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Determination of lactic acid bacteria in Kaşar cheese and identification by Fourier transform infrared (FTIR) spectroscopy

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Lactic acid bacteria (LAB) arise in Kaşar cheese, an artisanal pasta filata cheese produced in Turkey from raw milk without starter addition or pasteurized milk with starter culture. In this study, 13 samples of Kaşar cheese that were produced from raw milk were used as reference materials. LAB were characterized by using phenotypic, API and Fourier transform infrared (FTIR) spectroscopy methods. One hundred and fifty-seven (157) strains were isolated from 13 cheese samples, and identification test was performed for 83 strains. At the end of the study, a total of 22 *Lactococcus* sp., 36 *Enterecoccus* sp. and 25 *Lactobacillus sp.* were determined in the isolated strains by phenotypic identification. After identification with FTIR spectroscopy and statistical analysis, expected results were not taken by FTIR spectroscopy due to higher correlation (> % 99) obtained with more than one reference culture. Fewer number of reference strains was a limitation of the analysis. Therefore, identification should be made with more reference bacteria in the FTIR analysis and should also be supported with molecular techniques.

Key words: Kaşar cheese, lactic acid bacteria, identification, Fourier Transform Infrared (FTIR).

INTRODUCTION

Fourier transform infrared (FTIR) spectroscopy is used for characterization of many microorganisms. It is useful at the serogroup, species and genus level, data acquisition is faster than in PAGE and other techniques and preparation is simple and quick. FTIR spectroscopy and cluster analysis of bacterial cells is a promising tool not only for identification and classification purposes based upon bacterial IR spectra alone, but also as an additional aid to support conventional methods for clarifying relationships, especially within poorly classified taxa (Helm et al., 1991). Phenotypic and biochemical methods such as morphological and physiological properties, API CHL50 kit are useful at genus-species level but they are insufficient at sub species level for characterization of LAB (Kıran and Osmanağaoğlu, 2011). Molecular genetic techniques, such as randomly

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amplified polymorphic DNA analysis PCR-based methods have been successfully used to identify LAB at the subspecies level but it is difficult to adapt them for their use in routine laboratories due to their high costs and the requirement for highly skilled personnel (Kirschner et al., 2001). It is important to be able to identify and characterize LAB in the dairy industry with rapid, reliable and cheap methods (Amiel et al., 2000).

Kaşar is a semi-hard "pasta filata" (plastic curd) cheese which is produced by being boiled and kneaded after curd of the cheese is acidified (Üçüncü, 2008). Kaşar is one of the most produced and consumed cheese type in Turkey and in some of Balkan and European countries (with different names like Kaşkaval, Kassari, Kachkawaj) (Halkman and Halkman, 1991). Kaşar is produced in Turkey from raw milk without starter addition or pasteurized milk with addition starter culture. Therefore, Kaşar cheese produced from raw milk is fermented by lactic acid bacteria originated from the milk. The number of systematic studies aiming determination of starter culture in Kaşar cheese is very few (Akyüz, 1978; Tunail, 1978; Halkman and Halkman, 1991). The use of the right starter cultures is essential and is important to produce Kaşar cheeses with a constant high quality.

The main purpose of the present study was to compare biochemical identification methods with FTIR cluster method for identification of LAB isolated from Kaşar cheese. Also, the FTIR spectra of the isolates were compared to a spectrum library to obtain FTIR identification. Two methods were applied in this study in order to evaluate the relevance and mutual agreement between methods, and the capacity of each method to accurately identify different LAB species present in Kaşar cheese.

MATERIALS AND METHODS

Isolation of lactic acid bacteria from cheese samples and identification tests

A total of 13 Kaşar cheese samples supplied from various factories were used as reference materials under laboratory conditions. The reference strains for the characterization of the isolates from samples with FTIR spectroscopy were provided by Izmir Institute of Technology (IIT), Department of Food Engineering. Biochemical characterization was performed in order to identify isolates using carbohydrates. BD BBL ™ Crystal ™ Gram-Positive ID kits were used for the isolates that were determined as cocci. BioMerieux API 50 CHL (Ref. 50410 to 10 × 10 ml) test kits were used for the isolates that were determined as bacilli. Appropriate dilutions of cheese samples were prepared using the isolation of bacteria, and were plated in agar culture media. Isolation of *Lactococcus* sp. was performed by plating M17 (Merck) medium at 28°C for 24 to 48 h. While *Lactobacilli* sp. was isolated by plating MRS agar (Merck) medium at 30°C for 48 h, isolation of *Enterecoccus* sp. was carried out by using NB (Merck) medium at 37°C for 24 to 48 h at incubation (Tunail et al., 2001).

Morphological characteristics, catalase activities, litmus milk reductions and gram reactions of the isolates were performed as the physiological analyzes. Biochemical tests (growing such conditions 10, 15, 45°C, 6.5% NaCl, 4% NaCl, pH 9.2 to 9.6, 0.1 to 0.3% metylen blue reduction, Voges-Proskauer reaction, forming ammoniac from arginine, citrate reduction, 0.04% tellurite reduction, forming gas from glocuse and sugar fermentation tests) were carried out according to the Sherman classification (Sandine et al., 1962; Cowan and Steel, 1966; Harrigan and McCance, 1966; Tunail, 1978; Sürmeli, 1979; Tunail and Köşker, 1986; Salminen and Wright, 1993; Holt et al., 1994; Tunail et al., 2001). API 20 was applied for isolation of *Enterecoccus* sp. and *Lactococcus* sp. After the basic analysis, sugar and API50CHL tests were conducted for lactobacilli (Tunail et al., 2001).

