimum | 3.24 | 0.82 | 2.63 |
| Maximum | 3.92 | 1.82 | 4.45 |
| Mean | 4.96 | 1.73 | 4.70 |

## Discussion

It was determined that lactobacilli and pediococci were highly present in the microbial flora of pickle samples Yıldız [13]. Lactobacillus (47.9%) and Pediococcus (52.03%) were identified as Lactobacillus (47.9%) and the dominant species was *P. pentosaceus* (43 isolates) followed by *L. plantarum* (23 isolates), L. brevis (15 isolates), *P. damnosus* (13 isolates), L. collinoides (10 isolates), L. pentosus (9 isolates), *Pediococcus spp* (8 isolates) and L. buchneri (2 isolates). *P. pentosaceus* and *L. plantarum* bacterial species were isolated from cabbage and cucumber pickles [15]. In the identification of 41 traditionally produced home-made pickles from different regions by molecular method, it was reported that 100 of the bacteria were lactobacilli, 66 were lactococci and 10 were enterococci [16]. It has been determined in different studies that *Leuconostoc mesenteroides*, *L. plantarum*, *L. brevis*, *L. brevis*, *L. pentosus* and *P. pentosaceus* constitute the microbial flora of cabbage, cucumber and olive fermentations [11,17]. Lactic acid bacteria were isolated and identified from 17 traditional pickle samples collected from different provinces of Turkey and the functional characteristics of the isolates were characterised. As a result of genotypic differentiation, 21 strains belonging to 9 different species were identified. It was stated that Lactobacillus plantarum strain was more numerous [18]. It was observed that the distribution of lactic acid bacteria species in pickle samples was similar to the studies [11,13,15,17].

It was determined that the dominant flora present in the home pickle samples belonged to L. plantarum and *P. pentosaceus*. Since *L. plantarum* has higher acid tolerance than other lactic acid bacteria, it completes the last stage of natural fruit and vegetable fermentation. The growth and fermentation activity of *L. plantarum* in cucumber and cabbage fermentations greatly influences the microbial stability and quality of the final product [19]. The Total Aerob Mesophilic Bacteria (TAMB) counts of pickle samples were determined as 3.08 Log CFU/ml, the lowest 3.08 Log CFU/ml, the highest 5.94 Log CFU/ml and the average 4.59 Log CFU/ml. The highest number of TAMB was counted in mixed 2 pickles, followed by mixed 1 and mixed 4 pickles, respectively. The lowest TAMB count was determined in pepper pickle 1. The number of TAMBs was determined as 2.39-4.34 Log CFU/mL (mean 4.02 log CFU/mL) in bean pickle samples made by the traditional method specific to the Black Sea Region [20]. In 41 traditional home pickle samples from different regions, the average number of TAMB was determined as 5.33 Log CFU/ml, yeast-mould number as 4.45 Log CFU/ml, Lactobacillus bacteria as 4.45 log cfu/ml and Lactococcus bacteria as 3.13 Log CFU/ml [16]. Yıldız [13]. In pickle samples produced by traditional method, the number of TAMB in the range of 3.04-6.36 Log CFU/ml (average 4.84 Log CFU/ml), the number of yeast moulds in the range of 4.57 Log CFU/ml the number of lactobacilli bacteria in the range of 5.81-8.09 (average 6.65 Log CFU/ml) and the number of lactococci bacteria in the range of 2.27 Log CFU/ml. The average TAMB counts determined in pickle samples were close to the value of Yıldız [13]. and lower than the value determined by Dündar [16] in pickle samples.