Identification of lactic acid bacteria with FTIR analysis

After growing the isolates in the appropriate media, they were centrifuged at 5000 r.p.m. The pellet was washed and later lyophilized according to procedures previously described by Irudayaraj et al. (2002), Kuleaşan and Başyiğit Kılıç (2007) and Başyiğit Kılıç (2009).

Preparation of samples for FTIR spectroscopy

Ten milligram of lyophilized cultures was crushed together with approximately 300 mg of KBr in agate mortar. The mixture was transferred into 10 mm diameter disks and placed under 10 tons/cm² pressures for 10 min. The disks were analyzed by FTIR (Perkin Elmer, Spectrum 100). Spectra samples between 4000 and 450 cm^{-1} wavelengths were taken in the analysis. Each sample was scanned 64 times at 4 cm^{-1} resolution. The study was conducted with two parallels (Başyiğit Kılıç, 2009).

Evaluation of absorbance data

After reading the absorbance values obtained from the range of 4000 to 450 cm^{-1} wavelengths, they were analyzed by the use of the Statistical Package for the Social Sciences. Similar rates of isolates were determined with the aid of Pearson correlation product moment coefficient at regions of 3000 to 2800, 1500 to 1400, 900 to 700 cm^{-1} wavelengths in order to identify and separate strains. Hierarchical cluster analysis was applied to the isolates whose similarity rates were over 99% and dendogram was drawn with Ward's algorithm (Naumann et al., 1990).

RESULTS AND DISCUSSION

Results of phenotypic analysis

A total of 157 isolates were obtained from 13 cheese samples. Physiological and biochemical analysis were further conducted with 83 isolates in terms of morphological characteristics, catalase activities, and gram reactions. Biochemical tests were carried out based on Sherman classification. Cocci that could grow under conditions such 10, 15, 45°C, 6.5% NaCl and pH 9.6 were identified as enterococci. The rest of the cocci strains were evaluated as lactococci.

Taking these criteria and their phenotypic characteristics into account, 36 of 83 isolates were identified as *Enterecoccus* sp. and 22 of them were identified as *Lactococcus sp*. The rest of the 25 isolates which were morphologically identified as bacilli were evaluated as

Lactobacillus sp., they were capable to grow at a temperature of 15°C. Seventeen (17) of 22 isolates which were identified as *Lactococcus sp*. was estimated as *Lactococcus lactis* ssp*. lactis* (*L. lactis*), and three of them was estimated as *L. lactis* ssp*. cremoris* (*Lactococcus cremoris*), and two of them was estimated as *L. lactis* ssp*. lactis biovar. diacetylactis* (*Lactococcus diacetylactis*). In contrast to expected, five of these isolates that was identified as *L. lactis* could be grown in pH 9. 6 medium conditions, the other five could be grown at 45°C temperature, and seven of them could also be grown in medium which had 6.5% NaCl additive. Similar atypical reactions, in which strains were growing in various conditions such as pH 9.6, 45°C and 6.5% NaCl, were also observed for *Lactococcus sp.* isolates in various studies such as that of Tunail et al. (2001) who worked on Feta cheese, Bulut (2003)'s work on Pottery cheese and Kırmacı (2010)'s work on Urfa cheese. Unexpected positive and negative reactions also occurred in the tests where *Lactococcus* sp*.* was used to distinguish as subspecies. The results were evaluated as the subspecies-specific atypical reactions. It was noticed that the five isolates which were identified as *L. diacetylactis* and *L. lactis* could not generate ammoniac from arginine. In Garde et al. (1999) study, 13 of 57 arginine positive isolates isolated from cheeses produced from raw milk, were determined as *L. cremoris* and 44 of them were determine as *L. lactis*. Our study found out that, two isolates identified as *L. cremoris* could grow in a medium of 4% NaCl additive.

As a result of biochemical tests which provide identification based on physiological characteristics and nutrient requirements of bacteria, it is known that bacteria give atypical reactions because of adaptation to different conditions. Tolerance of bacteria isolated from cheeses with high salt concentration or whose clot is boiled, increasing with increase temperature or salt concentrations, respectively. Hence, it is believed that these cultures can adapt to these environment. Due to the resistance of difficult circumstances of *Enterecoccus* sp*.,* 36 isolates which showed positive reaction particularly to conditions such as 6.5% NaCl, pH 9.6, and temperature of 10 to 45°C, were separated as *Enterecoccus* sp*.,* 27 of them were identified as *Enterococcus faecalis* (*E. faecalis*) due to the 0.04% telluride reduction. 12 of 27 *E. faecalis* isolates could produce CO_2 by using citrate. *Entrecoccus* sp. is used as a starter culture in foods due to their properties such as lipolytic and esterolytic activity, citrate utilization and synthesis of volatile aromatic compounds (Vuyst et al., 2002; Giraffa, 2003; Erginkaya et al., 2007). *Enterococcus faecium* and *E. faecalis* in particular, can contribute to provide flavor for cheese due to their ability of producing compounds such as acetaldehyde, ethanol, diacetyl and acetone (Franz et al., 2003; Giraffa, 2003; Erginkaya et al., 2007). Twenty-four (24) *Enterecoccus* sp*.* were identified as 14 *E. faecalis*, five *Enterococcus durans*, three *Enterococcus hirae*, two *E. faecium* by using Gram-positive ID kit. The 11 *E. faecalis* isolates were similarly identified through both biochemical and carbohydrate tests but three of them was evaluated as *E. hirae* (two isolates) and *E. durans* (one isolates) as a result of a biochemical (carbohydrate) test.

Biochemically, identified 25 lactobacilli strains were evaluated as 12 *Lactobacillus plantarum (Lb. plantarum)* and 13 *Lactobacillus* sp*.* as a result of sugar tests and 16 of them were evaluated as seven *Lb. plantarum,* 7 *Enterococcus curvatus* and two *Enterococcus fermentum* by using API 50 CHL kit. Seven isolates were identified as *L. plantarum* as a result of both biochemical and carbohydrate tests (API 50 CHL kit) (Sharpe, 1979; Balows et al., 1991; Durlu-Özkaya et al., 2001).

Results of FTIR spectroscopy analysis

Bacterial spectra were formed as a result of chemical bonds in bacteria which absorbed infrared rays. The study found that spectra of a total of 91 samples (83 bacterial isolates and eight reference cultures) were taken at the range of 4000 and 450 cm^{-1} wavelength and 4 cm^{-1} resolution. Each sample was scanned 64 times with two parallel. The most dominant absorbance peaks were observed at ~ 3400, ~ 1600, ~ 1300, ~ 1100, ~ 600 cm^{-1} wavelengths. These wavelengths are divided into five windows by Naumann et al. (1990): 1. P1, Fatty acid region I (3000 to 2800 cm^{-1}); 2. P2, Amide Region (1800 to 1500 cm^{-1}); 3. P3, Combined Region (1500 to 1200 cm^{-1}); 4. P4, Polysaccharide Region (1200 to 900 cm^{-1}); 5. P5, Correct Fingerprint Region (900 to 700 cm^{-1}) windows.

FTIR spectrum of one of the reference strain at range of 4000 to 450 cm^{-1} wavelengths are shown in Figure 1. Pegram (2007) determined the dominant peaks to consist of water (3500 cm^{-1}), amide bonds (1700 to 1500 cm^{-1}), fatty acids (1500 to 1400 cm^{-1}) and polysaccharides (1200 to 900 cm^{-1}) regions. The separation of the bacteria at species and subspecies level was performed considering the same peaks of parallel bacteria, and different peaks of different bacteria at different absorbance. The fingerprint spectra were observed to be different and it was determined that *Pediococcus pentosaceus*, *Pediococcus acidilacdici*, *L. lactis ssp. lactis*, *Lactobacillus casei 201*, *L. casei 202*, *Lactobacillus helveticus*, *Pediococcus fluorescens* B 52 and *P. fluorescens* AFT 29 bacteria gave different peaks at repeated spectra. Pearson correlation product moment coefficient was applied to samples at all the 4500 to 450 cm^{-1} regions, like the P1 (3000 to 2800 cm-1) fatty acids region, P2 (1800 to 1500 cm^{-1}) amide region, P3 (1500 to 1200 cm^{-1}) combined region, P4 (1200 to 900 cm^{-1}) polysaccharide region and P5 (900 to 700 cm^{-1}) fingerprint region in order to determine the extent of similarity between two parallel of isolates and reference