The highest number of Lactobacilli bacteria in the samples was determined in mixed 7, 5 and mixed 4 pickles. The lowest LAB count was determined in chilli pickles. The lowest Lactobacillus bacteria count of pickle samples was 5.63 Log CFU/ml, the highest was 7.5 Log CFU/ml and the average was 6.44 Log CFU/ml. The determined values were higher than the values found by (Odabaş and Metin [20]. in bean pickles (mean average 3.94 Log CFU/ml) and Dündar [16]. in different pickle samples. The highest number of lactococci bacteria cultured on M17 agar of pickle samples was found in sample 5 of mixed pickle samples, while the lowest number was found in pickle samples of pepper 1 and cucumber 3. The average number of Lactococcus bacteria in pickle samples was 2.32 Log CFU/ml, which was close to the value determined by Yıldız [13]. (average 2.27 Log CFU/ml) and lower than the value found by Dündar [16] (average 3.13 Log CFU/ml) in different pickle samples. The yeast-mould counts of the pickle samples varied between <1-4.73 Log CFU/ml, the average was 3.13 Log CFU/ml, these values belong to yeasts and no mould growth was observed. It is thought that the detection of mould growth is due to the pre-treatments such as washing during the preparation of raw materials and the anaerobic environment due to the presence of the samples in jars. The highest yeast count in pickle samples was determined in mixed 1 pickle, while the lowest yeast count was determined in pepper 1 and 2 pickles. No yeast was found in the mixed 4 and 6 pickle samples. The pH values of the pickle samples made at home by traditional method were between 3.24-3.92 (mean 3.56), acidity between 0.82-1.82% (mean 1.23% lactic acid) and salt ratios between 2.63-4.45% (mean 3.56%). The pH values of bean pickle samples made with the traditional method specific to the Black Sea Region were found to be between 3.40-3.65, and the salt amounts were between 3.98%-5.89% (Odabaş and Metin [20]. In 41 traditional home pickle samples from different regions, pH was found to be 3.81, % salt 3.26 and % acidity 0.62 on average [16].

Yıldız [13] Reported the pH values of pickle samples as pH 3.51 in the range of 3.39-3.96 and the average pH as 3.51 and the amount of salt in the range of 1.25-5.11% and the average amount of salt as 3.66%. İspirli and Dertli [18]. Stated that the pH values in pickle samples were in the range of 2.97-6.67. The lowest salt content of pickle samples was 2.63%, the highest salt content was 4.45% and the average salt content was

3.56%. The average salt content was close to the values found by Yıldız [13]. (average salt content 3.66%) and Dündar [16] (average 3.26%). When the pickle standards (TS 4200, TS 11112) are examined, it is stated that the amount of salt in the brine can be 6-7% at most, although it varies according to the type of pickled raw material [21,22]. Accordingly, it was determined that the salt amounts of the analysed pickle samples were below the values specified in the standards. According to the pickle standards (TS 4200, TS 4214), it is stated that the acidity amount should be at least 0.5% and at most 2.0%. Accordingly, it is seen that the total acidity amount of the pickle samples examined is within the range specified in the standards [21,23].

## Conclusion

This study was carried out to isolate and identify lactic acid bacteria and yeasts from homemade pickles samples produced by natural fermentation and to determine some chemical and microbiological properties. According to the results of microbiological analysis of homemade pickle samples, it was determined that lactic acid bacteria constituted the dominant flora in pickles. As a result of the evaluation, it was determined that 88 (67.69%) of the lactic acid bacteria in pickle samples were Lactobacillus strains and 42 (32.30%) were Pediococcus strains. Of the 88 Lactobacillus isolates, 33 were L. plantarum, 14 were L. brevis, 5 were *L. delbrueckii spp.* lactis, 11 were L. Pentosus, 6 were *L. buchneri*, 13 were *L. acidophilus* and 6 were *L. paracasei spp.* paracasei (4.62%). Of the 42 Pediococcus isolates, 23 were identified as *P. pentosaceus*, 11 as *P. damnosus* and 8 as *Pediococcus spp.* According to the results obtained, *L. plantarum* was the dominant species among lactobacilli and *P. pentosaceus* was the dominant species among pediococci in pickle samples. No mould growth was observed in any of the pickle samples examined. It is thought that the absence of mould growth may be due to the fact that the raw materials to be used in pickle production undergo pre-treatments such as washing and sorting during the preparation of pickles and the samples are stored in jars, providing an anaerobic environment. In addition, the development of lactic flora in pickles can be shown as a reason for the absence of mould in pickles.

As a result, it is thought that our traditional pickle samples show high LAB diversity and studies on the activities of some of these strains and their use in the industry, especially as bioprotective starters, will be carried out in the future to increase the variety of natural starter cultures and to intensify their industrial use.

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