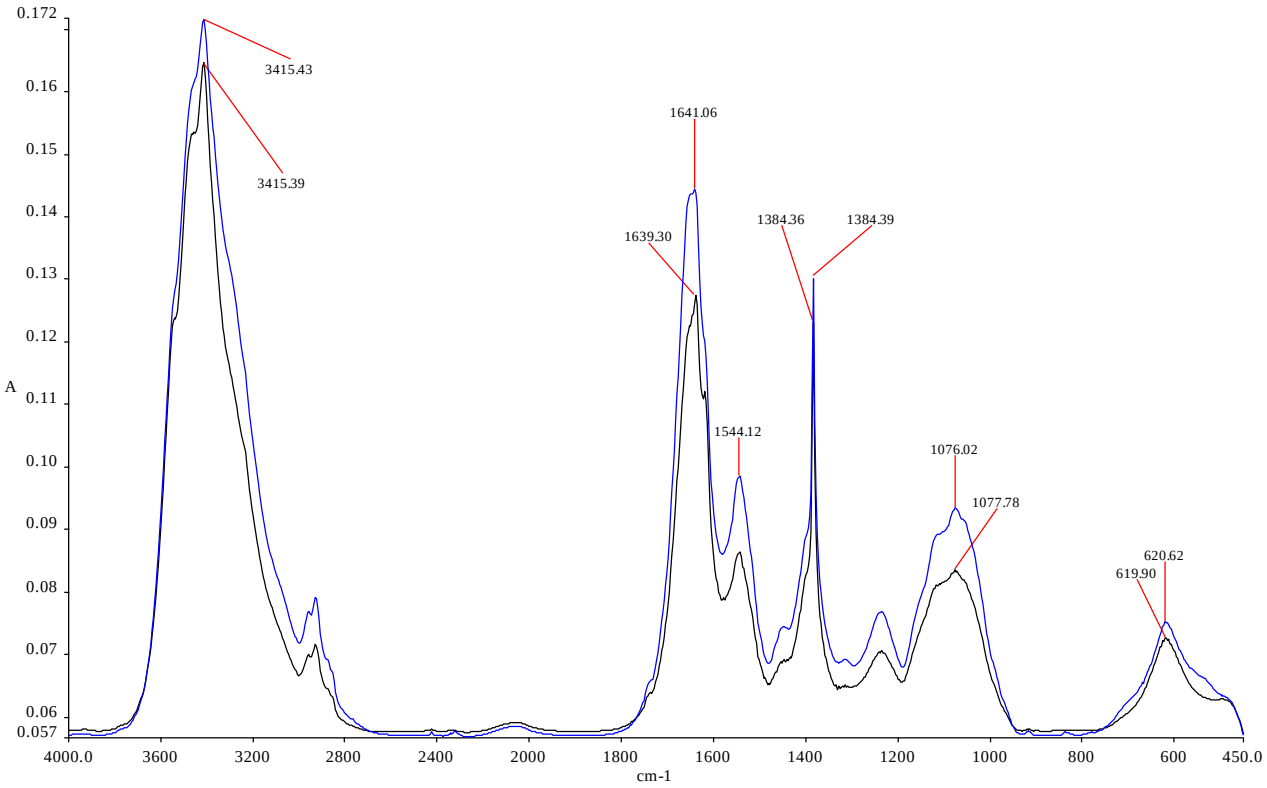

Figure 1. FTIR spectra of *L. lactis* at range of 4000 to 450 cm^{-1} wavelengths.

Table 1. Pearson correlations between the parallels of reference isolates at different wavelengths (Dziuba et al., 2007).

Reference strains	4000-450 cm^{-1} (%)	3000-2800 cm^{-1} (%)	1800-1500 cm^{-1} (%)	1500-1200 cm^{-1} (%)	1200-900 cm^{-1} (%)	900-700 cm^{-1} (%)
L. lactis	99.2	99.6	99.2	99.6	99.4	98.8
L. cremoris	99	99.4	83.2	99	96.9	35.3
L. diacetylactis	99.7	100	94	99.8	99.7	64
E. faecalis	97.6	99.7	99.2	97.4	100	99.7
E. faecium	99.1	99.5	94.5	98.4	98.6	94.5
Lb. plantarum	99.3	99.9	98.8	98.4	99.9	100
Lb. brevis	100	99.9	98.2	99.9	98.8	81.9
Str. thermophilus	96.2	98.2	94.8	96.1	95.7	91.1

strains samples (Table 1). Naumann et al. (1990) determined that the highest correlation was reached at the experiment with recurrence of reference strains at the following ranges; 3000 to 2800, 1500 to 1400 and 900 to 700 cm^{-1}. According to this study, correlation of the reference strains were found such as *L. lactis ssp. lactis* 99.9%, *L. lactis ssp. cremoris* 99.9%, *L. lactis ssp. lactis biovar. diacetylactis* 100%, *E. faecalis* 99.7%, *E. faecium* 99.9%, *Lactobacillus brevis* 99.9%, *L. plantarum* 99.9% and *Streptococcus thermophilus* 99.5%.

Similarity percentage of each isolates and each reference strains between their parallels were determined at the specified absorbance intervals (3000 to 2800, 1500 to 1400 and 900 to 700 cm^{-1}). Isolates and reference strains which have appropriate similarity rate (> 99%) with their parallels were averaged. Then, similarity percentages of averaged isolates with the averaged reference strains were determined by using Pearson correlation product moment coefficient. Isolates codes, biochemical tests results, API test results and isolates which gave the highest correlation ratios with the reference strains are given in Tables 2, 3 and 4.

According to Pearson correlation product moment coefficient, hierarchical clustering analysis was applied

Table 2. Comparison of the test results of *Lactococcus* sp.

Isolate	Biochemical tests results	Gram positive ID kit test results	API (%)	Pearson similarity (1. / 2. correlation)	r_{y1y2} (%)
İLc1	*L. lactis*	*L. lactis*	99.97	-	-
İLc2	*L. diacetylactis*	*L. lactis*	99.99	*E. faecalis* *L. lactis*	99.8 99.7
İLc3	*L.. cremoris*	-	-	*L. cremoris* *L. diacetylactis*	99.8 99.7
İLc4	*L. cremoris*	-	-	*E. faecalis* *L. lactis*	99.8 99.7
İLc5	*L. lactis*	*L. lactis*	99.99	-	-
iLc6	*L. lactis*	*L. lactis*	94.66	*L. cremoris* *L. diacetylactis*	99.7 99.6
İLc7	*L. lactis*	*L. lactis*	99.82	*L. cremoris* *L. diacetylactis*	99.7 99.7
İLc8	*L. lactis*	*L. cremoris*	94.66	*L. cremoris* *L. diacetylactis*	99.7 99.7
İLc9	*L. lactis*	*L. lactis*	99.49	*E. faecalis*	72.4
İLc10	*L. lactis*	*L. lactis*	99.95	*L. cremoris* *E. faecalis*	99.6 99.6
İLc11	*L. cremoris*	*Leuconostoc mesenteroides*	-	*L. lactis* *E. faecalis*	99.9 99.9
İLc12	*L. lactis*	*L. lactis*	99.99	*L. diacetylactis* *L. cremoris*	100 99.9
İLc13	*L. lactis*	*L. cremoris*	99.42	*L. diacetylactis, Str. thermophilus ve E. faecium*	99.9
İLc14	*L. lactis*	-	-	*E. faecalis* *L. lactis*	99.4 99.3
İLc15	*L. lactis*	*L. lactis*	91,21	*E. faecalis* *L. lactis*	97.2 96.7
İLc16	*L. lactis*	*L. lactis*	91.21	*L. lactis* *Str. thermophilus*	100
İLc17	*L. lactis*	-	-	-	
İLc18	*L. lactis*	*L. lactis*	99.99	*L. lactis* *E. faecalis*	95.6 95.6
İLc19	*L. lactis*	-	-	-	
İLc20	*L. diacetylactis*	-	-	*L. lactis* *Str. thermophilus ve E. faecalis*	100 99.9

Table 2. Contd.

İLc21	*L. lactis*	*L. lactis*	99.99	*E. faecalis* *L. lactis*	99.9 99.7
İLc22	*L. lactis*	*L. lactis*	99.99	*E. faecium* *L. diacetylactis,* *Str. thermophilus* *ve L. cremoris*	100 99.9

Table 3. Comparison of the tests results of *Enterecoccus* sp.

Isolate	Biochemical tests results	Gram positive ID kit test results	API (%)	Pearson similarity (1. / 2. correlation)	r_{y1y2} (%)
İE23	*Enterecoccus sp.*	-	-	*E. faecalis*	99.6
İE24	*Enterecoccus sp.*	*E. durans*	99.95	*E. faecium* *L. cremoris*	99.8
İE25	*E. faecalis*	*E. faecalis*	99.99	*L. diacetylactis* *L. cremoris*	99.8
İE26	*Enterecoccus sp.*	*E. durans*	99.99	*L. cremoris* *E. faecium* *L. diacetylactis*	99.8 99.7
İE27	*Enterecoccus sp.*	*E. durans*	99.99	*L. cremoris* *E. faecium* *L. diacetylactis*	99.7 99.6
İE28	*E. faecalis*	*E. faecalis*	99.99	*E. faecalis*	99.6
İE29	*Enterecoccus sp.*	*E. durans*	99.99	*E. faecalis* *L. lactis*	99.6 99.2
İE30	*E. faecalis*	-	-	*L. cremoris* *E. faecium* *L. diacetylactis*	100
İE31	*E. faecalis*	*E. faecalis*	99.87	*E. faecalis* *L. lactis*	99.7 99.7
İE32	*E. faecalis*	-	-	*E. faecalis* *L. lactis*	98.8 98.6
İE33	*Enterecoccus sp.*	-	-	*L. lactis* *Str. thermophilus*	100 99.9
İE34	*E. faecalis*	-	-	*L. lactis* *Str. thermophilus*	100
İE35	*E. faecalis*	*E. faecalis*	99.99	*Str. thermophiles* *L. lactis* *E. faecalis*	100 99.9
İE36	*E. faecalis*	*E. faecalis*	99.67	*Str. thermophiles* *L. lactis* *E. faecium*	100

Table 3. Contd.

İE37	*E. faecalis*	-	-	*E. faecalis* *L. lactis*	99
İE38	*E. faecalis*	-	-	*L. cremoris* *E. faecium* *L. diacetylactis*	100
İE39	*E. faecalis*	*E. faecalis*	99.67	*E. faecalis*	90
İE40	*E. faecalis*	*E. faecalis*	99.67	*E. faecalis* *L. lactis*	90.7 90.5
İE41	*E. faecalis*	-	-	*E. faecalis* *L. lactis*	99.9 99.8
İE42	*E. faecalis*	-	-	-	-
İE43	*E. faecalis*	*E. faecalis*	78.72	*E. faecalis* *L. lactis*	99.7 99.4
İE44	*E. faecalis*	*E. faecalis*	97.87	*L. cremoris, L. diacetylactis* *E. faecium* *Str. thermophilus*	100
İE45	*E. faecalis*	*E. faecalis*	99.97	*L. cremoris,* *E. faecium Str. thermophilus*	100
İE46	*E. faecalis*	*E. faecalis*	99.97	*E. faecalis*	99.8
İE47	*E. faecalis*	*E. faecalis*	99.97	*E. faecium Str. thermophilus*	100
İE48	*E. faecalis*	-	-	*L. diacetylactis*	100
İE49	*E. faecalis*	-	-	*L. diacetylactis L. cremoris*	100
İE50	*E. faecalis*	-	-	*L. diacetylactis*	100
İE51	*E. faecalis*	*E. hirae*	99.27	*L. lactis*	100
İE52	*E. faecalis*	*E. hirae*	98.88	-	-
İE53	*E. faecalis*	*E. durans*	99.95	*L. cremoris, L. diacetylactis* *E. faecium*	100
İE54	*E. faecalis*	*E. faecalis*	99.86	*E. faecalis* *L. lactis*	99.7 99.2
İE55	*E. faecalis*	*E. faecalis*	98.32	*L. cremoris,* *E. faecium*	99.8
İE56	*Enterecoccus sp.*	*E. faecalis*	99.96	*L. cremoris, L. diacetylactis* *E. faecium*	100

Table 3. Contd.

İE57	*Enterecoccus sp.*	*E. hirae*	98.59	*L. cremoris, L. diacetylactis* *E. faecium*	100
İE58	*Enterecoccus sp.*	*E. faecalis*	99.97	*L. cremoris,* *E. faecium*	99.7

Table 4. Comparison of the tests results of *Lactobacillus sp.*

Isolate	Biochemical tests results	Gram positive ID kit test results	API (%)	Pearson similarity (1. / 2. correlation)	r_{y1y2} (%)
İLb59	*Lactobacillus* sp.	*Lb. curvatus*	93.9	*Lb. plantarum* *Lb. brevis*	99.7 99.5
İLb60	*Lactobacillus* sp.	*Lb. curvatus*	99.9	*Lb. brevis* *Lb. plantarum*	99.9 99
İLb61	*Lactobacillus* sp.	-	-	*Lb. brevis* *Lb. plantarum*	100 98.9
İLb62	*Lb. plantarum*	*Lb. plantarum*	93.9	*Lb. brevis* *Lb. plantarum*	99.9 99.4
İLb63	*Lactobacillus sp.*	-	-	*Lb. brevis* *Lb. plantarum*	99.8 99.6
İLb64	*Lb. plantarum*	*Lb. plantarum*	93.9	-	-
İLb65	*Lb. plantarum*	*Lb. plantarum*	93.9	-	-
İLb66	*Lb. plantarum*	-	-	*Lb. brevis* *Lb. plantarum*	99.8 99.6
İLb67	*Lactobacillus* sp.	-	-	*Lb. brevis* *Lb. plantarum*	100 98.9
İLb68	*Lb. plantarum*	*Lb. plantarum*	93.9	*Lb. brevis* *Lb. plantarum*	99.9 99.3
İLb69	*Lactobacillus* sp.	-	-	*Lb. brevis* *Lb. plantarum*	99.9 99.5
İLb70	*Lb. plantarum*	-	-	*Lb. brevis* *Lb. plantarum*	99.9 99.4
İLb71	*Lb. plantarum*	-	-	*Lb. brevis* *Lb. plantarum*	100 99.1
İLb72	*Lb. plantarum*	-	-	*Lb. brevis* *Lb. plantarum*	99.9 99.2
İLb73	*Lb. plantarum*	-	-	*Lb. plantarum* *Lb. brevis*	99.8 99.5
İLb74	*Lactobacillus* sp.	*Lb. fermentum*	98.10	*Lb. brevis* *Lb. plantarum*	99.9 99.6

Table 4. Contd.

İLb75	*Lb. plantarum*	*Lb. plantarum*	93.9	*Lb. brevis* *Lb. plantarum*	100 99.2
İLb76	*Lactobacillus* sp.	*Lb. curvatus*	99.9	*Lb. plantarum* *Lb. brevis*	100 98.9
İLb77	*Lactobacillus* sp.	*Lb. fermentum*	98.10	*Lb. brevis* *Lb. plantarum*	99.9 99.2
İLb78	*Lb. plantarum*	*Lb. plantarum*	93.9	*Lb. brevis* *Lb. plantarum*	99.9 99.5
İLb79	*Lb. plantarum*	*Lb. plantarum*	93.9	*Lb. plantarum* *Lb. brevis*	96.8 92.7
İLb80	*Lactobacillus* sp.	*Lb. curvatus*	99.8	*Lb. brevis* *Lb. plantarum*	99.6 99.3
İLb81	*Lactobacillus* sp.	*Lb. curvatus*	99.8	*Lb. brevis* *Lb. plantarum*	99.8 98.9
İLb82	*Lactobacillus* sp.	*Lb. curvatus*	9.8	*Lb. brevis* *Lb. plantarum*	99.6 99
İLb83	*Lactobacillus* sp.	*Lb. curvatus*	99.8	*Lb. brevis* *Lb. plantarum*	99.8 99

to cultures whose similarity conditions were analyzed and isolates were divided into clusters by using the Ward method. Isolates evaluated as *Lactococcus sp.* were divided into two different clusters and while all of the reference strains were gathered in one cluster, 4 isolates (İLc4, İLc10, ILC3 and İLc2) formed a different cluster (Figure 2). Isolates considered to be *Enterecoccus* sp. were divided into four different clusters in two main clusters (Figure 3). While all of the reference strains were gathered in one main cluster, 7 isolates were collected in the other main cluster. Isolates evaluated as *Lactobacillus sp.* were divided two different clusters (Figure 4). While all of the reference strains were gathered in one cluster, 4 isolates (İLb80, İLb81, İLb82 and İLb83) formed a different cluster. Distances between isolates found in a different cluster outside the main cluster where the reference strains were found, were also considered. It was believed that those isolates might be originating from a different variety of isolates outside the known references.

Naumann et al. (1990) could successfully cluster *Staphylococcus*, *Streptococcus* and *Clostridium* at species level, at the ranges of 3000 to 2800, 1200 to 900, 900 to 700 cm^{-1} by applying cluster analysis and Pearson Pearson correlation product moment coefficient. Naumann et al. (1990) also applied cluster analysis to Clostridium type bacteria at the range of 1200 to 900 and 1500 to 1200 cm^{-1} region. In our study, appropriate fingerprint regions of reference strains could not be used to determine the statistical analysis due to inadequate reference strains been difficult to separate species to subspecies due to similar genotypic structure of LAB species. In order to identify distinctive fingerprint regions of LAB species using FTIR spectroscopy, it is thought that particular species are needed to compare with more than one reference strains according to different studies. For this reason, identification at species level using FTIR spectroscopy was not successfully carried out.

Conclusion

As a result, differences between the biochemical and the carbohydrate test results revealed the importance of the genotypic characterization at LAB's characterization. Twelve of 22 *Lactococcus* isolates were identified as *L. lactis* with biochemical and carbohydrate tests. However, this number was reduced to 6 following FTIR analysis. While 14 out of 36 *Enterecoccus* isolates were identified as *E. faecalis*, only 10 isolates were identified with FTIR analysis. Also, while 12 out of 25 *Lactobacillus* isolates were identified as *L. plantarum*, only four isolates were identified with FTIR analysis. 15.66% of total isolates were identified as *L. lactis*, 16.87% of total isolates were identified as *E. faecalis* and 8.43% of total isolates were identified as *L. plantarum*. It has been determined that in

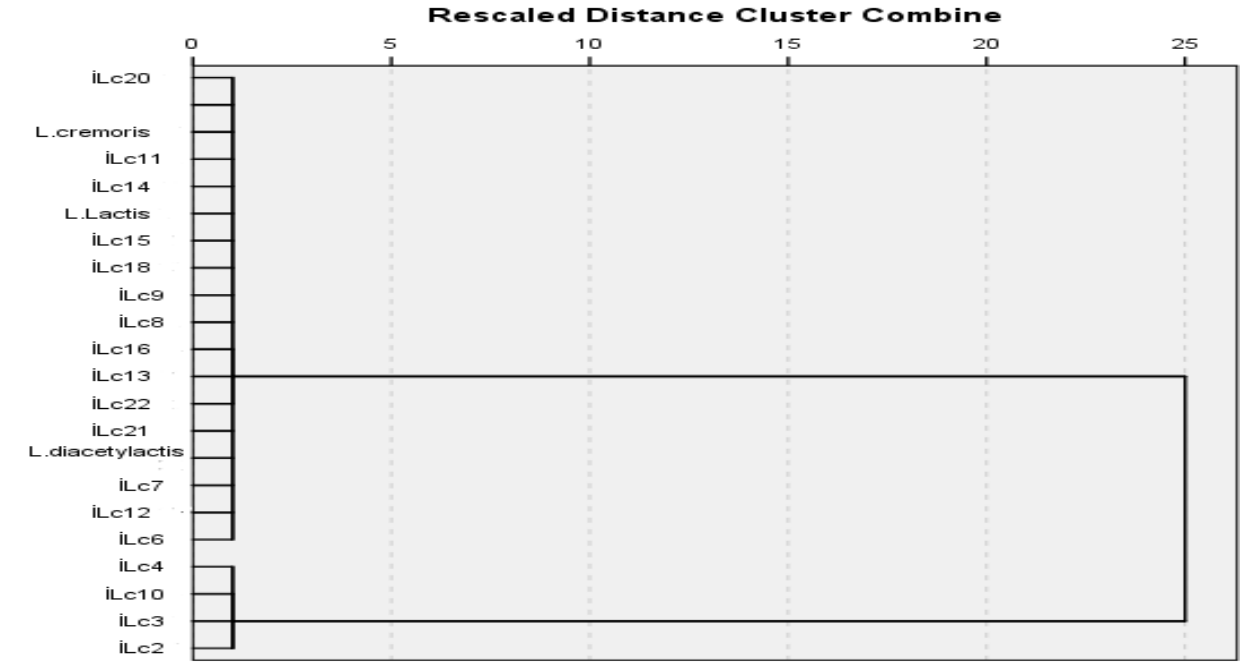

Figure 2. Dendogram of *Lactococcus sp.* by Ward's method.

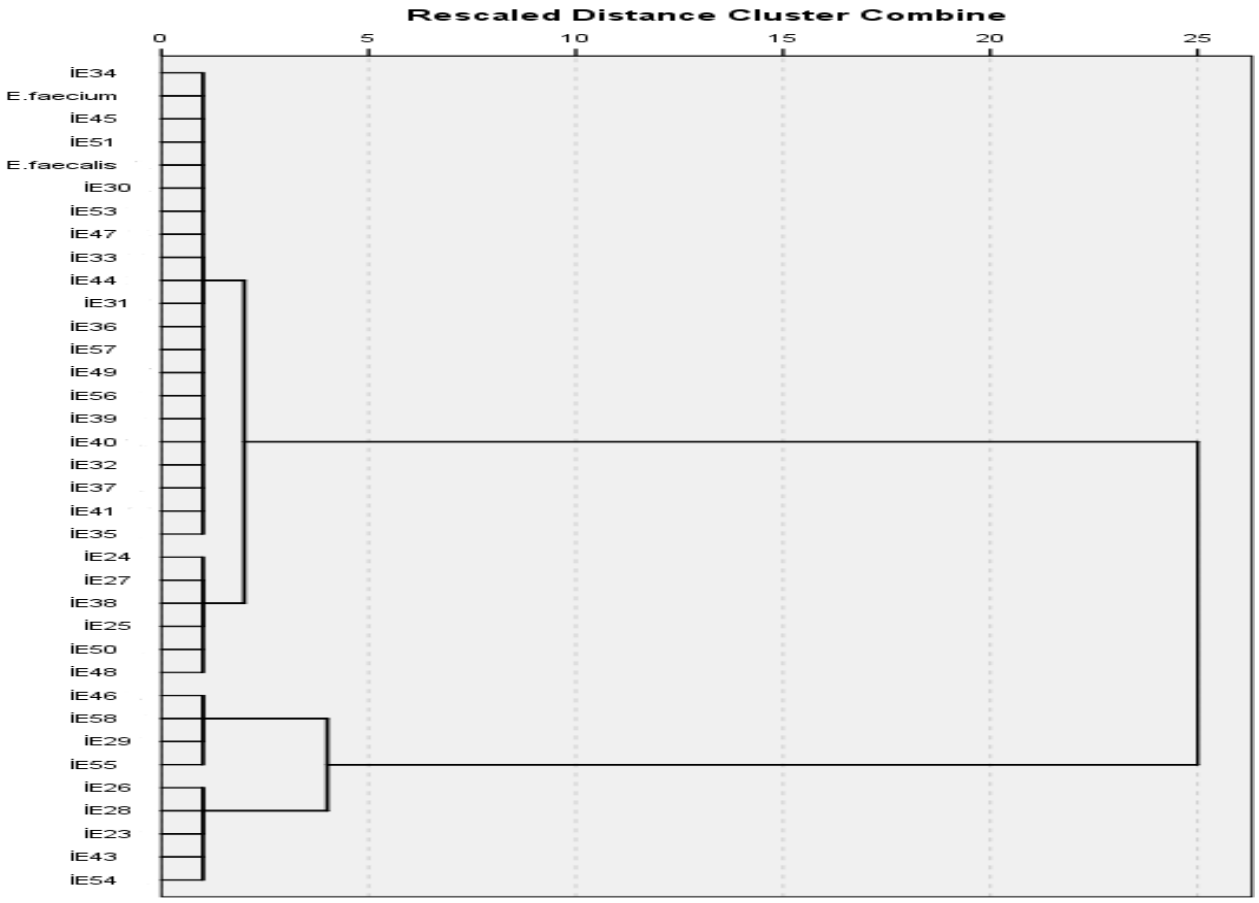

Figure 3. Dendogram of *Enterecoccus* sp. by Ward's method.

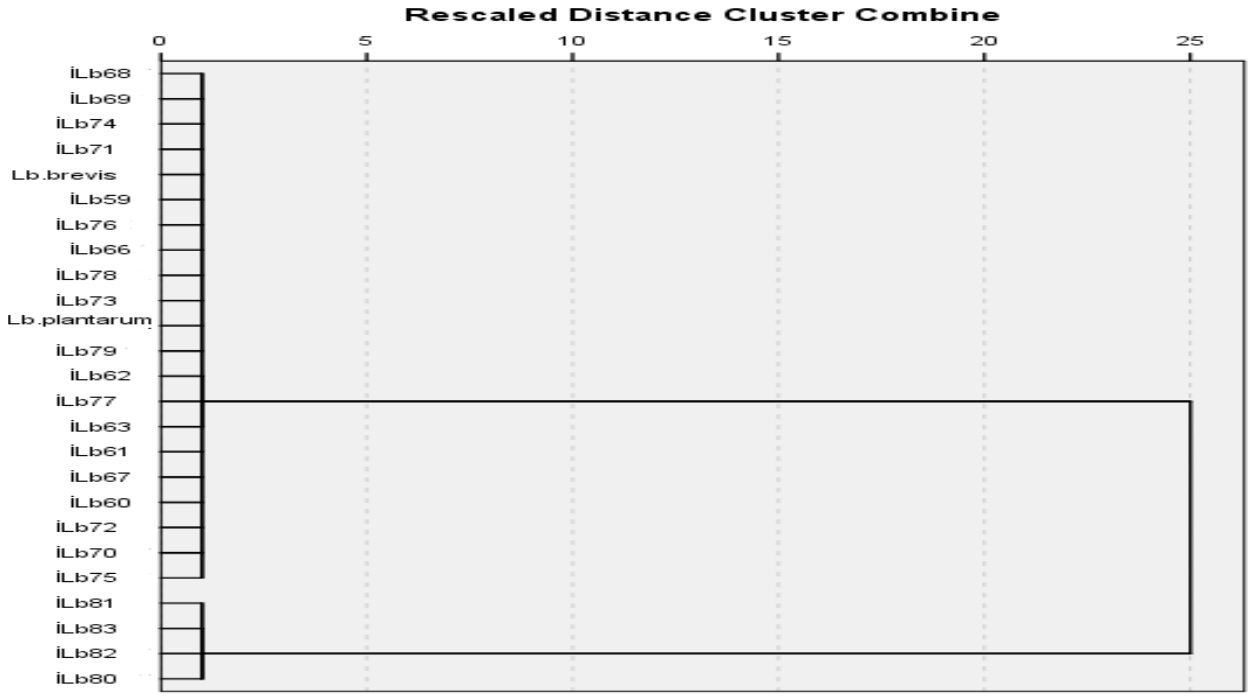

Figure 4. Dendogram of *Lactobacillus sp.* by Ward's method.

order to succeed in the identification of LAB with FTIR spectroscopy, a number of reference strains should be increased.

Conflict of interests

The authors did not declare any conflict of interest.

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